NELSON MANDELA UNIVERSITY

A comparison of different strategies to control pests and diseases in *Brassica* spp. production in the Western Cape

By

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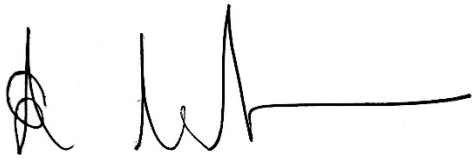
ABSTRACT

Brassica spp. are cultivated all over the world, commercial species include: cabbage, broccoli, kale, kohlrabi and turnip. In this study the focus was on broccoli (*Brassica oleracea)* production in the Western Cape province of South Africa and its economically important pests and diseases: sugar beet cyst nematode (*Heterodera schachtii*), diamondback moth (*Plutella xylostella)*, white blister (*Albugo candida*) and clubroot (*Plasmodiophora brassicae*), and the different methods to control these pests and diseases. The control methods focused on in this study included a commercial chemical control programme, a biological control programme and a holistic approach. Other factors were bio-fumigation and chemical fumigation and different crop rotation practices including rotation crops versus no rotation crops. The experimental design was a strip split plot design, with different pest and disease management strategies as the main plot treatment and fumigation and rotation treatment combinations arranged in strips across the main plot treatments. The main plot design was a randomized complete block with four programmes (Control, Holistic, Chemical and Biological) replicated four times and laid out in a Randomised Complete Block Design (RCBD). The treatment design of the strip plot factors was a 2x2 factorial with two fumigations (fumigated chemically and fumigated biologically) and two rotations (crop rotated and monoculture) randomly allocated across main plot treatments. Each experimental unit consisted of 40 plants. Plants were evaluated weekly for the incidence of white blister and diamondback moth. Incidences of clubroot and white blister infection of heads of broccoli were recorded 78 days after planting. Baseline soil samples were analysed to establish the soil chemical properties. Post-trial soil samples were also analysed to investigate the effect of the different practices and programmes on the soil chemical properties. Nematodes were extracted pre-trial and post-trial, and the effect of fumigation and crop rotation on plant parasitic nematodes and the nematode population diversity investigated. In the post-trial soil chemical analysis, a significantly higher

concentration of Na was recorded for the biological programme when crop rotation was included compared to the no rotation treatment. The concentration of K was also significantly higher in the no rotation compared to the rotation treatment. The nematode results showed very high numbers of bacterial feeders in all the samples. Overall nematode diversity was lacking and showed very few fungal feeders, omnivores and predators. The nematode indices for all of the samples showed that nematodes were highly enriched and unstructured. Of the plant parasitic nematodes, only *Heterodera* spp. were obtained in the pre-trial analysis, and incidences of these nematodes were lower at the end of the trial. Low numbers of other plant parasitic nematodes viz. *Pratylenchus*, *Paratrichodorus* and *Tylenchorhynchus* were reported for the post-trial analysis of the soil. Because of the low numbers of plant parasitic nematodes, it was not possible to analyse the data statistically. With regard to diamondback moth and the fungal diseases, crop rotation and fumigation did not significantly affect the incidence of white blister and diamondback moth. A significant "days after planting by control programme" interaction was reported for the incidence of white blister on foliage and the incidence of diamondback moth. All three control programmes significantly reduced the incidence of diamondback moth with the chemical programme being significantly more effective than the other two programmes. All three programmes also significantly reduced the incidence of white blister on foliage and the holistic and biological programmes significantly reduced the incidence of white blister on broccoli heads with the holistic programme being significantly more effective than the biological programme. There was no clubroot infection in the trial for any of the treatments. Results of this study showed that it is possible to manage diseases and pests of broccoli using a holistic approach. However, long term trials are needed to confirm the results obtained in this study.

DECLARATION

I, Abraham Johannes van Niekerk, student number, 241372995, hereby declare that all the work contained in this dissertation, for the degree *Master of Technology*, is my own work and that it has not previously been submitted for assessment of completion of any postgraduate qualification to another University or for another qualification.



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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ARC	Agricultural Research Council
BAC	Bacterivores
Bio-Fum	Biological Fumigation
Biol	Biological
Che	Chemical treatment
Ct	Control
DAFF	Department of Agriculture, Forestry and Fisheries
DBM	Diamondback moth
DDAC	Didecyldimethylammoniumchloride
DDT	Dichlorodiphenyltrichloroethane
EI	Enrichment Index
EU	European Union
EPN	Entomopathogenic Nematodes
FAO	Food and Agricultural Organization
FNR	Fumigation and no crop rotation
FR	Fumigation and crop rotation
Fum	Fumigation
FUN	Fungivores
GLM	General Linear Models
GSL	Glucosinolate
Ha/ha	Hectare
ICP	Inductive Coupled Plasma
IPM	Integrated Pest Management
ITC	Isothiocyanate
LSD	Least Significant Difference
LWCM	Large White Cabbage Moth
MB	Methyl bromide
MITC	Methyl isothiocyanate
MS	Metam sodium
NN	Bio-fumigation and no crop rotation
NR	Bio-fumigation and crop rotation
OMNI	Omnivores
PPN	Plant Parasitic Nematodes
PRED	Predators
RCBD	Randomised Complete Block Design
RE	Root exudate feeders
Rot	Rotation
SBCN	Sugar Beet Cyst Nematode
SI	Structure Index
StatsSA	Statistics South Africa
WB	White blister
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background

Brassicaceae refers to the mustard family of flowering plants, composed of 338 genera and 3700 species. Many of the plants are commercially farmed, especially the genus *Brassica* which includes cabbage, broccoli, kale, kohlrabi and turnip (Encyclopaedia Britannica, 2017). The genus *Brassica* is regarded as the most economically important of the *Brassicaceae* family (Fourie *et al.,* 2016). In South Africa *Brassica* vegetables are important crops with 160,000 t/annum produced commercially in 1998 and in 2007 40,000 t/annum was produced just in the Western Cape province (StatsSA, 2007; Waladde *et al.,* 2001). *Brassica* vegetables are grown by 80% of small-scale rural farmers in South Africa and it is considered a staple diet for many people (Waladde *et al.,* 2001). The consumption of *Brassica* vegetables is increasing (Kfir, 1997). As this is an import vegetable for the small-scale rural and commercial farmers, the pest and disease management of this crop is extremely important. But in what way should farmers protect this valuable crop? Chemical pesticides often seem to be the only means to control economically important pests and diseases (Malais & Ravensburg, 2003). Balancing effective pest and disease control with environmental and human safety issues is an ongoing challenge for research. Strategies to reduce chemical use in South African agriculture are increasingly seen as important, for human and environmental health, and to ensure food security (Government Gazette, 2010).

"Pesticides are widely used to control the growth and proliferation of undesirable organisms that, if left unchecked, would cause significant damage to forests, crops, stored food products, ornamental and landscape plants, and building structures. The use of pesticides in both agricultural and non-agricultural settings provides important benefits to society, contributing to an abundant supply of food and fiber and to the control of a variety of public health hazards and nuisance pests. Owing to the fact that they are designed to be biologically

active, pesticides have potential to cause undesirable side effects. These include adverse effects on workers, consumers, community health and safety, groundwater, surface waters, and non-target wildlife organisms. In addition, pesticide use raises concerns about the persistence and accumulation of pesticides in food chains quite distant from the original point of use, and about the role of certain pesticides in causing reproductive failure and endocrine system abnormalities in both wildlife and humans, and other species that are not their intended target. It is therefore important to control the use of pesticides, by carefully weighing the benefits that they confer against any possible adverse effects" (Government Gazette, 2010).

Different crop protection strategies of *Brassica* spp., production need to be explored to ensure a more holistic and sustainable way of providing food. Food security is the number one concern in Africa, with the high numbers of malnourished and extremely poor people. Not everybody has access to technology, tools and costly chemical pest control products, thus more research needs to be done, to help people with limited tools and technology to produce healthy and sustainable food and income. One of the biggest challenges for integrated pest management or a holistic approach to pest and disease management, is the integration of chemical and biological approaches to control these pests and diseases. That is because there are limited biological products that can be applied, and the cost of these biological products often exceeds the cost of chemical products (Nofemela, 2013).

The current study aims at providing sustainable holistic solutions to crop protection of *Brassica* spp. pests and diseases.

1.2. *Brassica* spp. pests and diseases in South Africa

There are many economically significant pests and diseases of this crop; in this study the main focus will be sugar beet cyst nematode, *Heterodera schachtii*, diamondback moth, *Plutella xylostella*, white blister of *Brassica* spp. caused by *Albugo candida*, and clubroot of *Brassica* spp. caused by *Plasmodiophora brassicae*. However, in South Africa limited new research has

been done on these pests and diseases, and publications on these topics in South Africa are few and far between (Nofemela & Kfir, 2005; Morris & Knox-Davies, 1980; Sereda *et al.,* 1997; Daiber, 1991). These pests and diseases can cause major economic loss to small-scale rural and commercial farmers, with total crop loss being reported (Ploch *et al.,* 2010). The first list of recorded diseases of fruit and vegetables in the Western Cape province of South Africa was published in 1922. This list contained 98 genera, 148 identified species and 20 fungi not specifically identified. *Albugo candida* (white blister) and *Plasmodiophora brassicae* (clubroot) were both published on this list (Van der Byl, 1922).

1.2.1 Important plant parasitic nematodes in South Africa

Nematodes are microscopic worm-like organisms that are found in fresh water, marine and terrestrial environments, and are associated with the soil water layer. They represent several trophic groups and play important roles in ecosystems and ecological processes, and respond rapidly to environmental disturbance (Du Preez *et al.,* 2018).

Plant parasitic nematodes (PPN), cause damage to horticultural and agricultural crops, through cell destruction, as vectors for viruses or by secondary bacterial and fungal infections as a result of their physical damage to roots (Hooks *et al.,* 2010). Many crops, as well as non-cultivated plants, such as weeds and ornamental plants are susceptible hosts for PPN (Mashele *et al.,* 2017).

In South Africa *Meloidogyne* spp. are among the most important soilborne pests and cause economic loss to a wide range of agricultural crops (Daneel *et al.,* 2018). Plant parasitic nematodes (PPN) are economically important pests worldwide, especially *Meloidogyne* spp. (root knot nematodes) (Manfort *et al.,* 2007). The top three nematode groups in the world, as well as in South Africa are: *Meloidogyne* (root knot nematode), *Heterodera* and *Globodera* (cyst nematode), and *Pratylenchus* (lesion nematode). Ten of the 98 *Meloidogyne* spp. are

classified as agricultural pests (Fourie *et al.,* 2016). In greenhouse trials conducted in the United States of America, broccoli showed the least susceptibility to *Meloidogyne incognita* and *Meloidogyne javanica* infestations, although infestations have been reported in the field (Mc Sorley & Frederick, 1995).

A 1999 - 2001 survey of rural, home, community and school gardens in South Africa, concluded that in 49 out of the 51 gardens evaluated, root knot nematode was the major cause of crop loss (Mashela *et al.,* 2017). This is a major problem as many people rely on these gardens for food security and income. In commercial South African vegetable production, crop losses of up to 10% can be attributed to nematodes (Mashela *et al.,* 2017). Chemical control of PPN with nematicides has been the main focus of control. Nematicides are extremely toxic to ground water, humans, birds and non-target organisms, expensive and very difficult to apply (Fourie *et al.,* 2016). These chemical nematicides are successful but not suitable for small scale farmers. Therefore alternative methods of control need to be researched, as was done in this trial.

1.2.2 Sugar beet cyst nematode, *Heterodera schachtii* in Philippi in the Western Cape

According to Sheila Storey, owner of Nemlab, a commercial nematode laboratory in Klapmuts, Western Cape, who has studied this PPN intensively, the sugar beet cyst nematode (SBCN), is the most common PPN on vegetable crops grown and a major pest in the area of Philippi in the Western Cape. The most economically important PPN on *Brassica* spp., especially cauliflower, cabbage and broccoli is the SBCN, with recorded crop losses of up to 30% (Daiber, 1991).

The SBCN is extremely difficult to control. Its most important host is sugar beet, but yield losses have been reported on different vegetables, including beetroot (*Beta vulgaris),* and usually where cruciferous vegetables such as: cabbage, cauliflower and broccoli are grown

Daiber, 1991). There is a wide range of weed species that act as successful host plants, the most common of the weed species are: wild mustard (*Sisymbrium* spp.), chick weed (*Stellaria media*), shepherd's purse (*Capsella bursapastoris*), pigweed (*Amaranthus deflexus*), and common purslane (*Portulaca oleracea*). A commonly occurring weed that is also an excellent host is from the *Chenopodium* spp., commonly known as "white goosefoot" or "wit hondebossie". Vegetables infected with this species include spinach and beetroot (Daiber, 1991; Storey, 2018, Personal communication, 19 November).

Crop rotation is an excellent farming practice but it will not control SBCN. It will however help to supress the nematode but only if non-host plants are cultivated. Studies have shown that a six course rotation system can be successful, although there have been rotations with hosts that only prove successful after ten years. (Daiber, 1991; S. Storey, 2018, Personal communication, 19 November). The non-host plants are the following: carrot (*Daucus carota*), lettuce (*Lactuca sativa*), celery (*Apium graveolens*), leeks, chives, garlic, onion (*Allium* spp.), melon, cucumber, (*Cucumin* spp.), pumpkin, squash, marrow (*Cucurbita* spp.), potatoes (*Solanum tuborosum*), beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), maize (*Zea mays*), rye (*Secale cereale*), oats (*Avena sativa*) and barley (*Hordeum vulgare*). Leguminous crops were reported to be the best rotational crop to use, with clover (*Trifolium*) and lucerne (*Medicago sativa*) reducing SBCN numbers significantly. Barley is almost as effective as lucerne (Daiber, 1991; Storey, 2018, Personal communication, 19 November).

Although it has a wide range of hosts SBCN does not need a host to survive. It survives by staying dormant in the soil for months until a suitable host is present. It can spread rapidly through different fields by means of farming equipment, run off water, or when vegetables are harvested (Daiber, 1991; S. Storey, 2018, Personal communication, 19 November).

Chemical soil fumigation will only protect the host for a while, and does not produce significant results as the SBCN is well protected by the cyst. And it has been reported that chemically

fumigated fields have a higher number of SBCN at the end of the growing season while untreated fields have had yield losses of 65% and as high as 90% (Daiber, 1991; Storey, 2018, Personal communication, 19 November).

Farmers cannot just rely on nematicides to eradicate nematodes in the soil completely. The most recommended means of control is with a good crop rotation system in conjunction with a chemical fumigant (Daiber, 1991; Storey, 2018, Personal communication, 19 November).

1.2.3 Diamondback moth, *Plutella xylostella,* in *Brassica* spp. in South Africa

Diamondback moth, *Plutella xylostella,* (DBM) a cosmopolitan pest is a major pest of *Brassica* spp. in South Africa (Sereda *et al.,* 1997). It is considered the most damaging pest of *Brassica* spp. around the world (Kfir, 2001). There has been some speculation about the origin of DBM that it might have originated in South Africa but there is also an accepted theory that its origin is from the Mediterranean region of Europe. The exact origin has never been established (Kfir, 2005). Limited research on DBM was done in South Africa for almost 60 years, but due to major outbreaks of the pest it has raised some renewed interest (Kfir, 2001).

Although the pest status in South Africa is considered to be lower than in other parts of the world, it still causes some serious damage (Smith & Villet, 2001). In some cases the infestation in South Africa was so high that farmers applied chemical insecticides twice a week (Waladde *et al.,* 2001). Control of DBM in South Africa depends heavily on chemical insecticides (Sereda *et al.,* 1997). This is a major problem considering the pest's ability to develop resistance (Sereda *et al.,* 1997). Various broad spectrum chemical insecticides are used to control DBM, and intensive applications have led to resistance being reported around the world (Waladde *et al.,* 2001). DBM was the first pest to develop resistance to dichlorodiphenyltrichloroethane (DDT). There have also been some reports of DBM resistance to biological insecticide *Bacillus*

thuringiensis (Guo *et al.,* 2015). Cross resistance to multiple insecticides has been reported in South Africa, and new chemical insecticides are developed continuously due to the high levels of resistance (Kfir, 2005). In South Africa resistance to organophosphates and synthetic pyrethroid insecticides has been reported (Waladde *et al.,* 2001). However, with all these reports on resistance to chemical insecticides it is still the primary means to control DBM (Sereda *et al.,* 1997; Nofemela, 2013).

Due to DBM resistance to chemical insecticides, biological control methods have become very important in controlling DBM (Smith & Villet, 2001). A holistic approach to DBM control needs to be encouraged, and a similar approach needs to be adopted in South Africa to control DBM and limit its resistance to chemical insecticides (Waladde *et al.,* 2001), but the effectiveness of biological control to reduce DBM populations remains unknown (Kfir, 2001).

1.2.4 White blister of *Brassica* spp. in South Africa

White blister was first recorded in 1921 in the Western Cape (Van der Byl, 1922). In 1931, a second publication on plant diseases in South Africa reported white blister in the Western and Southern Cape and Natal provinces of South Africa, and mentioned that it had become wide spread in just ten years, since the first publication was released (Doidge & Bottomley, 1931). Since then there has been very little research published on white blister of *Brassica* in South-Africa, although international literature is readily available. It still causes considerable economic damage in Philippi in the Western Cape province (Serfontein, 2018, Personal communication, 19 November).

1.2.5 Clubroot of *Brassica* spp. in South Africa

Although clubroot was first officially recorded in 1913 in the Western Cape province, it has passed almost unnoticed since 1898 in the Western Cape's vegetable producing areas: Brackenfell, Kuilsrivier, Bottelary and Stellenbosch, and was commonly known as "dikvoet". In 1913 clubroot disease in South Africa was identified to be caused by the fungus, *Plasmodiophora brassicae*. No previous authentic record was recorded before this date. The disease was not brought under the attention of agricultural experts before 1913, and this led to the disease becoing firmly established and causing considerable agronomic and economic losses before anybody could take the necessary steps to control it. Clubroot was first recorded in Scotland in 1789, but it was only in 1878 that Russian botanist Woronin of St. Petersburg identified the cause of clubroot to be a microorganism which invades the roots that he named *Plasmodiophora brassicae* (Woron). According to Woronin, clubroot caused £50,000 loss around St. Petersburg city alone in 1876. The disease was also recorded in Britain, France, Germany, Belgium and the United States of America (Pole- Evans, 1913).

By 1931, *Plasmodiophora brassicae* occurred in the Western and Southern Cape, Transvaal and the Orange Free State (Doidge & Bottomly, 1931).

1.3 Research questions and objectives

This thesis investigates and reports on different crop protection strategies in *Brassica* spp. production. This study analysed the effects of a chemical, biological and a holistic approach to control plant parasitic nematodes, diamondback moth, white blister and clubroot in *Brassica oleracea*. The effects of monoculture and crop rotation as well as the effect of biological and chemical fumigation will be investigated on plant parasitic nematodes and soil chemical properties. This thesis investigates crop protection of *Brassica oleracea* production; Catherine Eckert is investigating water use efficiency and N'wa Jama Mashele and Marike Swanepoel the production of *Brassica* spp. in long-term trials at Nelson Mandela University on the George campus. The timeline for the research is presented in Table 1.

Table 1: Timeline of experimental site events

OBJECTIVE	DATE
Biological and Chemical Fumigation	January 2015
Plant 1st *Brassica oleracea* seedlings	February 2015
Apply different crop protection strategies	Feb - Apr 2015
1st Evaluation	May 2015
Plant 1st rotation crop. Radish	June 2015
Plant 2nd rotation crop. Green bean.	July 2015
Plant 2nd *Brassica oleracea* seedlings	September 2015
Apply different crop protection strategies	Sep - Nov 2015
Evaluate crop protection strategies	November 2015

The Nelson Mandela comparative organic long-term farming systems research trials (known as the Mandela Trials) are reported in outline by Auerbach (2018), and are described in detail in Auerbach (Forthcoming).

To understand the context of this trial, Chapter 1 provides a background of different plant parasitic nematodes, pests and diseases of *Brassica* vegetables in South Africa. It presents the general challenges of controlling these pests and diseases. Chapter 2 presents a literature review of the pests and diseases and different strategies to control them: chemical, biological and a holistic approach. There is also a focus on chemical and biological fumigation, monoculture

and crop rotation. Chapter 3 presents a description of the trial site, the methodology used for the research and the baseline study. Chapter 4 presents the results of the different crop protection, fumigation and crop rotation strategies in this trial. This chapter aims to provide a preliminary result to the research question. Chapter 5 presents the conclusions derived from the trial as well as recommendations for future research. Substantial research has been done in other countries regarding different crop protection strategies for *Brassica* vegetable plant parasitic nematodes, pests and diseases, but limited research has been done in South Africa. The baseline study presents the focus of this research study.

CHAPTER 2: LITERATURE REVIEW

2.1 Vegetable production in the Western Cape

According to Risenga Maluleka, Statistician General of South Africa, the South African Statistical Service (StatsSA) published agricultural statistics on a yearly basis from 1918 to 1980. After 1994 it was decided that an agricultural census will only be conducted every five years. After the censuses of 2002 and 2007, there should have been a census conducted in 2012, however the Department of Agriculture, Forestry and Fisheries (DAFF) did not have enough funds to conduct the census. The latest census on agriculture was to have been conducted in 2017, but it was postponed until 2018. The Statistician General of South Africa has confirmed that the census that will be conducted in 2018 will reflect data from 1 July 2016 – 30 June 2017 and will only be available in 2019. The agricultural census of 2017 will be submitted to the Food and Agricultural Organization of the United Nations (FAO) as part of the World Programme of the 2020 Agricultural Census (Kruger, 2018). For these reasons the newest statistics available on agriculture in South Africa are from the agricultural census of 2007 published by StatsSA.

Some 35% of people residing in South-Africa live below the poverty line; most of these communities depend on home vegetable and community gardens for food. With the limited available land, there are many agricultural production challenges caused by limited crop rotation. This leads to soil degradation, and an increase of pests and diseases, which again has a negative effect on sustainable food security (Mashela *et al.,* 2017).

Vegetable production in the Western Cape forms an important part of job creation, and food security for many South Africans. Therefore research needs to be conducted on pests and diseases in vegetable production and the different strategies to control these pests and diseases economically and in an environmentally friendly manner. It is of utmost importance that we

understand the pests and diseases in order to make the right choices in protecting our crops, our jobs and our food source (StatsSA, 2007).

Table 2: Vegetable production in the Western Cape (StatsSA, 2007)

HORTICULTURAL PRODUCTS	Planted ha	Production in metric tons	Gross Farm Income	R/ha	Yield (t/ha)
Potatoes	15,631	502,499	R 492,290,000	R 31,494	32.15
Sweet potatoes	307	5,893	R 8,196,000	R 26,697	19.20
Green Mealies and Sweetcorn	273	2,269	R 7,713,000	R 28,253	8.31
Beetroot	239	8,353	R 8,614,000	R 36,042	34.95
Tomatoes	3,151	41,632	R 108,985,000	R 34,587	13.21
Onions	4,861	148,929	R 260,682,000	R 53,627	30.64
Pumpkins	1,843	43,802	R 60,315,000	R 32,727	23.77
Carrots	931	34,057	R 54,828,000	R 58,892	36.58
Cabbage	845	39,453	R 35,899,000	R 42,484	46.69
Mushrooms	43	1,964	R 35,579,000	R 827,419	45.67
Green Beans	582	6,298	R 15,712,000	R 26,997	10.82
Other Vegetables	2,912	108,609	R 151,768,000	R 52,118	37.30

In the Western Cape 934 758 ton vegetables are produced on 28 913 ha with a gross income of R1.2 billion per annum (StatsSA, 2007).

Table 3: Total income of vegetable production industry in R'000 (StatsSA, 2007)

Province	Total income							
	Horticulture	Potato	Tomato	Onion	Carrot	Cabbage	Green beans	Other veg
Eastern Cape	1,290,983	180,661	95,655	2,723	5,414	13,743	1,236	991,551
Free State	890,073	725,786	877	26,883	15,820	12,411	6,182	102,114
Gauteng	962,282	36,657	4,655	252	83,630	16,593	7,716	812,779
KwaZulu-Natal	916,898	200,608	26,085	2,246	8,527	76,053	4,821	598,558
Limpopo	3,040,295	479,635	628,713	87,669	1,548	7,096	14,237	1,821,397
Mpumalanga	1,956,486	226,039	33,342	9,849	6,590	14,310	51,943	1,614,413
North West	573,758	106,474	73,097	18,092	21,240	11,194	14,126	329,535
Northern Cape	1,164,837	143,036	5,137	50,000	11,137	973	1,120	953,434
Western Cape	8,285,015	492,290	108,985	260,682	54,828	35,899	15,712	7,316,619
South Africa	**19,080,627**	**2,591,186**	**976,546**	**458,396**	**208,734**	**188,272**	**117,093**	**14,540,400**

Table 4: Total hectare of vegetable production per province (StatsSA, 2007)

	Production in hectares							
Province	**Total**	**Potato**	**Tomato**	**Onion**	**Carrot**	**Cabbage**	**Green beans**	**Other veg**
Eastern Cape	7383	4770	1183	54	177	568	77	554
Free State	19487	16874	46	517	475	414	557	604
Gauteng	4430	578	38	9	1268	478	755	1304
KwaZulu-Natal	7492	3876	392	63	187	1396	436	1142
Limpopo	17413	8526	4711	2163	32	237	479	1265
Mpumalanga	7037	3640	500	39	226	523	976	1133
North West	6492	3071	655	463	707	362	353	881
Northern Cape	4889	2619	31	860	1204	69	24	82
Western Cape	28913	15631	3151	4861	931	845	582	2912
South Africa	**103536**	**59585**	**10707**	**9029**	**5207**	**4892**	**4239**	**9877**

The data in Tables 2, 3 and 4 showed that the total cabbage production in the Western Cape was 845 ha planted making it the second largest cultivation of cabbages in South Africa. The income generated from the 845 ha, amounted to R36,000,000 for the year. An average production of 47 t/ha that generated an income of R43,000 /ha.

Table 5: Total employment by the agricultural industry per province (StatsSA, 2007)

	Skilled		**Unskilled**		**Seasonal**	
Province	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Eastern Cape	2007	5249	5717	15848	10684	16894
Free State	2009	9404	5852	27379	17963	20905
Gauteng	1218	2406	5379	7086	3464	3030
KwaZulu-Natal	3542	8116	21183	27907	19766	13983
Limpopo	2696	4823	13982	18412	17415	12764
Mpumalanga	2816	7321	11885	22976	20762	14999
North West	1559	5371	6472	22673	12978	16011
Northern Cape	1235	4549	3106	13005	20961	27163
Western Cape	10519	20048	18235	35788	52910	54769
South Africa	**27601**	**67287**	**91811**	**191074**	**176903**	**180518**
Total Employment	**735194**					

2.2 Summary of the vegetable production industry of the Western Cape

Vegetable production in South Africa plays an important role in the income of the country. The total vegetable production income for South Africa was R19.1 billion for 2007 as illustrated by Table 3. Table 4 explains the cultivated area to be a massive 103,536 ha produced by the vegetable production industry of South Africa (StatsSA, 2007).

It is clear that the Western Cape province of South Africa has the biggest contribution to income per province of South Africa, at a total of R8.3 billion for vegetable production. The planted hectares for vegetable production in the Western Cape province of South Africa is 28,913 ha (StatsSA, 2007), that is 28% of the country's total vegetable production. The agricultural industry of South Africa employs 735,194 people, and 192,269 of these employees are employed in the Western Cape province, which is a total 26% of the total (StatsSA, 2007).

2.3 Plant parasitic nematodes

Nematodes are an important component of the biological community in agricultural soil. Plant parasitic nematodes (PPN) are widely studied because of the damage they cause to root systems which leads to yield reduction in crops (Stirling *et al.,* 2017). These nematodes cause significant economic losses to a wide variety of crops worldwide. Overall losses per year have been estimated to exceed US$ 10 billion, including 10 – 20% yield reductions in several vegetable crops (Hooks *et al.,* 2010). Georgia State in the United States of America is one of the country's biggest vegetable producers. It was estimated that the damage caused by pests and diseases, with one of the biggest contributing factors being nematode damage, amounted to US$ 44.3 million per annum (Manfort *et al.,* 2007).

Some symptoms of damage by PPN include: poor foliage, knots on roots and stunted growth, which leads to poor yields (Kruger *et al.,* 2015). These infestations can lead to secondary

infections of other soilborne and foliar diseases (Devran *et al.,* 2017). Over 98 *Meloidogyne* spp. are found worldwide and almost all vascular plants on earth can be hosts to these nematodes (Ntalli & Caboni, 2017).

Plant parasitic nematodes was considered an economically important pest, until the introduction of chemical control in the 20th century (Stirling *et al*, 2017). Plant parasitic nematodes were successfully controlled with chemical fumigants, organophosphates and carbamate nematicides. There is, however, a high environmental risk with the use of these chemical products (Stirling *et al.,* 2017). Methyl bromide (MB) has been used by producers since the 1930's as a broad spectrum fumigant, to control nematodes effectively in vegetables (Manfort, 2007). Non-selective MB formulations were the preferred products to control these pests, but they have been banned in developed countries since 2006 (Ntalli & Caboni, 2017). After the initial ban of MB several other chemical fumigants (nematicides, organophosphates, carbamates and soil fumigants) were withdrawn (Fourie *et al*., 2016), due to the fear of toxicity and the negative impact on animals, humans and the environment (Daneel *et al.,* 2018).

Yield reductions caused by PPN became higher after the ban of MB (Hooks *et al.,* 2010), and the pressure on other chemical products has forced the agricultural industry to seek different control methods for soilborne pests and diseases especially to control PPN (Manfort, 2007). Therefore more target specific methods are being developed, with a lower impact on the environment and soil biology. The main focus of the industry is to produce healthy, economically sustainable crops with limited impact on the environment (Ntalli *et al.,* 2017). This shift has driven the industry to a more holistic approach to agriculture and created a gap for biological, holistic or at least a more environmentally friendly approaches to control PPN (Daneel *et al*., 2018; Fourie *et al.,* 2016; Stirling *et al.,* 2017).

2.4 Free–living nematodes

Nematodes are microscopic wormlike organisms that are found in fresh water, marine and terrestrial environments and are associated with the soil water layer (Du Preez *et al.,* 2018); they are even found in polar regions, deep ocean sediments and hot sulphuric volcanic springs (Swart, 2011). For one billion years these highly specialized microorganisms have been in existence, they are thus considered to be of the earliest and most diverse animals on earth (Wang *et al.,* 2006). There have been many studies on plant parasitic nematodes, because of their economic importance to agriculture, but there is a gap in the literature available when it comes to beneficial nematodes (Neher & Powers, 2005). The majority of soil nematodes fulfil beneficial roles in ecosystem processes and are not parasites or pests (Storey, 2015).

Nematodes (free-living and PPN) may be the most useful group as a soil health indicator (Neher, 2001). The food source of beneficial nematodes ranges from a diverse array of sources including; bacteria, fungi, algae, protozoa, other nematodes and invertebrates such as small insects (Neher & Powers, 2005). This means that predatory and entomopathogenic nematodes can be used to control other PPN and insect pests (Yeates *et al.,* 1993). Nematodes can be divided into different trophic groups, or feeding groups, as classified by the nematode's feeding habit (Yeates *et al.,* 1993). The different nematode trophic groups are as follows (Storey, 2015):

- Herbivores: feed with their stylets, and feed on plant roots, they are plant parasitic nematodes.
- Bacterivores: feed with a hollow tube, and feed on bacteria.
- Fungivores: feed by puncturing the hyphae with a stylet, and feed on fungi.
- Omnivores: feed on more than one type of food source; organic material, etc.
- Predators: feed by puncturing food source with a tooth, feed on other nematodes.
- Entomopathogenic Nematodes (EPN): have no stylet, feed on insects and bacteria.

Nematodes play an important role in soil biogeochemistry and to the soil food web by regulating the behaviour of the microbial community as well as decomposition of organic materials and nutrient mineralization (Neher & Powers, 2005; Storey, 2015; Ugarte & Zaborski, 2014). Beneficial nematodes break down organic material; bacterial and fungal feeding nematodes are the most abundant of all the nematodes found in soils. They contribute directly to the available nitrogen in the soil by converting organic nitrogen to inorganic nitrogen, through their feeding. Nitrogen in the form of protein is consumed and released in the form of ammonium, this process is known as mineralization. Nematodes absorb greater amounts of nutrients, especially carbon and nitrogen (in the form of protein) than they require. The remainder is excreted into the soil, and is then available as a food source for microbes and as nitrogen in ammonium for plants (Ferris & Bongers, 2006; Neher & Powers, 2005; Storey, 2015; Ugarte & Zaborski, 2014). In laboratory trials it was shown that more ammonium nitrogen was available when bacterivores and fungivores were present as opposed to their absence (Trofymow & Coleman, 1982). It is estimated that in conventional and integrated crop production bacterivores and fungivores contribute 8-19% of nitrogen mineralization (Beare, 1997).

Different nematode species are found in different soil conditions (Yeates *et al.,* 2009). Nematodes are extremely responsive to changes in the delicate soil ecosystem and thus are useful bio-indicators of soil chemical and physical disturbance. The composition of the nematode community is determined by: composting, mulching, fertilising, water drainage, toxic substances such as heavy metals, pesticides, soil type, season, crop, soil moisture and soil organic matter (Yeates *et al.,* 1993; Storey, 2015).

Nematodes, being commonly found and easy to sample, make good bio-indicators of soil health. If there is a spike in bacterivores or fungivores this indicates that there is a rise in soil bacteria and soil fungi (Bongers, 1990; Ferris *et al.,* 2001). The plant parasitic nematodes or

herbivores contribute to the food web structure as well with their direct feeding (Ferris & Bongers, 2006). It is possible to determine the indication of carbon flow through an important herbivore nematode channel and channels mediated by bacteria and fungi with a single soil sample (Ferris & Congers, 2006). Nematodes as indicators of soil biology provide a means of measuring the shift in nematode population diversity over time (Neher, 2001). Biological features reinforce nematodes as an indicator. They possess a permeable cuticle, which allows different reactions to pollutants and corresponds with restorative capacity of soil ecosystems (Saly & Ragala, 1984). Some nematodes have resistant stages such as cryptobiosis or cysts which allows them to survive in unfavourable soil conditions (Bongers, 1999). Nematodes possess heat shock proteins. These proteins are enhanced to protect them when they are exposed to heat stress, toxic metal ions or organic toxins (Guven, *et al.,* 1994; Hashmi, *et al.,* 1997; Kammenga, *et al.,* 1998; Kammenga, *et al.,* 2000).

2.5 Diamondback moth, *Plutella xylostella*

Plutella xylostella, diamondback moth (DBM), may have originated from Europe or South Africa, although it has been recorded in 128 countries worldwide. It is found wherever *Brassica* spp. are cultivated, and is considered the most widely distributed of all Lepidoptera (Dennill & Pretorius, 1995; Saeed *et al.,* 2010). This is the most important pest of *brassicas* worldwide (Reddy *et al.,* 2004). It is estimated that DBM causes US$ 4 billion in losses annually (Zalucki *et al.,* 2012). With its cosmopolitan distribution DBM has been found from the cold Himalayan Mountains to the dry Ethiopian region (Marchioro *et al.,* 2017). This worldwide distribution is made possible by the pest's tolerance to high temperatures as well as its high migratory capacity. Thus suitable environmental conditions are exploited (Marchioro *et al.,* 2017). Understanding pest behaviour, susceptible hosts, reproduction and detection is important in managing economically important pests (Sarfaz *et al.,* 2006). Serious damage by DBM occurs

in the second and third instar of the larvae, which feed on the leaves, altering photosynthesis and leading to yield loss, and reduction in size and product quality (Correa – Caudros *et al.,* 2016). Farmers have experienced problems with controlling DBM, due to its short lifecycle and there has been some recorded resistance to chemical insecticides (Harris *et al.,* 1999). There have been situations where growers were forced to plough in all of their standing crop, in spite of applying multiple insecticides, as the pest could not be controlled. This exceptional status of *P. xyslostella* is due to the diversity and abundance of its host plants, lack of natural enemies and its high reproductive rate, with up to 20 generations in one year, as well as its insecticide resistance potential (Saeed *et al.,* 2010; Marchioro *et al.,* 2017).

Chemical insecticides are still the preferred method of control for DBM. The reason why chemical control is so popular is because of its practicality, speed and efficiency in population control, but continuous application has contributed to the problem of resistance (Peres *et al.,* 2017). Controlling DBM with pesticides has become more difficult all over the world due the use of single potent toxicants over a long period of time, and resistance to almost all the recommended chemical insecticides has developed (Ghosal *et al.,* 2015).

The preferred chemical insecticides that were used were organophosphates, carbamates and pyrethroids, but their continued use has rendered them ineffective in controlling DBM (Correa - Caudros *et al.,* 2016). The resistance of *P. xylostella* has made it economically impractical to farm with *Brassica* spp. in certain parts of the world. This has forced the industry to investigate a more holistic approach in controlling this major pest of *Brassica* spp. (Marchioro *et al.,* 2017). The resistance to chemical insecticides has also allowed for alternatives to be explored including *Bacillus thuringiensis* (Kfir, 2001). Attempts at biological control as an alternative to reduce populations of *P. xylostella* found that entomopathogenic fungi and nematodes (EPN) were effective. Using *Beauvaria bassiana* for biological control showed promising results, but the mortality is only achieved over a long period of time (9 – 15 days). Entomopathogenic

nematodes, however, can cause a 91% mortality in just 48 hours (Correa–Caudros *et al.,* 2016). According to Sarfraz *et al*., (2006) different management strategies need to be explored.

2.6 White blister of *Brassica* spp.

Albugo candida is the pathogen that causes white blister or white rust of crucifers. It is found on almost all *Brassica* spp., including the cultivated vegetable and oil seed brassicas. The fungus can produce two types of infection, local or systemic (Santos & Dias, 2004). There have been 17 different races reported of *A. candida* across the different *Brassica* spp. (Barbetti *et al.,* 2016). White blister was always regarded as a minor disease of *Brassica* spp. but that has changed, following severe outbreaks reported in the UK, Netherlands, France, Spain and Portugal and on brussels sprouts, broccoli, cauliflower and cabbages (Santos & Dias, 2004). Yield loss of up to 60% has been recorded in some *Brassica* spp. In India, combined infection of *Brassica juncea* leaves and inflorescences caused yield loss of up to 90%, with 63% of this loss through systemic damage (Kaur *et al.,* 2011). This disease has increased in significance in recent years with total crop loss being reported in certain instances (Ploch *et al.,* 2010).

Albugo candida is an obligate pathogen and is considered to be ancient compared to downy mildew. It is believed that white blister was introduced with the cruciferous crops (Kaur, 2013). The downy mildew pathogen, *Peronospora parasitica,* commonly co-occurs with white blister and even asymptomatic colonisation by *P. parasitica* will speed up the infection by *A. candida* thus increasing disease severity (Barbetti *et al.,* 2016). The localized disease characteristics can be described as the formation of white to cream coloured zoosporongial pustules on cotyledons, leaves, stems and inflorescences. It occurs on all plant parts that contain chlorophyll (Kaur *et al.,* 2011). The systemic disease characteristics are caused by oospores in mature stagheads.

Stagheads refers to the extensive distortion, hypertrophy, hyperplasia and sterility of inflorescence. An obligate parasite can only develop on living host tissue, where it produces sexual sporangia or zoospores and thick wall sexual spores. The pathogen survives as oospores in crop residues and perennial mycelium in living host tissue, which develop in distorted swellings and galls including stagheads, and in infected pods and stems. These overwintering spores are quite hardened against drying and extreme temperatures and they are responsible for the long-term survival and are liberated when a suitable host is planted (Kaur, 2013).

A disease epidemic can be established by only a few infected plants that serve as the primary source of infection (Kaur, 2013). The first symptoms will appear 5 – 20 days after infection, with a new crop of sporangia released 3 – 14 days after the first infection to start the second disease cycle, and in cool wet conditions it can complete its cycle every 8 – 10 days (Kaur, 2013). *A. candida* is spread by planting seeds that have been contaminated with oospores, by wind and rain and perennial mycelium in infected live plants (Kaur, 2013).

A. candida has a wide host range which complicates disease control (Choi *et al.,* 2011; Kaur & Savisithamparam, 2011). Chemical control is quite difficult and only a few products are registered, which are extremely expensive and often farmers cannot afford to apply these.
At present there are no alternatives to chemical control against *A. candida*, and this means of control is reported with limited success. Disease resistant cultivars would be the more environmentally friendly and more holistic approach towards control, thus reducing pesticide usage and resistance (Santos & Dias, 2004). This is still the most efficient and cost effective means of control of *A. candida* (Barbetti *et al.,* 2016). More research on this disease and its control is needed, since white blister is now an economically important disease which is poorly understood (Ploch *et al.,* 2010).

2.7 Clubroot disease of *Brassica* spp.

Clubroot is caused by *Plasmodiophora brassicae*, which is an obligate soilborne plant pathogen of *Brassica* spp. that can cause massive economic loss in production if not controlled (Irani *et al.,* 2018; Koike, 2003). Clubroot is one of the most important diseases of *Brassica* spp. and can be found in all *Brassica* production areas worldwide (Labrador Morales *et al.,* 2013). The disease is of global importance and has been reported to cause yield loss of up to 15% although 100% losses have been recorded with severe infections.

The lifecycle of *P. brassica* has two phases. In the primary phase, resting spores in the soil start to germinate as soon as there is a host and soil conditions are optimal. The spores then penetrate a suitable host's root hair, in the form of zoospores. In the second phase, secondary plasmodia form in the cortex of the root, producing galls. These galls prevent the root from functioning normally, and lead to yield loss, as normal functions such as nutrient and water uptake cannot take place (Irani *et al.,* 2018). Clubroot disease is sporadically found in soil with some plants seriously diseased and some neighbouring plants having no symptoms at all. This can be evident in the uneven distribution of *P. brassicae* throughout the field (Zhoa *et al.,* 2017).

P. brassicae in mature secondary plasmodia form resting spores that can survive for a long time in the soil since they are long lived and resistant to severe environmental conditions, making it impossible to prevent the disease with chemical treatment or crop rotation (Irani *et al.,* 2018). The resting spores, which can remain viable for over 15 years in the soil in absence of a host, make it a very persistent pathogen. The average half-life of the spores is 3.5 years and rotation as a control method is therefore not a viable option (Mc Grann *et al.,* 2017).

Different fungicides, biological controls and soil fumigants have been tested for the control of clubroot disease in *Brassica* spp. but their field efficacy has been inconsistent (Mc Grann *et al.,* 2017). Given the ineffectiveness of traditional chemical control methods, alternative approaches to managing clubroot disease like biological control have been the most promising.

Considerable research has been done in this regards with different bacteria, fungi and crop rotations; although *Trichoderma harzianum* shows potential against *P. brassicae* very little recorded research has been done (Yu *et al.,* 2015). Clubroot resistant varieties have provided effective control against the disease in the production of different *Brassica* spp. (Mc Grann *et al.,* 2017). The evolution of the pathogen has resulted in *P. brassicae* populations that can overcome this method of control as well (Mc Grann *et al.,* 2017). Although this is a problem disease, use of resistant cultivars is the most effective method to control clubroot disease (Irani *et al.,* 2018).

2.8 Crop Rotation

Monoculture refers to planting the same crop on the same piece of land year after year. It has not been very successful, as non-leguminous crops usually exhaust nitrogen in the soil leading to yield reduction (Encyclopeadia Britannica, 2018a). Practicing monoculture can lead to a loss of soil fertility, productivity and higher pest and disease rates (Tshikala *et al.,* 2018). In general crop rotations are known to build soil organic matter (Campbell, 2015), improve soil structure, control soil erosion (Tshikala *et al.,* 2018), reduce soilborne pests and diseases and suppress PPN (Hooks, 2010; Larkin *et al.,* 2014; Mall *et al.,* 2018).

When monoculture of a certain crop is practiced, pests and diseases including plant parasitic nematodes, are likely to increase because of the reliable host that is present (Campbell, 2015). Some pathogens can survive in the soil for a very long time, for example the bean anthracnose fungus may remain viable in soil for three years (Campbell, 2015). Resting spores of clubroot can remain viable for over 15 years in the soil in absence of a host, which makes it a very persistent pathogen (Mc Grann *et al.,* 2017). That is why it is important to have a rotation programme (Campbell, 2015). Crop rotation will therefore be serving as a break in the host-pest cycle, these crops can be referred to as disease suppressive crops (Larkin *et al.,* 2014).

One study found that tomatoes in a monoculture programme experienced early blight at a rate of 3% in year one, but increased rapidly to 74% blight in year three (Campbell, 2015). Crops in the *Brassica* spp. family used in rotations have been observed to reduce soilborne diseases, pathogens and PPN, and to improve soil health and crop yield (Larkin *et al.,* 2014; Mall *et al.,* 2018). It is therefore important that farmers are educated on crop rotation management to lead to more sustainable agriculture (Tshikala *et al.,* 2018).

2.9 Chemical control of pests and diseases

"Pesticides are widely used to control the growth and proliferation of undesirable organisms that, if left unchecked, would cause significant damage to forests, crops, stored food products, ornamental and landscape plants, and building structures. The use of pesticides in both agricultural and non-agricultural settings provides important benefits to society, contributing to an abundant supply of food and fibre and to the control of a variety of public health hazards and nuisance pests. Owing to the fact that they are designed to be biologically active, pesticides have potential to cause undesirable side effects. These include adverse effects on workers, consumers, community health and safety, groundwater, surface waters, and non-target wildlife organisms. In addition, pesticide use raises concerns about the persistence and accumulation of pesticides in food chains quite distant from the original point of use, and about the role of certain pesticides in causing reproductive failure and endocrine system abnormalities in both wildlife and humans, and other species that are not their intended target. It is therefore, important to control the use of pesticides, by carefully weighing the benefits that they confer against any possible adverse effects" (Government Gazette, 2010).

Lack of knowledge has led farmers to believe that pests and diseases can only be controlled with chemical pesticides (Khan & Damalas, 2015). Alternatives to chemical pest control solutions, that are less harmful to people and the environment, while still effectively controlling

pests, are of utmost importance in the modern world of crop protection (Khan & Damalas, 2015). Chemical pesticides need to be understood and not just applied, as many problems can occur with incorrect use of chemicals (Safaz *et al.,* 2006). Chemical pesticides are widely used and very popular because they provide a cheap and effective way for farmers to control various pests and diseases (Mall *et al.,* 2018). Human and environmental safety, and resistance of pests and diseases to chemicals are just a few factors playing a role in the use of chemicals (Macharia *et al.,* 2005).

In the USA, a first world country, 20,116 people are hospitalized every year for pesticide poisoning (Khan & Damalas, 2015). The World Health Organization has reported 3 million acute poisoning events every year (Khan & Damalas, 2015). Dependence on chemical control has led to pest resistance being reported worldwide (Dennill & Pretorius, 1995). Uneducated and uncontrolled use of chemical pesticides has resulted in an increase in resistance of pests to pesticides, thus alternatives to solely using chemical control of pests and diseases should be explored (Khan & Damalas, 2015).

2.10 Chemical soil fumigation

Historically, soil used to be chemically fumigated with methyl bromide for the control of soilborne pathogens (Ntalli *et al.,* 2017). Methyl bromide, a highly toxic and persistent substance, has been banned worldwide in the last few years (Manfort *et al.,* 2007), and a gap in the industry has emerged in seeking a suitable replacement such as 1.3–Dichloropropene (Shi *et al.,* 2009), for the control of multiple soilborne pests and diseases including weeds, fungi, bacteria and nematodes (Wang *et al.,* 2006). Other replacements for MB are the following: metam sodium (MS) (Sederholm *et al.,* 2017), chloropicrin and dimethyl disulphide (Guo *et al.,* 2017), methyl iodide, propargyl bromide (Wang *et al.,* 2006) and calcium cyanamide (Shi *et al.,* 2009). These alternatives to MB still need to be studied for their impact

on soil ecology, as some of these may be as devastating as MB (Wang *et al.,* 2006). The use of soil fumigants is strictly regulated because of environmental and safety concerns.

The most common soil fumigants now used in vegetable production are chloropicrin and MS (Guo *et al.,* 2017). As a soil fumigant MS is the third most used pesticide in the USA (Selderholm *et al.,* 2017). Metam sodium salt is hydrolysed, when it comes in contact with water, to methyl isothiocyanate (MITC), a volatile toxic gas which is applied as a broad spectrum pesticide for its herbicidal, fungicidal and insecticidal qualities. Unfortunately MS can have adverse effects on soil biology, especially on soil micro-organisms that are responsible for plant nutrient uptake, nitrogen transformation and pollutant degradation. Recovery of these microbial populations takes time (Selderholm *et al.,* 2017).

Calcium cyanamide is also one of the possible replacement products for MB, and is generally used as a fertilizer, but it has some fungicidal, herbicidal and insecticidal qualities. Reports state that calcium cyanamide is effective in the control of *P. brassicae* (cause of clubroot disease of brassica) (Shi *et al.,* 2009). Calcium cyanamide has some fungicidal and nematicidal properties (Watson, 1915), and is sold in the EU as a fertilizer without national regulations as it consists of 19% N and >50% Ca, thus giving the product liming qualities as well as supplying nitrogen. When calcium cyanamide comes into contact with soil moisture it decomposes to hydrogen cyanamide and hydrated lime (Donald *et al.,* 2004). Hydrogen cyanamide has fungicidal and nematicidal properties (Donald *et al.,* 2004; Watson, 1915), and is a perfect alternative for liming and a slow release nitrogen source which has herbicidal and fungicidal properties (Tremblay *et al.,* 2005).

Considering the long-term effect of chemical fumigation on the sustainability of vegetable production, there is an urgent need for research into different fumigation and bio-fumigation alternatives (Guo *et al.,* 2017). The decrease in soil microbial populations with the use of MS has proven to be devastating (Sederholm *et al.,* 2017). Given the need to maintain soil health

through management practices, non-chemical alternatives to soil fumigation should be explored (Wang *et al.,* 2006).

2.11 Biological control of pests and diseases

Biological control refers to controlling pests and diseases with living organisms. A natural enemy is introduced into the environment of the pest, where it multiplies and becomes effective in reducing or controlling the pest (Encyclopeadia Britannica, 2018b).

It relies on predation, parasitism, herbivory, or other natural mechanisms, but typically also involves an active human management role. It can be an important component of a holistic approach to pest and disease management (FAO, 2018). Using registered biological pesticides should be as effective in controlling pests and disease in *Brassica* spp. production as ordinary pesticides, provided that certain application needs are met, as biological products can be more sensitive to apply (Collier & van Steenwyk, 2004). It is known that various Lepidoptera pests can be controlled with *B. thuringiensis,* a soil-living bacterium (Correa–Caudros *et al.,* 2016).

2.12 Biological soil fumigation

"Bio-fumigation, as originally defined, is the use, in agriculture, of the toxicity of *Brassica* crop residues to control plant parasitic nematodes and soilborne plant pathogens" (Motisi *et al.,* 2010).

Non-selective MB formulations were the preferred products to control these pests, but they have been banned in developed countries since 2006 (Ntalli *et al.,* 2017). The product was used to control soilborne pathogens and weeds, and to avoid loss of yield in crops associated with monoculture practices. The effect of chemical fumigation products on management of soilborne pathogens, the environment and soil biology has led to the search for environmentally friendlier products as alternatives (Wang *et al.,* 2014). Incorporating cruciferous plant residues

into the soil, bio-fumigation, has been recorded as an alternative to chemical fumigation, furthermore, the incorporation of a legume cover crop into soil can also help to increase soil fertility (Wang *et al.,* 2006). Bio-fumigation refers to the suppression of soilborne pathogens through toxins released by decomposing organic matter. The volatile chemicals that are released during this process have some fungal, bacterial and nematode control properties (Wang *et al.,* 2014). The potential mode of action of *Brassica* spp. as biological control for PPN are the following; production of nematoxic glucosinolates (GSL) products like isothiocyanate (ITC) (Fourie *et al.,* 2016).

Bio-fumigation can be carried out by incorporating *Brassica* spp. plant residues into the soil. Some *Brassica* spp. produce ITC, a natural origin hydrolysis product of GSL which originates from *Brassica* spp. including rape, mustard, canola, cabbage and broccoli, which has a toxic effect on soilborne pests and diseases (Ntalli *et al.,* 2017). Glucosinolates present in cells of *Brassica* spp. can be hydrolysed by myrosinase enzyme to produce ITC, a natural fumigant (Kruger *et al.,* 2015; Omirou *et al.,* 2011). Isothiocyanates in hydrolysed brassica organic material has fumigation properties like methyl isothiocyanate (MITC) in metam sodium (Selderholm *et al.,* 2017). The GSL in *Brassica* spp. plants is biologically inactive, after tissue disruption and incorporation into the soil they are hydrolysed by myrosinase to a few byproducts including ITC which are most toxic to soilborne pathogens (Omirou *et al.,* 2011). Adequate soil moisture is needed for the hydrolyse process to take place (Ntalli *et al.,* 2017). The *Brassica* spp. with the highest GSL contents includes: *B. sativus* (radish), *B. rapanus* (turnip), *B. napus* (oil seed rape), *B. juncea* (mustard species) and *B. oleracea* (broccoli, cauliflower, brussel sprouts and cabbage) (Bennet *et al.,* 2006; Fourie *et al.,* 2016).

Glucosinolate levels vary in the different *Brassica* spp., the phenological stage (inflorescence) and amount of organic material produced, slashed and incorporated into the soil are the two most important factors contributing to the success of the bio-fumigation (Bellostas *et al.,* 2004;

Fourie *et al.,* 2016). Limiting factors of biological fumigation with *Brassica* spp., are: choosing a *Brassica* sp. with a low GSL contents, phenological stage of the plant (inflorescence stage provides highest level of GSL), method of tissue maceration and tissue incorporation into the soil, soil temperature and soil moisture (Fourie *et al.,* 2016). Covering the soil with clear plastic after the *Brassica* has been incorporated is known as soil solarisation. This prevents the volatile nematicidal compounds from escaping, it leads to a higher soil temperature, faster decomposition of organic material and limits soil moisture loss (Fourie *et al.,* 2016; Ploeg & Stapleton, 2001).

It is important to remember that the microbes in soil are an important part of the soil ecosystem. Decomposition of organic material, nutrient cycling, pollutant degradation and formation of humic substances are all part of the make-up of a healthy soil (Omirou *et al.,* 2011). Bio-fumigation is constantly explored as the preferred alternative to fumigation, but the adoption of this practice has been limited due to the gap in the knowledge and mechanisms for disease suppression and control (Wang *et al.,* 2014).

There have been recordings of soilborne disease and weeds suppression with *Brassica* spp., in a crop rotation programme, such as the pathogen, *Rhizoctonia solani. Brassica* spp. cover crops have been used for their bio-fumigation qualities in a holistic approach to crop protection with PPN (Omirou *et al.,* 2011). Plant pathogens in the genera *Fusarium, Pyrenochaeta, Sclerotinia* and *Verticillium,* that belong to the largest group of true fungi, the *Ascomycetes,* can be controlled by bio-fumigation (Omirou *et al.,* 2011). Although the use of *Brassica* crops as an alternative for chemical fumigation in a biological control programme has not always been effective (Fourie *et al.,* 2016), some research has shown variable and significant control (Fourie *et al.,* 2016; Henderson, *et al.,* 2009). This controversial topic again provides a need to research the effect of *Brassica* spp. as a biological means to control PPN (Fourie *et al.,* 2016).

However, the effect of bio-fumigation on non-target organisms also needs to be investigated (Wang *et al.,* 2014). Some research shows a reduction in EPN as a result of the biological fumigation. Other trials show an increase in bacterivores and fungivores (Fourie *et al.,* 2016; Henderson *et al.,* 2009; Ramirez *et al.,* 2009).

Controlling PPN in the long-term with a single strategy is rarely successful. The controlling strategies should be combined with the integration of different biological control methods, crop rotation, and host plant resistance, to reduce nematode populations and optimize sustainable crop production (Fourie *et al.,* 2016).

Using a *Brassica* spp. as a bio-fumigant can be very successful if done correctly, but the cost and time it takes to produce this crop should be measured against what they put back into the soil, as they have no other economic return to the farmer (Fourie *et al.,* 2016).

2.13 A holistic approach to pest and disease management

A holistic approach to pest and disease management systematically tries to reduce pest and disease numbers, on the target plant, and contributes to long-term sustainability by combining judicious use of biological, cultural, physical and chemical control tools in a way that minimizes the risks of pesticides to human health and the environment (Bajwa & Kogan, 2002). Understanding that an "holistic approach" is a system is extremely important in managing pests and diseases (Way & van Emden, 2000). An adaptable range of pest control methods is explored, which is cost effective whilst being environmentally acceptable and sustainable (Way & van Emden, 2000). A farmer who manages diseases and pests by means of a holistic approach reduces the effects of chemical use (Nga & Kumar, 2008). Famers need to manage an ecosystem, as chemical use can eliminate the natural enemies as well as pests (Macharia *et al.,* 2005). A holistic approach aims at using the minimum amount of chemical pesticides

needed to control a pest, with the incorporation of non-insecticidal control whenever possible (Finch & Collier, 2000). Because of the limited availability of products for biological control and the difficulty in registering these products, a balance should be found between chemical and biological products, which can be used together for resistance management, crop protection and to be economically justifiable.

CHAPTER 3: METHODS AND MATERIALS

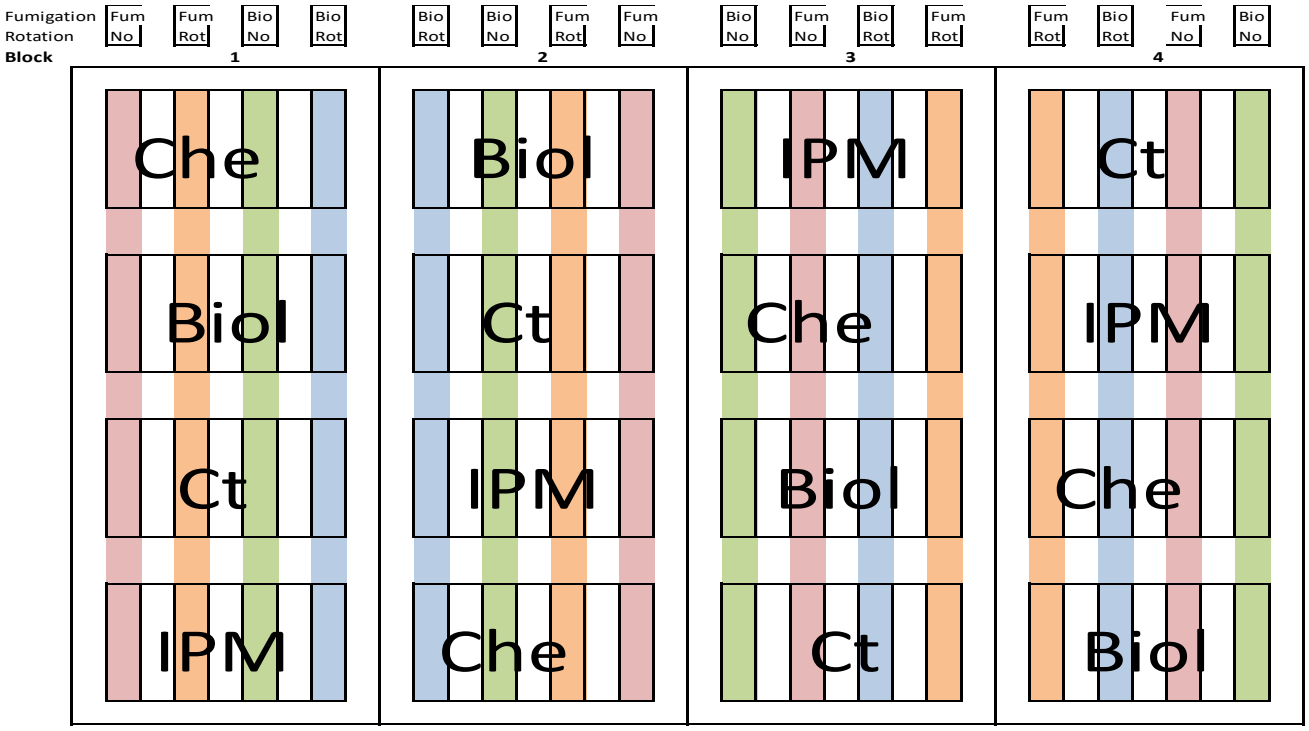
3.1 Background

The land for this study was situated at a commercial vegetable farm in Philippi (34°01'33.46" S 18°32'31.47" E with an elevation of 22 m above sea level) in the heart of the Western Cape vegetable production area known as the Cape Flats. Similar methods of biological control were incorporated on the Mandela Trials at the George Campus of Nelson Mandela University.

3.2 Trial layout and design

The experimental design was a strip split plot design, with different pest and disease management strategies as the main plot treatment and fumigation and rotation treatment combinations arranged in strips across the main plot treatments. The main plot design was a randomized complete block with four, management programmes (Control, Holistic, Chemical and Biological) replicated four times and laid out in a Randomised Complete Block Design (RCBD). The treatment design of the strip plot factors was a 2x2 factorial with two fumigations (fumigated chemically and fumigated biologically) and two rotations (crop rotated and monoculture) randomly allocated across main plot treatments (Table 6). Each experimental unit consisted of 40 plants.

Table 6: Trial layout (IPM refers to a holistic approach programme)



Analysis of variance (ANOVA) was performed according to the experimental design, using GLM (General Linear Models) Procedure of SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC, USA). A Shapiro-Wilk test was performed to test for normality of variables assessed (Shapiro & Wilk, 1965). The least significant difference (LSD) was calculated at the 5% level to compare treatment means (Ott & Longnecker, 1998). A probability level of 5% was considered significant for all significance tests.

In Tables 6 and 7: Fumigation Fum, refers to the semi-permanent bed of soil being treated with chemical fumigation. Fumigation Bio, refers to the semi-permanent bed of soil being treated with biological fumigation. Rotation Yes, refers to the crops in the semi-permanent bed cultivated with a crop rotation programme. Rotation No, refers to the crops in the semi-permanent bed cultivated as a monoculture crop. IPM, refers to a holistic management programme; Che, refers to chemical treatment; Biol, refers to biological treatment and Ct, refers to control treatment.

Table 7: Statistical layout of the research trial

Randomised Block Design for Programme Main Plot Effect

Block	1	2	3	4
	3	4	2	1
	4	1	3	2
	1	2	4	3
	2	3	1	4

where

Nr	Programme
1	Control
2	IPM
3	Chemical
4	Biological

Randomised order of Fumigation x Rotation Treatment combinations in strips over Programme main plots

3		4		1		2	2		1		4		3	1		3		2		4	4		2		3		1

where

Nr	Fumigation	Rotation
1	Bio	Not
2	Bio	Rotated
3	Fumigated	Not
4	Fumigated	Rotated

Anova

Source	d.f.
Block	4-1=3
Programme	4-1=3
Error (a)	(4-1)(4-1)=9
Fumigation	2-1=1
Rotation	2-1=1
FumxRot	(2-1)(2-1)=1
Error (b)	3(4-1)=9
ProgxFum	(4-1)(2-1)=3
ProgxRot	(4-1)(2-1)=3
ProgxFumxRot	(4-1)(2-1)(2-1)=3
Error (c	9(4-1)=27
Total	4x4x2x2 -1=63

3.3 Establishing soil chemical properties

One representative soil sample of the trial site was sampled before the trial commenced to establish the baseline chemical properties. After the trial was conducted soil samples were taken, this time soil from two replicates of each treatment (32 different samples) on which the vegetables were cultivated. Samples were taken separately to establish the effect different fumigation, rotation and cultivation practices had on the chemical properties.

Soil was analysed by the Department of Soil Science at Elsenburg, Stellenbosch.

Analysis of the soil chemical properties was established by the following methods:

- pH in KCl
- Acidity: Exchangeable acidity potassium sulphate (K_2SO_4)
- P: Olsen method – (Inductive Coupled Plasma, ICP)

- K, Ca, Mg, Na, S: Ammonium EDTA, (NH_4) EDTA, extract (ICP)
- Cu, Zn, Mn: Ammonium EDTA extract (ICP)
- Boron: Calcium Chloride extract (ICP)
- Carbon: Walkley-Black method

An inductive coupled plasma instrument was used for analysis of cations and micro elements, where soil was mixed with a reagent and the solution filtered. The solution was then placed in the ICP and the analysis done. The soil extract was measured against solutions of known concentration of the analytes (F. Redeers, 2018, Personal communication, 20 November).

The method used to analyse soil pH indicates the activity of hydrogen ions in a soil suspension in 1 mol/dm³ KCl. The pH meter was calibrated before the procedure. 10g of dried soil was placed in a glass beaker and 25 cm³ KCl solution (1 mol/dm³) added. The contents was then rapidly stirred for 10 seconds, and allowed to stand for 50 minutes, then stirred again and allowed to stand for another 10 minutes. After this procedure the pH was measured with a calibrated pH meter, by placing the electrodes in the mixture.

The exchangeable acid was determined with the Eksteen method, using K_2SO_4 (potassium sulphate). 10g of dried soil was placed in an extraction bottle and 25 ml K_2SO_4 solution (0.5 mol/litre) added. The contents was then shaken for 60 minutes. 4 drops of superflock N-100 (Cyanimide) solution was then added. The mixture was then filtered and rinsed through the filter paper up to 100ml by adding a small amount of K_2SO_4. 50 ml was then titrated with 0.01 mol/litre NaOH (sodium hydroxide). The end point, where all the acid was neutralized by the NaOH, was detected by the pink colour of phenolphthalein base indicator.

Exchangeable acidity (Eksteen, 1969).

= (v – v´) ml x factor x 0.01 mol/litre x 100ml/50ml x 100g/10g
= 0.2 x factor (v - v´) m mol H^+/100g soil.

For Phosphorous the Olsen method was used as described by Lindsay & Moreno (1960), by placing dried and refined soil in an extraction bottle. 1 g of phosphate free charcoal was then added, and 50 cm^3 $NaHCO_3$ (Sodium bi-carbonate) solution. It was then shaken for 30 minutes at 180 oscillations per minute, the solution was then filtered through Whatman no 40 filter paper. 5 cm^3 was pipetted into 25 cm^3 flasks, 2.5 ml/dm^3 H_2SO_4 was then added to bring the pH to 5. 10 cm^3 de-ionised water followed by 4 cm^3 colour reagent. The absorbance of the solution was then determined on a spectrophotometer at a wavelength of 882 nm.

c = mg/dm^3 P in the extract
mg/kg P in soil = c x 50/2.5.

The cations (Ca, Mg, K, Na, S) as well as micro-elements (Zn, Cu, Mn) were determined by $(NH_4)_2$ EDTA (di-ammonium (EDTA)) extraction method and analysed by ICP. Dry soil was crushed and refined to a fineness of ≤ 1mm. 5g of air-dried soil was placed in an extraction bottle. 15 cm^3 0.02 mol/dm^3 $(NH_4)_2$ EDTA was then added to the soil. The mixture was shaken for 60 minutes in a reciprocating shaker at a constant temperature of 20 ± 2 °C, the sample was then centrifuged for 5 minutes at 2000 rpm and filtered through Whatman no. 40 paper. For manganese (Mn) 5g soil was used with 50 cm^3 $(NH_4)_2$ EDTA.

Standards used:

Zinc: Calibration standards ranging from 0.5 - 2 $\mu g/cm^3$ Zn in in 0.02 mol/dm^3 $(NH_4)_2$ EDTA solution.

Copper: Calibration standards ranging from 1 - 10 $\mu g/cm^3$ Zn in in 0.02 mol/dm^3 $(NH_4)_2$ EDTA solution.

Manganese: Calibration standards ranging from 1 – 4 µg/cm³ Zn in in 0.02 mol/dm³ $(NH_4)_2$ EDTA solution.

Elemental content of sample = w µg/cm³
Total extractant = 15 cm³ representing 5 g soil
mg/kg Zn/Cu/Mn in soil = 15 x w / 5

For the macro-elements (K, Ca, Mg, Na, S) 5 g soil was used with 50 cm³ $(NH_4)_2$ EDTA and analysed by ICP using the methods described by Beyers & Coetzer, 1971.

Standards used:

K, Ca, Mg, Na, S: Calibration standards ranging 1 – 400 µg/cm³ in 0.02 mol/dm³ $(NH_4)_2$ EDTA solution.

Elemental content of sample = w µg/cm³
Total extractant = 50 cm³ representing 5 g soil
mg/kg Zn/Cu/Mn in soil = 50 x w / 5.

Boron was analysed by ICP using the methods described by Bingham (1982). Boron was extracted from the soil by boiling 25 g of air dried and refined soil with 50 cm³ 0.02 mol/dm³ $CaCl_2$ (Calcium chloride) for 15 minutes. It was then filtered through a Whatman no 41 filter paper. 1 cm³ was pipetted onto an evaporation dish, and 4 cm³ curcumin-oxalic acid solution added and mixed. It was placed on a waterbath at 55°C to evaporate to dryness. Then 25 cm³ ethanol was added, and the solution was filtered through Whatman no 40 paper. The boron content of the solution was read using a spectrophotometer set at 540 nm.

Boron content of sample = b µg/cm³
Total extractant = 50 cm³ 0.02 mol/dm³ $CaCl_2$ solution
mg/kg B in soil = b x 50 / 25.

To establish the carbon levels of the soils, the Walkley-Black method was used as described by Allison & Black (1965). Air dried soil was ground to pass through a 0.42 mm sieve. Between 0.5 – 1 g of top soil and 2 – 4 g of subsoil was used to contain between 10 – 20 mg of carbon.

10 ml 1N $K_2Cr_2O_7$ (anhydrous potassium dichromate) was added swirled and 20 ml concentrated H_2SO_4 (sulphuric acid) and mixed thoroughly. The mixture was then heated to 135°C, and cooled on asbestos in a fume cupboard. When the mixture was cooled, 200 ml deionised water was added. $FeSO_4$ (Ferrous sulphate) was then titrated with an automatic titrator.

$$2Cr_2O_7^{2-} + 3C + 16H^+ \rightarrow 4Cr^{3+} + 8H_2O + 3CO_2\uparrow$$

1 ml of 1 N Dichromate solution is equivalent to 3 mg of carbon.

Where the quality and normality of the acid/dichromate mixture used are as stated in the method, the percentage carbon was determined from the following:

Where:

N = Normality of $K_2Cr_2O_7$ solution

T = Volume of FeSO4 used in sample titration (ml)

S = Volume of FeSO4 used in blank titration (ml)

ODW = Oven-dry sample weight (g)

Organic carbon (%)
= (0.003 g x N x 10 ml x (1 T/S) x100) / ODW
= 3 (1− *T/S*) / W.

3.4 Nematode extraction

One representative soil sample, consisting of 32 subsamples, 30 cm deep and with a "zig-zag pattern" at regular intervals, on the total trial site was sampled. The subsamples was then

thoroughly mixed and 2 kg of this soil was analysed by Nemlab before the trial was conducted to establish the baseline nematode population.

After the trial was conducted, at harvest, soil samples were evaluated. Four subsamples in each of the sixteen semi-permanent beds in which the vegetables were cultivated were mixed and analysed separately to establish the effects that different fumigation, rotation and cultivation practices had on the nematode population. The soil samples were analysed by Nemlab for PPN and free-living nematodes.

Extraction of nematodes from soil was accomplished by means of Cobb's decanting and sieving method (Cobb, 1918). A 250 ml volume of soil was washed through a coarse mesh sieve with an aperture of 2 mm into a 5 l bucket. The sieve served to remove stones and plant material from the samples and was also used to break up clods. Water was added to the bucket to increase the volume to 5 l, and the soil was brought into suspension by stirring and allowed to settle for 60 seconds. Thereafter, the suspension was poured through a bank of sieves consisting of the following apertures from top to bottom: 90 µm, 53 µm, 53 µm, and 45 µm. The residue collected on each sieve was transferred to a 250 ml beaker. The 5 l bucket was filled for a second time and the process repeated, but with a settling time of 30 seconds. Subsequently, the suspension was poured through the sieves and the residue transferred to the same 250 ml beaker previously mentioned. Samples were cleared by means of a modified Baermann funnel (Cobb, 1918). The technique used required samples to be poured onto a watch glass through a two-ply paper towel supported on a coarse-meshed plastic screen. The plastic screen was contained within a metal dish. Water was added to the container until the residue on the paper towel was thoroughly wet, but not immersed. The modified funnel was left undisturbed for 48 hours, after which the filter was removed and discarded, and the suspension poured into a 250 ml beaker for examination.

Nematode suspensions extracted from soil were allowed to settle for one hour in the 250 ml beaker, after which the volume was adjusted to 50 ml, and then transferred to a 100 ml beaker. The nematodes were once more allowed to settle for 60 min, after which the excess water was siphoned off to 20 ml, by using a thin plastic tube. A small fish pump was used to blow air through the nematode suspension to agitate the sample. A pipette was used to add a 1 ml suspension to a 1 ml graduated slide for counting. Two slides were counted and the mean number of nematodes for each sample determined. Nematodes were counted using a compound microscope.

Temporary slides were made for identification of the nematodes. A 1 ml nematode suspension was placed on a microscope slide with a grid pattern (0.5 × 0.5 mm) drawn on the back of the slide, using a permanent marker. Use of such a grid pattern facilitated the identification process, by preventing confusion as to which nematodes on the slide had been identified. The suspension was then covered by a 52 × 22 mm rectangular cover slide, which was held over a gentle flame for several seconds to heat kill the nematodes. Clear nail-polish was used to seal the cover slide. The first 100 nematodes were identified to family level. The process was repeated for each sample.

Identification to family level was done using the following books: *A Guide to Plant and Soil Nematodes of South Africa* (Heyns, 1971); *Soil and Freshwater Nematodes* (Goodey, 1963); and *The Nematodes of the Netherlands* (Bongers, 1994). The 'Interactive Diagnostic Key to Plant-parasitic, Free-living and Predaceous Nematodes' from the Nematology laboratory of the University of Nebraska Lincoln, which is available from their identification website (http://nematode.unl.edu/konzlistbutt.htm), was also used as an aid in the identification. Conventionally, identification of nematodes is accomplished mainly by the morphology of the oesophagus, in combination with other characteristics. Nematodes were identified with the aid of a compound microscope.

3.5 Diamondback moth infestation assessment

The number of plants infested with Diamondback moth larvae was assessed weekly from 7 days after transplant to 78 days after transplant at harvest. Using assessment keys can be a quick, simple and successful way of assessing the percentage of disease present. It can be used on leaves, individual plants or in small sample areas (James, 1971).

3.6 White blister severity assessment

White blister severity was measured weekly by assessing all the plants in the whole block from 7 days after transplant to 78 after transplant at harvest. (James, 1971).

A disease rating scale of 0-6, was used to determine the percentage infection of the block where:

0 = 0%
1 = 1 - 10%
2 = 11 - 25%
3 = 26 - 50%
4 = 51 – 75%
5 = 76 – 99%
6 = 100%

3.7 Clubroot infection assessment

The number of plants infected with clubroot was assessed at harvest. Whole root systems were removed from all the plants and washed. 10 plants per plot was used in the assessment, 640 plants in total in the trial site. Clubroot severity was assessed on a scale from 0 – 3 per Jordan & Gevens (2011).

0 = 0% clubbed
1 = only lateral roots clubbed
2 = < 50% of tap root clubbed
3 = > 50 % of tap root clubbed

3.8 Cultivation practices

Enhancing the quality of the soil is dependent on the physical, chemical and biological properties of the soil (Buneman *et al.,* 2018). The main focus of agriculture is yield maximization. Poor agricultural practices and excessive use of nitrogen fertilizer cause a decline in the soil fertility. Thus a beneficial crop rotation system is critical in *Brassica* spp. production, (Ahmad *et al.,* 2014). Rotation crops need to be researched as it may contribute to improving soil quality and fertility (Messinga *et al.,* 2015).

Brassicas such as broccoli, cauliflower and cabbage are heavy feeders, because these plants extract a large amount of nutrients out of the soil (Venetta, 2011). For this reason the broccoli (*Brassica oleracea)* cultivar Star 2204, was planted first in the season. In the second planting of broccoli the cultivar Parthenon was planted as it performs better during cooler months. Broccoli was planted at a density of 30 000 plants/ha. Radish (*Raphanus sativus)* although *Brassicaceae* are root vegetables, and part of the mustard family. Radishes are only in the soil for a short period of 4-6 weeks, depending on the weather. They are good rotation crops and do not take excessive amounts of nutrient out of the soil, as they are light feeders (Albert, 2014). The radish cultivar Cherry Belle was planted. The seeding density for radishes was 10 kg seed/ha. Root vegetables and high nitrogen are not compatible, since high nitrogen levels cause lush foliage at the expense of the edible root (Growveg.com, 2015). Nitrogen needs to be fixed in the soil in a rotation programme, after the heavy and light feeders. Legumes form nodules on the roots where *Rhizobia* (nitrogen-fixing bacteria) establish themselves, and fix nitrogen (Masson-Biovin *et al.,* 2009). The legume, green bean (*Phaseolus vulgaris)* cultivar Douglas was planted. The seeding density for legumes is 170 000 – 200 000 seeds/ha. A heavy feeder (broccoli) then followed the nitrogen fixing legume (Venetta, 2011).

The following rotation programme was used:

Broccoli → root vegetable, radish → green bean → broccoli.

The no rotation programme was broccoli monoculture.

The trial was established on 0.0896 ha (32 m x 28 m = 896 m²). The planting density was 30 000 *Brassica oleracea* plants/ha, and there were 2688 *B. oleracea* plants planted for this trial. Each of the foliar spray programmes (Chemical, Biological, Holistic, Control) had 16 treatments x 5 m² plot x 6 plants/m² = 480 plants per foliar spray programme.

Soil was cultivated before the planting of the *brassica*, and a commercial chemical fertilizer programme was applied on all the plots. Soil cultivation can have the following advantages; it is a form of weed control, reduces soilborne pathogens, creates structure, and helps to retain moisture (McCullen, 2000). All vegetables (rotation crops and *brassica*) for this study, were planted in semi-permanent beds, and needed to be cultivated.

Ploughing was avoided in this study; the beds were only ripped and tilled. Avoiding ploughing saved cultivation time, labour, maintenance and fuel costs. The semi-permanent beds in which the vegetables were planted were bedded up. This leads to better water drainage, less waterlogging, less soil compaction, and fertilizer is not lost by being worked in too deeply; organic matter is higher, soil structure is improved which leads to better root development (McCullen, 2000).

3.9 Chemical control programme

The chemical control programme and time of application is summarized in Table 8 and the products were: calcium cyanamide, metham sodium, didecyldimethylammonium chloride, alpha-cypermethrin, azoxystrobin + chlorothalonil, tebuconazole, chlorfenapyr and chlorotraniliprole + lambda-cyhalothrin.

Calcium cyanimide is a slow release calcium + nitrogen that has fumigation properties. It was used in conjunction with MS which is registered as a fumigant to control PPN and soil fungi. Didecyldimethylammonium chloride (DDAC), is used throughout the world as a contact

fungicide and as a sanitation product (K. Serfontein, 2018, Personal communication, 19 November). It was applied with every chemical spray application as DDAC which is an excellent disease resistance management product. Alpha-cypermethrin is a suspension concentrate insecticide for the control of cutworms (*Agiotis* spp.), bollworm (*Helicoverpa armigera)* and DBM (*P. xylostella)* larvae in *Brassicas* (van Zyl, 2010a, b). Systemic insecticides are used in transplanting to protect young plants against insect pests, until such a time as plants are big enough to start spraying against disease.

Azoxystrobin + chlorothalonil is a suspension concentrate fungicide with systemic, translaminar and contact properties for the control of whiteblister (*A. candida)* on *Brassica.* Chlorothalonil is a suspension concentrate contact fungicide for the control of whiteblister on *Brassica.* Tebuconazole is an emulsion in water systemic fungicide for the control of downy mildew, *Peronospora brassicae* (van Zyl, 2010a). *Hyalopernospora*, a new genus, which accommodates several other *Peronospora* spp., parasitic on *Brassicae* (Constantinescu & Fathi, 2002).

Chlorfenapyr is a suspension concentrate translaminar insecticide with stomach and contact activity for the control of DBM larvae and large white cabbage moth (LWCM) (*Crocidolomia pavonana*) larvae. Chlorantraniliprole + lambda-cyhalothrin (pyrethroid), is a translaminar encapsulated suspension flowable concentrate with contact and stomach action for the control of DBM, LWCM, cutworm and bollworm (van Zyl, 2010b).

Table 8: The chemical control programme used in the trial

Application Timing	Problem	Recommendation			Notes
		Product	Active Ingredient	Dosage	
Fumigation	Nematodes, Soilborne diseases	Perlka + herbifume	Calium cyanamide + metam sodium	500kg/ha + 900ml/ha	90ml Herbifume in 10L water, drench seedbed with 1l/m² (9ml/m²/l)
Before Planting	Seedling Diseases (*Rhizoctonia, Pythium, Fusarium*)	Sporekill drench	Didecyldimethylammonium chloride	50ml/100l	
Planting	Diamondback moth, Cutworms	Fastac	Alpha-cypermethrin	1ml/5ml/100m	Drench over plants. 4 days witholding period
14 days after planting	Downy mildew, White blister	Amistar Opti	Azoxystrobin + Chlorothalonil	600ml/100l	Only 3 sprays. 7 day intervals. 7 days witholding period
		Bravo	Chlorothalonil	400ml/100l	Only 2 sprays. 7 day intervals. 7 days witholding period
		TebuCure	Tebuconazole	75ml/100l	Only 5 sprays. 7 day intervals. 7 days witholding period
					Choose chemicals to repeat and rotate
Every 7 - 14 days later	Diamondback moth and lepidopterous pests	Hunter	Chlorfenapyr	60ml/100l	Only 4 sprays. 14 day intervals. 7 days witholding period
		Ampligo	Chlorotaniliprole + lambda cyhalothrin	40ml/500l	Only 4 sprays. 14 day intervals. 3 days witholding period
		Fastac	Alpha-cypermethrin	7ml/100l	Only 2 sprays. 14 day intervals. 4 days witholding period
Headforming until harvest	Diamondback moth	Hunter	Chlorfenapyr	60ml/100l	Only 4 sprays. 14 day intervals. 7 days witholding period
		Ampligo	Chlorotaniliprole + lambda cyhalothrin	40ml/500l	Only 4 sprays. 14 day intervals. 3 days witholding period
		Fastac	Alpha-cypermethrin	7ml/100l	Only 2 sprays. 14 day intervals. 4 days witholding period
	Downy mildew, White blister	Amistar Opti	Azoxystrobin + Chlorothalonil	600ml/100l	Only 3 sprays. 7 day intervals. 7 days witholding period
		Bravo	Chlorothalonil	400ml/100l	Only 2 sprays. 7 day intervals. 7 days witholding period
		TebuCure	Tebuconazole	75ml/100l	Only 5 sprays. 7 day intervals. 7 days witholding period
Notes:					
1. Add Nufilm P 30ml/100l (3ml/1l) to spray mixture					
2. Watervolume = 500l/Ha					

3.10 Biological control programme

The biological control programme and time of application is summarized in Table 9 and the products included were: Caliente 199, Nemat arugula, *T. harzianum*, *Paecilomyces lilacinus*, *Bacillus thuringiensis* ssp. *kurstaki* and azadirachtin.

Caliente 199 mustard and Nemat arugula were planted six months before the broccoli was planted, and four weeks before planting broccoli, at inflorescence stage, the bio-fumigation plants was incorporated into the soil. The decomposing organic matter helped fumigate the soil biologically (bio-fumigation) (Valdes *et al.,* 2012).

T. harzianum a wettable powder inoculant was applied for the control of root diseases as a drench in combination with *Paecilomyces lilacinus,* a wettable spore concentrate and fungal nematicide. According to Cheah and Page (1997), *Trichoderma* spp. can be used to control clubroot. *Trichoderma* spp. also facilitate the absorption of nutrients (Mazhabi *et al.,* 2010), and vegetable juices with *T. harzianum* can be applied as a foliar spray for the control of foliar diseases (van Zyl, 2010b). *Beauveria bassiana* is a biological control agent, registered for the control of DBM. *Bacillus thuringiensis* ssp. *kurstaki* is registered for the control of lepidopterous pests of *Brassica* (van Zyl, 2010a). Adzadirachtin isolates from the seeds of the neem tree *Adzadirachta indica* L. has been used to control various pests in vegetables (Darabian & Yarahmadi, 2017).

Table 9: The biological control programme used in the trial

Application Timing	Problem	Recommendation			Notes
		Product	Active Ingredient	Dosage	
Fumigation	Nematodes, Soilborne diseases	Caliente Mustard + Nemat Aragula		10kg/ha	
		Trichoplus	*Trichoderma harzianum*	250g/Ha (0.5g/l)	Drench every 3 - 4 weeks
		PL Gold	*Paecilomyces liliacinus*		
Before Planting	Seedling Diseases (*Rhizoctonia, Pythium, Fusarium*)	Trichoplus	*Trichoderma harzianum*	250g/Ha (0.5g/l)	Drench seedlings every 7 days up to planting
Planting	Soilborne Diseases and Nematodes	Trichoplus	*Trichoderma harzianum*	250g/Ha (0.5g/l)	Drench every 3 - 4 weeks
		PL-Gold	*Paecilomyces liliacinus*	2kg/Ha (4g/l)	Drench every 3 - 4 weeks
Planting	Diamondback moth, Cutworms	Broadband	*Beauvaria bassiana*	1l/ha (2ml/l)	Spray 7 day intervals
		Bio-Insek	*Beauvaria bassiana*	1l/ha (2ml/l)	Spray 7 day intervals
		BetaPro	*Bacillus thuringiensis*	320g/ha (0.7g/l)	Spray 7 day intervals
14 days after planting	Downy mildew, White blister	Bio-Impilo	Fermented *Trichoderma harzianum*	500ml/100l	Spray 7 day intervals
		Bio-Tricho	*Trichoderma harzianum*	500ml/100l	Spray 7 day intervals
	Diamondback moth, Cutworms	Broadband	*Beauvaria bassiana*	1l/ha (2ml/l)	Spray 7 day intervals
		Bio-Neem	Azadirachtin	500ml/100l	Spray 7 day intervals
		BetaPro	*Bacillus thuringiensis*	320g/ha (0.7g/l)	Spray 7 day intervals
Headforming until harvest	Diamondback moth, Cutworms	Broadband	*Beauvaria bassiana*	1l/ha (2ml/l)	Spray 7 day intervals
		BetaPro	*Bacillus thuringiensis*	320g/ha (0.7g/l)	Spray 7 day intervals
	Downy mildew, White blister	Bio-Impilo	Fermented *Trichoderma harzianum*	500ml/100l	Spray 7 day intervals
Notes:					
1. Add Nufilm P 30ml/100l (3ml/1l) to spray mixture					
2. Watervolume = 500l/Ha					

3.11 Holistic control programme

The following holistic approach programme is a combination of the chemical and biological active ingredients discussed.

Table 10: The holistic control programme used in the trial (referred to as an integrated pest management programme)

Application Timing	Problem	Recommendation			Notes
		Product	Active Ingredient	Dosage	
Fumigation	Nematodes	Pertka + Herbifume	Calcium Cyanamide + Metam Sodium	500g/Ha + 900ml/Ha	90ml Herbifume/100 water, drench seedbed with 1l/m² (9ml/m²/l)
Before Planting	Seedling Diseases (Rhizoctonia, Pythium, Fusarium)	Trichoplus	*Trichoderma harzianum*	250g/Ha	Every 7 days
Planting	Soilborne Diseases and Nematodes	Trichoplus	*Trichoderma harzianum*	250g/Ha (0.5g/l)	Drench over plants every 3 - 4 weeks
		PL-Gold	*Paecilomyces lilacinus*	2kg/Ha (4g/l)	Drench over plants every 3 - 4 weeks
		Broadband	*Beauveria bassiana*	1l/Ha (2ml/l)	Spray with 7 day intervals
Planting	Diamondback moth, Cutworms	Ampligo	Chlorantraniliprole + Lambda - Cyhalothrin	200ml/500l (0.4ml/l)	Only 4 applications, 14 day intervals, 3 day witholding period
		BetaPro	*Bacillus thuringiensis*	320g/Ha (0.7g/l)	Spray with 7 day intervals
14 days after planting	Downy mildew, White blister	Copper hydroxide + Sulphur	Copper hydroxide + Sulphur	500ml/100l (5ml/1l)	Spray with 7 day intervals
		Broadband	*Beauveria bassiana*	1l/Ha (2ml/l)	Spray with 7 day intervals
	Diamondback moth, Cutworms	Ampligo	Chlorantraniliprole + Lambda - Cyhalothrin	200ml/500l (0.4ml/l)	Only 4 applications, 14 day intervals, 3 day witholding period
		BetaPro	*Bacillus thuringiensis*	320g/Ha (0.7g/l)	Spray with 7 day intervals
Headforming until harvest	Diamondback moth, Cutworms	Broadband	*Beauveria bassiana*	1l/Ha (2ml/l)	Spray with 7 day intervals
		Ampligo	Chlorantraniliprole + Lambda - Cyhalothrin	200ml/500l (0.4ml/l)	Only 4 applications, 14 day intervals, 3 day witholding period
		BetaPro	*Bacillus thuringiensis*	320g/Ha (0.7g/l)	Spray with 7 day intervals
	Downy mildew, White blister	Amistar Opti	Azoxystrobin + Chlorothalonil	600ml/100l (6ml/1l)	Only 3 applications, 7 day interval, 7 dat witholding period
		Bravo	Chlorothalonil	400ml/100l (4ml/1l)	Only 2 applications, 14 day interval, 14 day witholding period
		Tebucure	Tebuconazole	75ml/100l (0.75ml/1l)	Only 5 applications, 7 day interval, 7 day witholding period
Notes:					
1. Add Nuffilm P 30ml/100l (3ml/1l) to spray mixture					
2. Watervolume = 500l/Ha					

CHAPTER 4: RESULTS & DISCUSSION

4.1 Background

The different fumigation and rotation practices were evaluated, at the end of the trial period, for nematodes and soil chemical properties.

The different crop protection strategies were evaluated against different pest and diseases at weekly intervals. The following treatments were evaluated:

Control (No pest control programme applied).

Biological (A biological control spray programme applied).

Holistic (An integrated control spray programme applied).

Chemical (A chemical control spray programme applied).

All the evaluation methods were followed, as presented in the methods and materials section.

4.2 The effects of different crop production practices on soil properties

Data of the soil analyses are given in Appendix 2. Codes of soil samples are listed in Table 11.

Table 11: The codes of soil samples analysed

Foliar Programme	Fumigation	Crop rotation	Soil sample	Trial ID
Chemical	Chemical	No	1	1 A
Biological	Chemical	No	2	1 B
Control	Chemical	No	3	1 C
Holistic (IPM)	Chemical	No	4	1 D
Chemical	Chemical	Yes	5	2 A
Biological	Chemical	Yes	6	2 B
Control	Chemical	Yes	7	2 C
Holistic (IPM)	Chemical	Yes	8	2 D
Chemical	Biological	No	9	3 A
Biological	Biological	No	10	3 B
Control	Biological	No	11	3 C
Holistic (IPM)	Biological	No	12	3 D
Chemical	Biological	Yes	13	4 A
Biological	Biological	Yes	14	4 B
Control	Biological	Yes	15	4 C
Holistic (IPM)	Biological	Yes	16	4 D
Chemical	Biological	Yes	17	5 A
Biological	Biological	Yes	18	5 B
Control	Biological	Yes	19	5 C
Holistic (IPM)	Biological	Yes	20	5 D
Chemical	Biological	No	21	6 A
Biological	Biological	No	22	6 B
Control	Biological	No	23	6 C
Holistic (IPM)	Biological	No	24	6 D
Chemical	Chemical	Yes	25	7 A
Biological	Chemical	Yes	26	7 B
Control	Chemical	Yes	27	7 C
Holistic (IPM)	Chemical	Yes	28	7 D
Chemical	Chemical	No	29	8 A
Biological	Chemical	No	30	8 B
Control	Chemical	No	31	8 C
Holistic (IPM)	Chemical	No	32	8 D

The treatment programmes, soil fumigation and crop rotation did not significantly affect the soil chemical properties, except for crop rotation that significantly ($P = 0.0437$) affected the concentration of K with a significantly lower concentration (36.17 mg/kg) of K in soil where rotation was included compared to 64.13 mg/kg for the no rotation treatment. There was also a

significant (*P = 0.0128*) programme x crop rotation interaction for the concentration of Na in the soil. A significantly higher concentration of Na was recorded for the biological programme when crop rotation was included compared to the no rotation treatment (Table 12).

Table 12: Effect of management programme and crop rotation on the concentrations of Na in the soil

Crop rotation	Na (mg/kg)[z]			
	Control	Biological	Holistic	Chemical
Yes	57.3c	93.8a	65.3bc	69.0bc
No	75.5a-c	60.5bc	59.8c	80.3ab
[z]Means followed by the same letter in a particular row do not differ significantly at P = 0.05 (LSD = 20.41)				

Salinity in soil can be a result of many factors, the most common contributor of high salinity in soil is irrigation water (Shannon & Grieve, 1999). Sodium is the most important physiological threat to agricultural soils and crop production (FERTASA, 2016). Broccoli is a moderate Na-sensitive crop (Shannon & Grieve, 1999).

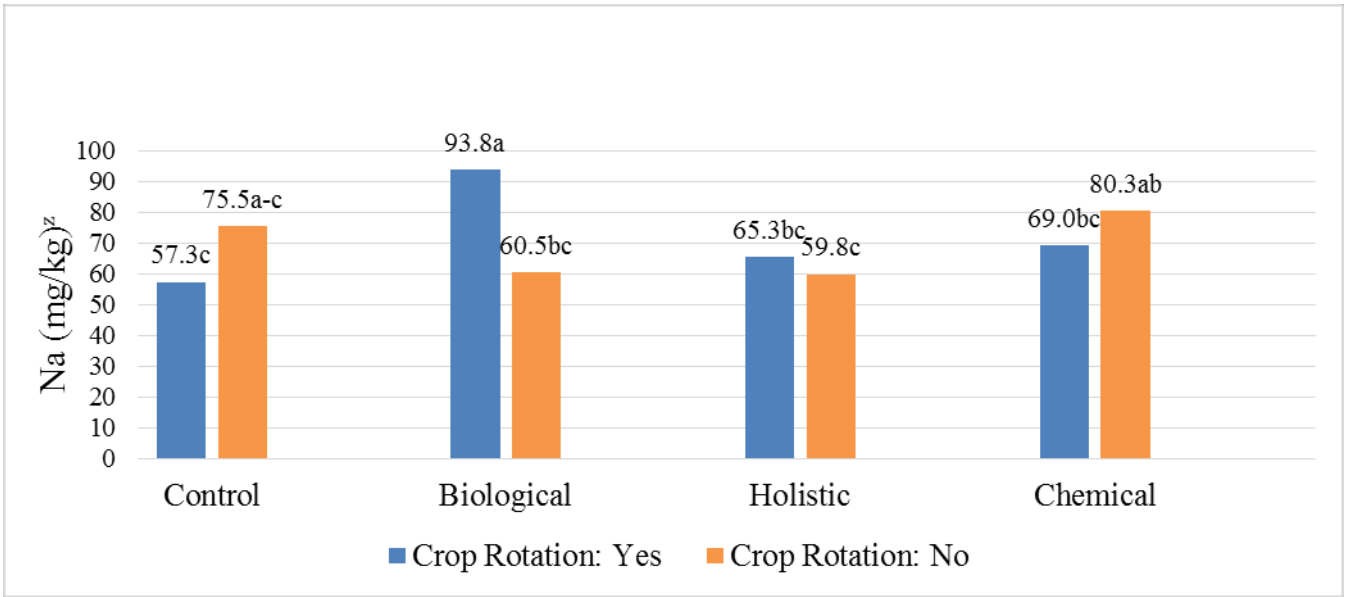


Figure 1: Effect of the management programme and crop rotation on the concentrations of Na in the soil.

There is some interesting significant statistical data in Table 12 that was generated from the trial, regarding crop rotation, no rotation, and the different foliar programmes followed. Long term trials are needed to determine if these trials will continue to produce the significant results obtained in this trial.

4.3 The effects of different methods of fumigation and cultivation practices on nematode population diversity

As the trial was conducted on a commercial vegetable farm in Philippi in the Western Cape, the previous crop on the trial site was spinach (*Spinacia oleracea*). Spinach is extremely susceptible to *Heterodera* (Daiber, 1991; S. Storey, 2018, Personal communication, 19 November) and this can explain why the baseline had a moderate infestation of *Heterodera,* as shown in Table 13 (Nemlab report, 23 September 2019).

The PPN that were found in the trial was *Heterodera* spp. in 9 of the 16 samples, *Paratrichodorus* spp. in 3 of the 16 samples, and *Pratylenchus* spp., and *Tylenchorhynchus* spp., from 1 of the 16 samples each (Table 14). The data in Table 13 refer to the pre-trial PPN counts, and serves as a baseline. The data in Table 14 refers to the post-trial PPN counts. Unfortunately there were not enough data to conduct statistical analyses on PPN, and the focus was shifted to the effect of fumigation and crop rotation on nematode population diversity.

Table 13: Baseline sample plant parasitic nematode (PPN) counts of the trial site

Block No	***Pratylenchus* (Root lesion)**	***Heterodera* (Cyst)**	***Paratrichodorus* (Stubby root)**	***Tylencho-rhynchus***	***Meloidogyne* (Root-knot)**	***Criconematinae* (Ring)**	***Helicotylenchus* (Spiral)**
Pre-Trial	0	270	0	0	0	0	0

Table 14: Post-trial plant parasitic nematode (PPN) counts per strip plot

Trial No.	*Pratylenchus* (Root lesion)	*Heterodera* (Cyst)	*Paratrichodorus* (Stubby root)	*Tylenchorhynchus* (Stunt)
FR 1-4	0	10	0	10
FR 2-4	0	0	10	0
FR 3-4	0	0	20	0
FR 4-4	0	40	0	0
FNR 1-4	0	80	10	0
FNR 2-4	0	0	0	0
FNR 3-4	0	0	0	20
FNR 4-4	0	60	0	0
NR 1-4	0	0	0	0
NR 2-4	0	0	0	0
NR 3-4	20	0	0	0
NR 4-4	0	10	0	0
NN 1-4	0	60	0	0
NN 2-4	0	20	0	0
NN 3-4	0	30	0	0
NN 4-4	0	80	0	0

All the programmes: Fumigation, Crop Rotation (FR), Fumigation, No Rotation (FNR), Bio-Fumigation, Rotation (NR) showed a decline in the PPN, whereas Bio-Fumigation, No Rotation (NN) had the highest PPN counts (Table 14). SBCN is always found were *Brassica* spp. are cultivated (Daiber, 1991). Unfortunately the *Heterodera* spp. analysed in this study were not identified to species level and it is therefore not possible to know whether the species identified, included SBCN.

There was a small random increase in *Paratrichodorus* spp., *Pratylenchus* spp., and *Tylenchorhynchus* spp. Unfortunately the time between samplings, from the first sample before trial was conducted and the last sample when the trial was harvested, was too short to see a drastic change. Long term trials are needed for significant differences to become apparent.

Table 15: Baseline nematode diversity analysis

Diversity	Count	Percentage
Plant Parasitic Nematodes (PPN)	732	24.00
Bacterivores (BAC)	2318	76.00
Fungivores (FUN)	0	0.00
Omnivores (OMNI)	0	0.00
Predators (PRED)	0	0.00
Root Exudate Feeders (RE)	0	0.00
TOTAL	**3050**	**100.00**

Table 15 shows baseline nematode diversity, while Figure 2 illustrates this graphically, showing that bacterivores made up 76% of the baseline, while PPN made up the balance (24%).

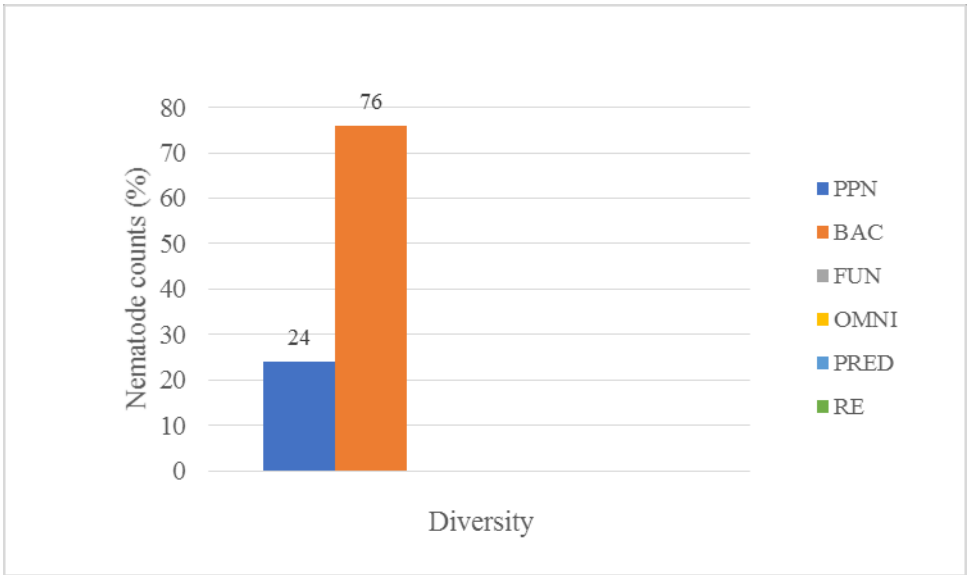


Figure 2: Baseline nematode diversity analysis

As the trial progressed, the soil remained dominated by PPN and bacterivores, but some diversity has developed, with omnivores, fungivores and root exudate feeders now apparent in most replications (Table 16).

The results in Table 16 are expressed in total percentage per strip plot and mean percentage per treatment of nematodes present.

The nematode trophic groups that were analysed in the trial are as follows:

Plant Parasitic Nematodes (PPN)

Bacterivores (BAC)

Fungivores (FUN)

Omnivores (OMNI)

Predators (PRED)

Root exudate feeders (RE)

Graphs are presented to illustrate:

- Baseline nematode diversity percentage (Figure 2)
- Effect of different soil programmes (FR, FNR, NR and NN) on mean nematode population diversity, combined for all four replications (Figure 3)

Table 16: Effect of different soil treatments (FR, FNR, NR and NN) on mean nematode population diversity

Diversity	Fumigation	Rotation	Percentage (%)				
			Rep 1	Rep 2	Rep 3	Rep 4	Mean
Plant parasites (PPN)	Chemical	Yes	5.02	13.95	12.47	10	10.36
Bacterivores (BAC)			87.92	76.74	77.53	57.98	75.04
Fungivores (FUN)			1.02	0	0	0	0.26
Omnivores (OMNI)			1.02	0	0	0	0.26
Predators (PRED)			0	0	0	0	0.00
Root exudate feeders (RE)			5.02	9.3	10	32.02	14.09
Plant parasites (PPN)	Chemical	No	9.00	4.00	1.00	10.00	6.00
Bacterivores (BAC)			90.00	85.01	94.99	76.00	86.50
Fungivores (FUN)			0.00	0.99	0.00	4.00	1.25
Omnivores (OMNI)			1.00	4.00	0.00	1.00	1.50
Predators (PRED)			0.00	0.00	0.00	0.00	0.00
Root exudate feeders (RE)			0.00	6.01	4.01	9.00	4.76
Plant parasites (PPN)	Biological	Yes	4.99	2.00	4.00	3.01	3.50
Bacterivores (BAC)			82.01	85.01	91.00	73.97	83.00
Fungivores (FUN)			0.00	1.00	0.00	1.98	0.75
Omnivores (OMNI)			4.00	1.00	0.00	3.02	2.01
Predators (PRED)			0.00	0.00	0.00	0.00	0.00
Root exudate feeders (RE)			9.00	11.00	5.00	18.01	10.75
Plant parasites (PPN)	Biological	No	5.00	14.33	8.56	5.03	8.23
Bacterivores (BAC)			92.02	79.59	73.56	83.98	82.29
Fungivores (FUN)			0.00	0.00	0.00	0.00	0.00
Omnivores (OMNI)			1.99	0.00	1.23	2.98	1.55
Predators (PRED)			0.00	0.00	0.00	0.00	0.00
Root exudate feeders (RE)			0.99	6.08	14.65	8.01	7.43

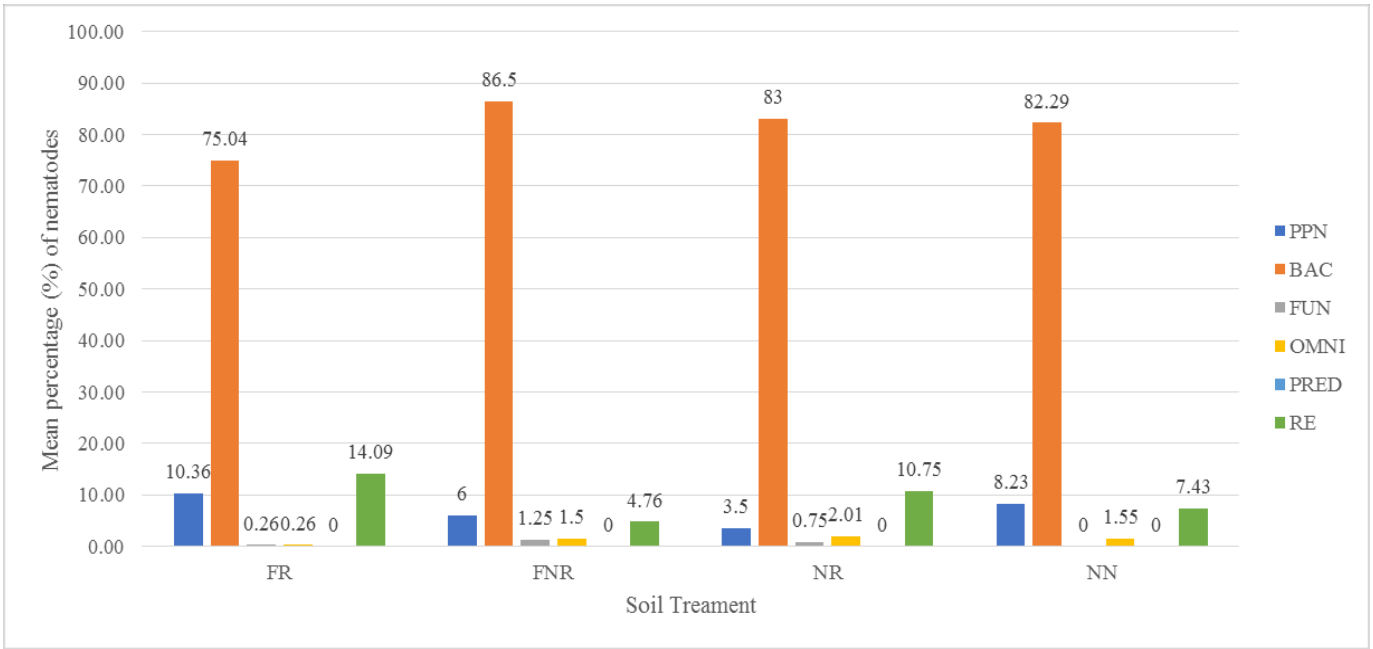


Figure 3: Effect of different soil treatments (FR, FNR, NR and NN) on mean nematode population diversity

Although it seems that there was a decline in the PPN present, there is too little data to produce a meaningful result. The baseline had a moderate infestation of PPN, where the post-trial results had a low to moderate infestation of PPN.

The results show very high numbers of bacterial feeders in all the samples, thus indicating high enrichment in the soil. The overall nematode diversity was lacking and showed very few fungal feeders, omnivores and predators. In some samples the root exudate feeder numbers were high, which could give an indication that the system is under stress and not well balanced in bio-diversity. The results in Table 16 showed high levels of variation between replications of the same treatment. This could relate to the statistical layout of the trial and the randomness of how nematodes are found in soil.

There were some results, unfortunately the time between samplings, from the first sample before the trial was conducted and the last sample when the trial was harvested, was too short to see a drastic change. Long term trials over a few seasons are needed for significant differences, in the decline of PPN and the increase of free-living nematodes, to be apparent.

4.4 The effects of different methods of fumigation and cultivation practices on nematode diversity in the soil with regards to the structure and enrichment indices (SI and EI)

One representative soil sample of the trial site was sampled as baseline sample before the trial was conducted to establish the nematode population diversity (Table 13).

After the trial was conducted soil samples were analysed, this time soil from the sixteen semi-permanent beds in which the vegetables were cultivated were analysed separately to establish the effects of different fumigation, rotation (x four reps) on the nematode diversity in the soil.

The structure index (SI), is represented on the X-axis. It reflects the composition of the soil food web structure. The abundance of a larger quantity of nematodes, higher up in the trophic levels, indicates the trophic connection to the system. The enrichment index (EI) on the Y-axis indicates when the soil food web becomes enriched by the adding of resources (compost, mulches, etc.). The EI measures the presence of opportunistic bacterivorous and fungivorous nematodes. Functional guild indicators are weighted according to growth and metabolic rates or resource consumption on the EI. Whereas the SI shows the sensitivity to soil disturbances. Vegetables would reflect in sector A and D on the graph, where soil disturbances take place (ploughing, rotavating, disking, etc.). These mechanical disturbances would prevent the development of a complex soil food web structure which would lead to a lower SI.

The enrichment index refers to the composting, mulching or living mulches in the soil (Ferris *et al*., 2001; Neher, 2001; Neher *et al*., 2004; Storey, 2015).

The nematodes as bio-indicators for structure and enrichment index can be categorized as:

A graphic representation of the structural and enrichment condition of the soil food web, the so-called Nematode Faunalyzer (Fig. 4) is based on the relative weighted abundance of the nematode feeding guilds (Storey, Nemlab., 2015). The results in Table 17 express the pre-trial, baseline nematode as bio-indicator for structure and enrichment index of the soil. Fig. 5 presents the pre-trial results.

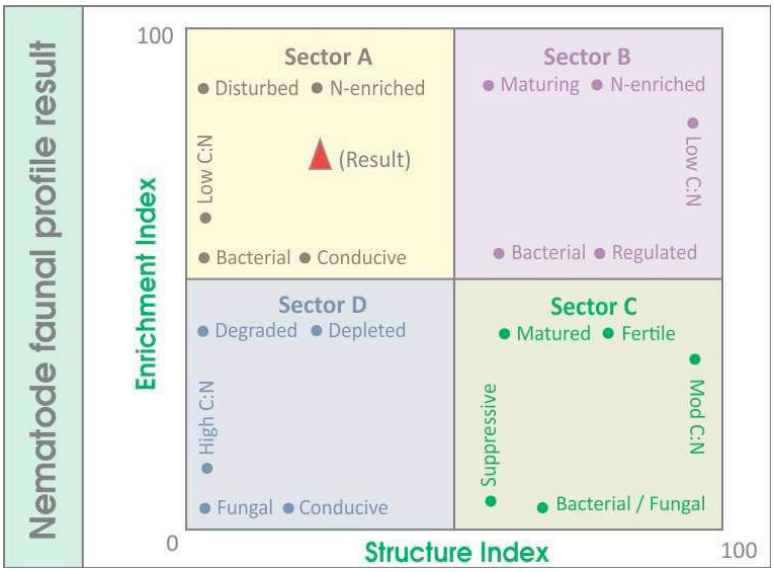


Figure 4: Nematode bio-indicator index (Storey, Nemlab., 2015)

The baseline results presented in Table 17 and Fig. 5 showed that the enrichment is sufficient as shown by the enrichment index.

Table 17: Baseline soil nematode bio-indicators

Pre-Trial Soil Nematode Bio-Indicator	
Nematode Bio-Indicator	Percentage
Structure Index (SI)	0.00
Enrichment Index (EI)	70.00

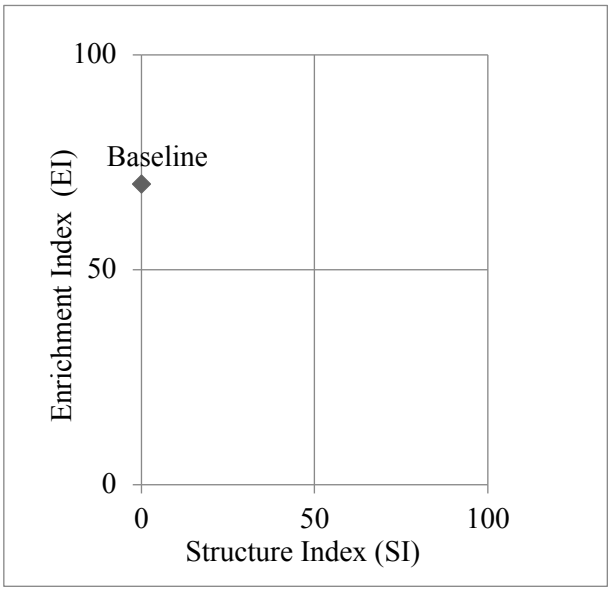


Figure 5: Baseline structure index (SI) and enrichment index (EI)

This indicates a presence of bacteriovorous and fungivorous nematodes. There are very few connections between the feeding groups and thus a soil food web structure is not present. There is a high number of bacterial feeders that are responsible for fast nutrient turnover. The nematode faunal profile lies in Sector A, which indicates a low C:N ratio, the soil is disturbed and nitrogen-enriched. A low SI and high EI is favourable for the development of soilborne diseases. This is typical of a vegetable producing soil. As mechanical disturbances would prevent the development of a complex soil food web structure.

The results in Table 18 express post-trial nematodes as bio-indicators for soil health and the structure and enrichment index of the soil. There are four replicates of the different soil treatments plotted on each graph.

Table 18: Effect of different soil treatments (FR, FNR, NR and NN), on mean structure index (SI) and enrichment index (EI)

Diversity	Fumigation	Rotation	Percentage (%)				
			Rep 1	Rep 2	Rep 3	Rep 4	Mean
Structure Index (SI)	Chemical	Yes	11.93	0	0	0	2.98
Enrichment Index (EI)			88.76	91.43	74.24	89.9	86.08
Structure Index (SI)	Chemical	No	19.00	41.02	0.00	28.57	22.15
Enrichment Index (EI)			94.50	91.67	91.80	96.60	93.64
Structure Index (SI)	Biological	Yes	37.23	21.03	0.00	34.48	23.19
Enrichment Index (EI)			89.07	95.00	71.43	90.31	86.45
Structure Index (SI)	Biological	No	21.52	0.00	31.03	15.93	17.12
Enrichment Index (EI)			89.68	93.03	95.93	57.14	83.95

Fig. 6 presents the effect of the following treatments on mean soil structure and enrichment index:

- Fumigation, Rotation (FR)
- Fumigation, No Rotation (FNR)
- Bio-Fumigation, Rotation (NR)
- Bio-Fumigation, No Rotation (NN)

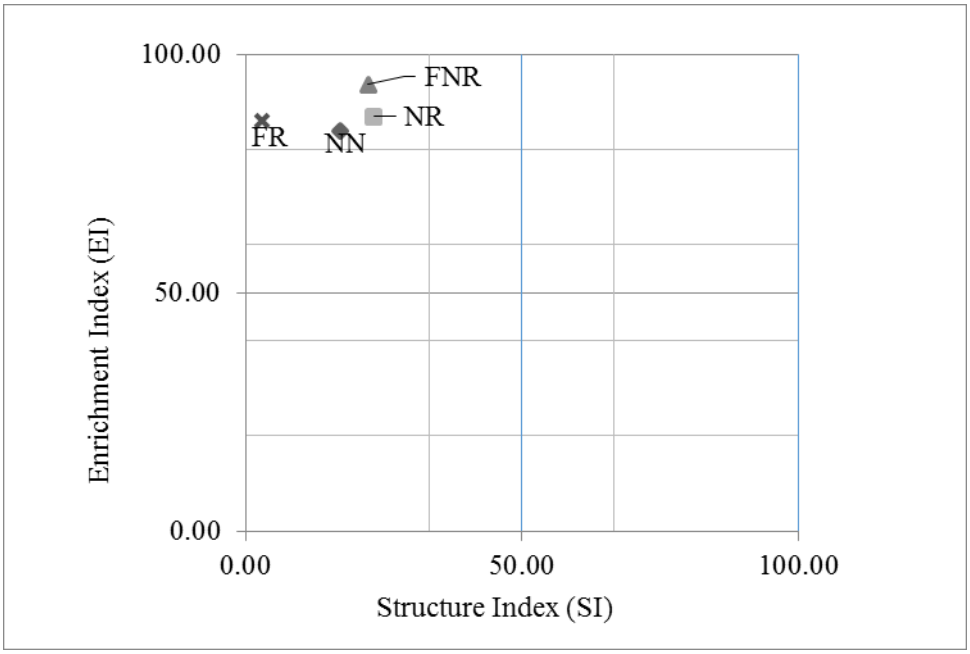


Figure 6: Effect of different soil treatments (FR, FNR, NR and NN), on mean structure index (SI) and enrichment index (EI)

Results presented in Table 18 and Fig. 6 show that the enrichment is sufficient as shown by the enrichment index. This indicates a presence of bacterivorous and fungivorous nematodes. There are very few connections between the feeding groups and thus a soil food web structure is not present. There is a high number of bacterial feeders that are responsible for fast nutrient turnover. The nematode faunal profile lies in Sector A, which indicates a low C:N ratio, the soil is disturbed and nitrogen-enriched. A low SI and high EI is favourable for the development of soilborne diseases. This is typical of a vegetable producing soil. As mechanical disturbances would prevent the development of a complex soil food web structure.

From the results the following can be concluded:

The nematode indices for all of the samples showed that it was highly enriched and unstructured. The structure index increased in some cases, but the time between samplings was too short to see a drastic change. Long term trials are needed to detect significant differences. Because of the soil disturbance the structure index would also not increase significantly.

4.5 The effects of different crop protection strategies on diamondback moth (DBM) *Plutella xylostella* in *Brassica oleracea* production

Results of the mean number of plants infested by DBM larvae are given in Table 19 (40 plants per treatment x four replications). There was a significant ($P < 0.0001$) days after planting x programme interaction for the diamondback moth larvae counts on plants.

Rotation system ($P = 0.5991$) and fumigation ($P = 0.2513$) did not significantly affect DBM incidence.

Table 19: Effect of different management programmes and days after planting on the mean number of diamondback moth larvae infested *Brassica oleracea* plants

Days after planting	Mean number of diamond back moth larvae infested plants[z]			
	Control	Biological	Holistic	Chemical
35	0.00c	0.00c	0.00c	0.00c
49	2.18b	2.50b	0.31c	0.00c
63	4.37a	2.93b	3.37b	0.00c
74	4.06a	3.00b	2.31b	0.18c
78	4.06a	3.00b	2.31b	0.18c
[z]Means followed by the same letter do not differ significantly at P = 0.05 (LSD = 0.8367)				

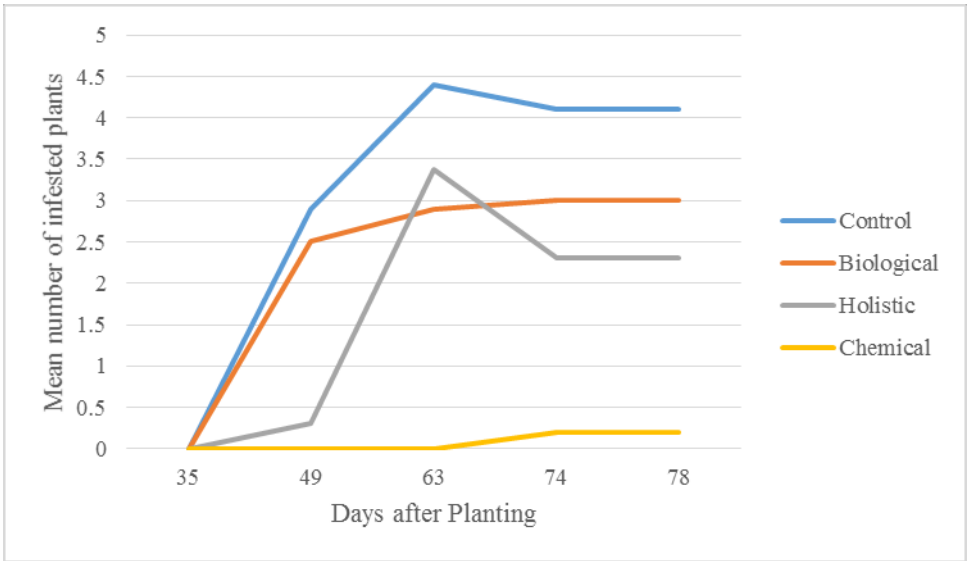


Figure 7: Effect of different management programmes and days after planting on the mean number of diamondback moth larvae infested *Brassica oleracea* plants.

4.6 White blister infection on the leaves of *Brassica oleracea*

White blister was noticed and evaluated weekly on the leaves of the *Brassica oleracea* plants. As mentioned in the Methods and Materials section, white blister severity was evaluated by assessing the whole plot. A disease rating scale of 0-6 was used. The four plots and four replications were scored according to the infection rate. This rating was then converted to a percentage of infection where:

0 = 0%
1 = 1 - 10%
2 = 11 - 25%
3 = 26 - 50%
4 = 51 – 75%
5 = 76 – 99%
6 = 100%

There was also a significant ($P < 0.0001$) days after planting x programme interaction for the severity of white blister.

Rotation system ($P = 0.8262$) and fumigation ($P = 0.0946$) did not significantly affect white blister severity.

Table 20: Effect of different management programmes and days after planting on severity of white blister on *Brassica oleracea*

Severity of whiteblister (% infection)[z]				
Days after planting	Control	Biological	Holistic	Chemical
35	12.75hi	7.84ij	1.71j	4.65j
49	36.43bc	29.56b-d	20.18f-h	13.46g-i
63	37.46ab	27.06d-f	28.90c-e	9.40ij
74	32.376b-d	21.43e-g	27.53d-f	8.62ij
78	44.84a	14.78g-i	7.90ij	9.75ij
[z]Means followed by the same letter do not differ significantly at P = 0.05 (LSD = 8.0651)				

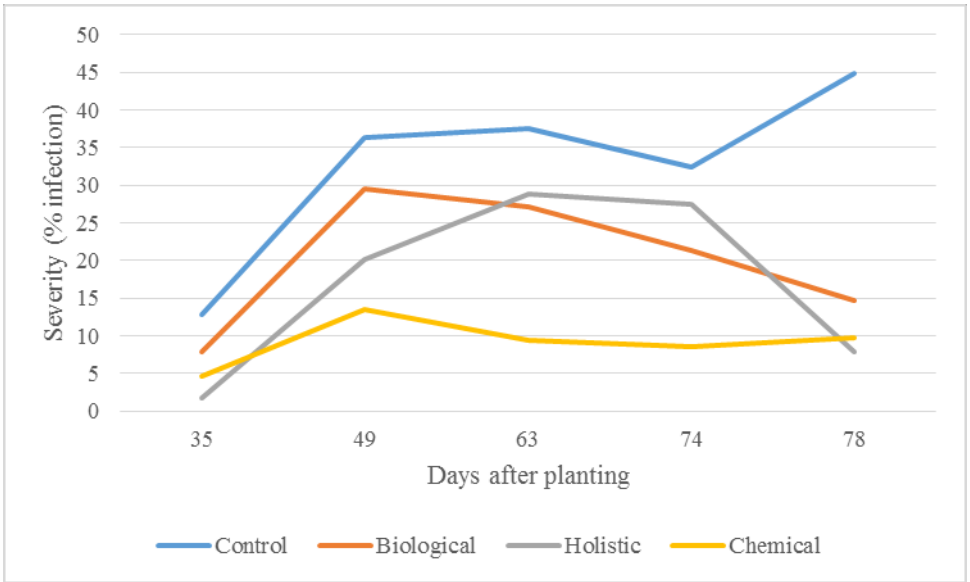


Figure 8: Effect of different management programmes and days after planting on the severity of white blister on *Brassica oleracea.*

4.7 White blister infection on the heads of *Brassica oleracea* at harvest

At harvest the infection of white blister on the heads of *Brassica oleracea* was evaluated.

If the head is infected the market value goes down, so much so that the crop may not be marketable, because of the deformation of the heads or stagheads discussed in the introduction.

If a head had a blister it was counted as infected, the results were converted to a percentage of total infected *Brassica oleracea* heads at harvest.

There were statistically significant differences between all the treatments, where the holistic approach gave significantly better control of white blister compared to the chemical control programme. The biological programme also produced significant control.

Management programmes significantly (*P = 0.0002*) affected the incidence of plants with white blister. Rotation system (*P = 0.6704*) and fumigation (*P = 0.4018*) did not significantly affect the incidence of white blister on heads.

Table 21: Effect of different management programmes on the incidence of white blister on *Brassica oleracea* heads

Programme	White blister incidence (Mean number of plants/10 plants)[z]
Control	4.18a
Biological	1.56b
Holistic	0.31c
Chemical	1.18bc
[z]Means followed by the same letter do not differ significantly at P = 0.05 (LSD = 1.1659)	

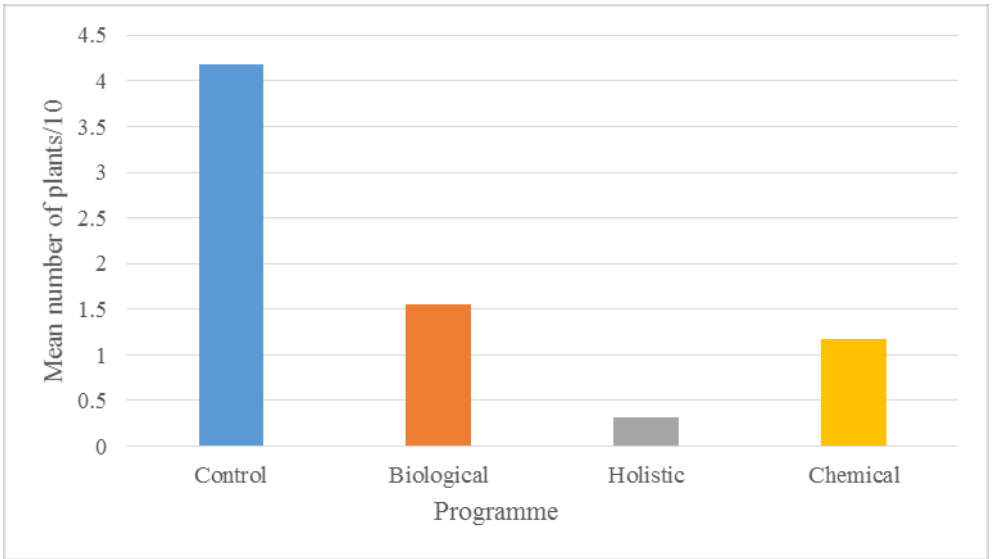


Figure 9: Effect of different management programmes on the incidence of white blister on *Brassica oleracea* heads.

Research on biological control has increased in the last few years, and with good reason. There are some biological products that can give significant control against pests and diseases.

As mentioned in the introduction, farmers still rely largely on chemical methods to control pests and diseases, but with increasing pressure from supermarkets and exporters, and pest and disease resistance to chemicals, different methods to control pests and diseases are now being explored.

In this study the results showed that chemical control was most effective against diamondback moth with 0.18 plants per block infested at harvest. In the introduction it was mentioned that diamondback moth has the ability to develop resistance against chemical products in a matter of a few seasons, if those chemical products are not used in a rotation and used with care and intelligence. The untreated control had 4.1 plants infested per block. There were no statistically significant differences on diamondback moth control between the biological and holistic approach programmes at harvest, although the infestation was lower for the holistic approach with an average of 2.3 infested plants, compared to the biological control programme for which an average of 3 infested plants were recorded. This shows that chemicals and biological products can be used in the same programme and against pests that are difficult to control; this is a good agricultural practice to use in controlling DBM.

White blister disease on the foliage was controlled effectively with the holistic approach and chemical programme, which had no significant difference and resulted in just 7.9% infection for the holistic approach and 9.8% for the chemical programme. The control plot had a 44% infection. The biological programme had 14.8% infection.

White blister infection at harvest gave interesting results. There were significant differences in all the treatments. In the control 4.2 plants were infected per 10 plants evaluated. In the biological programme 1.6 plants and in the chemical programme 1.2 plants were infected. The holistic approach programme resulted in significantly better control of white blister than the

chemical control programme, with only 0.3 plants infected per 10 plants assessed. One explanation for this is that because of the residues that chemical control products leave, there is a withholding period on all chemical products regarding how close to harvest you can apply a product. Biological products do not have an application withholding period and can be applied up until harvest.

There was no evidence of clubroot of *Brassica* spp., in any of the treatments.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

The data for this study were collected after only one rotation cycle. However, the results that were obtained showed some interesting trends that need to be further investigated in trials replicated at different locations and over different production seasons.

In the post-trial soil chemical analysis, the concentration of K was significantly lower in the rotation compared to the no rotation treatment. This could be due to the fact that plants uses more K to produce a good yield than nitrogen. Potassium plays an important role in plant health and nutrition; photosynthesis, regulating the stomata opening and closing, regulates CO_2 uptake. The results could therefore point out that more K was absorbed by the different crops in the rotation programmes than in the monoculture (FERTASA, 2016). A significantly higher concentration of Na was recorded for the biological programme when crop rotation was included compared to the no rotation treatment. Salinity in soil can be a result of many factors, the most common contributor of high salinity in soil is irrigation water (Shannon & Grieve, 1999). Unfortunately the irrigation water was not analysed. Long term trials would give more significant differences between the crop rotation and no crop rotation programmes on soil chemical properties.

There were some PPN nematodes extracted in the trial but unfortunately the numbers were too low to result in significant differences. There was, however, a decline in numbers of *Heterodera* spp., in all the post-trial results with the highest numbers of *Heterodera* found in the Bio-Fumigation, No Rotation (NN) programme. The time between samplings was too short to see a drastic change. Long term trials are needed to see significant differences. The nematode results showed very high numbers of bacterial feeders in all the samples, and indicated high enrichment. The overall nematode diversity was lacking and showed very few fungal feeders, omnivores and predators. In some samples the root exudate feeder numbers were high, which could give an indication that the system was under stress (Storey, 2015). The nematode indices

for all of the samples showed that it was highly enriched and unstructured. The structure index increased in some cases, but the time between samplings was too short to see a drastic change. Because of the soil disturbance the structure index would also not increase significantly. More research needs to be conducted on the effect of fumigation and crop rotation on nematodes in broccoli production.

Unfortunately there was no clubroot infection in the trial for any of the treatments. It was therefore not possible to evaluate the effect of the different treatments on clubroot of broccoli. The data collected relating to diamondback moth and white blister produced significant data; it is possible to produce a healthy crop with not only a chemical programme, but also with a more holistic approach. Unfortunately in South Africa there is limited data and few published peer reviewed articles on the matter of crop protection of pests and diseases of *Brassica* spp., available. This trial will need to be repeated for a longer period and under a range of conditions to determine the effects of various control methods on PPN, clubroot of *Brassica* spp., and to confirm the results obtained for white blister and diamondback moth.

From this study it can be concluded that further research on different crop protection strategies is needed to understand the control of pests and diseases of *Brassica* spp. The lack of registered biological products is a major problem in building a biological or holistic approach in crop protection programmes. A holistic approach would seem to be the best, for the environment and the farm labourers, as it minimizes the amount of chemical residues on edible crops.

The type of registered biological and chemical products that are compatible in a spray tank needs to be researched. As many of the biological products consist of fungi and bacteria, there are few chemical products that can be applied with these biological products, as the chemical products can destroy some of the biological products when they are mixed or applied at different intervals. Research is needed on compatibility of chemical and biological products.

According to Agri-Intel, South Africa's database of registered agricultural remedies, there are 81 registered chemical fumigants and nematicides for the control of PPN, and only 2 registered biological nematicides. There are over 142 chemical pesticides registered for DBM on *Brassica* spp. and only two registered biological products. For white blister there are 12 registered chemical pesticides available and no biological registered products. For clubroot there are no biological registered products available (Agri-Intel, 2018a, b, c, d).

The prices of the biological products exceed the prices of chemical products, and this is also a major problem in the industry. It is already expensive to farm, with high cost of diesel and implements, labour costs, water costs, fertilizer costs and the cost of crop protection products, and with the relatively low prices that farmers get for their crops at the market. The more biological products are researched and registered, the lower the prices of these products would become, assisting farmers to farm with safer, more environmentally friendly products, but still produce an economical yield and protecting their precious crops.

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APPENDICES

Appendix 1: Baseline soil chemical properties analysis

Soil Property															
Sample No	Trial ID	pH (KCl)	Resistance (ohm)	Ca cmol/kg)	Mg (cmol/kg)	Na (mg/kg)	K (mg/kg)	T-value (cmol/kg)	P (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	B (mg/kg)	S (mg/kg)	C (%)
1	Baseline	5.8	2890	6.57	0.53	15	15	7.21	800	4.81	47.79	30.08	0.14	3.8	0.72

Appendix 2: Post-trial soil chemical properties analysis

Soil Property																
Soil Sample	Sample No	Trial ID	pH (KCl)	Resistance (ohm)	Ca cmol/kg)	Mg (cmol/kg)	Na (mg/kg)	K (mg/kg)	T-value (cmol/kg)	P (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	B (mg/kg)	S (mg/kg)	C (%)
1	2079	1 A	6.7	320	11.93	1.45	130	46	14.07	902	6.75	79.56	44.5	0.54	51	0.92
2	2080	1 B	6.5	490	11	1.27	118	72	12.98	974	6.55	78.58	43.11	0.48	24	0.94
3	2081	1 C	6.2	450	9.32	1.27	117	47	11.23	928	6.39	72.15	43.18	0.59	25	0.96
4	2082	1 D	6.2	430	9.79	1.14	102	51	11.51	970	5.32	60.96	38.26	0.47	32	1.01
5	2083	2 A	6.4	600	11.33	1.12	72	37	12.87	910	6.29	81.14	42.75	0.38	20	0.96
6	2084	2 B	5.9	460	9.52	0.98	118	20	11.07	956	5.97	74.25	39.45	0.37	25	0.84
7	2085	2 C	6.1	1680	7.78	0.7	48	22	8.75	827	5.12	60.99	33.77	0.24	3.6	0.82
8	2086	2 D	5.7	780	6.66	1.03	48	44	8.02	817	5.69	59.1	40.56	0.34	6.3	0.84
9	2087	3 A	6.5	1380	11.26	1.22	61	20	12.81	907	6.59	85.82	41.62	0.28	5.2	0.94
10	2088	3 B	5.8	890	8.31	0.82	42	74	9.51	852	6.13	77.7	40.83	0.26	7.2	0.86
11	2089	3 C	5.6	570	7.06	0.65	62	31	8.07	795	5.39	63.39	36.27	0.27	11	0.85
12	2090	3 D	5.5	1300	6.95	0.6	45	127	8.08	804	5.08	57.84	32.22	0.22	5.8	0.8
13	2091	4 A	6.5	1470	10.91	1.29	65	25	12.56	975	6.32	74.66	39.15	0.27	5.1	0.98
14	2092	4 B	5.9	480	8.36	0.91	106	44	9.85	892	5.88	66.95	36.66	0.33	30	0.94
15	2093	4 C	5.7	420	7.81	0.87	77	40	9.13	866	5.77	67.52	38.88	0.38	19	0.84
16	2094	4 D	6.1	1490	8.22	0.79	56	34	9.35	853	5.13	58.17	36.24	0.31	5.9	0.9
17	2095	5 A	5.6	1330	7.78	0.74	44	40	8.82	845	5.36	63.68	36.46	0.25	5	1.27
18	2096	5 B	6.4	1020	9.94	1.21	66	21	11.5	930	7.37	79.08	41.27	0.46	6.9	0.84
19	2097	5 C	6.2	1630	8.41	0.72	39	47	9.43	835	5.79	69.1	37.56	0.16	3	0.82
20	2098	5 D	5.3	360	6.96	0.66	68	46	8.58	790	5.66	64.1	34.8	0.3	16	0.88
21	2099	6 A	6.2	1720	7.51	0.71	55	23	8.53	790	5.68	59.64	37.15	0.22	3.8	1.37
22	2100	6 B	6.5	1560	10.81	1.43	41	68	12.6	926	6.75	76.87	41.48	0.21	3.6	0.92
23	2101	6 C	5.7	620	8.36	0.76	43	233	9.91	903	5.89	70.68	39.16	0.23	6.9	1.31
24	2102	6 D	5.9	1860	7.81	0.72	40	52	8.85	857	5.65	62.98	38.69	0.21	3.3	0.86
25	2103	7 A	5.2	380	7.02	0.84	95	38	8.99	847	5.74	62.65	35.73	0.32	22	0.86
26	2104	7 B	5.9	700	9.4	0.88	85	50	10.79	954	6.62	74.77	40.09	0.28	14	0.92
27	2105	7 C	5.7	1150	7.86	0.66	65	37	8.91	904	6.6	71.85	41.98	0.27	5.8	0.82
28	2106	7 D	5.8	740	7.98	0.86	89	34	9.32	909	7.5	80.56	54.43	0.34	13	0.82
29	2107	8 A	5.8	830	7.44	0.84	75	78	8.82	819	6.06	62	37.95	0.41	14	0.82
30	2108	8 B	6.4	1310	10.43	1.01	41	58	11.78	923	7.17	77.16	40.73	0.18	4.1	1.03
31	2109	8 C	6.4	870	8.87	0.92	80	26	10.21	880	6.51	72.72	38.16	0.24	14	0.99
32	2110	8 D	6.5	1620	8.43	0.86	52	20	9.58	823	5.92	66.2	37.32	0.17	3.8	0.9

Appendix 3: Baseline plant parasitic nematode (PPN) diversity analysis of the trial site

Lab Nr	Blok Nr	*Pratylenchus* (Root lesion)	*Meloidogyne* (Root-knot)	*Criconematinae* (Ring)	*Helicotylenchus* (Spiral)	*Paratrichodorus* (Stubby root)	*Heterodera* (Cyst)	*Tylenchorhynchus* (Stunt)
H022-2445-14	Trial	0	0	0	0	0	270	0

Appendix 4: Post-trial plant parasitic nematode (PPN) diversity analysis per strip plot

Trial No.	Sample No.	Analysis	Saprophytes	*Pratylenchus* (Root lesion)	*Meloidogyne* (Root-knot)	*Criconematinae (Ring)*	*Helicotylenchus* (Spiral)	*Heterodera* (Cyst)	*Paratrichodorus* (Stubby root)	*Tylenchorhynchus* (Stunt)
FR 1-4	3134	300cc soil	710	0	0	0	0	10	0	10
FR 2-4	3135	300cc soil	290	0	0	0	0	0	10	0
FR 3-4	3136	300cc soil	70	0	0	0	0	0	20	0
FR 4-4	3137	300cc soil	450	0	0	0	0	40	0	0
FNR 1-4	3138	300cc soil	1020	0	0	0	0	80	10	0
FNR 2-4	3139	300cc soil	640	0	0	0	0	0	0	0
FNR 3-4	3140	300cc soil	570	0	0	0	0	0	0	20
FNR 4-4	3141	300cc soil	360	0	0	0	0	60	0	0
NR 1-4	3142	300cc soil	720	0	0	0	0	0	0	0
NR 2-4	3143	300cc soil	590	0	0	0	0	0	0	0
NR 3-4	3144	300cc soil	330	20	0	0	0	0	0	0
NR 4-4	3145	300cc soil	320	0	0	0	0	10	0	0
NN 1-4	3146	300cc soil	970	0	0	0	0	60	0	0
NN 2-4	3147	300cc soil	410	0	0	0	0	20	0	0
NN 3-4	3148	300cc soil	660	0	0	0	0	30	0	0
NN 4-4	3149	300cc soil	360	0	0	0	0	80	0	0

Appendix 5: Post-trial, free-living nematode diversity of different trophic groups

(Lab# 3134) Fumigation + Rotation 1			(Lab# 3135) Fumigation + Rotation 2			(Lab# 3136) Fumigation + Rotation 3			(Lab# 3137) Fumigation + Rotation 4		
Plant Parasitic Nematodes (PPN)	94	5.02	Plant Parasitic Nematodes (PPN)	120	13.95	Plant Parasitic Nematodes (PPN)	96	12.47	Plant Parasitic Nematodes (PPN)	99	10.00
Bactivores (BAC)	1645	87.92	Bactivores (BAC)	660	76.74	Bactivores (BAC)	597	77.53	Bactivores (BAC)	574	57.98
Fungivores (FUN)	19	1.02	Fungivores (FUN)	0	0.00	Fungivores (FUN)	0	0.00	Fungivores (FUN)	0	0.00
Omnivores (OMNI)	19	1.02	Omnivores (OMNI)	0	0.00	Omnivores (OMNI)	0	0.00	Omnivores (OMNI)	0	0.00
Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00
Root Exudate Feeders (RE)	94	5.02	Root Exudate Feeders (RE)	80	9.30	Root Exudate Feeders (RE)	77	10.00	Root Exudate Feeders (RE)	317	32.02
Total	**1871**	**100.00**	**Total**	**860**	**100.00**	**Total**	**770**	**100.00**	**Total**	**990**	**100.00**
(Lab# 3138) Fumigation + No Rotation 1			(Lab# 3139) Fumigation + No Rotation 2			(Lab# 3140) Fumigation + No Rotation 3			(Lab# 3141) Fumigation + No Rotation 4		
Plant Parasitic Nematodes (PPN)	524	9.00	Plant Parasitic Nematodes (PPN)	149	4.00	Plant Parasitic Nematodes (PPN)	34	1.00	Plant Parasitic Nematodes (PPN)	310	10.00
Bactivores (BAC)	5237	90.00	Bactivores (BAC)	3170	85.01	Bactivores (BAC)	3222	94.99	Bactivores (BAC)	2356	76.00
Fungivores (FUN)	0	0.00	Fungivores (FUN)	37	0.99	Fungivores (FUN)	0	0.00	Fungivores (FUN)	124	4.00
Omnivores (OMNI)	58	1.00	Omnivores (OMNI)	149	4.00	Omnivores (OMNI)	0	0.00	Omnivores (OMNI)	31	1.00
Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00
Root Exudate Feeders (RE)	0	0.00	Root Exudate Feeders (RE)	224	6.01	Root Exudate Feeders (RE)	136	4.01	Root Exudate Feeders (RE)	279	9.00
Total	**5819**	**100.00**	**Total**	**3729**	**100.00**	**Total**	**3392**	**100.00**	**Total**	**3100**	**100.00**
(Lab# 3142) Bio Fumigation + Rotation 1			(Lab# 3143) Bio Fumigation + Rotation 2			(Lab# 3144) Bio Fumigation + Rotation 3			(Lab# 3145) Bio Fumigation + Rotation 4		
Plant Parasitic Nematodes (PPN)	288	4.99	Plant Parasitic Nematodes (PPN)	104	2.00	Plant Parasitic Nematodes (PPN)	118	4.00	Plant Parasitic Nematodes (PPN)	55	3.02
Bactivores (BAC)	4731	82.01	Bactivores (BAC)	4428	85.01	Bactivores (BAC)	2694	91.00	Bactivores (BAC)	1347	73.97
Fungivores (FUN)	0	0.00	Fungivores (FUN)	52	1.00	Fungivores (FUN)	0	0.00	Fungivores (FUN)	36	1.98
Omnivores (OMNI)	231	4.00	Omnivores (OMNI)	52	1.00	Omnivores (OMNI)	0	0.00	Omnivores (OMNI)	55	3.02
Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00
Root Exudate Feeders (RE)	519	9.00	Root Exudate Feeders (RE)	573	11.00	Root Exudate Feeders (RE)	148	5.00	Root Exudate Feeders (RE)	328	18.01
Total	**5769**	**100.00**	**Total**	**5209**	**100.00**	**Total**	**2960**	**100.00**	**Total**	**1821**	**100.00**
(Lab# 3146) Bio Fumigation + No Rotation 1			(Lab# 3147) Bio Fumigation + No Rotation 2			(Lab# 3148) Bio Fumigation + No Rotation 3			(Lab# 3149) Bio Fumigation + No Rotation 4		
Plant Parasitic Nematodes (PPN)	171	5.00	Plant Parasitic Nematodes (PPN)	139	14.33	Plant Parasitic Nematodes (PPN)	125	8.56	Plant Parasitic Nematodes (PPN)	96	5.03
Bactivores (BAC)	3147	92.02	Bactivores (BAC)	772	79.59	Bactivores (BAC)	1104	75.56	Bactivores (BAC)	1604	83.98
Fungivores (FUN)	0	0.00	Fungivores (FUN)	0	0.00	Fungivores (FUN)	0	0.00	Fungivores (FUN)	0	0.00
Omnivores (OMNI)	68	1.99	Omnivores (OMNI)	0	0.00	Omnivores (OMNI)	18	1.23	Omnivores (OMNI)	57	2.98
Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00	Predators (PRED)	0	0.00
Root Exudate Feeders (RE)	34	0.99	Root Exudate Feeders (RE)	59	6.08	Root Exudate Feeders (RE)	214	14.65	Root Exudate Feeders (RE)	153	8.01
Total	**3420**	**100.00**	**Total**	**970**	**100.00**	**Total**	**1461**	**100.00**	**Total**	**1910**	**100.00**

Appendix 6: Post-trial nematode bio-indicator index

(Lab# 3134) Fumigation + Rotation 1		(Lab# 3135) Fumigation + Rotation 2		(Lab# 3136) Fumigation + Rotation 3		(Lab# 3137) Fumigation + Rotation 4	
Structure Index (SI)	11.93	Structure Index (SI)	0.00	Structure Index (SI)	0.00	Structure Index (SI)	0.00
Enrichment Index (EI)	88.76	Enrichment Index (EI)	91.43	Enrichment Index (EI)	74.24	Enrichment Index (EI)	89.90
(Lab# 3138) Fumigation + No Rotation 1		(Lab# 3139) Fumigation + No Rotation 2		(Lab# 3140) Fumigation + No Rotation 3		(Lab# 3141) Fumigation + No Rotation 4	
Structure Index (SI)	19.00	Structure Index (SI)	41.02	Structure Index (SI)	0.00	Structure Index (SI)	28.57
Enrichment Index (EI)	94.50	Enrichment Index (EI)	91.67	Enrichment Index (EI)	91.80	Enrichment Index (EI)	96.60
(Lab# 3142) Bio Fumigation + Rotation 1		(Lab# 3143) Bio Fumigation + Rotation 2		(Lab# 3144) Bio Fumigation + Rotation 3		(Lab# 3145) Bio Fumigation + Rotation 4	
Structure Index (SI)	37.23	Structure Index (SI)	21.03	Structure Index (SI)	0.00	Structure Index (SI)	34.48
Enrichment Index (EI)	89.07	Enrichment Index (EI)	95.00	Enrichment Index (EI)	71.43	Enrichment Index (EI)	90.31
(Lab# 3146) Bio Fumigation + No Rotation 1		(Lab# 3147) Bio Fumigation + No Rotation 2		(Lab# 3148) Bio Fumigation + No Rotation 3		(Lab# 3149) Bio Fumigation + No Rotation 4	
Structure Index (SI)	21.52	Structure Index (SI)	0.00	Structure Index (SI)	31.03	Structure Index (SI)	15.93
Enrichment Index (EI)	89.68	Enrichment Index (EI)	93.03	Enrichment Index (EI)	95.93	Enrichment Index (EI)	57.14

Appendix 7: Post-trial nematode diversity analysis per strip plot

Lab nr	Blok nr	*Rhabditidae*	*Panagrolaimidae*	*Cephalobidae*	*Diplogasteridae*	*Aphelenchidae*	*Aphelenchoididae*	*Monhysteridae*	*Dorylaimidae*	*Tylenchidae*	*Criconematidae*	*Heteroderidae*	*Heteroderidae (Glob)*	*Trichodoridae*
		Ba	Ba	Ba	Ba	Fu	Fu	Ba	Om	Re	Pl	Pl	Pl	Pl
3134	**FR 1**	879	0	542	224	19	0	0	19	94	0	0	94	0
3135	**FR 2**	340	0	160	140	0	0	20	0	80	20	0	80	20
3136	**FR 3**	250	0	347	0	0	0	0	0	77	0	0	77	19
3137	**FR 4**	99	20	178	277	0	0	0	0	317	0	0	99	0
3138	**FNR 1**	3259	0	989	989	0	0	0	58	0	0	0	524	0
3139	**FNR 2**	1753	0	783	597	0	37	37	149	224	37	0	112	0
3140	**FNR 3**	2204	0	848	170	0	0	0	0	136	0	0	34	0
3141	**FNR 4**	2077	0	186	93	124	0	0	31	279	31	0	279	0
3142	**NR 1**	3058	0	1558	115	0	0	0	231	519	0	115	173	0
3143	**NR 2**	3699	0	729	0	0	52	0	52	573	0	0	104	0
3144	**NR 3**	858.4	0	1657.6	177.6	0	0	0	0	148	0	0	118.4	0
3145	**NR 4**	892	0	382	73	36	0	0	55	328	0	0	55	0
3146	**NN 1**	2052	0	992	103	0	0	0	68	34	0	0	171	0
3147	**NN 2**	436	0	178	158	0	0	0	0	59	0	99	20	20
3148	**NN 3**	748	0	160	196	0	0	0	18	214	0	0	125	0
3149	**NN 4**	401	0	1203	0	0	0	0	57	153	0	0	96	0

Appendix 8: Last day evaluation data of crop protection programmes on diamondback moth (DBM), white blister and clubroot on broccoli

Evaluation Date	Days after planting	Plant Stage	Block#	Strip within Block	Posision within Strip	Plot#	Unit#	Program	Fumigation	Rotation	# Plants with Bollworm	# Boll worm in traps	# Plants with DBM	# DMB in traps	# Plants with Clubroot	White Blister Class per block	# White blister/ 10 Harvestable heads
4/24/2015	78	45	1	1	1	1	1	Chemical	Fumigation	No	0	0	0	19	0	2	2
4/24/2015	78	45	1	1	2	1	2	Biological	Fumigation	No	0	0	4	19	0	0	0
4/24/2015	78	45	1	1	3	1	3	Control	Fumigation	No	0	0	6	19	0	4	5
4/24/2015	78	45	1	1	4	1	4	IPM	Fumigation	No	0	0	2	19	0	0	0
4/24/2015	78	45	1	2	1	2	5	Chemical	Fumigation	Rotation	0	0	0	19	0	1	1
4/24/2015	78	45	1	2	2	2	6	Biological	Fumigation	Rotation	0	0	2	19	0	1	1
4/24/2015	78	45	1	2	3	2	7	Control	Fumigation	Rotation	0	0	5	19	0	5	7
4/24/2015	78	45	1	2	4	2	8	IPM	Fumigation	Rotation	0	0	3	19	0	0	0
4/24/2015	78	45	1	3	1	3	9	Chemical	No	No	0	0	0	19	0	1	1
4/24/2015	78	45	1	3	2	3	10	Biological	No	No	0	0	3	19	0	2	2
4/24/2015	78	45	1	3	3	3	11	Control	No	No	0	0	6	19	0	3	3
4/24/2015	78	45	1	3	4	3	12	IPM	No	No	0	0	5	19	0	0	0
4/24/2015	78	45	1	4	1	4	13	Chemical	No	Rotation	0	0	0	19	0	1	1
4/24/2015	78	45	1	4	2	4	14	Biological	No	Rotation	0	0	4	19	0	2	2
4/24/2015	78	45	1	4	3	4	15	Control	No	Rotation	0	0	5	19	0	3	4
4/24/2015	78	45	1	4	4	4	16	IPM	No	Rotation	0	0	3	19	0	0	0
4/24/2015	78	45	2	1	1	5	17	Biological	No	Rotation	0	0	5	19	0	1	1
4/24/2015	78	45	2	1	2	5	18	Control	No	Rotation	0	0	3	19	0	4	6
4/24/2015	78	45	2	1	3	5	19	IPM	No	Rotation	0	0	4	19	0	1	1
4/24/2015	78	45	2	1	4	5	20	Chemical	No	Rotation	0	0	1	19	0	0	0
4/24/2015	78	45	2	2	1	6	21	Biological	No	No	0	0	5	19	0	3	3
4/24/2015	78	45	2	2	2	6	22	Control	No	No	0	0	3	19	0	3	5
4/24/2015	78	45	2	2	3	6	23	IPM	No	No	0	0	4	19	0	1	1
4/24/2015	78	45	2	2	4	6	24	Chemical	No	No	0	0	1	19	0	3	4
4/24/2015	78	45	2	3	1	7	25	Biological	Fumigation	Rotation	0	0	4	19	0	2	2
4/24/2015	78	45	2	3	2	7	26	Control	Fumigation	Rotation	0	0	5	19	0	4	6
4/24/2015	78	45	2	3	3	7	27	IPM	Fumigation	Rotation	0	0	3	19	0	1	1
4/24/2015	78	45	2	3	4	7	28	Chemical	Fumigation	Rotation	0	0	0	19	0	3	3
4/24/2015	78	45	2	4	1	8	29	Biological	Fumigation	No	0	0	4	19	0	1	1
4/24/2015	78	45	2	4	2	8	30	Control	Fumigation	No	0	0	4	19	0	3	3
4/24/2015	78	45	2	4	3	8	31	IPM	Fumigation	No	0	0	3	19	0	0	0
4/24/2015	78	45	2	4	4	8	32	Chemical	Fumigation	No	0	0	0	19	0	1	1
4/24/2015	78	45	3	1	1	9	33	IPM	No	No	0	0	3	19	0	0	0
4/24/2015	78	45	3	1	2	9	34	Chemical	No	No	0	0	0	19	0	1	1
4/24/2015	78	45	3	1	3	9	35	Biological	No	No	0	0	3	19	0	0	0
4/24/2015	78	45	3	1	4	9	36	Control	No	No	0	0	1	19	0	3	4
4/24/2015	78	45	3	2	1	10	37	IPM	Fumigation	No	0	0	1	19	0	1	1
4/24/2015	78	45	3	2	2	10	38	Chemical	Fumigation	No	0	0	0	19	0	0	0
4/24/2015	78	45	3	2	3	10	39	Biological	Fumigation	No	0	0	2	19	0	2	2
4/24/2015	78	45	3	2	4	10	40	Control	Fumigation	No	0	0	3	19	0	3	4
4/24/2015	78	45	3	3	1	11	41	IPM	No	Rotation	0	0	2	19	0	0	0
4/24/2015	78	45	3	3	2	11	42	Chemical	No	Rotation	0	0	1	19	0	0	0
4/24/2015	78	45	3	3	3	11	43	Biological	No	Rotation	0	0	1	19	0	0	0
4/24/2015	78	45	3	3	4	11	44	Control	No	Rotation	0	0	3	19	0	3	4
4/24/2015	78	45	3	4	1	12	45	IPM	Fumigation	Rotation	0	0	4	19	0	0	0
4/24/2015	78	45	3	4	2	12	46	Chemical	Fumigation	Rotation	0	0	0	19	0	0	0
4/24/2015	78	45	3	4	3	12	47	Biological	Fumigation	Rotation	0	0	2	19	0	2	2
4/24/2015	78	45	3	4	4	12	48	Control	Fumigation	Rotation	0	0	5	19	0	4	6
4/24/2015	78	45	4	1	1	13	49	Control	Fumigation	Rotation	0	0	1	19	0	2	2
4/24/2015	78	45	4	1	2	13	50	IPM	Fumigation	Rotation	0	0	0	19	0	0	0
4/24/2015	78	45	4	1	3	13	51	Chemical	Fumigation	Rotation	0	0	0	19	0	2	2
4/24/2015	78	45	4	1	4	13	52	Biological	Fumigation	Rotation	0	0	2	19	0	2	2
4/24/2015	78	45	4	2	1	14	53	Control	No	Rotation	0	0	4	19	0	3	3
4/24/2015	78	45	4	2	2	14	54	IPM	No	Rotation	0	0	0	19	0	0	0
4/24/2015	78	45	4	2	3	14	55	Chemical	No	Rotation	0	0	0	19	0	1	1
4/24/2015	78	45	4	2	4	14	56	Biological	No	Rotation	0	0	3	19	0	2	2
4/24/2015	78	45	4	3	1	15	57	Control	Fumigation	No	0	0	6	19	0	3	3
4/24/2015	78	45	4	3	2	15	58	IPM	Fumigation	No	0	0	0	19	0	1	1
4/24/2015	78	45	4	3	3	15	59	Chemical	Fumigation	No	0	0	0	19	0	1	1
4/24/2015	78	45	4	3	4	15	60	Biological	Fumigation	No	0	0	2	19	0	3	3
4/24/2015	78	45	4	4	1	16	61	Control	No	No	0	0	5	19	0	2	2
4/24/2015	78	45	4	4	2	16	62	IPM	No	No	0	0	0	19	0	0	0
4/24/2015	78	45	4	4	3	16	63	Chemical	No	No	0	0	0	19	0	1	1
4/24/2015	78	45	4	4	4	16	64	Biological	No	No	0	0	2	19	0	2	2