Nabil El-Wakeil[1,2], Christa Volkmar[2]

Efficacy of entomopathogenic nematodes against the frit fly *Oscinella frit* (L.) (Diptera: Chloropidae) on spring wheat

Zur biologischen Regulation der Fritfliege *Oscinella frit* (L.) durch entomopathogene Nematoden an Sommerweizen

Abstract

The efficiency of three species of entomopathogenic nematodes (EPNs) (*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) and one pyrethroid (lambda-cyhalothrin) were evaluated to control frit fly, *Oscinella frit* (L.) in the laboratory and field on two wheat varieties (Triso and Sakha 93) during 2009 and 2010. This is the first research studying the efficiency of EPNs against *O. frit*. Within seven days in laboratory tests, up to 100% mortality was observed in *O. frit* larvae with all nematodes and the pyrethroid; however, the pyrethroid was only 100% effective when high concentrations and young pest larvae were used. In laboratory tests, *H. bacteriophora* was more efficacious than *S. carpocapsae* against *O. frit,* while the latter was more efficient in field experiments. In 2009 field experiments, *Steinernema carpocapsae* was more efficacious than *S. feltiae* and *H. bacteriophora*; however, in 2010 *S. carpocapsae* and *S. feltiae* were more efficient than *H. bacteriophora*. Weather parameters affect the infestation percentages of *O. frit* and the efficiency of EPNs as well. Weather conditions in 2009 were warmer and with less rain (14.0°C and 0.9 mm) compared to 2010 (12.0°C and 3.1 mm) for mean temperature and rainfall, respectively. Thus, infestation percentages were greater in 2009 because weather conditions negatively affected oviposition and newly hatched larvae in 2010. In 2009 and 2010 the yield index was greater in Triso than Sakha 93 variety in weight of grains/ha. It can be concluded that λ-cyhalothrin, *S. feltiae* and *S. carpocapsae* were the most efficacious in *O. frit* control and consequently provided the greatest yields. These results confirmed that EPNs can be used as biocontrol agents in Integrated Pest Management (IPM) programs of *O. frit*.

Key words: *Oscinella frit*, spring wheat, biological control, λ-cyhalothrin, yield

Zusammenfassung

Die Larven der Fritfliege können während der Blattentwicklung und Bestockungsphase beträchtliche Schäden an Sommerweizen hervorrufen. Aufgrund der Diskussion um die Beizproblematik wurde nach alternativen Bekämpfungsmaßnahmen in der Frühphase der Pflanzenentwicklung gesucht. Deshalb wurde in den Jahren 2009 und 2010 eine Untersuchung zum Befall des deutschen Wechselweizens Triso und des ägyptischen Sommerweizens Sakha 93 mit *Oscinella frit* (L.) und deren Regulierung durchgeführt. Es wurden die entomopathogenen Nematoden *Steinernema feltiae*, *Steinernema carpocapsae* und *Heterorhabditis bacteriophora* sowie das Pyrethroid Karate Zeon auf ihr Potential zur Regulation der Larvenpopulation im Labor sowie unter Freilandbedingungen getestet.

Im Labor erfolgte die Untersuchung befallener Pflanzenproben aus den behandelten Freilandparzellen auf Vorhandensein und Vitalität der Fritfliegenlarven zur Bestimmung der Mortalitätsrate. Des Weiteren wurden befallene Pflanzen aus unbehandelten Randparzellen entnommen und aus ihnen Larven präpariert, welche dann in Petri-

Institute

Pests and Plant Protection Dept. National Research Center, Dokki, Cairo, Egypt[1]

Institute of Agricultural and Nutritional Sciences, Martin Luther-University Halle-Wittenberg, Germany[2]

Correspondence

Dr. Nabil El-Wakeil, Institute of Agricultural and Nutritional Sciences, Martin Luther-Univeristy Halle-Wittenberg, Betty Heimann Str. 3, 06120 Halle (Saale) Germany, E-Mail: nabil.el-wakeil@landw.uni-halle.de

Accepted

30 November 2012

schalen mit den Nematodenarten sowie Karate Zeon unter Verwendung der vollen, der halben und einem Viertel der Aufwandmenge über 3 Termine auf ihre Vitalität untersucht wurden. Es herrschten konstante Temperaturbedingungen von 25 ± 2°C. In diesem Versuch zeigte *H. bacteriophora* eine größere Effizienz als *S. carpocapsae*, im Freiland war *S. carpocapsae* erfolgreicher. Die Ergebnisse bestätigen Literaturangaben, die auf höhere Temperaturansprüche von *H. bacteriophora* hinweisen. Das Regulationspotential von *S. feltiae* lag unter denen der anderen Nematodenarten. In Abhängigkeit von der Konzentration und dem Larvenstadium konnten im Labor bei den Nematoden wie auch bei Karate Zeon bis zu 100% Larvenmortalität erreicht werden. Die Spezies *H. bacteriophora* als effektivste Nematodenart im Labor erreichte bei Konzentrationen von 250 (¼), 500 (½) und 1000 (volle Aufwandmenge) infektiösen Nematoden pro ml eine Larvenmortalität bei L2-Larven von 74, 86 und 88% nach einem Tag sowie 90, 98 und 100% nach 7 Tagen. Bei den L3-Larven war das Ergebnis nach einem Tag um ca. 20% geringer, nach 7 Tagen wurde aber die gleiche Mortalität erzielt. Karate Zeon brachte im Vergleich zu *H. bacteriophora* denselben Erfolg.

Weiterhin wurde zweimal wöchentlich der sichtbare Schaden durch die Fritfliegenlarven am Sommerweizen bonitiert und befallene Pflanzen wie bereits dargestellt im Labor untersucht. Die Schädigung der Pflanzen (%) war 2009 höher als 2010, offensichtlich beeinflussten die ungünstigen abiotischen Bedingungen 2010 das Befallsgeschehen negativ. Der Durchschnitt der bereits sichtbaren Schadsymptome im Freiland betrug vor der Behandlung 1,5% bei der deutschen und 2,3% bei der ägyptischen Varietät und erhöhte sich bis zum 14. Tag nach der ersten Applikation auf 24,7 bzw. 40,0%. Dieser signifikante Unterschied zwischen den Sommerweizenvarietäten ist auf die schnellere Entwicklung der deutschen Sorte zurückzuführen. Nach der zweiten Applikation reduzierten sich die sichtbaren Symptome. Weiterhin konnten signifikante Unterschiede der Behandlungen zur Kontrolle bezüglich Larvenmortalität, Befallsreduktion und Ertrag erkannt werden. Die Ergebnisse belegen in beiden Jahren, dass die Möglichkeit der effizienten Regulation mit entomopathogenen Nematoden im Sinne des Biologischen Pflanzenschutzes besteht.

Stichwörter: Fritfliege, Sommerweizen, biologische Bekämpfung, λ-Cyhalothrin, Ertrag

1 Introduction

Frit fly *Oscinella frit* (L.) (Diptera: Chloropidae) is a stemborer that can cause considerable damage in newly sown spring cereals. Spring wheat is susceptible to *O. frit* infestation at early growth stages. Early studies have shown that damage on autumn-sown cereals is lower at late sowing dates due to low temperatures after seedling emergence (Clements et al., 1990) Plants with one or two unfolded leaves, growth stage 11 and 12 are most susceptible to attack in wheat according to growth stages (Meier, 2001). At later growth stages, seedlings are less susceptible because they are more difficult for the larvae to penetrate (Jonasson, 1977).

Oscinella frit is an economic pest to wheat, barley, oats, rye and other cereal grains in many places over the world: Italy, Sweden and Germany (Henderson and Clements, 1979; Larsson, 1984; El-Wakeil et al., 2009). Frit fly overwinters as a larva within the stems of cereals (Lindblad, 1999) and pupates in spring. Adults emerge in early summer and migrate by flight from overwintering sites to spring cereals where the females oviposit (Tolley and Niemzcyk, 1988). Large population fluctuations occur among years; the numbers of spring migrants vary up to 20-fold (Lindblad and Sigvald, 1999). On wheat seedlings, the females preferably lay eggs behind coleoptiles (Jonasson, 1977). After hatching, the larvae penetrate the plant and destroy the main shoot. Damaged plants produce small panicles which mature late causing high yield losses (Lindblad and Sigvald, 1999). Synthetic pyrethroids applied at early growth stages have been shown to control *O. frit* (Larsson, 1984), and significantly increase yields when measured across multiple locations (Clements et al., 1990; El-Wakeil et al., 2009). Because insecticides are known to cause many problems to humanity and the environment, many studies are looking for controlling Dipterans with alternative environmentally friendly methods (i.e. biological control); specifically entomopathogenic nematodes (EPNs), which serve as alternatives for chemical insecticides (Toledo et al., 2005; Hussein et al., 2006; Oestergaard et al., 2006).

Genera *Steinernema* and *Heterorhabditis* are pathogenic to many insects attacking many economic crops in the world (Poinar, 1990). They are symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively that are harbored in the intestine of the third-stage infective juvenile (IJ) (Ciche et al., 2006). Entomopathogenic nematodes have potential use for not only against insects in soil and cryptic habitats, but also against leaf-feeding insects (Hussein et al., 2006; Saleh et al., 2009). Půža and Mráček (2005) stated that broad host range of EPNs in many habitats was predominantly represented by dipteran larvae. Studies were conducted on *Tipula* species (Peters and Ehlers, 1994; Gerritsen et al., 1998). *Steinernema feltiae* is known for its high virulence to a number of small-sized hosts such as families Sciaridae and Phoridae (Jess et al., 2006); especially the young host larvae which are the most appropriate stage for EPNs to penetrate and develop. Susurluk (2008) reported that some *Steinernema* species were more efficient than *Heterorhabditis bacteriophora* in low temperatures. Although heterorhabditids are endemic to warmer climates, the upper thermal limits and temperature optima for reproduction of *H. bacteriophora* and *H. megidis* were cooler than that of some of the steinernematids (Grewal et al., 1994). Recommended application rates vary with application time, different nematode strains and different fly species in different growing conditions, all lead to different recommended doses (Fenton et al., 2002).

The main objective of this work is to investigate the possibility of controlling *O. frit* with EPNs and lambda (λ-) cyhalothrin by: (1) studying infectivity of two steinernematids, one heterorhabditid and chemical toxicity of λ-cyhalothrin against *O. frit* larvae in the laboratory, (2) assessing the role of EPNs and λ-cyhalothrin in suppressing *O. frit* populations and their effect on spring wheat yield.

2 Materials and Methods

2.1 Materials

2.1.1 Nematodes. EPNs for lab and field trials (*S. feltiae*, *S. carpocapsae* and *H. bacteriophora*) were obtained from the commercial company e-nema GmbH (Raisdorf, Germany) that had been cultured in liquid media according to the method described by EHLERS (2001).

2.1.2 Karate. The pyrethroid insecticide Karate SC 9.4% (lambda-cyhalothrin) was used at the rate of 75 ml/200L water/ha (7.5 g active ingredient; Active ingredient is 100 g/l lambda-cyhalothrin and 1,2-benzisothiazolin-3-one) to control *O. frit* (ANONYMOUS, 2008).

2.2 Efficiency of EPNs & λ-cyhalothrin on frit fly O. frit in laboratory

Water suspensions of the studied EPNs (*S. feltiae*, *S. carpocapsae* and *H. bacteriophora*) were prepared at serial concentrations of 0, 250, 500 and 1000 infective juveniles (IJs)/ml. Λ-cyhalothrin was also sprayed using three concentrations; 0.375, 0.188 and 0.094 µl/ml (1, ½, and ¼ field rates, respectively) plus control. Five larvae of *O. frit* (2nd instar (L2) as well as five larvae 3rd instar (L3)) were separately placed in a 10-cm diameter Petri dish furnished with filter paper and treated with 1 ml of assigned nematode or λ-cyhalothrin concentrations. Pieces of wheat stems were added for larval feeding. Each experiment was replicated 10 times, including five larvae per replicate. The dishes were kept at 25 ± 2°C and recorded the larval mortality for L2 and L3 after 1, 3 and 7 days. *O. frit* larvae dead in EPNs treatments were inspected by microscope to determine whether or not these larvae were infected with the EPNs. The mortality percents were corrected according to Abbott's formula (ABBOTT, 1925).

2.3 Spring wheat plots

The experiments were conducted in Julius Kühn field (sandy loam soil) in Halle University, Germany. An Egyptian wheat variety (Sakha 93) commonly cultivated in the Delta region of Egypt, and is known to be resistant against drought. In addition, a high quality German variety (Triso); both were selected for these experiments, which were cultivated at end of March in 2009 and 2010. The experimental area was a randomized complete block design with four blocks. In each block, variables (5 treatments and 2 wheat varieties) were distributed randomly; each treatment was replicated four times. The experimental unit was the 1.5 × 8m plots. Weather conditions were warmer and dryer in 2009 (14.0°C and 0.9 mm) than 2010 (12.0°C and 3.1 mm) for mean temperature and rainfall, respectively from the middle of April to the end of May (local meteorological station – Kühn field in Halle/Saale, Germany, N 51.48°; E 11.96°).

2.4 Field experiments

2.4.1 Treatments. The EPNs and λ-cyhalothrin were sprayed in the field on 23rd April and 7th May 2009, and 29th April and 13th May 2010 at growth stages 11 and 20, respectively (MEIER, 2001). Nematode suspensions of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* were sprayed with application dose 2.5×10^9 IJs/ha, and applied to wheat plants naturally infested with *O. frit*. Lambda-cyhalothrin at the rate of 75 ml/200L water/ha (7.5 g active ingredient) was sprayed using a knapsack sprayer fitted with one nozzle. Care was taken to avoid insecticide drifts among plots (ANONYMOUS, 2008). The control plots were only sprayed with water.

2.4.2 Frit fly infestation. Inspection of infestation was carried out just before spraying and 3, 7, 10, 15 days after both sprays by investigating 10 plants randomly from each plot (total 40 plants/treatment: All sampled plants were examined visually for infestation symptom (yellow or missing central leaf:

2.4.3 Frit fly larval mortality. Frit fly larvae were counted by dissecting 10 infested tillers in each plot (40 plants/treatment: These larvae were observed whether they were live or dead to calculate the mortality percents after 3 and 10 days of both sprays. Dead larvae were observed once again in nematode treatments after 7 days to confirm that the death was due to EPNs by dissecting them.

2.4.4 Yield. The mature kernels of each plot were harvested and weighed to estimate wheat yield for each treatment. Finally, that value was converted to the yield in kilograms per hectare.

2.5 Statistical analyses

Mortality percents obtained from laboratory experiment were corrected according to Abbott's formula (ABBOTT, 1925). Data of infestation and mortality percents were arcsine square-root transformed. The difference among treatments was analyzed by Generalized Linear Model (GLM), ANOVA, Factorial Design; treatments (EPNs and λ-cyhalothrin) as the main effect; interactions between treatments, investigation dates and wheat varieties were analyzed using Statistix 9 program (THOMAS and MAURICE, 2008). Also, percent infestation data were analyzed by a repeated measures design. The F-test assumes that the within-group variances are the same for treatments and varieties. The null hypothesis of these tests is that different treatments and varieties are equal. A large F-test and corresponding small p-value (say, smaller than 0.05) is evidence that there are differences, by using Tukey's test to compare means of treatments and varieties.

3 Results

3.1 Effect of EPNs and λ-cyhalothrin on mortality percents of frit fly in laboratory

There were significant differences ($df = 2, 27$, $F = 10.15$, $P = 0.005$) in percent mortality caused by *S. feltiae* vs L2 of *O. frit*. They reached 62, 78 and 80% after one day post application with concentrations 250, 500 and 1000 IJs, respectively. These percents increased daily to reach 86, 96 and 100% after 7 days (Fig. 1A). In the case of L3, there was also significant difference ($df = 2, 27$, $F = 12.84$, $P = 0.0001$) which resulted in 50, 64, and 68% mortality at the 3 doses on day one post treatment that reached to 80, 90 and 96% mortality by day 7 (Fig. 1B). The percent mortality was lower on L3 than L2, indicating some tolerance to L3.

Percent mortality significantly differed ($df = 2, 27$, $F = 16.43$, $P = 0.0011$) among different concentrations in *S. carpocapsae* replicates. The percent mortality of L2 of *O. frit* was 58, 70 and 76% on the 1st day application of 250, 500 and 1000 IJs, respectively. These percents increased daily reaching to 82, 92 and 100% after 7 days (Fig. 1A). The percent mortality was significantly higher ($df = 2, 53$, $F = 9.35$, $P = 0.003$) on L2 than L3, because the later was more tolerant to EPNs, wherever *S. carpocapsae* caused percent mortality 44, 56 and 62% on the 1st day post treatment; while they reached 74, 90 and 100% on the 7th day (Fig. 1B).

The percent mortality by *H. bacteriophora* on L2 reached 74, 86 and 88% on the 1st day post treatment of 250, 500 and 1000 IJs, respectively. The respective percent mortality increased daily to reach 90, 98 and 100% after 7 days (Fig. 1A). While L3 has slightly more tolerance, thus the percent mortality was significantly lower ($df = 2, 27$, $F = 10.27$, $P = 0.0005$), L3 percent mortality was 52, 68 and 76% on the 1st day and reached 90, 94 and 100% after 7 days, respectively (Fig. 1 B).

Λ-cyhalothrin caused a significantly higher percent mortality than EPNs on L2 ($df = 3, 35$, $F = 8.97$, $P = 0.002$) with the percent mortality reaching 68, 86, and 100% on day one post treatment at doses of 0.375, 0.188 and 0.094 µl of λ-cyhalothrin, respectively. The respective percent mortality increased daily to reach 90, 100 and 100% after 7 days (Fig. 1A). There was a significant difference ($df = 2, 27$, $F = 10.34$, $P = 0.005$) in percent mortality caused by λ-cyhalothrin vs L3. This percent mortality was 54, 72 and 94% after one day and reached to 82, 100 and 100% on the 7th day, respectively (Fig. 1 B).

Generally, there were significant differences ($df = 4, 89$, $F = 3.57$, $P = 0.0095$) between λ-cyhalothrin and EPNs; the percent mortality was higher on the 1st day with λ-cyhalothrin than EPNs treatments. There were significant differences ($df = 2, 53$, $F = 6.72$, $P = 0.0025$) in percent mortality caused by the three EPNs species. *H. bacteriophora* caused a greater percent mortality than *S. feltiae* and *S. carpocapsae* in laboratory experiment. Λ-cyhalothrin affected more than EPNs to control *O. frit*.

3.2 Field experiments

3.2.1 Frit fly infestation percents.

A 2009

After the first spray, the percent infestation significantly differed between treatments ($df = 4, 99$, $F = 11.36$, $P = 0.0001$); they were higher in control than the treated plots. There was a significant difference ($df = 3, 79$, $F = 24.57$, $P = 0.0002$) between λ-cyhalothrin and EPNs treatments (these percents were lower in λ-cyhalothrin than EPNs plots: Among EPNs nematodes, there was a significant difference ($df = 2, 59$, $F = 4.67$, $P = 0.0131$), where the lowest infestations were recorded in *S. carpocapsae* plots compared to *S. feltiae* and *H. bacteriophora*, especially in Sakha 93 variety (Fig. 2A). Data analyses showed that there were significant differences between wheat varieties ($df = 1, 6$, $F = 64.73$, $P = 0.002$); the infestation

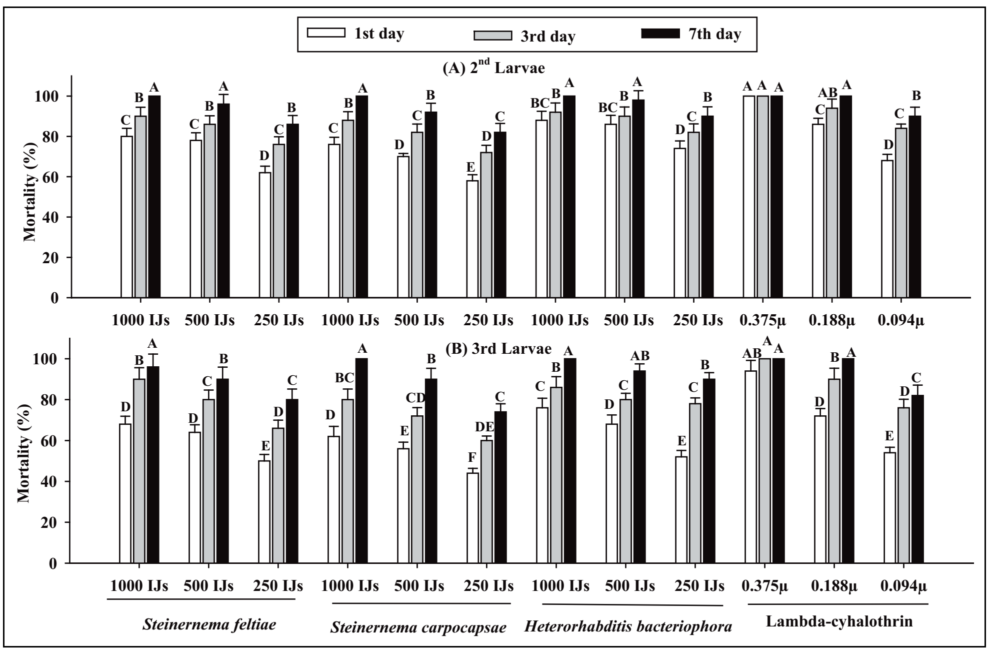

Fig. 1. Effects of entomopathogenic nematodes and Lambda-cyhalothrin on larval percent mortality of (A) 2nd and (B) 3rd larval instars of *Oscinella frit* in laboratory. Different letters indicate significant differences.

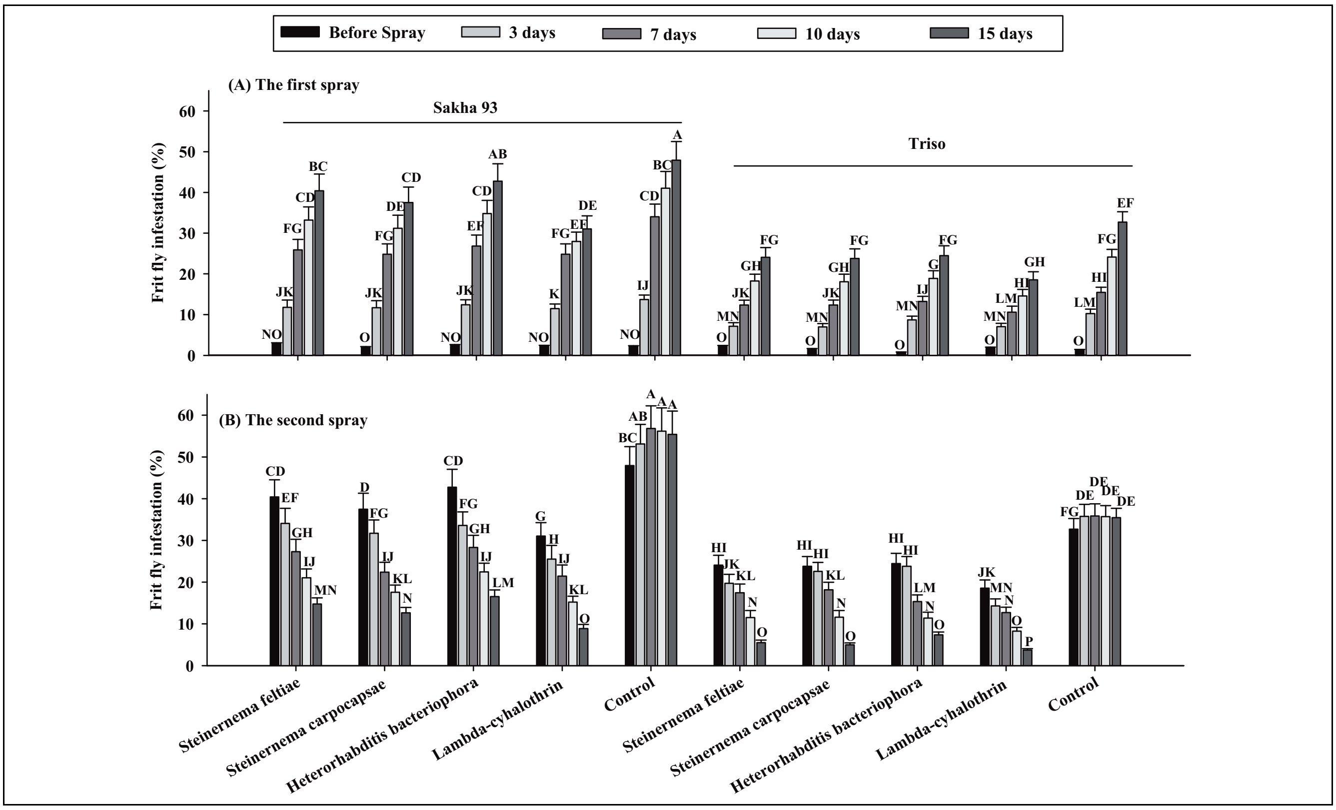

Fig. 2. Effects of entomopathogenic nematodes and Lambda -cyhalothrin on *Oscinella frit* percent infestation before and after 3, 7, 10 and 15 days: (A) 1st spray on 23 April and (B) 2nd spray on 7 May 2009 in two wheat varieties. Different letters indicate significant differences.

percents in Sakha 93 variety were higher than Triso variety (Fig. 2A).

After the second spray, percent infestation significantly differed (df = 4, 99, F = 43.50, P = 0.001) between treatments; where λ-cyhalothrin and *S. carpocapsae* were more efficient in reducing the percent infestation than *S. feltiae* and *H. bacteriophora*. The percent infestation of *O. frit* reduced regularly with days post treatment (Fig. 2B). The analyses of data showed that there were significant differences between wheat varieties (df = 1, 6, F = 67.38, P = 0.003); the Sakha 93 variety had received infestation percents higher than Triso variety (Fig. 2B).

B 2010

There were significant differences in percent infestation (df = 4, 99, F = 25.18, P = 0.002) between treatments; where λ-cyhalothrin had the lowest percent infestation, followed by *S. feltiae* and *S. carpocapsae* which had the greatest infestation than *H. bacteriophora* after the first spray. The percent infestation significantly differed (df = 1, 6, F = 29.58, P = 0.0016) between varieties; Sakha 93 had infested tillers more than Triso (Fig. 3A).

After the second spray, the differences between treatments were significant (df = 4, 99, F = 4.05, P = 0.0044), the percent infestation was higher in control than the treated plots. There was a significant difference (df = 3, 79, F = 3.76, P = 0.014) between λ-cyhalothrin and EPNs treatments (these percents were lower in λ-cyhalothrin than EPNs plots: Among EPNs nematodes, there was a significant difference (df = 2, 59, F = 6.64, P = 0.0025), where the highest infestations were recorded in *H. bacteriophora* plots compared to *S. carpocapsae* and *S. feltiae*; the later two species have the same percent infestation in the 2010 (Fig. 3B: Frit fly infestation was reduced with days after the second spray. The percent infestation significantly differed between varieties (df = 1, 6, F = 10.08, P = 0.0192); they were lower in Triso than in Sakha 93 variety (Fig. 3B).

3.2.2 Frit fly larval mortality percents.

A 2009

After the first spray, the larval percent mortality was significantly different (df = 2, 23, F = 34.75, P = 0.0001) among EPNs treatments after 3 and 10 days, where percent mortality after 10 days were higher than after 3 days. There was no significant difference (df = 1, 7, F = 5.02, P = 0.061) between two dates in λ-cyhalothrin plots. In Sakha 93 variety, *S. carpocapsae* achieved greater larval percent mortality 60% after 10 days than other treatments, while λ-cyhalothrin caused 53.3% on 10th day (Fig. 4A). In Triso variety, λ-cyhalothrin accomplished the highest larval percent mortality 53.3% on both dates, followed by *S. carpocapsae* which had caused mortality (37 and 43.3%) after 3 and 10 days, respectively (Fig. 4A).

After the second spray, there were significant differences between percent mortality after 3 and 10 days (df = 2, 23, F = 54.45, P = 0.0001) in EPNs plots, where mortality after 10 days was greater than after 3 days. The larval

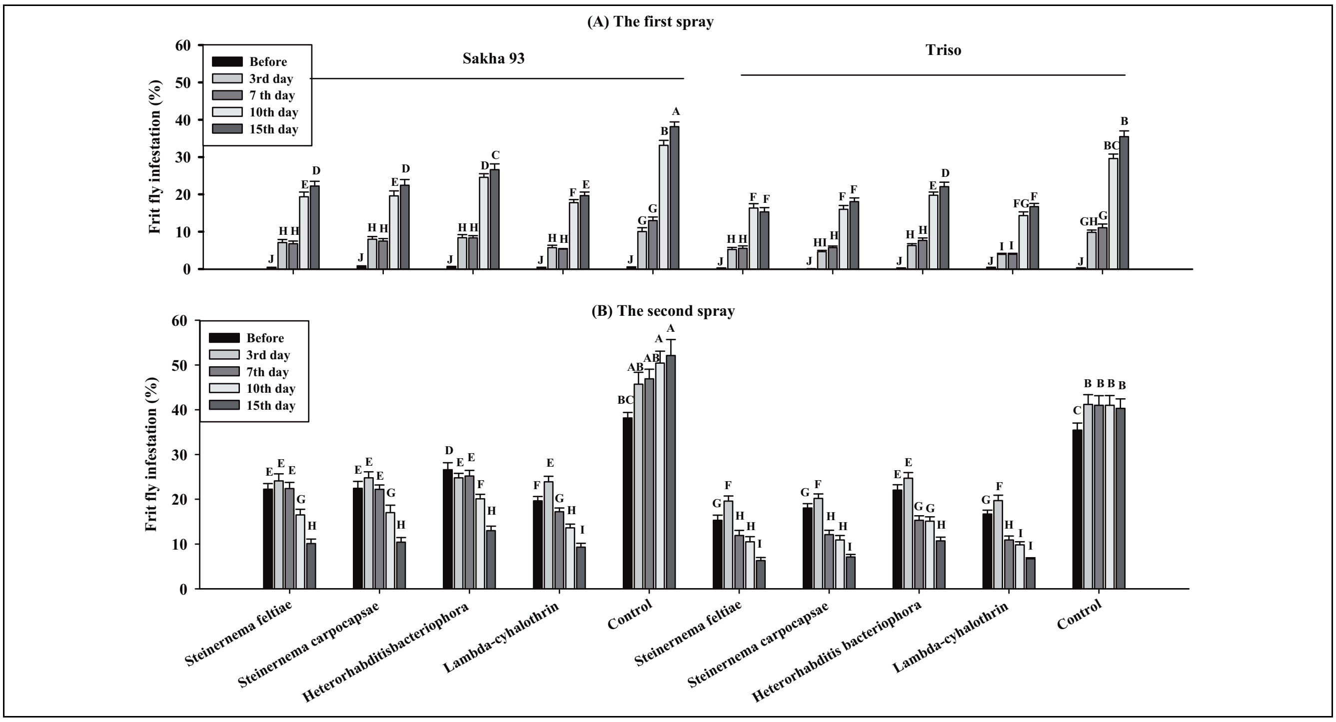

Fig. 3. Effects of entomopathogenic nematodes and Lambda-cyhalothrin on *Oscinella frit* percent infestation before and after treatments in two wheat varieties: (A) 1st spray on 29 April and (B) 2nd spray on 13 May 2010. Different letters indicate significant differences.

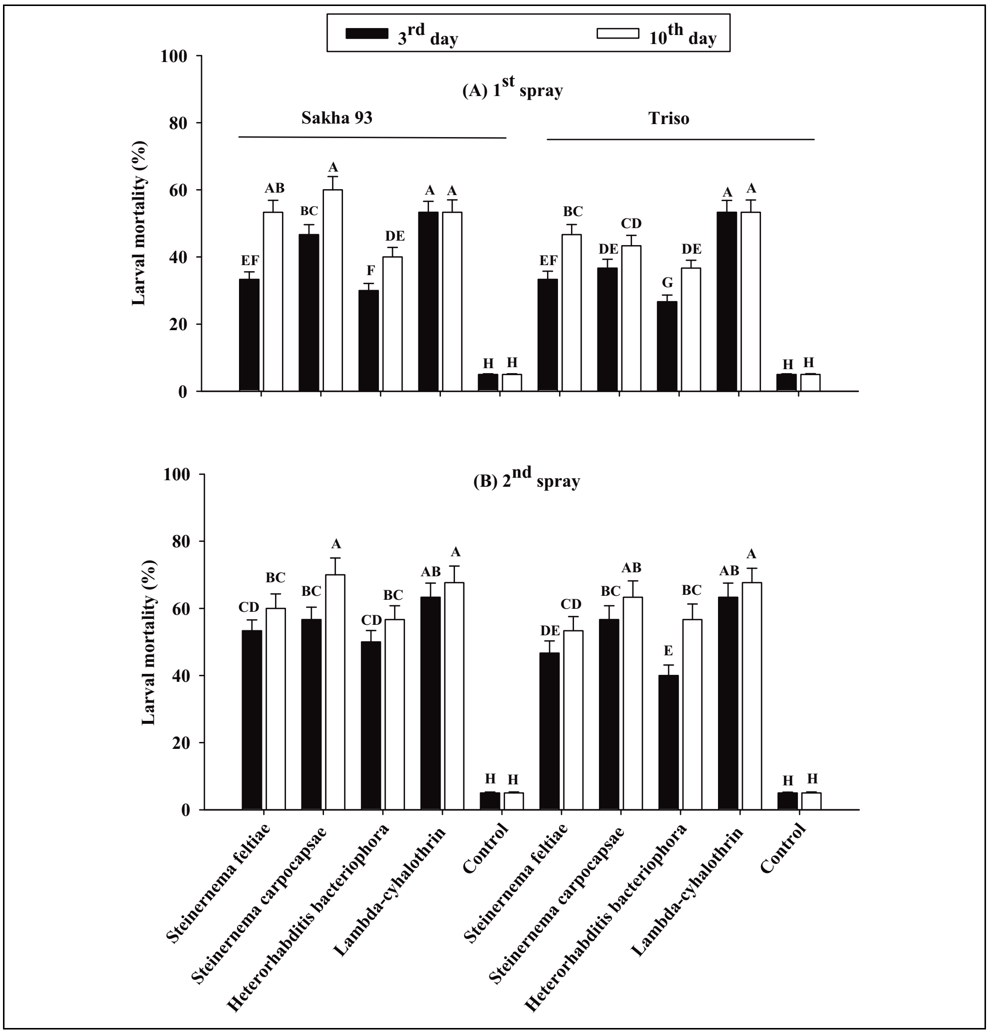

Fig. 4. Mean ± SE of larval percent mortality in infested tillers in two spring wheat varieties after entomopathogenic nematodes and Lambda-cyhalothrin treatments: (A) 1st spray on 23 April and (B) 2nd spray on 7 May 2009. Different letters indicate significant differences.

mortality did not differ significantly (df = 1, 7, F = 4.34, P = 0.0757) between two dates in λ-cyhalothrin plots. The highest larval mortality was recorded in *S. carpocapsae* plots; 70% after 10 days than other treatments, while λ-cyhalothrin had caused 68% mortality after 10 days, respectively in Sakha 93 variety (Fig. 4B). While in Triso variety, the greatest percent mortality was recorded in λ-cyhalothrin (63 and 68%) after 3 and 10 days, followed by *S. carpocapsae* which caused 57 and 63.3% mortality after 3 and 10 days, respectively (Fig. 4B).

B 2010

Steinernema species (*feltiae* and *carpocapsae*) achieved the same mortality level in 2010. There were significant differences in larval percent mortality (df = 4, 39, F = 36.18, P = 0.0001) among treatments after the 1st spray; the highest mortality was found in treated compared to control plots. Among EPNs treatments, percent mortality was significantly higher (df = 2, 23, F = 19.88, P = 0.002) in *S. feltiae* and *S. carpocapsae* than *H. bacteriophora*. There were significant differences (df = 1, 7, F = 135.13, P = 0.0011) in larval mortality between the investigation dates (3rd and 10th); the highest mortality was recorded on the 10th day compared to 3rd day (Fig. 5A).

After the 2nd spray, the larval percent mortality was significantly different (df = 4, 39, F = 94.51, P = 0.001) between treated and control plots; λ-cyhalothrin had the highest mortality percents, followed by both *Steinernema* species compared to the others (Fig. 5B). There were significant differences between percent mortality (df = 2, 23, F = 57.25, P = 0.001) among EPNs treatments after 3 and 10 days; where mortality after 10 days was higher than after 3 days. The highest larval mortality was recorded in *S. carpocapsae* and *S. feltiae* plots than *H. bacteriophora* (Fig. 5B).

3.2.3 Yield.

A 2009

These results suggest that all treatments enhance yield relative to the control. Analysis showed significant differences (df = 4, 4, F = 16.50, P = 0.0094) between treatments and control. There were significant differences (df = 3, 3, F = 29.75, P = 0.0099) between λ-cyhalothrin and EPNs species. There were other significant differences (df = 2, 2, F = 23.79, P = 0.0403) among EPNs species, where the yield in *S. carpocapsae* plot was higher than in other EPNs species (Fig. 6A). Within the Triso variety, the highest yield was recorded in λ-cyhalothrin treatment (5339 kg/ha),

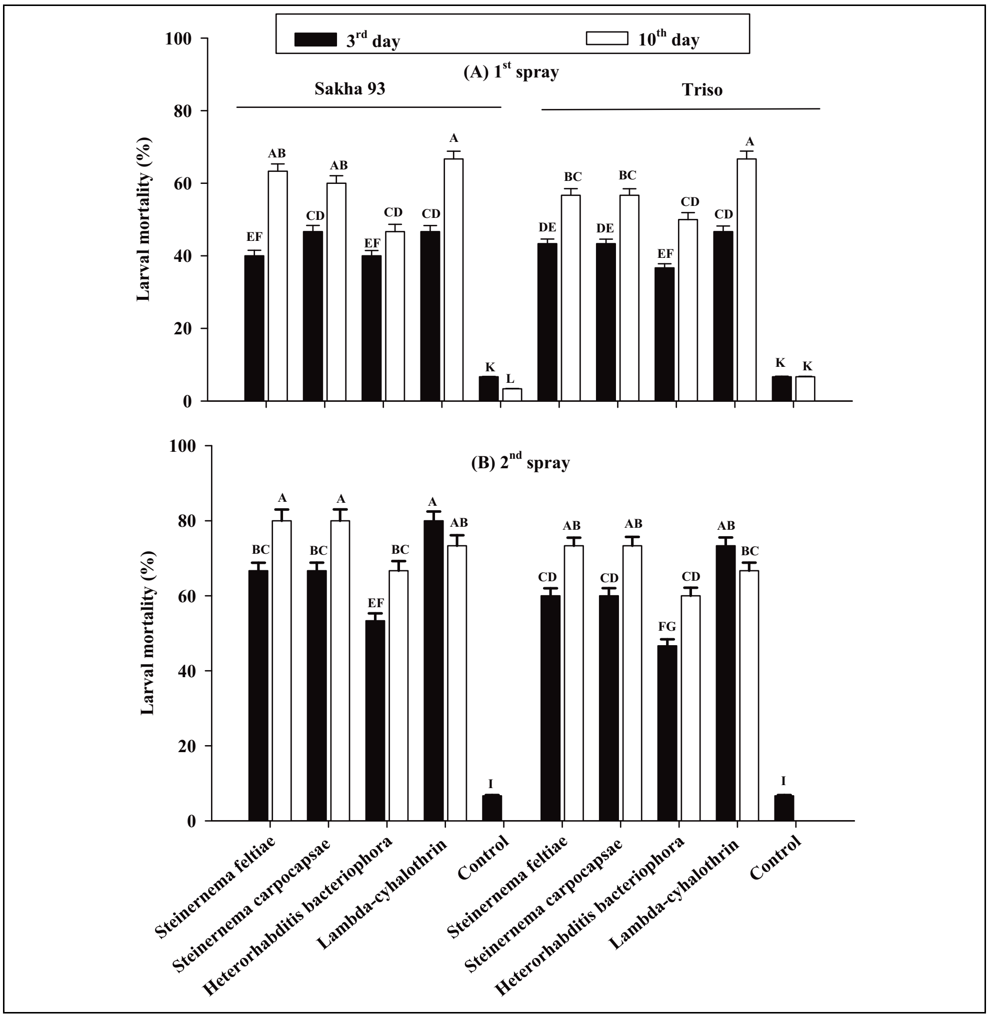

Fig. 5. Mean ± SE of larval percent mortality in infested wheat tillers in two spring wheat varieties after entomopathogenic nematodes and Lambda-cyhalothrin treatments: (A) 1st spray on 29 April and (B) 2nd spray on 13 May 2010. Different letters indicate significant differences.

followed by *S. carpocapsae* (4776 kg/ha), then *S. feltiae* and *H. bacteriophora* were almost equal (4235 and 4244 kg/ha, respectively), while control plots had the lowest yield (3823 kg/ha). Within the Sakha 93 variety, the highest yield was also found in λ-cyhalothrin treatment (4365 kg/ha), followed by *S. carpocapsae* (3505 kg/ha), *S. feltiae* and *H. bacteriophora* were equivalent (3127 and 3105 kg/ha, respectively), while control plots had the least yield (2875 kg/ha). Yield in λ-cyhalothrin and *S. carpocapsae* plot was higher than other treatments (Fig. 6A).

B 2010

Spring wheat yield in 2010 was lower than the 2009 season. There were significant differences ($df = 4, 4$, $F = 10.13$, $P = 0.00228$) between treated plots and control; the treated had higher yields than control plots. The yield quantities significantly differed ($df = 3, 3$, $F = 23.47$, $P = 0.0139$) between λ-cyhalothrin and EPNs species. There were also significant differences ($df = 2, 2$, $F = 21.28$, $P = 0.0449$) among EPNs species, where the yield in *S. carpocapsae* and *S. feltiae* plots had higher yield than *H. bacteriophora* (Fig. 6B). Within the Triso variety, the highest yield was recorded in λ-cyhalothrin treatment (4863 kg/ha), followed by *S. carpocapsae* (4196 kg/ha) which was almost equal as *S. feltiae* (4123 kg/ha), then came *H. bacteriophora* (3817 kg/ha); while control plots had the lowest yield (3504 kg/ha). Within the Sakha 93 variety, the highest yield was also found in λ-cyhalothrin treatment (4080 kg/ha), followed by *S. carpocapsae* (3495 kg/ha) and *S. feltiae* which almost equivalent (3473 kg/ha), and *H. bacteriophora* (3126 kg/ha); whereas control plots had the lowest yield (2968 kg/ha) (Fig. 6B). Yield in λ-cyhalothrin *S. carpocapsae* and *S. feltiae* plots were higher than *H. bacteriophora*. Mean of yield was higher in the Triso variety (4100 kg/ha) than in the Sakha 93 variety (3428 kg/ha) (Fig. 6B).

4 Discussion

Three EPNs of the genera *Heterorhabditis* and *Steinernema* were tested for their ability to control *O. frit*. In the laboratory, up to 100% mortality in the pest larvae could be achieved with all tested EPNs as well as the chemical insecticide when higher concentrations and younger pest larvae were used. To our knowledge, this is the first study to address the efficiency of EPNs against *O. frit*. No literature citations were found on the effect of EPNs on *O. frit*. In their work on controlling larvae of the cabbage fly *Delia radicum* with EPNs, Chen et al. (2003) noticed sim-

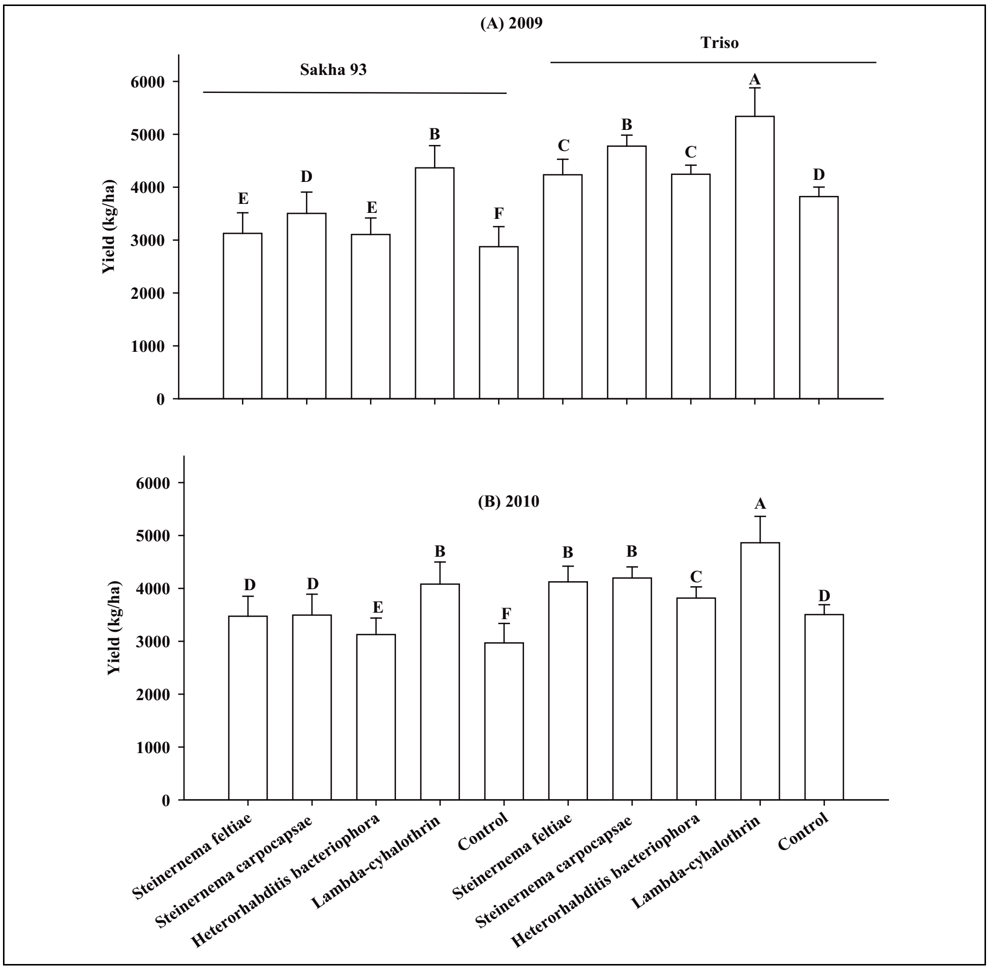

Fig. 6. Effects of *Oscinella frit* control treatments on spring wheat yield (kg/ha) (A) 2009 and (B) 2010. Different letters indicate significant differences.

ilar results for insect mobility. Our results also agree with Toledo et al. (2005), who stated that the highest tolerance to nematode infection occurs in older instar larvae (i.e. L3) of *Anastrepha* species. Nevertheless, Oestergaard et al. (2006), in their work on leatherjackets of *Tipula paludosa* (Diptera: Nematocera) using *Steinernema* spp., reported that application of nematodes will only be successful and economically feasible during earlier larval instars.

Among EPNs nematodes, in 2009 *S. carpocapsae* caused the most significant reductions in percent infestation, followed by *S. feltiae* and *H. bacteriophora*. While in 2010, *S. feltiae* and *S. carpocapsae* had the same efficiency and both were also significantly to *H. bacteriophora*. This indicated that *H. bacteriophora* was ineffective under field conditions from the middle of April to the end of May, while *S. carpocapsae* and *S. feltiae* were effective at similar temperatures. However, this suggests that the efficacy of EPNs was best evaluated through field trials that embody a broader range of influencing factors than were usually incorporated into bioassays. Also, this may be due to behavioural differences such as mobility and their ability to survive under natural field conditions between the two nematode genera *Steinernema* and *Heterorhabditis* as reported in other studies by Molyneux (1985).

The damaged-tiller counts were considered as indicators for infestation rates. Treatments applied showed a reduction in *O. frit* damage relative to the control. These results corresponded with Clements et al. (1990) who stated that synthetic pyrethroids are the most effective chemicals used in controlling *O. frit*. The percent infestation in Triso was lower than Sakha 93 variety; this may be due to the Triso variety growing faster than the Sakha 93 variety early in the growing season as mentioned by El-Wakeil et al. (2009).

Larval mortalities in λ-cyhalothrin treatments and *S. carpocapsae* were higher than other treatments in 2009; whereas in 2010, *S. carpocapsae* and *S. feltiae* achieved the same larval mortality levels and also were still better than *H. bacteriophora*. This may be due to weather conditions which were warmer and dryer in 2009 than 2010, as confirmed in the other study by El-Wakeil and Volkmar (2011). These results indicate that *S. carpocapsae* can be used to control *O. frit* in spring wheat field, because, *O. frit* begins egg laying in the early to middle part of April. The L2 larvae were found until early May and L3 came in middle to end of May. At that time, temperatures were below the threshold necessary for *H. bacteriophora* to be an effective biological control agent; therefore efficacy of *H. bacteriophora* was lower than other EPNs species. The efficacy of *S. carpocapsae* against *O. frit* started to increase at 13°C as reported by Gerritsen et al. (1998) in other studies, thus it seems to be better adapted to control insect pests as in case of *O. frit*. The larval mortality was higher in Sakha 93 than Triso variety; because the percent infestation was also high, therefore it was easily for EPNs to find and parasitize *O. frit* larvae.

Yield in 2009 was higher than that in 2010; this was due to weather conditions which affected infestation of *O. frit*. These experiments indicated that *O. frit* infestation affected yield of both wheat varieties. Preference of *O. frit* for Sakha 93 over Triso variety has been demonstrated when assessed by numbers of larvae recovered from wheat tillers, similar results were recorded by Henderson and Clements (1979) and Mowat and Jess (1985). Frit fly larvae affected the yield, while the inverse relationship was found between their numbers and yield. The use of chemicals to control *O. frit* larvae led to an increase in yield as confirmed by French et al. (1988) in winter cereals. It can be assumed that EPNs application, especially *S. carpocapsae* or *S. feltiae* could give good response attainable by *O. frit* control. It was possible that some of the yield responses observed may have been due to treatments reducing *O. frit* damage.

Finally, reducing infestation and increasing larval mortality in treatments of EPNs was achieved by application dose 2.5×10^9 IJs/ha; which should be applied to ensure that ample numbers of nematodes come in contact with *O. frit* to provide acceptable control, according to Georgis (1990) recommendations. These results confirmed that yield improvements were mainly due to the control of *O. frit*. The analysis of yield data suggests that all applications enhanced yield. These results indicated that these treatments were efficacious on *O. frit* as alternative control methods. The potential of EPNs as biological control agents for *O. frit* should be incorporated into integrated pest management programs for the production of wheat to keep the environment safe and clean.

Acknowledgements

This work was financially supported by Martin Luther-University Halle-Wittenberg. We gratefully acknowledge the constructive comments on this manuscript by Dr. N. Schmidt (Pioneer Hi-Bred, USA), Prof. B. Vinson (Texas A&M University, USA) and Prof. M. Saleh (NRC, Egypt: The authors appreciate Prof. R. Ehlers (e-Nema GmbH, Kiel University, Germany) for providing us nematodes.

References

Abbott, W.S., 1925: A method of computing the effectiveness of an insecticide. Journal of Economic Entomology **18**, 265-267.

Anonymous, 2008: List of authorized plant protection products in Germany with information on terminated authorizations. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 56. Aufl. 2008, ISSN 0178-059X.

Chen, S., X. Han, M. Moens, 2003: Biological control of *Delia radicum* with entomopathogenic nematodes. Applied Entomology and Zoology **38**, 441-448.

Ciche, T.A., C. Darby, R.-U. Ehlers, S. Forst, H. Goodrich-Blair, 2006: Dangerous liaisons: the symbiosis of entomopathogenic nematodes and bacteria. Biological Control **38**, 22-46.

Clements, R.O., B.R. Bentley, E.J. Asteraki, 1990: Origin of frit-fly (*Oscinella* spp.) attack on newly sown grass, effect of pesticides on frit-fly larvae and benefit of using glyphosate herbicide pre-ploughlng. Crop Protection **9**, 105-110.

Ehlers, R.-U., 2001: Mass production of entomopathogenic nematodes for plant protection. Applied Microbiology and Biotechnology **56**, 623-633.

El-Wakeil, N.E., C. Volkmar, 2011: Effect of weather conditions on frit fly (*Oscinella frit,* Diptera: Chloropidae) activity and infestation levels in spring wheat in central Germany. Gesunde Pflanzen, published online (DOI: 10.1007/s10343-011-0255-9).

El-Wakeil, N.E., A.A. Sallam, C. Volkmar, 2009: Ecological studies on frit fly *Oscinella frit* (L.) and its control in summer wheat in Central Germany. Mitteilungen Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie **17**, 215-220.

Fenton, A., R.L. Gwynn, A. Gupta, R. Norman, J.P. Fairbairn, P.J. Hudson, 2002: Optimal application strategies for entomopathogenic nematodes: Integrating theoretical and empirical approaches. Journal of Applied Ecology **39**, 481-492.

French, N., D.B.R. Nichols, A.J. Wright, D.I. Green, D.B. Green, J.S. Fenlon, 1988: Chemical control of frit fly on winter cereals sown after grass. Annual of Applied Biology **113**, 245-251.

Georgis, R., 1990: Formulation and application technology. In Gaugler, R., H.K. Kaya (eds.): Entomopathogenic nematodes in biological control. Boca Raton, FL, CRC Press, pp. 173-191.

Gerritsen, L.J.M., G.L. Wiegers, P.H. Smits, 1998: Pathogenicity of new combinations of *Heterorhabditis* spp. and *Photorhabdus luminescens* (*Xenorhabdus luminescens*) against *Galleria mellonella* and *Tipula oleracea*. Biological Control **13**, 9-15.

Grewal, P.S., S. Selvan, R. Gaugler, 1994: Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology **19**, 245-253.

Henderson, I.F., R.O. Clements, 1979: Differential susceptibility to pest damage in agricultural grasses. Journal of Agricultural Science, Cambridge **13**, 465-472.

Hussein, M.A., N.E. El-Wakeil, T.N. El-Sebai, 2006: Susceptibility of melon fruit fly, *Dacus ciliatus* (Diptera) to entomopathogenic nematodes and to insecticides. International Journal of Nematology **16**, 13-18.

Jess, S., H. Schwiser, M. Kilpatrick, 2006: Mushroom applications. In: Grewal, P.S., R.-U. Ehlers, D.I. Shapiro-Ilan (eds.): Nematodes as biocontrol agents. Wallingford, CABI Publishing, pp. 191-213.

Jonasson, T., 1977: Frit fly *Oscinella frit* oviposition on oat seedlings: Ecological significance of the host plant selection. Oikos **29**, 104-111.

Larsson, H.A., 1984: Frit fly (*Oscinella frit* L.) damage in oats and its chemical control by synthetic pyrethroids. Zeitschrift für Angewandte Entomologie **97**, 470-480.

Lindblad, M., 1999: Density and mortality of overwintering *Oscinella frit* and other oscinellids in Swedish grasslands. International Journal of Pest Management **45**, 23-28.

Lindblad, M., R. Sigvald, 1999: Frit fly infestation of oats in relation to growth stage and weather conditions at oviposition. Crop Protection **18**, 517-521.

Meier, U. (ed.) 2001: Growth stages of mono-and dicotyledonous plants. 2. ed., Federal Biological Research Centre for Agriculture and Forestry, 158 p.

Molyneux, A.S., 1985: Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. at various temperatures and their subsequent infectivity for insects. Revue de Nématologie **8**, 165-170.

Mowat, D.J., S. Jess, 1985: A comparison of insecticide treatments for the control of frit fly, *Oscinella frit* (L.), in seedling ryegrass. Grass and Forage Science **40**, 251-256.

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

Peters, A., R.-U. Ehlers, 1994: Susceptibility of leatherjackets (*Tipula paludosa* and *T. oleracea*) to the entomopathogenic nematode *Steinernema feltiae*. Journal of Invertebrate Pathology **63**, 163-171.

Poinar, G.O. Jr., 1990: Biology and taxonomy of Steinernematidae and Heterorhabditidae. In Gaugler, R., H.K. Kaya (eds.): Entomopathogenic nematodes in biological control. Boca Raton, FL, CRC Press, pp. 23-61.

Půža, V., Z. Mráček, 2005: Seasonal dynamics of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* as a response to abiotic factors and abundance of insect hosts. Journal of Invertebrate Pathology **89**, 16-122.

Saleh, M.M.E., K.A.A. Draz, M.A. Mansour, M.A. Hussein, M.F.M. Zawrah, 2009: Controlling the sugar beet beetle *Cassida vittata* with entomopathogenic nematodes. Journal of Pest Science **82**, 289-294.

Susurluk, A., 2008: Potential of the entomopathogenic nematodes *Steinernema feltiae*, *S. weiseri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris*. Journal of Pest Science **81**, 221-225.

Thomas, C.R., S.C. Maurice, 2008: Statistix 9, Ninth Edition, Managerial Economics McGraw-Hill/Irwin. (ISBN: 0073402818, More information at http://www.statistix.com).

Toledo, J., J.E. Ibarra, P. Liedo, A. Gómez, M.A. Rasgado, T. Williams, 2005: Infection of *Anastrepha ludens* (Diptera: Tephritidae) larvae by *Heterorhabditis bacteriophora* under laboratory and field conditions. Biocontrol Science & Technology **15**, 627-634.

Tolley, M.P., H.D. Niemzcyk, 1988: Seasonal abundance, oviposition activity, and degree-day prediction of adult frit fly (Diptera: Chloropidae) occurrence on turfgrass in Ohio. Environmental Entomology **17**, 855-862.