Biological control of tomato leaf miner *Tuta absoluta* using entomopathogenic nematodes

By

Taha Omar Ali Ben Husin

A Thesis Submitted for the Degree of Doctor of Philosophy

School of Biology

Faculty of Science, Agriculture and Engineering

Newcastle University

United Kingdom

January 2017

Abstract

Increasing resistance to chemical insecticides in field populations of tomato leafminer, *Tuta absoluta*, has motivated research on alternative control measures. Biological control with entomopathogenic nematodes (EPNs) can be an alternative, particularly against larval instars. In foliar application, EPNs encounter many factors that affect their survival and efficacy adversely. This thesis has investigated: (1) the efficacy of some commercial EPNs against different stages of *Tuta absoluta* (larvae, pupae and adults) in Petri dish, leaf and soil bioassays, (2) factors affecting EPNs on tomato leaves and (3) impact of some organic and non-organic adjuvants on efficacy of EPNs at 75 ± 10 % and 45 ± 10 % RH. Results showed high adult and larval mortality of *Tuta absoluta* using *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* in dish and leaf bioassays in optimum conditions (> 90 % RH). *S. feltiae* was the most virulent species, followed by *S. carpocapsae* then *H. bacteriophora.* Larval susceptibility increased throughout larval development. Pupae in contrast were less susceptible to nematode infection. In soil, *S. feltiae* and *S. carpocapsae* were significantly more virulent than *H. bacteriophora* against fourth larval instar when they drop to pupate in the soil and against adults when they are emerging from pupae. All factors studied (relative humidity, temperature, repeat application, method of application and time for EPNs to enter a leaf) affected the efficacy of EPNs to some extent, but relative humidity (RH) was the most important factor. EPNs' efficacy and survival decreased as RH declined. The addition of Barricade® II and xanthan gum to aqueous suspensions of *S. feltiae* and *S. carpocapsae* resulted in increasing their efficacy and survival. What is more, they reduced the loss of nematodes into the soil in run-off by depositing them on the leaf and they assured even distribution of nematodes in the spray tank by preventing them from settling down. Moreover, Barricade® II (1.5 %) reduced the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf. Barricade® II is the candidate adjuvant to be used with *S. feltiae* for foliar application against *T. absoluta*.

_____

Dedication

I would like to dedicate this work to my parents, my wife,

daughter (Jenna) and my siblings

أهدي هذا العمل الى والدي ووالدتي وزوجتي وابنتي (جنى)

وأخوتي وأخواتي

_____

_____

ACKNOWLEDGMENT

_____

Roger Furness for his help with computer issues;

Mark Bendall for his laboratory safety advice;

All the staff and students who supported me.

Last but most importantly, I reserve the greatest acknowledgement for my mother and my wife, Amina Irhouma, for their love, support and patience.

Table of Contents

_____

_____

List of Tables

_____

List of Figures

_____

_____

_____

_____

_____

_____

Chapter 1 General introduction

1.1 Tomato leafminer (TLM) *Tuta absoluta*

1.1.1 Classification of **Tuta absoluta**

Tuta absoluta, also known as the tomato leafminer, is classified as follows:

Phylum Arthropoda, class Insecta, order Lepidoptera, suborder Glossata, superfamily Gelechioidea, family Gelechiidae, subfamily Gelechiinae, tribe Gnorimoschemini and species *Tuta absoluta* (USDA–APHIS, 2011).

The generic assignment of *Tuta absoluta* has been revised repeatedly; initially, it was defined as *Phthorimaea absoluta* by Meyrick (1917) based on a single adult male found in Peru. It was subsequently described as *Gnorimoschema absoluta* by Clarke (1965), *Scrobipalpula absoluta* by Povolny (1964) and Becker (1984) cited in USDA–APHIS (2011), *Scrobipalpuloides absoluta* by Povolny (1987) and finally as *Tuta absoluta* by Povolny (1994).

1.1.2 Origin and distribution of **Tuta absoluta**

Tuta absoluta is believed to be indigenous to Central America; it has subsequently spread to South America where it has been considered a pest in Argentina, Brazil, Bolivia, Colombia, Chile, Ecuador, Peru, Paraguay, Venezuela and Uruguay since the early 1980s (Barrientos *et al.*, 1998; Estay, 2000) cited in Urbaneja *et al.* (2013). It is considered that the propagation of *Tuta absoluta* through South America was facilitated by fruit trade (Cáceres, 1992) cited in Urbaneja *et al.* (2013).

The pest continued to spread and reached Europe in 2006, where it was first detected in the northern part of Castellón de la Plana in eastern Spain, and continued to spread throughout the Spanish Mediterranean region where it caused severe damage (Desneux *et al.*, 2010). By 2009, the pest had reached other Mediterranean countries such as Italy, France, Greece, Portugal, Morocco, Algeria and Tunisia (EPPO, 2008b; EPPO, 2008c; EPPO, 2009a; EPPO, 2009d; EPPO, 2009b; EPPO, 2010; EPPO, 2011a). The pest had also reached more northern European countries such as Germany, the Netherlands, Switzerland and the UK (EPPO, 2009c; Potting, 2009; EPPO, 2010). By 2010, *Tuta absoluta* had reached central Europe,

having been reported in Albania, Bulgaria and Romania; and Lithuania (EPPO, 2009d; EPPO, 2010; Ostrauskas and Ivinskis, 2010) and also the Middle East, having been reported in Bahrain, Iraq, Jordan, Kuwait, Saudi Arabia and Syria (EPPO, 2011a). Since 2010, this pest has also been reported in many other Middle Eastern, African and Asian countries reaching the far east to India and the far south and south east of Africa (Nigeria and Tanzania) (EPPO, 2011a; EPPO, 2011b; Baniameri and Cheraghian, 2012; EPPO, 2012a; EPPO, 2012b; EPPO, 2012c; EPPO, 2012d; EPPO, 2013a; EPPO, 2013b; Brevault *et al.*, 2014; EPPO, 2014a; EPPO, 2014b; *Tuta absoluta Information Network*, *2014;* EPPO, 2015) (Fig. 1-1). It is assumed that the pest spread mainly through tomato trading, including trade in accidentally infested tomatoes in the aforementioned countries (Desneux *et al.*, 2011). For example, the pest was caught in a light trap in Denmark, which is not known for tomato growing (Buhl *et al.*, 2010). However, spread through flight and wind have been also suggested (Desneux *et al.*, 2011).

Studies conducted on *Tuta absoluta* species in Argentina (Flores *et al.*, 2003) and the Mediterranean and South America (Cifuentes *et al.*, 2011) have discovered that different *Tuta absoluta* populations share a common gene pool regardless of being geographically distant and that they are able to adapt to different environments. The studies also confirmed that the pest originated in South America and subsequently spread towards the Mediterranean region (Cifuentes *et al.*, 2011).

Figure 1-1 Current distribution (beginning of 2016) of *Tuta absoluta* in newly invaded areas after 2006. Source of picture *Tuta absoluta* (2016a).

1.1.3 Description and life cycle of **Tuta absoluta**

The life cycle of *Tuta absoluta* consists of four developmental stages: adult, egg, larva and pupa (Urbaneja *et al.*, 2013).

Adults

Adult moths measure approximately 1 cm in length with a wingspan of around 1 cm and present long, filiform antennae (Vargas, 1970) cited in USDA–APHIS (2011). They are mottled grey (Estay, 2000) cited in USDA–APHIS (2011) with the anterior wings spotted black (Urbaneja *et al.*, 2013) (Fig 1.2). Differences between males and females are not largely apparent, but males tend to have narrower, posteriorly pointed abdomens in comparison with females, in which abdomens are wider and bulkier. In addition, abdominal scales are grey in males and cream in females (Vargas, 1970) cited in USDA–APHIS (2011).

Adult longevity is influenced by environmental conditions (Urbaneja *et al.*, 2013). The lifespan of adult females ranges between 10 and 15 days and that of adult males ranges between 6 and 7 days (Estay, 2000) cited in Tropea Garzia *et al.* (2012). The earlier eclosion of females means they are sexually mature as soon as the males eclode (Fernandez and

_____

Montagne, 1990) cited in Tropea Garzia *et al.* (2012). Adults disperse by means of flight and are usually hidden throughout plants during the daytime, exhibiting great activity in the early mornings and courtship and mating behaviour at dusk as well as predominantly daytime oviposition (Uchôa-Fernandes *et al.*, 1995). *Tuta absoluta* carry out short, ground level flights when stimulated and are very much enticed by sources of light, especially blue lights. Mating occurs within a few hours of emergence for males and roughly within 20-22 hours for females. Females do not mate more than once a day and can mate up to 6 times in a lifespan, where each mating session lasts around 4- 5 hours (Estay, 2000) cited in Tropea Garzia *et al.* (2012).

Figure 1-2 Adult of *Tuta absoluta*. Source of picture *Tuta absoluta* (2016b).

Eggs

Eggs are cylindrical in shape, either laid individually or in small groups but hardly ever in bulk. Freshly laid eggs are creamy white in colour and change to a yellow followed by a yellow- orange colour towards hatching (Estay, 2000) cited in USDA–APHIS (2011). Eggs measure approximately 0.383 mm long by 0.211 mm wide (Fig. 1-3). As eggs mature, they undergo what is known as the blackhead stage whereby they turn dark and the larval head capsule outline is visible through the chorion (Vargas, 1970) cited in USDA–APHIS (2011). Most of the eggs are laid seven days after first mating, when females lay over 70 % of their eggs during daytime hours; one female is able to lay as many as 260 eggs throughout its lifetime (Uchôa-Fernandes *et al.*, 1995) cited in Desneux *et al.* (2010). Hosts are determined by adults through tomato leaf volatiles that attract them for oviposition (Proffit *et al.*, 2011). Eggs can be found on all aerial parts of the plant (Estay, 2000) cited in USDA–APHIS (2011).

_____

_____

Figure 1-3 Eggs of *Tuta absoluta* laid on tomato leaf. Source of picture *Tuta absoluta* (2016b).

According to an experiment conducted by Torres *et al.* (2001), preferred oviposition site varies according to plant phenological stage. Before flowering, eggs are laid on all plant structures including leaves, petioles and main stems with a preference for leaves. The apical part of the plant was preferred followed by the median and basal parts. Egg viability is about 90 %. After flowering, eggs are laid equally among leaves and petioles on all three plant parts (apical, median and basal parts) with an egg viability of 90 %. After fruiting, the majority of oviposition occurs on the leaves and petioles on the apical and median plant parts, with a significant inclination to oviposit on apical parts. Leaves are preferred to petioles and flowers and no eggs are laid on the main stem, with an egg viability of about 94 %. No preference is shown for oviposition on lower or upper side of the leaf in all phenological stages.

Preferred oviposition sites are leaves (7 %), leaf veins and stem margins (21 %), sepals (5 %) and immature fruits (1 %) (Estay, 2000) cited in Urbaneja *et al.* (2013); oviposition is only conceivable on immature tomato fruits (Monserrat, 2009) cited in Urbaneja *et al.* (2013).

Larvae

The larval stage consists of four instars that differ in size and colour (Estay, 2000). Larval instar is best determined by measuring the head capsule diameter. Measurements of larval instars (body length) are c. 1.6, 2.8, 4.7 and 7.7 mm for first, second, third and fourth instars respectively (Estay, 2000) cited in USDA–APHIS (2011) (Fig. 1-4). First instar larvae are cream in colour with a dark head. Larval colour then changes to a deep green colour; the fourth instar changes to a pinkish colour followed by creamy white again as they purge their stomach contents towards pupation (USDA–APHIS, 2011).

_____

Figure 1-4 Larvae of *Tuta absoluta* feeds on tomato leaf. Source of picture *Tuta absoluta* (2016b).

Larvae typically emerge in the morning and roam around for 5- 40 min before they start mining (Fernandez and Montagne, 1990) cited in Tropea Garzia *et al.* (2012). Young larvae feed and develop by penetrating leaves, stems, growing tips, flowers, and immature fruits producing noticeable mines and galleries; developed instars are also able to feed on mature fruits. As larvae increase in size, mines and galleries expand accordingly (Vargas, 1970) cited in USDA–APHIS (2011). Larvae tend to stay inside mines with the exception of the second instar, which may emerge at cooler temperatures (Torres *et al.*, 2001). The epidermis usually remains undamaged as larvae only feed on mesophyll tissues (Desneux *et al.*, 2010). However, in severe cases, all leaf tissue may be consumed and skeletonized leaves and frass are left over. Larvae may also tie leaves together or spin silk shelters in them (Vargas, 1970) cited in USDA–APHIS (2011). Larvae feed constantly and do not enter diapause given adequate nutrition and suitable climatic conditions (Fernandez and Montagne, 1990; Uchôa-Fernandes *et al.*, 1995; Viggiani *et al.*, 2009) cited in Tropea Garzia *et al.* (2012).

According to Torres *et al.* (2001), before and after flowering and before fruiting, first and fourth larval instars prefer apical and median plant parts whereas after fruiting, first instar larvae prefer apical and median plant parts while fourth instar larvae are present on all three plant parts.

Pupae

Once the fourth instar larvae are ready to pupate, they refrain from eating, empty their guts from food (USDA–APHIS, 2011) and, using a silk thread, move to the soil where they pupate and complete development (Urbaneja *et al.*, 2007) cited in Garcia-del-Pino *et al.* (2013).

_____

Although the pupation process can also take place on all above–ground parts of the plant (Torres *et al.*, 2001). Pupae are cylindrical in shape and measure c. 4.3 mm and 1.1 mm in diameter (Urbaneja *et al.*, 2013) (Fig. 1-5). Freshly formed pupae are green in colour and change to dark brown towards maturation (Estay, 2000) cited in USDA–APHIS (2011). They are sheltered by a thin, silky cocoon covered with specks of earth and debris in the case of those located in the soil (Uchôa-Fernandes *et al.*, 1995) cited in Tropea Garzia *et al.* (2012). Preferred pupation location is affected by environmental conditions; it occurs mainly in the soil and on leaves with a small fraction taking place in sheltered locations including stems and fruits (Fernandez and Montagne, 1990; Uchôa-Fernandes *et al.*, 1995; Viggiani *et al.*, 2009) cited in Tropea Garzia *et al.* (2012).

Regarding pupation taking place on plants, throughout the three phenological stages, the majority of pupation takes place on the leaves and in the apical plant part. Before flowering, when plants have an average of five or six leaves, there is significantly higher ratio of pupae present on the apical part (1.43), followed by 0.67 on the median part and 0.10 on the basal part. After flowering and fruiting, pupation levels on apical and median plant parts are similar and are higher than that present on basal parts. Overall, in all three phenological stages, the majority of pupation takes place in the soil, followed by the main stem after fruiting (Torres *et al.*, 2001).

Tuta absoluta is capable of overwintering in the form of eggs or pupae, and adults depending on the condition that food is available, pupae being the most dominant when temperature is low (Sannino and Espinosa, 2010) cited in Tropea Garzia *et al.* (2012). However, adults can be found any time of the year in the Mediterranean region (Vercher *et al.*, 2010) cited in Tropea Garzia *et al.* (2012). It is unknown whether this species is able to enter diapause (USDA–APHIS, 2011).

_____

_____

Figure 1-5 Pupa of *Tuta absoluta*. Source of picture *Tuta absoluta* (2016b).

Tuta absoluta is considered a species that has several generations each year, with a significant reproductive potential which facilitates rapid growth of pest populations (Pereyra and Sánchez, 2006). It is able to adapt to a wide range of temperatures (EPPO, 2005) and is capable of completing a life cycle in 22 to 73 days depending on environmental conditions; a life cycle can be completed on tomato leaves in 22 days at temperatures between 25 and 27°C (Barrientos *et al.*, 1998) cited in Urbaneja *et al.* (2013). Thus, *Tuta absoluta* could potentially complete up to 12 generations per year (EPPO, 2005). It is reported to be able to complete 10-12 generations per year in the Mediterranean climate (EPPO, 2005). In South America, there may be 10-12 generations per year (Barrientos *et al.*, 1998) cited in Urbaneja *et al.* (2013); there can be 6-9 generations per annum in Sao Paulo in Brazil (Michereff Filho *et al.*, 2000a) and 7-8 generations per annum in Arica Valley, Chile (Vargas, 1970) cited in USDA–APHIS (2011). A maximum of 5 generations per year were noted in Argentina (EPPO, 2005), with up to 13 generations per annum predicted in Spain (Vercher *et al.*, 2010) cited in Tropea Garzia *et al.* (2012) and up to 9 generations per annum in Italy (Sannino and Espinosa, 2010) cited in Tropea Garzia *et al.* (2012).

On average, *Tuta absoluta* is able to complete a lifecycle in about 24 days at 27.1°C, 40 days at 19.7°C, 76 days at 14°C (Barrientos *et al.*, 1998) cited in Tropea Garzia *et al.* (2012), and 29 days at a constant temperature of 25°C under laboratory conditions (Vargas, 1970) cited in USDA–APHIS (2011).

_____

_____

1.1.4 Host range

T. absoluta is categorised as an oligophagous insect attacking plants from the Solanaceae family (Muszinski *et al.*, 1982) cited in Siqueira *et al.* (2000a). Its main and preferred host plant is tomato (*Solanum lycopersicum* L.), including all varieties, whether grown outdoors or in a greenhouse, destined for fresh market or for processing and regardless of tomato plant development stage (Lopez, 1991; Apablaza, 1992) cited in Urbaneja *et al.* (2009).

T. absoluta is also able to feed, develop and reproduce on other cultivated Solanaceae including potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.), tobacco (*Nicotiana tabacum* L.), sweet pepper (*S. muricatum* L.) (Vargas, 1970; Campos, 1976) cited in Desneux *et al.* (2010) and pepper (*Capsicum anuum* L.) (EPPO, 2005). It will also develop and reproduce on wild Solanaceae including silver nightshade (*S. eleagnifolium* L.), black nightshade (*S. nigrum* L.), *S. sisymbriifolium* Lam., *S. bonari*ense L., *Lycopersicum puberulum* Ph., *S. saponaceum*, jimson weed (*Datura stramonium* L.), tree tobacco (*N. glauca* Graham), long- spined thorn apple (*Datura ferox* L.), (Garcia and Espul, 1982; Larraín, 1986) cited in Desneux *et al.* (2010), *S. lyratum* Thumberg, *Lycopersicon hirsutum* L., *Lycium chilense* Mill, *S. puberulum* Phil. (EPPO, 2005) and for the first time in Sudan on *S. dubium* (Mohamed *et al.*, 2015).

The tomato leafminer has also been reported on non- solanaceous plants including French bean (*Phaseoulus vulgaris* L.), cape gooseberry (*Physalis peruviana L.*) (EPPO, 2009c; Tropea Garzia, 2009), (*Lycium* sp.) and (*Malva* sp.) (Caponero, 2009) cited in Desneux *et al.* (2010) and for the first time on watermelon (*Citrullus lanatus*) in the Cucurbitaceae family, physic nut (*Jatropha curcas*) in the Euphorbiaceae family, spiny amaranth (*Amaranthus spinosus*) in the Amaranthaceae family and Ramtouk (*Xanthium brasilicum*) in the Asteraceae family in Sudan (Mohamed *et al.*, 2015).

It has been reported that *Tuta absoluta* is able to complete its lifecycle on aubergine (*S. melongena*), potato (*S. tuberosum*), slender nightshade (*S. gracilius*), *S. bonariense* and sticky nightshade (*S. sisymbriifolium*). However, progress is interrupted on cultivated tobacco (*N. tabacum*) and winter cherry (*S. pseudocapsicum*) at the first and second larval instars (Galarza, 1984), although a newer study claims that full development is possible on cultivated tobacco (*N. tabacum*) (Cardozo *et al.*, 1994) cited in USDA–APHIS (2011).

_____

_____

Although coverage of the damage of this pest in Europe has focussed mainly on tomato, it has also been reported to cause direct and indirect damage to potato and pepper (Viggiani *et al.*, 2009) in addition to severe damage to eggplant in Italy (Ministero delle Politiche Agricole Alimentari e Forestali, 2009) and potatoes in France (Maiche, 2009) last three references cited in USDA–APHIS (2011). In potato, *Tuta absoluta* damages above ground parts, such that roots are not directly affected. However, feeding on leaves may cause a decrease in potato yield (Pereyra and Sánchez, 2006). The fact that *Tuta absoluta* is able to inhabit such a wide range of wild plants means that it can multiply and propagate in new regions even when there is a lack of cultivated host plants (Cifuentes *et al.*, 2011).

1.1.5 Damage of **Tuta absoluta**

Tuta absoluta is considered an invasive pest due to its rapid development and propagation capability (Desneux *et al.*, 2010). Furthermore, it is able to attack tomato designated for fresh or processed use (Lopez, 1991; Apablaza, 1992) cited in Desneux *et al.* (2010) regardless of developmental stage (Estay 2000) cited in Desneux *et al.* (2010) with all above-ground parts of plant are appropriate for development (Bogorni *et al.*, 2003) cited in (Tropea Garzia *et al.*, 2012). After hatching, in order to feed, larvae either create a shelter by binding together leaves or young shoots (Pastrana, 1967) cited in USDA–APHIS (2011), or directly penetrate and mine leaves, apical buds, stems, flowers and young fruit creating noticeable, uneven mines and galleries and leaving behind dark remains, making invasions easily noticeable. Galleries increase in size in conjunction with larval growth and development (Vargas, 1970). In potato, *Tuta absoluta* larvae mine leaves and tubers (Pastrana, 2004) cited in USDA–APHIS (2011).

In tomato leaves, mines formed in mesophyll through larval feeding result in the reduction of photosynthetically active surface, which reduces tomato yield (Lopez, 1991; Apablaza, 1992) cited in Desneux *et al.* (2010). In extreme cases, larvae may even exhaust all the leaf flesh, leaving behind a skeletonised leaf and plenty of frass. In addition, second, third and fourth instar larvae usually bind leaves together or spin silk shelters in leaves (Vargas, 1970) cited in USDA–APHIS (2011). On shoots, larvae usually gain access through the apical part or from the angle enclosed by the petioles and the leaves. They are also able to bind new shoots together (Vargas, 1970) cited in USDA–APHIS (2011). Mines bored in stems affect plant development and could potentially lead to necrosis (Lopez, 1991; Apablaza, 1992) cited in

_____

Desneux *et al.* (2010). Seedlings are particularly vulnerable and are likely to die when larvae develop inside the main stem (Pereyra and Sánchez, 2006).

Tuta absoluta larvae are also able to attack flowers; however, damage is not as significant as that which is caused on fruit, which can be attacked as soon as it is formed. Early and late instars are associated with developing and mature fruits respectively (USDA–APHIS, 2011). *T. absoluta* larvae tend to enter the fruit under the calyx (which can make primary detection problematic) (Tropea Garzia *et al.*, 2012), through the terminal part or through other fruit parts that are in contact with the rest of the plant. They damage the fruit by creating mines in the tissue, resulting in frass- congested tunnels, causing the fruit to rot on the plant or drop to the ground (USDA–APHIS, 2011). Although fruits are only attacked during substantial infestations, once they are attacked, even slight injury can lead to severe consequences (FERA, 2009), including a decrease in produce and quality and thus commercial value and even entire crop failure (Desneux *et al.*, 2010). In addition to affecting the appearance of fruits, wounds caused by larval feeding activity make fruits susceptible to invasion by secondary pathogens resulting in fruit rot, especially bacteria, which are capable of permeating damaged tissue (Tropea Garzia *et al.*, 2012), as well as fungi (FERA, 2009). Furthermore, plant development is impaired particularly as larvae feed directly on the growing tip (Lopez, 1991; Apablaza, 1992) cited in Desneux *et al.* (2010).

1.1.6 Economic importance of **Tuta absoluta**

The destruction caused by *T. absoluta* is economically significant (Desneux *et al.*, 2010). This pest has been described as the main restriction on the production of tomato in South America (Bahamondes and Mallea, 1969; Quiroz, 1978) cited in Ferrara *et al.* (2001); it is considered a major pest of tomato in Bolivia, Argentina, Chile, Brazil, Uruguay, Ecuador, Colombia, Peru, Venezuela and Paraguay where it causes up to 100 % damage to tomato plants (Lopez, 1991; Apablaza, 1992) cited in Desneux *et al.* (2010).

Tomato is the world's main vegetable crop after potato. The top ten primary tomato growing countries are India, USA, China, Turkey, Italy, Egypt, Iran, Spain, Mexico and Brazil. In 2009, approximately 152 M tons of fresh tomatoes were produced globally on 4.4 M ha. Tomato production has increased dramatically in 10 years, namely by 40 %, Asia and China being responsible for 84 % and 63 % of this surge respectively (Desneux *et al.*, 2011).

T. absoluta was confined to South America for approximately 40 years, where as little as 3.1 % of cultivated surface as well as 5 % of global tomato production were infested. However, due to its invasion of Spain in 2006 and rapid spread thereafter until 2011, the worldwide impact imposed by *T. absoluta* has increased considerably whereby 21.5 % of cultivated surface and 27.2 % of tomato production were infested, that is, 0.95M ha and 41M tons respectively. By 2011, 84.9 % or 3.7 M ha of tomato cultivated surface, and 87.4 % or 133.7 M tons of tomato produce were endangered by *T. absoluta* worldwide (Desneux *et al.*, 2011). Based on the current known distribution (Fig. 1-1), *T. absoluta* is currently present in nine out of ten primary tomato producing countries (India, Turkey, Egypt, Iran, Italy and Spain) (EPPO, 2008a; EPPO, 2009c; Kilic, 2010; Temerak, 2011; Baniameri and Cheraghian, 2012; EPPO, 2015) and is now threatening the first leading tomato-producing country in Asia (China) (Desneux *et al.*, 2011).

The need to control *T. absoluta* has led to a significant increase in tomato production costs at both field and post- harvest stages (USDA–APHIS, 2011). This is particularly true in the case of insecticide use. For instance, soon after *T. absoluta* was first discovered in the Mediterranean basin, up to 15 *T. absoluta* - specific insecticides were added to Integrated Pest Management (IPM). In Spain, an additional 450 € was incurred in pest control costs per ha for each harvest season. *T. absoluta* control in Argentina is primarily based on defensive applications of up to 16 distinct neurotoxic pesticides, once or twice a week (Sanchez *et al.*, 2009); in central Argentina, this pest is responsible for 46 % of pest management costs between September and December, and 70 % between January and May, accruing costs between US $80 and $460 per ha (Strassera, 2009) cited in Desneux *et al.* (2011). It is projected that future *T. absoluta* invasions would increase annual tomato pest control costs by approximately 240 – 410 M € and US $ 487 M according to the Spanish and Argentinean examples respectively (Desneux *et al.*, 2011). Thus, a global infestation would lead to significant economic as well as environmental concerns (Pimentel *et al.*, 1992), and over-use of insecticides by tomato farmers may result in numerous undesirable side-effects (Peterson and Higley, 1993; Weisenburger, 1993; Riquelme Virgala *et al.*, 2006; Arno and Gabarra, 2011; Biondi *et al.*, 2012b).

1.1.7 Control of **Tuta absoluta**

Control of *T. absoluta* in its area of origin still relies mainly on the use of chemical insecticides in both field and glasshouse, regardless of the search for other control methods

_____

(Guedes and Picanço, 2012). Effective chemical control is difficult because of the ability of this pest to develop resistant strains and because of the feeding behaviour of larvae in which they are protected from contact with insecticides inside the mines on leaves, fruits and stems (Guedes and Picanço, 2012; Terzidis *et al.*, 2014). Alternative control methods include semiochemical, cultural, resistance and biological methods (Urbaneja *et al.*, 2013) and much investigation has been directed towards biological methods. Integrated Pest Management (IPM) programmes, which include chemical, biological and cultural procedures, are also under development in numerous South American countries as well as in newly invaded areas (USDA–APHIS, 2011).

1.1.8 Cultural control strategies for **Tuta absoluta**

Cultural control methods for *T. absoluta* include activities such as sanitation, destruction of host plants, crop rotation and the designation of host free periods and solarisation of soil.

A. Sanitation

T.absoluta populations may be carried over from infected plants that remain in fields or greenhouses following harvest. The pest may also reach these locations through the movement of infected plants. Thus, stringent sanitation recommendations need to be adhered to in tomato production sites including nurseries and greenhouses (USDA–APHIS, 2011).

a. Nurseries and greenhouse tomato production

In tomato producing greenhouses and nurseries, adequate access control should be established, such as the installation of self-closing doors and covering openings and windows with insect mesh that is at most 1.6 mm in order to prevent entry or exit of *T. absoluta* adults (InfoAgroSystem, 2009) cited in USDA–APHIS (2011). Also, pots, carts and greenhouse apparatus should be examined and cleaned carefully before they are transferred to other locations. In addition, stems and leaves should be inspected regularly for signs like eggs, mines, larvae, frass or other destruction, and fruits as well as the undersides of calyces should be inspected for piles of frass, which are indicative of larval access holes (Mallia, 2009) cited in USDA–APHIS (2011). Furthermore, infected plant parts or plants should be eliminated, particularly at the start of the growing process; remains should be discarded cautiously such that they are kept in sealed containers prior to being dispatched to a waste management authority (InfoAgroSystem, 2009) cited in USDA–APHIS (2011). Additionally, solanaceous

_____

_____

weeds local to infected greenhouses should be eliminated in order to inhibit the potential growth of a population (Abbes *et al.*, 2012b). In addition, greenhouse staff should check their clothing for eggs, larvae and inactive *T. absoluta* adults (USDA–APHIS, 2011).

b. Field grown tomatoes

In tomato growing fields, the efficient post-harvest destruction of crop remains disturbs the life cycle of *T. absoluta* in that the immature stages residing within the plant material are killed. Also, high pressure washing or steam should be used to clean mechanical harvesting and tilling equipment following utilisation in infected fields. In addition, all resources such as harvesting containers, field boxes, carts etc. should be cleaned and examined prior to their transfer to different areas. Moreover, the whole plot should be thoroughly destroyed, for instance by ploughing or burning, in the event that *T. absoluta* is identified, in order to disturb its life cycle and propagation. This applies at any point during the growing cycle (USDA–APHIS, 2011).

B. Destruction of Wild and Cultivated Hosts

In order to inhibit the accumulation of *T. absoluta* populations, wild host plants growing in the vicinity (within a radius of 50 metres) of infected greenhouses, packing stations and fields should be eliminated and destroyed (USDA–APHIS, 2011).

C. Other cultural controls

In order to preclude pest carry-over, solanaceous hosts should not be alternated with tomato, therefore alternation with non-solanaceous crops is one of the cultural control techniques for this pest. Additional techniques are ploughing, suitable irrigation and fertilisation, destruction of infected plants as well as post-harvest plant remains and disposing of infected remainders (USDA–APHIS, 2011). In greenhouses, soil solarisation eradicates pupae on the ground (Abbes *et al.*, 2012b).

A six week host-free period between growing vulnerable crops in the same location has been recommended in open field environments by MARM (2008) cited in USDA–APHIS (2011); in the winter, this gap should be increased to 8 weeks given that *T. absoluta* develops at a slower rate. If further procedures are employed to destroy pupae in the soil, the host-free gap can be shortened (MARM, 2008) cited in USDA–APHIS (2011).

_____

1.1.9 Using pheromones

Sex pheromones are volatile compounds released by females to attract males of the same species for mating (Howse, 1998b). The main mate-seeking approach of around 120,000 known species of moth involves male upwind flight in the direction of a female-released sex pheromone to which they are attracted (Carde and Minks, 1995). Sex pheromones are prevalently and effectively utilised to detect and monitor pest populations. They are also utilised in mating disruption and mass annihilation applications in order to control populations of insect pests (Witzgall *et al.*, 2010).

A. Detection and Monitoring

Presence of a given insect species as well as commencement of its seasonal flight period are precisely determined by catches in synthetic pheromone lure–baited traps, thus the capture of adults in pheromone traps determines whether insecticides should be used due to its connection with damage caused by larvae and loss of harvest (Witzgall *et al.*, 2010), In regards to *T. absoluta*, action thresholds are 100 males in a day in each pheromone trap in Chile (Desneux *et al.*, 2010), and 45 ± 19.50 per day in Brazil (Benvenga *et al.*, 2007).

Essentially, a pheromone monitoring system comprises an attractant source and a trap that are positioned in a certain way (Howse, 1998a).

In relation to trap design, for purposes of monitoring *T. absoluta,* pheromone lures are primarily combined with Delta traps (Russell IPM, 2012b), such that the pheromone lure is suspended above the trap, which is composed of an open-ended, triangular paper or plastic unit in which a removable adhesive attachment is placed. Efficacy of traps also appears to be influenced by colour, such that light coloured traps catch fewer males than dark ones (Uchôa-Fernandes *et al.*, 1995).

Regarding the attractant source of *T. absoluta*, a more recent formulation which combines 0.8 mg of the two pheromone elements ([3*E*, 8*Z*, 11Z]-3,8,11-tetradecatrien-1-yl acetate or TDTA and [3*E*, 8*Z*]-3,8- tetradecadien-l-yl acetate or TDDA) (Megido *et al.*, 2013) and provides extra advantages over the regular 0.5 mg lure, including a greater catch capability and a continuous pheromone release in hotter environments for a longer duration, is offered by Russel IPM Ltd (Chermiti and Abbes, 2012; Russell IPM, 2012b).

_____

In regards to trap position, an association exists between plant height, trap height and captures of *T. absoluta* males (Uchôa-Fernandes *et al.*, 1995), such that trap height must always be under 1 m and co-ordinated with plant growth stage and take account of the fact that the majority of moths occur higher in the canopy (Coelho and França, 1987; Laore, 2010) cited in Megido *et al.* (2013).

Regarding trap density, it is commonly recommended to use one trap per hectare and two to four traps per hectare for greenhouses that are smaller and larger than 2500 m² respectively (Fredon, 2009; Laore, 2010) cited in Megido *et al.* (2013). A trap density of two traps per hectare is recommended by Russel IPM for crops in small holdings and in field where the land is not level and one trap for every two hectares in larger fields with even land (Russell IPM, 2012a).

B. Mating disruption

The mating disruption approach involves inundating the area with a synthetic female pheromone to induce sexual disorientation in males in order to inhibit mating and thus decrease pest populations (Witzgall *et al.*, 2010; Cocco *et al.*, 2013). A field experiment conducted on small plots using about 50 g per hectare of sex pheromone in Brazil produced above 85 % orientation disruption in *T. absoluta* males. Damage to fruit and leaflets was not reduced through the pheromone treatment though, possibly as a result of the relocation of females that had already mated to the region, the dense pest population or the dose and composition of the synthetic pheromone (Michereff Filho *et al.*, 2000b). However, an experiment conducted in Spanish greenhouses on mating disruption of *T. absoluta* indicates that mating disruption can provide control of this pest provided that the respective greenhouses are adequately isolated such that moth entry is inhibited (Navarro-Llopis *et al.*, 2010) cited in USDA–APHIS (2011).

C. Mass annihilation

Lure and kill and mass trapping are methods of mass annihilation, and involve the luring of insects into a trap or target that is integrated with insecticides (Witzgall *et al.*, 2010).

In relation to mass trapping, pheromone-baited traps are densely and strategically placed within crops for mass trapping purposes (Jones, 1998). The pest's mating process is affected by the imbalance of the male to female proportion due to the entrapment of a significant

_____

_____

number of adult males (Witzgall *et al.*, 2010; USDA–APHIS, 2011). The recommended density of pheromone traps for *T. absoluta* is about 45 traps per hectare in open environments and 23 traps per hectare within greenhouses (Bolckmans, 2009). In mass trapping, water traps provide a higher trapping capability compared to Delta traps, require less maintenance and are less susceptible to dust in comparison with light and Delta traps and are the main type of pheromone trap utilised for the mass trapping of *T. absoluta* (Salas, 2004) cited in Megido *et al.* (2013).

The lure and kill approach consists of a lure for males and/or females that is paired with a high volume trap, or integrated with an insecticide. This can be applied in two ways: firstly, using a formulation which combines an attractant with an insecticide, secondly, by the integration of the insecticide and the attractant into a single medium for use as an inclusive application (Witzgall *et al.*, 2010). A formulation of 3 % cypermethrin and 0.3 % sex pheromone is manufactured for the management of *T. absoluta* (Al-Zaidi, 2010) cited in Megido *et al.* (2013).

1.1.10 Tomato resistance

Although resistance to *T. absoluta* occurs predominantly in wild tomato plants, it has also been detected in hybrids obtained through the crossbreeding of marketable and wild genotypes in addition to marketable genotypes (Urbaneja *et al.*, 2013). A study of plant resistance of 22 tomato genotypes in a greenhouse found an antagonistic effect for *Lycopersicon hirsutum* f. *typicum* and a non-preference effect for *L. hiristum* f. *glabratum* and *L. pennellii* resulting in a large degree of *T. absoluta* resistance in the latter variety (França *et al.*, 1984) cited in Urbaneja *et al.* (2013). In addition, a large degree of resistance was recorded for *L. peruvianum, L. hirsutum* and *L. pimpinellifolium* (Barona *et al.*, 1989) cited in Urbaneja *et al.* (2013). In contrast to larvae reared on a vulnerable *L. esculentum* (Santa Cruz Kada) cultivar, those reared on *L. hirsutum* f. *glabratum* exhibited inferior levels of fecundity and viability in addition to lighter pupae and extended development duration (Giustolin and Vendramim, 1996) cited in Urbaneja *et al.* (2013).

Mortality of 100 % was achieved through feeding larvae of this pest an artificial diet combined with a blend of particular concentrations of allelochemicals 2-tridecanone and 2-undecanone, 2-T and 2-U respectively, which are obtained from *L. hirsutum* f. *glabratum* trichomes of type VI (Giustolin and Vendramim, 1996), cited in Urbaneja *et al.* (2013).

_____

_____

Larval mortality has been found to increase with plant age as well as length of the larval period; this is thought to be due to the possible corresponding increase of trichome density and hence the level of 2-T (Leite *et al.*, 2001). There is a correlation between greater 2-T levels and non-preference for laying eggs as well as feeding resistance (Labory *et al.*, 1999).

1.1.11 Chemical control

The use of chemical pesticides has undoubtedly enhanced agricultural production (Grewal and Georgis, 1999). For many years, synthetic pesticides have been largely responsible for the regulation of agricultural pests and the decline of diseases transmitted by insects (Weinzierl and Henn, 1991). Generally speaking, $4 worth of crop is rescued with each $1 spent on pesticide control, and it is projected that there would be a 10 % increase in pest-related losses in the absence of pesticide use, reaching up to 100 % in some cases (Pimentel *et al.*, 1992).

Chemical pesticides have been the major means of pest and weed control since the 1940s, in both developed and developing countries (Flint and van den Bosch, 1981; Whitten and Oakeshott, 1991). Organochlorines and organophosphates are examples of chemical insecticides that were produced and widely used in the early 1950s; due to ease of application by means of spraying, they were effective at killing a vast variety of pests at low cost. However, organochlorine use was subsequently banned following the detection of its continuation in the environment and its consequent residues in water and in the food chain (Römbke and Moltmann, 1996). Nevertheless, new generations of synthetic pesticides were then developed and are still utilised, in an attempt to prevent crop damage caused by invertebrate pests and diseases (Altieri and Nicholls, 2003). By the end of the 1980s, approximately 4,000 registered pesticides weighing 30,000 tons were used to manage agricultural insect pests in the UK each year (Best and Ruthven, 1995). Annual agricultural pesticide costs amount to approximately £500 million in the United States (Altieri and Nicholls, 2003). In addition, annual worldwide pesticide use was valued at a cost of $20 billion, weighing around 2.5 million tons, between the years 1980 and 2000 (Hajek, 2004). Thus, it is evident that the use of synthetic insecticides against agricultural insect pests has increased significantly and that humans are becoming reliant on them (Pretty and Hine, 2005).

T. absoluta was originally controlled using organophosphates, which were then exchanged for pyrethroids in the 1970s. This was followed by alternating applications of cartap and pyrethroids/thiocyclam in the 1980s, which were effective at regulating the pest. In the 1990s,

_____

new insecticides such as abamectin, acylurea, chlorfenapyr, spinosad and tebufonozide were used against outbreaks (Lietti *et al.*, 2005). Since the 1980s, development of resistance has resulted in a decline in efficacy in the use of organophosphates in Brazil and Chile, in addition to abamectin, cartap and permethrin in Brazil (Siqueira *et al.*, 2000a; 2000b; 2001) and pyrethroids in Argentina (Lietti *et al.*, 2005) and Chile (Salazar and Araya, 1997) cited in Urbaneja *et al.* (2013).

Nevertheless, more recent generations of insecticides have been effective against *T. absoluta* (USDA–APHIS, 2011). In Spain, indoxacarb, imidacloprid and deltamethrin were used against *T. absoluta* larvae (USDA–APHIS, 2011). In Italy, chlorpyrifos and pyrethrins were used (Garzia *et al.*, 2009). In Malta, abamectin, imidacloprid, indoxacarb, lufenuron and thiacloprid were suggested for epidemics (USDA–APHIS, 2011). In France, use of indoxacarb was suggested (FREDON-Corse, 2009). In the north eastern tomato cultivating region of Brazil, use of chlorfenapyr and phenthoate was suggested, whereas in the southern, south eastern and savannah regions, use of cartap, chlorfenapyr, phenthoate, methamidophos, and indoxacarb was suggested (USDA–APHIS, 2011). Triflumuron was suggested for use as part of an Integrated Pest Management programme against larvae of *T. absoluta* in Argentina (Riquelme Virgala *et al.*, 2006) cited in USDA–APHIS (2011). In Spain, temporary use of pesticides emamectin, flubendiamide, metaflumizone and chlorantraniliprole against this pest was legalised for a maximum of 120 days after March 15, 2010, as used management techniques were inadequate in some parts of the country (MARM, 2010) cited in USDA–APHIS (2011). Relatively novel insecticides etofenprox and rynoxapir have been described as being effective and selective (Araujo-Gonçalves, 2010; Astor, 2010; Espinosa, 2010; López *et al.*, 2010; Robles, 2010; Torné *et al.*, 2010) cited in Urbaneja *et al.* (2013). Moreover, 10 novel pyrethroid molecules were found to be very efficient against *T. absoluta* in Brazil, potentially reaching 100 % larval mortality (Silverio *et al.*, 2009).

1.1.12 Disadvantages of Chemical control

 In spite of the acknowledged benefits of using chemical pesticides, their use has been a major cause for concern in the modern day (Edwards, 1993) due to negative consequences such as the harm they pose to human health and the environment (Pimentel *et al.*, 1992; Peterson and Higley, 1993; Weisenburger, 1993; Ji *et al.*, 2001), insect resistance (Brattsten *et al.*, 1986; Heckel, 2012) and harm to non- target organisms, including beneficial ones used in Integrated Pest Management programmes (Pimentel *et al.*, 1992; Riquelme Virgala *et al.*, 2006; Desneux

_____

et al., 2007; Alavanja and Bonner, 2012; Biondi *et al.*, 2012b; Biondi *et al.*, 2013). In addition, the use of chemical pesticides may lead to increased pest populations as well as compromising the efficacy of IPM programmes due to decreased efficacy of the chemical element (Terzidis *et al.*, 2014). These complications are intensified with respect to the control of *T. absoluta* considering that the traditional control strategy of this pest is based on chemical insecticide applications in South America (Branco *et al.*, 2001; Lietti *et al.*, 2005; Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2010; Reyes *et al.*, 2012) and in newly invaded areas (Desneux *et al.*, 2010; Desneux *et al.*, 2011; Gonzalez-Cabrera *et al.*, 2011; Urbaneja *et al.*, 2012; Konus, 2014). The scale of problems associated with use of pesticides has led to the introduction of, and rise in the use of non-chemical control approaches, such as mechanical, physical, cultural, biological and biotechnological methods and the use of pheromones for the control of *T. absoluta* (USDA–APHIS, 2011; Abbes *et al.*, 2012a; Cabello *et al.*, 2012; Cagnotti *et al.*, 2012; Chailleux *et al.*, 2013; Megido *et al.*, 2013; Urbaneja *et al.*, 2013; Salem and Abdel-Moniem, 2015) and other pests (DeBach, 1991; Van Driesche and Bellows, 1996; Rechcigl and Rechcigl, 1998; Hajek, 2004). Furthermore, there has been a major global change in pest control, including control of *T. absoluta*. While chemical control has traditionally been the primary defence practice, it is now considered that it should only be used as a final option in the event that defensive procedures are insufficient and informed field observation points to probable economic damage (Urbaneja *et al.*, 2013).

A. Human health and environmental issues

The volume of pesticide release into the environment has increased by approximately 1900 % between 1930 and 1980 (Pimentel, 2000), yet the use of chemical pesticides gives rise to significant negative health and environmental implications (Pimentel *et al.*, 1992; Edwards, 1993; Weisenburger, 1993), which has been a concern and a subject of debate since the 1960s (Peterson and Higley, 1993). The concept of pesticides is rather contradictory given that they aim to kill a particular species; the fact that organisms have common biochemical and physiological characteristics makes it difficult to come up with pesticides that provide an adequate safety margin between target and non-target organisms, such as humans and animals (National Research Council, 2000). Some natural as well as synthetic chemicals have been classed as carcinogens (Ames *et al.*, 1990). Globally, annual totals of pesticide poisoning incidents that are hospitalised, fatal and result in chronic illness amount to 3,000,000, 220,000 and 750,000 respectively (Hart and Pimentel, 2002). Furthermore, in the United States, the

_____

annual rate of poisonings by pesticides amounts to 10,000-20,000 out of 2 million agricultural workers (NIOSH, 2012).

Human exposure to pesticides occurs via air, water, dermal contact or food consumption (National Research Council, 2000), including eggs and milk (Pimentel *et al.*, 1992); consequently, collective exposure may be substantially higher than exposure through food consumption. In addition, humans can be exposed to residues of many different pesticides at the same time, as produce may be treated with many different pesticides, and through the consumption of a variety of foods. In addition, accidental consumption of pesticides may occur, such as through the consumption of seeds set for planting, and pesticide products may be mistaken for food. Industrial exposure to pesticides is usually by means of inhalation or dermal contact, and impacts those participating in pesticide production, transportation and application (National Research Council, 2000). Occupational repercussions such as pesticide poisoning and death are more prevalent in developing countries due to the lack of awareness of pesticide dangers, inadequate labelling of pesticides, illiteracy, insufficient protective clothing and washing amenities as well as insufficient and lack of enforcement of safety standards (Pimentel *et al.*, 1992). Moreover, it is problematic to make inferences regarding the toxicity of pesticides, as knowledge of the target pest and chemical structure do not necessarily provide an accurate projection of the taxonomic hazard (National Research Council, 2000).

Synthetic pesticides are often persistent and their remains may contaminate air, water, wildlife and food (Snelson, 1978; Peterson and Higley, 1993). Once a pesticide is placed into soil, it can either stay in place or move into the air, water runoff or soil water. During rainfall and irrigation, pesticides may also be transported by runoff and can consequently be transferred to surface water. Furthermore, once a pesticide has reached soil-pore water, it may then transfer to a saturated zone (aquifer) and thus pollute groundwater (National Research Council, 2000).

Following application, further residues can pass into the air through wind erosion and volatilisation, the consequent deposits including particles and vapours, can travel downwind. These residues may be deposited through processes like rainfall, snowfall, fog coalescence washout, vapour exchange with surfaces and fallout of particles. Furthermore, degradation may also occur in addition to the downwind processes such that the products of decomposition may accompany the initial chemical deposit in additional transport and

deposition (National Research Council, 2000). Thus, all humans in all locations are prone to some level of pesticide remains, whether from food, water or the atmosphere (Pimentel *et al.*, 1992).

B. Pesticide effects on non-target organisms

Overall, chemical pesticides work efficiently in combatting a vast range of insects as well as other invertebrates, each of which has a role to play in the ecosystem, thus local extinction will inevitably disturb natural balance. Many species such as predators and parasites act as population regulators. Several studies have reported on the adverse consequences of extensive use of chemical pesticides, including mortality, on non-target organisms (Hajek, 2004). The application of pesticides to combat a particular pest results in scarcity of the natural enemies of that pest. Consequently, in the event of a re-invasion, the respective natural enemies will not be present and thus a sudden increase in pest population would occur (Hajek, 2004). Furthermore, once natural enemies are eliminated, secondary pests of crops may prevail due to the significant decrease in their natural enemies. For instance, the European spruce sawfly *Gilpinia hercyniae* Hartig (Hymenoptera: Diprionidae), which was under natural biological regulation, emerged as a secondary pest once DDT was applied against another pest, the spruce budworm *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) (Hajek, 2004).

According to Yardim and Edwards (1998) up to 91.5 % population reduction of some predatory arthropods was recorded after applications of insecticides, fungicides and herbicides in tomato agroecosystems. Arno and Gabarra (2011) stated that indoxacarb induced a mortality of 28 % and 77 % of the predators *Macrolophus pygmaeus* (nymph stage) and *Nesidiocoris tenuis* (female) respectively. The insecticides thiacloprid and metaflumizone and the fungicide copper hydroxide were found to cause 100 %, 80 % and 58 % mortality respectively to nymphs of a common generalist predator in Mediterranean agroecosystems, *Macrolophus pygmaeus* (Martinou *et al.*, 2014). Barros *et al.* (2015) reported that nine insecticides used in tomato crops (cartap, abamectin, chlorfenapyr, beta-cyfluthrin, etofenprox, permethrin, methamidophos, phenthoate and Spinosad) caused high mortality to the predatory wasps *Protonectarina sylveira*e and *Polybia scutellaris* for 30 days after the treatment. The three major insecticides used to control *T. absoluta* in Spain are azadirachtin, spinosad and indoxacarb (Arno and Gabarra, 2011). While such insecticides are effective against lepidopteran pests (Mordue *et al.*, 1998; Wing *et al.*, 2000), a study regarding the

lethal and sublethal effects of these insecticides shows adverse effects on predators such as *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) which are considered the most valuable predators in the Mediterranean area (Arno and Gabarra, 2011), despite the claims by Schmutterer (1990), Williams *et al.* (2003), and Galvan *et al.* (2005) that such insecticides do not have much of an effect on natural enemies.

In addition to the side effects of insecticides, Biondi *et al.* (2013) tested the lethal and sublethal effects of some bioinsecticides used to control *T. absoluta* on the parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) (adult and pupal stages) and found that Spinosad caused 100 % and 80 % mortality of adults and pupae respectively. Moreover, several sublethal consequences occurred despite the low acute toxicity levels of the majority of the bioinsecticides (abamectin, azadirachtin and borax plus citrus oil (Prev-AmH). Furthermore, the application of neurotoxic insecticides emamectin benzoate and abamectin considerably impacted the biocontrol activity of females that survived after exposure to residues (1 hour and 10 days) as well as emerged females from treated pupae (Biondi *et al.*, 2013). Similarly, Abbes *et al.* (2015) examined the lethal and sublethal effects of two spinosyns (Spinetoram and Spinosad), which are widely used and authorized for organic farming, on the same parasitoid and reported adult mortality up to 100 %, and that that the demographic growth index of the parasitoid was estimated to be significantly lower after exposure to the two bioinsecticides. In the same way, Biondi *et al.* (2012a) tested the lethal and sublethal effects of the bioinsecticide abamectin on the main natural enemy of many arthropods in agricultural and natural ecosystems, the predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) and found that it induced nearly 100 % mortality up to 14 days after the application.

C. Resistance to chemical insecticides

Resistance to insecticides is prevalent; by 1996, over 600 species of plant-feeding insect pests had become resistant to insecticides (Sharma *et al.*, 2001). Resistance within insect populations develops with numerous applications of the same insecticide throughout several generations, such that vulnerable individuals are eliminated and resistant ones persist and go on to reproduce generations against which the respective insecticide is no longer effective (Riley and Sparks, 2009). Factors such as pesticide dosage, application frequency and pest characteristics influence the development of insecticide resistance (Regupathy, 1995). Arthropods which produce numerous offspring and multiple annual generations are more prone to becoming resistant to chemical insecticides (Metcalf, 1982). In addition, cross-

resistance may occur, such that insects may develop resistance to more than one insecticide within the same chemical family whereby resistance to a given insecticide dictates resistance to another, where the pest has not necessarily been exposed to the second insecticide (Zhang *et al.*, 1997).

The ability of *T. absoluta* to develop resistant strains and the fact that larvae of this pest are protected from contact with insecticides inside mines in leaves, fruits and stems (Terzidis *et al.*, 2014) have prompted frequent and extensive use of insecticides, which has stimulated the development of insecticide resistance in this pest in its area of origin (Siqueira *et al.*, 2000a; Haddi *et al.*, 2012; Reyes *et al.*, 2012; Gontijo *et al.*, 2013; Silva *et al.*, 2015) and in newly invaded areas (Haddi *et al.*, 2012; Roditakis *et al.*, 2013; Konus, 2014; Roditakis *et al.*, 2015). Extreme changes in tomato production have happened since the introduction of *T. absoluta* into new areas in South American countries, including a sudden upsurge of insecticide use in the early 1980s (Guedes and Picanço, 2012). Since then resistance of *T. absoluta* was identified to many insecticides including pyrethroids, abamectin, cartap, methamidophos, permethrin, deltamethrin, acephate, triflumuron, diflubenzuron, teflubenzuron, indoxacarb and bifenthrin in Brazil (Siqueira *et al.*, 2000a; Siqueira *et al.*, 2000b; Branco *et al.*, 2001; Siqueira *et al.*, 2001; Silva *et al.*, 2011), abamectin, deltamethrin, pyrethroids and cartap in Argentina (Salazar and Araya, 1997; Lietti *et al.*, 2005) and deltamethrin, esfenvalerate, lambda-cyhalothrin, mevinphos and methamidophos in Chile (Salazar and Araya, 1997; Salazar and Araya, 2001) cited in Terzidis *et al.* (2014). Consequently, new insecticides were registered and extensively utilised, especially in Brazil, such as chlorfenapyr, insect growth regulators, diamides, indoxacarb and spinosyns (Guedes and Picanço, 2012; Guedes and Siqueira, 2012). However, there have also been records of resistance in Chile and Brazil for the bioinsecticide spinosad, which is utilised in organic and conventional tomato fields, and which has become one of the leading compounds used against this pest (Reyes *et al.*, 2012; Gontijo *et al.*, 2013). In addition, resistance-related control failures were reported as being possible for cypermethrin, chlorpyriphos and metaflumizone in Greece (Roditakis *et al.*, 2013). Furthermore, resistance of *T. absoluta* populations native to Sicily (Italy) to diamide insecticides such as flubendiamide and chlorantraniliprole was recorded for the first time (Roditakis *et al.*, 2015). Also, an experiment regarding the predisposition of five *T. absoluta* strains collected from Europe and Brazil to pyrethroids using insecticide bioassays showed a large degree of resistance to lambda cyhalothrin and tau fluvalinate in all five strains (Haddi *et al.*, 2012).

More recent groups of insecticides are fairly effective against this pest, but there is a need for the implementation of resistance management regimes. A sustainable and efficient approach involves use of insecticides with different modes of action in rotation (USDA–APHIS, 2011).

1.1.13 Biological control

All creatures are susceptible to natural enemies such as predators, parasites and pathogens including bacteria, viruses and fungi, which regulate populations; this is referred to as "natural control". A wide variety of biological control agents exists for the regulation of invertebrate pests; these are classified as pathogens, parasites or predators functionally and as nematodes, mites or microorganisms including bacteria, viruses and fungi, taxonomically; managing pests through use of their natural enemies is referred to as "biological control" (Hajek, 2004). Biological control has been defined as *"The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be"* (Eilenberg *et al.*, 2001).

The three main approaches for the application of biological control are classical, conservation and augmentation biological control (DeBach, 1991). Classical biological control has been defined as "*the intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control*" therefore requiring no additional involvement of humans (Eilenberg *et al.*, 2001).

Conservation biological control is defined as *"Modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests"* This approach is different from the other two approaches in that it does not involve release of natural enemies. It involves the enhancement of natural enemies through protection and provision of resources as well as limited and rational use of insecticides (Eilenberg *et al.*, 2001).

Augmentation biological control is such that the aim is not to establish the introduced agent long-term and includes inundative and inoculative control, which are used when populations of natural enemies are lacking or inadequate (Hajek, 2004). Inundative biological control has been defined as "*the use of living organisms to control pests when control is achieved exclusively by the organisms themselves that have been released*" (Eilenberg *et al.*, 2001) and is applied when prompt pest management is necessary (Hajek, 2004). Examples include the

_____

introduction of microbial pathogens, parasitoid wasps or entomopathogenic nematodes in large volumes in an attempt to counteract insect pests (Waage and Hassell, 1982; Lacey *et al.*, 2001). Inoculative control though, has been defined as "*the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not that it will do so permanently*" (Eilenberg *et al.*, 2001), and is applied when long term management is necessary (Hajek, 2004).

In comparison with chemical pesticides, biological control can serve as a safe, efficient and environmentally friendly approach for managing pest insects, in that it does not harm non-target organisms and beneficial insects, or result in toxic remains (Harris and Dent, 2000). Furthermore, it is sustainable and incurs lower production costs in comparison with chemical pesticides (Van Emden and Service, 2004).

1.1.14 Biological control of Tuta absoluta

Biological control is a major component in *T. absoluta* IPM programmes (Haji *et al.*, 2002) cited in Urbaneja *et al.* (2013). The necessity of alternative management approaches for *T. absoluta* is reinforced due to the side effects of pesticides on arthropods that are beneficial (Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2007; Arno and Gabarra, 2011; Biondi *et al.*, 2012a; 2012b; 2013; Martinou *et al.*, 2014; Gontijo *et al.*, 2015) as well as the resistance of *T. absoluta* to insecticides (Siqueira *et al.*, 2000a; Haddi *et al.*, 2012; Konus, 2014; Roditakis *et al.*, 2015). There have been numerous records of *T. absoluta* natural enemies in its area of origin. Spontaneous, native enemies of this pest have also been recorded since its discovery in the Mediterranean. Investigation is in progress to establish whether such enemies can be used against this pest in biological control programmes in areas that have been infected recently (Urbaneja *et al.*, 2013).

In South America, at least 87 generalist natural enemy species of *T. absoluta* have been recorded (Desneux *et al.*, 2010). In North Africa and Europe, *T. absoluta* is attacked naturally by several parasitoids and predators, some of which are used in IPM programmes, particularly indigenous Miridae. In the Western Palaearctic area, at least 70 natural enemy species of this pest have been recorded on plants grown under different systems (protected and open-field including wild plants) (Zappalà *et al.*, 2013).

_____

1.1.15 Parasitoids

In South America, approximately 50 *T. absoluta* parasitoids have been recorded, the majority of which parasitise larvae and eggs; records of pupal and adult parasitoids are scarce and non-existent respectively. Some of these species are widely disseminated throughout South America, whereas others are geographically constrained to a particular area. An extensive list of these parasitoids can be found in Desneux *et al.* (2010). *T. absoluta* relevant *Trichogramma* species (egg parasitoids) are varied and numerous throughout South America, including at least 10 species, many of which are mass-produced and utilised in inundative biological control applications. Larval parasitoids reported for this pest are varied and include species within families Braconidae, Tachinidae, Eulophidae, Bethylidae and Ichneumonidae, with particularly important parasitoids within the first two families, of which some parasitoids have been utilised in biological control applications (Miranda *et al.*, 2005; Desneux *et al.*, 2010). Pupal parasitism of at least 30 % has been recorded. There are no records of parasitoids for adults of this pest (Desneux *et al.*, 2010).

In North Africa, Europe and the Middle East, at least 50 parasitoids were recorded for eggs and early instars of *T. absoluta* in areas that have been invaded recently, where 28 species were from the Eulophidae family. An extensive list of these parasitoids can be found in Zappalà *et al.* (2013). Eggs of the pest were naturally attacked by parasitoids *Trichogramma bourarachae* Pintureau & Babault and *Trichogramma achaeae* Nagaraja & Nagarkatti in Tunisia and France respectively as well as by other *Trichogramma* parasitoids that were unidentified in Italy, Egypt, Spain, France, Algeria and Iran (Zappalà *et al.*, 2013).

1.1.16 Predators

In South America, at least 37 predators have been recorded for eggs, larvae, pupae and adults of *T. absoluta*. A list of these predators can be found in Desneux *et al.* (2010). In tomato fields, a vast array of *T. absoluta* predators has been reported. *Cycloneda sanguinea* (Coleoptera: Coccinellidae) (larva), Araneidae, *Anthicus sp.* (Coleoptera: Anthicidae), Staphylinidae (adults), *Orius sp.* and *Xylocoris sp.* (Hemiptera: Anthocoridae), Phlaeothripidae (Thysanoptera) and Formicidae (Hymenoptera) were found in Brazil (Miranda *et al.*, 2005). In addition, further varied arthropods have been recorded (Desneux *et al.*, 2010).

Recently in newly attacked Western Palaearctic countries, there have been reports of fifteen species of arthropod preying on *T. absoluta.* A list of these predators can be found in (Zappalà *et al.*, 2013). Ten species of these predators are members of order Hemiptera, and in increasing order of numbers, are of the Nabidae, Anthocoridae and Miridae families. Mirids within the Dicyphini tribe are the species which has propagated most extensively. In three countries, *Macrolophus pygmaeus* (Rambur) has been reported to prey on early instars and eggs of *T. absoluta.* In addition, *Nesidiocoris tenuis* (Reuter) was found naturally in open fields and protected crops virtually throughout the year in eleven countries (Zappalà *et al.*, 2013).

1.1.17 Entomopathogens

Entomopathogens are those organisms that infect insects and induce diseases like bacteria, fungi, viruses and nematodes (Lacey *et al.*, 2001; Hajek, 2004).

Throughout the world, Coleopteran, Dipteran and particularly Lepidopteran orders are managed using formulations containing a bacterium, *Bacillus thuringiensis* (*Bt*) containing formulations (Srinivasa Rao *et al.*, 2015)**.** In Spain, weekly application of such formulations have controlled *T. absoluta* efficiently in greenhouse and open-field evaluations. All larval instars were susceptible, particularly the first. In contrast to controls, a 90 % decrease in loss was achieved using a *Bt*-formula at a concentration of 180.8 MIU l^{-1} (million International Units per litre) (Gonzalez-Cabrera *et al.*, 2011). This pest was also managed in cases where invasion is substantial during the course of the season of cultivation using a lower concentration of 90.4 MUI L^{-1} of the same formulation weekly (Gonzalez-Cabrera *et al.*, 2011). In open-field tests in Italy, the damage to the production of marketable fruits was reduced below 30 %, which was similar to control treatment (three synthetic insecticides: Indoxacarb, Emamectin and Metaflumizone), by combining azadirachtin and *B. thuringiensis* var. *kurstaki* (Lo Bue *et al.*, 2012). Other investigation showed that combining *Bt* with the fungus *Beauveria bassiana* resulted in a higher efficacy than that achieved through the sole use of the fungus, although the degree of fruit protection differed throughout the investigation (Torres Gregorio *et al.*, 2009), cited in (Urbaneja *et al.*, 2012). In addition to the *Bt* that is commercially available, two *Bt* strains have been isolated from Chile which demonstrate higher levels of larval toxicity than commercial ones (Niedmann and Meza-Basso, 2006) cited in (Desneux *et al.*, 2010).

On the other hand, investigations have been conducted using entomopathogenic fungi against *T. absoluta*. Rodríguez *et al.* (2006a) tested the virulence of *B. bassiana* (Bb) isolate Qu-B912 and *Metarhizium anisopliae* var. *anisopliae* isolate Qu-M558 on the third larval instar of this pest by spraying spore suspensions directly on larvae and reported more than 90 % mortality, but when third instar larvae were fed on Bb treated leaves, 68 % mortality was recorded. The same pathogen isolates were reported to induce 80 % and 60 % egg mortality respectively (Rodríguez *et al.*, 2006b). In a subsequent study, Pires *et al.* (2010) reported more than 95 % egg mortality using the *M. anisopliae* isolate. The eggs in this study were reported to be more vulnerable than first instar larvae. Giustolin *et al.* (2001a) reported synergistic and additive effects between *Beauveria bassiana* and a resistant tomato plant (*Lycopersicon hirsutum* f. *glabratum*) in a laboratory experiment. These authors reported 40 % to 80 % mortality of different larval instars eleven days post application, when larvae were fed on leaves of the resistant tomato plant treated with the Bb. This induced a higher mortality than the other treatments in which larvae were fed on leaves of susceptible tomato plants (*L. esculentum*) treated with the Bb, larvae fed on the resistant tomato plant and larvae fed on the susceptible tomato plant.

Pupae of *T. absoluta* were reported to be very susceptible to the fungus *M. anisopliae* (Metschnikoff) Sorokin (100 % mortality) for the first time using the rate recommended by the manufacturer, when this fungus was applied with irrigation water in laboratory tests in Spain (Contreras *et al.*, 2014).

1.1.18 Integrated Pest Management (IPM)

The concept of IPM was first endorsed during the 1960s as a result of chemical insecticide failures, particularly in cotton production (Gullan and Cranston, 2005b). IPM then developed as an outcome of initiatives which aimed to decrease total reliance on synthetic pesticides to control pests. IPM has been defined as "*A pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible, and maintains the pest populations at levels below those causing economically unacceptable damage or loss*" (FAO, 1967).

In the European Union, IPM is defined as "*The rational application of a combination of biological, biotechnical, chemical, cultural or plant-breeding measures, whereby the use of*

plant protection products is limited to the strict minimum necessary to maintain the pest population at levels below those causing economically unacceptable damage or loss" through Directive 91/414/EEC (EC, 2007)**.** The definition of IPM was then extended by the FAO in 2012, with emphasis on the economic, social and environmental elements of pest management and was as follows: "*the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms*" (FAO, 2012).

Biological control methods that rely on the use of organisms such as parasitoids, predators and pathogens that are particularly effective against a particular pest have become increasingly popular as major components of IPM programmes (Van Driesche and Bellows, 1996). Entomopathogenic nematodes (EPNs) are exceptional substitutes for chemical pesticides and now function as an effective part of IPM in various environments. EPNs have been assessed against almost 200 pests, almost all of which can be efficiently managed in field conditions. EPNs are particularly interesting due to their wide range of hosts and their safety to all vertebrates, most non-target invertebrates and in the food supply, ease of mass production (both *in vivo* and *in vitro*) as well as their ability to find and kill insects in soil and in cryptic environments like plant roots and tree trunks that most chemical pesticides are unable to reach. EPNs have been widely adopted due to ease of application through ordinary pesticide spray tools and irrigation systems. The use of EPNs in IPM regimes is further enhanced by their compatibility with various agrochemicals including fungicides, insecticides, herbicides, miticides, plant growth regulators, spray adjuvants and wetting agents. Moreover, some pesticides can contribute additive effects and even synergistic effects, enhancing EPN efficiency (Grewal *et al.*, 2005b; Grewal, 2012; Lacey and Georgis, 2012). Integrated Pest Management (IPM) of *T. absoluta* is recommended as it is impossible to control it with a single method (Urbaneja *et al.*, 2013).

Thus, the most effective strategy for the control of *T. absoluta* is based on the avoidance of epidemics as well as long-term and economic control, including biological, biotechnological, cultural and chemical methods, where pesticides should only be used when defensive

procedures are insufficient and informed observation indicates a likely economic threat (Urbaneja *et al.*, 2013).

IPM programmes are under development in various countries in an attempt to manage *T. absoluta* invasions. The majority of such programmes involve monitoring pest populations, efficient means of prevention and control, and the utilisation of pesticides when required. Biological control is also applied subject to availability (USDA–APHIS, 2011). For example, IPM components utilised against the tomato leafminer in Spain include mass-trapping of adults in advance of planting, removal of crop remains from soil, application of imidacloprid through irrigation water 8-10 days post planting, application of spinosad or indoxacarb on the detection of this pest and removal of crop remains instantly following the harvesting of the last fruits (Junco and Herrero, 2008) cited in USDA–APHIS (2011).

In Argentina, the use of IPM in greenhouse grown tomatoes against *T. absoluta* has been evaluated by INTA (Instituto Nacional de Tecnología Agropecuaria) since the late 1990s and has achieved encouraging outcomes (Botto, 1999) cited in USDA–APHIS (2011). The approach involves, the use of pheromone traps for early detection of adults and visual inspection of plants, particularly for eggs, once the first eggs are detected and/ or the first adults are trapped, *Trichogrammatoidea bactrae* is released inundatively, release of egg parasitoids followed by or integrated with the use of *Bacillus thuringiensis* to control larvae and safety-focussed usage of compatible pesticides if essential.

In Tunisia, IPM approaches in greenhouse include use of insect-proof screens on doors of glasshouses, solarisation of the soil to kill the pupae, stopping the emergence of adults from pupae by covering the soil with plastic screens, destroying secondary host plants inside and near glasshouses, eradicating infested leaves and secondary shoots, use of sex pheromone water traps (one trap per 500 m^2), closing the door of the infested glasshouses after gathering in of the crop to stop the adults from immigrating to open-field crops, balanced use of soft (insecticides that cause the mortality of fewer than 25 % of the beneficial organisms present) and organic insecticides and release of the egg parasitoid wasp *Trichogramma cacoeciae* and the predatory mirid *Nesidiocoris tenuis* Reuter (prefers young larvae and eggs), if possible (Abbes *et al.*, 2012b).

Since the current study uses entomopathogenic nematodes, this group of pathogens is reviewed in more detail below.

1.2 Entomopathogenic Nematodes

Nematodes are cylindrical in shape and lengthwise measure from 0.1 mm to many metres. They possess muscular, digestive, reproductive, nervous and excretory systems, where the latter two are simple. They do not have respiratory or circulatory systems (Koppenhöfer, 2007). Numerous species of nematode have pathogenic, parasitic or phoretic relationships with insects. Those with parasitic relationships come from 23 families of nematode. Those with possibility for biological insect management include Steinernematidae and Heterorhabditidae (Order: Rhabditida), Tetradonematidae and Mermithidae (Order: Stichosomida) and Phaenopsitylenchidae, Allantonematidae and Sphaerulariidae (Order: Tylenchida) (Koppenhöfer, 2007). The only entomopathogenic nematodes (EPNs) that are utilised as microbial insecticides are the Steinernematidae and Heterorhabditidae, apart from the inoculative use of a tylenchid, *Deladenus siricidicola*, in Australia against a woodwasp species (Bedding, 1993). Other species of nematode have less possibility for microbial control due to restricted pathogenicity and/ or culturing issues (Koppenhöfer, 2007).

Steinernematidae and Heterorhabditidae have been found in soils worldwide and are pathogens that are unable to survive extensively outside their hosts. The presence of suitable hosts may predominantly restrict their dispersal (Smits, 1996; Hominick, 2002). These nematodes are, according to species of host and nematode, capable of killing hosts within one to four days, which is enabled by their mutualistic relationship with bacteria of genera *Photorhabdus* and *Xenorhabdus* that are carried in the intestines of Heterorhabditidae and Steinernematidae respectively, and are hence called entomopathogenic nematodes. The biology of all species of EPN that have been discovered appears to be similar (Koppenhöfer, 2007).

EPNs have been utilised in biological control applications, particularly classical, since the 1930s. In the USA for instance, Japanese beetle (*Popillia japonica*) was managed through the use of the EPN *S. glaseri*. Investigation on the use of EPNs for biological pest control became subdued due to the introduction of chemical pesticides (Smart, 1995) and remained as such until the 1960s due to the lack of regulation, low cost and high efficacy of chemical control methods. Following the acknowledgement of the adverse consequences of pesticides on the environment, research on biological control gained momentum (Adams and Nguyen, 2002). EPNs then resurfaced in the 60s and 70s as means of biological control; investigation was predominantly on *Neoaplectana carpocapsae,* now known as *Steinernema carpocapsae*.

Investigation on mass EPN manufacture in bioreactors followed in the 1980s (Kaya *et al.*, 1993). Recently, short-term storage-friendly formulations of numerous species of EPN have been manufactured and are publicly accessible (Hazir *et al.*, 2003). Steinernematidae and Heterorhabditidae have been used in augmentative, classical and conservational biological control applications; investigation has focussed predominantly on their inundative use (Grewal *et al.*, 2005b).

1.2.1 Biology of steinernematid and heterorhabditid nematodes

The life cycle of steinernematid and heterorhabditid nematodes comprises an egg, four larval (juvenile) stages and an adult stage. The third juvenile stage is referred to as the infective juvenile (IJ) or dauer larva and is the free-living non-feeding and non-developing stage (Poinar and Leutenegger, 1968; Glazer, 1992; Grewal *et al.*, 2002b). It has a carbohydrate energy reserve and an extra cuticle layer which is retained from the second stage for protection. It is able to survive and persist in adverse conditions of moisture, temperature, and aeration outside the host until it finds a new one (Poinar and Leutenegger, 1968; Glazer, 1992; Grewal *et al.*, 2002b; Mohan, 2015). Steinernematid and heterorhabditid IJs carry bacteria of genera *Xenorhabdus* and *Photorhabdus* in their intestines respectively. IJs are able to locate an appropriate host, which they enter through natural openings including the mouth, anus and spiracles (Koppenhöfer, 2007). Heterorhabditid nematodes and some steinernematid nematodes are also known to directly cut through thinner parts of the cuticle (Peters and Ehlers, 1994). Once the IJs have entered the host, bacteria are released after the hemocoel is invaded by IJs. The bacteria spread within the host and kill it through septicaemia, within 24-48 hours of nematode invasion, and disintegrate its tissue. The IJ feeds and develops on this tissue and on the bacteria which grow rapidly (Grewal and Georgis, 1999). In addition, the symbiotic bacteria produce nematicidal (Hu *et al.*, 1999), bactericidal and fungicidal antibiotics to deter secondary infection of the host (Hu and Webster, 2000; Forst and Clarke, 2002). In return, IJs safeguard symbiotic bacteria from environmental factors and transfer them into the host (Grewal and Georgis, 1999). Two to four days later, according to nematode species and host, having passed through the fourth juvenile stage, the IJ matures into an adult (Kaya, 1990). Steinernematid adults are mainly amphimictic (males and females are involved in cross-fertilization), with the exception of *Steinernema hermaphroditum* in which there are hermaphrodites (males and females are not involved in cross-fertilization) in the first generation, whereas in heterorhabditids, the first generation of adults within the infected host

_____

are hermaphrodites and subsequent generations are amphimictic (Stock *et al.*, 2004). Thus, in *Steinernema,* nematodes of both sexes are required for a complete life cycle to occur while in Heterorhabditidae, a single infective juvenile is sufficient (Griffin *et al.*, 2005). Reproduction occurs for a few (2-3) generations within the cadaver for a duration of 7-15 days until resources are exhausted. IJs then emerge from the cadaver in pursuit of a new host (Kaya, 1990). A single infected host can result in hundreds of IJs based on the host (Griffin *et al.*, 2005) (Fig. 1-6).
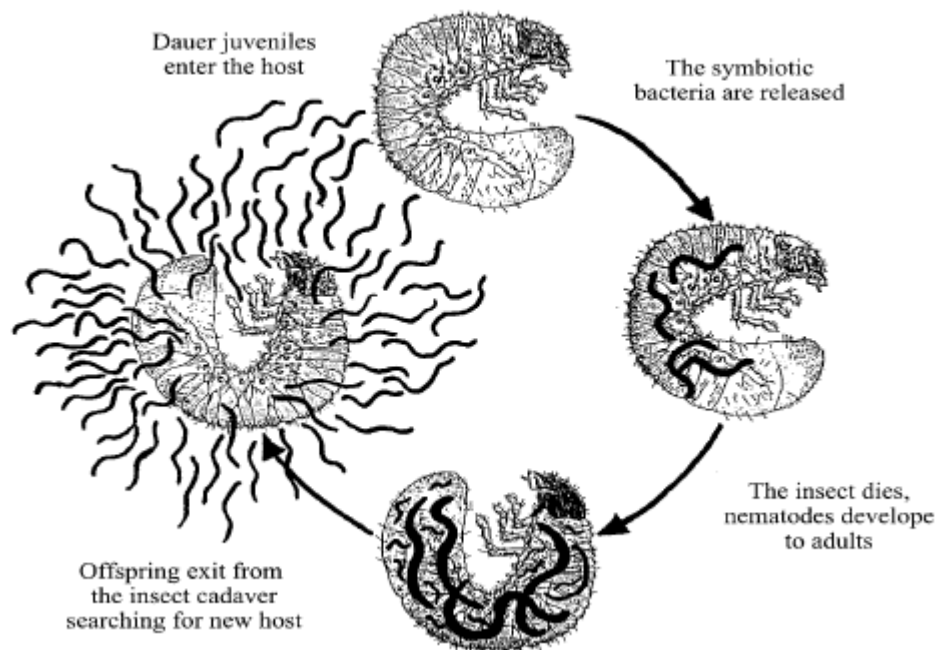
Figure 1-6 Life cycle of entomopathogenic nematodes in infected larva of a scarabaeid beetle. Source of picture (Ehlers, 2001).

Infection of insects by nematodes is apparent as hosts become flabby after death and change colour in accordance with the respective bacteria. Throughout nematode development, insects killed by *Heterorhabditis* decrease in flaccidness while those killed by *Steinernema* remain flaccid. For example, larvae of wax moth *Galleria mellonella* killed by *Heterorhabdita* become green, yellow, orange, red, brick-red or purple in colour, while those killed by *Steinernema* become a brownish colour which ranges from ochre to near black. Nematodes can be observed within the cadaver if its cuticle is transparent. Cadavers do not decay or emit

_____

_____

decaying smells; and while their bodies do not liquefy, they do eventually deteriorate (Koppenhöfer, 2007).

1.2.2 Nematode movement and host location

IJ activities include dispersal, host searching, host identification and infection (Lewis, 2002). Nematodes are classified as 'ambushers' or 'cruisers' depending on the foraging strategy of their IJs. Nematode foraging strategy is significant in that it determines the suitability of a given host including the position of the IJ within the substrate and the type of host they are expected to encounter (Gaugler *et al.*, 1997a; Griffin *et al.*, 2005). Ambushers wait for a host to pass by and tend to pursue mobile insects close to the surface of the substrate (Griffin *et al.*, 2005; Lewis *et al.*, 2006). Examples include *S. scapterisci* and *S. carpocapsae* (Campbell and Gaugler, 1993; Campbell and Gaugler, 1997). Ambushers are also able to nictate, which enables them to attach to a passing host; this involves standing upright by raising most of their bodies (Campbell and Gaugler, 1993; Lewis *et al.*, 2006). *S. carpocapsae* and *S. scapterisci* are known for their nictating behaviour which may last hours at one time (Campbell and Kaya, 2002; Griffin *et al.*, 2005). Jumping has also been reported in some *Steinernema* spp. (Campbell and Kaya, 1999; Campbell and Kaya, 2000); this enables them to travel relatively significant distances, which enables their spread, and can also serve as an attacking strategy such that IJs throw themselves at their target host (Campbell and Kaya, 1999; Lewis *et al.*, 2006).

Cruisers move through the medium in search of a host and tend to pursue fairly sedentary insects at different depths within the substrate (Griffin *et al.*, 2005; Lewis *et al.*, 2006). Examples include *H. megidis, H. bacteriophora, S. glaseri* and *S. kraussei* (Campbell and Gaugler, 1997; Lewis, 2002). Nematodes that do not comply with either criterion are categorised as 'intermediates', which are associated with insects on a continuum from sedentary to mobile (Griffin *et al.*, 2005; Lewis *et al.*, 2006). Examples include *S. feltiae* and *S. riobrave* (Campbell and Gaugler, 1997; Griffin *et al.*, 2005).

IJs are attracted to hosts through signs such as vibrations caused by host movement, as well as host waste including faecal matter and carbon dioxide (Grewal *et al.*, 1993; Lewis *et al.*, 1993; Torr *et al.*, 2004). Given that cruisers are able to travel towards their hosts, they seem to be more sensitive to such cues in comparison with their ambushing counterparts (Grewal *et al.*, 1994b). Once a host has been infected, it becomes more or less appealing to other

_____

nematodes within the same or different species due to volatiles that are given off (Grewal *et al.*, 1997).

1.3 Factors affecting success of entomopathogenic nematodes for above ground applications

The success of EPN application is influenced by biotic factors such as host range and nematode searching strategy, as well as abiotic factors such as temperature, desiccation, exposure to ultraviolet light, means of application and agrochemicals, the most important factors being desiccation, temperature, lethality against the respective pest and searching strategy (Lacey and Georgis, 2012).

1.3.1 Biotic factors

EPN success is influenced by several biotic factors mainly concerning the co-ordination of a suitable nematode with the respective pest. Factors include pathogenicity of the nematode towards the target pest, nematode foraging strategy and persistence and tolerance to the environment (Shapiro *et al.*, 2000; Shapiro-Ilan *et al.*, 2006b; Shapiro-Ilan *et al.*, 2012). In addition, when EPNs are combined with biotic agents, neutral, positive or negative effects such as additive, synergistic and antagonistic impacts can be observed (Barbercheck and Kaya, 1990; Kaya *et al.*, 1995; Koppenhofer and Kaya, 1997; Ansari *et al.*, 2004; Shapiro-Ilan *et al.*, 2004; Ansari *et al.*, 2008; Shapiro-Ilan *et al.*, 2012). Furthermore, competition amongst EPNs (intra-specific) and competition between EPNs and microbes (inter-specific) may also occur, affecting EPN success (Alatorre-Rosas and Kaya, 1990; Alatorre-Rosas and Kaya, 1991).

Host suitability is an important factor in the determining EPN pathogenicity, thus, in order to achieve adequate control, it is necessary to select the appropriate nematode species as well as strain, given that noteworthy differences in virulence exist between different EPN strains of the same species (Grewal *et al.*, 2002a; Grewal *et al.*, 2002b; Grewal *et al.*, 2004; Koppenhofer *et al.*, 2004; Grewal *et al.*, 2005a; Wright *et al.*, 2005).

Given that EPN species vary in relation to their host searching behaviour, EPN host range is further restricted by foraging strategy (Gaugler *et al.*, 1997b; Grewal *et al.*, 2005a; Griffin *et al.*, 2005; Lacey and Georgis, 2012). Using nematodes that ambush to suppress sedentary insect pests such as leafminers would result in control failure in foliar application, since it is

_____

highly unlikely that the nematode would encounter larvae inside the mines on leaves. For instance, Williams and Walters (2000) reported that a single application of *S. feltiae* (Nemasys®) (5000 infective juveniles (IJs)/ ml) at > 90 % RH induced a mean mortality of 82 % of the leafminer *Liriomyza huidobrensis* in trials on lettuce at a commercial greenhouse. This was higher than the results obtained by Broadbent and Olthof (1995) who reported 61 % and 75 % mortality of the leafminer *Liriomyza trifolii* induced by *S. carpocapsae* "All" and "Pye" strains respectively at > 90 % RH in similar field trials carried out on chrysanthemum using double the concentration (10,000 IJs/ ml) used by Williams and Walters (2000). This superior efficacy of *S. feltiae* could be explained by the habitat adaption (sedentary) and its behaviour (an intermediate forager that employs both an ambush and a cruise foraging strategy) compared to *S. carpocapsae* (an ambush forager that exploits a 'sit and wait' strategy). Another example of the significance of host finding behaviour (or foraging strategy) is *S. carpocapsae,* which shows poor performance against the larvae of the scarab beetle or white grub (Coleoptera: Scarabaeidae) although it is highly effective against numerous caterpillars. On this occasion, the inability to control white grubs may be explained by ambushing nematode behaviour near the surface of the soil and sedentary habitat of the white grub (Forschler and Gardner, 1991). In addition, cruiser-foraging EPN species such as *H. bacteriophora* are consequently less suitable for utilisation against surface feeding pests that are highly mobile; examples include armyworms and cutworms (Grewal *et al.*, 2005a).

Nematode and host developmental stage are also important factors influencing effectiveness of EPN use. Given that infective juveniles do not feed during the energy- consuming host searching process (particularly for cruisers), older infective juveniles have reduced energy reserves, which reduces their infectivity. According to a laboratory experiment, IJs with exhausted reserves were unable to kill larvae of the mealworm beetle *Tenebrio molitor* when they were required to search for them, although they were able to kill them when they were put into contact with the pests and thus a search was not required (Vänninen, 1990).

Nematode efficacy may also fluctuate according to the developmental stage of a given insect pest and within a given stage (different larval instars). For instance, susceptibility of adults and larvae of Lepidoptera is higher than that of lepidopteran pupae (Kaya and Hara, 1980; Kaya and Grieve, 1982; Kaya, 1985) and the susceptibility of larvae to nematode infection increases with larval development (Kaya, 1985; Journey and Ostlie, 2000; Bélair *et al.*, 2003).

_____

_____

Therefore, host developmental stage should be taken into account in order to improve EPN effectiveness in foliar applications.

In addition, nematode performance may be compromised by intra-specific competition between EPNs. This can occur when a host is invaded by too many infective juveniles of the same species at the same time, and impacts nematode fitness, survival of offspring and recycling (Alatorre-Rosas and Kaya, 1990; Alatorre-Rosas and Kaya, 1991). Moreover, nematode performance may be compromised by inter-specific competition between EPNs, such as that between the heterorhabditid and steinernematid species, which are normally unable to co-exist in the same host due to incompatibility resulting from differences in their mutualistic bacteria (Alatorre-Rosas and Kaya, 1990; Alatorre-Rosas and Kaya, 1991). It has been claimed that no benefit is achieved by the use of two EPN species in a single inundative application against a given soil pest (Alatorre-Rosas and Kaya, 1990). Furthermore, competition with entomopathogenic microbes could also potentially influence nematode efficacy (Alatorre-Rosas and Kaya, 1990; Alatorre-Rosas and Kaya, 1991).

Additive, synergistic or antagonistic effects on EPN efficacy can be observed when control agents are combined. Additive effects are observed when there is no interaction between agents and they operate independently of one another, synergistic effects take place when the combined action of two agents produces a superior outcome to that of the combination of the outcomes of the agents acting separately, and antagonistic effects are observed when interactions between the agents produce results that are less effective than those where an additive effect has taken place (Koppenhöfer, 2005). Synergism of EPNs with other entomopathogens was reported with *Metarhizium anisopliae* (Ansari *et al.*, 2008; Ansari *et al.*, 2010; Monteiro *et al.*, 2013), *Bacillus thuringiensis* (Koppenhofer *et al.*, 1999; Oestergaard *et al.*, 2006), and *Beauveria brongniartii* (Choo *et al.*, 2002), as well as antagonism with *Beauveria bassiana* (Darissa and Iraki, 2014).

1.3.2 Abiotic factors

Several abiotic factors such as desiccation (Glazer and Navon, 1990; Glazer, 1992; Baur *et al.*, 1995; Williams and MacDonald, 1995; Mason and Wright, 1997; Wright *et al.*, 2005; De Waal *et al.*, 2013) temperature (Molyneux, 1985; Grewal *et al.*, 1994b; Grewal, 2002; Wright *et al.*, 2005) and ultraviolet radiation (Gaugler and Boush, 1978; Gaugler *et al.*, 1992; Nickle and Shapiro, 1994; Wright *et al.*, 2005) affect the efficacy of EPNs, the most important of

_____

which is normally desiccation (Glazer and Navon, 1990; Baur *et al.*, 1995; Mason and Wright, 1997; Grewal, 2002; Wright *et al.*, 2005). These factors are more pronounced in open field environments as opposed to protected environments and are particularly challenging in foliar applications (Wright *et al.*, 2005).

A. Desiccation

The success of EPN applications is largely dependent upon the presence of moisture, as infective juveniles require enough water in order to move freely on the leaf surface in order to find and enter the mines of leafminers and thus infect them with ease (Tomalak *et al.*, 2005). However, excessive use of water can lead to reduced efficacy due to EPN formulation runoff (Glazer *et al.*, 1992). High relative humidity is necessary in order to maintain moisture levels and is consequently essential for the success of above-ground EPN applications (Wright *et al.*, 2005). In cases where desiccation occurs progressively, infective juveniles are able to persist and adapt by entering an inactive stage as they have time to do so (Womersley, 1990; Koppenhöfer, 2007). This usually occurs in soils in which the relative humidity in the pores is almost 100 % and excludes areas that are close to the soil surface in sandy soils that have low levels of organic material (Koppenhöfer, 2007).

In the case of the Codling Moth *Cydia pomonella*, once the IJs are applied, they must reach the cryptic environment in which the host larvae are located, penetrate the encompassing cocoon, and enter the pests before the environment dries (Lacey *et al.*, 2006). It is consequently useful to choose an EPN strain which uses an active foraging strategy such that it is able to detect and enter the pest more quickly, resulting in a reduced post- application wetting period (Lacey *et al.*, 2006). It is also recommended not to apply EPNs when weather is windy as this accelerates drying time thus decreasing EPN effectiveness (Unruh and Lacey, 2001). In addition, applying EPNs in the late afternoon or early evening may lead to a decrease in desiccation and thus extends nematode infectivity (Lello *et al.*, 1996).

B. Temperature

Temperature is a significant factor affecting EPN efficacy (Grewal, 2002; Wright *et al.*, 2005). EPN species differ in relation to temperature range adequate for survival and infectivity, which is influenced by EPN indigenous habitat as well as place of origin (Kaya, 1990; Lacey and Georgis, 2012). For instance, nematodes *H. megidis, S. feltiae*, and *Heterorhabditis* are tolerant of lower temperatures while *H. indica*, *S. glaseri*, and *S. riobrave*

_____

are somewhat tolerant of higher temperatures (Kung *et al.*, 1991; Grewal *et al.*, 1994b; Shapiro *et al.*, 2000; Shapiro-Ilan *et al.*, 2012). Depending on the duration, prolonged exposure to temperatures below 0 °C and above 40 °C is fatal to the majority of EPN species. Adequate performance of the majority of commercial EPNs tends to occur at temperatures between 20 and 30 °C, with the exception of *S. feltiae* and *S. riobrave*, which are effective and extremely effective at temperatures between ca. 12 and 25 °C, and 25 and 35 °C respectively. EPNS usually become lethargic at low temperatures (<10–15 °C) and become deactivated at higher temperatures (> 30–40 °C) (Glazer, 2002; Koppenhöfer, 2007).

An association exists between temperature and relative humidity, such that the joint effect influences the success of foliar EPN applications against leafminers. For instance, foliar applications of *S. feltiae* and *Heterorhabditis* spp. were recorded to be efficient against second stage larvae of *L. huidobrensis* at 20°C and > 80 % relative humidity. In addition, *S. feltiae* was found to be similarly efficient against the same larvae between the temperatures of 10 °C and 30 °C, where optimal efficacy was attained at > 90 % relative humidity (Williams and MacDonald, 1995; Tomalak *et al.*, 2005). The All strain of *S. carpocapsae* also achieved mortality levels of over 50 % in leafminer *L. trifolii* at 22 °C and 95 % relative humidity (Olthof and Broadbent, 1992; Tomalak *et al.*, 2005). However, the effect of high temperature on EPNs can be avoided by applying nematodes at dusk (Gaugler and Boush, 1978; Macvean *et al.*, 1982; Mason and Wright, 1997).

C. UV light

UV radiation significantly affects survival of IJs. For instance, IJs die rapidly as a result of several minutes of exposure to light at an approximate frequency of 300 nm. They are able to endure direct sunlight for a maximum of 30 minutes before their infectivity and survival are negatively affected. *Steinernema carpocapsae* is vulnerable to shorter UV (254 nm), but is more resilient to longer UV (366 nm) in comparison to *H. bacteriophora*, indicating that it may be more efficient in biocontrol applications on uncovered surfaces (Gaugler and Boush, 1978; Gaugler *et al.*, 1992). However, the effect of solar radiation on nematodes can be avoided by applying nematode at dusk (Gaugler and Boush, 1978; Macvean *et al.*, 1982; Mason and Wright, 1997).

_____

_____

1.4 Foliar application

The use of entomopathogenic nematodes has been particularly successful against insect stages occurring in the soil. However, very good results have also been achieved through applications of EPNs against leafminers, thrips, whiteflies and on wood-boring insects in tree trunks, as well as in lure and kill procedures against pests like cockroaches and houseflies, and also against human and animal pests like fleas and lice (Grewal, 2012).

The EPN host range also comprises a wide range of significant foliar feeding insect pests (Grewal *et al.*, 2005b; Wright *et al.*, 2005). However, EPN efficacy in above- ground applications has been found to be the lowest in foliar habitats (Lacey and Georgis, 2012) and commercialisation of EPNs for use against pests on foliage has been uncommon and generally unproductive (Shapiro-Ilan *et al.*, 2006a) due to limiting factors such as desiccation (Lello *et al.*, 1996; Grewal, 2002), temperature (Grewal *et al.*, 1994b; Grewal, 2002) and ultraviolet radiation (Gaugler and Boush, 1978; Gaugler *et al.*, 1992; Grewal, 2002). Results for foliar EPN fluctuate in open field environments (Mason and Wright, 1997; Unruh and Lacey, 2001; Tomalak *et al.*, 2005; Wright *et al.*, 2005). Nevertheless, there have been encouraging results for foliar EPN applications in protected environments (Tomalak *et al.*, 2005). For instance, under commercial circumstances, greenhouse trials have demonstrated that a maximum of 80 % control can be achieved using *S. feltiae* against leafminers and thrips on ornamentals, and against agromyzid leafminers (*Chromatomyia syngensiae, Liriomyza huidobrensis* and *Liriomyza bryoniae*) on tomato and lettuce (Tomalak *et al.*, 2005; Wright *et al.*, 2005). EPNs are also potentially effective against other insect pests on a foliar target such as *Liriomyza trifolii* and *Bemisia tabaci*. However, all such successful cases required a relative humidity of ≥ 80-90 % (Wright *et al.*, 2005). One approach for decreasing the influence of desiccation, temperature and UV radiation and thus extending nematode survival and infectivity involves the application of EPNs at dusk (Gaugler and Boush, 1978; Macvean *et al.*, 1982; Lello *et al.*, 1996; Mason and Wright, 1997).

Although the implications of sunlight exposure can be lessened by applying nematodes in the early evening (Gaugler and Boush, 1978; Lello *et al.*, 1996; Mason and Wright, 1997), maintaining a relative humidity of > 80 % as well as free water on leaf surfaces has been more challenging to attain (Grewal, 2002). The use of different adjuvants with anti-desiccant (Glazer, 1992; Broadbent and Olthof, 1995; Baur *et al.*, 1997; Piggott *et al.*, 2000; Williams and Walters, 2000; Head *et al.*, 2004; Schroer and Ehlers, 2005; Llacer *et al.*, 2009; Lacey *et*

_____

_____

al., 2010; Shapiro-Ilan *et al.*, 2010; De Waal *et al.*, 2013) or UV-protective properties (Nickle and Shapiro, 1994; Baur *et al.*, 1997) with EPNs can improve nematode survival and efficacy to different extents on above ground application. However, further efforts are required in order to boost post-application survival especially on foliage.

Adjuvants can be described as "*material added to a tank mix to aid or modify the action or the physical characteristics of the mixture*" (ASTM Standards E1519). A distinction is made between two types of adjuvant: formulation adjuvants, sometimes referred to as additives or inerts, which constitute part of the formulation, and spray adjuvants, also referred to as tank mixing additives or simply adjuvants, which are added to the water in the spraying tank in addition to the formulation itself prior to application (Hochberg, 1996; Krogh *et al.*, 2003). Spray adjuvants exist under several categories, such as surfactants, spreaders, stickers, humectants and wetting agents (Hoffmann *et al.*, 2008).

Enhanced control of leafminers and thrips on ornamentals has been recorded through use of a polymer-based preparation of *S. feltiae* (Wright *et al.*, 2005). The deposition rate of infective juveniles applied to Chinese cabbage leaves by spinning disc was raised considerably with the addition of non-ionic surfactants (Crovol L27, Crovol L40 and Triton X-100) or antidesiccants (glycerol and Croduvant) (Mason *et al.*, 1998). In a laboratory experiment, formulation of xanthan gum and the surfactant Rimulgan® (both at 0.3 % concentration) was reported to cause a reduction in EPN runoff on cabbage plants and a considerable increase in EPN efficacy through decreasing surface tension and improving binding features. This formulation leads to quicker EPN infection through the assistance of EPN movement on foliage as well as the decrease of pest mobility. Use of this formulation resulted in a 50 % rise in efficacy under laboratory conditions, although EPNs persisted for a maximum of 10 h (Schroer and Ehlers, 2005). Shapiro-Ilan *et al.* (2010) observed a significant increase in the efficacy of *S. carpocapsae* "All" strain in aboveground application on barks of tree against the lesser peachtree borer, *Synanthedon pictipes* (Grote & Robinson) using Barricade® II. The authors reported that a treatment of *S. carpocapsae* suspended in water followed by a post-application covering of Barricade® II induced 70 % and 100 % mortality of *Synanthedon pictipes*. Llacer *et al.* (2009) reported a mortality of 80 % and 98 % in curative and preventive treatment respectively of the red palm weevil, *Rhynchophorus ferrugineus*, using *Steinernema carpocapsae* in a chitosan formulation.

_____

_____

Regarding *T. absoluta*, Batalla-Carrera *et al.* (2010) reported that two foliar applications of three nematode species indigenous to Spain (*Steinernema feltiae* (strain Bpa), *S. carpocapsae* (strain B14) and *Heterorhabditis bacteriophora* (strain DG46)) with the addition of 0.05 % of the oil adjuvant Addit® (Koppert), induced mortality of 92 %, 88.5 % and 76.3 % respectively of larvae of *T. absoluta* on naturally infested tomato leaves in a pot experiment under controlled conditions. Similarly, Jacobson and Martin (2011) reported high efficacy (> 90 % mortality) using an aqueous suspension of the commercial nematode species *S. feltiae* (Nemasys®) against larvae of *T. absoluta* at favourable conditions. But these authors observed lower nematode efficacy using an aqueous suspension of the same nematode species against larvae of *T. absoluta* in two separate trials undertaken on commercial organic tomato crops in Horticilha, Cilha Queimada, Alcochete, Portugal (50 % and 43 % overall mean mortality in first and second trial respectively). The low efficacy in the first trial was attributed by these authors to poor application in one of the plots due to some nozzles being temporarily blocked and the authors suggested that the overall mortality would rise to 56 % if the data from that plot were excluded. The result of the second trial was attributed to the unfavourable environmental conditions for nematode activity (65-74 % RH). The authors suggested that the efficacy of nematode could be increased by addition of adjuvants to the spray mixture and by improving the spray technique.

It has been claimed that despite the overall improvement in nematode effectiveness achieved through use of adjuvants, such an improvement is regarded as being inadequate for endorsement for foliar application (Grewal, 2002; Lacey and Georgis, 2012). Thus, the agricultural use of nematodes to control foliar pests necessitates formulation, application methodology and spray system optimisation (Bélair *et al.*, 2005). Additionally, due to a number of factors that interact with each other including target insect pest, host plant and method of applications, no single adjuvant is appropriate for all conditions. Therefore, it is important to consider these factors when testing possible adjuvants (Mason *et al.*, 1998).

1.5 Aims of the study

The overall aim of this study was to evaluate the use of EPNs as biocontrol agents of *T. absoluta*.

The precise objectives of this study are discussed in separate chapters of the thesis:

- To determine the efficacy of commercial EPNs against different stages of *Tuta absoluta*,

_____

- To study factors affecting nematode efficacy against larvae of *Tuta absoluta* on tomato leaves, and

- To evaluate adjuvants for their potential to increase efficacy of EPNs on tomato leaves against larvae of TLM *Tuta absoluta.*

Chapter 2 Efficacy of entomopathogenic nematodes against *Tuta absoluta*

Abstract

Tomato leafminer, *Tuta absoluta*, is one of the most serious lepidopteran pests of tomato plants. The larvae of this pest can cause losses of up to 100 % by attacking leaves, fruits, stems and flowers. Current control strategies for *T. absoluta* primarily involve the use of insecticides. Increasing resistance to chemical insecticides in field populations of *Tuta absoluta* has motivated research on alternative control measures. Biological control with entomopathogenic nematodes (EPNs) can be an alternative or one component of the Integrated Pest Management programme for this pest. This chapter reports investigations into the virulence of some commercial EPNs against different stages of *Tuta absoluta* (larvae, pupae and adults) in Petri dish, leaf and soil bioassays. Results showed high adult and larval mortality using *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* in dish bioassays without difference in efficacy between nematode species or in susceptibility between larval instars. Pupae in contrast were less susceptible to nematode infection. Differences in nematode infectivity and larval instar susceptibility were found in leaf bioassays (> 95 % RH). *S. feltiae* was the most virulent species with mean mortality against all larval instars of 80 %, followed by *S. carpocapsae* (70 %) then *H. bacteriophora* (57 %). LC_{50} and LT_{50} values of *S. feltiae* against different larval instars were the lowest followed by *S. carpocapsae* then *H. bacteriophora*. Larval susceptibility increased throughout larval development. In soil experiments, *S. feltiae* and *S. carpocapsae* were significantly more virulent than *H. bacteriophora* against the fourth larval instar when they drop to pupate in soil and against adults when they are emerging from pupae. The pupal stage was less susceptible to nematode infection in soil too.

_____

2.1 Introduction

One of the most serious lepidopteran pests of tomato plants (*Lycopersicon esculentum* L.) in South America is the tomato leafminer (TLM), *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) (EPPO, 2005; Urbaneja *et al.*, 2013). Since its introduction into Europe in 2006, being first detected in the northern part of Castellón de la Plana in eastern Spain, it has continued to spread rapidly across Europe, northern Africa and the Middle East where it immediately reached damaging levels and became a serious pest of tomatoes grown in field and greenhouse (Desneux *et al.*, 2010; Guenaoui *et al.*, 2013; Urbaneja *et al.*, 2013; Brevault *et al.*, 2014). This pest has now been reported in many Middle Eastern, African and Asian countries (EPPO, 2011a; EPPO, 2011b; Baniameri and Cheraghian, 2012; EPPO, 2012a; EPPO, 2012b; EPPO, 2012c; EPPO, 2012d; EPPO, 2013a; EPPO, 2013b; Brevault *et al.*, 2014; EPPO, 2014a; EPPO, 2014b; EPPO, 2015). Besides tomato plants, this pest can also live on other cultivated host plants from the family Solanaceae such as sweet pepper (*Solanum muricatum*), aubergine (*S. melongena*), potato (*S. tuberosum*) and tobacco (*Nicotiana tabacum*) (Vargas, 1970; Campos, 1976) cited in Desneux *et al.* (2010) and Urbaneja *et al.* (2013) as well as on non-cultivated Solanaceae (solanaceous weeds) such as *S. nigrum*, *S. eleagnifolium*, *S. bonariense*, *S. sisymbriifolium*, *S. saponaceum*, and *Lycopersicum puberulum*. Furthermore, *Datura ferox*., *D. stramonium* and *N. glauca* are other naturally available host plants for this pest (Garcia and Espul, 1982; Larraín, 1986) cited in Desneux *et al.* (2010) and Urbaneja *et al.* (2013). Moreover, alternative hosts of this pest have been reported since its entrance to Europe, such as bean (*Phaseolus vulgaris*) (EPPO, 2009c), *Malva* sp. and *Lycium* sp. in Italy (Caponero, 2009) cited in Desneux *et al.* (2010) and Cape gooseberry (*Physalis peruviana*) in a greenhouse in Sicily (Tropea Garzia, 2009).

The life cycle of *T. absoluta* consists of four development stages: adult, eggs, larva and pupa. Male and female adults mate multiple times. Eggs are laid on all aerial parts of tomato plants. Eggs hatch in a few days and newly emerged larvae enter leaves, stems or fruits, where they feed and develop through four different larval instars and as a result produce visible mines and galleries which expand as larvae develop and increase in size. Yield is reduced as a result of the damage happening to the leaves when larvae feed on the mesophyll which affects the photosynthetic capacity of the crop. Fruits can be invaded by secondary pathogens which enter from the galleries produced by larvae. General development of plants is altered due to the mines formed on the stems. Finally, direct feeding of larvae on the growing tips can result

_____

in reducing and stopping plant development (Desneux *et al.*, 2010). Pupation occurs in the soil when larvae reach the last instar. After a few days, when the development of pupae is completed, adults emerge from soil (Urbaneja *et al.*, 2007) cited in Garcia-del-Pino *et al.* (2013).

The management of *T. absoluta* has commonly relied on the use of chemical insecticides in South America (Branco *et al.*, 2001; Lietti *et al.*, 2005; Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2010; Reyes *et al.*, 2012) and in the newly invaded areas (Desneux *et al.*, 2010; Desneux *et al.*, 2011; Gonzalez-Cabrera *et al.*, 2011; Urbaneja *et al.*, 2012; Konus, 2014). Effective chemical control is difficult because of the ability of this pest to develop resistant strains and because of the feeding behaviour of larvae in which they are protected from contact with insecticides inside the mines on leaves, fruits and stems (Terzidis *et al.*, 2014). This has prompted frequent and extensive use of insecticides, which stimulated the development of insecticide resistance in *T. absoluta* in its area of origin (Siqueira *et al.*, 2000a; Haddi *et al.*, 2012; Reyes *et al.*, 2012; Gontijo *et al.*, 2013; Silva *et al.*, 2015) and in the newly invaded areas (Haddi *et al.*, 2012; Konus, 2014; Roditakis *et al.*, 2015) and raised concern about the negative impacts of insecticides on non-target organisms (some of which are beneficial) that are used in integrated pest management programmes in tomato crops (Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2007; Arno and Gabarra, 2011; Biondi *et al.*, 2012a; 2012b; 2013; Martinou *et al.*, 2014; Gontijo *et al.*, 2015) as well as the negative impact of insecticides on the environment, users and consumers (Pimentel *et al.*, 1992; Peterson and Higley, 1993; Weisenburger, 1993). Other non-chemical control methods for *T. absoluta*, including using parasitoids, predators, pheromones, entomopathogenic fungi and bacteria, have been attempted with varied levels of success (Desneux *et al.*, 2010; USDA–APHIS, 2011; Urbaneja *et al.*, 2013). *T. absoluta* is a challenging pest to control; therefore alternative biocontrol agents are urgently needed. Additionally, the available information on its behaviour and control revealed that it is impossible to control it with one method and an integrated pest management programme (IPM) should be used in order to successfully control this pest (Urbaneja *et al.*, 2013).

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are an important group of biological control agents for various insect pests that are economically important (Grewal *et al.*, 2005b; Mohan, 2015). These EPNs are capable of killing hosts within 24-48 hours of nematode invasion, which is enabled by their mutualistic

relationship with bacteria of the genera *Photorhabdus* and *Xenorhabdus* that are carried in the intestines of Heterorhabditidae and Steinernematidae respectively (Koppenhöfer, 2007). Once the infective juveniles (IJs) have entered the haemocoel of the host, bacteria spread within the host and kill it through septicaemia (Grewal and Georgis, 1999). EPNs are considered a good component of IPM programmes due to their desirable characteristics. They are characterised by a wide range of pest insect hosts, can actively search or sit and wait for a host, kill insects quickly (24-48 h), can be applied by traditional application equipment, do not harm all vertebrates, most non-target invertebrates and the food supply, ease of mass production (both *in vivo* and *in vitro*) and compatibility with many agrochemicals, biological insecticides and other biocontrol agents (Koppenhöfer, 2005; Lacey and Georgis, 2012). EPNs have already been used, with mixed success, against a wide range of insect pests in a diversity of habitats (Kaya and Gaugler, 1993; Lacey *et al.*, 1993; Mason and Wright, 1997; Belair *et al.*, 1999; Williams and Walters, 2000; Bélair *et al.*, 2003; Bélair *et al.*, 2005; Tomalak *et al.*, 2005; Shapiro-Ilan *et al.*, 2010; Bellini and Dolinski, 2012; Shapiro-Ilan *et al.*, 2015).

There have been few attempts to test the efficacy of EPNs against *T. absoluta* and these studies used native EPNs. For example, Batalla-Carrera *et al.* (2010) reported that three nematode species (*Steinernema feltiae* strain Bpa, *S. carpocapsae* strain B14 and *Heterorhabditis bacteriophora* strain DG46), which are indigenous to Spain, were able to find and kill larvae of *T. absoluta* on naturally infested tomato leaves in pot experiments under greenhouse conditions. Additionally, these authors found that adults emerged from surviving pupae were susceptible and infected by EPNs, whereas pupae were less susceptible to nematode infection in Petri dish bioassays. Garcia-del-Pino *et al.* (2013) tested the efficacy of soil treatment with the three indigenous nematode species mentioned above against larvae, pupae and adults of *T. absoluta* and found that last instar larvae of *T. absoluta* were highly susceptible to all three nematode species when they drop to the ground and that emerging adults were susceptible to *S. carpocapsae,* but not to *S. feltiae*, whereas pupae were less susceptible to *S. carpocapsae* and *S. feltiae* infection. Furthermore, results of trials undertaken by Jacobson and Martin (2011) in commercial organic tomato crops in Portugal using the commercial nematode species *S. feltiae* (Nemasys®) showed that this nematode species induced mean larval mortality of less than 51 % in two separate trials.

There are a number of biotic and abiotic factors that are critical for successful EPN application. Among the most important biotic factors is that the target insect pest must be

treated with the most effective nematode. Virulence, environmental tolerance, host finding and persistence are factors that must be taken into account when choosing the suitable nematode. Additionally, nematode rate must be considered for effective control (Shapiro-Ilan *et al.*, 2002; Shapiro-Ilan *et al.*, 2006b; Shapiro-Ilan *et al.*, 2012). Moreover, nematode efficacy may vary between the developmental stages of *T. absoluta* and also within a given stage (e.g. larvae). This has been reported in other lepidopteran pests in which larvae and adults were more susceptible to nematode infection than pupae (Kaya and Hara, 1980; Kaya and Grieve, 1982) and the susceptibility of larvae increases as the larvae develop (Kaya, 1985; Journey and Ostlie, 2000; Bélair *et al.*, 2003).

The aim of this work was to test the efficacy of the three commercial EPN species *Steinernema feltiae* (Nemasys®), *Steinernema carpocapsae* (Nemasys® C) and *Heterorhabditis bacteriophora* (Nemasys® H), which are distributed as biological agent by BASF plc to many countries such as UK, Europe, USA, Canada and New Zealand, against *T. absoluta* under laboratory conditions. The objectives were (1) to test the efficacy of EPNs against different stages of *T. absoluta* in Petri dish bioassays; (2) to test the efficacy of EPNs against different larval instars of *T. absoluta* in leaf bioassays in order to select the most virulent nematode species for further investigations; and (3) to test the efficacy of EPNs against last instar larvae when they drop to the soil to pupate and the adults when they emerge from the soil in order to select the most virulent nematode species that can be applied to the soil to control this pest.

2.2 Materials and methods

2.2.1 Source of Insects

A *Tuta absoluta* culture was established from pupae which were obtained from Professor Denis Wright at Imperial College in London and maintained in a chamber under quarantine conditions in the DEFRA quarantine laboratory at Newcastle University at 25 ± 5 °C with an 18:6 hours (h) light:dark (L:D) regime using high pressure sodium lamps and at 45-88 % RH. Adults were reared in an insect rearing tent "BugDorm" 60 × 60 × 60 cm (l-w-h) and provided with 5 % sugar solution to feed on. To obtain eggs, fresh tomato leaves were cut from the petiole and immersed in a 125 ml polyethylene narrow neck bottle filled with a 20 % nutrient solution (Canna Hydro Vega nutrients solution) and placed in the adult cage. Leaves were replaced daily with fresh leaves and those with eggs were placed separately into Perspex

_____

cages 25 × 25 × 25 cm (l-w-h) for rearing the larvae (each cage was used to hold a certain larval instar). Fresh leaves were provided to the larvae to feed on whenever required. When larvae reached the fourth larval instar, leaves were transferred to an insect rearing tent "BugDorm" 60 × 60 × 60 cm (l-w-h) to obtain the pupae and adults. Pupation occurred on leaves, tissue paper that was placed in the cage and under the trays that carried the polyethylene bottles. Once the adults emerged they were transferred to a new adult cage using a Fulton mechanical aspirator to continue the breeding cycle of the insect.

2.2.2 Tomato plants

The insect was reared on leaves of tomato plants *Solanum lycopersicum* "Moneymaker" variety (4-6 weeks old) which were grown at Newcastle University Field Station (Close House) and provided weekly. They were grown under greenhouse conditions in individual pots (13 cm diameter) using J Arthur Bowers John Innes growing medium Number 2 loam based compost at 20-25 °C and 18 hours day length. Extra lighting was provided during periods of shorter natural day length using high pressure sodium lamps.

2.2.3 Source of entomopathogenic nematodes (EPNs)

Commercial formulations of *Steinernema feltiae* (Nemasys), *Steinernema carpocapsae* (Nemasys C) and *Heterorhabditis bacteriophora* (Nemasys H) were provided by BASF plc (Becker Underwood, UK previously) as a moist paste and were used in all experiments unless otherwise specified.

2.2.4 Determination of different larval instars of Tuta absoluta

T. absoluta has four different larval instars (Giustolin *et al.*, 2002). Each instar lasts for a certain period of time during its life cycle which depends on the environmental conditions. In order to determine these larval instars under our laboratory conditions to obtain the required larval instar for each experiment, a study to differentiate between larval instars was carried out. With the aid of a camel hair brush ten newly hatched larvae obtained from the laboratory culture were placed on a tomato leaflet which was cut from the petiole and wrapped with moist cotton wool to keep it fresh. The leaflets were then placed in vented plastic Petri dishes (9 cm × 1.6 cm), which were covered with their lids and kept at 25 ± 5 °C with an 18:6 L:D regime using high pressure sodium lamps and 45-88 % RH. Whenever required, fresh leaflets were provided to feed the larvae inside the Petri dishes and the cotton wool was wetted with

_____

_____

water to keep the leaflets fresh. Sixty-three plates were used, each with 10 larvae (total of 630 newly hatched larvae). Daily, twenty to thirty-eight larvae were extracted from the mines using a fine needle and a camel hair brush with the aid of a dissecting microscope, killed in hot water and fixed in 70 % alcohol. These procedures were continued until pupation. Subsequently, widths of larval head capsules (HC) were measured using a dissecting microscope equipped with a calibrated ocular micrometre to determine the larval instars according to Dyar's rule (Gullan and Cranston, 2005a). Widths were measured as the distance between the most distant lateral sides of HC margins (Fig. 2-1). Before running an experiment, a sample of ten larvae was taken from the larvae cage that represented a certain larval instar and their head capsule widths were measured in order to ensure that they were the required larval instar.
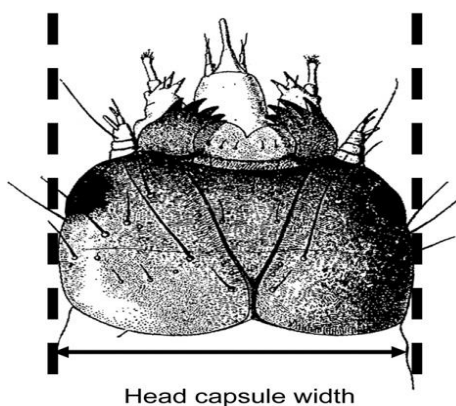
Figure 2-1 Principle of head-capsule measurement. Source of picture Delbac et al. (2010).

2.2.5 Preparing and adjusting concentration of nematodes

Nematode suspensions were prepared using tap water and stored at 7 °C no longer than 30 days before use. Prior to use, nematodes were allowed to acclimatise in an incubator at 25 ± 0.5 °C for 24 h and were examined microscopically to ensure a viability of over 80 %. Nematode concentration was adjusted by altering dilution, using the method described by Glazer and Lewis (2000). The following formula was used to calculate the adjustment and the final counts were within ± 10 % of the needed concentration:

$$[(i/c) - 1] \times V = V_a$$

_____

_____

where i = initial concentration 50 μl, c = final concentration 50 μl, V = volume of the suspension (ml),
and V_a = the amount of water (ml) to be added (if positive) or to be removed (if negative) from the
suspension.

Petri dish and culture tube bioassays

2.2.6 Efficacy of EPNs against different larval instars of Tuta absoluta

Efficacy of *S. feltiae* (Nemasys), *S. carpocapsae* (Nemasys C) and *H. bacteriophora*
(Nemasys H) was evaluated against first, second, third and fourth larval instars in culture tube
bioassays in order to investigate if the nematodes were able to kill *T. absoluta* larvae as well
as to distinguish virulence between nematode species against different larval instars. One
millilitre of either water (control) or nematodes suspended in water (treatment) was applied
into culture tubes (30 ml crystal clear polystyrene with fitted polyethylene cap, Fisher
Scientific) lined with 8.5 cm diameter Whatman No 1 filter paper (area = 56.8 cm^2).
Nematodes were applied at a rate of 50 ± 5 IJs cm^{-2}. In order to obtain first, second, third and
fourth larval instars, larvae in the rearing cages (as mentioned above) were left until they
reach the required larval instar. Afterwards, they were extracted directly from the mines on
infested tomato leaves using a fine needle and a camel hair brush with the aid of a binocular
dissecting microscope and placed in the culture tube. Ten larvae were placed in each tube.
The tubes were closed with their caps and kept in an incubator at 25 ± 0.5 °C in darkness and
larval mortality was recorded after 48 h. A larva was scored dead if it failed to respond to
mechanical stimulation. There were five replicate tubes for treatments (n = 5) with the
corresponding control treatments (n = 5). Moreover, the number of IJs per square centimetre
required to kill 50 % of *T. absoluta* larvae (LC$_{50}$) of each nematode species was determined
against different larval instars. The experimental procedure was as above except that
nematodes were used at five different rates: 0, 1 ± 0.1, 5 ± 0.5, 15 ± 1.5, 25 ± 2.5 and 50 ± 5
IJs cm^{-2}. In addition, the time required by the three EPNs to kill 50 % of *T. absoluta* larvae
(LT$_{50}$) was investigated with the third larval instar. The experimental procedures were as
above except that larval mortality was checked every 8 h up to 48 h. Independent samples
were used for each time point. Nematodes were used at a rate of 50 ± 5 IJs cm^{-2}. There were
five replicate tubes for each time point (n = 5) with the corresponding control treatment (n =
5).

_____

2.2.7 Efficacy of EPNs against adults of **Tuta absoluta**

Efficacy of the three nematode species was evaluated against adults of *T. absoluta* in culture tube bioassays. Experimental procedures were as in the previous experiment (2.2.6) except that adults aged 24 to 48 h were used, and nematodes were used at a rate of 50 ± 5 IJs cm^{-2}. Adults were taken from the adult cage using a mouth aspirator and were sedated in order to transfer them to test tubes by placing the aspirator for 3 - 5 minutes in a basket filled with ice. They were then transferred to the bioassay tube. Adult mortality was recorded after 48 h.

2.2.8 Efficacy of EPNs against pupae of **Tuta absoluta**

Efficacy of the three nematode species was evaluated against pupae of *Tuta absoluta* in Petri dish bioassays. One millilitre of nematodes suspended in tap water was applied onto 8.5 cm diameter plastic Petri dishes (area = 56.8 cm^2) lined with Whatman No 1 filter paper. Ten pupae aged 24 to 48 h were placed in each Petri dish. Nematodes were used at a rate of 50 IJs ± 5 cm^{-2}. The control treatment received water only. The Petri dishes were sealed with parafilm to avoid dehydration and kept in an incubator at 25 ± 0.5 °C in darkness. After 48 h of exposure, the pupae were transferred to new vented Petri dishes and air dried by blowing cool air in order to fix and kill the nematodes on pupae surfaces. Petri dishes were then covered with their lids and kept in the rearing chamber until the emergence of adults. The number of emerged and non-emerged adults was recorded. There were five replicate Petri dishes for each treatment (n = 5) with the corresponding control treatment (n = 5).

Leaf bioassays

2.2.9 Experiment arena

The experiment arena in all leaf bioassays in this chapter and the other chapters consisted of a 3.25 l clear plastic container (Fig. 2-2) filled either with 400 ml water (to obtain > 90 % relative humidity (RH)), 400 ml of saturated salt solution of calcium chloride hexahydrate (to obtain 45 ± 10 % RH) which was prepared according to Winston and Bates (1960) or 400 ml of 85 % glycerine solution (to obtain 75 ± 10 % RH) which was prepared according to Grover and Nicol (1940). In order to obtain second, third and fourth larval instars, larvae were left in the larvae rearing cages (as mentioned above) until they reached the required larval instar. Afterwards they were extracted directly from the mines on infested tomato leaves using a fine needle and a camel hair brush with the aid of a binocular dissecting microscope. In order to

_____

obtain the first larval instar, eggs laid on leaves by females were used. The larvae or eggs were then placed on the upper side of fresh tomato leaves. To keep these leaves fresh, they were immersed in a 125 ml narrow neck bottle filled with a 20 % nutrients solution (Canna Hydro Vega nutrients solution). The leaves containing larvae were left for 24 hours and those containing eggs were left for 48 h to develop well-formed mines before EPN treatment. The leaves were then sprayed on both sides (unless otherwise specified) using a 100 ml spray bottle with nematodes (at the desired concentration) suspended either in tap water until run off or suspended in tap water and adjuvant (at the desired concentration) until a layer of the suspension was formed. Control treatments received water only (unless otherwise specified). Afterwards, the leaves were transferred into the clear plastic containers which were filled with one of the solutions mentioned above depending on RH required in the experiment. The containers were then closed with their lids and kept in an incubator at 25 ± 0.5 °C in darkness in a randomized block design and numbers of dead and live larvae were recorded after 48 h unless otherwise specified. A larva was scored dead if it failed to respond to mechanical stimulation. RH was confirmed by hanging a RH and temperature data logger, type LASCAR EL-USB-2, on the lid of the container. The number of IJs on the leaf surface area was determined following the method described by Glazer and Navon (1990). Leaflets were sprayed with a known concentration of nematodes suspended either in tap water or tap water and adjuvants (at the desired concentration). Immediately after spraying, each leaflet was dipped into 20 ml of water in a separate Petri dish. The leaflet was shaken thoroughly in the water suspension to ensure that all IJs were removed to the water. IJs were counted under a binocular dissecting microscope and the number of IJs per square centimetre for each leaflet was calculated by dividing the total number of IJs washed from a leaflet by the total surface area of this leaflet. Five leaflets were used as replicates. Leaflet surface area was determined using a grid counting method. Each leaflet was placed on 1 cm grid paper and its outline was traced using a pencil. Subsequently, the number of square centimetres was counted and the area of the partial squares was estimated.

2.2.10 Efficacy of EPNs against different larval instars of **Tuta** **absoluta** *in leaf bioassays at > 95 % RH*

Efficacy of the three nematode species was evaluated against first, second, third and fourth larval instars in leaf bioassays in favourable conditions (> 95 % RH) to investigate if the nematodes were able to penetrate tomato leaves and kill *Tuta absoluta* larvae inside tunnels as

_____

_____

well as to distinguish virulence between nematode species against different larval instars on tomato leaves. The experiment arena and procedures were as described in section 2.2.9 with some exceptions. Ten to twenty-five larvae were placed on each leaf. The leaves were then sprayed with either water (control) or nematodes suspended in water at a rate of 60 ± 6 IJs cm^{-2}. There were four replicate containers for treatments ($n = 4$) with the corresponding control treatments ($n = 4$). Furthermore, LC_{50} of the three nematode species was determined against different larval instars. The experimental procedures were as above except that nematodes were used at five different rates: $0, 5 \pm 0.5, 15 \pm 1.5, 30 \pm 3$ and 60 ± 6 IJs cm^{-2}. In addition, LT_{50} of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) was determined against the third larval instar. The experimental procedures were as above except that leaves were sprayed either with water (control) or nematodes suspended in water at a rate of 60 ± 6 cm^{-2} (treatment) and larval mortality was checked every 8 h up to 48 h. Independent samples were used for each time point. There were four replicate containers for each time point ($n = 4$) with the corresponding control treatment ($n = 4$).
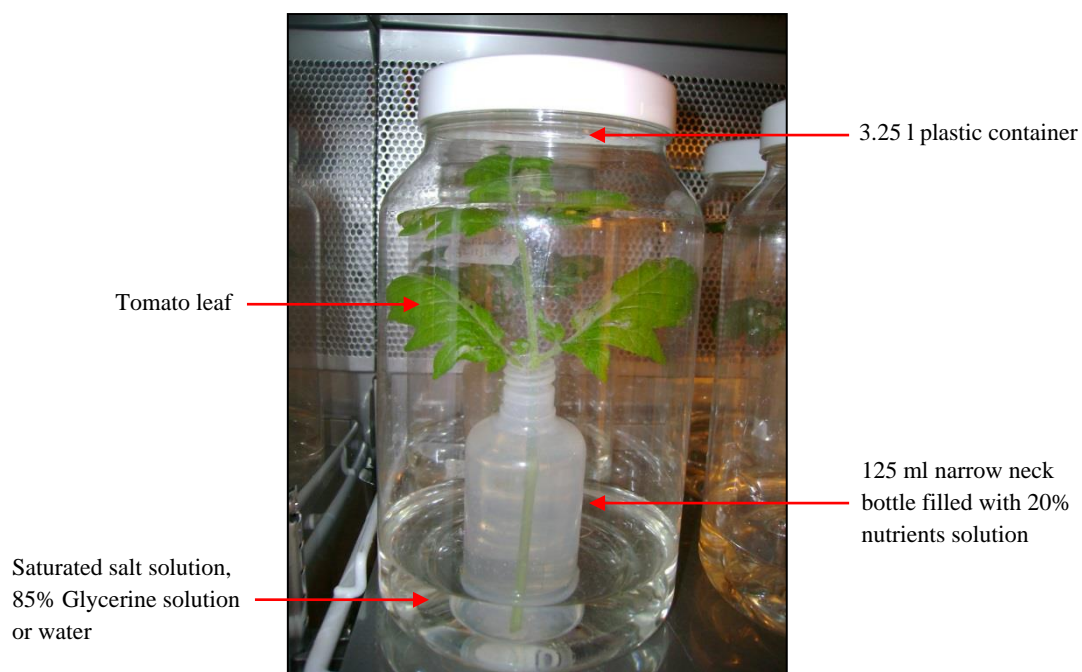
Figure 2-2 The experiment arena which consisted of a 3.25 l clear plastic container filled either with 400 ml water (to obtain > 90 % RH), 400 ml of saturated salt solution (calcium chloride hexahydrate) (to obtain 45 ± 10 % RH) or 400 ml of 85 % glycerine solution (to obtain 75 ± 10 % RH).

_____

_____

Soil bioassays

2.2.11 Efficacy of EPNs against larvae of **Tuta absoluta** *in soil*

Efficacy of the three nematode species in soil against the fourth larval instar was investigated to determine if they were able to kill larvae in the soil as they are pupating. The experiment arenas were 550 ml clear round plastic containers (11.5 cm diameter × 8 cm) filled with 100 g garden loam soil taken from Newcastle University Field Station (Close House). The soil was autoclaved, oven dried, and passed through a 1.66 mm aperture sieve. Nematodes at a rate of 50 ± 5 IJs cm^{-2} were applied onto the soil surface of each plastic container in 10 ml of water. The final moisture content was 10 % (w/w). Twenty-four hours later, fifteen fourth larval instar *T. absoluta* were placed on the soil surface of each plastic container. The control treatment received water only. Trapping adhesive (Tangle-Trap®) located on the lids of the plastic containers was used to capture emerging adults from the soil in order to protect them from continued exposure to nematodes during the first flight. Eleven days after nematode application, the number of adult moths caught in the trapping adhesive was recorded. There were five replicate containers for each nematode species with the corresponding control treatments.

2.2.12 Efficacy of EPNs against pupae and adults of **Tuta absoluta** *in soil*

Efficacy of the three nematode species in soil was investigated against the pupae and the adults emerging from the soil. Experimental procedures were carried out as in the larval experiment above except that, before nematode application, fifteen fourth larval instar *T. absoluta* were placed on the soil surface. After six days, when all larvae had pupated, one millilitre of nematodes suspended in water was applied to the soil surface of each plastic container. Additionally, the presence of nematodes was confirmed by dissecting the adults caught in the trapping adhesive (the emerged adults).

2.3 Statistical analysis

Data were analysed using Minitab® 16.1.0 (© 2010 Minitab Inc.) in order to:

- compare the efficacy of the three nematode species against different stages of *T. absoluta* (larvae, pupae and adults) in culture tube bioassays,

_____

- compare the efficacy of the three nematode species against different larval instars of *T. absoluta* in leaf bioassays (corrected percentage of larval mortality was angular transformed before analysis), and

- compare the efficacy of the three nematode species against larvae, pupae and adults in a soil experiment.

Percentage mortality was corrected for percentage control mortality using Schneider-Orelli's formula (Püntener, 1981) and tested for normality. Subsequently a General Linear Model (GLM) was used to test significant differences between treatments. Afterwards, Tukey's multiple range tests were performed to separate means. In addition, LC_{50} and LT_{50} values for nematode species were computed by probit analysis (Finney, 1971) in Minitab® 16.1.0.

2.4 Results

2.4.1 Determination of different larval instars of Tuta absoluta

Under the laboratory conditions, *Tuta absoluta* completed its life cycle in about 28 to 31 days. Each day after hatching larvae were assigned to a particular instar by measuring the head capsule width. The percentage of larvae in each instar was plotted for each day (Fig. 2-3). The first, second, third and fourth group were representative of the first, second, third and fourth larval instar respectively, while the fifth group was representative of the pupa stage. The time required for this development was 1 to 2 days from hatching for the first larval instar, 3 to 6 days for the second larval instar, 7 to 10 days for the third larval instar and 11 to 16 days for the fourth larval instar.
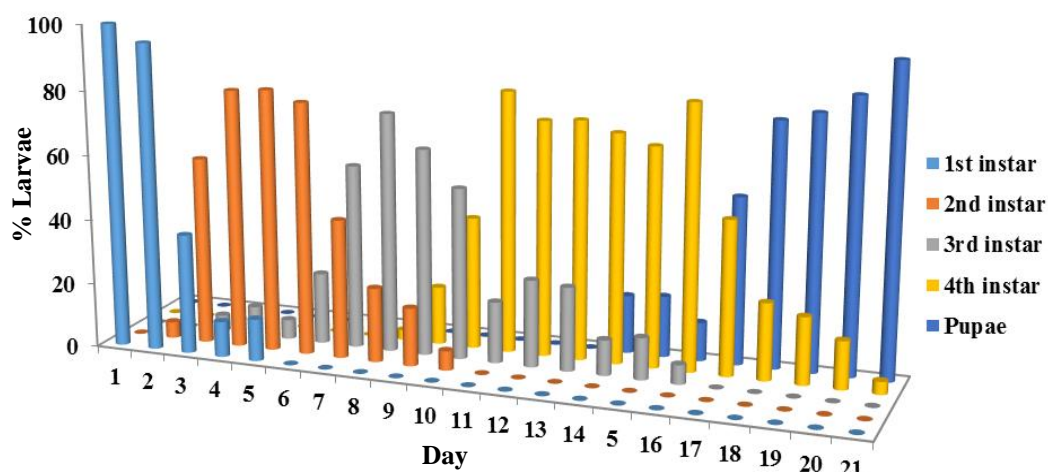
Figure 2-3 The percentage of larvae in different instars each day after hatching. Larvae were assigned to instars based on measurement of the larval head capsule width tested in laboratory at 25 ± 5 °C with an 18:6 h light: dark regime using high pressure sodium lamps and at 45-88 % RH.

Petri dish and culture tube bioassays

2.4.2 Efficacy of EPNs against larvae, pupae and adults of **Tuta absoluta**

The results of Petri dish and culture tube bioassays revealed that the three nematode species were effective in killing different larval instars and adults of *T. absoluta,* but the pupal stage was less susceptible to nematode infection (Fig. 2-4). No significant differences in the efficacy between nematode species were observed (GLM: $F_{2, 72}$ = 0.95; P > 0.05), whereas there were significant differences in the susceptibility between different stages of *T. absoluta* (GLM: $F_{5, 72}$ = 185.96; P < 0.05). Larval instars had significantly higher mortality than adults and pupae. The mean mortality of the second, third and fourth larval instars was 100 % and that of the first larval instar was 96 %, with no significant differences in the susceptibility between these larval instars (P > 0.05). Adults recorded significantly higher mortality than pupae (P < 0.05) with mean mortality of 88.5 % while mean mortality of pupae was 24 % (Fig. 2-4). Although there were no significant differences in the susceptibility between different larval instars treated with 50 IJs cm^{-2} (Fig. 2-4), LC_{50} values of the three nematode species decreased with the development of larvae (Table 2-1). On the other hand, the LT_{50} of the three nematode species against third instar larvae varied, with *S. feltiae* being the quickest species to kill larvae followed by *S. carpocsae* and *H. bacteriophora* (Table 2-2).

_____
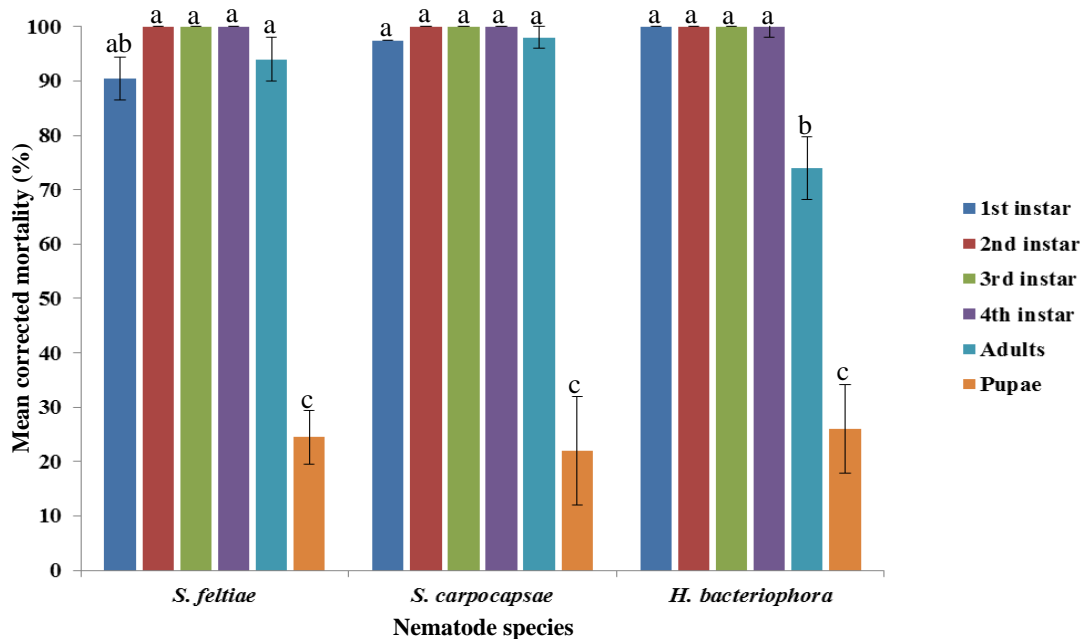
Figure 2-4 Mean corrected mortality of different stages of *T. absoluta* 48 h after exposure to *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* (50 ± 5 IJs cm^{-2}) suspended in water in Petri dish and culture tube bioassays at 25 ± 0.5 °C. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 5).

Table 2-1 LC$_{50}$ values of three EPNs applied in water against different larval instars of *T. absoluta* in culture tube bioassays at 25 °C.

Nematode species	Larval instars / LC$_{50}$ / Fiducial limits (95 %)										
	1st			2nd			3rd			4th		
	LC$_{50}$[a]	Fiducial limits		LC$_{50}$	Fiducial limits		LC$_{50}$	Fiducial limits		LC$_{50}$	Fiducial limits	
		lower	upper		lower	upper		lower	upper		lower	upper
S. feltiae	4.6	3.3	6.2	3.3	2.7	3.9	2.0	1.7	2.3	1.8	1.5	2.0
S. carpocapsae	4.4	3.3	5.5	4.3	3.2	5.4	2.4	1.8	3.1	2.1	1.7	2.6
H. bacteriophora	4.8	3.5	6.1	5.0	4.1	6.0	3.2	2.5	4.0	3.0	2.5	3.6

[a] LC$_{50}$ value = number of IJs per cm^2 needed to reach 50 % mortality

Table 2-2 LT$_{50}$ of three EPNs applied in water at a rate of 50 ± 5 IJs cm^{-2} against 3rd larval instar of *T. absoluta* in culture tube bioassays at > 95 % RH and 25 °C.

Nematode species	LT$_{50}$[a]	Fiducial limits (95 %)	
		Lower	Upper
S. feltiae	6.0	4.9	7. 0
S. carpocapsae	8.1	6.2	9.9
H. bacteriophora	12.0	8.3	15.0

[a] LT$_{50}$ = time needed (hours) by IJs to cause 50 % mortality

Leaf bioassays

2.4.3 Efficacy of EPNs against different larval instars at > 95 % RH

The results of leaf bioassays showed that the three nematode species were able to penetrate tomato leaves and kill *T. absoluta* larvae inside the tunnels. In contrast to culture tube bioassays, there were significant differences in efficacy between the three nematode species (GLM: $F_{2, 36} = 32.40$; $P < 0.05$) applied at 50 IJs cm^{-2}. *S. feltiae* was the most virulent species with mean mortality against all larval instars of 80 %, followed by *S. carpocapsae* (70 %) and *H. bacteriophora* (57 %). Significant differences were also observed in the susceptibility between different larval instars (GLM: $F_{3, 36} = 20.88$; $P < 0.05$). Third and fourth larval instars were most susceptible to nematode infection followed by first and second larval instars, with no significant differences in the susceptibility either between third and fourth larval instars or between first and second larval instars ($P > 0.05$) (Fig. 2-5). Similarly to culture tube bioassays, the LC$_{50}$ values of the three nematode species against different larval instars decreased with the development of larvae, but these values were higher than those in culture tube bioassays (Table 2-3). As with the culture tube bioassays, the LT$_{50}$ of the three nematode species against the third larval instar varied, with *S. feltiae* being the quickest species to kill larvae followed by *S. carpocapsae* and *H. bacteriophora*, but the LT$_{50}$ of each nematode species was slightly higher than in the culture tube bioassays (Table 2-4).
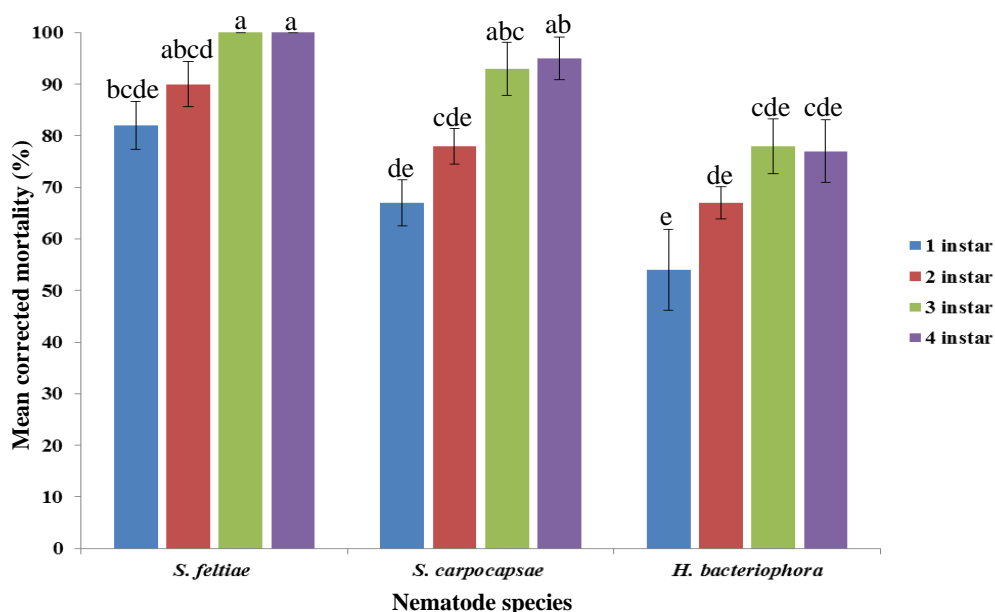
Figure 2-5 Mean corrected mortality of different stages of *T. absoluta* 48 h after exposure to *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* (60 ± 6 IJs cm^{-2}) applied in water in leaf bioassays at > 95 % RH and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

Table 2-3 LC$_{50}$ values of three EPNs applied in water against different larval instars of *T. absoluta* in leaf bioassays at > 95 % RH and 25 °C.

Nematode species	Larval instars / LC$_{50}$ / Fiducial limits (95 %)											
	1st			2nd			3rd			4th		
	LC$_{50}$[a]	Fiducial limits		LC$_{50}$	Fiducial limits		LC$_{50}$	Fiducial limits		LC$_{50}$	Fiducial limits	
		lower	upper		lower	upper		lower	upper		lower	upper
S. feltiae	16.2	12.4	21.4	6.4	4.8	8.2	4.3	3.2	5.5	3.7	2.8	4.7
S. carpocapsae	21.7	16.6	29.2	9.0	6.6	11.9	6.4	4.8	8.2	5.9	4.3	7.6
H. bacteriophora	26.1	18.2	41.2	13.6	9.7	18.7	11.2	7.9	15.3	10.1	7.0	14.0

[a] LC$_{50}$ value = number of IJs per cm^2 needed to reach 50 % mortality

Table 2-4 LT$_{50}$ of three EPNs applied in water at a rate of 60 ± 6 IJs cm^{-2} against 3rd larval instar of *T. absoluta* in leaf bioassays at > 95 % RH and 25 °C.

Nematode species	LT$_{50}$ [a]	Fiducial limits (95 %)	
		lower	upper
S. feltiae	7.5	6.2	8.9
S. carpocapsae	10.0	7.3	12.5
H. bacteriophora	14.0	10.0	17.3

[a] LT$_{50}$ = time needed (hours) by IJs to cause 50 % mortality

Soil bioassays

2.4.4 Efficacy of EPNs against larvae, pupae and adults of Tuta absoluta

The results of the soil experiment revealed that the three nematode species were able to kill the last instar larvae of *T. absoluta* when they drop to the soil to pupate as well as killing the adults when they emerge from the soil. In contrast, the pupal stage was less susceptible to nematode infection (Fig 2.6). There were significant differences in efficacy between the three nematode species against different stages of *T. absoluta* (GLM: $F_{2, 36}$ = 15.56; $P < 0.05$) with *S. carpocapsae* and *S. feltiae* significantly more virulent than *H. bacteriophora.* Significant differences were also observed in susceptibility between the different stages of *T. absoluta* (GLM: $F_{2, 36}$ = 288.59; $P < 0.05$) with fourth larval instar and adults being significantly more susceptible than pupae (Fig 2.6).

_____
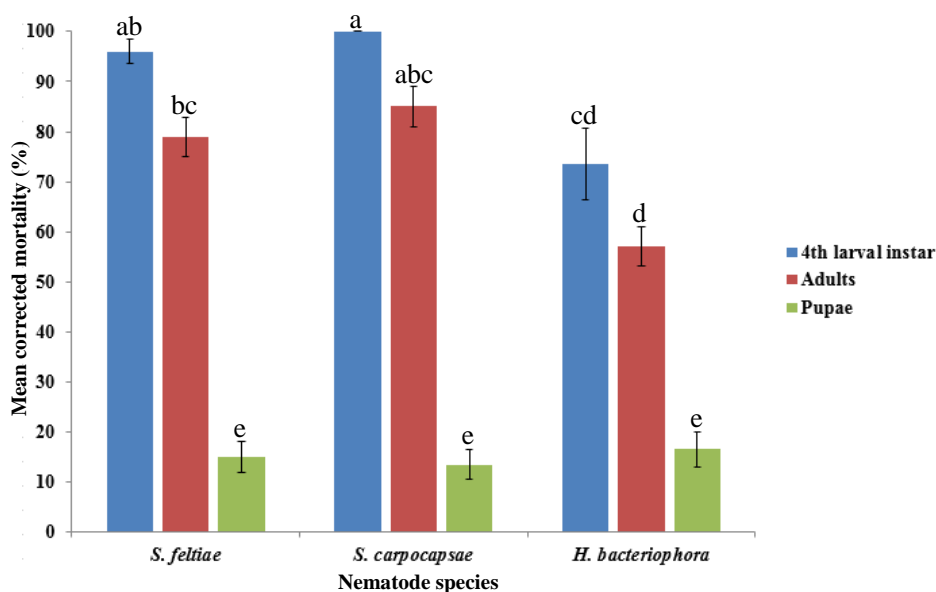
Figure 2-6 Mean corrected mortality of different stages of *T. absoluta* after exposure to *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* (50 ± 5 IJs cm^{-2}) in soil at 25 ± 0.5 °C. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 5).

2.5 Discussion

The results of the culture tube bioassays showed that the three commercial EPN species were effective in killing different instar larvae and adults of *T. absoluta* when they were continuously exposed to EPNs (48 h) (Fig. 2-4). These results are consistent with those of Batalla-Carrera *et al.* (2010), who found that the third and fourth larval instars of *T. absoluta* were highly susceptible to continuous exposure (48 h) to similar nematode species native to Spain, but different strains (*Steinernema feltiae* "Bpa" strain, *S. carpocapsae* "B14" strain and *Heterorhabditis bacteriophora* "DG46" strain), in Petri dish bioassays filled with soil and the adults were susceptible when they were emerging from pupae in Petri dish bioassays lined with filter paper. The authors reported 86.6 % larval mortality with *S. carpocapsae* and 100 % with *H. bacteriophora* and *S. feltiae* as well as 6.7 % adult mortality with *H. bacteriophora* and 40 % with *S. feltiae* and *S. carpocapsae* when nematodes were used at a rate of 50 IJs cm^{-2}. The findings of the current study are also in agreement with those obtained on other insect pests from the same order but different families. For example, Henneberry *et al.* (1996) demonstrated that larvae of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), were highly susceptible to infection by *S. carpocapsae* (Weiser) and *S. riobravis* Cabanillas, Poinar and Raulston and adults were moderately susceptible to nematode infection (34 % and 27 % respectively) during their emergence from soil treated with these nematode species when they were continuously exposed to the EPNs. Furthermore,

_____

Mbata and Shapiro-Ilan (2005) reported that the susceptibility of adults of Indian meal moth *Plodia interpuctella* Hübner (Lepidoptera: Pyralidae) to continuous exposure to different heterorhabditid nematodes (in Petri dish bioassays lined with filter paper) was higher than that of larvae (in plastic cups bioassays filled with Indian meal moth's rearing medium).

The three EPN species were able to penetrate tomato leaves and infect larvae of *T. absoluta* inside the mines when applied at a rate of 60 IJs cm^{-2} at > 95 % RH but, unlike culture tube bioassays, there were significant differences in efficacy between the three nematode species against all larval instars. *S. feltiae* was the most virulent species followed by *S. carpocapsae* then *H. bacteriophora* (Fig. 2-5). These results are similar to those obtained by Jacobson and Martin (2011), who treated tomato leaflets infested with medium sized larvae of *T. absoluta*, which were placed in trays under favourable conditions, with an aqueous suspension of two of the same commercial nematode species used in the present study (*S. feltiae* (Nemasys®) and *S. carpocapsae* (Nemasys C®)), and reported high efficacy of > 90 % mortality. The results of this study are also in accord with those obtained by Batalla-Carrera *et al.* (2010), who reported that similar nematode species indigenous to Spain (mentioned above) were effective in finding and killing larvae of *T. absoluta* inside the mines on tomato leaves but, in contrast to our results, their results showed no significant differences in the efficacy between nematode species in both leaf bioassays and in pot experiments under greenhouse condition. A possible explanation for these results may be that they treated older larval instars than those we treated. For instance, in the leaf bioassay the authors did not determine the age of the larvae that had been treated, whereas in the pot experiment, the authors infested the tomato plants by placing first, second and third larval instars on their leaves and incubated the plants for 5-7 days at 15-40 °C to enable the larvae to develop well-formed tunnels before EPN treatments. In this period of time and under these conditions, the larvae can reach the third and fourth instar according to our results (Fig. 2-3) and the results of Giustolin *et al.* (2001b), and these ages are more susceptible than younger larval instars. This is confirmed by the results of the present work, which revealed that there were significant differences in susceptibility between different larval instars in leaf bioassays, with third and fourth larval instars being most susceptible to nematode infection followed by first and second instars (Fig 2.5). The LC$_{50}$ values of the three nematode species decreased throughout larval development (Tables 2-1 and 2-..3). Similar results were obtained by Bari and Kaya (1984), Kaya (1985) and Bélair *et al.* (2003), who reported that the susceptibility of other lepidopteran pests to nematode infection increased with larval age. The possible reasons behind this could be that

_____

the small size of larvae may have hindered nematodes entering through the normal infection routes (mouth, spiracle, or anus) or that younger larvae may produce smaller amounts of attractants such as CO_2 or kairomones, which makes it more difficult for nematodes to locate them (Kaya, 1985). However, in contrast to these results, Glazer and Navon (1990) demonstrated that susceptibility of larvae of the cotton bollworm *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) to nematode infection decreased with the development of larvae. These results emphasise the importance of evaluating the efficacy of EPNs against different larval instars separately in order to have a clear idea about the expected control level. What is more, the present study also showed that the LT_{50} of the three nematode species against third instar larvae in culture tube and leaf bioassays differed, with *S. feltiae* being the fastest species to kill larvae followed by *S. carpocapsae* then *H. bacteriophora*. A possible explanation for this could be differences in the virulence mechanisms among nematode species (Dowds and Peters, 2002). In addition, the LT_{50} in leaf bioassays was somewhat higher than those in the culture tube bioassays (Tables 2-2 and 2-4). This might be due to the difficulties that EPNs encountered in finding larvae inside the mines compared with those exposed on filter paper.

The lower efficacy of *S. carpocapsae* compared to *S. feltiae* against larvae on leaves in the current work could be due to the difference in the foraging strategies of the two nematode species. *S. carpocapsae* is an ambush forager (exploiting a 'sit and wait' strategy), which is known to target highly mobile insect pests at the soil or substrate surface (Gaugler *et al.*, 1980; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997; Griffin *et al.*, 2005), whereas *S. feltiae* is an intermediate forager that employs both an ambush and a cruise foraging strategy (Gaugler *et al.*, 1980; Grewal *et al.*, 1994a). A forager nematode actively moves through the soil or substrate to locate sedentary and slow-moving insects by detecting the carbon dioxide or other volatiles released by the insects (Gaugler *et al.*, 1980; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997; Griffin *et al.*, 2005). Therefore, the intermediate forager *S. feltiae* is more effective at finding the larvae of *T. absoluta* inside the mines than the ambush forager *S. carpocapsae*. The high efficacy of the intermediate forager *S. feltiae* compared to the ambush forager *S. carpocapsae* against a sedentary insect pest was also reported by Williams and Walters (2000), who reported that a single application of *S. feltiae* (Nemasys®) (5000 IJs/ ml) at > 90 % RH induced mean mortality of 82 % of the leafminer *Liriomyza huidobrensis* (Blanchard) in trials on lettuce at a commercial greenhouse. This was higher than the results obtained by Broadbent and Olthof (1995), who reported 61 % and 75 % mortality of the leafminer *Liriomyza trifolii* induced by *S. carpocapsae* "All" and "Pye" strains respectively at

_____

> 90 % RH in similar field trials carried out on chrysanthemum using double the concentration (10,000 IJs/ ml) used by Williams and Walters (2000). On the other hand, the low performance of *H. bacteriophora* compared to *S. feltiae* and *S. carpocapsae* in this study may be explained by the fact that *Heterorhabditis* species have poor survival on foliage compared to *Steinernema* species (Hara *et al.*, 1993). These authors found that *Heterorhabditis* species, which did well in a Petri dish bioassay, induced low leafminer (*Liriomyza trifolii*) mortality on leaves of bean (< 33 %) compared to the steinernematids*, S. carpocapsae* and *S. feltiae* (> 63 %) under greenhouse conditions (70-98 % RH) and attributed this to the poor survival of *Heterorhabditis* spp. in the foliar environment with high RH as compared to steinernematids.

The outcomes of the present investigation are also in agreement with studies conducted on other leafminers from a different order (Diptera: Agromyzidae). For example, Williams and Walters (2000) reported that foliar application of *S. feltiae* was very effective in killing the larval stage of three different species of leafminer (*Liriomyza huidobrensis*, *L. bryoniae* and *Chromatomyia syngenesiae*) on vegetables (lettuce, tomato) and ornamentals under greenhouse conditions. Also, Williams and MacDonald (1995) demonstrated that *S. feltiae* and *Heterorhabditis* spp. were effective against second instar larvae of *L. huidobrensis* on tomato leaves. Furthermore, Hara *et al.* (1993) stated that *S. carpocapsae* "All" strain and *S. feltiae* "MG-14" strain induced mean mortality of 69 % and 67 % respectively to *L. trifolii* on bean plants in a foghouse. A common attribute of the current and the above mentioned studies is that high relative humidity (80-90 % or greater) was needed to obtain high control levels.

The soil experiment showed that the three nematode species are able to kill fourth instar larvae of *T. absoluta* in soil as they are pupating as well as the adults when they emerge from pupae (infected during the period of time they emerge from the soil without continuing exposure to EPNs). *S. carpocapsae* and *S. feltiae* were significantly more virulent than *H. bacteriophora* against both larvae and adults (Fig 2.6). These findings are in accord with those of Batalla-Carrera *et al.* (2010), who stated that similar nematode species native to Spain (mentioned above) were very efficient at killing the fourth instar larvae of *T. absoluta* in a Petri dish bioassay filled with soil but without significant differences between nematode species, as well as that *S. feltiae* and *S. carpocapsae* were more virulent than *H. bacteriophora* against adults of *T. absoluta* when they emerged from pupae when they were continuously exposed to EPNs in a Petri dish bioassay filled with soil. A possible explanation

_____

_____

for the differences in efficacy between nematode species against fourth instar larvae in the present study, compared to the work of Batalla-Carrera *et al.* (2010), could be the differences in virulence among nematode strains used in the two studies (Dowds and Peters, 2002). For instance, *H. bacteriophora* in our study induced 73 % larval mortality whereas in their study it induced 100 % larval mortality. Moreover, the observations of adults' susceptibility to nematode infection in the current investigation are in agreement with those of Kaya and Grieve (1982), who stated that the adults of beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) were susceptible to *S. carpocapsae* as they were emerging from soil in Plexiglas tube bioassays.

However, the results of the current soil experiment are in contrast with those of Garcia-del-Pino *et al.* (2013), who tested the efficacy of soil treatment with the same nematode species, native to Spain, tested by Batalla-Carrera *et al.* (2010) against larvae, adults and pupae of *T. absoluta*. They found that *S. carpocapsae* and *H. bacteriophora* were significantly more effective than *S. feltiae* in killing fourth instar larvae. This could be due to the differences in virulence among nematode strains used in the two studies. The results of Garcia-del-Pino *et al.* (2013) are also contrary to the findings of Batalla-Carrera *et al.* (2010), who found that the three nematode species were equally effective in killing the fourth instar larvae. This contrast is not understandable since the latter authors used the same nematode strains that were used by the former ones. On the other hand, Garcia-del-Pino *et al.* (2013) reported that *S. carpocapsae* induced significantly higher adult mortality (79 %) than *S. feltiae* (0.5 %) during the period of time that *T. absoluta* emerge from the soil. These results are in disagreement with our results (85 % for *S. carpocapsae* and 79 % for *S. feltiae*) and with the findings of Batalla-Carrera *et al.* (2010), who reported that both nematode species induced equal adult mortality (40 %) when they were in continuous exposure to EPNs. A possible explanation for the differences between the results of the current study and those of Garcia-del-Pino *et al.* (2013) could be differences in virulence among nematode strains used in the two studies, whereas the reason behind the differences in the findings of Batalla-Carrera *et al.* (2010) and those of Garcia-del-Pino *et al.* (2013) might be differences in the length of exposure period required by each nematode species to penetrate and infect a host or infection behaviour (Griffin *et al.*, 2005). For example, Batalla-Carrera *et al.* (2010) tested the effect of exposure time on nematode efficacy and found that the larval mortality induced by *S. feltiae* after 1 and 3 h exposure periods (25 % and 64 % respectively) was less than mortality induced by *S. carpocapsae* (56 % and 87 % respectively). From these results we can see that *S. carpocapsae*

_____

"B14" strain induced higher larval mortality than *S. feltiae* "Bpa" strain at these two short exposure periods. Therefore, since the adults in the experiment of Batalla-Carrera *et al.* (2010) were continuously exposed to EPNs, unlike the adults in the experiment of Garcia-del-Pino *et al.* (2013), this could explain why *S. feltiae* and *S. carpocapsae* gave the same level of adult mortality (40 %). Therefore, we can say that the time adults took to leave the soil after emergence from pupae in the study of Garcia-del-Pino *et al.* (2013) might not be long enough for successful penetration and infection by *S. feltiae* "Bpa" strain as it requires a longer exposure period than *S. carpocapsae* "B14" strain. This explanation is supported by the results of Yoder *et al.* (2004), who reported that young (< 1 week) *S. carpocapsae* "All" strain had high penetration (27 %) compared to young *S. feltiae* "UK" strain (4 %) when exposed for short periods (4 h), but when exposed for 64 h, old (2-4 weeks) *S. feltiae* (57 %) exceeded young and old *S. carpocapsae* penetration (45 % and 4 % respectively).

In contrast to larvae and adults, the current investigation revealed that the pupal stage was less susceptible to nematode infection in both Petri dish bioassays lined with filter paper and in soil experiments (Fig. 2-4 and 2-6). These results are consistent with those of Batalla-Carrera *et al.* (2010) and Garcia-del-Pino *et al.* (2013), who demonstrated that the pupal stage of *T. absoluta* was hardly infected with similar nematode species indigenous to Spain (mentioned above). These results are also in line with those of Kaya and Hara (1981) who found that soil- or litter-pupating lepidopterous insects generally are not highly susceptible to continuous exposure to *S. carpocapsae* in Petri dish bioassays lined with filter paper. The authors reported pupal mortality of 18.5 %, 8 %, 2.5 %, 26.5 %, 6 %, 26 %, 13 % and 0 % for potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae), saltmarsh caterpillar *Estigmene acrea* (Lepidoptera: Arctiidae), fall webworm *Hyphantria cunea* (Lepidoptera: Arctiidae), bollworm *Heliothis zea* (Lepidoptera: Noctuidae), bollworm *H. zea* (Lepidoptera: Noctuidae), armyworm *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), redhumped caterpillar *Schizura concinna* (Lepidoptera: Notodontidae) and tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae) respectively. Also, the results of the current study are in accord with those of Henneberry *et al.* (1995), who reported that the pupae of the pink bollworm *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) were not susceptible to continuous exposure to *S. carpocapsae* and *S. riobravis* in Petri dish bioassays lined with filter paper unless the pupa was injured. The reason behind the low susceptibility of pupae to nematode infection was attributed to the lack of entry routes (mouth and anus) (Lindegren *et al.*, 1993; Henneberry *et al.*, 1995).

_____

In conclusion, EPNs tested in this investigation are effective in killing the larvae of *T. absoluta* on foliage in optimum conditions (> 95 % RH), fourth instar larvae pupating in the soil and the adults during their emergence from soil. Therefore, in order to control this pest, foliar and soil applications of theses EPNs would be two good approaches to consider. The larvae on leaves can be controlled by the foliar application whereas the surviving larvae that pupate in the soil as well as the emerging adults from the soil can be controlled by the soil application. Applying EPNs to soil to control adults when they are emerging from pupae has already been suggested by Garcia-del-Pino *et al.* (2013) to control this pest using Spanish strains of EPNs and by Kaya and Grieve (1982) to control the beet armyworm *S. exigua* (Lepidoptera: Noctuidae). However, the current results were obtained under optimum conditions for EPNs (> 95 % RH and 25 ± 0.5 °C) in the laboratory and they still need to be confirmed by conducting trials under greenhouse and field conditions where the environmental conditions are harsher, especially on leaves. Accordingly, before doing that, there is a need to study and determine the factors affecting these EPNs when controlling this pest and to find methods and techniques to reduce the adverse effects. The next chapter will deal with this matter and investigate some factors affecting EPNs' efficacy on tomato leaves.

_____

Chapter 3 Factors affecting entomopathogenic nematode efficacy against larvae of *Tuta absoluta* on tomato leaves

Abstract

Tomato leafminer, *Tuta absoluta*, is the most serious pest of tomato plants. Damage happens as a result of the larvae feeding on all above ground parts of the plant, especially leaves. EPNs are being investigated as a management option for *T. absoluta*. In foliar application, EPNs encounter many factors that affect their survival and efficacy adversely. This chapter reports investigations into some of these factors; relative humidity (RH), temperature, method of application, number of applications and time required by EPNs to enter a leaf in whole leaf bioassays on tomato leaves, which provide conditions similar to the natural environment. Results revealed that all factors affected EPNs' efficacy to some extent. RH was the most important factor. EPNs' efficacy and survival decreased as RH declined. *S. feltiae* was the most effective species. It induced the highest larval mortality at the three ranges of RH followed by *S. carpocapsae* then *H. bacteriophora*. *H. bacteriophora* was excluded from further experiments because of its low efficacy. *S. carpocapsae* survived better at low RH than *S. feltiae*. Both nematode species induced similar mortality at 25 °C, but *S. feltiae* was more virulent at lower temperatures (15 and 20 °C) while *S. carpocapsae* was more virulent at higher temperatures (30 and 35 °C). Two-surface treatments (spraying nematode suspension on both leaf surfaces) induced higher larval mortality than single-surface treatments (spraying nematode suspension on lower or upper leaf surface). Mortality of larvae increased with the increase in number of applications. *S. feltiae* was more effective than *S. carpocapsae* and required fewer applications to induce significant mortality. First and second larval instars required additional applications compared to third and fourth larval instars. Time required by EPNs to enter a leaf differed between the two nematode species, being shorter for *S. feltiae* (6 h) than *S. carpocapsae* (12 h).

3.1 Introduction

The tomato leafminer (TLM) *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae) is the most serious pest of tomato (EPPO, 2005; Desneux *et al.*, 2010; Urbaneja *et al.*, 2013). Adults emerge from pupae, mate and females start laying eggs on all above ground parts of plants. After hatching, neonates enter leaves, stems or fruits, where they feed and develop and produce visible mines and galleries. Yield is reduced as a result of (1) the decrease in photosynthetic capacity of the crop due to damage to the mesophyll caused by the feeding of larvae, (2) the fruit rot that happens due to the invasion of secondary pathogens which enter fruits through mines produced by larvae, (3) the death of the plant cells due to the galleries produced by larvae, (4) the decrease and the stopping of plant development due to the direct feeding of larvae on the growing tips and (5) the alteration in the general development of the plant due to the mines formed on the stems. Depending on temperature, *T. absoluta* may be able to complete twelve generations per year (EPPO, 2005; Desneux *et al.*, 2010; USDA–APHIS, 2011; Urbaneja *et al.*, 2013).

Methods for managing *T. absoluta* have been described in Chapter 2. Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are parasites with a wide host range and are potentially useful biological control agents for *T absoluta*. Due to the desirable characteristics of EPNs they are considered an important group of biological control agents for several insect pests (Grewal *et al.*, 2005b; Lacey and Georgis, 2012). Nevertheless, successful control of *T. absoluta* using EPNs on foliage is not easy because the nematode efficacy is reduced due to a number of detrimental environmental factors such as desiccation (Glazer and Navon, 1990; Glazer, 1992; Baur *et al.*, 1995; Williams and MacDonald, 1995; Mason and Wright, 1997; De Waal *et al.*, 2013), ultra-violet radiation (Gaugler and Boush, 1978; Gaugler *et al.*, 1992; Nickle and Shapiro, 1994) and extreme temperature (Molyneux, 1985; Grewal *et al.*, 1994b). Generally, the most important among these factors is desiccation (Begley, 1990; Glazer and Navon, 1990; Glazer, 1992; Baur *et al.*, 1995; Mason and Wright, 1997; Bélair *et al.*, 2003; Head *et al.*, 2004; Wright *et al.*, 2005). In addition to the environmental factors, there are other factors that interact with each other and affect nematode efficacy on leaves such as the specific insect pest (e.g., type of feeding, activity), host plant (e.g., hairy or non-hairy surface) and application method (e.g., spinning disc or hydraulic nozzles) (Mason *et al.*, 1998).

_____

Nematodes require a thin water film to survive and move freely on the leaf surface to locate and enter the mines where larvae of *T. absoluta* live. The duration of this water film, before the leaf surface dries, is critical. Thus high relative humidity (RH) after application is required for successful foliar application of nematodes (Glazer and Navon, 1990; Glazer, 1992; Broadbent and Olthof, 1995; Koppenhoefer, 2000; Wright *et al.*, 2005). Plants have different rates of water loss from the leaf surface depending on the physical characteristics of their leaves (rough and smooth leaves). This was found to affect nematode survival, where survival of nematodes on leaves of tomato and soybean was found to be higher than survival of nematodes applied to leaves of cotton, pepper and bean as well as in filter paper (Glazer, 1992). Moreover, if high infection rate is reliant on the high density of the nematodes, the nematode suspension has to stick strongly to the leaf surface (Macvean *et al.*, 1982). Head *et al.* (2004) stated that the mortality of nematodes applied on poinsettia leaves (which have relatively few hairs and waxy surfaces) was significantly lower than those recorded on tomato, cucumber, chrysanthemum and verbena leaves. The authors attributed this to the fact that the leaves of the latter plants were better in maintaining high surface RH, as well as that the leaf characteristics of poinsettia might have reduced the adhesion of the spray suspension.

It is also useful to choose nematode species that have the ability to search for hosts because the shorter the time nematodes take to locate and enter the mines after application, the more protection they gain from detrimental environmental factors (Hara *et al.*, 1993; Williams and Walters, 1994). EPNs have different foraging behaviours that affect their efficacy, ranging from ambush to cruise foraging. For example, *Steinernema carpocapsae* and *S. scapterisci* are ambushers that exploit a 'sit and wait' strategy which enables them to target highly mobile insect pests at the soil or substrate surface. During foraging, ambushers are also able to nictate, which enables them to attach to a passing host; this involves standing upright by raising most of their bodies out of the soil or the substrate. In contrast, *Heterorhabditis* spp. and *S. glaseri* are cruiser foragers that never nictate and actively move through the soil or substrate to locate sedentary and slow-moving insects by detecting the carbon dioxide or other volatiles released by the insects. On the other hand, *S. riobrave* and *S. feltiae* employ both ambush and cruise foraging strategy (intermediate foragers) and are able to parasitize both sedentary and mobile insect pests (Gaugler *et al.*, 1980; Campbell and Gaugler, 1993; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997; Lewis, 2002; Griffin *et al.*, 2005; Mohan, 2015). Therefore, choosing the right nematode species will reduce the obstacles to attaining a high level of *T. absoluta* control.

_____

After a foliar application the period of time that nematodes need to enter a leaf is a factor that affects the efficacy of nematodes against leafminers. When nematodes enter the mines they are protected from environmental constraints and are able to seek and infect larvae (Hara *et al.*, 1993; Williams and Walters, 1994; Williams and MacDonald, 1995; Bélair *et al.*, 2005). Williams and Walters (1994) found that the time required by *S. feltiae* to successfully enter the leaf tissue and consequently achieve high mortality of the late second/ early third instar larvae of the dipteran leafminer *Liriomyza huidobrensis* at 15 and 25 °C and at high relative humidity is 10 hours, whereas with younger larvae (first/second instar), *S. feltiae* took a longer time to achieve the same mortality during the first 24 hours. This was attributed to the less extensively developed mines produced by these instars compared to the late second/ early third instar.

The goal, when controlling a leafminer, is to increase the quantity of nematodes on the leaf surfaces to allow the nematodes to find a mine entry. Since the infectivity of nematodes usually only lasts for a few hours and the redistribution of nematodes on leaves is limited, coating and positioning of nematodes on leaves is critical (Bélair *et al.*, 2005; Wright *et al.*, 2005). The efficacy of different spray methods such as hydraulic nozzles (flat-fan and full-cone), ultra-low-volume spinning disc applicators and higher output (flow rate) nozzles was investigated. The results showed that higher output (flow rate) nozzles gave the best deposition of nematodes on leaves as well as superior insect control in laboratory investigations (Lello *et al.*, 1996). Applying nematodes more than once when using a hand sprayer could result in better coverage that deposits nematodes near the entrances of mines where they can infect larvae.

Timing of nematode application is an important factor because nematode efficacy is affected by the age of the larval instar (Kaya, 1985; Williams and Walters, 1994; Journey and Ostlie, 2000; Bélair *et al.*, 2003) Williams and Walters (2000) reported that a repeat application after 24 h of *S. feltiae* induced higher leafminer *(Chromatomyia syngenesiae)* mortality than repeat applications at 48 h and 72 h. They suggested that second and early third instar larvae were more susceptible than older third instar leafminer larvae and prepupae. The authors also reported that a repeat application of *S. feltiae* after 96 h resulted in significantly higher leafminer (*Liriomyza bryoniae*) mortality than a repeat after 24 h and they suggested that the 96 h treatment was targeting the second instar larvae which were more susceptible than first instar larvae presented at 24 h treatment. The same authors stated that applying a repeat of a

_____

lower dose (1000 *S. feltiae*/ ml) to first instar larvae was as effective as applying a higher concentration (5000 *S. feltiae*/ ml). They suggested that applying nematodes to the more susceptible stage is more important than applying nematodes at a higher rate.

EPN species vary in their performance according to the temperature (Griffin, 1993; Grewal *et al.*, 1994b). Depending on the duration, prolonged exposure to temperatures below 0 °C and above 40 °C is fatal to the majority of EPN species. Adequate performance of the majority of commercial EPNs tends to occur at temperatures between 20 and 30 °C. EPNs usually become lethargic at low temperatures (<10–15 °C) and become deactivated at higher temperatures (> 30–40 °C) (Glazer, 2002; Koppenhöfer, 2007). Some nematode species such as *Heterorhabditis megidis, S. carpocapsae, S. feltiae* and other *Heterorhabditis* species are most infective in a range between 20 and 30 °C as they are adapted to moderate temperatures (Dunphy and Webster, 1986; Saunders and Webster, 1999; Glazer, 2002), while others such as *S. kraussei* can infect insects at temperatures below 10 °C as they prefer low temperature (Mracek *et al.*, 1998; Willmot *et al.*, 2002) and *S. riobrave* reproduces at 32 °C and infects at up to 39 °C (Grewal *et al.*, 1994b).

The aim of this work was to determine some factors that affect EPNs' efficacy against larvae of *T. absoluta* on tomato leaves. The objectives were to determine (1) effect of RH on efficacy of EPNs against larvae of *T. absoluta* on tomato leaves, (2) effect of RH on lethal concentration and lethal time of EPNs against larvae of *T. absoluta* on tomato leaves, (3) effect of RH on survival of EPNs on tomato leaves, (4) effect of temperature on efficacy of EPNs against larvae of *T. absoluta* on tomato leaves, (5) effect of number of applications of EPNs against larvae of *T. absoluta* on tomato leaves at different RH, (6) effect of method of application on efficacy of EPNs at different RH and (7) time required by EPNs to enter a leaf at different RH.

3.2 Materials and methods

3.2.1 Effect of RH on efficacy of EPNs against larvae of Tuta absoluta *on tomato leaves*

The impact of RH on infectivity of *S. feltiae* (Nemasys), *S. carpocapsae* (Nemasys C) and *H. bacteriophora* (Nemasys H) was investigated for each nematode species at > 95 %, 75 ± 10 % and 45 ± 10 % RH against 1st, 2nd, 3rd and 4th larval instars of *T. absoluta*. The experimental arena was prepared as described in section 2.2.9. Ten to twenty-five larvae were placed on each leaf depending on larval instar used. Leaves were then sprayed with nematodes

_____

suspended in water at a rate of 60 ± 6 IJs cm^{-2}. The control treatment received water only. There were four replicate containers for each treatment (n = 4) with the corresponding control treatments (n = 4). Additionally, the difference in the efficacy of the three nematode species at the three ranges of RH mentioned above was investigated against the 3rd larval instar.

3.2.2 Effect of RH on lethal concentrations (LC) and lethal time (LT) of EPNs against larvae of Tuta absoluta on tomato leaves

The influence of RH on the concentration of the three nematode species, tested in 3.2.1, which kills 50 % of *T. absoluta* larvae (LC$_{50}$) was tested at > 95 %, 75 ± 10 % and 45 ± 10 % RH against 3rd larval instars. The experiment arena was prepared as described in section 2.2.9. Nematodes were suspended in tap water at five different rates: $0, 5 \pm 0.5, 15 \pm 1.5, 30 \pm 3$ and 60 ± 6 IJs cm^{-2}. Ten to fifteen larvae were placed on each leaf. There were four replicate containers for each nematode concentration (n = 4). Additionally, the effect of RH on the time required by the three nematode species to kill 50 % of *T. absoluta* larvae (LT$_{50}$) was investigated at > 95 %, 75 ± 10 % and 45 ± 10 % RH against the third larval instar. The experimental arena was prepared as described in section 2.2.9. Ten to fifteen larvae were placed on each leaf. Leaves were then sprayed with water (control) or nematodes suspended in water at a rate of 60 ± 6 IJs cm^{-2} (treatment). Larval mortality was checked every 8 h up to 48 h. Independent samples were used for each time point. There were four replicate containers for each time point (n = 4) with the corresponding control treatment (n = 4).

3.2.3 Effect of RH on survival of IJs of EPNs on tomato leaves

The impact of RH on the survival of IJs of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) on tomato leaves was investigated at > 95 %, 75 ± 10 % and 45 ± 10 % RH. The experiment arena was prepared as described in section 2.2.9 except that leaves were not infested with larvae. Leaves were sprayed with nematodes suspended in tap water at a rate of 60 ± 6 IJs cm^{-2}. There were four replicate containers (n = 4). After incubation for 0, 2, 4, 7, 24 or 48 h, one leaflet from each replicate was sampled. IJ persistence was measured by washing each leaflet into a separate counting chamber and checking IJ survival by counting the number of living IJs after 24 h. Nematodes were examined under a dissecting microscope and IJs not moving when mechanically stimulated using a fine needle were recorded as dead. Nematode survival was calculated as the number of living nematodes as a proportion of the

total number of nematodes washed from leaves. Differences in the total number of nematodes washed from leaves between time points were used in later analysis.

3.2.4 Effect of temperature on efficacy of EPNs against larvae of **Tuta absoluta** *on tomato leaves*

The effect of temperature on the efficacy of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) was investigated against 3rd larval instar *T. absoluta* at > 95 % RH. Six different temperatures were tested: 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C. The experimental arena was prepared as described in section 2.2.9. Ten to fifteen larvae were placed on each leaf. Leaves were then sprayed with nematodes suspended in tap water at a rate of 60 ± 6 IJs cm^{-2}. Control treatments received water only. There were four replicate containers for each temperature (n = 4) with the corresponding control treatments (n = 4).

3.2.5 Effect of number of applications of EPNs against larvae of **Tuta absoluta** *on tomato leaves at different RH*

The effect of the number of applications on the efficacy of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) against first, second, third and fourth larval instars of *T. absoluta* was investigated at 75 ± 10 % RH and 45 ± 10 %. The experimental arena was prepared as described in section 2.2.9. Ten to twenty-five larvae were placed on each leaf depending on larval instar used. Leaves were then sprayed with nematodes suspended in tap water at a rate of 60 ± 6 IJs cm^{-2}. Four different frequencies of application were used: spraying leaves once (at the beginning (0 hours)), twice (0 h and after 24 h), three times (0 h, after 24 h and after 48 h) or four times (0 h, after 24 h, after 48 h and after 72 h). Control treatments received water only. There were four replicate containers for each treatment (n = 4) with the corresponding control treatments (n = 4).

3.2.6 Effect of method of application on efficacy of EPNs at different RH

Influence of method of application on efficacy of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) was investigated at > 95 % RH, 75 ± 10 % and 45 ± 10 % against the 3rd larval instar. The experiment arena was prepared as described in section 2.2.9. Ten to fifteen larvae were placed on each leaf. Leaves were sprayed with nematode suspended in tap water at a rate of 60 ± 6 IJs cm^{-2}. Control treatments received water only. Three different application methods (spraying mode) were applied; (1) spraying upper leaf surface, (2) spraying lower

_____

leaf surface, or (3) spraying both leaf surfaces. There were four replicate containers for each spraying mode (n = 4) with the corresponding control treatments (n = 4).

3.2.7 Time required by EPNs to enter a leaf

The time required by *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) to enter a leaf was investigated at > 95 %, 75 ± 10 % and 45 ± 10 % RH against the 3rd larval instar. The experiment arena was prepared as described in section 2.2.9. Ten to fifteen larvae were placed on each leaf. Leaves were then sprayed with nematodes suspended in tap water at a rate of 60 ± 6 IJs cm^{-2}. Control treatments received water only. At intervals from 1 to 24 h, four leaves were removed from the containers. In order to fix the IJs on the leaf surface, the leaves were immediately dried by blowing cool air across them until no surface moisture was apparent. Each dried leaf was wrapped with moist cotton wool to keep it fresh and placed in a vented plastic Petri dish (9 cm × 1.6 cm) and larval mortality was checked 48 h later. There were four replicate containers for each time interval (n = 4) with the corresponding control treatments (n = 4).

3.3 Statistical analysis

Data were analysed using Minitab® 16.1.0 (© 2010 Minitab Inc.). To test the effect of RH on efficacy of each nematode species against the four larval instars and to test the efficacy of the three nematode species against the third larval instar at various RH values, percentage larval mortality was corrected for percentage control mortality using Schneider-Orelli's formula (Püntener, 1981) and tested for normality. Subsequently a General Linear Model (GLM) was used to test significant differences between treatments. Afterwards, Tukey's multiple range tests were performed to separate means. LC$_{50}$ values and LT$_{50}$ of nematode species at each RH were computed by probit analysis (Finney, 1971) in Minitab® 16.1.0.

To test the effect of temperature on efficacy of nematode species, larval percentage mortalities were corrected for control percentage mortalities using Schneider-Orelli's formula (Püntener, 1981). The effect of temperature on efficacy of *S. feltiae* was analysed using the non-parametric Scheirer-Ray-Hare Test followed by the non-parametric Mann-Whitney-U Test to test significant differences between treatments. This test was used because the assumptions of normality were not met. The effect of temperature on efficacy of *S. carpocapsae* was analysed using GLM. Corrected larval percentage mortalities were transformed (arcsine of the square root) before analysis. GLM was used to test significant

_____

_____

differences between treatments. Later, Tukey's multiple range test was performed to separate means.

To test the effect of number of applications on efficacy of EPNs, the effect of method of application on efficacy of EPNs and the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf; larval percentage mortalities were corrected for control percentage mortalities using Schneider-Orelli's formula (Püntener, 1981) and tested for normality. One-way ANOVA tests were used to test for significant differences between treatments. Afterwards, Tukey's multiple range tests were performed to separate means.

To compare between the survival of *S. feltiae* and *S. carpocapsae* after 7, 24 and 48 h at various RH on tomato leaves percentage survival data was tested for normality and GLM was used to test for differences between treatments. Afterwards, Tukey's multiple range tests were performed to separate means.

3.4 Results

3.4.1 Effect of RH on efficacy of EPNs against larvae of Tuta absoluta *on tomato leaves*

In general, relative humidity had an obvious impact on efficacy of the three tested nematode species (*S. feltiae, S. carpocapsae* and *H. bacteriophora*). The efficacy of the three nematode species against all larval instars of *T. absoluta* decreased with the decrease in RH. Nematode efficacy was highest at > 95 % RH followed by 75 ± 10 % and 45 ± 10 % RH. Mortality of different larval instars induced by the three nematode species varied, being highest in the fourth larval instar followed by third, second and first larval instars. *S. feltiae* recorded the highest mortality against different larval instars at the three ranges of RH compared to *S. carpocapsae* and *H. bacteriophora* (Figs. 3-1, 3-2 and 3-3).

Efficacy of *S. feltiae* against all larval instars ranged from 10 % to 100 %, being most effective at > 95 % RH followed by 75 ± 10 % and 45 ± 10 % RH. There were significant differences in its efficacy at different RH (GLM: $F_{2, 36}$ = 1306; $P < 0.05$) and against different larval instars (GLM: $F_{3, 36}$ = 74; $P < 0.05$) (Fig. 3-1).

The efficacy of *S. carpocapsae* against all larval instars ranged from 3.5 % to 97 % being most effective at > 95 % RH followed by 75 ± 10 % and 45 ± 10 % RH. There were

_____

significant differences in its efficacy at different RH (GLM: $F_{2,\ 36} = 1167$; $P < 0.05$) and against different larval instars (GLM: $F_{3,\ 36} = 48$; $P < 0.05$) (Fig. 3-2).

Efficacy of *H. bacteriophora* against all larval instars ranged from 1 % to 83 %, being most effective at > 95 % RH followed by 75 ± 10 % and 45 ± 10 % RH. There were significant differences in its efficacy at different RH (GLM: $F_{2,\ 36} = 746$; $P < 0.05$) and against different larval instars (GLM: $F_{3,\ 36} = 9$; $P < 0.05$) (Fig. 3-3).
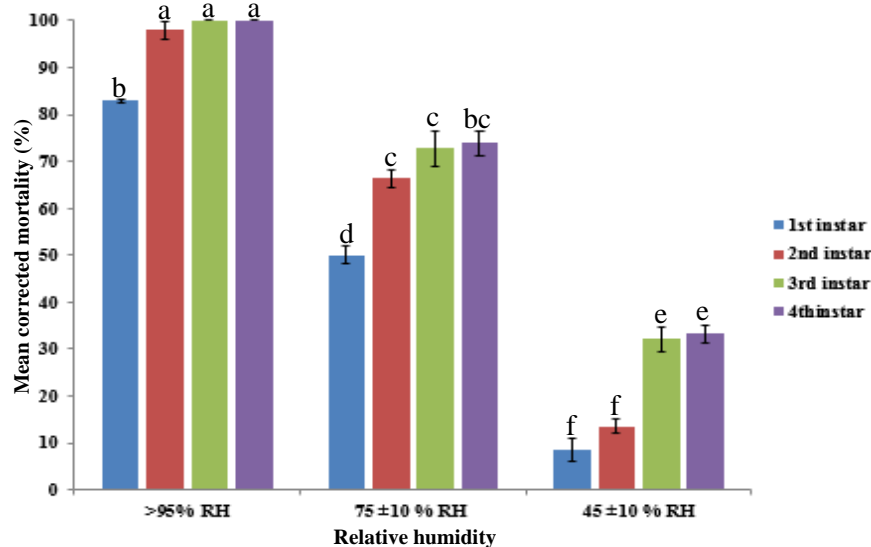
Figure 3-1 Mean corrected mortality of different larval instars of *T. absoluta* 48 h after application of *S. feltiae* (60 IJs ± 6 cm^{-2}) in water at different RH values and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P <0.05 (n = 4).
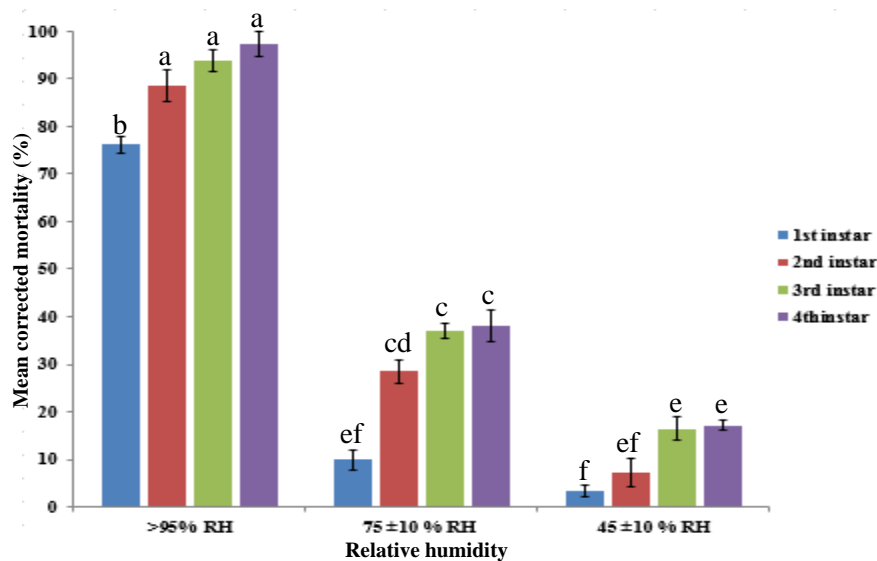
Figure 3-2 Mean corrected mortality of different larval instars of *T. absoluta* 48 h after application of *S. carpocapsae* (60 IJs ± 6 cm^{-2}) in water at different RH values and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P <0.05 (n = 4).
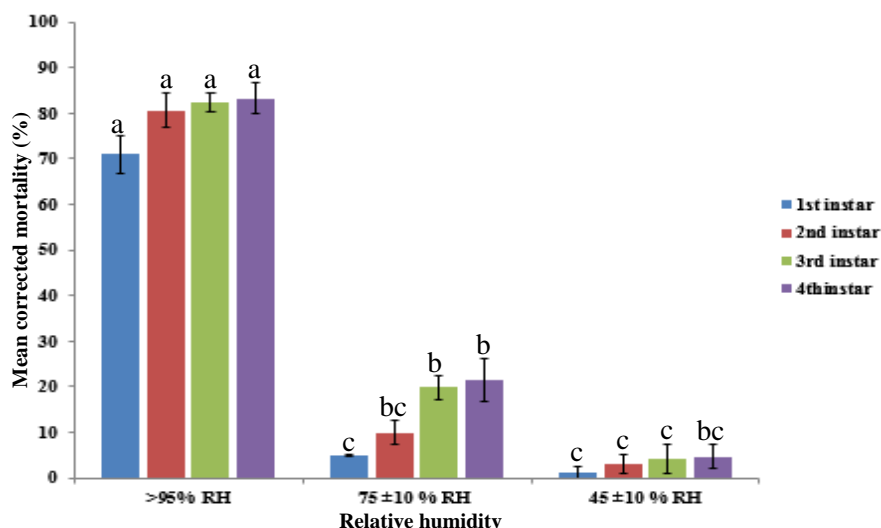
Figure 3-3 Mean corrected mortality of different larval instars of *T. absoluta* 48 h after application of *H. bacteriophora* (60 IJs ± 6 cm^{-2}) in water at different RH values and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

the efficacy of the three nematode species against the third larval instar at the three ranges of RH was compared, results showed that there were significant differences in efficacy between the nematode species (GLM: $F_{2, 27}$ = 128; *P* < 0.05) (Fig 3-4). At > 95 % RH, *S. feltiae* caused 100 % larval mortality, which was significantly (*P* < 0.05) higher than the 82.3 % mortality induced by *H. bacteriophora* but not significantly (*P* > 0.05) higher than the 93.8 % mortality caused by *S. carpocapsae*. At 75 ± 10 % RH, *S. feltiae* killed 72.8 % of larvae, which was significantly (*P* < 0.05) higher than the 37.1 % and 19.9 % larval mortality induced by *S. carpocapsae* and *H. bacteriophora* respectively. Although mortality at 45 ± 10 % RH was low (less than 35 %) for all nematode species, mortality induced by *S. feltiae* (32.1 %) was significantly (*P* < 0.05) higher than mortality induced by *S. carpocapsae* (16.5 %) and *H. bacteriophora* (4.3 %).
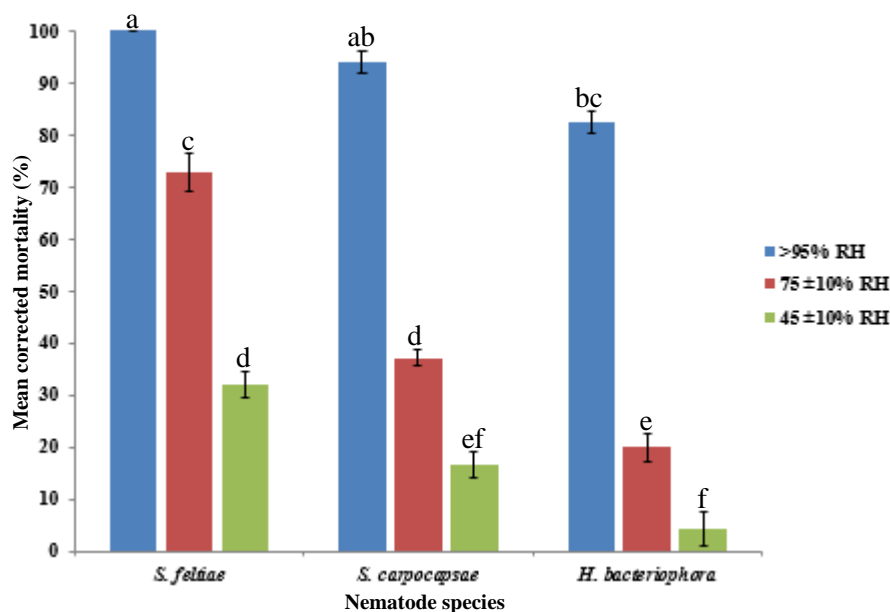
Figure 3-4 Mean corrected mortality of 3rd larval instar of *T. absoluta* 48 h after application of three EPNs (60 ± 6 IJs cm^{-2}) in water at three different RH values and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P <0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 3-1 to 3-3.

3.4.2 Effect of RH on lethal concentrations (LC) and lethal time (LT) of EPNs against larvae of **Tuta absoluta** *on tomato leaves*

LC$_{50}$ and LT$_{50}$ of *S. feltiae, S. carpocapsae* and *H. bacteriophora* were affected by the decrease in RH (Table 3-1). LC$_{50}$ values of *S. feltiae* increased from 4.3 IJs/cm^2 at > 95 % RH to 17 and 100 IJs/cm^2 at 75 ± 10 % and 45 ± 10 % RH respectively, whereas LC$_{50}$ values of *S. carpocapsae* increased from 6.4 IJs/cm^2 at > 95 % RH to 35 and 160 IJs/cm^2 at 75 ± 10 % and 45 ± 10 % RH respectively. Likewise, LC$_{50}$ values of *H. bacteriophora* increased from 11.2 IJs/cm^2 at > 95 % RH to 153 IJs/cm^2 at 75 ± 10 % RH, while at 45 ± 10 % RH LC$_{50}$ values were not calculated because larval mortality was less than 10 %.

Similarly, LT$_{50}$ of *S. feltiae* increased from 7.5 h at > 95 % RH to 29 and 53 h at 75 ± 10 % and 45 ± 10 % RH respectively, whereas LT$_{50}$ of *S. carpocapsae* increased from 10 h at > 95 % RH to 49 and 72 h at 75 ± 10 % and 45 ± 10 % RH respectively. Also, LT$_{50}$ of *H. bacteriophora* increased from 14 h at > 95 % RH to 63 h at 75 ± 10 % RH, while at 45 ± 10 % RH LT$_{50}$ was not calculated because larval mortality was less than 10 % (Table 3-1).

Table 3-1 Effect of different levels of RH on LC_{50} values (IJs/cm^2) and LT_{50} (hours) of EPNs applied in water on 3rd larval instar of *T. absoluta* on tomato leaves.

Nematode species	RH	LC_{50} (IJs/cm^2)	Fiducial limits (95 %)		LT_{50} (hours)	Fiducial limits (95 %)	
			lower	upper		lower	upper
S. feltiae	> 95 %	4.3	3.2	5.5	7.5	6.2	8.9
	75 ± 10 %	17.2	13.5	21.8	29.6	27.0	32.3
	45 ± 10 %	100*	60	285	53*	46.0	65.0
S. carpocapsae	> 95 %	6.4	4.8	8.2	10.0	7.3	12.5
	75 ± 10 %	35	26.5	50.1	49*	43.4	57.0
	45 ± 10 %	160*	86	1000	72*	58	113
H. bacteriophora	> 95 %	11.2	7.9	15.3	14.0	10.0	17.3
	75 ± 10 %	153*	86	496	63*	53	853
	45 ± 10 %	×	×	×	×	×	×

(x) LC_{50} and LT_{50} were not calculated because larval mortality was less than 18 %.
(*) LC_{50} and LT_{50} were calculated when mortality was below 50 % and above 18 %.

3.4.3 Effect of RH on survival of IJs of S. feltiae *and* S. carpocapsae *on tomato leaves*

In general *S. carpocapsae* survived better than *S. feltiae* but survival of both nematode species decreased as RH decreased and as time passed (Fig. 3-5). *S. carpocapsae* survived more than 48 h at > 95 % and 75 ± 10 % RH and 42 h at 45 ± 10 % RH, whereas *S. feltiae* survived more than 48 h at > 95 % RH, 41 h at 75 ± 10 % RH and 20 h at 45 ± 10 % RH. Survival of *S. carpocapsae* decreased over 48 h from 100 % to 89 % at > 95 % RH, 100 % to 19 % at 75 ± 10 % RH and 100 % to 0 % at 45 ± 10 % RH, whereas survival of *S. feltiae* decreased over 48 h from 100 % to 80 % at > 95 % RH and from 100 % to 0 % at 75 ± 10 % and 45 ± 10 % RH. Furthermore, LT_{50} values for *S. carpocapsae* were higher than those for *S. feltiae* at the three ranges of RH (Table 3-2).
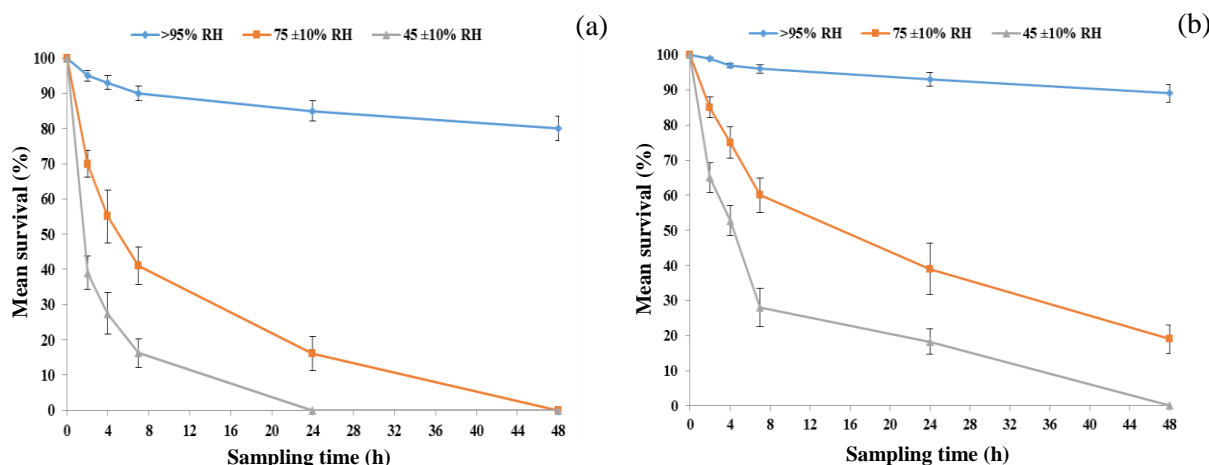
Figure 3-5 Mean survival over 48 h of IJs (60 ± 6 IJs cm^{-2}) of (a): *S. feltiae* and (b): *S. carpocapsae* applied in water on tomato leaves at various RH values and 25 ± 0.5 °C. Error bars indicate standard errors (n = 4).

Table 3-2 LT$_{50}$ of *S. feltiae* and *S. carpocapsae* applied in water on tomato leaves at three different RH values.

Nematode species	RH	LT$_{50}$ (hours)	Fiducial limits (95 %)	
			lower	upper
S. feltiae	> 95 %	508	276	1213
	75 ± 10 %	6.2	5.8	6.6
	45 ± 10 %	3.1	3.1	3.3
S. carpocapsae	> 95 %	1324	550	5382
	75 ± 10 %	14.0	12.9	15.3
	45 ± 10 %	5.5	5.1	5.9

When survival of *S. feltiae* and *S. carpocapsae* was compared after 7, 24 and 48 h at the three RH values tested, significant differences were observed in survival between the two nematode species (GLM: $F_{1, 54} = 53$; $P < 0.05$), the three ranges of RH (GLM: $F_{2, 54} = 750$; $P < 0.05$) and the three tested times (GLM: $F_{2, 54} = 64$; $P < 0.05$) (Fig. 3-6). Mean survival of *S. carpocapsae* (49 %) was significantly ($P < 0.05$) higher than survival of S. *feltiae* (36 %). Survival of *S. feltiae* and *S. carpocapsae* at > 95 % and 45 ± 10 % RH was similar and did not differ significantly ($P > 0.05$) between time points, whereas at 75 ± 10 % RH survival of *S. carpocapsae* was significantly ($P < 0.05$) higher than survival of *S. feltiae* at each time point (Fig. 3-6).

_____
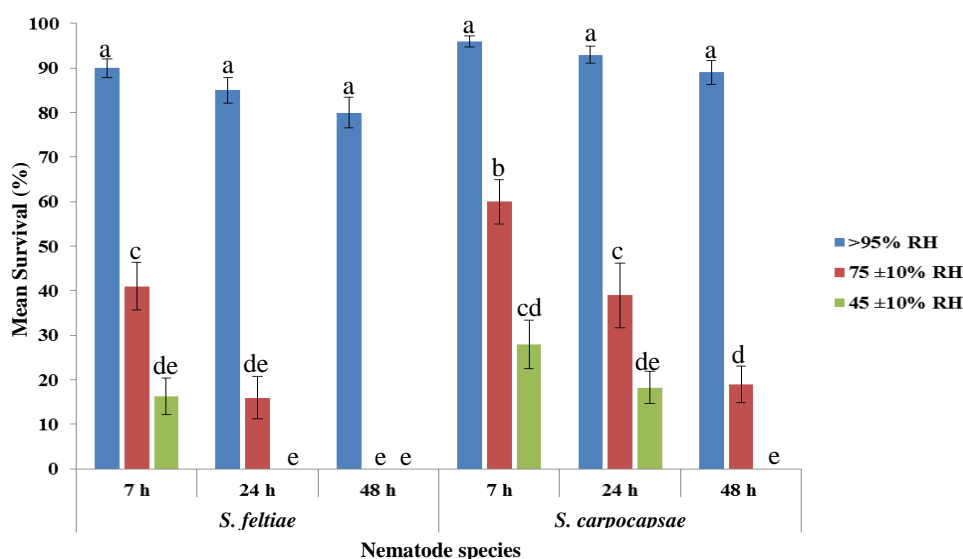
Figure 3-6 Mean survival of IJs (60 ± 6 IJs cm^{-2}) of *S. feltiae* and *S. carpocapsae* applied in water after 7, 24 and 48 h on tomato leaves at various RH values and 25 ± 0.5 °C. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4). Data used in the analysis here were from the experiment shown in Figure 3-5.

3.4.4 Effect of temperature on efficacy of *S. feltiae* and *S. carpocapsae* against 3rd larval instar of *Tuta absoluta* on tomato leaves

There were significant differences in the efficacy of *S. feltiae* (Sheirer-Ray-Hair Test; $\chi^2_5 = 74.5$; $P < 0.05$) and in the efficacy of *S. carpocapsae* (GLM: $F_{5, 72} = 198$; $P < 0.05$) at the tested temperatures. *S. feltiae* caused the highest larval mortality (above 95 %) at 15, 20 and 25 °C while at 30 °C mortality was slightly lower (80 %). Larval mortality at 10 °C increased sharply as the time passed, reaching 94 % after 96 h, whilst mortality at 35 °C was very low (13 %). *S. feltiae* required 48 h at 15 and 20 °C, 24 h at 25 and 30 °C and 96 h at 10 °C to induce the highest larval mortality recorded at each of these temperatures (Fig. 3-7). On the other hand, *S. carpocapsae* induced the highest larval mortality (above 91 %) at 20, 25, 30 and 35 °C, while at 15 °C mortality was slightly lower (86 %). Larval mortality at 10 °C was very low (12 %). *S. carpocapsae* required 24 h at 25, 30 and 35 °C and 96 h at 15 and 20 °C to induce the highest larval mortality recorded at each of these temperatures (Fig. 3-8).

_____
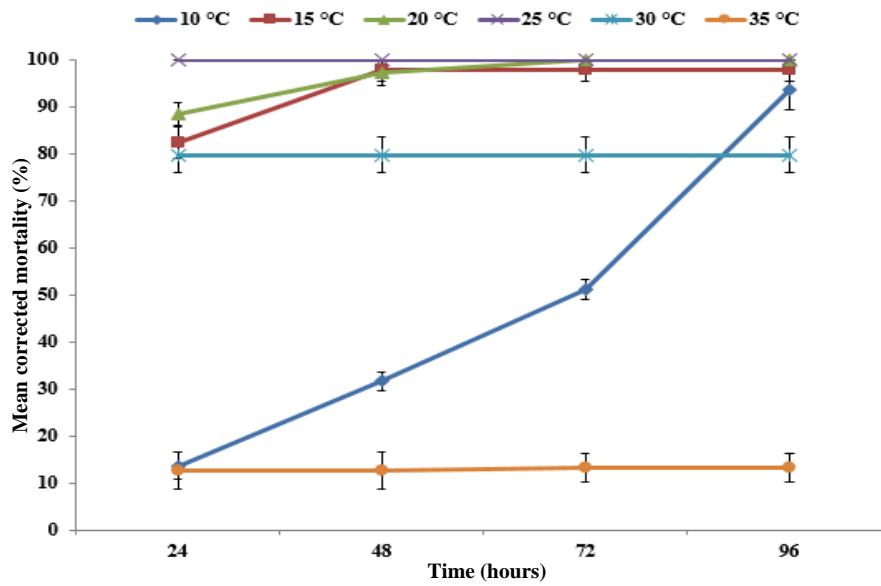
Figure 3-7 Mean (± SE) corrected mortality of 3rd larval instar of *T. absoluta* at different temperatures after application of *S. feltiae* (60 ± 6 IJs cm^{-2}) on tomato leaves in water at > 95 % RH (n = 4).
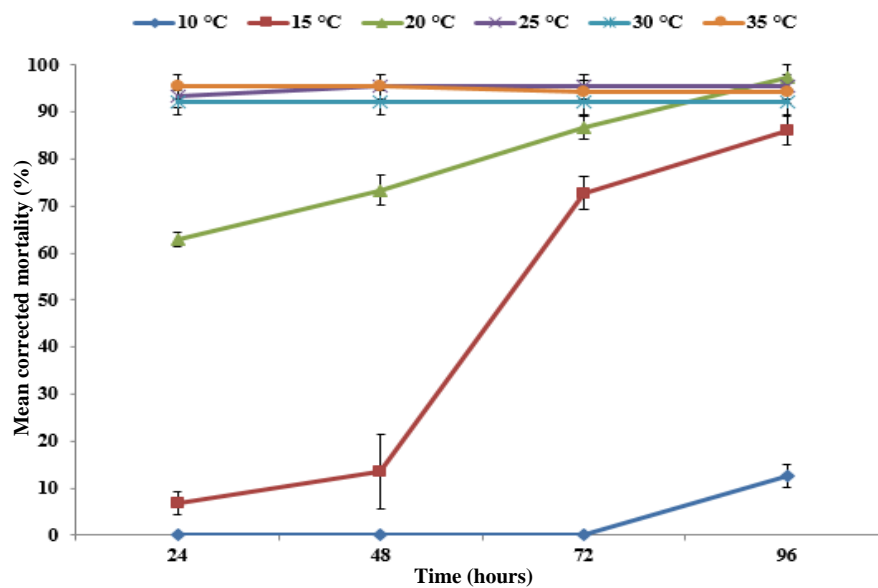
Figure 3-8 Mean (± SE) corrected mortality of 3rd larval instar of *T. absoluta* at different temperatures after application of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) on tomato leaves in water at > 95 % RH (n = 4).

When the two nematode species were compared in the same analysis with larval mortality recorded after 72 h, the results showed that there were no significant differences in mean efficacy between *S. feltiae* and *S. carpocapsae* (GLM: $F_{1, 36}$ = 0.02; *P* > 0.05), whereas there were significant differences in the efficacy of *S. feltiae* and *S. carpocapsae* at the six tested temperatures (GLM: $F_{5, 36}$ = 263; *P* < 0.05). *S. feltiae* induced the highest larval mortality at 15, 20 and 25 °C, whereas *S. carpocapsae* induced the highest larval mortality at 20, 25, 30

_____

_____

and 35 °C. *S. feltiae* caused larval mortality of 51 %, 98 % and 100 % at 10, 15 and 20 °C respectively, which was significantly ($P < 0.05$) higher than 0 %, 73 % and 87 % induced by *S. carpocapsae* at the same temperatures respectively. At 25 °C *S. feltiae* and *S. carpocapsae* caused mortality of 100 % and 95 % respectively but there was not a significant ($P < 0.05$) difference between the two nematode species. On the other hand, *S. carpocapsae* induced mortality of 92 % and 96 % at 30 and 35 °C respectively, which was significantly ($P < 0.05$) higher than 80 % and 13 % mortality induced by *S. feltiae* at the same temperatures respectively (Fig. 3-9).
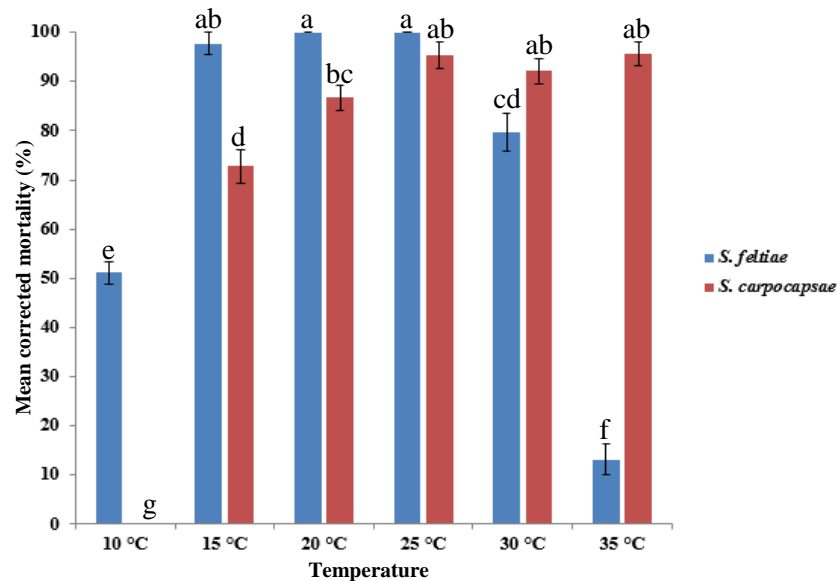
Figure 3-9 Mean (± SE) corrected mortality of 3rd larval instar of *T. absoluta* 72 h after application of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) in water at different temperatures and > 95 % RH. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 3-7 and 3-8.

3.4.5 Effect of number of applications of EPNs against larvae of **Tuta absoluta** *on tomato leaves at different RH*

In general, four applications gave significantly higher larval mortality of *T. absoluta* on tomato leaves at 45 ± 10 % RH and 75 ± 10 % RH than other frequencies of application except for *S. feltiae* at 75 ± 10 % RH, in which three and four applications (against the first and second larval instars) and two, three and four applications (against the third and fourth larval instars) gave the highest larval mortality with no significant differences ($P > 0.05$). There were significant differences in mortality between numbers of applications of *S. feltiae* at 75 ± 10 % RH against the first larval instar (One-Way ANOVA: $F_{3, 12} = 31$; $P < 0.05$), the second larval instar (One-Way ANOVA: $F_{3, 12} = 84$; $P < 0.05$), the third larval instar (One-

_____

_____

Way ANOVA: $F_{3, 12}$ = 18; $P < 0.05$) and the fourth larval instar (One-Way ANOVA: $F_{3, 12}$ = 17; $P < 0.05$). These significant differences were between one, two and three and four applications with the first and second larval instars and between one application and the other applications with the third and fourth larval instars. The best treatments with *S. feltiae* at this RH were three and four applications to the first and second larval instars and two, three and four applications to the third and fourth larval instars (Fig. 3-10).
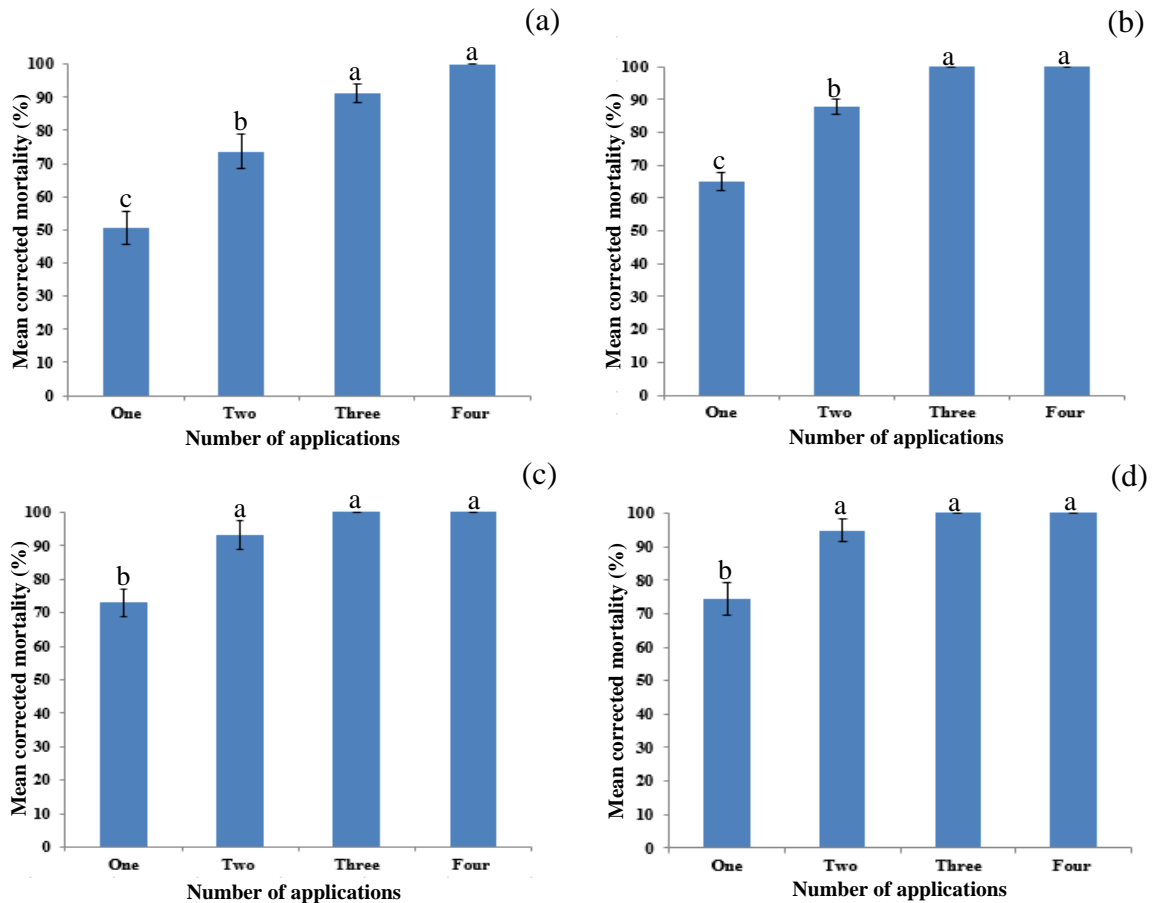
Figure 3-10 Mean corrected mortality at 75 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) in water. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

There were also significant differences in mortality between numbers of applications of *S. feltiae* at 45 ± 10 % RH against the first larval instar (One-Way ANOVA: $F_{3, 12}$ = $P < 0.05$), the second larval instar (One-Way ANOVA: $F_{3, 12}$ = $P < 0.05$), the third larval instar (One-Way ANOVA: $F_{3, 12}$ = 32; $P < 0.05$) and the fourth larval instar (One-Way ANOVA: F = 35; $P < 0.05$). These significant differences were between each treatment and all the others with the first and second larval instars and between four applications, three applications and the other numbers of applications with the third and fourth larval instars. The best treatment with

_____

_____

S. feltiae at this RH was four applications in which mean corrected larval mortality was 92 %, 91 %, 74 % and 50 % for the fourth, third, second and first larval instars respectively (Fig. 3-11).
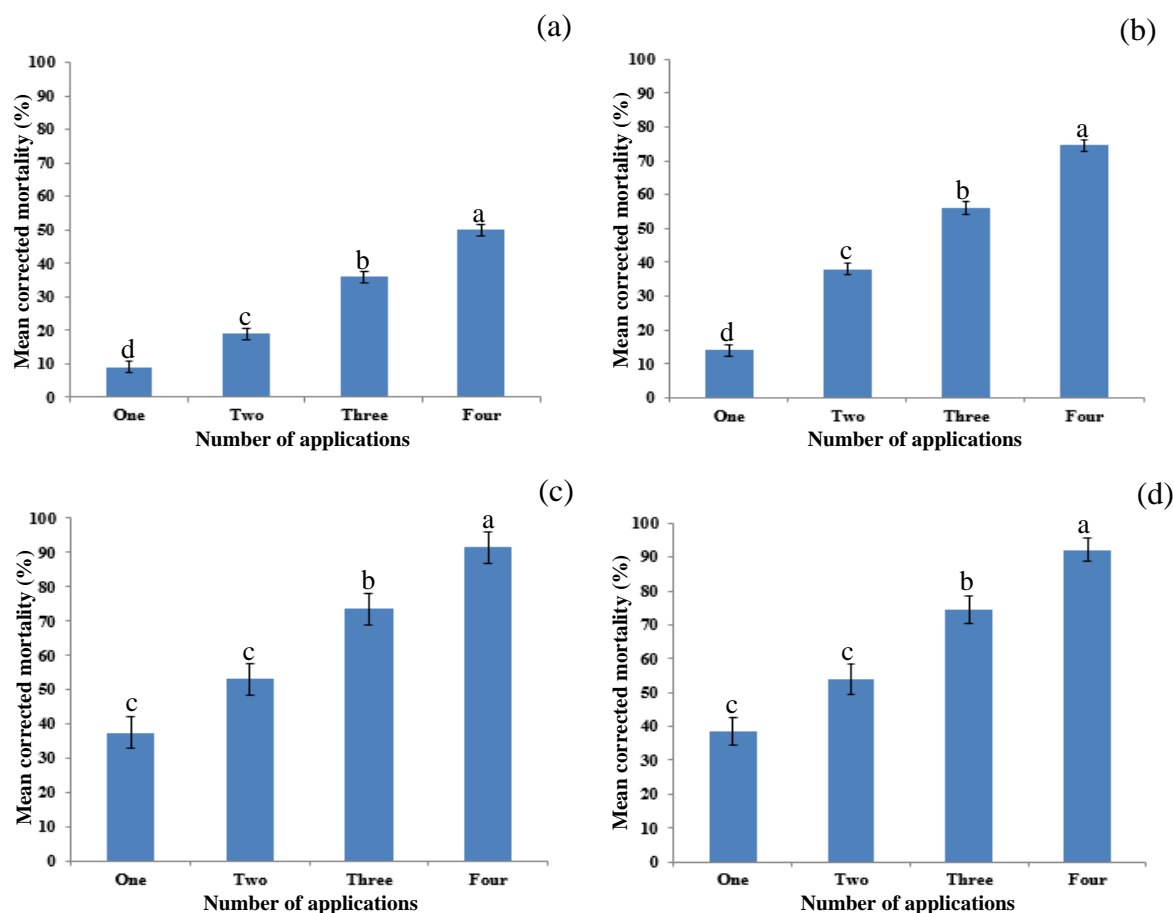
Figure 3-11 Mean corrected mortality at 45 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different number of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) in water. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

On the other hand, although larval mortality was low, there were significant differences between treatments with *S. carpocapsae* at 75 ± 10 % RH against the first larval instar (One-Way ANOVA: $F_{3, 12} = 64$; $P < 0.05$), the second larval instar (One-Way ANOVA: $F_{3, 12} = 61$; $P < 0.05$), the third larval instar (One-Way ANOVA: $F_{3, 12} = 53$; $P < 0.05$) and the fourth larval instar (One-Way ANOVA: $F_{3, 12} = 51$; $P < 0.05$). These significant differences were between each treatment and all the others with the first, second and third larval instars and between three and four applications and the other numbers of applications with the fourth larval instar. The best treatment of *S. carpocapsae* at this RH was four applications with the third, second and first larval instars, in which mean corrected larval mortality was 87 %, 75 %

_____

and 100 % respectively, and four and three applications with the fourth larval instar in which mean corrected larval mortality was 100 % and 87 % respectively (Fig. 3-12).
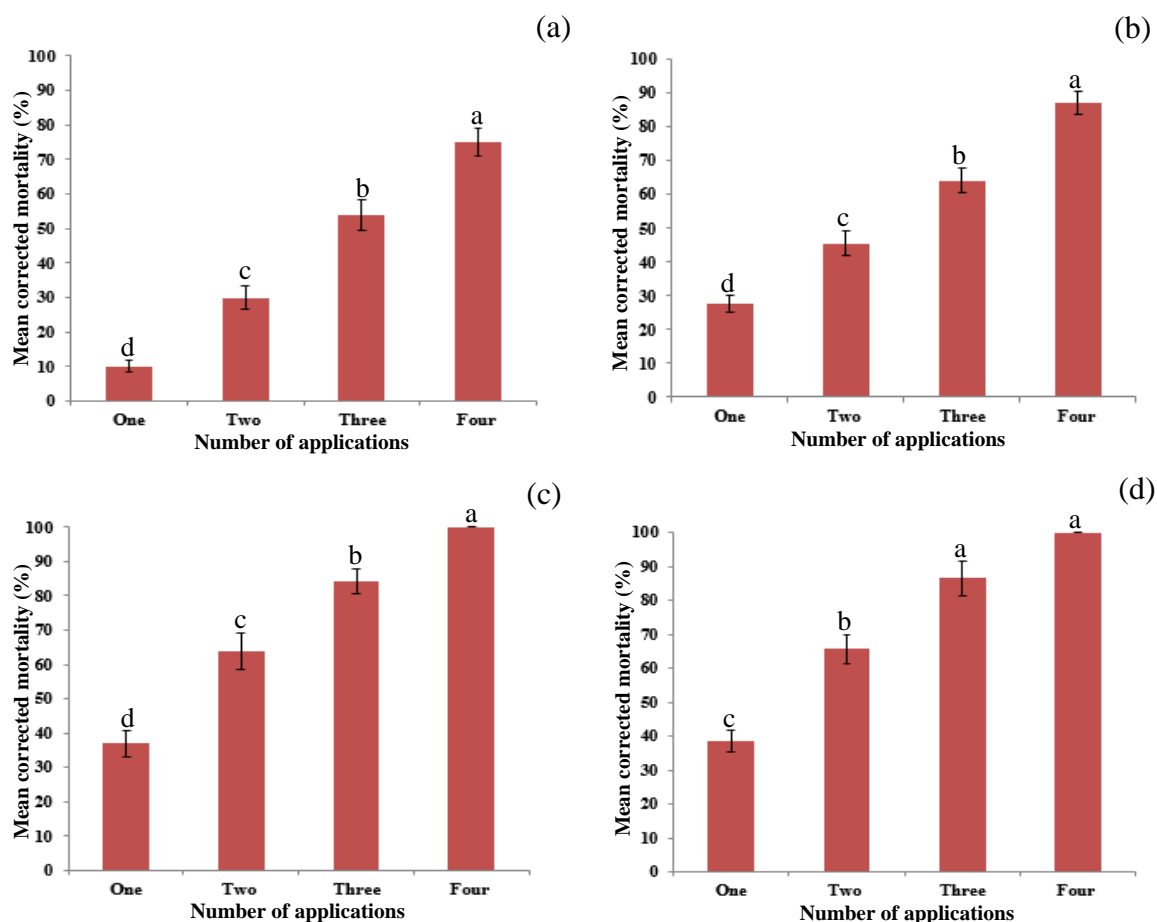
Figure 3-12 Mean corrected mortality at 75 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different number of applications of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) in water. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

Additionally, there were significant differences between treatments with *S. carpocapsae* at 45 ± 10 % RH against the first larval instar (One-Way ANOVA: $F_{3,\,12} = 20$; $P < 0.05$), the second larval instar (One-Way ANOVA: $F_{3,\,12} = 38$; $P < 0.05$), the third larval instar (One-Way ANOVA: $F_{3,\,12} = 15$; $P < 0.05$) and the fourth larval instar (One-Way ANOVA: $F_{3,\,12} = 23$; $P < 0.05$). These significant differences were between four applications and one and two applications with the first and third larval instars, between four applications and the other numbers of applications with the second larval instar and between four and three applications

_____

and the other numbers of applications with the fourth larval instar. The best treatment of *S. carpocapsae* at this RH was four applications, in which mean corrected larval mortality was 54 %, 52 %, 41 % and 26 % for the fourth, third, second and first larval instar respectively (Fig. 3-13).
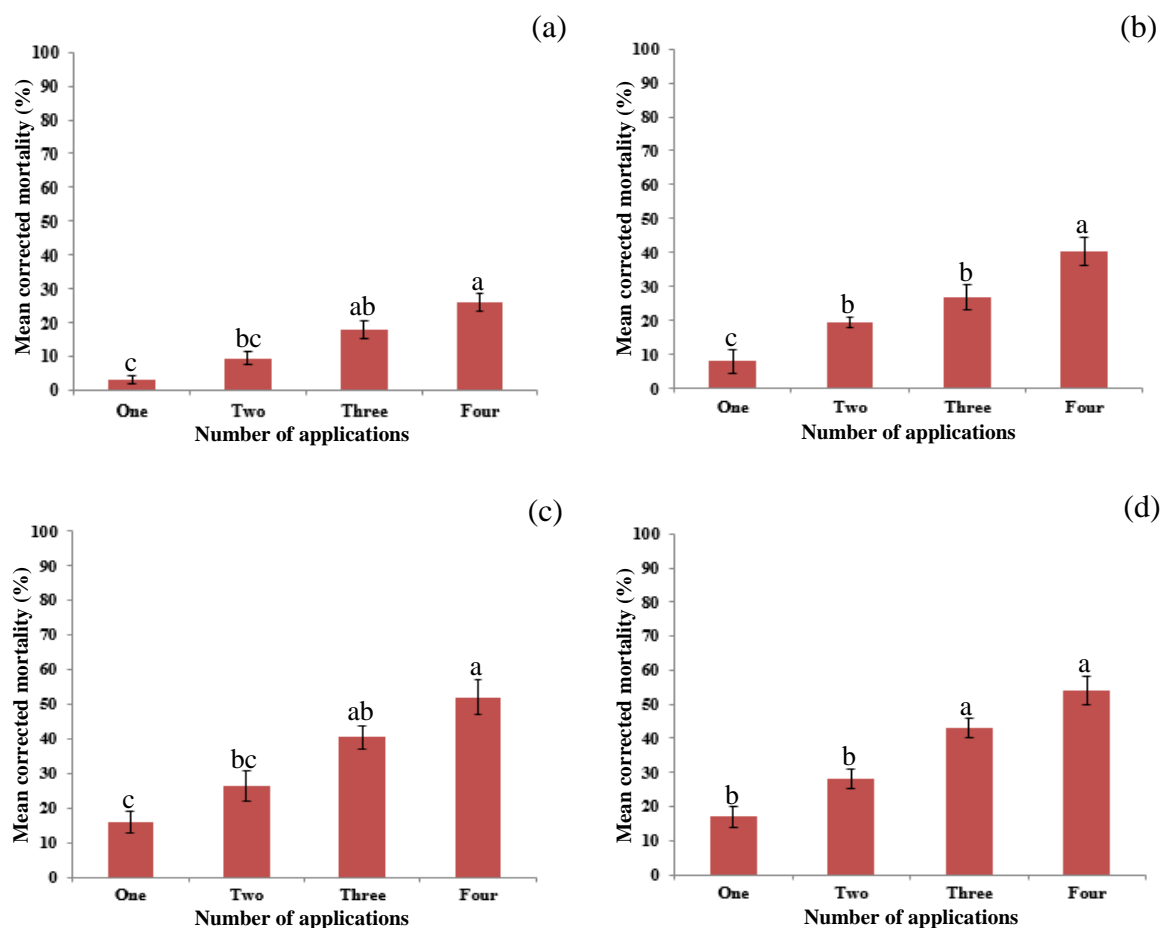
Figure 3-13 Mean corrected mortality at 45 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different number of applications of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) in water. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

3.4.6 Effect of method of application on efficacy of EPNs at different RH

In general, spraying nematode suspensions on both leaf surfaces gave higher larval mortality than spraying them on the lower or upper surface. There were significant differences between treatments with *S. feltiae* at > 95 % RH (One-Way ANOVA: $F_{2, 9} = 25$; $P < 0.05$) and between treatments with *S. carpocapsae* (One-Way ANOVA: $F_{2, 9} = 7$; $P < 0.05$). Two surface treatment and lower surface treatment gave significantly ($P < 0.05$) higher larval mortality than upper surface treatment using *S. feltiae* and *S. carpocapsae* (Fig. 3-14).

Similarly, there were significant differences between treatments with *S. feltiae* (One-Way ANOVA: $F_{2, 9} = 6$; $P < 0.05$) and between treatments with *S. carpocapsae* (One-Way ANOVA: $F_{2, 9} = 7$; $P < 0.05$) at 75 ± 10 % RH. Two-surface treatments gave significantly ($P < 0.05$) higher larval mortality than upper surface treatment but not significantly ($P > 0.05$) higher than lower surface treatment using *S. feltiae* and *S. carpocapsae* (Fig. 3-15).

(a)   (b)
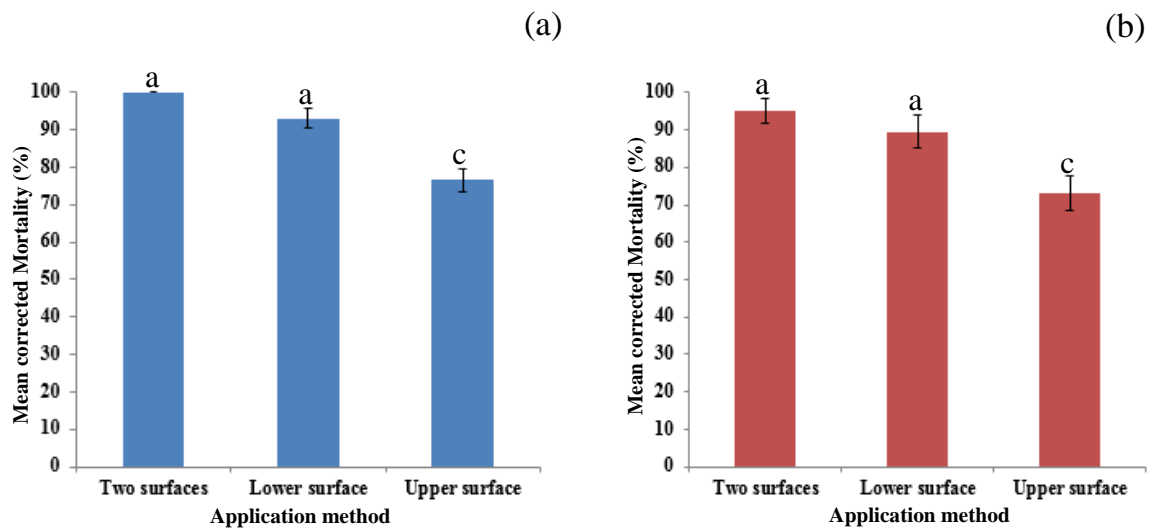
Figure 3-14 Mean corrected mortality at > 95 % RH and 25 ± 0.5 °C of 3rd larval instar of *T. absoluta* 48 h after application by different methods of (a): *S. feltiae* (b): *S. carpocapsae* at 60 ± 6 IJs cm^{-2} in water. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

(a)   (b)
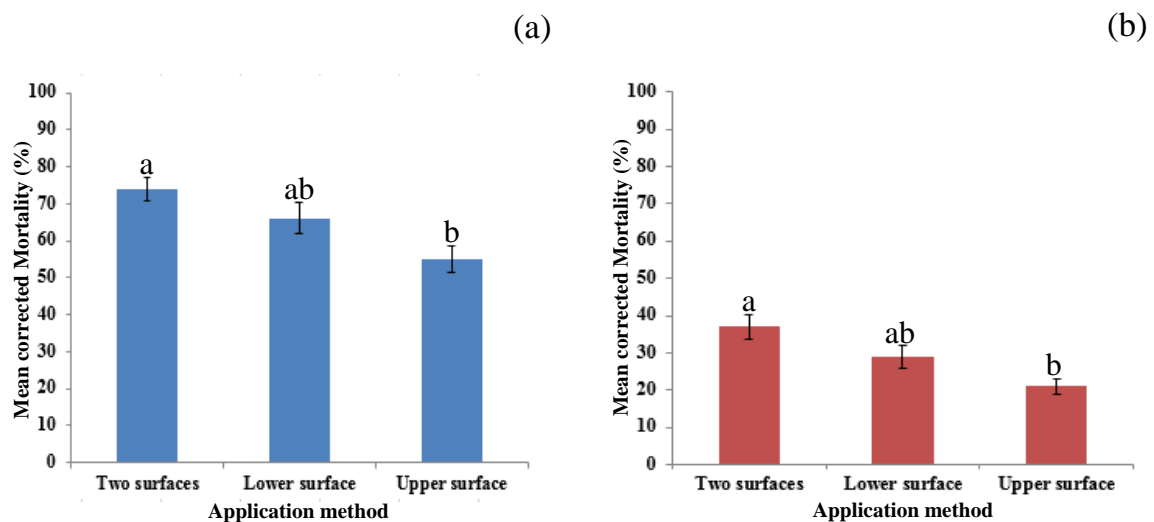
Figure 3-15 Mean corrected mortality at 75 ± 10 % RH and 25 ± 0.5 °C of 3rd larval instar of *T. absoluta* 48 h after application by different methods of (a): *S. feltiae* (b): *S. carpocapsae* at 60 ± 6 IJs cm^{-2} in water. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

Likewise, there were significant differences between treatments with *S. feltiae* (One-Way ANOVA: $F_{2, 9} = 19$; $P < 0.05$) and between treatments with *S. carpocapsae* (One-Way ANOVA: $F_{2, 9} = 7$; $P < 0.05$) at 45 ± 10 % RH. Although mortality was low at this RH, two-surface treatment gave significantly ($P < 0.05$) higher larval mortality than the other treatments using *S. feltiae* and significantly ($P < 0.05$) higher larval mortality than upper surface treatment but not significantly ($P > 0.05$) higher than lower surface treatment using *S. carpocapsae* (Fig. 3-16).
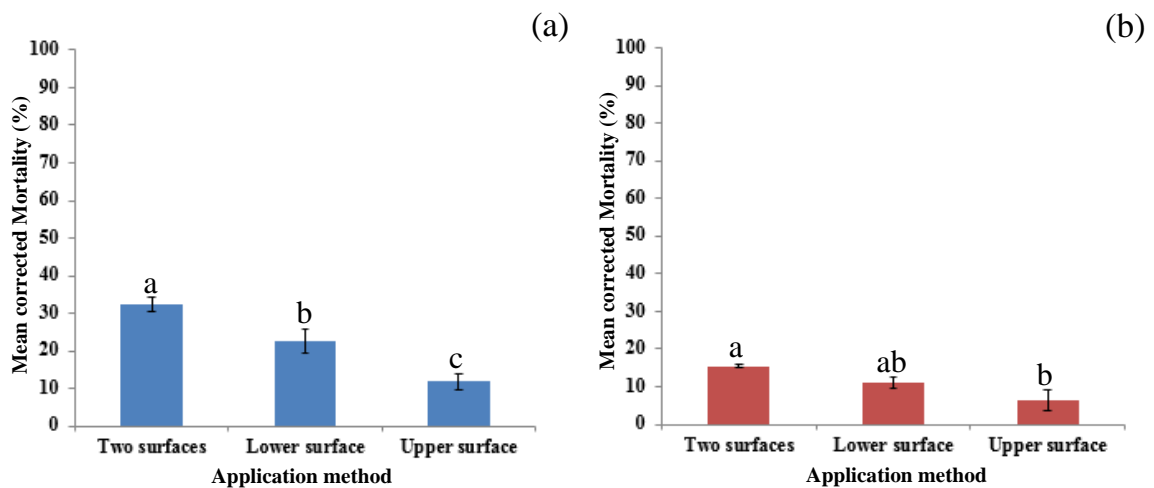
Figure 3-16 Mean corrected mortality at 45 ± 10 % RH and 25 ± 0.5 °C of 3rd larval instar of *T. absoluta* 48 h after application by different methods of (a): *S. feltiae* (b): *S. carpocapsae* in water at 60 ± 6 IJs cm^{-2}. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

3.4.7 Time required by EPNs to enter a leaf

Using *S. feltiae,* there were significant differences between fixing times at > 95 % RH (One-Way ANOVA: $F_{4, 15} = 32$; $P < 0.05$), at 75 ± 10 % RH (One-Way ANOVA: $F_{4, 15} = 20$; $P < 0.05$) and at 45 ± 10 % RH (One-Way ANOVA: $F_{4, 15} = 7$; $P < 0.05$). These differences were between 1 h fixing time and the other fixing times at > 95 % RH and between 1 and 3 h fixing times and the other fixing times at 75 ± 10 % RH and at 45 ± 10 % RH, with differences in the efficacy of *S. feltiae* at each level of RH being highest at > 95 % followed by 75 ± 10 % and 45 ± 10 % RH (Fig. 3-17).

On the other hand, using *S. carpocapsae*, there were significant differences between fixing times at > 95 % RH (One-Way ANOVA: $F_{4, 15} = 40$; $P < 0.05$), at 75 ± 10 % RH (One-Way ANOVA: $F_{4, 15} = 20.5$; $P < 0.05$) and at 45 ± 10 % RH (One-Way ANOVA: $F = 4.5$; $P < 0.05$). These differences were between 1 and 3 h fixing time and the other fixing times at > 95 % RH and between 1, 3 and 6 h fixing times and the other fixing times at 75 ± 10 % RH and at 45 ± 10 % RH, with differences in the efficacy of *S. carpocapsae* at each level of RH being highest at > 95 % followed by 75 ± 10 % and 45 ± 10 % RH (Fig. 3-18).

(a)   (b)

(c)
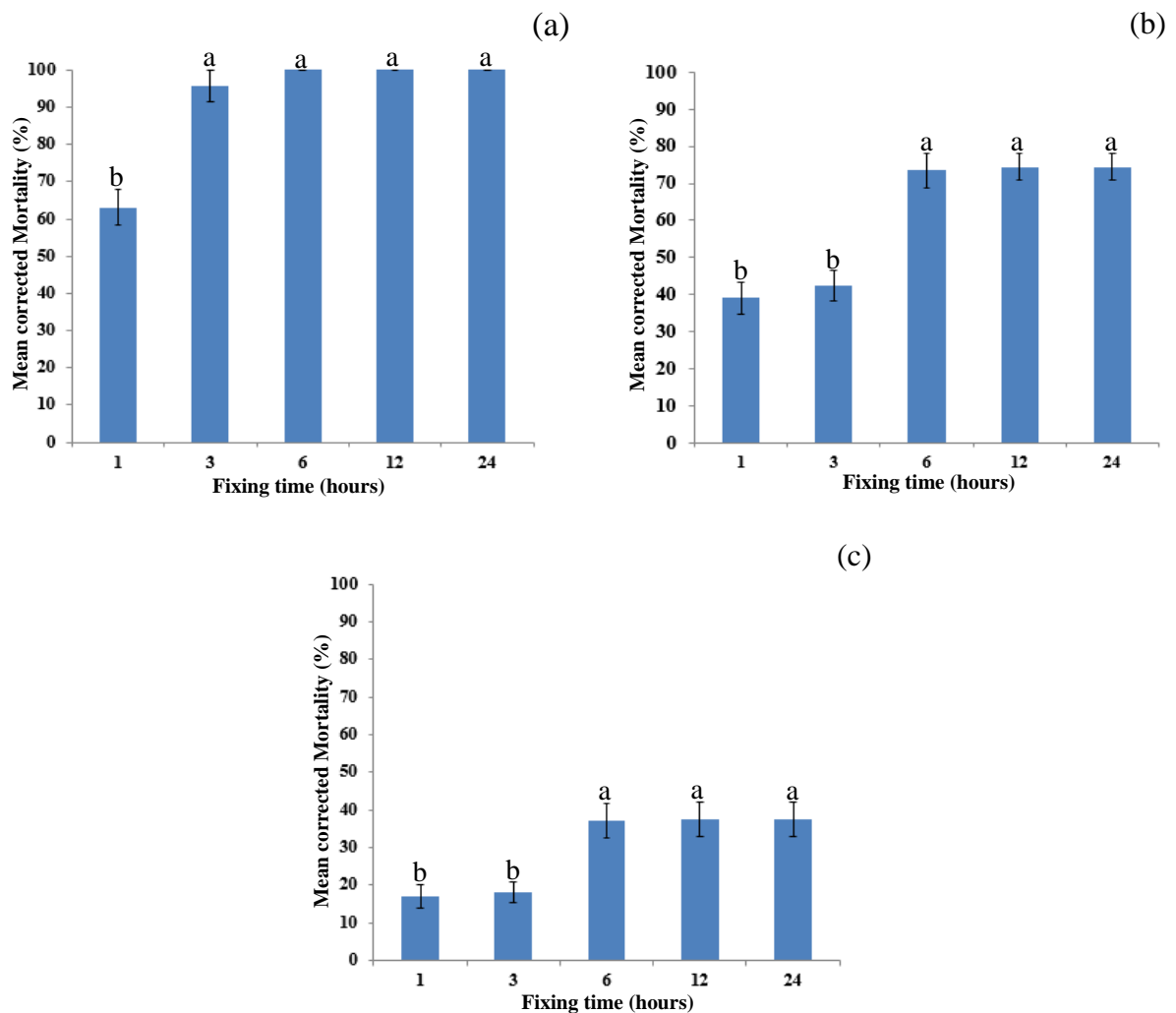
Figure 3-17 Mean corrected mortality of 3ʳ larval instar of *T. absoluta* after 48 h of fixing *S. feltiae* (60 ± 6 IJs cm^{-2}) applied in water after various times on tomato leaves at (a): > 95 %; (b): 75 ± 10 %; (c): 45 ± 10 % RH and 25 ± 0.5 °C. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).
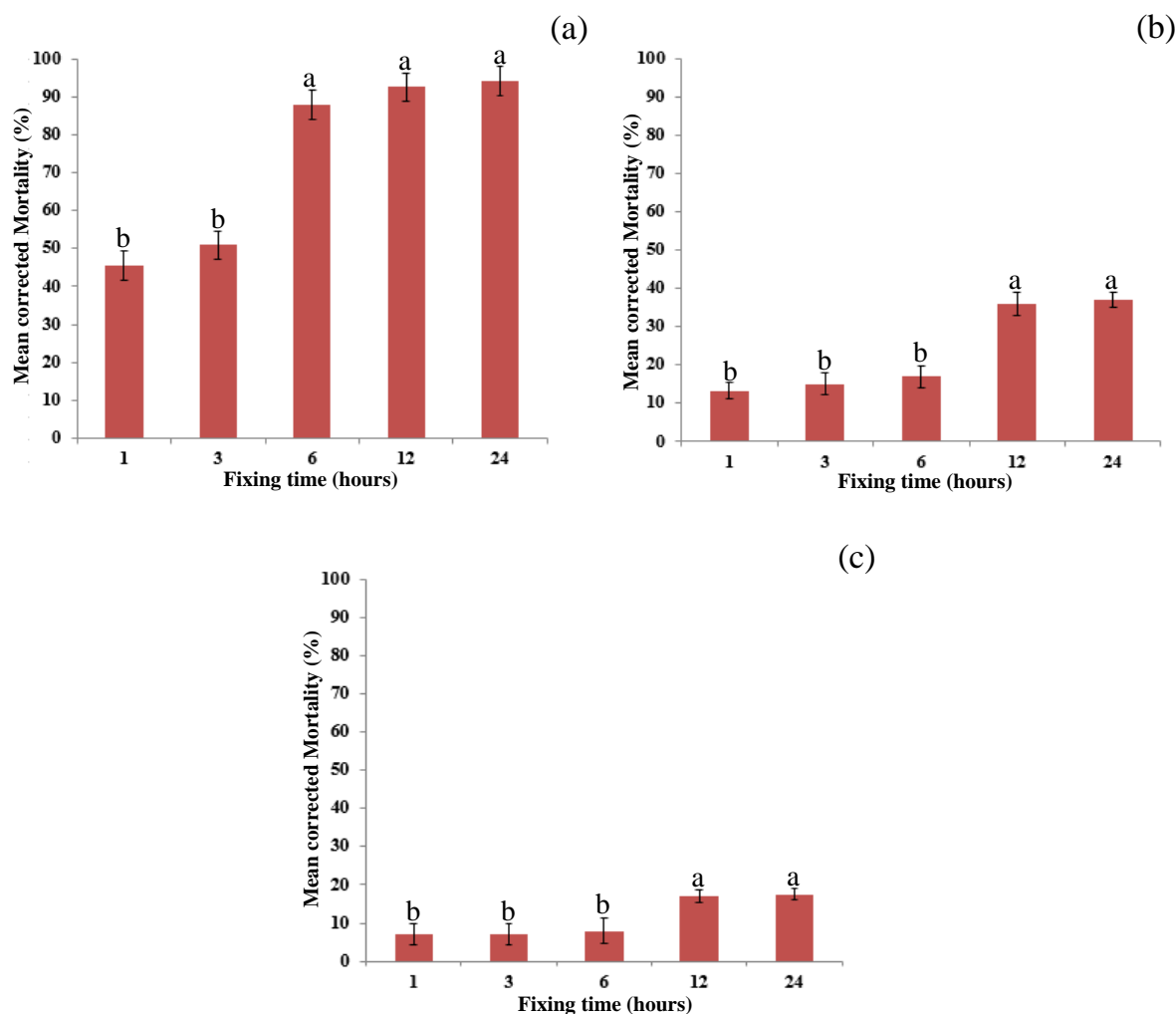
Figure 3-18 Mean corrected mortality of 3^r larval instar of *T. absoluta* after 48 h of fixing *S. carpocapsae* (60 ± 6 IJs cm^{-2}) applied in water after various times on tomato leaves at (a): > 95 %; (b): 75 ± 10 %; (c): 45 ± 10 % RH and 25 ± 0.5 °C. Bars indicated with the same letter (mean ± SE) do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

3.5 Discussion

The results of the current study revealed that relative humidity had a direct effect on efficacy of the EPNs. The efficacy of the three nematode species against larvae of *T. absoluta* decreased with the decrease in RH. Nematode efficacy was highest at > 95 % RH followed by 75 ± 10 % then 45 ± 10 % RH (Figs. 3-1, 3-2 and 3-3). These findings are in accord with those of Jacobson and Martin (2011), who reported high efficacy (> 90 % mortality) using an aqueous suspension of the same commercial nematode species used in the current study (*S. feltiae* (Nemasys®)) against larvae of *T. absoluta* in favourable conditions. However, they observed lower nematode efficacy using an aqueous suspension of the same nematode species against larvae of *T. absoluta* in two separate trials undertaken in commercial organic tomato

crops at Horticilha, Cilha Queimada, Alcochete, Portugal (50 % and 43 % overall mean mortality in first and second trial respectively). The low efficacy in the first trial was explained by these authors as due to poor application in one of the plots due to some nozzles being temporarily blocked and the authors suggested that the overall mortality would rise to 56 % if the data from that plot were excluded. The result of the second trial was attributed to the unfavourable environmental conditions for nematode activity (65-74 % RH). Similarly, Baur *et al.* (1998) reported a larval mortality of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) of 62-87 % using *Steinernema carpocapsae* "All" strain suspended in water in Petri dish bioassays lined with filter paper. However, only 41 % mean mortality was achieved in two experiments conducted at farms in Hawaii with more challenging environmental conditions using a foliar application of the nematode species mentioned above with 0.2 % vol.: vol. of the wetting agent Ag-98 (Sigma, Columbia, MO). Comparable results were obtained by Glazer and Navon (1990), who found that an aqueous suspension of *S. feltiae* "All" strain gave a mean mortality of 77 % with different larval instars of the cotton bollworm *Heliothis armigera* (Lepidoptera: Noctuidae) in Petri dish bioassays, whereas in harsher environmental conditions in a greenhouse (50–70 % RH and 24-28 °C), *S. feltiae* "Pay" strain and *S. feltiae* "All" strain induced only 15 % and 0 % larval mortality respectively on leaves of bean seedlings. In the same way, Glazer *et al.* (1992) recorded 22 % control of *H. armigera* on bean seedlings grown in 4-litre plastic pots in greenhouse conditions (60-70 % RH and 24-26 °C) and 23 % control of the Egyptian cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) on two-month-old cotton plants grown in 12-litre pots in a greenhouse (75 % RH and 20-29 °C), using aqueous suspensions of *S. carpocapsae* "Mexican" strain.

The results of the current investigation showed that the rate of nematode survival is related to RH with nematode survival decreasing as RH decreased. In addition, *S. carpocapsae* tolerated desiccation better than *S. feltiae* (Fig. 3-6). These results are consistent with those of Bélair *et al.* (2003) who found that survival rate of *S. carpocapsae* "All" strain on cabbage leaves was higher than survival of *S. feltiae* "UK" strain at 70 % RH in growth chambers. They are also in line with those of Glazer and Navon (1990), who recorded extreme reduction in *S. feltiae* "All" strain survival (80 % after 4 h and 0 % after 8 h exposure) on foliage of bean plants at 50-70 % RH under greenhouse conditions. Furthermore, similar results were obtained by Glazer (1992), who reported a reduction in survival of *S. carpocapsae* "Mexican" strain by 40 % and 70 % at 80 % and 60 % RH respectively after 6 hours exposure on filter papers. The

_____

author recorded a reduction in survival of the same nematode species by 39 % at 65 % RH after 4 h exposure on tomato leaves under greenhouse conditions.

When comparing the efficacy of the three nematode species against third larval instar at the three ranges of RH, the results presented here showed significant differences in efficacy between the species, with *S. feltiae* the most effective species (68 % mean mortality at the three RH) followed by *S. carpocapsae* (49 %) then *H. bacteriophora* (36 %) (Fig. 3-4). These findings were supported by the results discussed above, of the effect of RH, in which the LC_{50} and LT_{50} of *S. feltiae* were the lowest followed by *S. carpocapsae* then *H. bacteriophora* (Table 3-1). Thus, *H. bacteriophora* was excluded from further investigations. Despite the fact that *S. carpocapsae* tolerates desiccation better than *S. feltiae*, the latter species induced higher mortality. The reason behind this might be the difference in foraging strategies of the two nematode species (discussed in Chapter 2 of this thesis). *S. feltiae* required less time to locate and enter the mines than *S. carpocapsae* and consequently gained protection from desiccation inside the leaf tissue. This explanation is supported by the results obtained from the experiment on time required by nematodes to enter a leaf at 75 ± 10 and 45 ± 10 % RH, in which *S. feltiae* required less time (6 h) to enter a leaf and induce significant higher larval mortality compared to *S. carpocapsae* (12 h) when nematodes were suspended in water. However, *H. bacteriophora* was not included in the experiment of the effect of RH on nematode survival due to its poor performance even at high RH (> 95 %) and due to its high LC_{50} and LT_{50} values compared to the other two nematode species (Fig. 3-4 and Table 3-1). A possible explanation for this could be because of its poor survival on foliage (Hara *et al.*, 1993) (discussed in Chapter 2 of this thesis).

The amount of time that nematodes need to enter a leaf is an important factor which determines their efficacy after foliar application, because once the nematode enter the mines, they are protected from detrimental environment conditions and are able to seek and infect larvae (Hara *et al.*, 1993; Williams and Walters, 1994; Williams and MacDonald, 1995; Bélair *et al.*, 2005). The time nematodes required to enter a leaf in the present investigation (Figs. 3-17 and 3-18) was shorter than that observed by Williams and Walters (1994) who found that *S. feltiae* required 10 hours to successfully enter the leaf tissue and consequently cause high mortality of the late second/ early third instar larvae of the dipteran leafminer *Liriomyza huidobrensis* at temperatures of 15-25 °C and at high relative humidity. These results may be explained by the fact that the behaviour of the two pests is different. With *Liriomyza*,

_____

_____

nematodes enter the mines through the holes made by the female on leaves during oviposition. Larvae are unable to move between leaves and all larval instars occur inside the mines (Ameixa *et al.*, 2007). In contrast, in *T. absoluta,* nematodes enter the mines through the larger holes produced by larvae when they penetrate the leaves, which can be easily used by nematodes to enter the mines and thus avoid desiccation and infect the larvae more rapidly.

The findings of the effect of temperature on nematode efficacy revealed that both nematode species induced similar mortality at 25 °C and that *S. feltiae* was more virulent at lower temperatures (15 and 20 °C), while *S. carpocapsae* was more virulent at higher temperatures (30 and 35 °C) (Figs. 3-7, 3-8 and 3-9). Several other investigations have revealed that infectivity of nematodes was affected by temperature (Molyneux, 1984; 1985; Kaya, 1990; Grewal *et al.*, 1994b; Mason and Wright, 1997; Bélair *et al.*, 2003). Molyneux (1984) reported that the survival of *S. feltiae* at high temperature was poor. Bélair *et al.* (2003) found that *S. feltiae* was more effective than *S. carpocapsae* at 15 and 20 °C against second instar larvae of the imported cabbageworm *Artogeia rapae* and that at 30 and 35 °C, infectivity of *S. feltiae* strains declined and infectivity of *S. carpocapsae* remained unaffected compared to 25 °C. Similarly, Ratnasinghe and Hague (1998) reported that infectivity of *S. carpocapsae* against the diamondback moth (*Plutella xylostella*) was optimum at a temperature between 20 and 30 °C. A possible explanation for the high efficacy of *Steinernema carpocapsae* (ambush forager) at high temperature might be because it resides near the soil surface to ambush passing hosts and thus is adapted to tolerate high temperature and desiccation (Georgis and Poinar, 1983; Kaya, 1990; Campbell and Gaugler, 1993; Bélair *et al.*, 2003), whereas the reason behind the low survival of *S. feltiae* (intermediate forager) is that the food reserved is quickly depleted because of high activity level and respiration (Bélair *et al.*, 2003). Therefore, since the results of the current study revealed that *S. feltiae* is significantly more effective than *S. carpocapsae,* it seems that its use as a biological agent against this pest will be limited to countries that have a temperate climate. In countries where temperature is high, *S. feltiae* will not be effective and accordingly it is important to find and test other nematode species (indigenous or commercially available species) that are tolerant to high temperature for their potential as biological agents against *T. absoluta* in these and other countries with a similar climate.

The observations of the effect of number of applications on nematode efficacy in the current study showed that *S. feltiae* was more efficient than *S. carpocapsae* and required fewer

_____

_____

applications to induce significant mortality as well as that four applications gave significantly higher larval mortality than other frequencies of application at 45 ± 10 % RH and 75 ± 10 % RH, with some exceptions for *S. feltiae* at 75 ± 10 % RH (Figs. 3-10 – 3-13). The findings of the current work also revealed that first and second larval instars required additional applications to achieve significant mortality compared to third and fourth larval instars. These results may be explained by the fact that first and second larval instars are less susceptible to nematode infections (discussed in Chapter 2 of this thesis). Thus it is important to target the third and fourth larval instar when controlling this pest using EPNs. Williams and Walters (2000) reported that a 24 h repeat application of a dose one tenth (1000 *S. feltiae*/ml) of the original concentration of the single dose (10,000 *S. feltiae*/ml) at < 90 % RH induced higher leafminer (*Chromatomyia syngenesiae*) mortality than repeat applications at 48 h and 72 h. The authors attributed this to the differences in susceptibility of larval instars present at the time of application. Young larvae produce small amounts of attractants such as CO_2 and kairomones compared to old larvae, which makes it more difficult for nematodes to locate them inside the mines (Kaya, 1985). If the nematodes are deposited away from the entrance of the mines, it might be more difficult to them to locate these attractants. Therefore, the increase in nematode efficacy in the current study can be attributed to the increase in nematode density and coverage on leaf surfaces after each application, which enabled them to locate larvae inside the mines because they were deposited near the entrances of these mines, so that they parasitised the remaining live larvae.

When the effect of method of application on nematode efficacy was evaluated, results showed that spraying nematode suspension on both leaf surfaces gave the highest larval mortality, followed by spraying on the lower then the upper leaf surface, and that nematode efficacy reduced as RH decreased, with *S. feltiae* being more effective than *S. carpocapsae* (Figs. 3-14 – 3-16). A possible explanation for the high efficacy of two-surface treatment and lower-surface treatment compared to upper-surface treatment might be that, firstly the RH on the lower surface of leaves is higher than on the upper surface (Yarwood and Hazen, 1944; Sombardier *et al.*, 2009), and secondly the number of larvae penetrating through the lower leaf surface was higher than the number penetrating through the upper leaf surface. This was noticed when larvae were placed on leaves when preparing the experiment arena (personal observation). Consequently, nematodes were able to find and kill the larvae using the entry holes produced by larvae more effectively in two surface treatment and lower surface treatment than in upper surface treatment. In natural infestations (in field or in greenhouse), a

_____

_____

high percentage of neonate larvae will probably penetrate the lower leaf surface because females were found to prefer laying their eggs on the underside of the leaves (Ulle" 1996) cited in Torres *et al.* (2001). However, Torres *et al.* (2001) found that the females did not show a preference to lay their eggs on the upper or lower leaf surfaces. These findings suggest that it is important to spray and cover both leaf surfaces, especially the lower leaf surface, in order to attain the highest possible larval mortality. So, the sprayer nozzle should be set at the right angle and at the right height to ensure complete coverage of the leaves. Choosing the right sprayer and the right nozzle can also be important in this regard (Beck *et al.*, 2013; Beck *et al.*, 2014).

In conclusion, all factors studied in the current investigation affected nematode efficacy to a certain extent. Environmental factors are difficult to control and to manipulate, thus extra attention should be paid to these factors when applying nematodes under greenhouse or field conditions. Amongst all factors tested, RH was the most important and reduced nematode survival and efficacy significantly as it decreased. Therefore, it is important to enhance nematode survival and consequently efficacy when RH is low. This can be achieved by addition of adjuvants to nematode suspensions (Glazer, 1992; Mason *et al.*, 1998; Bélair *et al.*, 2003; Schroer *et al.*, 2005b). Consequently, in the next chapter we test the potential of some adjuvants to increase nematode survival and efficacy in harsh conditions (low RH). Temperature ($\geq$ 35 °C) also affected the efficacy of the most virulent species (*S. feltiae*). The effect of solar radiation on nematodes was not tested in recent work as its effects can be avoided together with the high temperature by applying nematodes at dusk (Gaugler and Boush, 1978; Macvean *et al.*, 1982; Mason and Wright, 1997). The impact of solar radiation can also be reduced by addition of stilbene brighteners such as Blankophor BBH (Nickle and Shapiro, 1994; Baur *et al.*, 1997).

_____

Chapter 4 Evaluation of adjuvants for their potential to increase efficacy of *S. feltiae* and *S. carpocapsae* on tomato leaves against larvae of *Tuta absoluta*

Abstract

Results from the previous chapter showed that RH was the most important factor that affects EPNs' efficacy on tomato leaves. As RH declined, EPNs' efficacy and survival decreased. The addition of adjuvants to nematode suspensions can increase leaf coverage and persistence of the nematodes on leaves. In this chapter we tested some organic and non-organic adjuvants to assess whether they improve nematode performance at 75 ± 10 % and 45 ± 10 % RH. Results showed that Barricade® II and xanthan gum were the best two adjuvants. The addition of these two adjuvants to aqueous suspensions of *S. feltiae* and *S. carpocapsae* resulted in increasing nematode efficacy and survival, reducing the loss into the soil in run-off by depositing nematodes on the leaf, assuring their even distribution in the spray tank by preventing them from settling and decreasing their LC_{50} and the LT_{50} significantly compared to the control (nematode suspended in water). *S. feltiae* was more virulent than *S. carpocapsae*. Results also showed that Barricade® II (1.5 %) reduced the time required by the two nematode species to enter a leaf, with *S. feltiae* being faster to enter the mines than *S. carpocapsae.* Barricade® II (1 % and 1.5 %) also reduced the number of applications required to reach the highest mortality of different larval instars with differences in the efficacy between nematode species and in the susceptibility of different larval instars. The addition of Barricade® II (1.5 %) to nematode suspension increased the efficacy of *S. feltiae* and *S. carpocapsae* using each method of application. Barricade® II is the candidate adjuvant to be used with *S. feltiae* for foliar application against *T. absoluta.* Xanthan gum is hardly soluble in cold water. When it is added to nematode suspension, the mixture becomes too thick and not sprayable using a hand sprayer and it is nutritious to microbes and can encourage the growth of plant pathogens.

_____

4.1 Introduction

The rapid spread of tomato leafminer (TLM) *Tuta absolut*a to new areas has resulted in intensive and extensive use of insecticides to control this invasive pest (Branco *et al.*, 2001; Lietti *et al.*, 2005; Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2007; Desneux *et al.*, 2010; Desneux *et al.*, 2011; Reyes *et al.*, 2012; Urbaneja *et al.*, 2012; Konus, 2014). In addition to the negative impact of insecticides on non-target organisms (some of which are beneficial), the interruption to existing IPM programs in tomato crops (Riquelme Virgala *et al.*, 2006; Desneux *et al.*, 2007; Arno and Gabarra, 2011; Biondi *et al.*, 2012b; Biondi *et al.*, 2013; Martinou *et al.*, 2014; Gontijo *et al.*, 2015) and the negative impact on the environment, users and consumers (Pimentel *et al.*, 1992; Peterson and Higley, 1993; Weisenburger, 1993), the intensive and extensive use of insecticides has led to the development of insecticide resistance in *T. absoluta* (Siqueira *et al.*, 2000a; Haddi *et al.*, 2012; Reyes *et al.*, 2012; Gontijo *et al.*, 2013; Konus, 2014; Roditakis *et al.*, 2015). To reduce further development of resistance against insecticides as well as to successfully control this pest, an effective biological control agent should be considered as well as using different control methods within an IPM program (Urbaneja *et al.*, 2013).

Entomopathogenic nematodes (EPNs) are an important group of biological control agents, for various economically important insect pests, that can be used alone to control *T. absoluta* or as part of an IPM program (Grewal *et al.*, 2005b). EPNs have already been tested to control above ground insect pests, but control on leaves was less effective compared to cryptic habitats (Mason and Wright, 1997; Williams and Walters, 2000; Unruh and Lacey, 2001; Bélair *et al.*, 2003; Bélair *et al.*, 2005; Cabanillas *et al.*, 2005; Tomalak *et al.*, 2005; Wright *et al.*, 2005; del Pino and Morton, 2008; Barbosa-Negrisoli *et al.*, 2010). This is due to the environment factors which affect EPNs' efficacy especially in above ground applications. These factors are desiccation (Glazer and Navon, 1990; Glazer, 1992; Baur *et al.*, 1995; Williams and MacDonald, 1995; Mason and Wright, 1997; De Waal *et al.*, 2013), ultra-violet radiation (Gaugler and Boush, 1978; Gaugler *et al.*, 1992; Nickle and Shapiro, 1994) and extremes of temperature (Molyneux, 1985; Grewal *et al.*, 1994b). Desiccation is the most critical of these factors (Begley, 1990; Glazer and Navon, 1990; Glazer, 1992; Baur *et al.*, 1995; Mason and Wright, 1997; Bélair *et al.*, 2003; Head *et al.*, 2004). Ultra-violet radiation and extreme temperature can be avoided by applying nematodes in the evening (Gaugler and Boush, 1978; Macvean *et al.*, 1982; Mason and Wright, 1997) while the detrimental effect of

_____

_____

desiccation can be reduced by addition of adjuvants to nematode suspensions (Glazer, 1992; Mason *et al.*, 1998; Bélair *et al.*, 2003; Schroer *et al.*, 2005b; Bellini and Dolinski, 2012; Hussein *et al.*, 2012; Beck *et al.*, 2014; van Niekerk and Malan, 2015). Adjuvants are defined as "*material added to a tank mix to aid or modify the action or the physical characteristics of the mixture*" (ASTM Standards E1519). Spray adjuvants can be classified as stickers, surfactants, spreaders, deposition aids, activators, wetting agents, anti-foaming agents, humectants and drift reduction agents (Hoffmann *et al.*, 2008).

Batalla-Carrera *et al.* (2010) reported that two foliar applications of three nematode species indigenous to Spain (*Steinernema feltiae* (strain Bpa), *S. carpocapsae* (strain B14) and *Heterorhabditis bacteriophora* (strain DG46)) with the addition of 0.05 % of the oil adjuvant Addit® (Koppert), induced mortality of 92 %, 88.5 % and 76.3 % respectively of larvae of *T. absoluta* on naturally infested tomato leaves in a pot experiment under controlled conditions. In contrast, results of trials undertaken by Jacobson and Martin (2011) on commercial organic tomato crops in Portugal, using the commercial nematode species *S. feltiae* (Nemasys®) without addition of adjuvants, showed that this nematode species induced mean larval mortality less than 51 % in two separate trials. The low efficacy in the first trial was explained by these authors as due to poor application in one of the plots due to some nozzles being temporarily blocked and the authors suggested that the overall mortality would rise to 56 % if the data from that plot were excluded. The results of the second trial were attributed to the unfavourable environmental conditions for nematode activity (65-74 % RH). The authors suggested that the efficacy of nematodes could be increased by addition of adjuvants to the spray mixture and by improving the spray technique. Additionally, previous results (Chapter 3 of this thesis) showed that the efficacy of nematodes in aqueous suspension against larvae of *T. absoluta* at low RH was insufficient to induce high mortality of *T. absoluta*. For example, nematode efficacy against the third larval instar at 45 ± 10 % RH was 36 % for S. *feltiae* and 15 % for *S. carpocapsae*, whereas at 75 ± 10 % RH nematode efficacy was 74 % and 37 % for S. *feltiae* and *S. carpocapsae* respectively.

The addition of adjuvants to nematode suspensions can enhance nematode survival and activity on foliage (Glazer, 1992; Glazer *et al.*, 1992; Navon *et al.*, 2002; Bélair *et al.*, 2003; Schroer *et al.*, 2005b; van Niekerk and Malan, 2015). Traditional spraying equipment used for chemical control is usually used for foliar application of EPNs, which has some disadvantages. EPNs settle quickly to the bottom in a spray tank without stirring (Schroer *et*

_____

_____

al., 2005b; Brusselman *et al.*, 2010; van Niekerk and Malan, 2015). Adding adjuvants to nematode suspensions can retard the sedimentation of the nematodes to the bottom of spray tanks (Peters and Backes, 2003; Schroer *et al.*, 2005b; Brusselman *et al.*, 2010; Beck *et al.*, 2013; van Niekerk and Malan, 2015). Additionally, nematodes which are in spray droplets can run off from the leaf surface. Adding adjuvants to nematode suspensions can increase the adhesion of the suspension by reducing the surface tension of the droplets and consequently reduce the loss of nematodes into the soil by runoff (Bélair *et al.*, 2003; Schroer *et al.*, 2005b; Beck *et al.*, 2013). Adjuvants have been already reported to improve above ground control of various insect pests (Glazer *et al.*, 1992; Baur *et al.*, 1997; Mason *et al.*, 1998; Bélair *et al.*, 2003; Head *et al.*, 2004; Schroer and Ehlers, 2005; Schroer *et al.*, 2005b; Llacer *et al.*, 2009; Lacey *et al.*, 2010; Shapiro-Ilan *et al.*, 2010; Bellini and Dolinski, 2012; Shapiro-Ilan *et al.*, 2015; van Niekerk and Malan, 2015). However, adjuvants can also be detrimental to nematodes (Shapiro *et al.*, 1985; Arthurs *et al.*, 2004) and can restrict nematode movement (Mason *et al.*, 1998; Beck *et al.*, 2013), thus side effects of adjuvants on nematodes should always be tested before they are used. Additionally, due to a number of factors that interact with each other including specific insect pest, host plant and application method, no single adjuvant is appropriate for all conditions. Therefore, it is important to consider these factors when testing possible adjuvants (Mason *et al.*, 1998).

The aim of this work was to screen new adjuvants (organic and non-organic) not tested previously and older ones already shown to be effective for their potential to increase the efficacy of EPNs on foliage against tomato leafminer *T. absoluta* especially under unfavourable conditions (e.g. low RH). The objective were to test (1) the side-effects of these adjuvants on infective juveniles (IJs), larvae of TLM *Tuta absoluta* and tomato leaves, (2) the impact of adjuvants on efficacy of *S. feltiae* and *S. carpocapsae* against larvae of TLM *Tuta absoluta* on tomato leaves at different RH, (3) the impact of adjuvants on *S. feltiae* and *S. carpocapsae* deposition on tomato leaves, (4) the impact of adjuvants on *S. feltiae* sedimentation, (5) the impact of adjuvants on survival of *S. feltiae* and *S. carpocapsae* on tomato leaves at varied RH, (6) the impact of adjuvants on lethal concentration (LC) and on lethal time (LT) of *S. feltiae* and *S. carpocapsae* against third larval instar on tomato leaves, (7) the impact of adjuvants at high and low concentrations on number of applications of *S. feltiae* and *S. carpocapsae* against different larval instars on tomato leaves, (8) the impact of adjuvants on method of application of *S. feltiae* and *S. carpocapsae* and (9) the impact of adjuvants on the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf.

_____

4.2 Materials and methods

4.2.1 Adjuvants used in experiments

Adjuvants and suppliers are listed in Table 4-1. Eight of these adjuvants are organic adjuvants (Codacide, Natural oil, Green Cypress Ag Aid, Surfact 50, Stick-it Organic, Green Cypress EcoSpreader, Nu-Film P and xanthan gum) and one is not (Barricade® II). Xanthan gum is a food thickening agent (thickener) while the rest of the organic adjuvants are oils and non-ionic surfactants. Barricade® II is a liquid fire gel concentrate (acting as a thickener) that has been approved for use by the U.S. Forest Service. It is free of toxic nonylphenol ethoxylates (NPEs), ammonium phosphates and petroleum distillate oils, and it has received the Champion award from the US Environmental Protection Agency (EPA) (http://firegel.com/EPA_Champion.aspx).

4.2.2 Side-effects of adjuvants on infective juveniles (IJs), larvae of Tuta absoluta *and tomato leaves*

The compatibility of the adjuvants with *S. feltiae* (Nemasys), *S. carpocapsae* (Nemasys C), the third larval instar of *T. absoluta,* and tomato leaves was investigated. The IOBC guidelines compiled by the working group 'Pesticides and Beneficial Organisms' (Peters, 2003) were followed to investigate the effect of adjuvants on *S. feltiae* and *S. carpocapsae.* Different concentrations of adjuvants were prepared (Table 4-1) in 25 ml tap water containing 5000 infective juveniles (IJs) of *S. feltiae* or *S. carpocapsae* in 8.5 cm Petri dishes. IJ controls were kept in tap water. The Petri dishes were covered with their lids and placed in an air tight container (12 litre capacity) containing 200 ml water to provide > 95 % relative humidity (RH). Containers were then incubated in an incubator at 25 °C in darkness. After 24 h nematode survival was evaluated by taking samples of 100 nematodes at random. Infective juveniles not moving after mechanical stimulation using a fine needle were considered dead. The impact of the adjuvants on nematode infectivity was tested by incubating ten third larval instar of *T. absoluta* with ten IJs of *S. feltiae* or *S. carpocapsae* per larva. A culture tube (30 ml crystal clear polystyrene with fitted polyethylene cap, Fisher Scientific) lined with 8.5 cm Whatman No 1 filter paper was wetted with 0.5 ml tap water. Afterwards, 0.5 ml of each adjuvant-nematode suspension was added onto the filter paper. Controls were without nematodes. Tubes were then incubated at 25 ± 0.5 °C in darkness and were inspected for larval mortality after 48 h. There were 5 replicate tubes for each adjuvant concentration (n = 5)

_____

with the corresponding control treatments (n = 5). In addition to testing the side effect of each adjuvant alone, a combination of xanthan gum with sodium hydrogen carbonate and oxalic acid (each at 0.3 % and 1 % (w/w)) was tested too in order to increase the solubility of the xanthan gum in the nematode-water suspension.

In order to investigate possible side effects of adjuvants on larvae of *T. absoluta*, tomato leaves were prepared as described in section 2.2.9 and infested with 10 third larval instar larvae. Leaves were then sprayed with the highest concentration of each adjuvant solution (Table 4-1) and without EPNs and placed in Perspex cages (25 cm L × 25 cm W × 25 cm H). The Perspex cages were then incubated in a chamber at 25 ± 5 °C with an 18:6 h light:dark (L:D) regime using high pressure sodium lamps and 45-88 % RH. The control treatment received water only. Larval mortality was recorded after 48 h. There were five replicate leaves for each adjuvant (n = 5) with the corresponding control treatments (n = 5).

The compatibility of the different adjuvants with tomato plants was tested according to the methods of Baur *et al.* (1997). Five to six week old tomato plants were sprayed with different adjuvants. Surfactants were used at concentrations of 0.3 %, 3 % and 10 % (v/v), Barricade® II at concentrations of 0.3 %, 1 % and 1.5 % (v/v) and xanthan gum at concentrations of 0.15 %, 0.3 % and 1 % (w/v). Plants were then incubated in the same chamber mentioned above and examined 3 days after application and any detrimental effects on the plant were recorded.

_____

_____

Table 4-1 Organic and non-organic adjuvants tested for effects on nematode survival on tomato leaves and infectivity against third larval instar of *T. absoluta*. Suppliers, chemical, common use and tested concentrations are presented

Name	Supplier	Chemical	Common use	Concentration (%)
Codacide Oil	Microcide Ltd, UK	Oilseed Rape (Colza) Oil	Vegetable oil adjuvant	0.3, 1, 3 & 10
Natural Oil	Stoller Enterprises, Inc., USA	93 % Vegetable oil and 7 % inert ingredient	Non-ionic surfactant	0.3, 1, 3 & 10
Agriculture Aid	Brandt Monterey, Inc. USA	100 % Yucca schidigera extract	Spreader-activator	0.3, 1, 3 & 10
Surfact 50	Brandt Monterey, Inc. USA	35 % Yucca schidigera and citric acid and 65 % Constituents ineffective as adjuvants	Non-ionic surfactant and Acidifier	0.3, 1, 3 & 10
Stick-it Organic	Brandt Monterey, Inc. USA	81.8 % Pinene Polymers and 18.2 % Constituents Ineffective as Spray Adjuvants	Extender-sticker	0.3, 1, 3 & 10
Green Cypress EcoSpreader	Brandt Monterey, Inc. USA	Siloxane polyalkyleneoxide copolymer and polyalkyleneoxide	Organosilicone surfactant	0.3, 3 & 10
Nu-Film P	Miller Chemical and Fertilizer Corporation, USA	96 % Poly-1-p-Menthene and 4 % inert ingredient	Spreader-sticker	0.3, 3 & 10
Barricade® II	Barricade International, Inc. Hobe Sound FL, USA	Superabsorbent Polymer, Vegetable oil, emulsifier and Thickener (NPE-Free gel)	Fire retardant gel (Humectant)	0.3, 1 & 1.5
Xanthan gum	Bob's Red Mill	Polysaccharide secreted by the bacterium *Xanthmonas campestris*	Food thickening agent (Humectant)	0.15, 0.3 & 1

_____

4.2.3 Impact of adjuvants on efficacy of S. feltiae *and* S. carpocapsae *against larvae of* Tuta absoluta *on tomato leaves at different RH*

The adjuvants Codacide oil, Natural oil, Agriculture Aid, Surfact, Stick it Organic, Barricade II and xanthan gum were chosen to test their potential to improve nematode efficacy.

A. The impact of the adjuvants on efficacy of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) was investigated at 75 ± 10 % and 45 ± 10 % relative humidity (RH) against the third larval instar. The experiment arena was prepared as described in section 2.2.9. Codacide oil, Natural oil, Agriculture Aid, Surfact and Stick-it Organic were used at concentrations of 0.3 %, 1 % and 3 % (v/v) at both RH. Barricade® II was used at concentrations of 0.3 %, 1 %, and 1.5 % (v/v). Xanthan gum was used at concentrations of 0.15 %, 0.3 % and 1 %. Nematodes were used at a rate of 60 ± 6 IJs cm^{-2} for all experiments.

B. Additionally, 1.5 % Barricade II and 1 % xanthan gum were used against other larval instars (first, second and fourth). Ten to twenty-five larvae (depending on larval stage under test) were placed on each leaf. Leaves were then sprayed with each formulation-nematode suspension until runoff if surfactants were used or until they formed a layer if Barricade® II or xanthan gum was used. The spray was distributed over the leaves with the help of a camel hair brush when 0.3 % and 1 % xanthan gum was used because it was not distributed evenly using the sprayer due to its high viscosity. Control treatments received nematodes suspended in water alone. There were four replicate containers for each adjuvant concentration (n = 4 each) with the corresponding control treatments (n = 4).

4.2.4 Impact of adjuvants on S. feltiae *and* S. carpocapsae *deposition on tomato leaves*

The influence of adjuvants on the deposition of *S. feltiae* and *S. carpocapsae* spray on tomato leaves was assessed for the two best adjuvants (Barricade® II and xanthan gum) that had no side effects on nematodes, larvae of *T. absoluta* and tomato leaves and for their performance in increasing nematode efficacy. Methods for spraying, determining leaf surface area and nematode numbers per square centimetre were as described in section 2.2.9. Leaves were sprayed with nematodes suspended either in water (control) or water and Barricade® II (0.3 %, 1 % and 1.5 %) or xanthan gum (0.15 %, 0.3 % and 1 %) (treatments). Nematodes were used at a concentration of 2540 ± 254 IJs ml^{-1}. There were five replicate leaves for each adjuvant concentration (n = 5) with the corresponding control treatment (n = 5).

_____

4.2.5 Impact of adjuvants on S. feltiae *sedimentation*

The adjuvants that improved nematode efficacy were evaluated for their ability to retard sedimentation of *S. feltiae* in 500 ml spray mixture. Sedimentation was recorded using the method described by Peters and Backes (2003). Adjuvants were added to nematodes suspended in water at concentrations of 0.3 %, 1 % and 1.5 % (v/v) for Barricade® II and 0.15 %, 0.3 % and 1 % (w/v) for xanthan gum. Additionally, the surfactant Stick-it Organic was used, in order to compare it with the two thickeners, at concentrations of 0.3 %, 1 % and 3 % (v/v). Nematodes were used at a concentration of 1000 ± 100 IJs ml^{-1}. The suspension was stirred in a flask until nematodes were equally distributed in the spray mixture and then poured into a $10 \times 10 \times 8$ cm (l-w-h) plastic cup. Sedimentation time was estimated by sampling 100 µl of the suspension from 2 cm depth at 0, 5, 10, 15, 30 and 60 min after stirring of the suspension had been terminated. Nematode concentration was estimated by counting the number of nematodes in each sample. Five samples of 100 µl were taken as replicates for each adjuvant concentration (n = 5) with the corresponding control treatment (n = 5).

4.2.6 Impact of adjuvants on survival of S. feltiae *and* S. carpocapsae *on tomato leaves*

The ability of the best two adjuvants (Barricade® II and xanthan gum) to prolong activity of IJs of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) on tomato leaves was investigated at 75 ± 10 % and 45 ± 10 % RH. The experiment arena was prepared as described in section 2.2.9 except that leaves were not infested with larvae. Nematodes were used at a rate of 60 ± 6 IJs cm^{-2}. Tomato leaves were treated with nematodes suspended either in water (control) or in water and Barricade® II (1 % and 1.5 %) or in water and xanthan gum (0.3 % and 1 %). After incubation for 0, 2, 4, 7, 24 or 48 h, one leaflet from each replicate was sampled. IJ persistence was measured by washing each leaflet into a separate counting chamber and checking IJ survival by counting the number of living IJs after 24 h. Nematodes were examined under a dissecting microscope and IJs not moving when mechanically stimulated using a fine needle were recorded as dead. Nematode survival was calculated as the number of living nematodes as a proportion of the total number of nematodes washed from leaves. Differences in the total number of nematodes washed from leaves between adjuvant concentrations were used in later analysis. There were four replicate containers for each adjuvant concentration (n = 4) with the corresponding control treatments (n = 4).

_____

4.2.7 Impact of adjuvants on lethal concentration (LC) of S. feltiae *and* S. carpocapsae *against third larval instar of* Tuta absoluta *on tomato leaves*

Effects of Barricade® II and xanthan gum on LC_{50} values of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) were investigated against third larval instar at 75 ± 10 % and 45 ± 10 % RH. The experiment arena and procedures were as described in section 2.2.9 with some exceptions. Ten to fifteen larvae were placed on each leaflet. The leaves were then sprayed with nematodes suspended in water (control) or water and adjuvants (1.5 % Barricade® II (v/v) or 1 % xanthan gum (w/v)). Nematodes were used at five different rates: 0, 5 ± 0.5, 15 ± 1.5, 30 ± 3 and 60 ± 6 IJs cm^{-2}. Larval mortality was checked after 48 h. There were four replicate containers for each nematode concentration (n = 4). Furthermore, the effect of 1 % Barricade® II (v/v) and 0.3 % xanthan gum (w/v) on LC_{50} values of *S. feltiae* against third larval instar was investigated at both RH.

4.2.8 Impact of adjuvants on lethal time (LT) of S. feltiae *and* S. carpocapsae *against third larval instar of* Tuta absoluta *on tomato leaves*

Effects of Barricade® II and xanthan gum on LT_{50} of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) were investigated against the third larval instar at 75 ± 10 % and 45 ± 10 % RH. The experiment arena and procedures were as described in section 2.2.9 with some exceptions. Ten to fifteen larvae were placed on each leaflet. The leaves were then sprayed with nematodes suspended either in water (control) or water and adjuvants (1 % and 1.5 % Barricade® II (v/v) or 0.3 % and 1 % xanthan gum (w/v)). Nematodes were used at a rate of 60 ± 6 IJs cm^{-2}. Larval mortality was checked every 8 h up to 48 h. Independent samples were used at each time interval. There were four replicate containers for each time interval (n = 4) with the corresponding control treatment (n = 4).

4.2.9 Impact of Barricade® II on number of applications of S. feltiae *and* S. carpocapsae *against different larval instars of* Tuta absoluta *on tomato leaves*

Effects of 1.5 % Barricade® II (v/v) on the number of applications of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) were investigated at 75 ± 10 % and 45 ± 10 % RH against the first, second, third and fourth larval instars. The experiment arena and procedures were as described in section 2.2.9 with some exceptions. Ten to twenty-five larvae were placed on each leaf. The leaves were then sprayed with nematodes suspended either in water (control) or

water and 1.5 % Barricade II (v/v). Nematodes were used at a rate of 60 ± 6 IJs cm^{-2}. Four different frequencies of application were used: spraying leaves once (at the beginning (0 hours)), twice (0 h and after 24 h), three times (0 h, after 24 h and after 48 h) or four times (0 h, after 24 h, after 48 h and after 72 h). Control treatments (0 %) received nematodes suspended in tap water. There were four replicate containers for each treatment (n = 4) with the corresponding control treatments (n = 4). Additionally, in order to reduce adjuvant concentration, Barricade II was tested at lower concentrations of 0.5 % and 1 % (v/v) using *S. feltiae* at a rate of 60 ± 6 IJs cm^{-2} against the third larval instar at the two ranges of RH. Furthermore, in order to reduce nematode concentration, *S. feltiae* was tested at a lower rate of 30 ± 3 IJs cm^{-2} with 0.5 %, 1 % and 1.5 % Barricade II (v/v) against the third larval instar at the two ranges of RH.

4.2.10 Impact of adjuvants Barricade® II on method of application of S. feltiae *and* S. carpocapsae

Effects of Barricade$^®$ II on method of application of *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) were investigated at 75 ± 10 % and 45 ± 10 % RH against the 3rd larval instar. The experiment arena and procedures were as described in section 2.2.9 except that 1.5 % Barricade II (v/v) was added to nematode suspension. Nematodes were used at a rate of 60 ± 6 IJs cm^{-2}. Control treatments received nematodes suspended in water. Ten to fifteen larvae were placed on each leaf. Three different application methods (spraying modes) were used: (1) spraying upper leaf surface, (2) spraying lower leaf surface, or (3) spraying both leaf surfaces. There were four replicate containers for each spray mode (n = 4) with the corresponding control treatments (n = 4).

4.2.11 Impact of adjuvant Barricade® II on the time required by S. feltiae *and* S. carpocapsae *to enter a leaf*

Effects of Barricade$^®$ II on the time required by *S. feltiae* (Nemasys) and *S. carpocapsae* (Nemasys C) to enter a leaf were investigated at 75 ± 10 % and 45 ± 10 % RH against 3rd larval instar. The experiment arena and procedures were as described in section 2.2.9 except that 1.5 % Barricade$^®$ II (v/v) was added to nematode suspension. Nematodes were used at a rate of 60 ± 6 IJs cm^{-2}. Control treatments received nematodes suspended in water. Ten to fifteen larvae were placed on each leaf. At intervals from 1 to 24 h, four leaves were removed from the containers. In order to fix the IJs remaining on the leaf surface, the leaves were

_____

immediately dried by blowing cool air across them until no surface moisture was apparent. Each dried leaf was wrapped with moist cotton wool to keep it fresh and placed in a vented plastic Petri dish (9 cm $\times$ 1.6 cm) and larval mortality was checked 48 h later. There were four replicate containers for each time interval (n = 4) with the corresponding control treatments (n = 4).

4.3 Statistical analysis

All data was analysed using Minitab® 16.1.0 (© 2010 Minitab Inc.). If the data was not normally distributed, it was transformed (angular, square root or log base 10) before analysis of variance was performed (General Linear Model (GLM)). If after transformation data were still not normally distributed, non-parametric Tests were performed (Scheirer-Ray-Hare Test and Mann-Whitney-U Test). Statistical tests and type of transformation used in each experiment are shown in Table 4-2.

_____

Table 4-2 Statistical tests and types of transformation used in the experiments.

No.	Experiment type	Transformation used	Statistic test used
1	The impact of all adjuvants on efficacy of *S. feltiae* against 3rd larval instar at 75 ± 10 % RH.	Not applicable.	Non-parametric Scheirer-Ray-Hare Test was used and followed by the non-parametric Mann-Whitney U Test to test significant differences between treatments.
2	The impact of all adjuvants on efficacy of *S. feltiae* against 3rd larval instar at 45 ± 10 % RH.	No transformation.	A nested ANOVA was used to test significant differences between treatments. Afterwards, Tukey's multiple range test was performed to separate means.
3	The impact of all adjuvants on efficacy of *S. carpocapsae* against 3rd larval instar at 75 ± 10 % RH.	″	″
4	The impact of all adjuvants on efficacy of *S. carpocapsae* at 45 ± 10 % RH.	Not applicable.	Non-parametric Scheirer-Ray-Hare Test was used and followed by the non-parametric Mann-Whitney U Test to test significant differences between treatments.
5	To compare between the efficacy of *S. feltiae* and *S. carpocapsae* against 3rd larval instar using Barricade® II (0.3, 1 and 1.5 %) or xanthan gum (0.15, 0.3 and 1 %) at 75 ± 10 % RH.	Larval percentage mortality was angular transformation.	GLM was used to test significant differences between treatments. Afterwards, Tukey's multiple range test was performed to separate means.
6	To compare between the efficacy of *S. feltiae* and *S. carpocapsae* against third larval instar using Barricade® II (0.3, 1 and 1.5 %) or xanthan gum (0.15, 0.3 and 1 %) at 45 ± 10 % RH.	No transformation.	″

Continued

No.	Experiment type	Transformation used	Statistic test used
7	The impact of the best adjuvants at the best concentrations (1.5 % Barricade® II and 1 % xanthan gum) on efficacy of *S. feltiae* and *S. carpocapsae* against other larval instars (1st, 2nd, 3rd and 4th) at 75 ± 10 % RH.	Percentage mortality of 2nd and 4th larval instars was subjected to angular transformation.	"
8	The impact of the best adjuvants at the best concentrations on the efficacy of *S. feltiae* and *S. carpocapsae* against other larval instars (1st, 2nd, 3rd and 4th) at 45 ± 10 % RH.	No transformation.	"
9	The impact of adjuvants on deposition of *S. feltiae* or *S. carpocapsae* on tomato leaves (data was expressed as the number of IJs per 10 cm^2 washed from the leaves).	"	A nested ANOVA was used to test significant differences between treatments. Concentrations were nested within adjuvants. Afterwards, Tukey's multiple range test was performed to separate means.
10	The impact of adjuvants on sedimentation of *S. feltiae* (data was expressed as percentage of the total number of IJs sampled at 0 minute after stirring of the suspension had been terminated).	"	GLM was used to test significant differences between treatments. Afterwards, Tukey's multiple range test was performed to separate means.
11	The impact of adjuvants on survival of IJs of *S. feltiae* and *S. carpocapsae* on tomato leaves at 75 ± 10 % RH.	"	GLM was used to test significant differences between treatments, with adjuvant and adjuvant concentration as a single factor. Afterwards, Tukey's multiple range test was performed to separate means.

Continued

No.	Experiment type	Transformation used	Statistic test used
12	The impact of adjuvants on survival of IJs of *S. feltiae* and *S. carpocapsae* on tomato leaves at 45 ± 10 % RH.	Percentage survival of IJs after 4, 7 and 48 h was subjected to Log base 10 transformation.	″
13	To compare between values of LC_{50} of *S. feltiae* at 75 ± 10 % and 45 ± 10 % RH.	No transformation.	A nested ANOVA was used to test significant differences between treatments. Concentrations were nested within adjuvants. Afterwards, Tukey's multiple range test was performed to separate means
14	To compare between values of LC_{50} of *S. carpocapsae* at 75 ± 10 % and 45 ± 10 % RH.	″	One –way ANOVA was used to test significant differences between treatments. Afterwards, Tukey's multiple range test was performed to separate means.
15	To compare between LT_{50} of *S. feltiae* and *S. carpocapsae* at 75 ± 10 % RH.	LT_{50} of *S. feltiae* and *S. carpocapsae* were subjected to square root transformation.	A nested ANOVA was used to test significant differences between treatments. Concentrations were nested within adjuvants. Afterwards, Tukey's multiple range test was performed to separate means.
16	To compare between LT_{50} of *S. feltiae* and *S. carpocapsae* at 45 ± 10 % RH.	LT_{50} of *S. feltiae* and *S. carpocapsae* were subjected to log base 10 transformation.	″
17	The impact of 1.5 % Barricade® II on number of applications of *S. feltiae* and *S. carpocapsae* against different larval instars at 75 ± 10 % RH.	Percentage mortality of 1st, 2nd, 3rd and 4th larval instar using *S. carpocapsae* was subjected to angular transformation.	GLM was used to test significant differences between treatments. Afterwards, Tukey's multiple range test was performed to separate means

_____

Continued

No.	Experiment type	Transformation used	Statistic test used
18	The impact of 1.5 % Barricade® II on number of applications of *S. feltiae* and *S. carpocapsae* against different larval instars at 45 ± 10 % RH.	Percentage mortality of 3rd and 4th larval instar using *S. feltiae* was subjected to angular transformation.	″
19	The impact of lower concentration (0.5 and 1 %) of Barricade® II on number of applications of *S. feltiae* against 3rd larval instar at 75 ± 10 % RH.	No transformation.	″
20	The impact of lower concentration (0.5 and 1 %) of Barricade® II on number of applications of *S. feltiae* against 3rd larval instar at 45 ± 10 % RH.	Larval percentage mortality was subjected to angular transformation.	″
21	The impact of lower concentrations (0.5 and 1 %) of Barricade® II on number of applications of *S. feltiae* at lower concentration (30 ± 3 IJs cm^{-2}) against 3rd larval instar at 75 ± 10 % RH.	No transformation.	″
22	The impact of lower concentrations (0.5 and 1 %) of Barricade® II on number of applications of *S. feltiae* at lower concentration (30 ± 3 IJs cm^{-2}) against 3rd larval instar at 45 ± 10 % RH.	″	″
23	The impact of adjuvants on method of application of *S. feltiae* and *S. carpocapsae* against 3rd larval instar at 75 ± 10 % RH.	″	″
24	The impact of adjuvants on method of application of *S. feltiae* and *S. carpocapsae* against 3rd larval instar at 45 ± 10 % RH.	″	″

_____

Continued

No.	Experiment type	Transformation used	Statistic test used
25	The impact of adjuvants on the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf at 75 ± 10 % RH.	"	"
26	The impact of adjuvants on the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf at 45 ± 10 % RH.	"	"

_____

4.4 Results

4.4.1 Side-effects of adjuvants on infective juveniles (IJs), larvae of **Tuta absoluta** *and tomato leaves*

With three exceptions, tested adjuvants had no measurable effect on survival and infectivity of IJs, larvae of *T. absoluta* and tomato leaves. Green cypress EcoSpreader and Nu-Film P had a detrimental effect on tomato leaves. Using 0.3 % of EcoSpreader caused injuries on the leaf cuticle while effects extended to complete leaf wilt when concentrations of 3 % and 10 % were applied (Fig. 4-1 a and b). Nu-Film P had less detrimental effect at 0.3 % concentration than Green cypress EcoSpreader but 3 % and 10 % concentrations of this adjuvant caused injuries on the leaf cuticle surrounded by yellow areas (Fig. 4-2 a and b). The combination of xanthan gum with sodium hydrogen carbonate and oxalic acid had a detrimental effect on survival and infectivity of *S. feltiae* and *S. carpocapsae*. Survival of *S. feltiae* decreased from 95 % to 27 % and from 96 % to 10 % when 0.3 % and 1 % sodium hydrogen carbonate and oxalic acid were added to xanthan gum respectively. This was followed by reduction in the infectivity from 83 % to 40 % and from 87 % to 17 % respectively. On the other hand, survival of *S. carpocapsae* was decreased from 96 % to 39 % by addition of 1 % sodium hydrogen carbonate and oxalic acid to xanthan gum followed by reduction in the infectivity from 87 % to 53 %.

| (a) | (b) |

Figure 4-1 Detrimental effect caused by EcoSpreader 72 h after application. (a): 0.3 % applied to leaf of tomato; (b): Effect on tomato plants at 0, 0.3, 3 and 10 % (from left to right)

_____

<p style="text-align:center">(a)   (b)</p>

Figure 4-2 Detrimental effect caused by Nu-Film P 72 h after application on tomato leaves. (a): 3 %; (b): 10 %

4.4.2 Impact of adjuvants on efficacy of S. feltiae and S. carpocapsae against larvae of Tuta absoluta on tomato leaves at different RH

A. Effects on third instar larvae

Results revealed that, with the exception of Barricade$^®$ II and xanthan gum, none of the surfactants or oils increased EPNs' efficacy against the third larval instar at 75 ± 10 % and 45 ± 10 % RH compared to the control treated with nematodes suspended in water. The efficacy of *S. feltiae* was superior to that of *S. carpocapsae* and caused the highest larval mortality at all tested RH.

There were significant differences in the efficacy of *S. feltiae* at 75 ± 10 % RH with different adjuvants (Scheirer-Ray-Hare Test; $\chi_6^2 = 34.07$; $P < 0.05$) at different concentrations (Scheirer-Ray-Hare Test; $\chi_3^2 = 8.11$; $P < 0.05$). Larval mortality was increased significantly compared to the control by 1.25-fold and 1.36-fold by addition of 1 % and 1.5 % Barricade$^®$ II respectively and by 1.36-fold by addition of 1 % xanthan gum ($P < 0.05$). Adding 1 % xanthan gum gave significantly higher larval mortality than 1 % Barricade$^®$ II ($P < 0.05$), whereas there were no significant differences either between 0.3 % Barricade$^®$ II and 0.3 % xanthan gum or between 1.5 % Barricade$^®$ II and 1 % xanthan gum ($P > 0.05$) (Fig. 4-3). At 45 ± 10 % RH there were significant differences in efficacy of *S. feltiae* with different adjuvants (nested ANOVA: $F_{6, 84} = 97.75$; $P < 0.05$) at different concentrations (nested ANOVA: $F_{21, 84} = 35.52$; $P < 0.05$). Larval mortality was increased significantly compared to the control by 1.85-fold and 2.25-fold by addition of 1 % and 1.5 % Barricade$^®$ II respectively and by 2.22-fold by addition of 1 % xanthan gum ($P < 0.05$). Adding 1 % xanthan gum gave

_____

significantly higher larval mortality than 1 % Barricade® II ($P < 0.05$), whereas there were no significant differences either between 0.3 % Barricade® II and 0.3 % xanthan gum or between 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Fig. 4-4).

Figure 4-3 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. feltiae* (60 ± 6 IJs cm^{-2}) in (1): water (control), (2): water and 0.15 % xanthan gum, 0.3 % Surfactants and Oils or 0.3 % Barricade® II; (3): water and 0.3 % xanthan gum, 1 % Surfactants and Oils or 1 % Barricade® II; (4): water and 1 % xanthan gum, 3 % Surfactants and Oils or 1.5 % Barricade® II). Bars (mean $\pm$ SE) with the same letter do not differ significantly according to the Mann Whitney test at P < 0.05 (n = 4).

Figure 4-4 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. feltiae* (60 ± 6 IJs cm^{-2}) in (1): water (control), (2): water and 0.15 % xanthan gum, 0.3 % Surfactants and Oils or 0.3 % Barricade® II; (3): water and 0.3 % xanthan gum, 1 % Surfactants and Oils or 1 % Barricade® II; (4): water and 1 % xanthan gum, 3 % Surfactants and Oils or 1.5 % Barricade® II). Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

There were also significant differences in the efficacy of *S. carpocapsae* at 75 ± 10 % RH with different adjuvants (nested ANOVA: $F_{6, 84} = 56.07$; $P < 0.05$) at different concentrations (nested ANOVA: $F_{21, 84} = 19.54$; $P < 0.05$). Larval mortality was increased significantly compared to the control by 1.66-fold and 2.04-fold by addition of 1 % and 1.5 % Barricade® II respectively and by 1.51-fold and 2.07-fold by addition of 0.3 % and 1 % xanthan gum respectively ($P < 0.05$). Adding 1 % xanthan gum gave significantly higher larval mortality than 1 % Barricade® II ($P < 0.05$), whereas there were no significant differences either between 0.3 % Barricade® II and 0.3 % xanthan gum or between 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Fig. 4-5). At 45 ± 10 % RH there were significant differences in efficacy of *S. carpocapsae* with different adjuvants (Scheirer-Ray-Hare Test; $\chi^2_6 = 33.78$; $P < 0.05$) at different concentrations (Scheirer-Ray-Hare Test; $\chi^2_3 = 12.19$; $P < 0.05$). Larval mortality was increased significantly compared to the control by 1.81-fold, 2.83-fold and 3.65-fold by addition of 0.3 %, 1 % and 1.5 % Barricade® II respectively and by 2.21-fold and 3.68-fold by addition of 0.3 % and 1 % xanthan gum respectively ($P < 0.05$). Adding 1 % xanthan gum gave significantly higher larval mortality than 1 % Barricade II ($P < 0.05$) and

_____

0.3 % xanthan gum gave significantly higher larval mortality than 0.3 % Barricade II ($P <$ 0.05), whereas there were no significant differences between 1.5 % Barricade II and 1 % xanthan gum ($P > 0.05$) (Fig. 4-6).
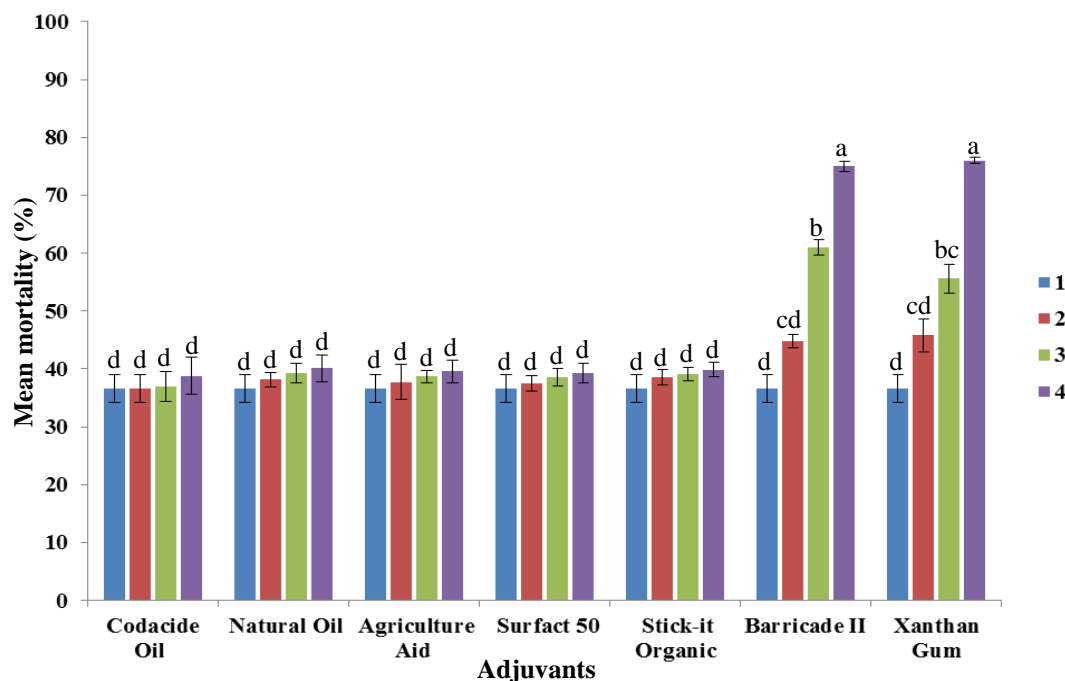
Figure 4-5 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) in (1): water (control), (2): water and 0.15 % xanthan gum, 0.3 % Surfactants and Oils or 0.3 % Barricade® II; (3): water and 0.3 % xanthan gum, 1 % Surfactants and Oils or 1 % Barricade® II; (4): water and 1 % xanthan gum, 3 % Surfactants and Oils or 1.5 % Barricade® II). Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).
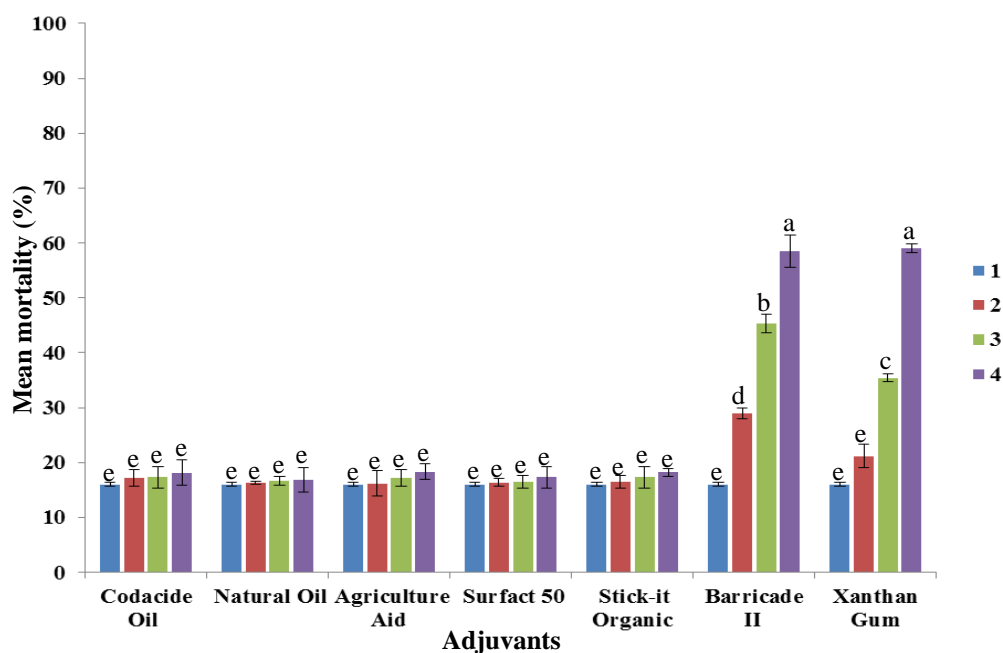
Figure 4-6 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) in (1): water (control), (2): water and 0.15 % xanthan gum, 0.3 % Surfactants and Oils or 0.3 % Barricade® II; (3): water and 0.3 % xanthan gum, 1 % Surfactants and Oils or 1 % Barricade® II; (4): water and 1 % xanthan gum, 3 % Surfactants and Oils or 1.5 % Barricade® II). Bars (mean $\pm$ SE) with the same letter do not differ significantly according to the Mann Whitney test at $P < 0.05$ (n = 4).

When the efficacy of the two nematode species against the third larval instar using either Barricade® II (0.3 %, 1 % and 1.5 %) or xanthan gum (0.15 %, 0.3 % and 1 %) at 75 ± 10 % RH was compared in the same analysis (but analysing the effects of the two adjuvants separately), results revealed that efficacy of the two nematode species increased with the increase in adjuvant concentration (Fig. 4-7 a and b). There were significant differences in efficacy between the two nematode species using Barricade II (GLM: $F_{1, 24} = 132.57$; $P < 0.05$) at different concentrations (GLM: $F_{3, 24} = 29.67$; $P < 0.05$) and using xanthan gum (GLM: $F_{1, 24} = 116.29$; $P < 0.05$) at different concentration (GLM: $F_{3, 24} = 25.75$; $P < 0.05$).

Efficacy of the two nematode species increased with the increase in adjuvant concentration. Efficacy of *S. feltiae* was significantly higher than efficacy of *S. carpocapsae* using the two adjuvants at all concentrations ($P < 0.05$). Adding 1 % or 1.5 % Barricade® II or 1 % xanthan gum to *S. feltiae* and 1.5 % Barricade® II or 1 % xanthan gum to *S. carpocapsae* increased their efficacy significantly compared to the control ($P < 0.05$) while the remaining adjuvant concentrations did not ($P > 0.05$). The highest larval mortality induced by *S. feltiae* was 100 % using 1.5 % Barricade® II or 1 % xanthan gum, while *S. carpocapsae* induced 75 % and 76

_____

% larval mortality using the same adjuvant at the same concentration respectively (Fig. 4-7 a and b).
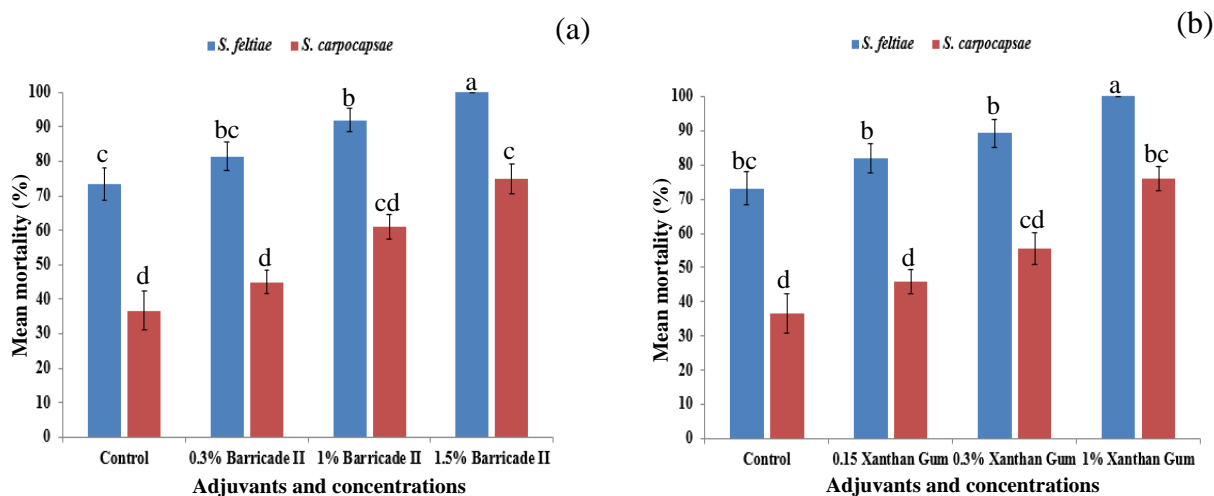
Figure 4-7 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water (control) or (a): water and Barricade® II (0.3 %, 1 % and 1.5 %); (b): water and xanthan gum (0.15 %, 0.3 % and 1 %). Bars (mean $\pm$ SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 4-3 and 4-5.

Again, when the efficacy of the two nematode species against the third larval instar using either Barricade® II (0.3 %, 1 % and 1.5 %) or xanthan gum (0.15 %, 0.3 % and 1 %) at 45 ± 10 % RH was compared in the same analysis, results revealed that efficacy of the two nematode species increased with the increase in adjuvant concentration (Fig. 4-8 a and b). There were significant differences in efficacy between the two nematode species using Barricade® II (GLM: $F_{1, 24} = 62.57$; $P < 0.05$) at different concentrations (GLM: $F_{3, 24} = 52.31$; $P < 0.05$) and using xanthan gum (GLM: $F_{1, 24} = 52.42$; $P < 0.05$) at different concentrations (GLM: $F_{3, 24} = 43.75$; $P < 0.05$). Efficacy of *S. feltiae* was significantly higher than efficacy of *S. carpocapsae* using the two adjuvants at all concentrations ($P < 0.05$). Adding 1 % or 1.5 % Barricade® II or 1 % xanthan gum to *S. feltiae* and *S. carpocapsae* increased their efficacy significantly compared to control ($P < 0.05$) while the other concentrations of adjuvant did not ($P > 0.05$). The highest larval mortalities recorded with *S. feltiae* were 84.5 % and 83.5 % using 1.5 % Barricade® II and 1 % xanthan gum respectively, whereas *S. carpocapsae* caused 58.5 % and 59.5 % larval mortality using the same adjuvant at the same concentrations respectively (Fig. 4-8 a and b).
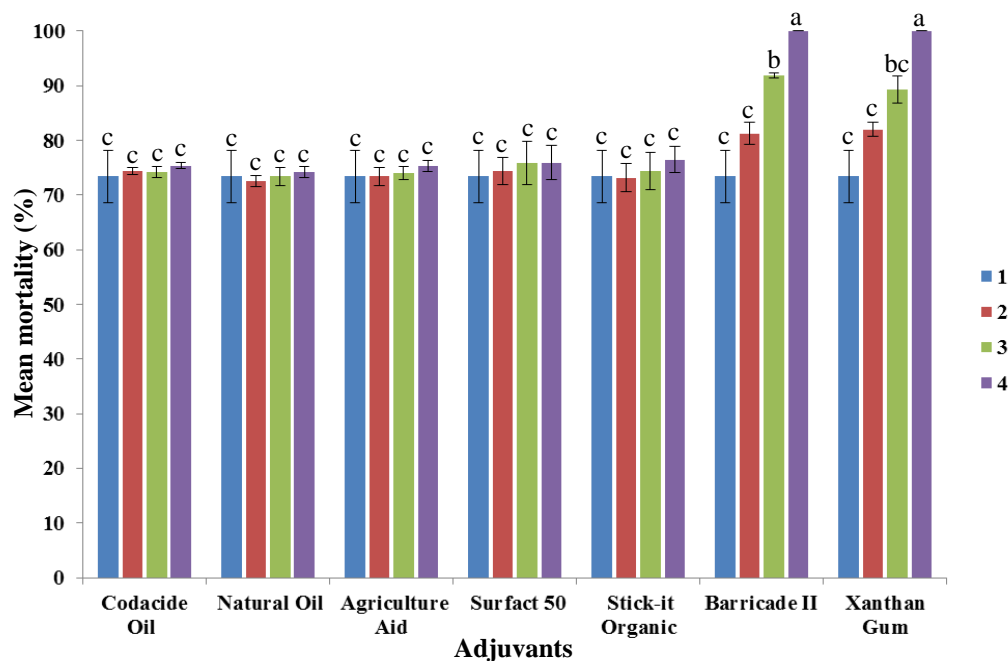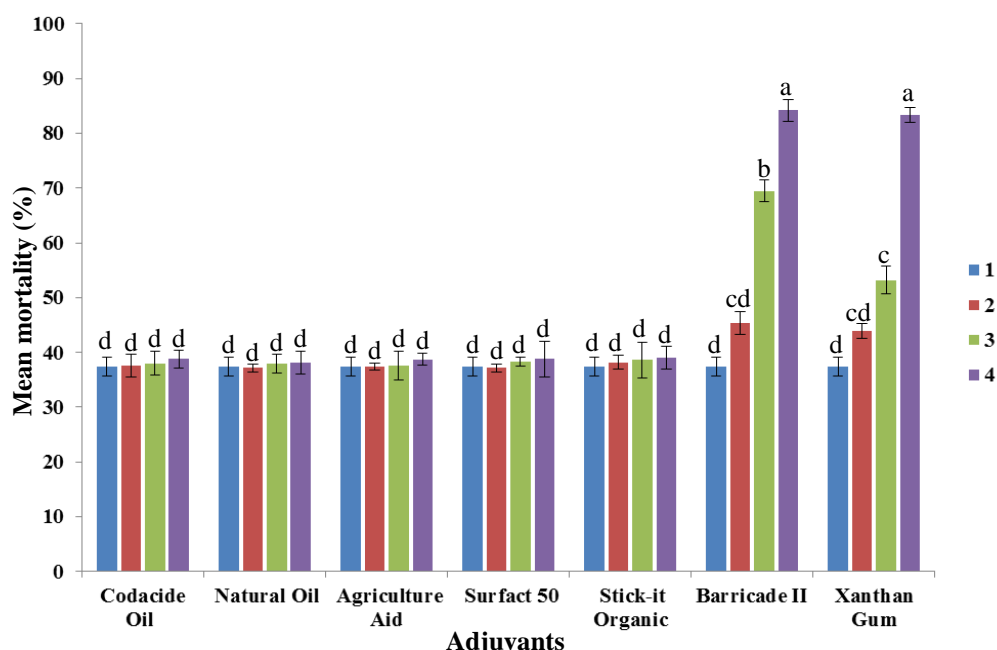
_____
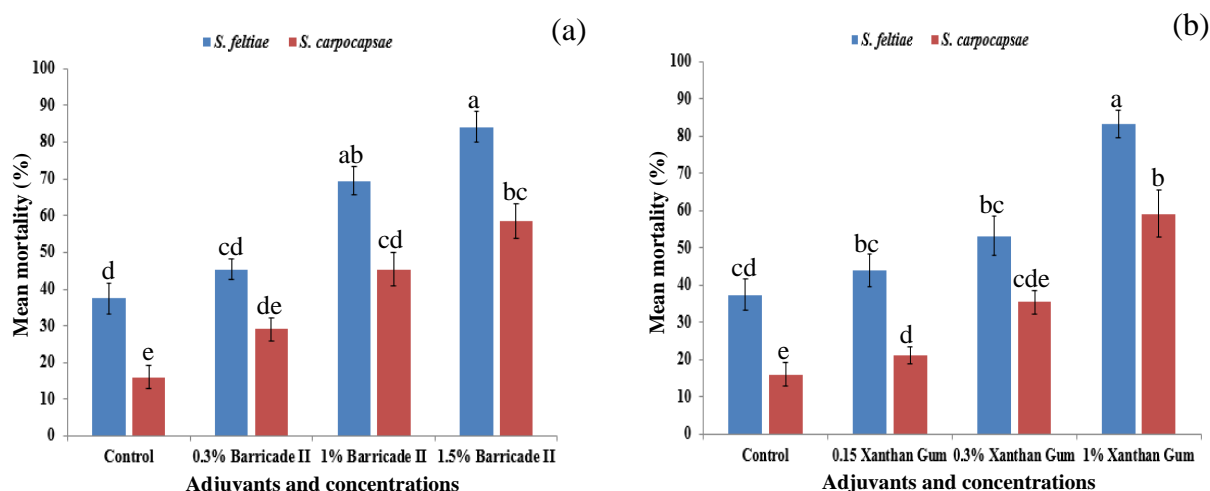
Figure 4-8 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after application of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water (control) or (a): water and Barricade® II (0.3 %, 1 % and 1.5 %); (b): water and xanthan gum (0.15 %, 0.3 % and 1 %). Bars (mean ± SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 4-4 and 4-6.

B. Effects on first, second and fourth instar larvae

Nematode efficacy against other larval instars (first, second and fourth) varied using the two best adjuvants, Barricade® II and xanthan gum, at the best concentrations, 1.5 % and 1 % respectively. *S. feltiae* induced higher larval mortality than *S. carpocapsae* against all larval instars at the two ranges of RH.

At 75 ± 10 % RH, the efficacy of *S. feltiae* and *S. carpocapsae* using 1.5 % Barricade® II or 1 % xanthan gum was significantly higher than the efficacy of control treatments against the fourth larval instar (GLM: $F_{1, 24}$ = 437.58; $P < 0.05$), the second larval instar (GLM: $F_{1, 24}$ = 116.99; $P < 0.05$) and the first larval instar (GLM: $F_{1, 24}$ = 126.38; $P < 0.05$), while efficacy of *S. feltiae* was significantly higher than efficacy of *S. carpocapsae* against the fourth larval instar (GLM: $F_{1, 24}$ = 356.02; $P < 0.05$), the second larval instar (GLM: $F_{1, 24}$ = 172.02; $P < 0.05$) and the first larval instar (GLM: $F_{1, 24}$ = 152.33; $P < 0.05$) (Fig. 4-9 a, b and c). There were no significant differences between the two adjuvants in increasing nematode efficacy against the fourth larval instar (GLM: $F_{1, 24}$ = 0.05; $P > 0.05$), the second larval instar (GLM: $F_{1, 24}$ = 0; $P > 0.05$) and the first larval instar (GLM: $F_{1, 24}$ = 0; $P > 0.05$). The fourth larval instar recorded the highest mortality followed by the second and first larval instars (Fig. 4-9 a, b and c).
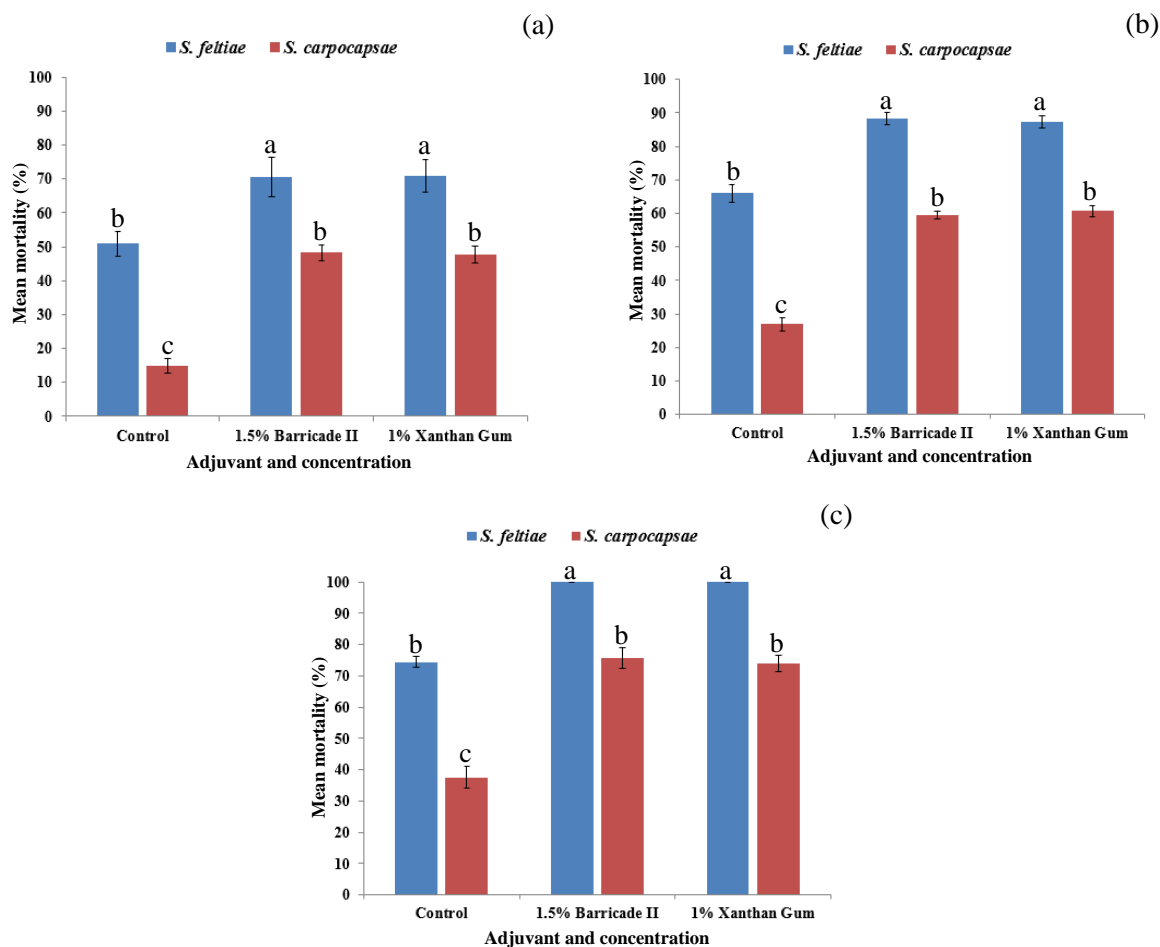
Figure 4-9 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 4th larval instars of *T. absoluta* 48 h after application of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water (control) or water and adjuvant (1.5 % Barricade® II or 1 % xanthan gum). Bars (mean $\pm$ SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

On the other hand, at 45 ± 10 % RH, although the efficacy of both nematode species did not exceed 26 % and 40 % against the first and second larval instar respectively, the efficacy of *S. feltiae* and *S. carpocapsae* using 1.5 % Barricade® II or 1 % xanthan gum was significantly higher than the efficacy of control treatments against the fourth larval instar (GLM: $F_{1, 24} = 494.59$; $P < 0.05$), the second larval instar (GLM: $F_{1, 24} = 270.38$; $P < 0.05$) and the first larval instar (GLM: $F_{1, 24} = 388.20$; $P < 0.05$), whereas efficacy of *S. feltiae* was significantly higher than efficacy of *S. carpocapsae* against the fourth larval instar (GLM: $F_{1, 24} = 119.66$; $P < 0.05$), the second larval instar (GLM: $F_{1, 24} = 45.36$; $P < 0.05$) and the first larval instar (GLM: $F_{1, 24} = 76.24$; $P < 0.05$) (Fig. 4-10 a, b and c). There were no significant differences between the two adjuvants in increasing nematode efficacy against the fourth larval instar (GLM: $F_{1, 24} = 0$; $P > 0.05$), the second larval instar (GLM: $F_{1, 24} = 0$; $P > 0.05$) and the first

larval instar (GLM: $F_{1, 24} = 0.02$; $P > 0.05$). The fourth larval instar recorded the highest mortality followed by the second and first larval instars (Fig. 4-10 a, b and c).
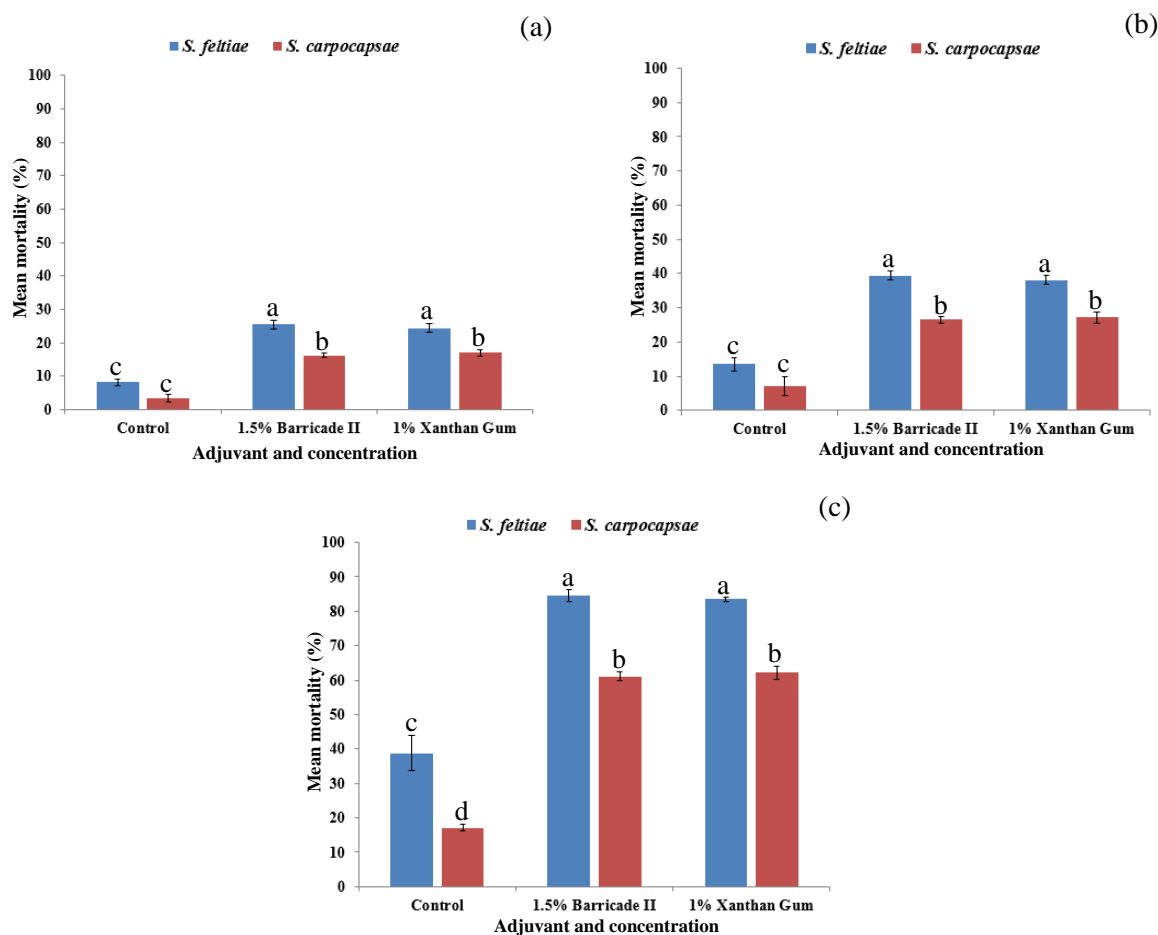
Figure 4-10 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 4th larval instars of *T. absoluta* 48 h after application of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water (control) or water and adjuvants (1.5 % Barricade® II or 1 % xanthan gum). Bars (mean $\pm$ SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

4.4.3 Impact of adjuvants on S. feltiae *and* S. carpocapsae *deposition on tomato leaves*

The addition of different concentrations of Barricade® II and xanthan gum to nematode suspensions gave significant differences in the numbers of nematodes present on leaf surfaces for *S. feltiae* (nested ANOVA: $F_{4, 28} = 13.90$; $P < 0.05$) and for *S. carpocapsae* (nested ANOVA: $F_{4, 28} = 13.05$; $P < 0.05$). Barricade® II at 1 % and 1.5 % and xanthan gum at 0.3 % and 1 % were able to enhance nematode adhesion on leaves significantly compared to the control ($P < 0.05$) (Fig. 4-11 a and b).
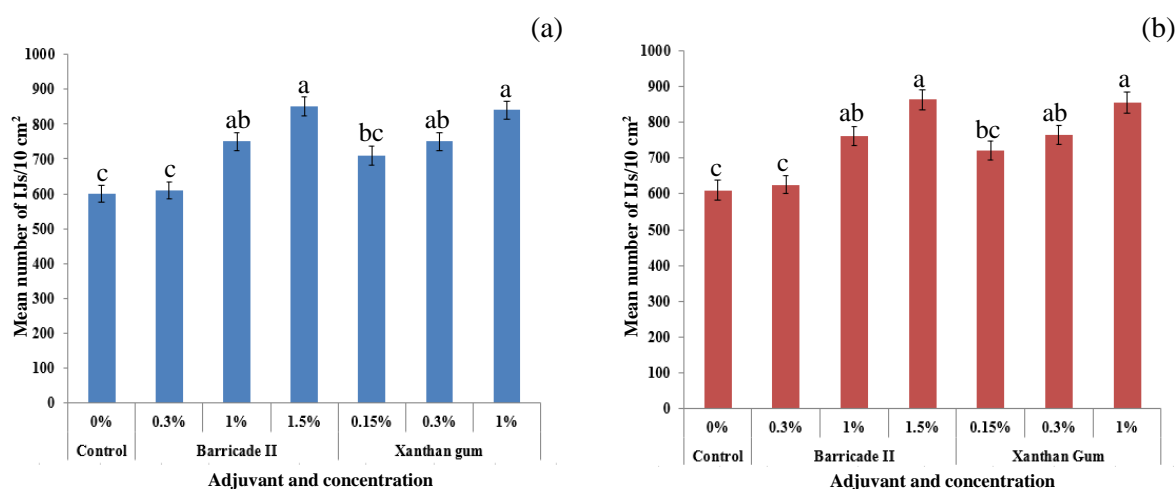
Figure 4-11 Mean number of IJs per 10 cm^2 stuck to tomato leaf surface after application of (a): *S. feltiae* and (b): *S. carpocapsae* (2540 $\pm$ 25 IJs ml^{-1}) either in water (control) or water and adjuvants at different concentrations. Bars (mean $\pm$ SE) with the same letter in each panel do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 5).

4.4.4 Impact of adjuvants on sedimentation of S. feltiae *on tomato leaves*

Sedimentation of *S. feltiae* in water (control) and in Stick-it Organic at all concentrations was fast. Five minutes after the stirring had stopped more than 60 % of the nematodes had passed beyond a depth of 2 cm. After 1 h no nematodes were recorded (Fig. 4-12 a). A general linear model for Barricade® II showed significant differences between concentrations (four levels; 0 %, 0.3 %, 1 % and 1.5 %) (GLM: $F_{3, 96}$ = 6081.29; P < 0.05) and times (five levels; 0, 5, 15, 30 and 60 min) (GLM: $F_{5, 96}$ = 872.39; P < 0.05) in retarding the sedimentation of *S. feltiae*. The interaction between concentrations and times was also significant (GLM: $F_{15, 96}$ = 279.50; P < 0.05). Barricade® II at 1 % and 1.5 % was able to retard sedimentation of *S. feltiae* significantly at all the time intervals, compared to the control and 0.3 % concentration (P < 0.05) (Fig. 4-12 b).

A general linear model for xanthan gum showed significant differences between concentrations (GLM: $F_{3, 96}$ = 4377.52; P < 0.05) and times (GLM: $F_{5, 96}$ = 285.81; P < 0.05) as well as significant interactions (GLM: $F_{15, 96}$ = 202.31; P < 0.05). Xanthan gum at all concentrations (0.15 %, 0.3 % and 1 %) was able to retard sedimentation of *S. feltiae* significantly at all the time intervals, compared to the control (P < 0.05) (Fig. 4-12 c).

(a)

(b)

(c)
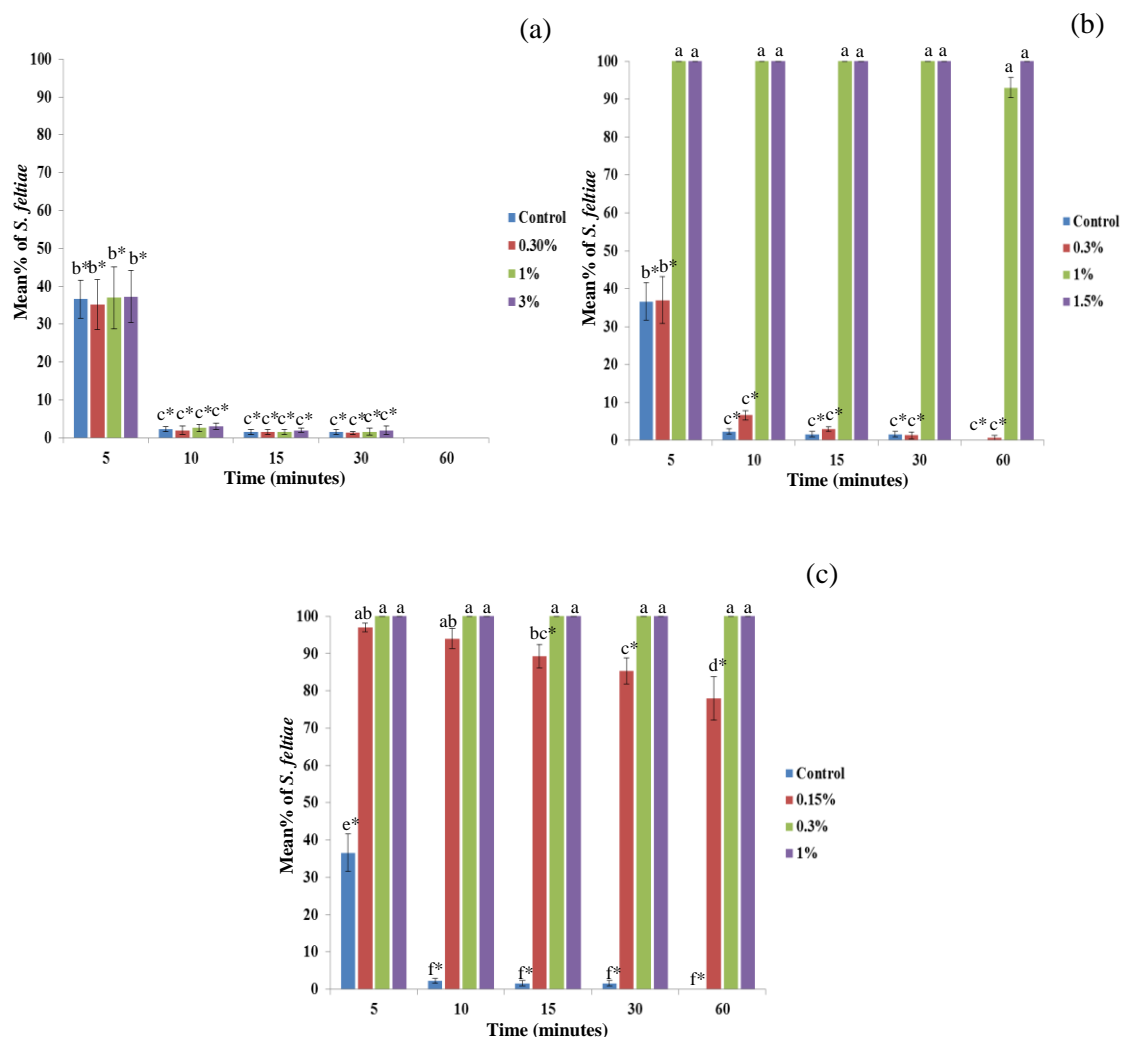
Figure 4-12 Sedimentation of *S. feltiae* suspended either in water or water and (a): Stick-it Organic; (b): Barricade® II and (c): xanthan gum at different concentrations. Sedimentation was measured as mean percentage (n = 5) of IJs recovered in 2 cm depth of suspensions at different periods after ending of mixing the nematode suspension. Reference set to 100 % was the number of nematodes recorded immediately after ending of mixing. Bars (mean ± SE) with the same letter do not differ significantly and those indicated with * differ significantly from the reference number according to Tukey's multiple range test at $P < 0.05$.

4.4.5 Impact of adjuvants on survival of S. feltiae *and* S. carpocapsae *on tomato leaves*

At 75 ± 10 % RH, survival of *S. feltiae* and *S. carpocapsae* decreased as time passed but noticeable improvement was observed with addition of Barricade® II (1 % and 1.5 %) and xanthan gum (0.3 % and 1 %) compared to the control (nematodes suspended in tap water), with differences in survival of nematodes between adjuvants at different concentrations (Figs. 4-13 and 4-14). For instance, survival of *S. feltiae* was increased after forty-eight hours by 22.5-fold, 44.5-fold, 53.5-fold and 55.5-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Fig. 4-13).

Additionally, LT_{50} of *S. feltiae* was increased from 9 h to 20.5, 35.5, 46 and 48 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Table 4-3). Similarly, survival of *S. carpocapsae* was increased after forty-eight hours by 2.28-fold, 3.44-fold, 3.89-fold and 3.92-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Fig. 4-14). Moreover, LT_{50} of *S. carpocapsae* was increased from 22.5 h to 38, 60.5, 64.5 and 67.5 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Table 4-3).
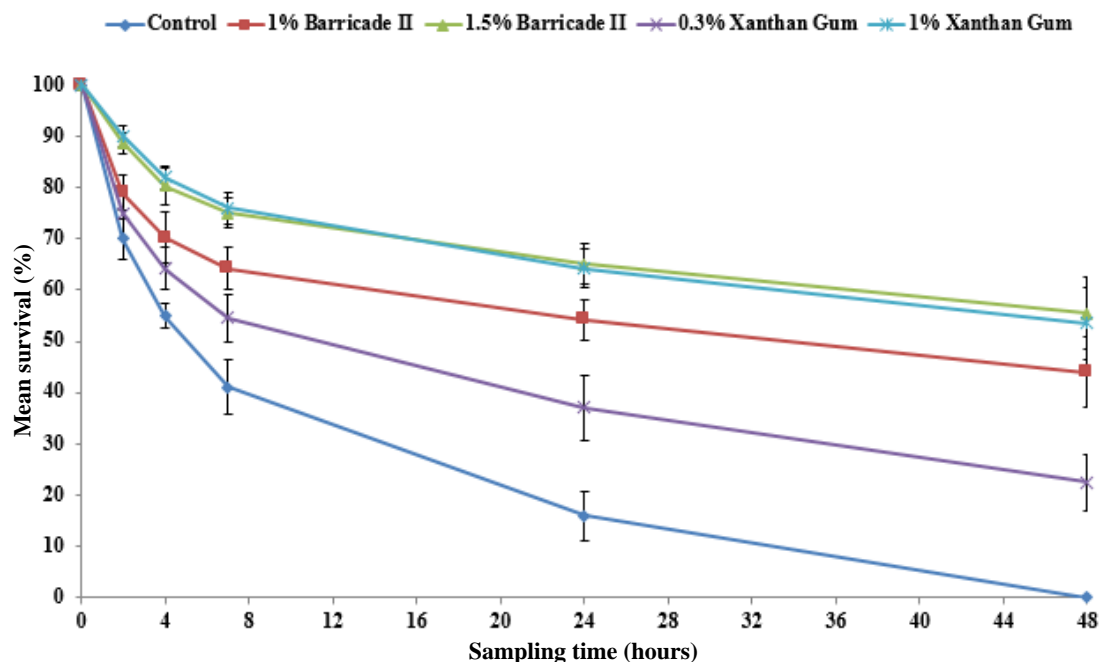
Figure 4-13 Mean survival over 48 h at 75 ± 10 % RH and 25 ± 0.5 °C of *S. feltiae* (60 ± 6 IJs cm^{-2}) applied on tomato leaves either in water (control) or water and adjuvants at different concentrations (treatments) . Error bars (mean $\pm$ SE) represent standard errors (n = 4).
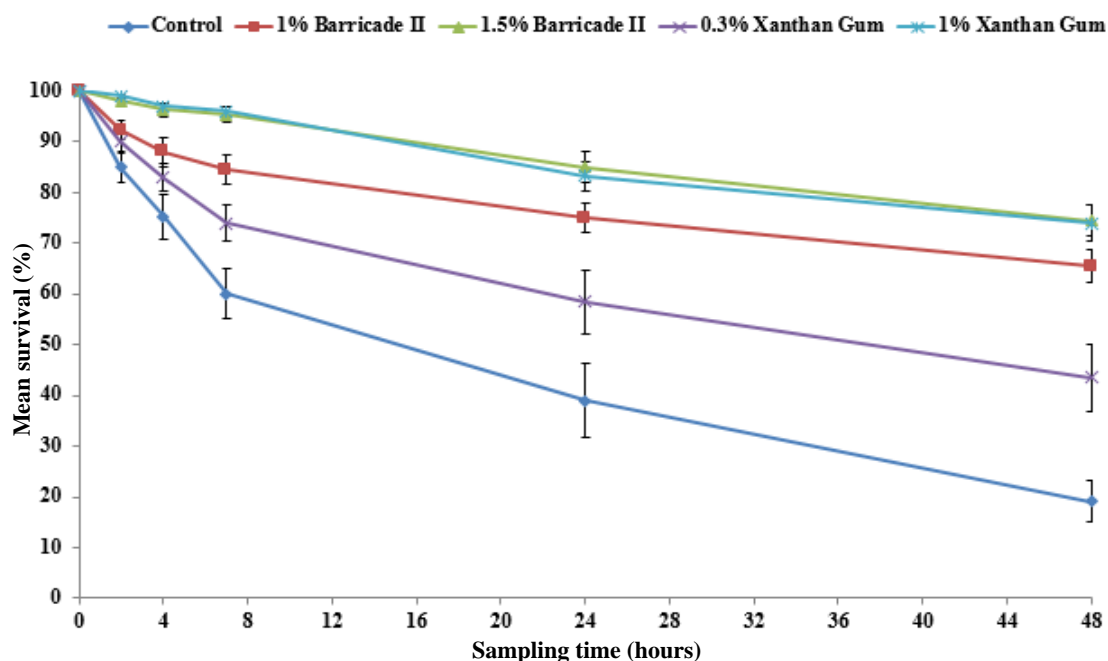
Figure 4-14 Mean survival over 48 h at 75 ± 10 % RH and 25 ± 0.5 °C of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) applied on tomato leaves either in water (control) or water and adjuvants at different concentrations (treatments). Error bars (mean $\pm$ SE) represent standard errors (n = 4).

Table 4-3 LT$_{50}$ at 75 ± 10 % RH of *S. feltiae* and *S. carpocapsae* applied at a rate of 60 ± 6 IJs cm^{-2} on tomato leaves either in water or water and adjuvants.

Nematode species	Adjuvant	Adjuvant Concentration (%)	LT$_{50}$	Fiducial limits (95 %)	
				lower	upper
S. feltiae	In water (Control)	0	6.2	5.8	6.6
	Barricade II	1	26.4	22.6	31.5
		1.5	54.4	44.0	70.1
	Xanthan gum	0.3	11.9	10.8	13.1
		1	50.2	41.4	63.2
S. carpocapsae	In water (Control)	0	14.0	12.9	15.3
	Barricade II	1	114	85	167
		1.5	171	124	261
	Xanthan gum	0.3	33.1	28.9	38.9
		1	135	103	191

When the two nematode species were compared, significant differences in survival between *S. feltiae* and *S. carpocapsae* at 75 ± 10 % RH were caused by addition of Barricade® II (1 % and 1.5 %) and xanthan gum (0.3 % and 1 %). Survival of *S. carpocapsae* was significantly higher than survival of *S. feltiae* after four hours (GLM: $F_{1, 30} = 74.05$; $P < 0.05$) with

significant differences between treatments (GLM: $F_{4,\,30} = 20.06$; $P < 0.05$) (Fig. 4-15 a), after seven hours (GLM: $F_{1,\,30} = 73.35$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4,\,30} = 33.53$; $P < 0.05$) (Fig. 4-15 b), after twenty-four hours (GLM: $F_{1,\,30} = 48.21$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4,\,30} = 35.84$; $P < 0.05$) (Fig. 4-15 c), and after forty-eight hours (GLM: $F_{1,\,30} = 37.60$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4,\,30} = 41.77$; $P < 0.05$) (Fig. 4-15 d). Survival of *S. feltiae* and *S. carpocapsae* was increased significantly compared to the control after four hours by addition of 1.5 % Barricade® II and 1 % xanthan gum ($P < 0.05$) and after seven, twenty-four and forty-eight hours by addition of 1 % and 1.5 % Barricade® II and 1 % xanthan gum ($P < 0.05$) (Fig. 4-15 a, b, c and d).
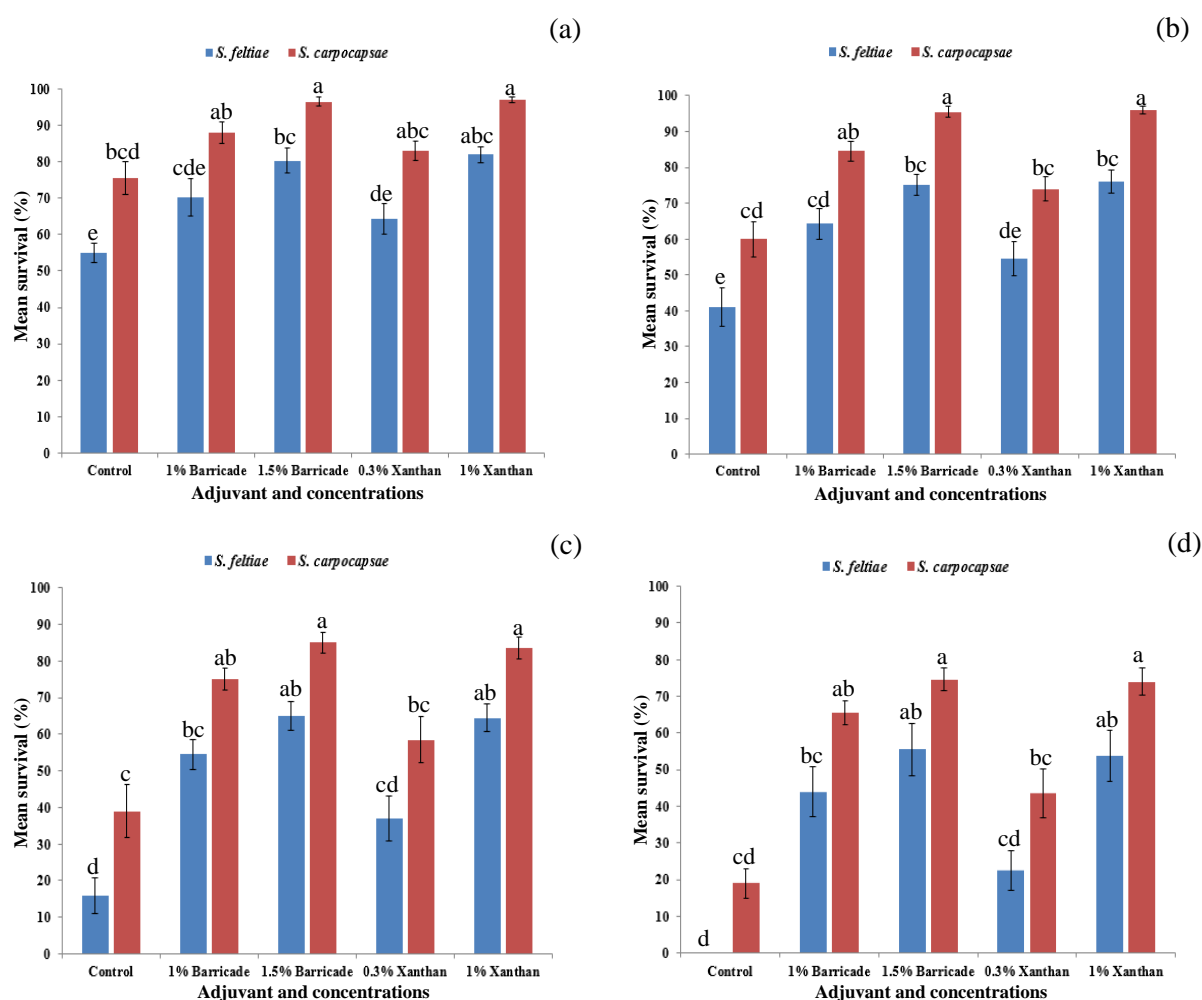
Figure 4-15 Mean survival at 75 ± 10 % RH and 25 ± 0.5 °C of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) applied on tomato leaves either in water (control) or water and adjuvants at different concentrations (treatments) after (a): 4 h, (b): 7 h, (c): 24 h and (d): 48 h. Bars (mean ± SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 4-13 and 4-14.

In contrast, at 45 ± 10 % RH survival of *S. feltiae* and *S. carpocapsae* decreased as time passed and obvious improvement was observed with addition of Barricade® II (1 % and 1.5 %) and xanthan gum (0.3 % and 1 %) compared to the control, with differences in survival between adjuvants at different concentrations, although nematode survival was less than at 75 ± 10 % RH (Figs. 4-16 and 4-17). Survival of *S. feltiae* was increased after forty-eight hours by 0-fold, 0-fold, 6.75-fold and 5.75-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Fig. 4-16). In addition, LT_{50} of *S. feltiae* was increased from 3 h to 4.5, 7, 12 and 12 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Table 4-4). Likewise, survival of *S. carpocapsae* was increased after forty-eight hours by 6.25-fold, 12.5-fold, 24-fold and 25-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively (Fig. 4-17). Moreover, LT_{50} of *S. carpocapsae* was increased from 8 h to 13, 17.5, 25.5 and 26 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1.5 % Barricade® II and 1 % xanthan gum respectively (Table 4-4).
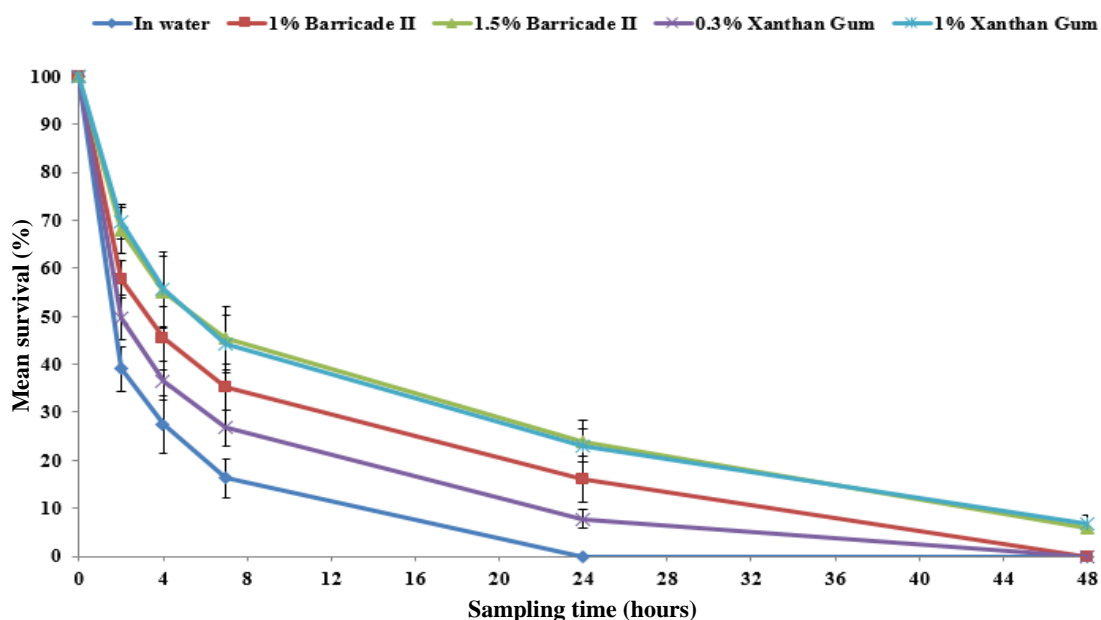
Figure 4-16 Mean survival over 48 h at 45 ± 10 % RH and 25 ± 0.5 °C of *S. feltiae* (60 ± 6 IJs cm^{-2}) applied on tomato leaves either in water (control) or water and adjuvants at different concentrations (treatments) . Error bars (mean + SE) represent standard errors (n = 4).
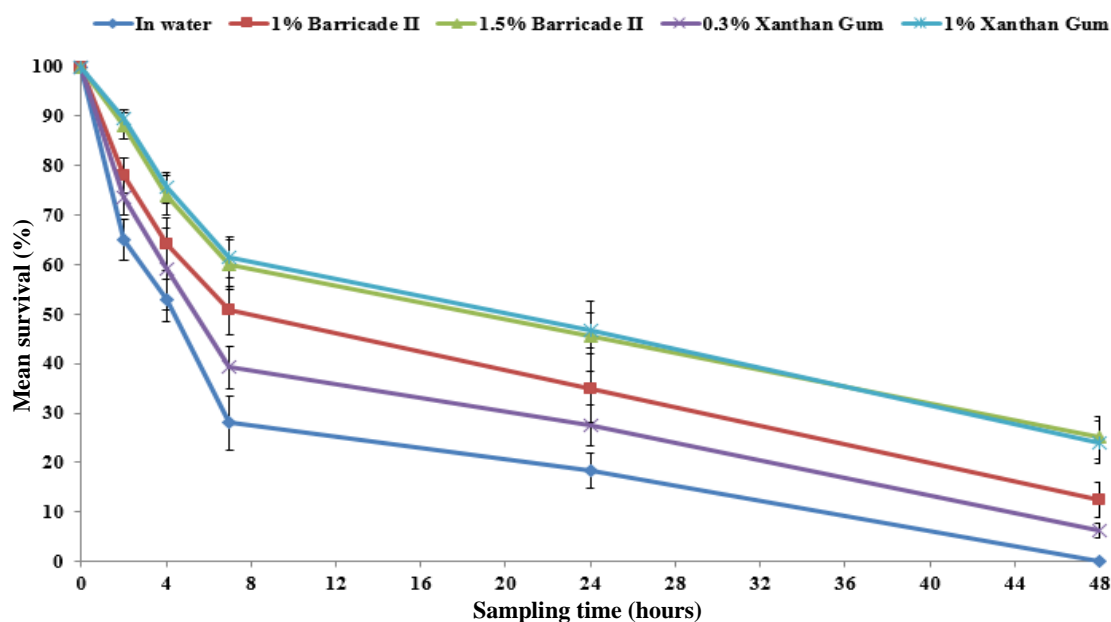
Figure 4-17 Mean survival over 48 h at 45 ± 10 % RH and 25 ± 0.5 °C of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) applied on tomato leaves either in water (control) or water and adjuvants at different concentrations (treatment) . Error bars (mean + SE) represent standard errors (n = 4).

Table 4-4 LT$_{50}$ at 45 ± 10 % RH of *S. feltiae* and *S. carpocapsae* applied at a rate of 60 ± 6 IJs cm^{-2} on tomato leaves either in water or water and adjuvants.

Nematode species	Adjuvant	Adjuvant Concentration (%)	LT$_{50}$	Fiducial limits (95 %)	
				lower	upper
S. feltiae	In water (Control)	0	3.1	3.0	3.3
	Barricade II	1	5.1	4.7	5.4
		1.5	7.1	6.6	7.7
	Xanthan gum	0.3	4.0	3.7	4.3
		1	7.2	6.7	7.8
S. carpocapsae	In water (Control)	0	5.5	5.1	5.9
	Barricade II	1	10.2	9.4	11.1
		1.5	16.5	15.1	18.3
	Xanthan gum	0.3	7.6	7.0	8.2
		1	17.1	15.5	18.8

When the nematode species were compared, significant differences in the survival between *S. feltiae* and *S. carpocapsae* at 45 ± 10 % RH were caused by addition of Barricade® II (1 % and 1.5 %) and xanthan gum (0.3 % and 1 %). Survival of *S. carpocapsae* was significantly higher than survival of *S. feltiae* after four hours (GLM: $F_{1, 30} = 28.45$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4, 30} = 6.44$; $P < 0.05$), seven hours (GLM: $F_{1, 30} = 16.65$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4, 30} = 13.33$;

$P < 0.05$), twenty-four hours (GLM: $F_{1,30} = 52.53$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4,30} = 12.49$; $P < 0.05$) and forty-eight hours (GLM: $F_{1,30} = 173.20$; $P < 0.05$) with significant differences between treatments (GLM: $F_{4,30} = 86.79$; $P < 0.05$). *S. feltiae* survival was increased significantly compared to the control after four, twenty-four and forty-eight hours by addition of 1.5 % Barricade® II and 1 % xanthan gum ($P < 0.05$) and after seven hours by addition of 1 % and 1.5 % Barricade® II and 1 % xanthan gum ($P < 0.05$) (Fig. 4-18 a, b, c and d), whereas survival of *S. carpocapsae* was increased significantly compared to the control after seven and twenty-four hours by addition of 1.5 % Barricade® II and 1 % xanthan gum ($P < 0.05$) and after forty-eight hours by addition of 1 % and 1.5 % Barricade® II and 0.3 % and 1 % xanthan gum ($P < 0.05$) (Fig. 4-18 a, b, c and d).
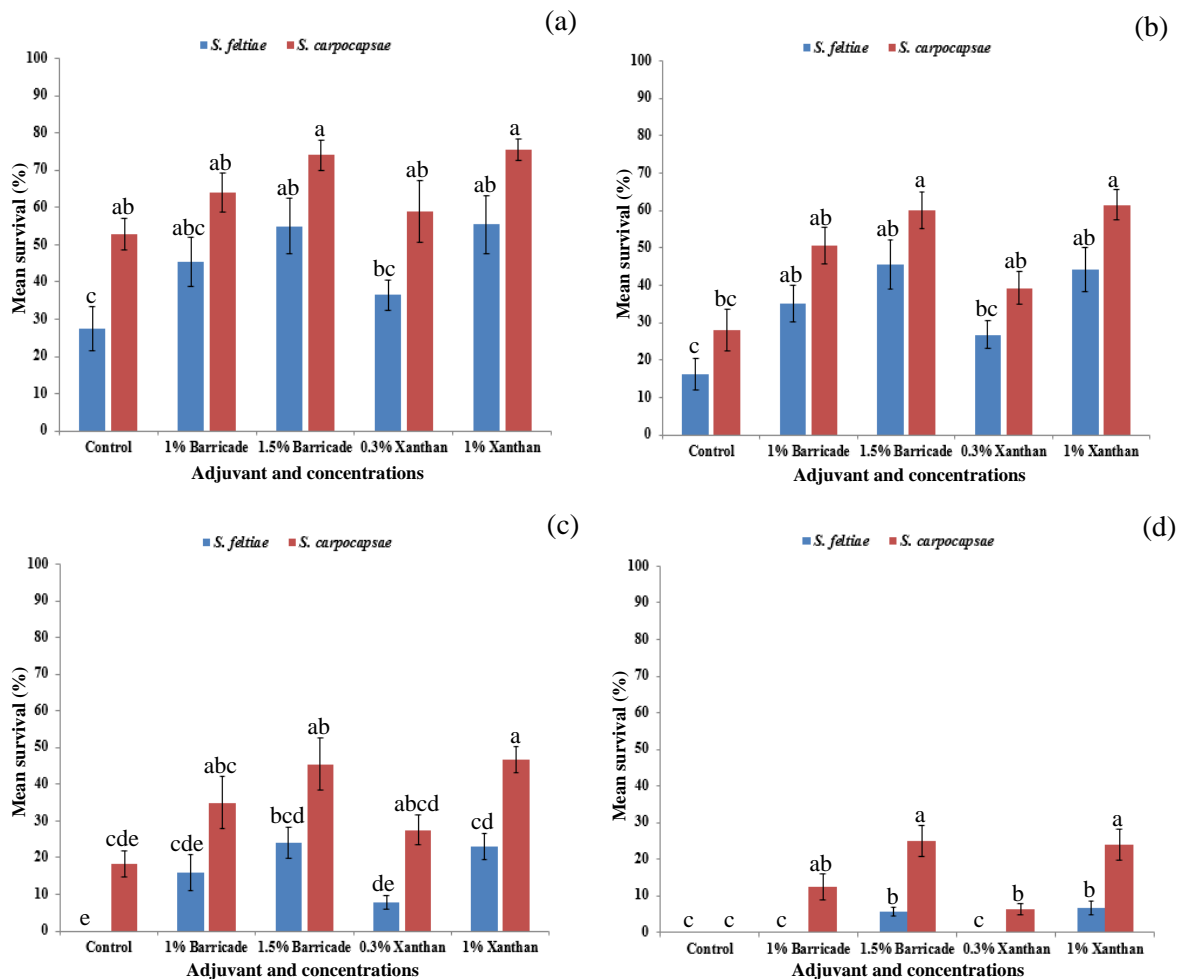
Figure 4-18 Mean survival at 45 ± 10 % RH and 25 ± 0.5 °C of *S. feltiae* and *S. carpocapsae* (60 ± 6 IJs cm^{-2}) applied either in water (control) or water and adjuvants at different concentrations (treatment) after (a): 4 h, (b): 7 h, (c): 24 h and (d): 48 h on tomato leaves. Bars (mean ± SE) in each panel with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4). Data used in the analysis here were from the experiment shown in Figures 4-16 and 4-17.

4.4.6 Impact of adjuvants on lethal concentration (LC) of S. feltiae and S. carpocapsae against third larval instar of Tuta absoluta on leaves

In general, both adjuvants decreased LC_{50} of *S. feltiae* and *S. carpocapsae* at 75 ± 10 % and 45 ± 10 % RH compared to the control, with differences among adjuvant concentrations and nematode species. High adjuvant concentrations were better than lower adjuvant concentrations in decreasing LC_{50} of both nematode species (Tables 4-5 and 4-6).

At 75 ± 10 % RH, significant differences were observed between the effectiveness of different adjuvant concentrations (zero concentration was considered as a control) in decreasing LC_{50} of *S. feltiae* (nested ANOVA: $F_{2, 10} = 15.27$; $P < 0.05$). LC_{50} of *S. feltiae* was decreased significantly compared to nematodes suspended in water (control) by 1.54-fold, 2.42-fold, 4.25-fold and 5.66-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum or 1.5 % Barricade® II respectively, with no significant differences between 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II or between 0.3 % xanthan gum and 1 % Barricade® II in decreasing LC_{50} ($P > 0.05$) (Table 4-5). Similarly at 45 ± 10 % RH, there were significant differences between the effectiveness of different adjuvant concentrations in decreasing LC_{50} of *S. feltiae* (nested ANOVA: $F_{2, 10} = 55.79$; $P < 0.05$). LC_{50} of *S. feltiae* was decreased significantly compared to the control by 3.22-fold, 5.55-fold, 11.11-fold and 12.5-fold by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1.5 % Barricade® II and 1 % xanthan gum respectively. Treatments differed significantly between each other in decreasing LC_{50} ($P < 0.05$) except between 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Table 4-5).

On the other hand, there were significant differences between treatments in LC_{50} of *S. carpocapsae* at 75 ± 10 % RH (one-way ANOVA: $F_{2, 8} = 126.75$; $P < 0.05$). LC_{50} of *S. carpocapsae* was decreased significantly compared to the control by 2.69-fold and 2.91-fold by addition of 1.5 % Barricade® II and 1 % xanthan gum respectively, with no significant differences between 1.5 % Barricade® II and 1 % xanthan gum in decreasing LC_{50} ($P > 0.05$) (Table 4-6). Likewise, there were significant differences between treatments in LC_{50} of *S. carpocapsae* at 45 ± 10 % RH (one-way ANOVA: $F_{2, 8} = 1455.36$; $P < 0.05$). LC_{50} of *S. carpocapsae* was decreased significantly compared to the control by 4.57-fold and 5-fold by addition of 1 % xanthan gum and 1.5 % Barricade® II respectively, with no significant differences between 1.5 % Barricade® II and 1 % xanthan gum in decreasing LC_{50} ($P > 0.05$) (Table 4-6).

Table 4-5 Impact of Barricade® II and xanthan gum on LC$_{50}$ values of *S. feltiae* against third larval instar of *T. absoluta* on tomato leaves at 75 ± 10 % and 45 ± 10 % RH and 25 ± 0.5 °C. Means of LC$_{50}$ (n = 3) followed by the same letters do not differ significantly according to Tukey's multiple range test at P < 0.05.

RH	Adjuvant	Adjuvant Concentration (%)	LC$_{50}$ [a]	Fiducial limits (95 %)	
				lower	upper
75 ± 10 %	In water (Control)	0	17.2 a[+]	13.5	21.8
	Barricade II	1	7.0 bc	5.2	9.0
		1.5	3.0 c	2.3	3.8
	Xanthan gum	0.3	10.9 b	8.3	13.9
		1	4.0 c	2.9	5.1
45 ± 10 %	In water (Control)	0	99.3* A[+]	59.8	285
	Barricade II	1	18.0 C	13	25.4
		1.5	9.3 D	6.4	12.9
	Xanthan gum	0.3	31.2 B	22.5	47.7
		1	8.1 D	5.8	10.7

[a] LC$_{50}$ values = number of IJs per cm^2 needed to reach 50 % mortality.
* LC$_{50}$ was calculated when mortality was above 18 % and below 50 %.
[+] Capital and small letters indicate separate statistical tests.

Table 4-6 Impact of Barricade® II and xanthan gum on LC$_{50}$ values of *S. carpocapsae* against third larval instar of *T. absoluta* on tomato leaves at 75 ± 10 % and 45 ± 10 % RH and 25 ± 0.5 °C. Means of LC$_{50}$ (n = 3) followed by the same letters do not differ significantly according to Tukey's multiple range test at P < 0.05.

RH	Adjuvant	Adjuvant Concentration (%)	LC$_{50}$ [a]	Fiducial limits (95 %)	
				lower	upper
75 ± 10 %	In water (Control)	0	34.9 a[+]	26.5	50.1
	Barricade II	1.5	13.0 b	9.6	17.1
	Xanthan gum	1	11.7 b	8.6	15.3
45 ± 10 %	In water (Control)	0	160* A[+]	86	1000
	Barricade II	1.5	31.8 B	23.4	47.3
	Xanthan gum	1	34.6 B	25.7	51.2

[a] LC$_{50}$ values = number of IJs per cm^2 needed to reach 50 % mortality.
* LC$_{50}$ was calculated when mortality was above 18 % and below 50 %.
[+] Capital and small letters indicate separate statistical tests.

4.4.7 Impact of adjuvants on lethal time (LT) of S. feltiae and S. carpocapsae against third larval instar of Tuta absoluta on tomato leaves

Overall, both adjuvants decreased LT$_{50}$ of *S. feltiae* and *S. carpocapsae* at 75 ± 10 % and 45 ± 10 % RH compared to the control, with differences among adjuvant concentrations and

_____

nematode species. High adjuvant concentrations were better than lower adjuvant concentrations in decreasing LT_{50} of both nematode species (Tables 4-7 and 4-8).

At 75 ± 10 % RH, there were significant differences between adjuvant concentrations in decreasing LT_{50} of *S. feltiae* (nested ANOVA: $F_{2, 10} = 29.47$; $P < 0.05$). LT_{50} of *S. feltiae* in water was 30 h. This was decreased significantly to 19, 14 and 13 h by addition of 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively ($P < 0.05$), while adding 0.3 % xanthan gum decreased LT_{50} to 25 h, which was not significantly different from the control ($P > 0.05$). Significant differences were observed between 1 % Barricade® II, 1.5 % Barricade® II and 0.3 % xanthan gum ($P < 0.05$) but not between 1 % xanthan gum and 1.5 % or 1 % Barricade® II ($P > 0.05$) (Table 4-7).

Similarly, at 45 ± 10 % RH, there were significant differences between adjuvant concentrations in decreasing LT_{50} of *S. feltiae* (nested ANOVA: $F_{2, 10} = 70.51$; $P < 0.05$). The LT_{50} of *S. feltiae* in water was 53 h. This was decreased significantly to 39, 31, 22 and 21 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively ($P < 0.05$). All adjuvant concentrations differed significantly between each other in decreasing LT_{50} ($P < 0.05$), except 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Table 4-7).

Table 4-7 Impact of Barricade® II and xanthan gum on LT_{50} of *S. feltiae* against 3rd larval instar of *T. absoluta* on tomato leaves at 75 ± 10 % and 45 ± 10 % RH and 25 ± 0.5 °C. Means of LT_{50} (n = 3) followed by the same letters do not differ significantly according to Tukey's multiple range test at P < 0.05.

RH	Adjuvant	Adjuvant Concentration (%)	LT_{50} [a]	Fiducial limits (95 %)	
				lower	upper
75 ± 10 %	In water (Control)	0	29.6 a[+]	27.0	32.3
	Barricade II	1	18.9 b	15.4	22.1
		1.5	12.9 c	10.1	15.0
	Xanthan gum	0.3	24.6 a	21.7	27.5
		1	14.0 bc	11.4	16.5
45 ± 10 %	In water (Control)	0	52.9* A[+]	46.1	65.3
	Barricade II	1	31.7 C	29.1	34.8
		1.5	21.3 D	18.4	24.0
	Xanthan gum	0.3	38.9 B	34.1	45.8
		1	21.8 D	19.1	24.4

[a] LT_{50} = time needed (hours) by IJs to cause 50 % mortality.
* LT_{50} were calculated when mortality was above 32 % and below 50 %.
[+] Capital and small letters indicating different statistical tests was carried out separately.

_____

_____

On the other hand, there were significant differences between adjuvant concentrations in decreasing LT_{50} of *S. carpocapsae* at 75 ± 10 % RH (nested ANOVA: $F_{2, 10} = 83.16$; $P < 0.05$). The LT_{50} of *S. carpocapsae* in water was 49 h. This was decreased significantly to 42, 36, 25 and 24 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively ($P < 0.05$). All adjuvant concentrations differed significantly between each other in decreasing LT_{50} ($P < 0.05$) except 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Table 4-8).

Similarly, there were significant differences between adjuvant concentrations in decreasing LT_{50} of *S. carpocapsae* at 45 ± 10 % RH (nested ANOVA: $F_{2, 10} = 126.50$; $P < 0.05$). LT_{50} of *S. carpocapsae* in water was 72 h. This was decreased significantly to 56, 48, 35 and 33 h by addition of 0.3 % xanthan gum, 1 % Barricade® II, 1 % xanthan gum and 1.5 % Barricade® II respectively ($P < 0.05$). All adjuvant concentrations differed significantly between each other in decreasing LT_{50} ($P < 0.05$) except 1.5 % Barricade® II and 1 % xanthan gum ($P > 0.05$) (Table 4-8).

Table 4-8 Impact of Barricade® II and xanthan gum on LT_{50} of *S. carpocapsae* against 3rd larval instar of *T. absoluta* on tomato leaves at 75 ± 10 % and 45 ± 10 % RH and 25 ± 0.5 °C. Means of LT_{50} (n = 3) followed by the same letters do not differ significantly according to Tukey's multiple range test at P < 0.05.

RH	Adjuvant	Adjuvant Concentration (%)	LT_{50} [a]	Fiducial limits (95 %)	
				lower	upper
75 ± 10 %	In water (Control)	0	48.6* a[+]	43.3	57.0
	Barricade II	1	35.8 c	32.8	39.6
		1.5	23.6 d	20.6	26.5
	Xanthan gum	0.3	41.5 b	35.7	51.0
		1	24.6 d	21.7	27.5
45 ± 10 %	In water (Control)	0	72.1* A[+]	58.0	113
	Barricade II	1	47.5 C	43.1	54.4
		1.5	33.0 D	29.5	37.3
	Xanthan gum	0.3	56.0 B	47.7	72.5
		1	34.9 D	31.7	38.9

[a] LT_{50} = time needed by IJs to cause 50 % mortality.
* LT_{50} values were calculated when mortality was above 18 % and below 50 %.
[+] Capital and small letters indicate separate statistical tests.

_____

4.4.8 Impact of Barricade® II on effectiveness of different numbers of applications of S. feltiae *and* S. carpocapsae *against different larval instars of* Tuta absoluta *on tomato leaves*

In general, adding adjuvants to nematode suspensions reduced the number of applications required to reach the highest mortality of different larval instars, with differences in efficacy between nematode species and in the susceptibility of different larval instars at the two ranges of RH.

When *S. feltiae* was used at a rate of 60 ± 6 IJs cm^{-2} with 1.5 % Barricade® II against different larval instars at 75 ± 10 % RH (Fig. 4-19), there were significant differences between numbers of applications of *S. feltiae* against the first larval instar (GLM: $F_{3, 24} = 47.25$; $P < 0.05$), the second larval instar (GLM: $F_{3, 24} = 54.67$; $P < 0.05$), the third larval instar (GLM: $F_{3, 24} = 18.18$; $P < 0.05$) and the fourth larval instar (GLM: $F_{3, 24} = 16.99$; $P < 0.05$), with significant differences between control (*S. feltiae* suspended in water) and treatment (*S. feltiae* suspended in water and 1.5 % Barricade® II) against the first larval instar (GLM: $F_{1, 24} = 20.35$; $P < 0.05$), the second larval instar (GLM: $F_{1, 24} = 38.85$; $P < 0.05$), the third larval instar (GLM: $F_{1, 24} = 31.45$; $P < 0.05$) and the fourth larval instar (GLM: $F_{1, 24} = 27.27$; $P < 0.05$). The highest larval mortality among different numbers of applications induced by *S. feltiae* in the control was after three and four applications against the first and second larval instars and after two, three and four applications against the third and fourth larval instars; and in the treatment was after two, three and four applications against the first and second larval instars ($P < 0.05$), with no significant differences between different numbers of applications against the third and fourth larval instars ($P > 0.05$). The significant differences in larval mortality between control and treatment were after one and two applications against the first and second larval instars and after one application against the third and fourth larval instars ($P < 0.05$) (Fig. 4-19).

At 45 ± 10 % RH (Fig. 4-20), there were significant differences between numbers of applications of *S. feltiae* against the first larval instar (GLM: $F_{3, 24} = 141.50$; $P < 0.05$), the second larval instar (GLM: $F_{3, 24} = 160.38$; $P < 0.05$), the third larval instar (GLM: $F_{3, 24} = 23.85$; $P < 0.05$) and the fourth larval instar (GLM: $F_{3, 24} = 25.32$; $P < 0.05$) with significant differences between control and treatment against the first larval instar (GLM: $F_{1, 24} = 263.46$; $P < 0.05$), the second larval instar (GLM: $F_{1, 24} = 216.51$; $P < 0.05$), the third larval instar (GLM: $F_{1, 24} = 157.62$; $P < 0.05$) and the fourth larval instar (GLM: $F_{1, 24} = 168.76$; $P < 0.05$).

_____

The highest larval mortality among different numbers of applications induced by *S. feltiae* was in the control after four applications against the first, second, third and fourth larval instars and in the treatment after four applications against the first larval instar, three and four applications against the second larval instar and two, three and four applications against the third and fourth larval instars ($P < 0.05$). The significant differences in larval mortality between control and treatment were after one, two, three and four applications against the first and second larval instars and after one, two and three applications against the third and fourth larval instars ($P < 0.05$) (Fig. 4-20).
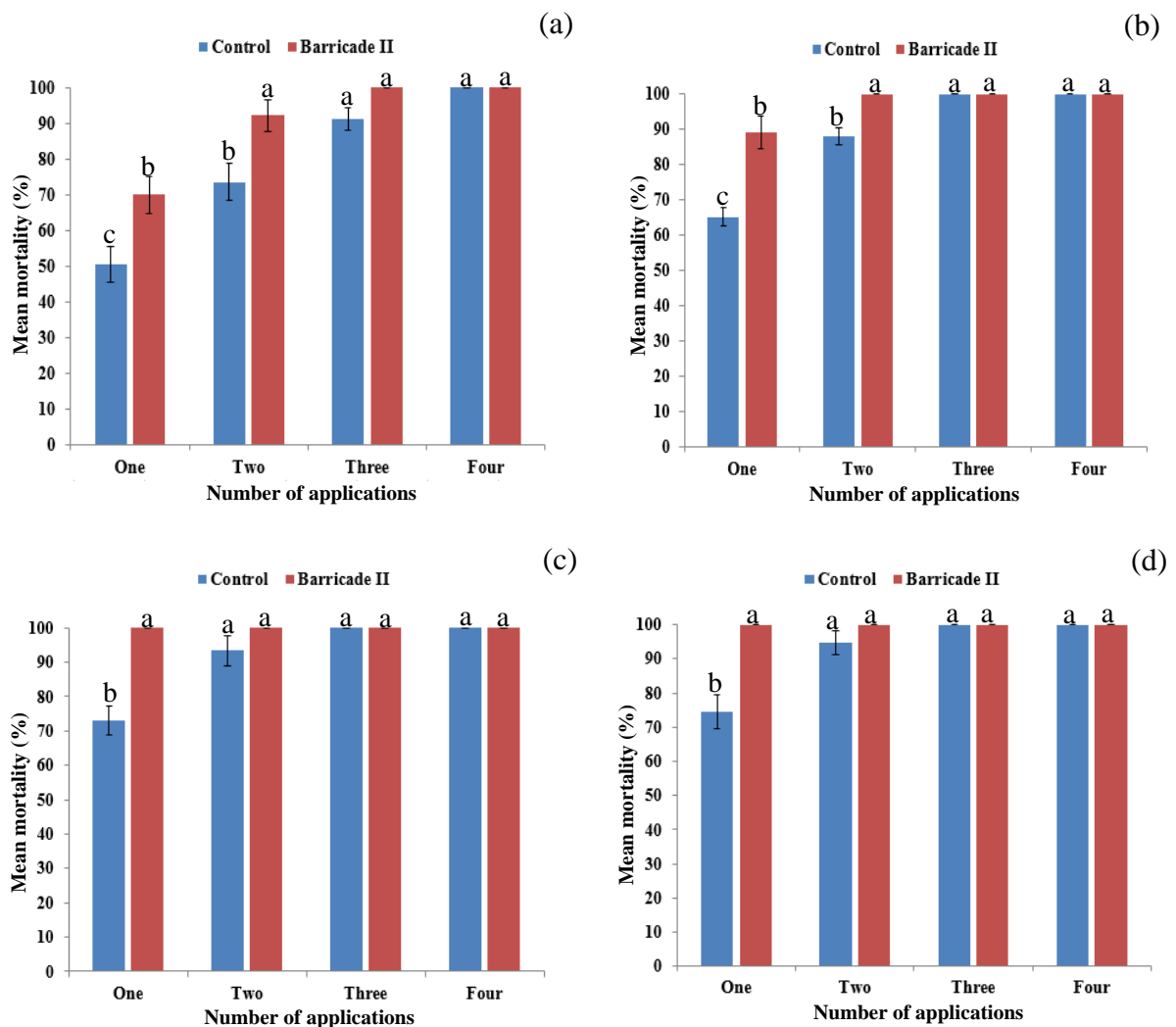
Figure 4-19 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) either in water or water and 1.5 % Barricade® II. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

_____

(a)   (b)

(c)   (d)
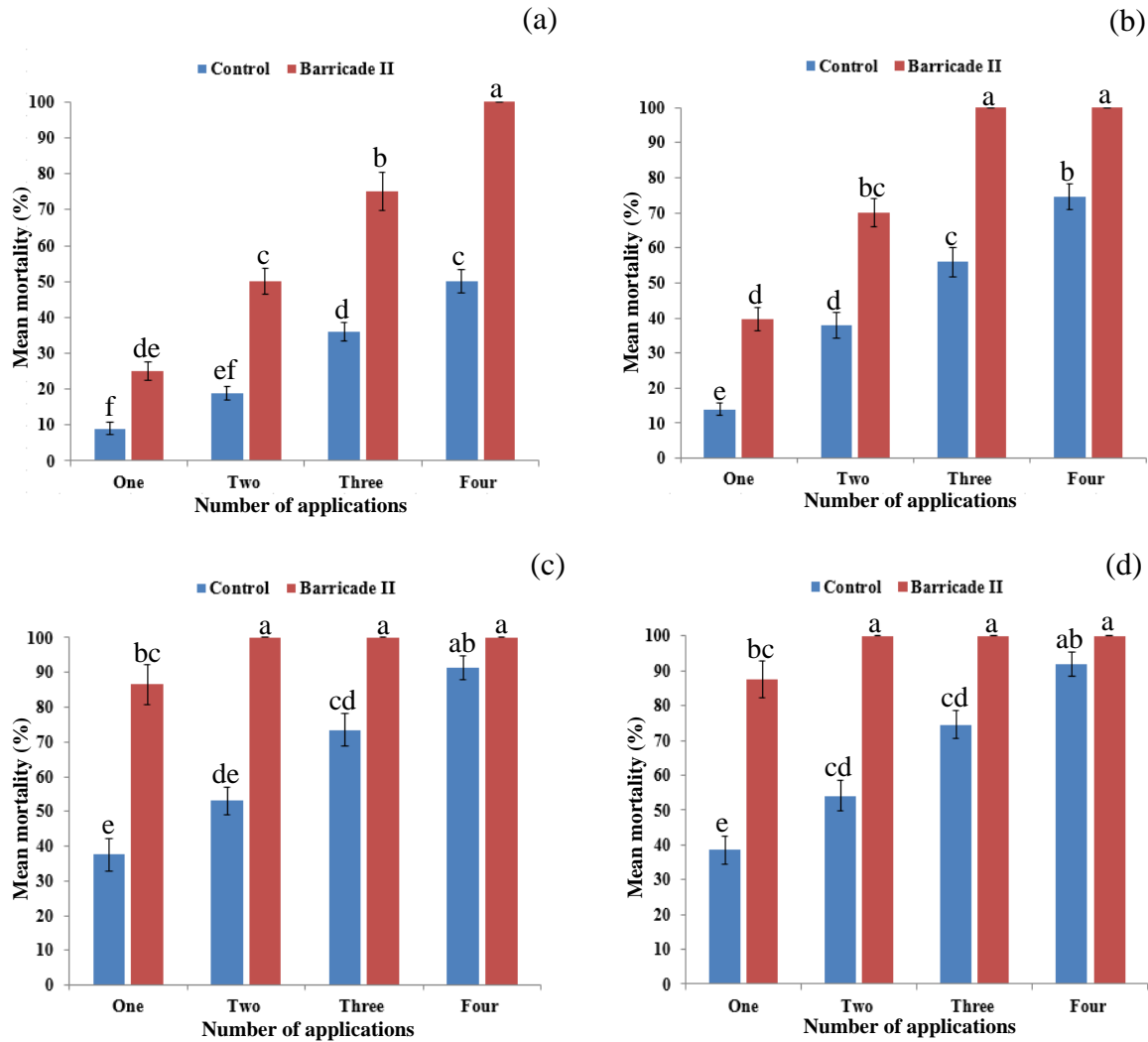
Figure 4-20 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) either in water or water and 1.5 % Barricade® II. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).

In contrast, when *S. carpocapsae* was used at a rate of 60 ± 6 IJs cm^{-2} with 1.5 % Barricade® II against different larval instars at 75 ± 10 % RH (Fig. 4-21), significant differences were observed between numbers of applications against the first larval instar (GLM: $F_{3,\ 24} = 120.71$; $P < 0.05$), the second larval instar (GLM: $F_{3,\ 24} = 65.79$; $P < 0.05$), the third larval instar (GLM: $F_{3,\ 24} = 75.64$; $P < 0.05$) and the fourth larval instar (GLM: $F_{3,\ 24} = 57.28$; $P < 0.05$), with significant differences between control (*S. carpocapsae* suspended in water) and treatment (*S. carpocapsae* suspended in water and 1.5 % Barricade® II) against the first larval instar (GLM: $F_{1,\ 24} = 229.69$; $P < 0.05$), the second larval instar (GLM: $F_{1,\ 24} = 115.31$; $P < 0.05$), the third larval instar (GLM: $F_{1,\ 24} = 69.44$; $P < 0.05$) and the fourth larval instar (GLM: $F_{1,\ 24} = 47.01$; $P < 0.05$). The highest larval mortalities among different numbers of

applications induced by *S. carpocapsae* were in the control after four applications (against the first, second, third and fourth larval instars), and in the treatment after four applications (against the first larval instar) and three and four applications (against the second, third and fourth larval instars) ($P < 0.05$). The significant differences in larval mortality between control and treatment were after one, two, three and four applications (against the first and second larval instars) and after one, two and three applications (against the third and fourth larval instars) ($P < 0.05$) (Fig. 4-21).

At $45 \pm 10\%$ RH (Fig. 4-22), there were significant differences between numbers of applications of *S. carpocapsae* against the first larval instar (GLM: $F_{3, 24} = 62.56$; $P < 0.05$), the second larval instar (GLM: $F_{3, 24} = 57.53$; $P < 0.05$), the third larval instar (GLM: $F_{3, 24} = 38.07$; $P < 0.05$) and the fourth larval instar (GLM: $F_{3, 24} = 50.05$; $P < 0.05$), with significant differences between control and treatment against the first larval instar (GLM: $F_{1, 24} = 151.70$; $P < 0.05$), the second larval instar (GLM: $F_{1, 24} = 141.50$; $P < 0.05$), the third larval instar (GLM: $F_{1, 24} = 310.20$; $P < 0.05$) and the fourth larval instar (GLM: $F_{1, 24} = 312.27$; $P < 0.05$). The highest larval mortalities among different numbers of applications induced by *S. carpocapsae* were in the control after four applications (against the first, second, third and fourth larval instars) ($P < 0.05$), with no significant differences between three and four applications ($P > 0.05$), and in the treatment after four applications (against first, second, third and fourth larval instars) ($P < 0.05$), with no significant differences between three and four applications ($P > 0.05$). The significant differences in mortality between control and treatment were after one, two, three and four applications against the first, second, third and fourth larval instars ($P < 0.05$) (Fig. 4-22).
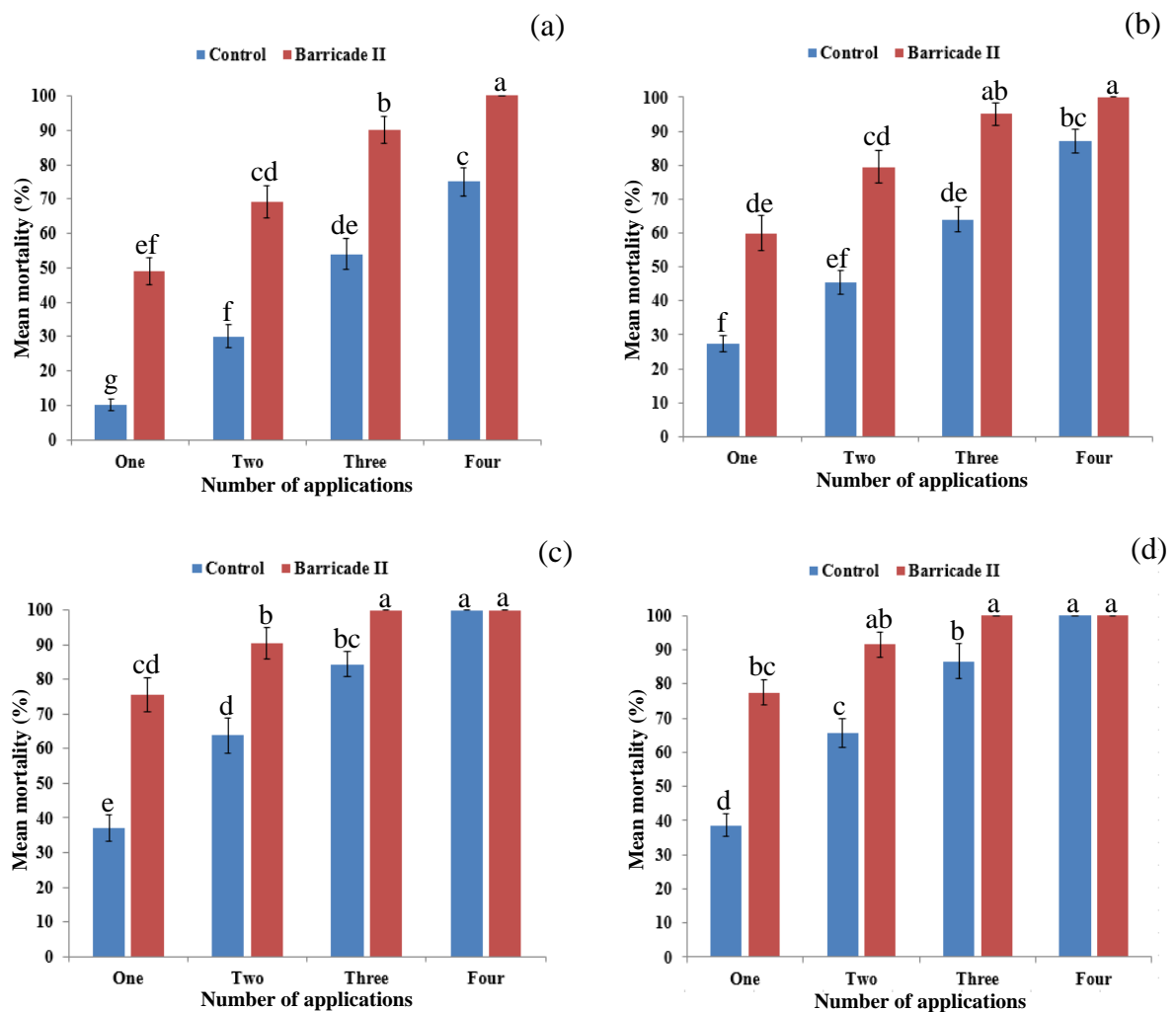
Figure 4-21 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different numbers of applications of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water or water and 1.5 % Barricade® II. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ (n = 4).
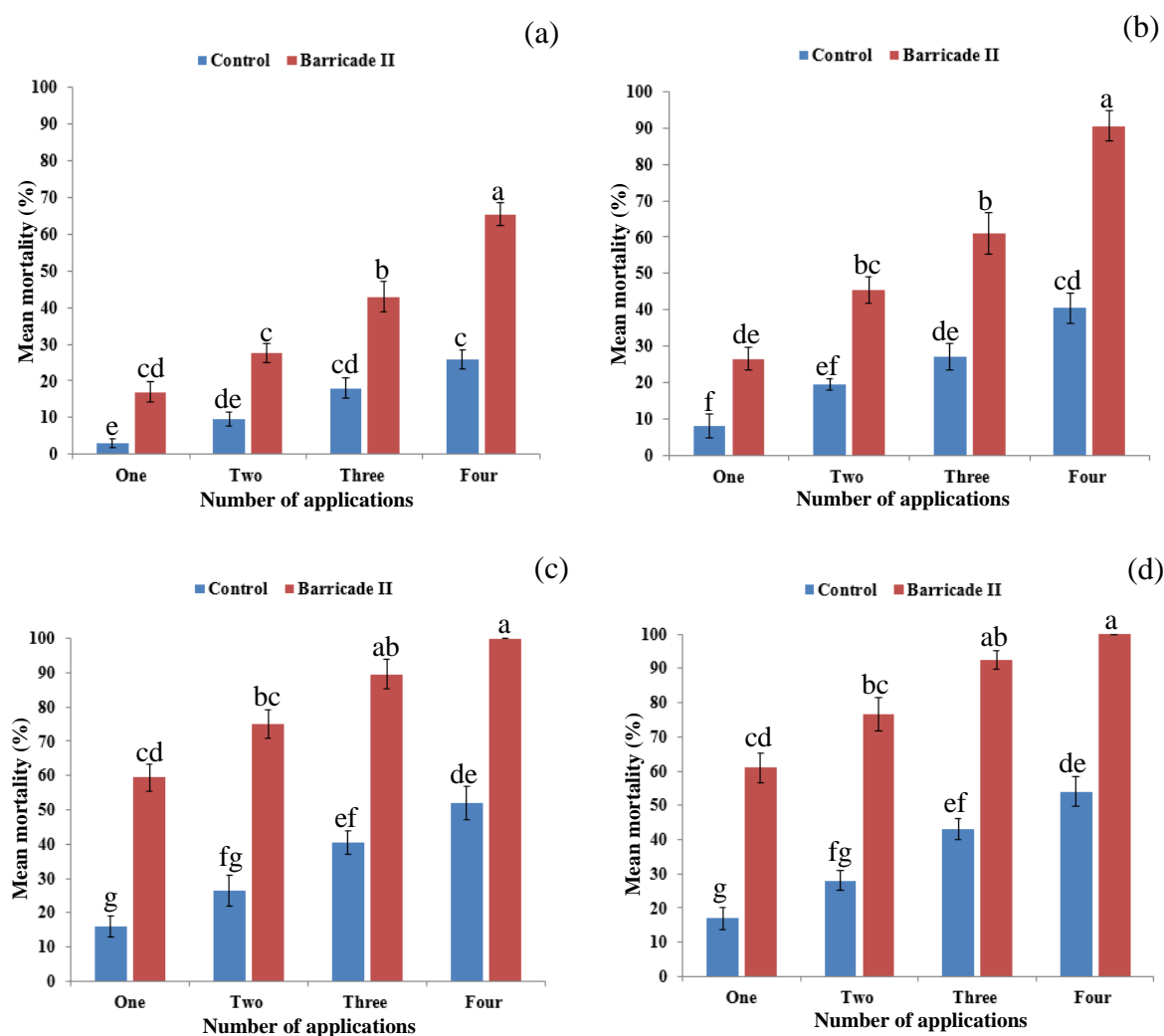
Figure 4-22 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of (a):1st; (b): 2nd; (c): 3rd and (d): 4th larval instars of *T. absoluta* 48 h after different numbers of applications of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water or water and 1.5 % Barricade® II. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

4.4.9 Impact of lower concentrations of Barricade II on effectiveness of different numbers of applications of S. feltiae (60 ± 6 IJs cm^{-2}) against third larval instars of Tuta absoluta on tomato leaves

When *S. feltiae* was used at a rate of 60 ± 6 IJs cm^{-2} with Barricade® II at lower concentrations (0.5 % and 1 %) against the third larval instar at 75 ± 10 % RH, there were significant differences between numbers of applications (GLM: $F_{3, 48} = 37.44$; $P < 0.05$) and different concentrations of Barricade® II (GLM: $F_{3, 48} = 12$; $P < 0.05$). After one application, Barricade® II at a concentration of 1.5 % gave significantly higher larval mortality than at 0.5 % and the control, whereas at 1 % it gave significantly higher larval mortality than the control only ($P < 0.05$). After two, three and four applications, no significant differences were observed between different concentrations including the control ($P > 0.05$) (Fig. 4-23).
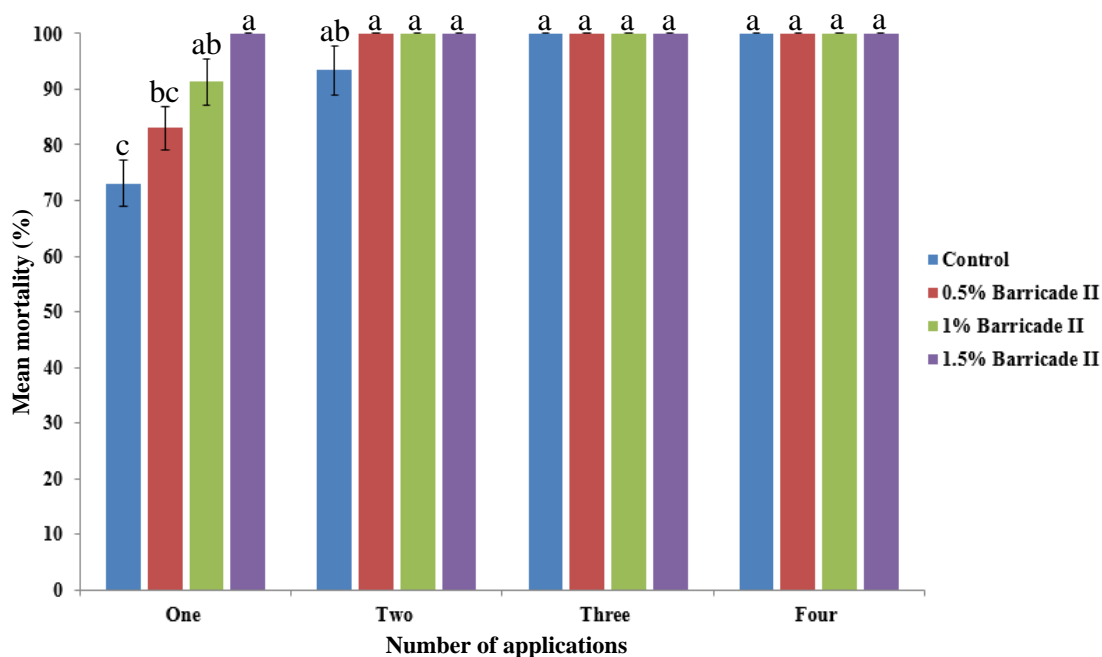
Figure 4-23 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) either in water or water and Barricade® II at different concentrations. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

On the other hand, when *S. feltiae* was used at a rate of 60 ± 6 IJs cm^{-2} with Barricade® II at lower concentrations as mentioned above against the third larval instar at 45 ± 10 % RH, there were significant differences between numbers of applications (GLM: $F_{3, 48}$ = 100.49; *P* < 0.05) and different concentrations of Barricade® II (GLM: $F_{3, 48}$ = 85.41; *P* < 0.05). After one and two applications, Barricade® II at a concentration of 1.5 % gave significantly higher larval mortality than at 0.5 %, at 1 % and the control, while at 1 % it gave significantly higher larval mortality than the control only (*P* < 0.05). After three applications, Barricade® II at concentrations of 1 % and 1.5 % gave significantly higher larval mortality than the other concentrations (*P* < 0.05), whereas after four applications there were no significant differences between different concentrations including the control (*P* > 0.05) (Fig. 4-24).
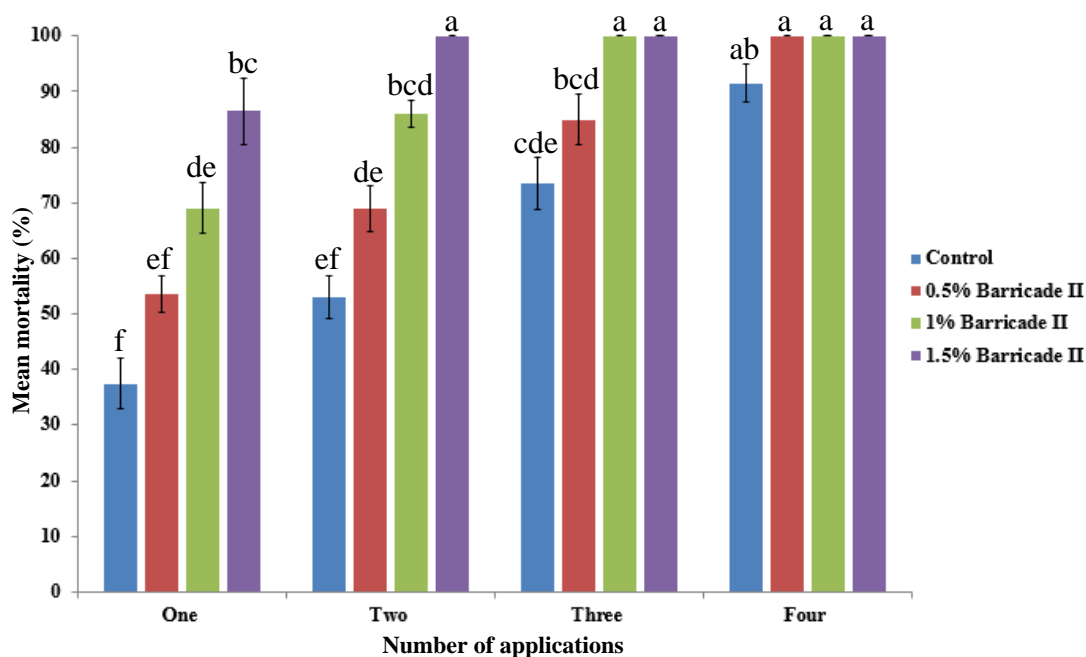
Figure 4-24 Mean mortality at 45 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (60 ± 6 IJs cm^{-2}) either in water or water and Barricade$^{®}$ II at different concentrations. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

4.4.10 Impact of lower concentrations of Barricade II on effectiveness of different numbers of applications of S. feltiae *at lower concentration (30 ± 3 IJs cm^{-2}) against third larval instars of* Tuta absoluta *on tomato leaves*

When *S. feltiae* was used at a lower rate of 30 ± 3 IJs cm^{-2} with Barricade$^{®}$ II at lower concentrations (0.5 % and 1 %) against the third larval instar at 75 ± 10 % RH, there were significant differences between numbers of applications (GLM: $F_{3, 48}$ = 81.42; *P* < 0.05) with significant differences between different concentrations of Barricade$^{®}$ II (GLM: $F_{3, 48}$ = 23; *P* < 0.05). After one application, Barricade$^{®}$ II at a concentration of 1.5 % gave significantly higher larval mortality than at 0.5 % and the control, whereas at 1 % it gave significantly higher larval mortality than the control only (*P* < 0.05). After two, three and four applications, no significant differences were observed between different concentrations including the control (*P* > 0.05), except for the control after two applications, in which larval mortality was significantly lower than with other concentrations (*P* < 0.05) (Fig. 4-25).
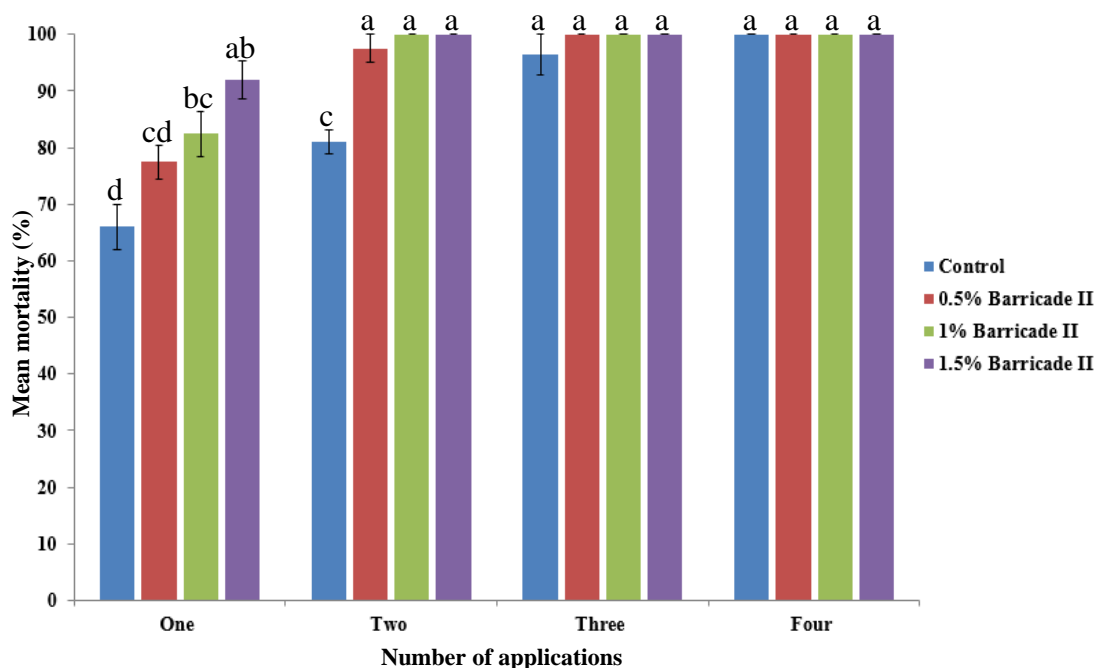
Figure 4-25 Mean mortality at 75 ± 10 % RH and 25 ± 0.5 °C of third larval instar of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (30 ± 3 IJs cm^{-2}) either in water or water and Barricade$^{®}$ II at different concentrations. Bars (mean $\pm$ SE) with the same letter do not differ significantly according to Tukey's multiple range test at $P < 0.05$ ($n = 4$).

In contrast, when *S. feltiae* was used at a lower rate of 30 ± 3 IJs cm^{-2} with Barricade$^{®}$ II at lower concentrations as mentioned above against the third larval instar at 45 ± 10 % RH, there were significant differences between numbers of applications (GLM: $F_{3, 48} = 96.44$; $P < 0.05$) with significant differences between different concentrations of Barricade II (GLM: $F_{3, 48} = 112.81$; $P < 0.05$). After one and three applications, Barricade$^{®}$ II at a concentration of 1.5 % gave significantly higher larval mortality than at 0.5 % and in the control, while at 1 % it gave significantly higher larval mortality than the control only ($P < 0.05$). After two applications 1.5 % Barricade$^{®}$ II gave significantly higher larval mortality than all other concentrations ($P < 0.05$), whereas after four applications, 1 % and 1.5 % Barricade II gave significantly higher larval mortality than the control only ($P < 0.05$) (Fig. 4-26).
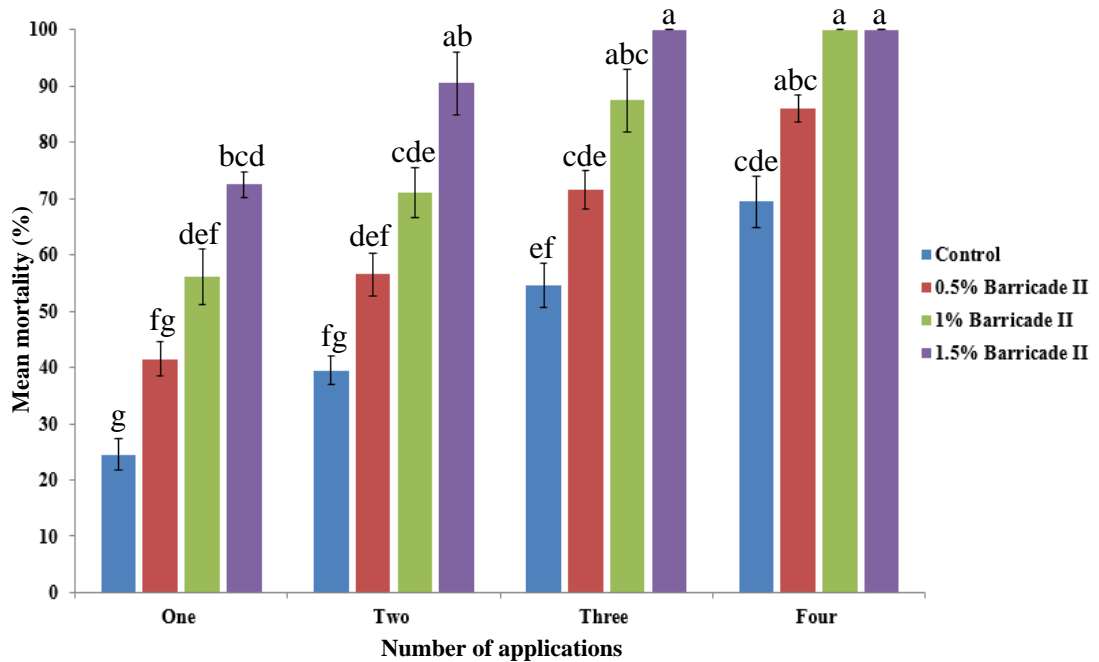
Figure 4-26 Mean mortality at 45 ± 10 % RH and 25± 0.5 °C of third larval instar of *T. absoluta* 48 h after different numbers of applications of *S. feltiae* (30 ± 3 IJs cm^{-2}) either in water or water and Barricade® II at different concentrations. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

4.4.11 Impact of Barricade II on effectiveness of different methods of application of S. feltiae *and* S. carpocapsae

Overall, adding 1.5 % Barricade® II (v/v) to nematode suspensions (treatment) increased nematodes' efficacy in each method of application compared to the control (nematodes suspended in water) with similarity in relative efficacy of the application methods (in inducing larval mortality) between control and treatment, in which two surface treatment was the best application method followed by lower surface treatment then upper surface treatment, with differences in the efficacy between the two nematode species at the two ranges of RH.

When *S. feltiae* was used, there were significant differences between control and treatment (GLM: $F_{1, 18}$ = 47.94; *P* < 0.05) and between methods of application (GLM: $F_{2, 18}$ = 18.20; *P* < 0.05) at 75 ± 10 % RH. These significant differences among application methods were between two surface treatment and upper surface treatment in both control and Barricade II treatment. The highest larval mortality induced by *S. feltiae* was 100 % using two surface treatment (Fig. 4-27 a). At 45 ± 10 % RH, significant differences were observed between

_____

control and treatment (GLM: $F_{1, 18}$ = 102.75; $P < 0.05$) and between methods of application (GLM: $F_{2, 18}$ = 18.72; $P < 0.05$). These significant differences among methods of application were between two surface treatment and upper surface treatment in the Barricade II treatment only, whereas there were no significant differences among methods of application in the control ($P > 0.05$). The highest larval mortality caused by *S. feltiae* was 80.5 % using two surface treatment (Fig. 4-27 b).
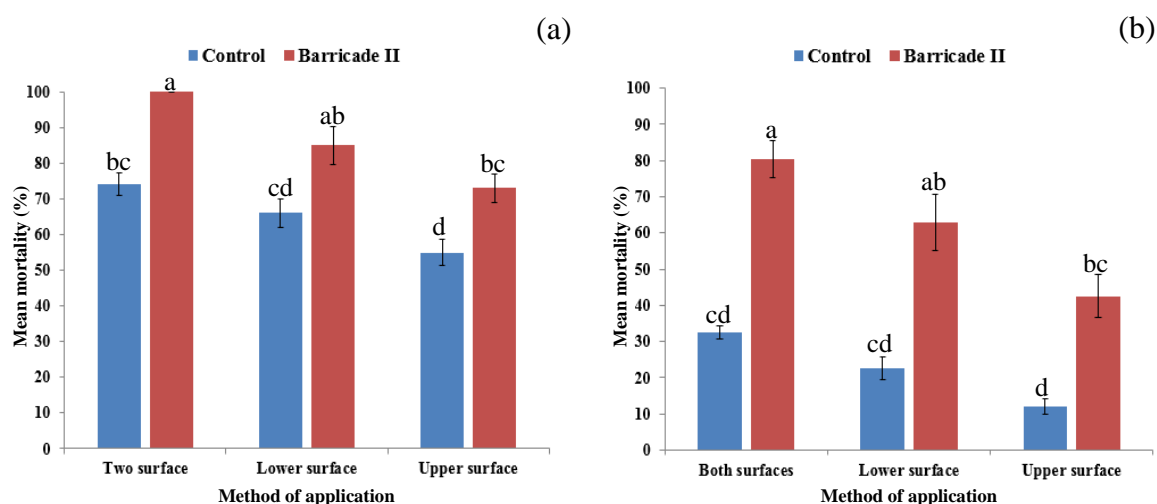
Figure 4-27 Mean mortality of 3rd larval instar of *T. absoluta* 48 h after different methods of application of *S. feltiae* (60 ± 6 IJs cm⁻²) either in water or water and 1.5 % Barricade® II at (a): 75 ± 10 % RH; (b): 45 ± 10 % RH and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

On the other hand, when *S. carpocapsae* was used, there were significant differences between control and treatment (GLM: $F_{1, 18}$ =127.62; $P < 0.05$) and between methods of application (GLM: $F_{2, 18}$ = 10.76; $P < 0.05$) at 75 ± 10 % RH. These significant differences among methods of application were between two surface treatment and upper surface treatment in the Barricade II treatment only, whereas there were no significant differences among methods of application in the control ($P > 0.05$). The highest larval mortality induced by *S. carpocapsae* was 75 % using two surface treatment (Fig. 4-28 a). At 45 ± 10 % RH, there were significant differences between control and treatment (GLM: $F_{1, 18}$ =260.05; $P < 0.05$) and between methods of application (GLM: $F_{2, 18}$ = 43.79; $P < 0.05$). The three methods of application were significantly different from each other in the Barricade II treatment, while there were no significant differences among methods of application in the control ($P > 0.05$). The highest

larval mortality induced by *S. carpocapsae* was 61.5 % using two surface treatment (Fig. 4-28 b).
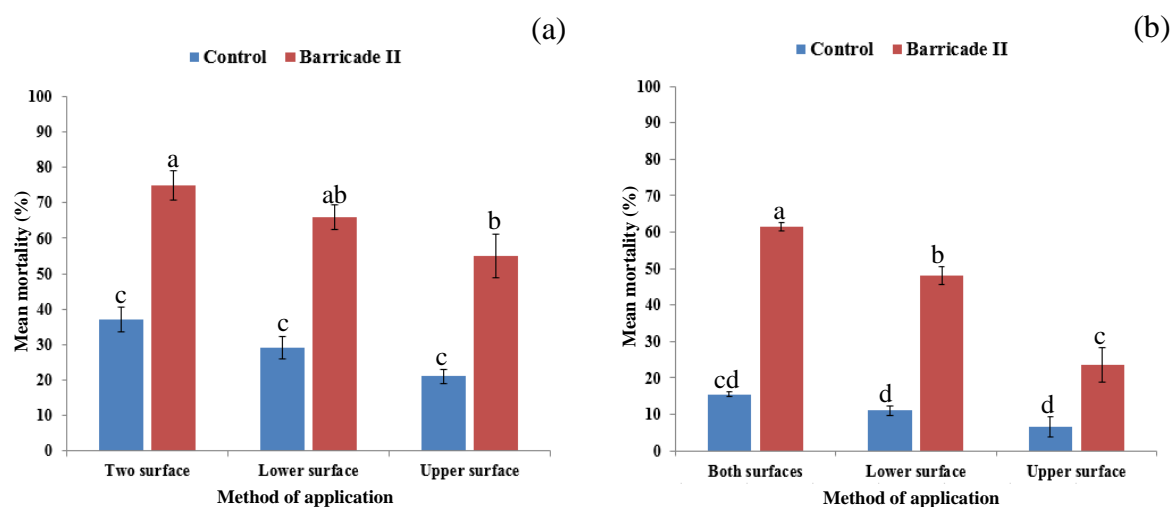
Figure 4-28 Mean mortality of 3rd larval instar of *T. absoluta* 48 h after different methods of application of *S. carpocapsae* (60 ± 6 IJs cm^{-2}) either in water or water and 1.5 % Barricade® II at (a): 75 ± 10 % RH; (b): 45 ± 10 % RH and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

4.4.12 Impact of Barricade® II on the time required by S. feltiae and S. carpocapsae to enter a leaf

Generally, adding 1.5 % Barricade® II (v/v) to nematode suspensions (Treatment) decreased the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf compared to the control (nematodes suspended in water) and increased nematode efficacy at each fixing time at the two ranges of RH.

When *S. feltiae* was used, there were significant differences between times (GLM: $F_{4, 30} = 34.91$; $P < 0.05$) and between control and treatment (GLM: $F_{1, 30} = 249.78$; $P < 0.05$) at 75 ± 10 % RH. These differences among times were between 1 and 3 h and 6, 12 and 24 h in the control and between 1 h and 3, 6, 12 and 24 h in the treatment (Fig. 4-29 a). At 45 ± 10 % RH, significant differences were observed between times (GLM: $F_{4, 30} = 16$; $P < 0.05$) and between control and treatment (GLM: $F_{1, 30} = 425.29$; $P < 0.05$). These differences among times were between 1 h and 3 h and 6, 12 and 24 h in the control and between 1 h and 3 h and 12 and 24 h in the treatment, whereas no significant differences were observed between 6 h and the other times in the treatment ($P > 0.05$) (Fig. 4-29 b).
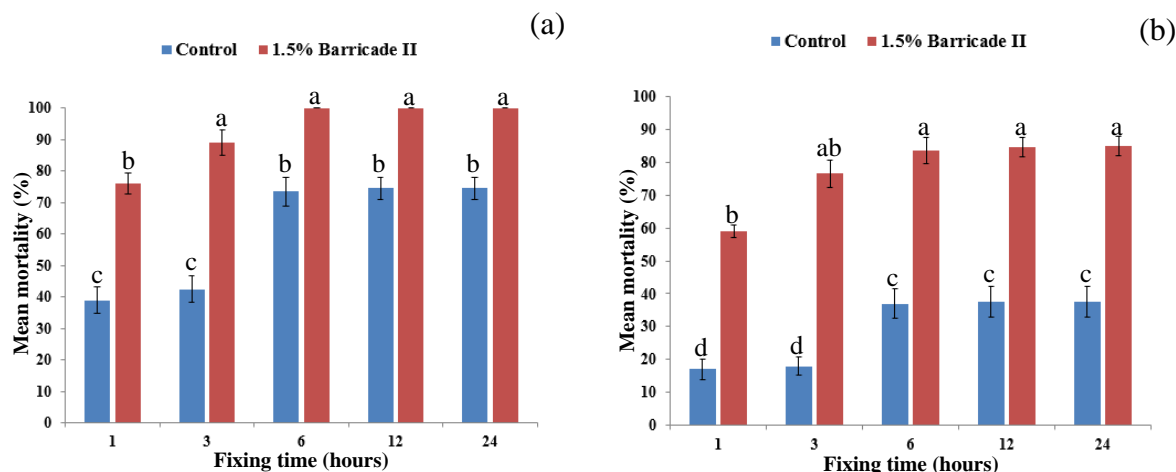
Figure 4-29 Mean mortality of 3rd larval instar of *T. absoluta* after 24 h caused by *S. feltiae* (60 ± 6 IJs cm⁻²) applied either in water or water and 1.5 % Barricade® II and fixed after various times on tomato leaves at (a): 75 ± 10 % RH; (b): 45 ± 10 % RH and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

On the other hand, when *S. carpocapsae* was used, there were significant differences between times (GLM: $F_{4, 30}$ = 38.46; P < 0.05) and between control and treatment (GLM: $F_{1, 30}$ = 317.05; P < 0.05) at 75 ± 10 % RH. These differences among times were between 1, 3 and 6 h and 12 and 24 h in the control and between 1 and 3 h and 6, 12 and 24 h in the treatment (Fig. 4-30 a). At 45 ± 10 % RH, significant differences were observed between times (GLM: $F_{4, 30}$ = 15.70; P < 0.05) and between control and treatment (GLM: $F_{1, 30}$ = 319.18; P < 0.05). These differences among times were between 1 and 3 h and 6, 12 and 24 h in the treatment (Fig. 4-30 b).
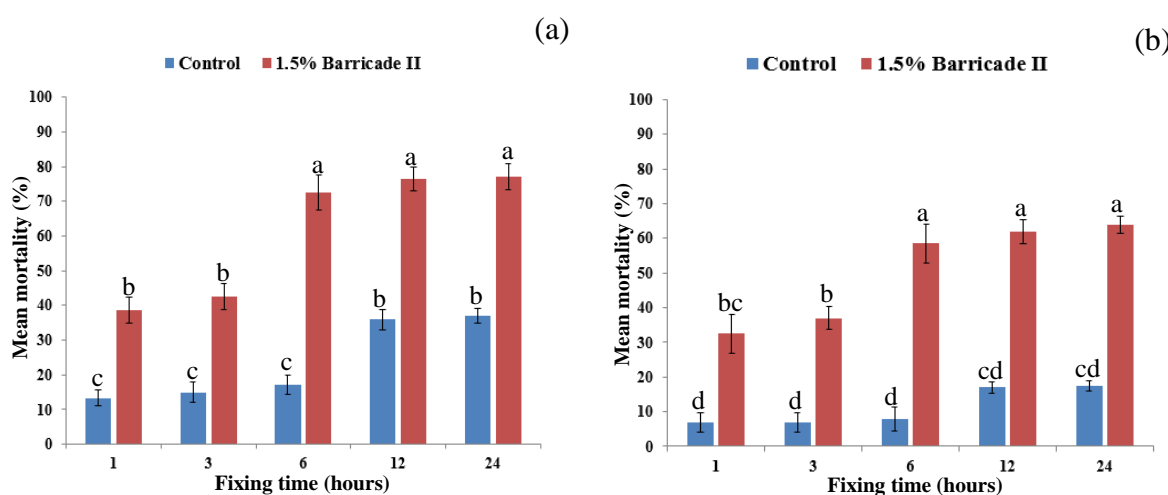
Figure 4-30 Mean mortality of 3rd larval instar of *T. absoluta* after 24 h caused by *S. carpocapsae* (60 ± 6 IJs cm⁻²) applied either in water or water and 1.5 % Barricade® II and fixed after various times on tomato leaves at (a): 75 ± 10 % RH; (b): 45 ± 10 % RH and 25 ± 0.5 °C. Bars (mean ± SE) with the same letter do not differ significantly according to Tukey's multiple range test at P < 0.05 (n = 4).

_____

4.5 Discussion

The findings of the current study revealed that Barricade® II and xanthan gum were the best two adjuvants in increasing nematode efficacy against larvae of *T. absoluta* on tomato leaves at the two ranges of RH (45 ± 10 % and 75 ± 10 % RH) by providing moisture to the nematodes, which enables them to survive, move, seek and invade *T. absoluta* larvae inside the mines, and that the efficacy of *S. feltiae* was superior to that of *S. carpocapsae*. Concentrations of 1.5 % Barricade® II and 1 % xanthan gum were the best two in increasing nematode efficacy significantly compared to the control and other concentrations of adjuvants used (Figs. 4-3 – 4-10). Using a higher concentration than 1.5 % of Barricade® II was not possible as the mixture became too thick and not sprayable using the hand sprayer. The increase in nematode efficacy when Barricade® II was used is similar to results reported by Shapiro-Ilan *et al.* (2010), who observed a significant increase in the efficacy of *S. carpocapsae* "All" strain in above ground application on barks of trees against the lesser peachtree borer, *Synanthedon pictipes*, using Barricade® II. The authors reported that a treatment of *S. carpocapsae* suspended in water followed by a post-application covering of Barricade® II induced 70 % and 100 % mortality of *Synanthedon pictipes*. In contrast, Lacey *et al.* (2010) reported that aqueous suspensions of *S. feltiae* or *S. carpocapsae* followed by a post-application covering of Barricade® II applied to cardboard bands infested with diapausing cocooned codling moth larvae and stapled onto trunks of apple trees resulted in only 22 % and 18.7 % mortality for *S. feltiae* and 27 % and 34.7 % mortality for *S. carpocapsae* in two trials. The low efficacy obtained by Lacey *et al.* (2010) was attributed to the insect pest species under examination and their habitats (borer larvae inside galleries where conditions are relatively moist due to frass and tree exudates at openings of galleries versus cocooned larvae on cardboard bands that are susceptible to drying).

The results of the present study also revealed that application of nematode-xanthan gum formulations against the third larval instar at 75 ± 10 % RH resulted in 89.5 % and 100 % mortality for *S. feltiae* and 56 % and 76 % mortality for *S. carpocapsae* by addition of 0.3 % and 1 % xanthan gum to nematode suspensions respectively (Figs. 4-3 and 4-5). Application at 45 ± 10 % RH resulted in 53 % and 83 % mortality for *S. feltiae* and 36 % and 59 % mortality for *S. carpocapsae* by addition of 0.3 % and 1 % xanthan gum to nematode suspensions respectively (Figs. 4-4 and 4-6). In comparison, results obtained by Schroer *et al.* (2005a) using *S. carpocapsae* with 0.3 % xanthan gum and 0.3 % surfactants against larvae of

_____

_____

the diamondback moth (DBM) *Plutella xylostella* showed mortality of 63 % and 71 % at 60 % and 80 % RH respectively in single cabbage leaf disk bioassays using 24-well plates. In another study, Schroer and Ehlers (2005) reported DBM larval mortality of 60 % and 80 % at 60 % and 80 % RH respectively when *S. carpocapsae* was used with surfactant-polymer formulation (containing 0.3 % xanthan gum and 0.3 % Rimulgan surfactant) in single cabbage leaf bioassays. This low efficacy of *S. carpocapsae* could be due to the target pest species and their habitats as well as to the differences in the foraging strategy of the two nematode species. For example, larvae of *Plutella xylostella* are active, feed on leaf surfaces and do not produce mines (Talekar and Shelton, 1993). Their behaviour makes them a good target, while they are moving on the leaf surfaces, for the ambush forager *S. carpocapsae* (which exploits a 'sit and wait' strategy), which is known to pursue mobile insects close to the surface of the soil or substrate (Gaugler *et al.*, 1980; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997; Griffin *et al.*, 2005). On the other hand, larvae of *T. absoluta*, which are less active, enter leaves, stems or fruits and produce mines and galleries where they feed and develop (USDA–APHIS, 2011). Therefore, it requires a cruiser forager nematode which actively moves through the medium to locate insects that do not move and those which move slowly by detecting the volatiles and carbon dioxide released by the insects (Gaugler *et al.*, 1980; Grewal *et al.*, 1993; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997; Griffin *et al.*, 2005). *S. feltiae* in this regard is an intermediate forager that employs both ambush and cruise foraging strategy (Gaugler *et al.*, 1980; Grewal *et al.*, 1994a; Campbell and Gaugler, 1997). Consequently, it is more effective in finding the larvae of *T. absoluta* inside the mines than *S. carpocapsae*. This explanation is supported by the results obtained from the investigation of the time required by nematodes to enter a leaf, which showed that *S. feltiae* required less time (3 h at > 90 % RH and 6 h at 45 ± 10 % and 75 ± 10 % RH) to enter a leaf and induce significant higher larval mortality than *S. carpocapsae* (6 h at > 90 % RH and 12 h at 45 ± 10 % and 75 ± 10 % RH) when both nematode species were suspended in water (Figs. 3-17 and 3-18). Furthermore, the high efficacy of the intermediate forager *S. feltiae* compared to the ambush forager *S. carpocapsae* against a sedentary insect pest was also reported by Lacey *et al.* (2010), who applied aqueous suspensions of *S. feltiae* or *S. carpocapsae* followed by a post-application of wood flour foam to cardboard bands infested with diapausing cocooned codling moth larvae and stapled onto trunks of apple tree. They found significantly higher mortality for S. *feltiae* (65.3 % and 97.4 %) than *S. carpocapsae* (45.4 % and 85.5 %) in two different trials. Moreover, Williams and Walters (2000) reported that a single application of *S.*

_____

feltiae (Nemasys®) (5000 IJs/ ml) at > 90 % RH induced mean mortality of 82 % of the leafminer *Liriomyza huidobrensis* in trials on lettuce in a commercial greenhouse. This was higher than the results obtained by Broadbent and Olthof (1995), who reported 61 % and 75 % mortality of the leafminer *Liriomyza trifolii* caused by *S. carpocapsae* "All" and "Pye" strain respectively at > 90 % RH in similar field trials carried out on chrysanthemum using double the concentration (10,000 IJs/ ml) used by Williams and Walters (2000).

Although xanthan gum increased nematode efficacy at lower concentrations than Barricade® II, there are some disadvantages which could preclude xanthan gum from being used with nematodes for foliar applications and make Barricade® II a better candidate for this purpose. The first disadvantage is that xanthan gum is hardly soluble in cold water and needs intensive stirring in order to dissolve; additionally, its solubility decreases as the concentration increases. Although the solubility problem of xanthan gum was solved by the addition of sodium hydrogen carbonate and oxalic acid (both at 0.3 %) to the formulation (Schroer *et al.*, 2005a), the current study showed that adding these two chemicals to nematode–xanthan gum formulation had a detrimental effect on survival and infectivity of *S. feltiae* (using concentrations of 0.3 % and 1 %) and on survival and infectivity of *S. carpocapsae* (using a concentration of 1 %). The second disadvantage is that at concentrations of 0.3 % and 1 % (at which xanthan gum increased nematode efficacy), the spray mixture becomes too thick and not sprayable using the hand sprayer, therefore this could happen with other sprayers used for nematode application under greenhouse and field conditions. For instance, van Niekerk and Malan (2015) did not evaluate xanthan gum at a concentration of 0.3 % in their research because the suspension did not pass through spray nozzles as it became too thick. The third disadvantage is that xanthan gum is nutritious to microbes and can encourage the growth of plant pathogens. On the other hand, even though Barricade® II contains a superabsorbent polymer suspended in vegetable oil (commonly used for home cooking and salad dressing) and two stabilisers, the first being an emulsifier in chocolate and the second a thickener in salad dressing, its use with nematodes as a spray on edible products such as tomato still needs to be authorised by national authorities in countries where it is going to be used. Barricade® II is the only liquid fire gel concentrate that has been approved for use by the U.S. Forest Service that it is free of toxic nonylphenol ethoxylates (NPEs), ammonium phosphates and petroleum distillate oils and has received the Champion award from the US Environmental Protection Agency (EPA) (http://firegel.com/EPA_Champion.aspx).

Nematodes require a thin water film to survive and move on the leaf surface freely to locate and enter the mines where larvae reside, thus high RH is required for successful foliar application of nematodes (Glazer and Navon, 1990; Glazer, 1992; Broadbent and Olthof, 1995; Koppenhoefer, 2000; Wright *et al.*, 2005). Desiccation, which is accelerated by low RH level, is a critical factor that affects nematode survival and mobility and prevents nematodes locating and parasitising larvae of *T. absoluta* inside the mines. The current work showed that Barricade® II and xanthan gum increased nematode survival significantly compared to the control at the two ranges of RH and that *S. carpocapsae* is more tolerant to desiccation compared to S. *feltiae* and survived longer (Figs. 4-13 - 4-18). In the same way, Bélair *et al.* (2003) reported a significant increase in *S. carpocapsae* "All" strain survival compared to water only by the addition of Corn Oil (0.9 %, 1.8 %, 3.6 %), Leafshield (3.0 %, 6.0 %, 12.0 %), Seaweed (0.1 %) and Agral (0.05 %) after 12 h exposure on cabbage leaves at 70 % RH. Correspondingly, Glazer (1992) recorded a significant increase in *S. carpocapsae* "Mexican" strain survival by the addition of antidesiccant "Folicote" (6 % w/w) after 6 h exposure in a Petri dish at 60 % RH (38-60 % increase in survival) and 80 % RH (10-20 % increase in survival) but not at 45 % RH. However, the addition of 1.5 % Barricade® II and 1 % xanthan gum in the current work increased survival of *S. feltiae* and *S. carpocapsae* significantly compared to the control at 45 ± 10 % and 75 ± 10 % RH even after 48 h exposure (Figs. 4-.15and 4-18). Additionally, adding 1.5 % Barricade® II to aqueous nematode suspension decreased the time required by *S. feltiae* and *S. carpocapsae* to enter a leaf compared to the control (aqueous nematode suspension without Barricade II) and increased nematode efficacy at each fixing time at the two ranges of RH. *S. feltiae* required less time to enter the leaves than *S. carpocapsae* (Figs. 4-29 and 4-30). For example, at 45 ± 10 % RH, the time required by S. *feltiae* in the control to enter the leaves and to induce significant larval mortality (37 %) was 6 h and its survival on leaves at this time (6 h) was 19 %. Addition of 1.5 % Barricade® II decreased the time required to enter the leaves and to induce significant larval mortality (77 %) to 3 h and increased its survival after 6 h to 48 %. The time required by *S. carpocapsae* in the control to enter the leaves and to induce significant larval mortality (17 %) was 12 h and its survival on leaves at this time (12 h) was 25 %. Addition of 1.5 % Barricade® II reduced the time required to enter the leaves to induce significant larval mortality (59 %) to 6 h increased its survival after this time (6 h) to 65 %. It is recommended to apply nematodes in the evening to provide high RH during the night (Gaugler and Boush, 1978). Therefore, since nematode-Barricade® II formulation increased nematode survival and reduced the time required by

_____

nematodes to enter the leaves, this will give the nematodes enough time and enable them to enter the leaves and gain protection after application before they are killed through exposure to more detrimental environmental conditions during the day.

The addition of adjuvants to nematode suspensions thickened and increased the adhesion of the suspension by reducing the surface tension of droplets (Beck *et al.*, 2013), and thus reduced the loss of nematodes into the soil by run-off by depositing the nematodes onto the leaves (Fig. 4-11). Barricade® II at concentrations of 1 % and 1.5 % and xanthan gum at concentrations of 0.3 % and 1 % were able to increase the number of *S. feltiae* and S. *carpocapsae* on leaves significantly compared to the control. These results match those observed by Schroer *et al.* (2005b), who reported a decrease in nematode loss on cabbage leaves from 70 % to less than 23 % when 0.2 % or 0.3 % xanthan gum was mixed with nematodes and spreading agent in a tank suspension. In contrast, the recent results are not in line with those reported by Beck *et al.* (2013), who reported that the addition of 0.3 % xanthan gum and some adjuvants (spreaders) to nematode suspensions did not increase the number of nematodes deposited on cauliflower leaf discs. A possible explanation for this may be that tomato leaves have different leaf surface characteristics (hairy leaves) compared to cabbage and cauliflower leaves (waxy leaves), which enable them to hold more nematode suspension as well as making them have less run-off. However, van Niekerk and Malan (2015) found that a combination of two adjuvants, Nu-Film-P® and Zeba®, significantly increased the average number of *H. zealandica* deposited on 2 cm^2 leaf discs of citrus (waxy leaves).

Sedimentation time is an important factor which should be considered when applying nematodes. Nematodes settle to the bottom of spray tanks when they are in aqueous suspension, causing uneven distribution in applications (Schroer *et al.*, 2005b; Brusselman *et al.*, 2010; van Niekerk and Malan, 2015). The results of the present investigation showed that the addition of adjuvants retarded sedimentation of nematodes in the suspension (Fig. 4-12). Without Barricade® II and xanthan gum more than 60 % of *S. feltiae* passed below a depth of 2 cm just five minutes after the stirring had stopped in the control and the Stick-it Organic treatment. No nematodes were recorded at 2 cm after 60 minutes. When Barricade® II (at 1 % and 1.5 %) and xanthan gum (at 0.3 % and 1 %) were added to the suspension, no signs of sedimentation were noticed after 1 h. Adding these adjuvants to a nematode suspension will secure an even distribution of nematodes in the spray tank and consequently even numbers of

_____

nematodes in spray droplets. These findings are consistent with those reported by Schroer *et al.* (2005b), who found that the addition of 0.05 % xanthan gum to *S. carpocapsae* suspension was effective at retarding sedimentation, with 70 % of the initial nematode number still in suspension after 1 h. The recent results are also in agreement with the results mentioned by van Niekerk and Malan (2015), who reported that 0.2 % xanthan gum was highly effective at retarding sedimentation, with 72 % of the initial nematode number still in suspension after 1 h.

The present investigations also showed that Barricade® II and xanthan gum decreased the LC_{50} and the LT_{50} of both nematode species significantly compared to the control (nematode suspended in water) at the two ranges of RH (Tables 4-5 – 4-8). The decrease in the LC_{50} reduces the number of nematodes required per square centimetre to kill the larvae, therefore ensuring high larval mortality when nematodes were used at a rate of 60 ± 6 IJs cm^{-2}, whereas the decrease in the LT_{50} from about 48 and 72 hours to about 24 hours can significantly reduce the damage to leaves caused by larvae, especially in cases of high infestations. The results obtained here using xanthan gum are in agreement with those obtained by Schroer *et al.* (2005a), who reported a decrease in LT_{50} of *S. carpocapsae* from 42 hours to 21 hours using 0.3 % xanthan gum and surfactant against *Plutella xylostella* at 80 % RH in single cabbage leaf disk bioassays using 24-well plates.

When Barricade® II and xanthan gum were tested at the best concentrations (1.5 % and 1 % respectively) to investigate their potential to increase the efficacy of *S. feltiae* and *S. carpocapsae*, results showed a significant increase in the efficacy of the two nematode species (Figs. 4-9 and 4-10). First and second larval instars are less susceptible to nematode infections (discussed in Chapter 2 of this thesis). Experiments to investigate the effect of number of applications with 1.5 % Barricade® II on nematode efficacy (Figs. 4-19 – 4-22) showed that *S. feltiae* was more efficient than *S. carpocapsae* and required fewer applications to induce significant mortality. First and second larval instars required additional applications compared to third and fourth larval instars. From these findings, it can thus be suggested that to reduce control costs and achieve high larval mortality when controlling *T. absoluta*, it is important to target the most susceptible larval instars (third and fourth). Williams and Walters (2000) reported that a 24 h repeat application of a dose of 1000 *S. feltiae*/ml at < 90 % RH induced higher leafminer *(Chromatomyia syngenesiae)* mortality than repeat applications at 48 h and 72 h and they attributed this to the differences in susceptibility of larval instars present at the

_____

time of application. Young larvae produce smaller amount of attractants such as CO_2 and kairomones than old larvae, which makes it more difficult for nematodes to locate them inside the mines (Kaya, 1985). If the nematodes are deposited away from the entrance of the mines, it might be more difficult for them to locate these attractants. Therefore, the increase in nematode efficacy can be attributed to the increase in nematode density and coverage on leaf surfaces after each application, which enabled them to locate larvae inside the mines by depositing them near the entrances of these mines so that they parasitised the remaining live larvae.

When Barricade® II was used at lower concentrations (0.5 % and 1 % v/v) to test the impact of repeat applications of *S. feltiae* (at a rate of 60 ± 6 IJs cm^{-2}) against the third larval instar, results showed that at 75 ± 10 % RH one application of 1 % Barricade® II was as effective as one application of 1.5 % Barricade® II in increasing mortality compared to the control and gave equal mortality to other repeat applications (two, three and four). In contrast, 0.5 % Barricade® II did not give a significant increase in mortality compared to the control with any number of applications (Fig. 4-23). At 45 ± 10 % RH, although one and two repeat applications of 1 % Barricade® II induced significantly higher larval mortality compared to the control, it required three repeat applications to induce equal mortality to 1.5 % Barricade® II. Again 0.5 % Barricade® II did not give any significant increase in mortality compared to the control with any number of applications (Fig. 4-24). When a similar experiment was carried out with *S. feltiae* at a lower rate (30 ± 3 IJs cm^{-2}) against third larval instars, results showed similar trends to those obtained above using the higher nematode rate (Figs. 4-25 and 4-26). This result is in agreement with those obtained by Williams and Walters (2000), who reported that a repeat application of a lower dose (1000 *S. feltiae*/ ml) to first instar larvae of the leafminer *Liriomyza bryoniae* was as effective as applying a higher concentration (5000 *S. feltiae*/ ml). Therefore, we can say that 1.5 % Barricade® II is the best concentration to be used with *S. feltiae* at a rate of 30 ± 3 IJs cm^{-2} to control *T. absoluta*, especially when RH is low (< 75 %), whereas a concentration of 1 % Barricade® II with *S. feltiae* at a rate of 30 ± 3 IJs cm^{-2} would give good control if the RH is quite high (≥ 75 %).

When the impact of Barricade® II (1.5 %) on method of application was tested, results showed that, at the two ranges of RH, the addition of this adjuvant to nematode suspensions increased the efficacy of *S. feltiae* and *S. carpocapsae* using each method of application (spraying upper leaf surface, spraying lower leaf surface or spraying both leaf surfaces), with

_____

S. feltiae more effective than *S. carpocapsae* in inducing larval mortality (Figs. 4-27 and 4-28). Two surface treatments did not give significantly higher mortality than lower surface treatment, whereas two surface treatment did give significantly higher mortality than upper surface treatment. Although the differences between two surface treatment and lower surface treatment were not significant they were consistent. For example, at 75 ± 10 % RH, the mortality induced by *S. feltiae* using two surface treatment and lower surface treatment was 100 % and 85 % respectively, whereas at 45 ± 10 % RH, mortality was 81 % and 63 % respectively. The reason behind the high efficacy of two surface treatment and lower surface treatment compared to upper surface treatment was discussed in Chapter 3 of this thesis. From these results we can conclude that spraying and covering both leaf surfaces is important to achieve high larval mortality of *T. absoluta,* therefore the sprayer nozzle should be set at the right angle and at the right height to ensure complete coverage of the leaves. Choosing the right sprayer and the right nozzle can also be important in this regard (Beck *et al.*, 2013; Beck *et al.*, 2014).

In conclusion, enhancing nematode survival on leaf surfaces by adding adjuvants to nematode suspensions (nematode-adjuvant formulation) improves the effectiveness of entomopathogenic nematodes for the control of above-ground insect pests such as leafminers and other foliage-feeding insect pests whether alone or within an IPM program. The addition of Barricade® II to a nematode suspension increases nematode efficacy by producing ideal conditions for nematode host seeking and invasion, increases nematode survival, reduces the time required by nematodes to enter a leaf, reduces loss of nematodes into the soil by run-off by depositing them on the leaf by lowering the surface tension and increasing the adhesion of nematode suspension, assures an even distribution of nematodes by preventing them from settling in the spray tank and decreases the LC_{50} and the LT_{50} of nematodes. Yet to our knowledge, this study is the first to report the use of Barricade® II as a single application (nematode and Barricade® II mixed together). The alternative is two applications (nematode followed by adjuvant). Although the viability of Barricade® II in this study has been tested in whole leaf bioassays, which provide more realistic conditions in relation to the natural environment and under conditions unfavourable to EPNs (low RH), further research is required to determine whether this nematode-adjuvant formulation is viable to increase nematode efficacy under greenhouse and field conditions. If it is effective, we may be able to substitute or reduce the use of chemical insecticides and accordingly prevent or delay

development of strains of *T. absoluta* resistant to chemical insecticides or to Bt products, as has happened in other foliage-feeding lepidopteran insect pests (Tabashnik *et al.*, 1990; Liu *et al.*, 1995; 1996; Janmaat and Myers, 2003).

Chapter 5 General discussion and recommendations and future work

5.1 General discussion

In earlier studies the susceptibility of different stages of *Tuta absoluta* in different habitats (leaves and soil) to particular EPN species/strains has been tested (Batalla-Carrera *et al.*, 2010; Jacobson and Martin, 2011; Garcia-del-Pino *et al.*, 2013), and mortality levels varied depending on factors such as the susceptibility of target stage, virulence of nematode species/strains and relative humidity (RH) when EPNs were used against larvae on leaves. Therefore, the current investigation was started to test the potential use of some commercial EPNs (which are distributed as biological agents by BASF plc to the UK, USA, Canada, New Zealand and many other countries in Europe) for *T. absoluta* control from three different viewpoints: (i) Testing the efficacy of these EPNs against different stages of *T. absoluta* and determining the most virulent species/strains and the most susceptible stage (Chapter 2); (ii) Studying some factors affecting EPN's efficacy against larvae of *T. absoluta* on tomato leaves (Chapter 3); and (iii) evaluating adjuvants for their potential to increase EPNs' efficacy on tomato leaves against larvae of *T. absoluta* in unfavourable conditions (Low RH) (Chapter 4).

Pathogenicity levels of EPN species/strains differ against different insect pests (Williams and Walters, 2000; Bélair *et al.*, 2003; Andalo *et al.*, 2010; Barbosa-Negrisoli *et al.*, 2010) and against different stages of a given insect (Kaya, 1985; Journey and Ostlie, 2000; Giustolin *et al.*, 2001b; Bélair *et al.*, 2003). According to the present study (Chapter 2), efficacy of commercial EPN species/strains against larvae of *T. absoluta* on leaves varied. *Steinernema feltiae* was the most virulent species followed by *S. carpocapsae* then *Heterorhabditis bacteriophora.* Third and fourth larval instars were more susceptible to nematode infection than first and second larval instars. Furthermore, the difference in the foraging behaviour of the nematode species (i.e., cruisers vs. ambushers) affects their invading ability against larvae on leaves. The intermediate forager *S. feltiae* was more effective than the ambusher forager *S. carpocapsae* (Chapter 2, 3 and 4). In other investigations on foliar applications of EPNs against other leafminer pests, results showed that the intermediate forager *S. feltiae* (5000 IJs/ ml) was more effective (82 % efficacy) against *Liriomyza huidobrensis* in trials on lettuce at a commercial greenhouse (Williams and Walters, 2000) compared to the ambush forager *S. carpocapsae* "All" strain (10,000 IJs/ ml) against *Liriomyza trifolii* (61 % efficacy) in similar

field trials carried out on chrysanthemum (Broadbent and Olthof, 1995). Nevertheless, in our study, high efficacy (up to 100 %) of *S. feltiae* was obtained using a 2,100 IJs/ ml concentration only compared to the two mentioned studies. Species of the cruiser forager *Heterorhabditis* were reported to induce low leafminer (*Liriomyza trifolii*) mortality on leaves of bean compared to steinernematid species due to their poor survival on leaves (Hara *et al.*, 1993). Moreover, in soil bioassays, *S. feltiae* and *S. carpocapsae* were significantly more virulent than *H. bacteriophora* against fourth instar larvae of *T. absoluta* when they drop on the soil to pupate and against adults when they emerge from pupae (infected during the period of time before they emerge from the soil), and fourth larval instar and adults were significantly more susceptible than pupae (Chapter 2). Therefore, in order to control this pest, foliar and soil applications of these EPNs would be two good approaches to consider. The larvae on leaves can be controlled by the foliar application whereas the surviving larvae that pupate in the soil as well as the emerging adults from the soil can be controlled by the soil application. Applying EPNs to the soil to control adults when they are emerging from pupae has already been suggested by Garcia-del-Pino *et al.* (2013) to control this pest using Spanish strains of EPNs and by Kaya and Grieve (1982) to control the beet armyworm *S. exigua* (Lepidoptera: Noctuidae). Even though the present study tested available commercial EPN species, the possibility of finding more effective EPN species/strain is not excluded. Results of the current work suggest that future screening should focus on EPN species/strains that are virulent, have cruiser or intermediate foraging strategy and are tolerant to desiccation and high temperature (Chapter 3).

The efficacy of EPNs depends on various factors. The most important factor is RH. For example, keeping the leaf surface moist at least for a few hours after application is very important (Chapter 3). This is to enable EPNs to survive, move, seek and invade *T. absoluta* larvae and gain protection from detrimental environment conditions inside the mines. This was achieved under conditions unfavourable to EPNs (low RH) through the addition of Barricade® II and xanthan gum to aqueous suspensions of EPNs in whole leaf bioassays (Chapter 4). Similarly, Shapiro-Ilan *et al.* (2010) reported a significant increase in the efficacy of *S. carpocapsae* "All" strain when applications of this nematode strain were followed by a post-application covering of Barricade® II for control of the lesser peach tree borer, *Synanthedon pictipes* (Grote & Robinson) on bark of peach trees. Although we used the same adjuvant that these authors used, our experiment is different from theirs in that we mixed the

adjuvant with aqueous nematode suspensions. In this work, we proved that the nematodes were able to move out of the gel when Barricade® II was used at the highest possible concentration (1.5 %). To our knowledge, this study is the first to report using Barricade® II mixed with EPNs in a single application (Chapter 4). In the same way, xanthan gum mixed with Rimulgan surfactant (both at 0.3 % concentration) was reported to increase the efficacy of *S. carpocapsae* against larvae of the diamondback moth (DBM) *Plutella xylostella* in single cabbage leaf disks and single cabbage leaf bioassays (Schroer and Ehlers, 2005; Schroer *et al.*, 2005a).

When EPN suspension is sprayed on leaf surfaces, the droplets containing nematodes are either retained or deflected. The result depends on the droplets' physical properties (Tadros, 1987) and plant factors such as roughness of the leaf surface, the ability of the leaf surface to repel the water (hydrophobicity of the surface) and leaf orientation (De Ruiter *et al.*, 1990). The addition of Barricade® II and xanthan gum to aqueous suspensions of EPNs in the current study resulted in thickening and increasing the adhesion of the suspension by reducing the surface tension of droplets (Beck *et al.*, 2013). This reduced the loss of nematodes into the soil by run-off through depositing the nematodes onto the leaves, and accordingly increased the efficacy. By the same token, the addition of adjuvants (e.g. surfactants) to spray solutions applied on leaf surfaces that are rough or reflective such as pea and barley was reported to improve the mixture retention (Anderson *et al.*, 1987). Furthermore, adding xanthan gum and a spreading agent to aqueous suspension of EPNs increased their retention on cabbage leaves (Schroer *et al.*, 2005b). Also, van Niekerk and Malan (2015) found that a combination of two adjuvants, Nu-Film-P® and Zeba®, significantly increased the average number of *H. zealandica* deposited on leaf discs of citrus. On the other hand, the addition of Barricade® II and xanthan gum to aqueous suspensions of EPNs will increase the efficacy by securing an even distribution of nematodes in the spray tank and consequently even numbers of nematodes in spray droplets as well as minimise the need for agitating the tank mixture (Chapter 4). These results are consistent with those of Schroer *et al.* (2005b) and van Niekerk and Malan (2015) who reported that xanthan gum was highly effective at retarding sedimentation of EPNs.

Another factor that affected nematodes' efficacy was temperature. EPN species vary in their performance according to the temperature (Griffin, 1993; Grewal *et al.*, 1994b). Normally, nematodes become inactivated at low temperature (<10-15 °C) and high temperature (> 30-40

_____

°C) (Glazer, 2002). Results of the present investigation revealed that both nematode species induced similar mortality at 25 °C and that *S. feltiae* was more virulent at lower temperatures (15 and 20 °C), while *S. carpocapsae* was more virulent at higher temperatures (30 and 35 °C) (Chapter 3). Since the results of the current study showed that *S. feltiae* is significantly more effective than *S. carpocapsae* especially under unfavourable conditions (Chapter 3 and 4)*,* it seems that its use as a biological agent against this pest will be limited to countries that have a temperate climate conditions. In countries where temperature is high, *S. feltiae* will not be effective. For this reason it is important to look for and find other EPN species/strains (indigenous or commercially available) that perform better at high temperature for their potential as biological agents against *T. absoluta* in these countries and others with similar climate conditions.

An additional factor that influenced EPNs' efficacy was number of applications. Findings of the recent work revealed that the efficacy of EPNs increased with the increase in the number of applications, and that first and second larval instars required additional applications compared to third and fourth larval instars due to their low susceptibility to nematode infection (discussed in Chapters 2, 3 and 4). From these results, it can be suggested that it is more useful to target the most susceptible larval instars (third and fourth) in order to achieve a high level of mortality, but in reality this is not possible. In natural infestations, different larval instars will be present. Therefore, multiple applications of EPNs must be undertaken targeting to the period when the highest proportion of third and fourth instar larvae are present (if EPNs are going to be the only method for control). EPNs can also be combined with other biological control agents that are effective against young larval instars and eggs of *T. absoluta* such as *Bacillus thuringiensis* (Bt), parasitoids and predators (Giustolin *et al.*, 2001b; Desneux *et al.*, 2010; Gonzalez-Cabrera *et al.*, 2011; Zappalà *et al.*, 2013). For example, a commercial formulation, based on *Bacillus thuringiensis* (Bt), was found to be highly effective in reducing damage caused by first, second and third larval instars of *T. absoluta* and first larval instars showed highest susceptibility followed by second then third larval instars (Gonzalez-Cabrera *et al.*, 2011). So, using this formulation to target first and second larval instars and *S. feltiae* to target third and fourth larval instars would increase the efficacy in less time with fewer applications of EPNs. This can save time and money. Furthermore, larval parasitoids from families such as Eulophidae, Braconidae, Bethylidae and Ichneumonidae and predators from families such as Miridae, Anthocoridae, Nabidae, Coccinellidae, Araneidae

_____

_____

and Vespidae as well as the egg parasitoids *Trichogramma* spp. (Desneux *et al.*, 2010; Zappalà *et al.*, 2013) can also be combined with EPNs to increase the efficacy and might reduce costs compared to using multiple applications of EPNs only.

In order to reduce the cost of control, it might be useful to decrease the amount of adjuvant used and EPNs as well as the number of applications. For this purpose, experiments were carried out using different concentrations of the best adjuvant Barricade® II (0.5 %, 1 % and 1.5 %) with the best nematode species *S. feltiae* (60 ± 6 IJs cm^{-2}) in conditions unfavourable to EPNs (low RH) against third larval instars (Chapter 4). The outcomes of these experiments showed that at 75 ± 10 % RH, one application of 1 % Barricade® II was as effective as one application of 1.5 % Barricade® II in inducing significantly greater mortality compared to the control and equal mortality to other numbers of applications (two, three and four), whereas at 45 ± 10 % RH, two applications of 1.5 % Barricade® II was the best option. When a similar experiment was carried out with *S. feltiae* at a lower rate (30 ± 3 IJs cm^{-2}), results showed similar trends to those obtained above using the higher nematode rate. Equally, other studies reported that a repeat of a lower dose (1000 *S. feltiae*/ ml) was as effective against first instar larvae of the leafminer *Liriomyza bryoniae* as applying a higher concentration (5000 *S. feltiae*/ ml) (Williams and Walters, 2000). The low nematode rate used in the current study, 30 ± 3 IJs cm^{-2} (1050 ± 105 IJs ml^{-1}), was nearly equal to the concentration of the same nematode species (1000 IJs ml^{-1} *S. feltiae* (Nemasys®)) used by Jacobson and Martin (2011) against larvae of *T. absoluta* in two separate trials undertaken in commercial organic tomato crops in Portugal at 65-74 % RH without the addition of adjuvants to the nematode suspension. The results of these trials showed only 50 % and 43 % overall mean mortality in the first and second trial respectively. The authors also stated that treatment costs were comparable for EPNs and the bioinsecticide Spinosad. In comparison with our results, we achieved 83 % larval mortality at the same RH (75 ± 10 %) using one application, but at lower RH (45 ± 10 %) we required two applications, in which the cost would be higher compared to treatment with Spinosad. However, reliance on the use of Spinosad might be not a good option since resistance of this pest to this bioinsecticide was reported in Brazil and Chile (Reyes *et al.*, 2012; Gontijo *et al.*, 2013; Campos *et al.*, 2014). Besides, Spinosad was reported to have negative impacts on the most promising biological control agents of *T. absoluta* in the Mediterranean Basin (the predators *Nesidiocoris tenuis* and *Macrolophus pygmaeus* (Hemiptera: Miridae)) (Arno and Gabarra, 2011) and on adults and pupae of the

_____

_____

parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) (Biondi *et al.*, 2013) as well as on many other natural enemies (Biondi *et al.*, 2012b). This highlights the need for alternative effective safe control methods such as EPNs. Therefore, we can say that if EPNs are going to be the only method for control, 1.5 % Barricade® II is the best concentration to be used with *S. feltiae* at a rate of 30 ± 3 IJs cm^{-2} to control *T. absoluta* especially when RH is low (< 75 %), whereas a concentration of 1 % Barricade® II with *S. feltiae* at a rate of 30 ± 3 IJs cm^{-2} would give acceptable control if the RH is quite high ($\geq$ 75 %). The precise number of applications that might result in effective control in naturally infested tomato crops under greenhouse and field conditions will definitely depend on level of infestation together with other factors mentioned here. Thus, further investigations should be undertaken to determine this.

One more factor that affected infectivity of EPNs was the method of application. The results of the present study showed that spraying nematode suspension on both leaf surfaces gave the highest larval mortality followed by spraying nematodes on lower then upper leaf surface (Chapter 3 and 4). These findings suggest that it is important to spray and cover both leaf surfaces, especially the lower leaf surface, in order to attain the highest possible larval mortality. So, the sprayer nozzle must be set at the right angle and at the right height to ensure complete coverage of the leaves. Choosing the right sprayer and the right nozzle can also be important in this regard (Beck *et al.*, 2013; Beck *et al.*, 2014). However, in order to determine the best method of application, the right sprayer and the right nozzle to be used, more in-depth investigations are needed under greenhouse and field conditions to evaluate the impact of these issues on the infectivity of EPNs.

Although xanthan gum increased nematode efficacy at lower concentrations than Barricade® II, there are some disadvantages which could preclude xanthan gum from being used with nematodes for foliar applications and make Barricade® II a better candidate for this purpose. The first disadvantage is that xanthan gum is hardly soluble in cold water and needs intensive stirring in order to dissolve. Additionally, its solubility decreases as the concentration increases. The second disadvantage is that at concentrations of 0.3 % and 1 % (at which xanthan gum increased nematode efficacy), the spray mixture becomes too thick and not sprayable using the hand sprayer, therefore this could happen with other sprayers used for nematode application under green house and field condition. For instance, van Niekerk and Malan (2015) did not evaluate xanthan gum at a concentration of 0.3 % in their research

_____

because the suspension did not pass through spray nozzles as it became too thick. The third disadvantage is that xanthan gum is nutritious to microorganisms and might encourage the growth of plant pathogens. On the other hand, even though Barricade® II contains a superabsorbent polymer suspended in vegetable oil (commonly used for home cooking and salad dressing) and two stabilisers, the first being an emulsifier in chocolate and the second a thickener in salad dressing, its use with nematodes as a spray on edible products such as tomato still needs to be authorized by national authorities in countries where it is going to be used. However, Barricade® II is the only liquid fire gel concentrate that has been approved for use by the U.S. Forest Service that it is free of toxic nonylphenol ethoxylates (NPEs), ammonium phosphates and petroleum distillate oils and has received the Champion award from the US Environmental Protection Agency (EPA) (http://firegel.com/EPA_Champion.aspx).

In conclusion, the commercial EPN species tested in this investigation were effective in killing the larvae of *T. absoluta* on foliage, the fourth instar larvae as they were pupating in the soil and the adults during their emergence from soil. Moreover, all factors studied in the current work affected nematodes' efficacy to a certain extent. Environment factors among these factors are difficult to control and to manipulate, thus extra attention should be paid to these factors when applying nematodes under greenhouse and field conditions. Amongst all factors, RH was the most important, reducing nematode survival and efficacy significantly as it decreased. The addition of Barricade® II to nematode suspension increases nematode efficacy by producing ideal conditions for nematode host seeking and invasion, increases nematode survival, reduces the time required by nematodes to enter a leaf, reduces loss of nematodes into the soil by run-off by depositing them on the leaf by lowering the surface tension and increasing the adhesion of nematode suspension, assures an even distribution of nematodes by preventing them from settling in the spray tank and decreases the LC_{50} and the LT_{50} of nematodes. Although the viability of Barricade® II in this study has been tested in whole leaf bioassays, which provide conditions similar to the natural environment and under unfavourable conditions to EPNs (low RH) under laboratory conditions (due to quarantine restrictions), further research is required to determine whether this nematode-adjuvant formulation is able to increase nematode efficacy under greenhouse and field conditions where other factors are present and may interact. If it is effective, we may be able to substitute or reduce the use of chemical insecticides and accordingly prevent or delay development of

_____

strains of *T. absoluta* resistant to chemical insecticides or to Bt products, as has happened in other foliage-feeding lepidopteran insect pests (Tabashnik *et al.*, 1990; Liu *et al.*, 1995; 1996; Janmaat and Myers, 2003).

5.2 Recommendations and future work

- In this work we demonstrated that the addition of the adjuvant Barricade® II to aqueous suspensions of EPNs was very effective in increasing survival and efficacy of EPNs under unfavourable conditions (low RH) in whole leaf bioassays. Further work is required to determine whether this EPN-adjuvant formulation is viable to increase EPN efficacy under greenhouse and field conditions, where other factors (mentioned in Chapter 3) are present and may interact.

- *S. feltiae* was the most effective EPN species, but its efficacy was optimum at a temperature range between 15 and 25 °C. Further research is needed to test and screen for EPN species/strains that are virulent, have cruiser or intermediate foraging strategy and are tolerant to desiccation and high temperature.

- In the current study, we tested the efficacy of EPNs alone. Future investigations can be carried out using EPNs with other biological control agents such as Bt and entomopathogenic fungi in order to test their efficacy and to find out how they interact with each other (additively, synergistically or antagonistically).

_____

Chapter 6 References

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G., Chermiti, B. and Zappala, L. (2015) 'Combined Non-Target Effects of Insecticide and High Temperature on the Parasitoid Bracon nigricans', *Plos One*, 10(9).

Abbes, K., Harbi, A. and Chermiti, B. (2012a) 'Comparative study of 2 protection strategies against *Tuta absoluta* (Meyrick) in late open field tomato crops in Tunisia', *EPPO Bulletin*, 42(2), pp. 297-304.

Abbes, K., Harbi, A. and Chermiti, B. (2012b) 'The tomato leafminer *Tuta absoluta* (Meyrick) in Tunisia: Current status and management strategies', *EPPO Bulletin*, 42(2), pp. 226-233.

Adams, B.J. and Nguyen, K.B. (2002) 'Taxonomy and Systematics', in Gaugler, R. (ed.) *Entomopathogenic Nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 1-33.

Al-Zaidi, S. (2010) 'Manejo de *Tuta absoluta* mediante feromonas', *Phytoma España: La revista profesional de sanidad vegetal*, (217), p. 41.

Alatorre-Rosas, R. and Kaya, H.K. (1990) 'Interspecific competition between entomopathogenic nematodes in the genera Heterorhabditis and Steinernema for an insect host in sand', *Journal of Invertebrate Pathology*, 55(2), pp. 179-188.

Alatorre-Rosas, R. and Kaya, H.K. (1991) 'Interaction between two entomopathogenic nematode species in the same host', *Journal of Invertebrate Pathology*, 57(1), pp. 1-6.

Alavanja, M.C.R. and Bonner, M.R. (2012) 'Occupational pesticide exposures and cancer risk: A review', *Journal of Toxicology and Environmental Health-Part B-Critical Reviews*, 15(4), pp. 238-263.

Altieri, M.A. and Nicholls, C.I. (2003) 'Agroecology and the Search for a Truly Sustainable Agriculture, first ed'. University of California, Berkeley.

Ameixa, O., Almeida, L., Goncalves, A. and Neto, L. (2007) 'Feeding behavior of Liriomyza huidobrensis (Blanchard) and L-trifolii (Burgess) adults on bean leaves', *Journal of Insect Behavior*, 20(1), pp. 137-155.

Ames, B.N., Profet, M. and Gold, L.S. (1990) 'Nature's chemicals and synthetic chemicals: comparative toxicology', *Proceedings of the National Academy of Sciences*, 87(19), pp. 7782-7786.

Andalo, V., Santos, V., Moreira, G.F., Moreira, C.C. and Moino, A. (2010) 'Evaluation of entomopathogenic nematodes under laboratory and greenhouses conditions for the control of Spodoptera frugiperda', *Ciencia Rural*, 40(9), pp. 1860-1866.

Anderson, N.H., Hall, D.J. and Seaman, D. (1987) 'Spray retention: effects of surfactants and plant species', *Aspects of Applied Biology*, 14:233-243.

Ansari, M.A., Shah, F.A. and Butt, T.M. (2008) 'Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus*, control', *Entomologia Experimentalis Et Applicata*, 129(3), pp. 340-347.

Ansari, M.A., Shah, F.A. and Butt, T.M. (2010) 'The entomopathogenic nematode Steinernema kraussei and *Metarhizium anisopliae* work synergistically in controlling overwintering larvae of the black vine weevil, *Otiorhynchus sulcatus*, in strawberry growbags', *Biocontrol Science and Technology*, 20(1), pp. 99-105.

Ansari, M.A., Tirry, L. and Moens, M. (2004) 'Interaction between *Metarhizium anisopliae* CLO 53 and entomopathogenic nematodes for the control of *Hoplia philanthus*', *Biological Control*, 31(2), pp. 172-180.

Apablaza, J. (1992) 'La polilla del tomate y su manejo ', *Tattersal*, 79, pp. 12-13.

Araujo-Gonçalves, A. (2010) 'Alverde: la solución BASF para el control de *Tuta absoluta*', *Phytoma España*, 217, pp. 21-22.

Arno, J. and Gabarra, R. (2011) 'Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae)', *Journal of Pest Science*, 84(4), pp. 513-520.

Arthurs, S., Heinz, K.M. and Prasifka, J.R. (2004) 'An analysis of using entomopathogenic nematodes against above-ground pests', *Bulletin of Entomological Research*, 94(4), pp. 297-306.

ASTM Standards E1519 'Standard Terminology Relating to Agricultural Tank Mix Adjuvants' *Annual Book of ASTM Standards*. ASTM International, West Conschohocken, PA, www.astm.org.

Astor, E. (2010) 'Estrategias DuPont en el control de *Tuta absoluta*', *Phytoma España*, 217, pp. 107-111.

Bahamondes, L.A. and Mallea, A.R. (1969) 'Biología en Mendoza de *Scrobipalpula absoluta* (Meyrick) Povolny (Lepidoptera: Gelechiidae), especie nueva para la República Argentina', *Rev Fac Cs Agrarias*, 15(1), pp. 96-104.

Baniameri, V. and Cheraghian, A. (2012) 'The first report and control strategies of *Tuta absoluta* in Iran', *EPPO Bulletin*, 42(2), pp. 322-324.

Barbercheck, M.E. and Kaya, H.K. (1990) 'Interactions between *Beauveria bassiana* and the entomogenous nematodes, *Steinernema feltiae* and *Heterorhabditis heliothidis*', *Journal of Invertebrate Pathology*, 55(2), pp. 225-234.

Barbosa-Negrisoli, C.R.C., Negrisoli, A.S., Dolinski, C. and Bernardi, D. (2010) 'Efficacy of entomopathogenic nematodes (Nemata: Rhabditida) to control Brazilian apple leafroller Bonagota salubricola (Meyrick, 1937) (Lepidoptera: Tortricidae)', *Crop Protection*, 29(11), pp. 1274-1279.

Bari, M.A. and Kaya, H.K. (1984) 'Evaluation of the entomogenous nematode *Neoaplectana carpocapsae* (=*Steinernema feltiae*) Weiser (Rhabditida:Steinernematidae) and the bacterium *Bacillus thuringiensis* Berliner var. kurstaki for suppression of the artichoke plume moth (Lepidoptera:Pterophoridae)', *Journal of Economic Entomology*, 77(1), pp. 225-229.

Barona, H., Parra, A. and Vallejo, C.F.A. (1989) 'Evaluación de especies silvestres de Lycopersicon sp, como fuente de resistencia a *Scrobipalpula absoluta* (Meyrick) y su intento de transferencia a *Lycopersicon esculentum* Mill', *Acta agronômica*, 39(1-2), pp. 34-45.

Barrientos, Z.R., Apablaza, H.J., Norero, S.A. and Estay, P. (1998) 'Temperatura base y constante térmica de desarrollo de la polilla del tomate, *Tuta absoluta* (lepidoptera: gelechiidae)', *1998*, 25(3), p. 5.

Barros, E.C., Bacci, L., Picanco, M.C., Martins, J.C., Rosado, J.F. and Silva, G.A. (2015) 'Physiological selectivity and activity reduction of insecticides by rainfall to predatory wasps of *Tuta absoluta*', *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, 50(1), pp. 45-54.

Batalla-Carrera, L., Morton, A. and Garcia-del-Pino, F. (2010) 'Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions', *BioControl (Dordrecht)*, 55(4), pp. 523-530.

Baur, M.E., Kaya, H.K., Gaugler, R. and Tabashnik, B. (1997) 'Effects of adjuvants on entomopathogenic nematode persistence and efficacy against *Plutella xylostella*', *Biocontrol Science and Technology*, 7(4), pp. 513-525.

Baur, M.E., Kaya, H.K., Tabashnik, B.E. and Chilcutt, C.F. (1998) 'Suppression of diamondback moth (Lepidoptera : Plutellidae) with an entomopathogenic nematode (Rhabditida : Steinernematidae) and *Bacillus thuringiensis* Berliner', *Journal of Economic Entomology*, 91(5), pp. 1089-1095.

Baur, M.E., Kaya, H.K. and Thurston, G.S. (1995) 'Factors affecting entomopathogenic nematode infection of *Plutella xylostella* on a leaf surface', *Entomologia Experimentalis Et Applicata*, 77(3), pp. 239-250.

Beck, B., Brusselman, E., Nuyttens, D., Moens, M., Pollet, S., Temmerman, F. and Spanoghe, P. (2013) 'Improving foliar applications of entomopathogenic nematodes by selecting adjuvants and spray nozzles', *Biocontrol Science and Technology*, 23(5), pp. 507-520.

Beck, B., Brusselman, E., Nuyttens, D., Moens, M., Temmerman, F., Pollet, S., Van Weyenberg, S. and Spanoghe, P. (2014) 'Improving the biocontrol potential of entomopathogenic nematodes against Mamestra brassicae: effect of spray application technique, adjuvants and an attractant', *Pest Management Science*, 70(1), pp. 103-112.

Becker, V.O. (1984) 'Gelechiidae. In: Atlas of Neotropical Lepidoptera. Checklist: Part 1 Micropterigoidea-Immoidea. W. Junk. 112 pp.'.

Bedding, R. (1993) 'Biological control of Sirex noctilio using the nematode *Deladenus siricidicola*', in Bedding, R., Akhurst, R. and Kaya, H.K. (eds.) *Nematodes and the biological control of insect pests*. East Melbourne, Victoria, Australia: CSIRO Publishing. 173 p., pp. 11-20.

Begley, J.W. (1990) 'Efficacy against insects in habitats other than soil', in Gaugler, R. and Kaya, H.K. (eds.) *Entomopathogenic nematodes in biological control.* Boca Raton, FL: CRC Press, pp. 215-231.

Bélair, G., Fournier, Y. and Dauphinais, N. (2003) 'Efficacy of Steinernematid Nematodes Against Three Insect Pests of Crucifers in Quebec', *Journal of Nematology*, 35(3), pp. 259-265.

Belair, G., Vincent, C., Lemire, S. and Coderre, D. (1999) 'Laboratory and field assays with entomopathogenic nematodes for the management of oblique banded leafroller Choristoneura rosaceana (Harris) (Tortricidae)', *Journal of Nematology*, 31(4), pp. 684-689.

Bélair, G., Wright, D.J. and Curto, G. (2005) 'Vegetable and Tuber Crop Applications', in Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as biocontrol agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 255-264.

Bellini, L.L. and Dolinski, C. (2012) 'Foliar application of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) for the control of *Diatraea saccharalis* in greenhouse', *Semina-Ciencias Agrarias*, 33(3), pp. 997-1003.

Benvenga, S.R., Fernandes, O.A. and Gravena, S. (2007) 'Decision making for integrated pest management of the South American tomato pinworm based on sexual pheromone traps', *Horticultura Brasileira*, 25(2), pp. 164-169.

Best, G.A. and Ruthven, A.D. (1995) *Pesticides -- developments, impacts and controls*. Cambridge, UK: Royal Society of Chemistry. 180 pp.

Biondi, A., Desneux, N., Siscaro, G. and Zappala, L. (2012a) 'Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*', *Chemosphere*, 87(7), pp. 803-812.

Biondi, A., Mommaerts, V., Smagghe, G., Vinuela, E., Zappala, L. and Desneux, N. (2012b) 'The non-target impact of spinosyns on beneficial arthropods', *Pest Management Science*, 68(12), pp. 1523-1536.

Biondi, A., Zappala, L., Stark, J.D. and Desneux, N. (2013) 'Do Biopesticides Affect the Demographic Traits of a Parasitoid Wasp and Its Biocontrol Services through Sublethal Effects?', *Plos One*, 8(9) [Online]. Available at: <Go to ISI>://WOS:000325423500164 DOI: 10.1371/journal.pone.0076548 (Accessed: Sep).

Bogorni, P.C., Silva, R.A.d. and Carvalho, G.S. (2003) 'Consumo de mesofilo foliar por *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechidae) em três cultivares de *Lycopersicon esculentum* Mill', *Ciência Rural*, 33(1), pp. 07-11.

Bolckmans, K. (2009) *Integrated pest management of the exotic invasive pest Tuta absoluta*. In: International Biocontrol Manufacturers Association and Research Institute of Organic Agriculture, *Proceedings of the 4th Annual Biocontrol Industry Meeting Internationals, Lucerne, Switzerland*.

Botto, E.N. (1999) 'Control biológico de plagas hortícolas en ambientes protegidos', *Rev. Soc. Entomol. Argent*, 58, pp. 58-64.

Branco, M.C., F. H. França, M. A. Medeiros and J. G. T. Leall. (2001) 'Use of insecticides for controlling the South American tomato pinworm and the diamondback moth: a case study', *Horticultura Brasileira*, 19(1), pp. 60-65.

Brattsten, L.B., Holyoke Jr, C.W., Leeper, J.R. and Raffa, K.F. (1986) 'Insecticide resistance: Challenge to pest management and basic research', *Science*, 231(4743), pp. 1255-1260.

Brevault, T., Sylla, S., Diatte, M., Bernadas, G. and Diarra, K. (2014) '*Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): a new threat to tomato production in sub-Saharan Africa', *African Entomology*, 22(2), pp. 441-444.

Broadbent, A.B. and Olthof, T.H.A. (1995) 'Foliar Application of *Steinernema carpocapsae* (Rhabditida: Steinernelnatidae) To Control *Liriomyza trifolii* (Diptera: Agromyzidae) Larvae in Chrysanthemums', *Environmental Entomology*, 24(2), pp. 431-435.

Brusselman, E., Moens, M., Steurbaut, W. and Nuyttens, D. (2010) 'Evaluation of hydraulic, pneumatic and mechanical agitation for the spray application of Steinernema carpocapsae (Rhabditida: Steinernematidae)', *Biocontrol Science and Technology*, 20(4), pp. 339-351.

Buhl, O., Falck, P., Karsholt, O., Larsen, K. and Vilhelmsen, F. (2010) 'Records of microlepidoptera from Denmark in 2009 (Lepidoptera)', *Entomologiske meddelelser*, 78(2), pp. 101-116.

Cabanillas, H.E., Wright, R.J. and Vyas, R.V. (2005) 'Cereal, Fibre, Oilseed and Medicinal Crop Applications', in Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 265-279.

Cabello, T., Gallego, J.R., Fernandez, F.J., Gamez, M., Vila, E., Del Pino, M. and Hernandez-Suarez, E. (2012) 'Biological Control Strategies for the South American Tomato Moth (Lepidoptera: Gelechiidae) in Greenhouse Tomatoes', *Journal of Economic Entomology*, 105(6), pp. 2085-2096.

Cáceres, S. (1992) 'La polilla del tomate en Corrientes. Biología y control', *Estación Experimental Agropecuaria Bella Vista,* , p. 19.

Cagnotti, C.L., Viscarret, M.M., Riquelme, M.B., Botto, E.N., Carabajal, L.Z., Segura, D.F. and Lopez, S.N. (2012) 'Effects of X-rays on *Tuta absoluta* for use in inherited sterility programmes', *Journal of Pest Science*, 85(4), pp. 413-421.

Campbell, J.F. and Gaugler, R. (1993) 'Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae)', *Behaviour*, 126(3-4), pp. 155-169.

Campbell, J.F. and Gaugler, R.R. (1997) 'Inter-specific variation in entomopathogenic nematode foraging strategy: Dichotomy or variation along a continuum?', *Fundamental and Applied Nematology*, 20(4), pp. 393-398.

Campbell, J.F. and Kaya, H.K. (1999) 'How and why a parasitic nematode jumps', *Nature*, 397(6719), pp. 485-486.

Campbell, J.F. and Kaya, H.K. (2000) 'Influence of insect associated cues on the jumping behavior of entomopathogenic nematodes (*Steinernema* spp.)', *Behaviour*, 137, pp. 591-609.

Campbell, J.F. and Kaya, H.K. (2002) 'Variation in entomopathogenic nematode (Steinernematidae and Heterorhabditidae) infective-stage jumping behaviour', *Nematology*, 4, pp. 471-482.

Campos, M.R., Rodrigues, A.R.S., Silva, W.M., Silva, T.B.M., Silva, V.R.F., Guedes, R.N.C. and Siqueira, H.A.A. (2014) 'Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance', *PLoS ONE*, 9(8).

Campos, R.G. (1976) 'Control químico del "minador de hojas y tallos de la papa"(*Scrobipalpula absoluta* Meyrick) en el valle del Cañete', *Revista Peruana de Entomologia*, 19(1), pp. 102-106.

Caponero, A. (2009) 'Solanacee, Rischio in serre. resta alta l'attenzione alla tignola del pomodoro nelle colture protette ', *Colture Protette*, 10, pp. 96-97

Carde, R.T. and Minks, A.K. (1995) 'Control of Moth Pests by Mating Disruption: Successes and Constraints', *Annual Review of Entomology*, 40, pp. 559-585.

Cardozo, M.R., Lopez, M.B., Evert, M.T., Palacio, C., Yasuda, S., Sugiyama, H., Mori, K., Kajita, M. and Sato, T. (1994) 'Control integrado de la palomilla del tomate, *Scrobipalpula absoluta* (Meyrick, 1917)', *Ins. Agr. Nac. CAACUPE, Paraguay*.

Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R. and Desneux, N. (2013) 'Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest Tuta absoluta', *Journal of Pest Science*, 86(3), pp. 533-541.

Chermiti, B. and Abbes, K. (2012) 'Comparison of pheromone lures used in mass trapping to control the tomato leafminer *Tuta absoluta* (Meyrick, 1917) in industrial tomato crops in Kairouan (Tunisia)', *EPPO Bulletin*, 42(2), pp. 241-248.

Choo, H.Y., Kaya, H.K., Huh, J., Lee, D.W., Kim, H.H., Lee, S.M. and Choo, Y.M. (2002) 'Entomopathogenic nematodes (*Steinernema spp.* and *Heterorhabditis bacteriophora*) and a fungus *Beauveria brongniartii* for biological control of the white grubs, *Ectinohoplia rufipes* and *Exomala orientalis*, in Korean golf courses', *Biocontrol*, 47(2), pp. 177-192.

Cifuentes, D., Chynoweth, R. and Bielza, P. (2011) 'Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers', *Pest Management Science*, 67(9), pp. 1155-1162.

Clarke, J.F.G. (1965) 'Microlepidoptera of Juan Fernandez Islands', *Proceedings of the United States National Museum*, 117, pp. 1-105.

Cocco, A., Deliperi, S. and Delrio, G. (2013) 'Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique', *Journal of Applied Entomology*, 137(1-2), pp. 16-28.

Coelho, M.C.F. and França, F.H. (1987) 'Biologia, quetotaxia da larva e descrição da pupa e adulto da traça-do-tomateiro', *Pesquisa Agropecuária Brasileira*, 22(2), pp. 129-135.

Contreras, J., Mendoza, J.E., Martinez-Aguirre, M.R., Garcia-Vidal, L., Izquierdo, J. and Bielza, P. (2014) 'Efficacy of Enthomopathogenic Fungus *Metarhizium anisopliae* Against *Tuta absoluta* (Lepidoptera: Gelechiidae)', *Journal of Economic Entomology*, 107(1), pp. 121-124.

Darissa, O.M. and Iraki, N.M. (2014) 'Antagonistic interactions between the biocontrol agents *Beauveria bassiana* and *Heterorhabditis indica*', *Russian Journal of Nematology*, 22(1), pp. 23-29.

De Ruiter, H., Uffing, A.J.M., Meinen, E. and Prins, A. (1990) 'Influence of surfactants and plant species on leaf retention of spray solutions', *Weed Science*, 38(6), pp. 567-572.

De Waal, J.Y., Malan, A.P. and Addison, M.F. (2013) 'Effect of humidity and a superabsorbent polymer formulation on the efficacy of *Heterorhabditis zealandica* (Rhabditida: Heterorhabditidae) to control codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae)', *Biocontrol Science and Technology*, 23(1), pp. 62-78.

DeBach, P. (1991) *Biological control by natural enemies*. 2nd edn. Cambridge, England; New York: Cambridge University Press. p. 440.

del Pino, F.G. and Morton, A. (2008) 'Efficacy of Steinernema feltiae against the leek moth *Acrolepiopsis assectella* in laboratory and field conditions', *Biocontrol*, 53(4), pp. 643-650.

Delbac, L., Lecharpentier, P. and Thiery, D. (2010) 'Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths', *Crop Protection*, 29(6), pp. 623-630.

Desneux, N., Decourtye, A. and Delpuech, J.-M. (2007) 'The sublethal effects of pesticides on beneficial arthropods', *Annual Review of Entomology*, 52, pp. 81-106.

Desneux, N., Luna, M.G., Guillemaud, T. and Urbaneja, A. (2011) 'The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production', *Journal of Pest Science*, 84(4), pp. 403-408.

Desneux, N.D.N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narvaez-Vasquez, C.A., Gonzalez-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T. and Urbaneja, A. (2010) 'Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control', *Journal of Pest Science*, 83(3), pp. 197-215.

Dowds, B.C.A. and Peters, A. (2002) 'Virulence Mechanisms', in Gaugler, R. (ed.) *Entomopathogenic nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 80-98.

Dunphy, G.B. and Webster, J.M. (1986) 'Temperature effects on the growth and virulence of *Steinernema feltiae* Strains and *Heterorhabditis heliothidis*', *Journal of Nematology*, 18(2), pp. 270-272.

EC (2007) *European Union Policy for a Sustainable use of Pesticides: The Story Behind the Strategy*. Luxembourg: Office for Official Publications of the European Communities. http://ec.europa.eu/environment/ecolabel. Accessed 25 Apr 2014.

Edwards, C.A. (1993) 'The Impact of Pesticides on The Environment ', in Pimentel, D. and Lehman, H. (eds.) *The Pesticide question : environment, economics, and ethics*. New York: Chapman & Hall., pp. 13-46.

Ehlers, R.U. (2001) 'Mass production of entomopathogenic nematodes for plant protection', *Applied Microbiology and Biotechnology*, 56(5-6), pp. 623-633.

Eilenberg, J., Hajek, A. and Lomer, C. (2001) 'Suggestions for unifying the terminology in biological control', *Biocontrol*, 46(4), pp. 387-400.

EPPO (2005) 'Data sheets on quarantine pests: *Tuta absoluta*', *EPPO Bulletin*, 35(3), pp. 434-435.

EPPO (2008a) 'EPPO Reporting service - Pest & Diseases. No 1, Paris, 2008-01-01'.

EPPO (2008b) 'EPPO Reporting service - Pest & Diseases. No 7, Paris, 2008-07-01'.

EPPO (2008c) 'EPPO Reporting service - Pest & Diseases. No 9, Paris, 2008-09-01'.

EPPO (2009a) 'EPPO Reporting service - Pest & Diseases. No 1, Paris, 2009-01-01'.

EPPO (2009b) 'EPPO Reporting service - Pest & Diseases. No 3, Paris, 2009-03-01'.

EPPO (2009c) 'EPPO Reporting service - Pest & Diseases. No 8, Paris, 2009-08-01'.

EPPO (2009d) 'EPPO Reporting service - Pest & Diseases. No 9, Paris, 2009-09-01'.

EPPO (2010) 'EPPO Reporting service - Pest & Diseases. No 1, Paris, 2010-01-01'.

EPPO (2011a) 'EPPO Reporting service - Pest & Diseases. No 04, Paris, 2011-04-01'.

EPPO (2011b) 'EPPO Reporting service - Pest & Diseases. No 11, Paris, 2011-01-01'.

EPPO (2012a) 'EPPO Reporting service - Pest & Diseases. No 1, Paris, 2012-01-01'.

EPPO (2012b) 'EPPO Reporting service - Pest & Diseases. No 2, Paris, 2012-02-01'.

EPPO (2012c) 'EPPO Reporting service - Pest & Diseases. No 5, Paris, 2012-05-01'.

EPPO (2012d) 'EPPO Reporting service - Pest & Diseases. No 11, Paris, 2012-11-01'.

EPPO (2013a) 'EPPO Reporting service - Pest & Diseases. No 4, Paris, 2013-04-01'.

EPPO (2013b) 'EPPO Reporting service - Pest & Diseases. No 9, Paris, 2013-09-01'.

EPPO (2014a) 'EPPO Reporting service - Pest & Diseases. No 1, Paris, 2014-01-01'.

EPPO (2014b) 'EPPO Reporting service - Pest & Diseases. No 8, Paris, 2014-08-01'.

EPPO (2015) 'EPPO Reporting service - Pest & Diseases. No 2, Paris, 2015-02'.

Espinosa, P.J. (2010) 'CAL-EX AVANCE EW: nueva alternativa en la estrategia de lucha control *Tuta absoluta*', *Phytoma España*, 217, pp. 76-80.

Estay, P. (2000) 'Polilla del tomate *Tuta absoluta* (Meyrick). Instituto de Investigationes Agropecuarias, Centro Regional de Investigacion La Platina, Ministerio de Agricultura Santiago Chile.', [Online]. Available at: http://www.inia.cl/medios/biblioteca/informativos/NR25648.pdf (Accessed: April 13, 2012).

FAO (1967) *Report of the First Session of the FAO Panel of Experts on Integrated Pest Control*. Food and agriculture organization of the United Nations: Rome.

FAO (2012) *Integrated Pest Management*. http://www.fao.org/agriculture/crops/core-themes/theme/pests/ipm/en/. Accessed 15 October 2014.

FERA (2009) 'South American tomato moth *Tuta absoluta*'. March 5, 2012. Food and Environment Research Agency, Department for Environment Food and Rural Affairs. Accessed March 5, 2012. Available at: http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/tAbsoluta.pdf.

Fernandez, S. and Montagne, A. (1990) 'Biology of the tomato borer, *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae)', *Boletin de Entomologia Venezolana*, 5, pp. 89-99.

Ferrara, F.A.A., Vilela, E.F., Jham, G.N., Eiras, A.E., Picanco, M.C., Attygalle, A.B., Svatos, A., Frighetto, R.T.S. and Meinwald, J. (2001) 'Evaluation of the synthetic major component of the sex pheromone of *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae)', *Journal of Chemical Ecology*, 27(5), pp. 907-917.

Finney, D.J. (1971) *Probit analysis*. London, UK: Cambridge University Press, 331 pp.

Flint, M.L. and van den Bosch, R. (1981) *Introduction to integrated pest management*. New York: Plenum Press.

Flores, L.V., Gilardón, E. and Gardenal, C.N. (2003) 'Genetic Structure of Populations of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)', *BAG - Journal of Basic and Applied Genetics*, 15(2), pp. 47-54.

Forschler, B.T. and Gardner, W.A. (1991) 'Parasitism of *Phyllophaga hirticula* (Coleoptera: Scarabaeidae) by *Heterorhabditis heliothidis* and *Steinernema carpocapsae*', *Journal of Invertebrate Pathology*, 58(3), pp. 396-407.

Forst, S. and Clarke, D. (2002) 'Bacteria-Nematode Symbiosis', in Gaugler, R. (ed.) *Entomopathogenic nematology*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 58-77.

França, F.H., Maluf, W.R., Rossi, P.E.F., Miranda, J.E.C. and Coelho, M.C.F. (1984) 'Avaliação e seleção em tomate visando resistência a traça-do-tomateiro', *In: Congresso Brasileiro de Olericultura, 24*, Resumos. FCAV, Jaboticabal, Brazil, v.1-p.143.

FREDON-Corse (2009) 'Mesures de lutte contre Tuta absoluta. Fédération Régionale de Défense contre les Organismes Nuisibles de Corse. http://www.fredon-corse.com/standalone/1/CE5Bk98q7hNOOAd4qo4sD67a.pdf'.

Fredon (2009) *Mesures de lutte contre Tuta absoluta*. http://www.fredon-corse.com/standalone/1/CE5Bk98q7hNOOAd4qo4sD67a.pdf (Accessed: 10 Dec 2012).

Galvan, T.L., Koch, R.L. and Hutchison, W.D. (2005) 'Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera : Coccinellidae)', *Biological Control*, 34(1), pp. 108-114.

Garcia-del-Pino, F., Alabern, X. and Morton, A. (2013) 'Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect', *Biocontrol*, 58(6), pp. 723-731.

Garcia, M.F. and Espul, J.C. (1982) 'Bioecología de la polilla del tomate (*Scrobipalpula absoluta*) en Mendoza, República Argentina.', *Rev. Invest. Agropecuarias INTA (Argentina)* 18, pp. 135-146.

Garzia, G.T., Siscaro, G., Colombo, A. and Campo, G. (2009) 'Reappearance of *Tuta absoluta* in Sicily', *Informatore Agrario* 65(4), p. 71.

Gaugler, R., Bednarek, A. and Campbell, J.F. (1992) 'Ultraviolet inactivation of heterorhabditid and steinernematid nematodes', *Journal of Invertebrate Pathology*, 59(2), pp. 155-160.

Gaugler, R. and Boush, G.M. (1978) 'Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae* ', *Journal of Invertebrate Pathology*, 32(3), pp. 291-296.

Gaugler, R., Lebeck, L., Nakagaki, B. and Boush, G.M. (1980) 'Orientation of the entomogenous nematode, *Neoaplectana carpocapsae* to carbon dioxide ', *Environmental Entomology*, 9(5), pp. 649-652.

Gaugler, R., Lewis, E. and Stuart, R.J. (1997a) 'Ecology in the service of biological control: The case of entomopathogenic nematodes', *Oecologia*, 109(4), pp. 483-489.

Gaugler, R., Wilson, M. and Shearer, P. (1997b) 'Field release and environmental fate of a transgenic entomopathogenic nematode', *Biological Control*, 9(2), pp. 75-80.

Georgis, R. and Poinar, G.O. (1983) 'Effect of Soil Texture on the Distribution and Infectivity of Neoaplectana glaseri (Nematoda: Steinernematidae)', *Journal of Nematology*, 15(3), pp. 329-332.

Giustolin, T.A. and Vendramim, J.D. (1996) 'Efeito de duas espécies de tomateiro na biologia de *Scrobipalpuloides absoluta* (Meyrick)', *Anais Sociedad Entomologica Brasileira*, 23, pp. 511-517.

Giustolin, T.A., Vendramim, J.D., Alves, S.B. and Vieira, S.A. (2001a) 'Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) reared on two genotypes of tomato', *Neotropical Entomology*, 30(3), pp. 417-421.

Giustolin, T.A., Vendramim, J.D., Alves, S.B., Vieira, S.A. and Pereira, R.M. (2001b) 'Susceptibility of *Tuta absoluta* (Meyrick) (Lep, Gelechiidae) reared on two species of Lycopersicon to *Bacillus thuringiensis* var. kurstaki', *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie*, 125(9-10), pp. 551-556.

Giustolin, T.A., Vendramim, J.D. and Postali Parra, J.R. (2002) 'Número de ínstares larvais de *Tuta absoluta* (Meyrick) em genótipos de tomateiro', *Scientia Agricola*, 59, pp. 393-396.

Glazer, I. (1992) 'Survival and efficacy of *Steinernema carpocapsae* in an exposed environment', *Biocontrol Science and Technology*, 2(2), pp. 101-107.

Glazer, I. (2002) 'Survival Biology', in Gaugler, R. (ed.) *Entomopathogenic nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 170-187.

Glazer, I., Klein, M., Navon, A. and Nakache, Y. (1992) 'Comparison of efficacy of entomopathogenic nematodes combined with antidesiccants applied by canopy sprays against 3 cotton pests (Lepidoptera, Noctuidae)', *Journal of Economic Entomology*, 85(5), pp. 1636-1641.

Glazer, I. and Lewis, E.E. (2000) 'Bioassays for entomopathogenic nematodes.', in Navon, A. and Ascher, K.R.S. (eds.) *Bioassays of entomopathogenic microbes and nematodes.* Wallingford, UK: CABI Publishing, pp. 229-247.

Glazer, I. and Navon, A. (1990) 'Activity and persistence of entomoparasitic nematodes tested against *Heliothis armigera* (Lepidoptera, Noctuidae)', *Journal of Economic Entomology*, 83(5), pp. 1795-1800.

Gontijo, L.M., Celestino, D., Queiroz, O.S., Guedes, R.N.C. and Picanco, M.C. (2015) 'Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae)', *Florida Entomologist*, 98(1), pp. 59-64.

Gontijo, P.C., Picanco, M.C., Pereira, E.J.G., Martins, J.C., Chediak, M. and Guedes, R.N.C. (2013) 'Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*', *Annals of Applied Biology*, 162(1), pp. 50-59.

Gonzalez-Cabrera, J., Molla, O., Monton, H. and Urbaneja, A. (2011) 'Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)', *Biocontrol*, 56(1), pp. 71-80.

Grewal, P., Ehlers, R.-U. and Shapiro-Ilan, D. (2005a) 'Critical Issues and Research Needs for Expanding the Use of Nematodes in Biocontrol', in Grewal, P., Ehlers, R.-U. and Shapiro-Ilan, D. (eds.) *Nematodes as biocontrol agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 479-489.

Grewal, P., Ehlers, R.-U. and Shapiro-Ilan, D. (2005b) *Nematodes as biocontrol agents*. Wallingford, Oxfordshire, UK CABI Publishing, 505 pp.

Grewal, P. and Georgis, R. (1999) 'Entomopathogenic Nematodes', in Hall, F.R. and Menn, J.J. (eds.) *Biopesticides: Use and Delivery*. Totowa, NJ: Humana Press, pp. 271-299.

Grewal, P.S. (2002) 'Formulation and application technology', in Gaugler, R. (ed.) *Entomopathogenic Nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 265-287.

Grewal, P.S. (2012) 'Entomopathogenic Nematodes as Tools in Integrated Pest Management', in Abrol, D.P. and Shankar, U. (eds.) *Integrated pest management : principles and practice*. Wallingford, Oxfordshire, UK: CABI, pp. 162-236.

Grewal, P.S., Gaugler, R. and Selvan, S. (1993) 'Host recognition by entomopathogenic nematodes: Behavioral response to contact with host feces', *Journal of Chemical Ecology*, 19(6), pp. 1219-1231.

Grewal, P.S., Grewal, S.K., Malik, V.S. and Klein, M.G. (2002a) 'Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities', *Biological Control*, 24(3), pp. 230-237.

Grewal, P.S., Lewis, E.E. and Gaugler, R. (1997) 'Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts', *Journal of Chemical Ecology*, 23(2), pp. 503-515.

Grewal, P.S., Lewis, E.E., Gaugler, R. and Campbell, J.F. (1994a) 'Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes', *Parasitology*, 108(2), pp. 207-215.

Grewal, P.S., Power, K.T., Grewal, S.K., Suggars, A. and Haupricht, S. (2004) 'Enhanced consistency in biological control of white grubs (Coleoptera : Scarabaeidae) with new strains of entomopathogenic nematodes', *Biological Control*, 30(1), pp. 73-82.

Grewal, P.S., Selvan, S. and Gaugler, R. (1994b) 'Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction', *Journal of Thermal Biology*, 19(4), pp. 245-253.

Grewal, P.S., Wang, X. and Taylor, R.A.J. (2002b) 'Dauer juvenile longevity and stress tolerance in natural populations of entomopathogenic nematodes: is there a relationship?', *International Journal for Parasitology*, 32(6), pp. 717-725.

Griffin, C.T. (1993) 'Temperature responses of entomopathogenic nematodes - Implications for the success of biological-control programs ', *Nematodes and the Biological Control of Insect Pests*. Beijing, Peoples R China JUL, 1992. pp. 115-126. Available at: <Go to ISI>://WOS:A1993BA02L00014.

Griffin, C.T., Boemare, N.E. and Lewis, E.E. (2005) 'Biology and behaviour', in Grewal, P.S., Ehlers, R.-U. and Shapiro-Ilan, D.I. (eds.) *Nematodes as biocontrol agents.* Wallingford, Oxfordshire, UK CABI Publishing, pp. 47-64.

Grover, D.W. and Nicol, J.M. (1940) 'The vapour pressure of glycerine solutions at twenty degrees', *Journal of the Society of Chemical Industry*, 59, pp. 175-177.

Guedes, R.N.C. and Picanço, M.C. (2012) 'The tomato borer *Tuta absoluta* in South America: Pest status, management and insecticide resistance', *EPPO Bulletin*, 42(2), pp. 211-216.

Guedes, R.N.C. and Siqueira, H.A.A. (2012) 'The tomato borer *Tuta absoluta*: Insecticide resistance and control failure', *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 7, pp. 1-7.

Guenaoui, Y., Dahliz, A., Bensaad, R. and Ouezzani, K. (2013) 'Five years after the first record of *Tuta absoluta* (Meyrick) in Algeria, what do we expect from its native natural enemies?', *Fourth International Scientific Symposium*. Agrosym, 3-6 October 2013. pp. 678-682.

Gullan, P.J. and Cranston, P.S. (2005a) *The insects: an outline of entomology. Third edition*. Blackwell Publishing; Malden & Oxford, 505 pp.

Gullan, P.J. and Cranston, P.S. (2005b) 'Sensory systems and behaviour', in Gullan, P.J. and Cranston, P.S. (eds.) *The insects : an outline of entomology*. 3rd edn. Malden, MA: Blackwell Pub., pp. 85-111.

Haddi, K., Berger, M., Bielza, P., Cifuentes, D., Field, L.M., Gorman, K., Rapisarda, C., Williamson, M.S. and Bass, C. (2012) 'Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*)', *Insect Biochemistry and Molecular Biology*, 42(7), pp. 506-513.

Hajek, A.E. (2004) *Natural enemies: an introduction to biological control*. Cambridge: Cambridge University Press. p. 388

Haji, F.N.P., Prezotti, L., Carneiro, J.d.S., Alencar, J.A.d., Parra, J.R.P., Botelho, P.S.M., Corrêa-Ferreira, B.S. and Bento, J.M.S. (2002) 'Trichogramma pretiosum para o controle de pragas no tomateiro industrial', in Parra, J.R.P., Botelho, P.S.M., Ferreira, B.S.C. and Bento, J.M.S. (eds.) *Controle biológico no Brasil: parasitóides e predadores. São Paulo: Manole*. São Paulo, Brazil: Manole Ltda, pp. 477–494.

Hara, A.H., Kaya, H.K., Gaugler, R., Lebeck, L.M. and Mello, C.L. (1993) 'Entomopathogenic nematodes for biological control of the leafminer, *Liriomyza trifolii* (Dipt.: Agromyzidae)', *Entomophaga*, 38(3), pp. 359-369.

Harris, J. and Dent, D. (2000) *Priorities in biopesticide research and development in developing countries*. Wallingford, Oxfordshire, UK CABI. p. 70.

Hart, K. and Pimentel, D. (2002) 'Public health and costs of pesticides', in Pimentel, D. (ed.) *Encyclopedia of pest management*. New York: Marcel Dekker, pp. 677–679.

Hazir, S., Kaya, H.K., Stock, S.P. and Keskin, N. (2003) 'Entomopathogenic nematodes (Steinernematidae and heterorhabditidae) for biological control of soil pests', *Turkish Journal of Biology*, 27(4), pp. 181-202.

Head, J., Lawrence, A.J. and Walters, K.F.A. (2004) 'Efficacy of the entomopathogenic nematode, *Steinernema feltiae*, against *Bemisia tabaci* in relation to plant species', *Journal of Applied Entomology*, 128(8), pp. 543-547.

Heckel, D.G. (2012) 'Insecticide resistance after silent spring', *Science*, 337(6102), pp. 1612-1614.

Henneberry, T.J., Jech, L.F. and Burke, R.A. (1996) 'Pink bollworm adult and larval susceptibility to steinernematid nematodes and nematode persistence in the soil in laboratory and field tests in Arizona', *Southwestern Entomologist*, 21(4), pp. 357-368.

Henneberry, T.J., Lindegren, J.E., Jech, L.F. and Burke, R.A. (1995) 'Pink Boll worm (Lepidoptera: Gelechiidae), Cabbage Looper, and Beet Army worm (Lepidoptera: Noctuidae) Pupal Susceptibility to Steinernematid Nematodes (Rhabditida: Steinernematidae)', *Journal of Economic Entomology*, 88(4), pp. 835-839.

Hochberg, E.G. (1996) 'The market for agricultural pesticide inert ingredients and adjuvants', in Foy, C.L. and Fritchard, D.W. (eds.) *Pesticide Formulation and Adjuvant Technology*. Boca Raton, FL: CRC Press, pp. 203-208.

Hoffmann, W.C., Hewitt, A.J., Ross, J.B., Bagley, W.E., Martin, D.E. and Fritz, B.K. (2008) 'Spray Adjuvant Effects on Droplet Size Spectra Measured by Three Laser-Based Systems in a High-Speed Wind Tunnel', *Pesticide Formulations & Delivery Systems, 26 Vol: Reassessing Pesticide Technologies*. Dallas, TX 17-20 OCT 2005 pp. 60-71. Available at: <Go to ISI>://WOS:000260921200009.

Hominick, W.M. (2002) 'Biogeography', in Gaugler, R. (ed.) *Entomopathogenic Nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 115-143.

Howse, P. (1998a) 'Pheromones and behaviour', in Howse, P., Stevens, I. and Jones, O. (eds.) *Insect Pheromones and their Use in Pest Management*. London: Chapman and Hall, pp. 1-130.

Howse, P.E. (1998b) 'The role of pheromones in insect behaviour and ecology', in Howse, P., Stevens, I. and Jones, O. (eds.) *Insect Pheromones and their Use in Pest Management*. London: Chapman and Hall, pp. 38-68.

Hu, K. and Webster, J.M. (2000) 'Antibiotic production in relation to bacterial growth and nematode development in *Photorhabdus Heterorhabditis* infected *Galleria mellonella* larvae', *Fems Microbiology Letters*, 189(2), pp. 219-223.

Hu, K.J., Li, J.X. and Webster, J.M. (1999) 'Nematicidal metabolites produced by Photorhabdus luminescens (Enterobacteriaceae), bacterial symbiont of entomopathogenic nematodes', *Nematology*, 1, pp. 457-469.

Hussein, H.M., Adel, M.M. and Gelbic, I. (2012) 'Effectiveness of the entomopathogenic nematode *Steinernema feltiae* in agar gel formulations against larvae of the Colarado potato beetle, *Leptinotarsa decemlineata* (Say.) (Coleoptera: Chrysomelidae)', *Central European Journal of Biology*, 7(1), pp. 77-82.

InfoAgroSystem (2009) *La Polilla del Tomate (Tuta absoluta).* InfoAgro Systems, Inc. Madrid, Spain. http://www.infoagro.com/hortalizas/polilla_tomate_tuta_absoluta.htm (Accessed: 20 Nov 2009).

Jacobson, R. and Martin, G. (2011) 'A potential role for entomopathogenic nematodes within IPM of *Tuta absoluta* (Meyrick) on organic tomato crops', *IOBC/WPRS Bull*, 68, pp. 79-83.

Janmaat, A.F. and Myers, J. (2003) 'Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*', *Proceedings of the Royal Society B-Biological Sciences*, 270(1530), pp. 2263-2270.

Ji, B.T., Silverman, D.T., Stewart, P.A., Blair, A., Swanson, G.M., Baris, D., Greenberg, R.S., Hayes, R.B., Brown, L.M., Lillemoe, K.D., Schoenberg, J.B., Pottern, L.M., Schwartz, A.G. and Hoover, R.N. (2001) 'Occupational exposure to pesticides and pancreatic cancer', *American Journal of Industrial Medicine*, 39(1), pp. 92-99.

Jones, O. (1998) 'Practical applications of pheromones and other semiochemicals', in Howse, P., Stevens, I. and Jones, O. (eds.) *Insect Pheromones and their Use in Pest Management*. London: Chapman and Hall, pp. 263-355.

Journey, A.M. and Ostlie, K.R. (2000) 'Biological control of the western corn rootworm (Coleoptera : Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*', *Environmental Entomology*, 29(4), pp. 822-831.

Junco, F.R. and Herrero, J.M.C. (2008) 'Estrategias contra la polilla del tomate," *Tuta absoluta*", Meyrick', *Agricultura: Revista agropecuaria*, (903), pp. 70-74.

Kaya, H.K. (1985) 'Susceptibility of early larval stages of *Pseudaletia unipuncta* and *Spodoptera exigua* (Lepidoptera: Noctuidae) to the entomogenous nematode *Steinernema feltiae* (Rhabditida: Steinernematidae)', *Journal of Invertebrate Pathology*, 46(1), pp. 58-62.

Kaya, H.K. (1990) 'Soil ecology', in Gaugler, R. and Kaya, H.K. (eds.) *Entomopathogenic nematodes in biological control*. Boca Raton, FL: CRC Press, pp. 93–115.

Kaya, H.K., Bedding, R.A. and Akhurst, R.J. (1993) 'An Overview of Insect-Parasitic and Entomopathogenic Nematodes', in Bedding, R.A., Akhurst, R.J. and Kaya, H.K. (eds.) *Nematodes and the biological control of insect pests*. East Melbourne, Victoria 3002, Australia: CSIRO Publishing, pp. 1-16.

Kaya, H.K., Burlando, T.M., Choo, H.Y. and Thurston, G.S. (1995) 'Integration of entomopathogenic nematodes with *Bacillus thuringiensis* or pesticidal soap for control of insect pests', *Biological Control*, 5(3), pp. 432-441.

Kaya, H.K. and Gaugler, R. (1993) 'Entomopathogenic Nematodes', *Annual Review of Entomology*, 38, pp. 181-206.

Kaya, H.K. and Grieve, B.J. (1982) 'The nematode *Neoaplectana carpocapsae* and the beet armyworm *Spodoptera exigua*: Infectivity of prepupae and pupae in soil and of adults during emergence from soil', *Journal of Invertebrate Pathology*, 39(2), pp. 192-197.

Kaya, H.K. and Hara, A.H. (1980) 'Differential susceptibility of lepidopterous pupae to infection by the nematode *Neoaplectana carpocapsae* ', *Journal of Invertebrate Pathology*, 36(3), pp. 389-393.

Kaya, H.K. and Hara, A.H. (1981) 'Susceptibility of various species of lepidopterous pupae to the entomogenous nematode *Neoaplectana carpocapsae*', *Journal of Nematology*, 13(3), pp. 291-294.

Kilic, T. (2010) 'First record of *Tuta absoluta* in Turkey', *Phytoparasitica*, 38(3), pp. 243-244.

Konus, M. (2014) 'Analysing resistance of different *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) strains to abamectin insecticide', *Turkish Journal of Biochemistry-Turk Biyokimya Dergisi*, 39(3), pp. 291-297.

Koppenhoefer, A.M. (2000) 'Nematodes', in Lacey, L.A. and Kaya, H.K. (eds.) *Field manual of techniques in invertebrate pathology: application and evaluation of pathogens for control of insects and other invertebrate pests*. Dordrecht, Boston & London: Kluwer Academic Publishers, pp. 283-301.

Koppenhöfer, A.M. (2005) 'Compatibility and interactions with agrochemicals and other biocontrol agents', in Grewal, P.S., Ehlers, R.U. and ShapiroIlan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 363–381.

Koppenhöfer, A.M. (2007) 'Nematodes', in Lacey, L.A. and Kaya, H.K. (eds.) *Field manual of techniques in invertebrate pathology: application and evaluation of pathogens for control of insects and other invertebrate pests*. Dordrecht, The Netherlands: Springer, pp. 249–264.

Koppenhofer, A.M., Choo, H.Y., Kaya, H.K., Lee, D.W. and Gelernter, W.D. (1999) 'Increased field and greenhouse efficacy against scarab grubs with a combination of an entomopathogenic nematode and Bacillus thuringiensis', *Biological Control*, 14(1), pp. 37-44.

Koppenhofer, A.M., Fuzy, E.M., Crocker, R.L., Gelernter, W.D. and Polavarapu, S. (2004) 'Pathogenicity of *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and S-scarabaei (Rhabditida : Heterorhabditidae, Steinernematidae) against 12 white grub species (Coleoptera : Scarabaeidae)', *Biocontrol Science and Technology*, 14(1), pp. 87-92.

Koppenhofer, A.M. and Kaya, H.K. (1997) 'Additive and synergistic interaction between entomopathogenic nematodes and *Bacillus thuringiensis* for scarab grub control', *Biological Control*, 8(2), pp. 131-137.

Krogh, K.A., Halling-Sørensen, B., Mogensen, B.B. and Vejrup, K.V. (2003) 'Environmental properties and effects of nonionic surfactant adjuvants in pesticides: a review', *Chemosphere*, 50(7), pp. 871-901.

Kung, S.P., Gaugler, R. and Kaya, H.K. (1991) 'Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence', *Journal of Invertebrate Pathology*, 57(2), pp. 242-249.

Labory, C.R.G., Santa-Cecília, L.V.C., Maluf, W.R., Cardoso, M.G., Bearzoti, E. and Souza, J.C. (1999) 'Seleção indireta para teor de 2-tridecanona em tomateiros segregantes e sua relação com a resistência à traça-do-tomateiro', *Pesquisa agropecuária brasileira*, 34(5), pp. 723-739.

Lacey, L.A., Arthurs, S.P., Unruh, T.R., Headrick, H. and Fritts, R. (2006) 'Entomopathogenic nematodes for control of codling moth (Lepidoptera : Tortricidae) in apple and pear orchards: Effect of nematode species and seasonal temperatures, adjuvants, application equipment, and post-application irrigation', *Biological Control*, 37(2), pp. 214-223.

Lacey, L.A., Bettencourt, R., Garrett, F.J., Simoes, N.J. and Gaugler, R.H. (1993) 'Factors influencing parasitism of adult Japanese beetles,*Polillia japonica* (Col.: Scarabaeidae) by entomopathogenic nematodes', *Entomophaga*, 38(4), pp. 501-509.

Lacey, L.A., Frutos, R., Kaya, H.K. and Vail, P. (2001) 'Insect pathogens as biological control agents: Do they have a future?', *Biological Control*, 21(3), pp. 230-248.

Lacey, L.A. and Georgis, R. (2012) 'Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production', *Journal of nematology*, 44(2), pp. 218-25.

Lacey, L.A., Shapiro-Ilan, D.I. and Glenn, G.M. (2010) 'Post-application of anti-desiccant agents improves efficacy of entomopathogenic nematodes in formulated host cadavers or aqueous suspension against diapausing codling moth larvae (Lepidoptera: Tortricidae)', *Biocontrol Science and Technology*, 20(9), pp. 909-921.

Laore (2010) *Tignola o falena del pomodoro, Tuta absoluta (Meyrick, 1917), Povolny (1994): riconoscimento e lotta*. Cagliari, Italia: Laore, agenzia regionale per lo sviluppo in agricoltura http://www.sardegnaagricoltura.it/documenti/14_43_20100420134239.pdf (Accessed: 10 Dec 2012).

Larraín, P.S. (1986) 'Plagas del tomate. ', *IPA, La Platina* 39, pp. 30–35.

Leite, G.L.D., Picanco, M., Guedes, R.N.C. and Zanuncio, J.C. (2001) 'Role of plant age in the resistance of Lycopersicon hirsutum f. glabratum to the tomato leafminer *Tuta absoluta* (Lepidoptera : Gelechiidae)', *Scientia Horticulturae*, 89(2), pp. 103-113.

Lello, E.R., Patel, M.N., Matthews, G.A. and Wright, D.J. (1996) 'Application technology for entomopathogenic nematodes against foliar pests', *Crop Protection*, 15(6), pp. 567-574.

Lewis, E.E. (2002) 'Behavioural Ecology', in Gaugler, R. (ed.) *Entomopathogenic Nematology*. Wallingford, Oxfordshire, UK: CABI publishing, pp. 205-225.

Lewis, E.E., Campbell, J., Griffin, C., Kaya, H. and Peters, A. (2006) 'Behavioral ecology of entomopathogenic nematodes', *Biological Control*, 38(1), pp. 66-79.

Lewis, E.E., Gaugler, R. and Harrison, R. (1993) 'Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues', *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, 71(4), pp. 765-769.

Lietti, M.M.M., Botto, E. and Alzogaray, R.A. (2005) 'Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae)', *Neotropical Entomology*, 34(1), pp. 113-119.

Lindegren, J.E., Meyer, K.F., Henneberry, T.J., Vail, P.V., Jech, L.J.F. and Valero, K.A. (1993) 'Susceptibility of pink bollworm (Lepidoptera: Gelechiidae) soil associated stages to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae)', *Southwestern Entomologist*, 18(2), pp. 113-120.

Liu, Y.B., Tabashnik, B.E. and Johnson, M.W. (1995) 'Larval Age Affects Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae)', *Journal of Economic Entomology*, 88(4), pp. 788-792.

Liu, Y.B., Tabashnik, B.E. and PusztaiCarey, M. (1996) 'Field-evolved resistance to *Bacillus thuringiensis* toxin CryIC in diamondback moth (Lepidoptera: Plutellidae)', *Journal of Economic Entomology*, 89(4), pp. 798-804.

Llacer, E., de Altube, M.M.M. and Jacas, J.A. (2009) 'Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in Phoenix canariensis', *Biocontrol*, 54(4), pp. 559-565.

Lo Bue, P., Abbas, S., Peri, E. and Colazza, S. (2012) 'Use of biorational insecticides for the control of *Tuta absoluta* (Meyrick) infestations on open field tomato', *New Medit*, 11(4), pp. 39-41.

Lopez, E. (1991) 'Polilla del tomate: problema critico para la rentabilidad del cultivo de verano', *Empresa y Avance Agricola Volume*, 1, pp. 6-7.

López, J.M., Martín, L., López, A., Correia, R., González, F., Sanz, E., Gallardo, M. and Cantus, J.M. (2010) 'AFFIRM (Emamectina), una nueva arma contra la *Tuta absoluta* y otras orugas de lepidópteros', *Phytoma España*, 217, pp. 90-94.

Macvean, C.M., Brewer, J.W. and Capinera, J.L. (1982) 'Field tests of antidesiccants to extend the infection period of an entomogenous nematode, *Neoaplectana carpocapsae*, (Rhabditida,

Steinernematidae) against the Colorado Potato Beetle (Coleoptera, Chrysomelidae)', *Journal of Economic Entomology*, 75(1), pp. 97-101

Maiche, Z.A. (2009) 'La tuta attaque les champs de pomme de terre. El Watan-Le Quotidient Independant (March 2, 2009). Accessed March 2, 2010. http://www.elwatan.com/La-tuta-attaque-les-champs-de'.

Mallia, D. (2009) *Guidelines for the control and eradication of Tuta absoluta. Ministry for resources and rural affairs, Plant Health Department, Malta. Accessed November 4, 2009.* *http://www.agric.gov.mt/plant-health-deptprofile?l=1*.

MARM (2008) 'Medidas provisionales de control de la plaga de la polilla del tomate, "*Tuta Absoluta*" (Meyrick). Pages 6', in *Ministra de Medio Ambiente y Medio Rural y Marino-Dirección General de Recursos Agrícolas y Ganaderos-Subdirección General de Sanidad de la Producción Primaria*.

MARM (2010) 'Resolución de autorización excepcional para la comercialización de productos fitosanitarios para el control de Tuta absoluta en el cultivo de tomate', in *Ministra de Medio Ambiente y Medio Rural y Marino--Dirección General de Recursos Agrícolas y Ganaderos-Secretaria General de Medio Rural*.

Martinou, A.F., Seraphides, N. and Stavrinides, M.C. (2014) 'Lethal and behavioral effects of pesticides on the insect predator *Macrolophus pygmaeus*', *Chemosphere*, 96, pp. 167-173.

Mason, J.M., Matthews, G.A. and Wright, D.J. (1998) 'Screening and selection of adjuvants for the spray application of entomopathogenic nematodes against a foliar pest', *Crop Protection*, 17(5), pp. 463-470.

Mason, J.M. and Wright, D.J. (1997) 'Potential for the control of *Plutella xylostella* larvae with entomopathogenic nematodes', *Journal of Invertebrate Pathology*, 70(3), pp. 234-242.

Mbata, G.N. and Shapiro-Ilan, D.I. (2005) 'Laboratory evaluation of virulence of heterorhabditid nematodes to *Plodia interpunctella* Hubner (Lepidoptera : pyralidae)', *Environmental Entomology*, 34(3), pp. 676-682.

Megido, R.C., Haubruge, E. and Verheggen, F.J. (2013) 'Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review', *Biotechnologie Agronomie Societe Et Environnement*, 17(3), pp. 475-482.

Metcalf, R.L. (1982) 'Insecticides in Pest Management', in Metcalf, R.L. and Luckmann, W.H. (eds.) *Introduction to pest management*. 2nd edn. New York: John Wiley & Sons, pp. 217 - 277.

Meyrick, E. (1917) 'Descriptions of South American Micro-Lepidoptera', *Transactions of the Entomological Society of London*, 1917, pp. (1-52).

Michereff Filho, M., Vilela, E.F., Attygalle, A.B., Meinwald, J., Svatoš, A. and Jham, G.N. (2000a) 'Field trapping of tomato moth, *Tuta absoluta* with pheromone traps', *Journal of Chemical Ecology*, 26(4), pp. 875-881.

Michereff Filho, M., Vilela, E.F., Jham, G.N., Attygalle, A., Svatoš, A. and Meinwald, J. (2000b) 'Initial Studies of Mating Disruption of the Tomato Moth, Tuta absoluta (Lepidoptera:

Gelechiidae) Using Synthetic Sex Pheromone', *Journal of the Brazilian Chemical Society*, 11(6), pp. 621-628.

Ministero delle Politiche Agricole Alimentari e Forestali (2009) *Rinvenimento in Italia dell 'Organismo Nocivo Tuta absoluta*. Documento Tecnico per la Conoscenza e il Contenimento del Fitofago. 16. Accessed February 5, 2010. http://www.ssabasilicata.it/CANALI_TEMATICI/Difesa_Fitosanitaria/File_allegatiDocumento_tecnico_su_Tuta_absoluta_approvato_dal_CFN_del_23.06.2009.pdf.

Miranda, M.M.M., Picanço, M.C., Zanuncio, J.C., Bacci, L. and Silva, É.M.d. (2005) 'Impact of integrated pest management on the population of leafminers, fruit borers, and natural enemies in tomato', *Ciência Rural*, 35(1), pp. 204-208.

Mohamed, E.S.I., Mahmoud, M.E.E., Elhaj, M.A.M., Mohamed, S.A. and Ekesi, S. (2015) 'Host plants record for tomato leaf miner *Tuta absoluta* (Meyrick) in Sudan', *EPPO Bulletin*, 45(1), pp. 108-111.

Mohan, S. (2015) 'Entomopathogenic Nematodes and Their Bacterial Symbionts as Lethal Bioagents of Lepidopteran Pests', in Sowjanya Sree, K. and Varma, A. (eds.) *Biocontrol of lepidopteran pests : use of soil microbes and their metabolites*. Cham, Switzerland: Springer, pp. 273-288.

Molyneux, A.S. (1984) 'The influence of temperature on the infectivity of heterorhabditid and steinernematid nematodes for larvae of the sheep blowfly, *Lucilia cuprina*', *Proceedings of the Fourth Australian Applied Entomological Research Conference, Adelaide 24-28 September 1984. Pest control: recent advances and future prospects.* pp. 344-351. Available at: <Go to ISI>://ZOOREC:ZOOR12600034732.

Molyneux, A.S. (1985) 'Survival of infective juveniles of Heterorhabditis spp., and Steinernema spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects', *Revue de Nematologie*, 8(2), pp. 165-170.

Monserrat, A. (2009) 'La Polilla del Tomate *Tuta absoluta* en la Región de Murcia: Bases para su Control. Consejería de Agricultura y Agua, Murcia, Spain'.

Monteiro, C.M.O., Araujo, L.X., Matos, R.S., Golo, P.D., Angelo, I.C., Perinotto, W.M.D., Rodrigues, C.A.C., Furlong, J., Bittencourt, V. and Prata, M.C.A. (2013) 'Association between entomopathogenic nematodes and fungi for control of *Rhipicephalus microplus* (Acari: Ixodidae)', *Parasitology Research*, 112(10), pp. 3645-3651.

Mordue, A.J., Simmonds, M.S.J., Ley, S.V., Blaney, W.M., Mordue, W., Nasiruddin, M. and Nisbet, A.J. (1998) 'Actions of azadirachtin, a plant allelochemical, against insects', *Pesticide Science*, 54(3), pp. 277-284.

Mracek, Z., Becvar, S., Kindlmann, P. and Webster, J.M. (1998) 'Infectivity and specificity of Canadian and Czech isolates of Steinernema kraussei (Steiner, 1923) to some insect pests at low temperatures in the laboratory', *Nematologica*, 44(4), pp. 437-448.

Muszinski, T., Lavendowski, I.M. and Maschio, L.M.A. (1982) 'Constatação de *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae), como praga do tomateiro (*Lycopersicon esculentum* Mill.), no litoral do Paraná', *An. Soc. Entomol. Brasil*, 11, pp. 291-292.

National Research Council (2000) *The future role of pesticides in US agriculture*. Washington, D.C.: National Academy Press. 301 p.

Navarro-Llopis, V., Alfaro, C., Vacas, S. and Primo, J. (2010) 'Aplicación de la confusión sexual al control de la polilla del tomate *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae)', *Phytoma España: La revista profesional de sanidad vegetal*, (217), pp. 32-34.

Navon, A., Nagalakshmi, V.K., Levski, S., Salame, L. and Glazer, I. (2002) 'Effectiveness of entomopathogenic nematodes in an alginate gel formulation against lepidopterous pests', *Biocontrol Science and Technology*, 12(6), pp. 737-746.

Nickle, W.R. and Shapiro, M. (1994) 'Effects of eight brighteners as solar radiation protectants for *Steinernema carpocapsae*, All strain', *Journal of Nematology*, 26(4), pp. 782-784.

Niedmann, L.L. and Meza-Basso, L. (2006) 'Evaluación de cepas nativas de *Bacillus thuringiensis* como una alternativa de manejo integrado de la polilla del tomate (*Tuta absoluta* Meyrick; Lepidoptera: Gelechiidae) en Chile', *Agricultura Técnica*, 66(3), pp. 235-246.

NIOSH (2012) *A story of impact: NIOSH pesticide poisoning monitoring program protects farmworkers*. National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention, US Department of Health and Human Services. http://www.cdc.gov/niosh/docs/2012-108/pdfs/2012-108.pdf. Accessed 4 Feb 2016.

Oestergaard, J., Belau, C., Strauch, O., Ester, A., van Rozen, K. and Ehlers, R.U. (2006) 'Biological control of Tipula paludosa (Diptera : Nematocera) using entomopathogenic nematodes (Steinernema spp.) and Bacillus thuringiensis subsp israelensis', *Biological Control*, 39(3), pp. 525-531.

Olthof, T.H.A. and Broadbent, A.B. (1992) 'Evaluation of steinernematid nematodes for control of a leafminer, *Liriomyza trifolii*, in greenhouse chrysanthemums', *J. Nematol*, 24, p. 612.

Ostrauskas, H. and Ivinskis, P. (2010) 'Records of the Tomato Pinworm (*Tuta Absoluta* (Meyrick, 1917)) - Lepidoptera: Gelechiidae - in Lithuania', *Acta Zoologica Lituanica*, 20(2), pp. 151-155.

Pastrana, J.A. (1967) 'Una nueva plaga en papas y tomates. INTA Republica Argentina. Secretaria de Estado de Agricultura y Ganaderia de la Nacion. Instituto de Patologia Vegetal. Hoja Information 10'.

Pastrana, J.A. (2004) 'Los Lepidópteros Argentinos – sus plantas hospederas y otros sustratos alimenticios', *Sociedad Entomológica Argentina ediciones viii*, p. 334.

Pereyra, P.C. and Sánchez, N.E. (2006) 'Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)', *Neotropical Entomology*, 35(5), pp. 671-676.

Peters, A. (2003) 'Pesticide and entomopathogenic nematodes current status and future work', *IOBC, WPRS Bulletin*, 26, pp. 107-110.

Peters, A. and Backes, J. (2003) 'Impact of substrate conditions and application method on the efficacy of *Steinernema feltiae*', *IOBC WPRS Bulletin*, 26(1), pp. 151-158.

Peters, A. and Ehlers, R.U. (1994) 'Susceptibility of Leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the Entomopathogenic Nematode *Steinernema feltiae*', *Journal of Invertebrate Pathology*, 63(2), pp. 163-171.

Peterson, R.K.D. and Higley, L.G. (1993) 'Communicating Pesticide Risks', *American Entomologist*, 39(4), pp. 206-211.

Piggott, S.J., Wright, D.J., Matthews, G.A., Bcpc and Bcpc (2000) 'Polymeric formulation for the application of entomopathogenic nematodes against foliar pests', *Bcpc Conference: Pests & Diseases 2000, Vols 1-3, Proceedings*. Brighton, England, UK 13-16 NOV 2000 pp. 1063-1068. Available at: <Go to ISI>://WOS:000166809600163.

Pimentel, D. (2000) *CRC handbook of pest management in agriculture*. 2nd edn. Boca Raton: CRC Press, Volume 3.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. and Damore, M. (1992) 'Environmental and economic costs of pesticide use ', *Bioscience*, 42(10), pp. 750-760.

Pires, L.M., Marques, E.J., de Oliveira, J.V. and Alves, S.B. (2010) 'Selection of Isolates of Entomopathogenic Fungi for Controlling Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) and their Compatibility with Insecticides Used in Tomato Crop', *Neotropical Entomology*, 39(6), pp. 977-984.

Poinar, G.O., Jr. and Leutenegger, R. (1968) 'Anatomy of the infective and normal third-stage juveniles of *Neoplectana carpocapsae* Weiser (Steinernematidae: Nematoda)', *Journal of Parasitology*, 54, pp. 340-350.

Potting, R. (2009) *Pest risk analysis, Tuta absoluta, tomato leaf miner moth*. Plant protection service of the Netherlands, Ministry of of Agriculture, Nature and Food Quality, Wageningen, The Netherlands.

Povolny, D. (1964) 'Gnorimoschemini Trib. nov.-Eine neue Tribus der Familie Gelechiidae nebst Bermerkungen zu ihrer Taxonomie (Lepidoptera)', *Acta. Soc. Ent. Czechoslov.*, (Prague) 61, pp. 330-359.

Povolny, D. (1987) 'Gnorimoschemini of southern South America 3: the scrobipalpuloid genera (Insecta, Lepidoptera, Gelechiidae)', *Steenstrupia*, 13(1), pp. 1-91.

Povolny, D. (1994) 'Gnorimoschemini of southern South America 6: identification keys, checklist of Neotropical taxa and general considerations (Insecta, Lepidoptera, Gelechiidae)', *Steenstrupia*, 20(1), pp. 1-42.

Pretty, J. and Hine, R. (2005) 'Pesticide use and the environment', in Pretty, J.N. (ed.) *The pesticide detox : towards a more sustainable agriculture*. Sterling, VA, London: Earthscan, pp. 1-22.

Proffit, M., Birgersson, G., Bengtsson, M., Reis, R., Witzgall, P. and Lima, E. (2011) 'Attraction and Oviposition of *Tuta absoluta* Females in Response to Tomato Leaf Volatiles', *Journal of Chemical Ecology*, 37(6), pp. 565-574.

Püntener, W. (1981) *Manual for Field Trials in Plant Protection*. 2 edn. Basle, Switzerland: Ciba-Geigy Ltd, 205 pp.

Quiroz, E. (1978) 'Utilizacion de trampas con hembras virgenes de *Scrobipalpula absoluta* (Mayrick)(Lep., Gelechiidae) en estudios de dinamica de poblacion', *Agricultura tecnica*, 38, pp. 94-97.

Ratnasinghe, G. and Hague, N.G.M. (1998) 'The invasion, development and reproduction of Steinernema carpocapsae (Rhabditida : Steinernematidae) in the diamondback moth, Plutella xylostella (Lepidoptera : Yponomeutidae)', *Nematropica*, 28(1), pp. 1-6.

Rechcigl, J.E. and Rechcigl, N.A. (1998) *Biological and biotechnological control of insect pests*. Boca Raton, Fla.: Lewis Publishers. 374 p.

Regupathy, A. (1995) 'Biological control by natural enemy Helicoverpa armigera in India', *Challenging the Future: Proc. World Cotton Conference-I*. Brisbane, Australia, CSIRO, Melbourne, 14-17, Feb., pp. 556 - 562.

Reyes, M., Rocha, K., Alarcon, L., Siegwart, M. and Sauphanor, B. (2012) 'Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad', *Pesticide Biochemistry and Physiology*, 102(1), pp. 45-50.

Riley, D.G. and Sparks, A.N. (2009) *Insecticide resistance management for diamondback moth in Georgia*. The University of Georgia, College of Agricultural and Environmental Sciences and the U.S. Department of Agriculture Cooperating, Circular No.899, 1-11.

Riquelme Virgala, M.B., Botto, E.N. and Lafalce, C. (2006) 'Efficacy of insecticides against the 'tomato moth' *Tuta absoluta* (Lepidoptera: Gelechiidae) and their residual effects on the parasitoid *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae)', *Revista de la Sociedad Entomologica Argentina*, 65(3-4), pp. 57-65.

Robles, J.L. (2010) 'FENOS: nuevo insecticida de Bayer Cropscience para el control de *Tuta absoluta*', *Phytoma España*, 217, pp. 87-89.

Roditakis, E., Skarmoutsou, C. and Staurakaki, M. (2013) 'Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece', *Pest Management Science*, 69(7), pp. 834-840.

Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M. and Bassi, A. (2015) 'First report of *Tuta absoluta* resistance to diamide insecticides', *Journal of Pest Science*, 88(1), pp. 9-16.

Rodríguez, M., Gerding, M. and France, A. (2006a) 'Effectivity of entomopathogenic fungus strains on tomato moth *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) larvae', *Agricultura Técnica*, 66(2), pp. 159-165.

Rodríguez, M., Gerding, M. and France, A. (2006b) 'Entomopathogenic fungi isolates selection for egg control of tomato moth *Tuta absoluta* meyrick (Lepidoptera: gelechiidae) eggs', *Agricultura Tecnica*, 66(2), pp. 151-158.

Römbke, J. and Moltmann, J.F. (1996) *Applied ecotoxicology*. Boca Raton CRS Press, 282 p.

Russell IPM (2012a) *Tuta absoluta*. http://www.russellipm-agriculture.com/en/insect/tuta-absoluta (Accessed: 20 Oct 2015).

Russell IPM (2012b) *Tuta absoluta products*. http://new.russellipm-agriculture.com/solutions-standard.php?id_ctg=1&lang=en (Accessed: 20 Oct 2015).

Salas, J. (2004) 'Capture of *Tuta absoluta* (Lepidoptera: Gelechiidae) in traps baited with its sex pheromone', *Revista Colombiana de Entomología*, 30(1), pp. 75-78.

Salazar, E.R. and Araya, J.E. (1997) 'Detección de resistencia a insecticidas en la polilla del tomate', *Simiente*, 67, pp. 8-22.

Salazar, E.R. and Araya, J.E. (2001) 'Respuesta de la polilla del tomate, *Tuta absoluta* (Meyrick), a insecticidas en Arica', *Agricultura Técnica*, 61(4), pp. 429-435.

Salem, S.A. and Abdel-Moniem, A.S.H. (2015) 'Evaluation of non-traditional approaches for controlling tomato moth, *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae), a new invasive pest in Egypt', *Archives of Phytopathology and Plant Protection*, 48(4), pp. 319-326.

Sanchez, N.E., Pereyra, P.C. and Luna, M.G. (2009) 'Spatial Patterns of Parasitism of the Solitary Parasitoid Pseudapanteles dignus (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae)', *Environmental Entomology*, 38(2), pp. 365-374.

Sannino, L. and Espinosa, B. (2010) '*Tuta absoluta*, guide to knowledge and recent acquisitions for a suitable control', *L'Informatore Agrario,*, 46(1), pp. 1-113.

Saunders, J.E. and Webster, J.M. (1999) 'Temperature effects on Heterorhabditis megidis and Steinernema carpocapsae infectivity to Galleria mellonella', *Journal of Nematology*, 31(3), pp. 299-304.

Schmutterer, H. (1990) 'Properties and Potential of Natural Pesticides from the Neem Tree, Azadirachta Indica', *Annual Review of Entomology*, 35, pp. 271-297.

Schroer, S. and Ehlers, R.U. (2005) 'Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*)', *Biological Control*, 33(1), pp. 81-86.

Schroer, S., Yi, X.L. and Ehlers, R.U. (2005a) 'Evaluation of adjuvants for foliar application of *Steinernema carpocapsae* against larvae of the diamondback moth (*Plutella xylostella*)', *Nematology*, 7, pp. 37-44.

Schroer, S., Ziermann, D. and Ehlers, R.U. (2005b) 'Mode of action of a surfactant-polymer formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)', *Biocontrol Science and Technology*, 15(6), pp. 601-613.

Shapiro-Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D.L., Behle, R.W. and Dunlap, C.A. (2010) 'Efficacy of *Steinernema carpocapsae* for control of the lesser peachtree borer, *Synanthedon pictipes*: Improved aboveground suppression with a novel gel application', *Biological Control*, 54(1), pp. 23-28.

Shapiro-Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D.L. and Zaid, A. (2015) 'Field suppression of the peachtree borer, *Synanthedon exitiosa*, using *Steinernema carpocapsae*: Effects of irrigation, a sprayable gel and application method', *Biological Control*, 82, pp. 7-12.

Shapiro-Ilan, D.I., Gouge, D.H. and Koppenho¨fer, A.M. (2002) 'Factors affecting commercial success: Case studies in cotton, turf and citrus', in Gaugler (ed.) *Entomopathogenic Nematology*. Wallingford, Oxfordshire, UK: CABI Publishing, pp. 333–356.

Shapiro-Ilan, D.I., Gouge, D.H., Piggott, S.J. and Fife, J.P. (2006a) 'Application technology and environmental considerations for use of entomopathogenic nematodes in biological control', *Biological Control*, 38(1), pp. 124-133.

Shapiro-Ilan, D.I., Han, R.C. and Dolinksi, C. (2012) 'Entomopathogenic Nematode Production and Application Technology', *Journal of Nematology*, 44(2), pp. 206-217.

Shapiro-Ilan, D.I., Jackson, M., Reilly, C.C. and Hotchkiss, M.W. (2004) 'Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of *Curculio caryae* (Coleoptera : Curculionidae)', *Biological Control*, 30(1), pp. 119-126.

Shapiro-Ilan, D.I., Stuart, R.J. and McCoy, C.W. (2006b) 'A comparison of entomopathogenic nematode longevity in soil under laboratory conditions', *Journal of Nematology*, 38(1), pp. 119-129.

Shapiro, D.I., McCoy, C.W., Fares, A., Obreza, T. and Dou, H. (2000) 'Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus* (Coleoptera : Curculionidae)', *Environmental Entomology*, 29(5), pp. 1083-1087.

Shapiro, M., McLane, W. and Bell, R. (1985) 'Laboratory evaluation of selected chemicals as antidesiccants for the protection of the entomogenous nematode, *Steinernema feltiae* (Rhabditidae: Steinernematidae), against *Lymantria dispar* (Lepidoptera: Lymantriidae)', *Journal of Economic Entomology*, 78(6), pp. 1437-1441.

Sharma, H.C., Sharma, K.K., Seetharama, N. and Ortiz, R. (2001) 'Genetic transformation of crop plants: Risks and opportunities for the rural poor', *Current Science*, 80(12), pp. 1495-1508.

Silva, G.A., Picanco, M.C., Bacci, L., Crespo, A.L.B., Rosado, J.F. and Guedes, R.N.C. (2011) 'Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*', *Pest management science*, 67(8), pp. 913-20.

Silva, W.M., Berger, M., Bass, C., Balbino, V.Q., Amaral, M.H.P., Campos, M.R. and Siqueira, H.A.A. (2015) 'Status of pyrethroid resistance and mechanisms in Brazilian populations of Tuta absoluta', *Pesticide Biochemistry and Physiology*, 122, pp. 8-14.

Silverio, F.O., de Alvarenga, E.S., Moreno, S.C. and Picanco, M.C. (2009) 'Synthesis and insecticidal activity of new pyrethroids', *Pest Management Science*, 65(8), pp. 900-905.

Siqueira, H.A.A., Guedes, R.N.C., Fragoso, D.B. and Magalhaes, L.C. (2001) 'Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae)', *International Journal of Pest Management*, 47(4), pp. 247-251.

Siqueira, H.A.A., Guedes, R.N.C. and Picanco, M.C. (2000a) 'Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae)', *Journal of Applied Entomology*, 124(5-6), pp. 233-238.

Siqueira, H.Á.A., Guedes, R.N.C. and Picanço, M.C. (2000b) 'Insecticide resistance in populations of *Tula absoluta* (Lepidoptera: Gelechiidae)', *Agricultural and Forest Entomology*, 2(2), pp. 147-153.

Smart, G.C. (1995) 'Entomopathogenic nematodes for the biological control of insects', *Journal of Nematology*, 27(4), pp. 529-534.

Smits, P.H. (1996) 'Post-application persistence of entomopathogenic nematodes', *Biocontrol Science and Technology*, 6(3), pp. 379-387.

Snelson, J.T. (1978) 'The need for and principles of pesticide registration', *FAO PLANT PROT. BULL.*, 26(3), pp. 93-100.

Sombardier, A., Savary, S., Blancard, D., Jolivet, J. and Willocquet, L. (2009) 'Effects of leaf surface and temperature on monocyclic processes in Podosphaera aphanis, causing powdery mildew of strawberry', *Canadian Journal of Plant Pathology*, 31(4), pp. 439-448.

Srinivasa Rao, K.Y., Pattanayak, D. and Sreevathsa, R. (2015) 'Bt Insecticidal Crystal Proteins: Role in Insect Management and Crop Improvement', in Sowjanya Sree, K. and Varma, A. (eds.) *Biocontrol of lepidopteran pests : use of soil microbes and their metabolites*. Cham, Switzerland: Springer, pp. 53-70.

Stock, S.P., Griffin, C.T. and Chaerani, R. (2004) 'Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp (Nematoda : Steinernematidae), an entomopathogenic nematode from Indonesia, and its phylogenetic relationships with other members of the genus', *Nematology*, 6, pp. 401-412.

Strassera, M.E. (2009) *Analisis de la sustentabilidad de tres alternativas de manejo de plagas en tomate bajo cubierta en el Cinturo´nHortı´cola Platense (Analysis of sustainability among three pest management alternatives in greenhouse tomato at La Plata Horticultural Belt, Buenos Aires, Argentina)*. Master thesis, Fac Cs Agrarias y Forestales, National University of La Plata.

Tabashnik, B.E., Cushing, N.L., Finson, N. and Johnson, M.W. (1990) 'Field Development of Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae)', *Journal of Economic Entomology*, 83(5), pp. 1671-1676.

Tadros, T.F. (1987) 'Interactions at interfaces and effects on transfer and performance', *Aspects of Applied Biology*, 14:1-22.

Talekar, N.S. and Shelton, A.M. (1993) 'Biology, ecology, and management of the Diamondback Moth', *Annual Review of Entomology*, 38, pp. 275-301.

Temerak, S.A. (2011) 'The status ot *Tuta absoluta* in Egypt', *EPPO / IOPC / FAO / NEPP Joint International Symposium on management of Tuta absoluta (tomato borer, Lepidoptera: Gelechiidae) Conference in collaboration with the IRAC and IBMA*. Agadri, Morocco, November 16-18. p. 18.

Terzidis, A.N., Wilcockson, S. and Leifert, C. (2014) 'The tomato leaf miner (*Tuta absoluta*): Conventional pest problem, organic management solutions?', *Organic Agriculture*, 4(1), pp. 43-61.

Tomalak, M., Piggott, S. and Jagdale, G.B. (2005) 'Glasshouse applications', in Grewal, P.S., Ehlers, R.U. and ShapiroIlan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 147-166.

Torné, M., Martín, A. and Fernández, J. (2010) 'Spintor 480SC: eficacia natural', *Phytoma España*, 217, pp. 27-31.

Torr, P., Heritage, S. and Wilson, M.J. (2004) 'Vibrations as a novel signal for host location by parasitic nematodes', *International Journal for Parasitology*, 34(9), pp. 997-999.

Torres Gregorio, J., Argente, J.T.B., Díaz, M.Á. and Yuste, A. (2009) 'Aplicación de" *Beauveria bassiana*" en la lucha biológica contra" *Tuta absoluta*"', *Agrícola vergel: Fruticultura, horticultura, floricultura*, 28(326), pp. 129-132.

Torres, J.B., Faria, C.A., Evangelista, W.S. and Pratissoli, D. (2001) 'Within-plant distribution of the leaf miner *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology', *International Journal of Pest Management*, 47(3), pp. 173-178.

Tropea Garzia, G. (2009) '*Physalis peruviana* L. (Solanaceae), a host plant of *Tuta absoluta* in Italy', *IOBC/WPRS bulletin*, 49, pp. 231-232.

Tropea Garzia, G., Siscaro, G., Biondi, A. and Zappalà, L. (2012) '*Tuta absoluta*, a South American pest of tomato now in the EPPO region: Biology, distribution and damage', *EPPO Bulletin*, 42(2), pp. 205-210.

Tuta absoluta (2016a) *Tuta absoluta Distribution*. *Tuta absoluta* Information Network. Available at: http://www.tutaabsoluta.com/ (Accessed: 15th February 2016).

Tuta absoluta (2016b) *Tuta Absoluta Gallery*. *Tuta Absoluta* Information Network. Available at: http://www.tutaabsoluta.com/gallery (Accessed: 15th February 2016).

Tuta absoluta Information Network (*2014*) *Tuta absoluta information network 'Tomato leaf miner Tuta absoluta reported in Tanzania for the first time (2014-08-27)'*. Available at: http://www.tutaabsoluta.com/news/376/tomato-miner-tuta-absoluta-reported-in-tanzania-for-the-first-time.

Uchôa-Fernandes, M.A., Della Lucia, T.M.C. and Vilela, E.F. (1995) 'Mating, oviposition and pupation of Scrobipalpuloides absoluta (Meyr.) (Lepidoptera: Gelechiidae)', *Anais da Sociedade Entomologica do Brasil*, 24(1), pp. 159-164.

Ulle" , J.A. (1996) *Comportamento de oviposicao de Scrobipalpuloides absoluta (Meyrick) (Lepidoptera: Gelechiidae) em tomateiro de crescimento indeterminado*. MSc thesis. UNESP, Botucatu, Sao Paulo, Brasil.

Unruh, T.R. and Lacey, L.A. (2001) 'Control of codling moth, Cydia pomonella (Lepidoptera : Tortricidae), with *Steinernema carpocapsae*: Effects of supplemental wetting and pupation site on infection rate', *Biological Control*, 20(1), pp. 48-56.

_____

Urbaneja, A., Desneux, N., Gabarra, R., Arnó, J., González-Cabrera, J., Mafra-Neto, A., Stoltman, L., De Sene Pinto, A. and Parra, J.R.P. (2013) 'Biology, ecology and management of the south american tomato pinworm, *Tuta absoluta*', in *Potential Invasive Pests of Agricultural Crops*. CABI Publishing, pp. 98-125.

Urbaneja, A., Gonzalez-Cabrera, J., Arno, J. and Gabarra, R. (2012) 'Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin', *Pest Management Science*, 68(9), pp. 1215-1222.

Urbaneja, A., Monton, H. and Molla, O. (2009) 'Suitability of the tomato borer *Tuta absoluta* as prey for Macrolophus pygmaeus and Nesidiocoris tenuis', *Journal of Applied Entomology*, 133(4), pp. 292-296.

Urbaneja, A., Vercher, R., Navarro, V., Garcı´a Marı´, F. and Porcuna, J. (2007) 'La polilla del tomate, *Tuta absoluta*', *Phytoma Espan˘a* 194, pp. 16-23.

USDA–APHIS (2011) 'New Pest Response Guidelines: Tomato Leafminer (*Tuta absoluta*). USDA APHIS–PPQ–EDP Emergency Management, Riverdale, Maryland'.

Van Driesche, R.G. and Bellows, T.S. (1996) *Biological control*. New York: Chapman & Hall. 539 p.

Van Emden, H.F. and Service, M.W. (2004) *Pest and vector control*. Cambridge, UK; New York: Cambridge University Press. p. 349.

van Niekerk, S. and Malan, A.P. (2015) 'Adjuvants to improve aerial control of the citrus mealybug *Planococcus citri* (Hemiptera: Pseudococcidae) using entomopathogenic nematodes', *Journal of Helminthology*, 89(2), pp. 189-195.

Vänninen, I. (1990) *Proceedings and abstracts, Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia, 20-24 August 1990*. Department of Entomology, University of Adelaide. pp. 232.

Vargas, H. (1970) 'Observaciones sobre la biologia enemigos naturales de las polilla del tomate, *Gnorimoschema absoluta* (Meyrick)', *Depto. Agricultura, Universidad del Norte-Arica*, 1, pp. 75-110.

Vercher, R., Calabuig, A. and Felipe, C. (2010) 'Ecology, sampling and economic threshold of *Tuta absoluta* (Meyrick)]', *Phytoma España*, 217, pp. 23-26.

Viggiani, G., Filella, F., Delrio, G., Ramassini, W. and Foxi, C. (2009) '*Tuta absoluta*, new moth reported also in Italy', *L'Informatore Agrario,* , 2, pp. 66-68.

Waage, J.K. and Hassell, M.P. (1982) 'Parasitoids as biological control agents – a fundamental approach', *Parasitology*, 84(4), pp. 241-268.

Weinzierl, R. and Henn, T. (1991) 'Alternatives in insect management: biological and biorational approaches', *North central regional extension publication (USA)*.

Weisenburger, D.D. (1993) 'Human health effects of agrichemical use', *Human Pathology*, 24(6), pp. 571–576.

_____

Whitten, M.J. and Oakeshott, J.G. (1991) 'Opportunities for modern biotechnology in control of insect pests and weeds, with special reference to developing countries', *FAO Plant Protection Bulletin (FAO)*, 39, pp. 155-181.

Williams, E.C. and MacDonald, O.C. (1995) 'Critical factors required by the nematode *Steinernema feltiae* for the control of the leafminers *Liriomyza huidobrensis*, *Liriomyza bryoniae* and *Chromatomyia syngenesiae*', *Annals of Applied Biology*, 127(2), pp. 329-341.

Williams, E.C. and Walters, K.F.A. (1994) 'Nematode control of leafminer - Efficacy, Temperature and Timing ', *Brighton Crop Protection Conference - Pests and Diseases - 1994, Vols 1-3, Proceeding - Brighton Crop Protection Conference* Brighton, England 21-24 NOV 1994 British Crop Protection Council, pp. 1079-1084. Available at: <Go to ISI>://WOS:A1994BC92A00158.

Williams, E.C. and Walters, K.F.A. (2000) 'Foliar application of the entomopathogenic nematode *Steinernema feltiae* against leafminers on vegetables', *Biocontrol Science and Technology*, 10(1), pp. 61-70.

Williams, T., Valle, J. and Vinuela, E. (2003) 'Is the naturally derived insecticide Spinosad (R) compatible with insect natural enemies?', *Biocontrol Science and Technology*, 13(5), pp. 459-475.

Willmot, D.M., Hart, A.J., Long, S.J., Edmondson, R.N. and Richardson, P.N. (2002) 'Use of a cold-active entomopathogenic nematode Steinernema kraussei to control overwintering larvae of the black vine weevil Otiorhynchus sulcatus (Coleoptera: Curculionidae) in outdoor strawberry plants', *Nematology*, 4(8), pp. 925-932.

Wing, K.D., Sacher, M., Kagaya, Y., Tsurubuchi, Y., Mulderig, L., Connair, M. and Schnee, M. (2000) 'Bioactivation and mode of action of the oxadiazine indoxacarb in insects', *Crop Protection*, 19(8-10), pp. 537-545.

Winston, P.W. and Bates, D.H. (1960) 'Saturated Solutions For the Control of Humidity in Biological Research', *Ecology*, 41(1), pp. 232-237.

Witzgall, P., Kirsch, P. and Cork, A. (2010) 'Sex Pheromones and Their Impact on Pest Management', *Journal of Chemical Ecology*, 36(1), pp. 80-100.

Womersley, C.Z. (1990) 'Dehydration survival and anhydrobiotic potential', in Gaugler, R. and Kaya, H.K. (eds.) *Entomopathogenic nematodes in biological control*. Boca Raton, FL.: CRC Press, pp. 117–137.

Wright, D.J., Peters, A., Schroer, S. and Fife, J.P. (2005) 'Application Technology', in Grewal, P.S., Ehlers, R.U. and ShapiroIlan, D.I. (eds.) *Nematodes as Biocontrol Agents*. Wallingford, Oxfordshire, UK CABI Publishing, pp. 91-106.

Yardim, E.N. and Edwards, C.A. (1998) 'The influence of chemical management of pests, diseases and weeds on pest and predatory arthropods associated with tomatoes', *Agriculture Ecosystems & Environment*, 70(1), pp. 31-48.

Yarwood, C.E. and Hazen, W.E. (1944) 'The Relative Humidity at Leaf Surfaces', *American Journal of Botany*, 31(3), pp. 129-135.

_____

Yoder, C.A., Grewal, R.S. and Taylor, R.A.J. (2004) 'Rapid age-related changes in infection behavior entomopathogenic nematodes', *Journal of Parasitology*, 90(6), pp. 1229-1234.

Zappalà, L., Biondi, A., Alma, A., Al-Jboory, I.J., Arnò, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D., Guenaoui, Y., Shaltiel-Harpaz, L., Siscaro, G., Stavrinides, M., Tavella, L., Vercher Aznar, R., Urbaneja, A. and Desneux, N. (2013) 'Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies', *Journal of Pest Science*, 86(4), pp. 635-647.

Zhang, Y., Zhang, W., Han, X. and Li, X. (1997) 'Biochemical and physiological mechanisms of insecticide resistance in Helicoverpa armigera (Hubner)', *Acta Entomologica Sinica*, 40(3), pp. 247-253.

_____