CONTROL OF THE SWEET POTATO WEEVIL (*Cylas puncticollis*) WITH ENTOMOPATHOGENIC NEMATODES

By

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Submitted in Partial Fulfillment of the MSc Dissertation

in

Plant Pathology

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

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Pietermaritzburg, South Africa

December, 2023

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Dissertation Summary

Sweet potato (*Ipomoea batatas* L) is one of the important crops worldwide because of its high calorific value. Sweet potato weevils (SPW) are the major pest affecting sweet potato, leading to a massive yield loss annually. An initial goal was to evaluate the diversity, incidence and damage severity caused to sweet potato by SPW in fields and in storage in three provinces, and the knowledge of small scale farmers about SPW. A survey was conducted in the Gauteng, North West and Limpopo provinces. Most of the farmers (96%) planted sweet potato for income generation, 3% farm for home consumption and 1% farm for employment. About 64% farmers knew about SPW, 28% had no knowledge and 8% were not sure. The crop was worst affected by SPW (79%), with rats, porcupines and other pests posing less of a problem (12%, 8% and 1%, respectively). Farmers were affected by SPW throughout the year. Farmers agreed that SPW had a negative impact on their production, with 34% of them expressing this sentiment, 28 were not sure and 14% did not think that SPW affected their crop yields. About 67% lost a quarter of their yield annually, 27% of the farmers lost a third of their yield and 6% lost half of their yield. All the three provinces are affected by SPW. Despite the majority of the farmers being aware of SPW damage, they had little knowledge of how to control the pest.

Entomopathogenic nematodes (EPNs) have potential as biocontrol agents of economically important pests. This study was conducted to explore the potential of EPNs for the biological control of the SPW, *Cylas puncticollis* (Boheman) 1883, in an artificially infested field using larvae-infested sweet potato pieces placed in perforated containers. A total of six treatments were applied in the field, namely A - control, B - chemical insecticide, C - *Steinernema tophus* Cimen isolate ROOI 352 Formulation 1 with 2% Barricade® gel, D - *S. tophus* Formulation 2 (cadavers) E - *Heterorhabditis bacteriophora* Poinar, isolate SGI 245 Formulation 1 with 2% Barricade® gel, F - *H. bacteriophora* Formulation 2 (cadavers). After two weeks, larvae, pupae and adult SPW numbers were counted to determine the SPW populations. *Heterorhabditis bacteriophora* (Treatment F, cadaver formulation) was the most effective treatment with 0% live larvae and 36% dead larvae. Larvae, pupue and adult weevils were evaluated, Treatment F obtained 0% for live LPW and 48% for dead LPW. This short-term field trial demonstrated a successful reduction of all life stages of SPW by *H. bacteriophora*SGI 245. Further trials of this EPN for the control of SPW are needed throughout the crop production cycle.

Entomopathogenic nematodes (EPNs) are of current research interest because of their ability to kill insect pests as biocontrol agents. However, EPNs are sensitive to ultraviolet (UV) light, high temperatures and desiccation. The aim of one study was to analyse the survival and efficacy of two strains of EPNs, produced either *in vivo* or *in vitro,* and formulated in either Barricade® gel or Potassium polyacrylate hydrogel (PPH) formulations. EPNs were produced *in vivo* using *Galleria mellonella* (Fabricius) 1798 (the greater wax moth), and *in vitro* using an artificial medium containing ground, desiccated larvae of *Musca domestica* (Linnaeus), the housefly. IJs of the three selected strains of EPNs were suspended in 2% gel formulations of Barricade® gel or PPH by mixing 98 ml of water containing infective juveniles (1000 IJs / ml) with 2 g of Barricade® gel or PPH. The formulations were stored in 2 ml Eppendorf tubes held at 15°C. The survival of the IJs was then tested at two week intervals. The efficacy of formulated IJs was tested by infecting 10 mealworms with IJs (1000 IJs / ml), incubated for 72 hours at 25°C, and counting the number of dead mealworms at two weeks intervals. On Day One, the survival of the EPNs of both isolates, in both formulations, and the controls were 100%. After two weeks, the controls had zero IJs survival for all the isolates, produced either *in vivo* or *in vitro*. The three isolates in both the Barricade® gel and PPH formulations had a zero IJs survival after eight weeks. On Day One, the mortality of mealworms was 100% for all the formulations. Both formulations of the three isolates caused zero mortality of mealworms after eight weeks. The use of Barricade® gel and PPH in formulating three isolates of EPNs enhanced their survival for up to six weeks. The *in vitro*-produced EPNs had a higher level of survival than the *in vivo* produced EPNs. However, the *in vivo* production resulted in EPNs that were more effective at killing mealworms than the *in vitro* produced EPNs. However, these differences were marginal. Differences between the three strains of EPNs were also marginal.

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae, coupled with their symbiotic bacteria, are utilised for the biological control of a wide range of agricultural insect pests. Persistence of EPNs in the field is affected by a number of abiotic factors such as UV light, fluctuation in temperature and desiccation. The aim of this study was to assess the persistence of EPNs under field conditions, applied in two different formulations (cadaver formulation and Barricade® gel formulation). Persistence was assessed after one and two months post treatment application by baiting soil with the greater wax moth larvae (*Galleria mellonella*) and observing the insect mortality. *Heterorhabditis bacteriophora* (SGI 245) cadaver formulation showed better survival in all the five sites, followed by *H. bacteriophora* Barricade® gel formulation. Persistence declined drastically two months post

treatment from a maximum 100% to 0%. The findings suggest that EPNs post application survival in the field can be enhanced by both cadaver and Barricade® gel formulations.

Sweet potato weevils is one of the most important insect pests of sweet potato. The control of SPW is difficult due to the cryptic nature of the larvae, and night activity of the adults. In Africa, control of SPW relies primarily on the use of synthetic insecticides. However, the insect has developed some level of resistance against a wide range of insecticides. Entomopathogenic nematodes (EPN), which are cosmopolitan soil-borne entomopathogens, have gained interest as potential biological control agents of various economically important insect pests. The main aim of this study was to evaluate the potential of EPN isolates from the families Heterorhabditidae and Steinernematidae as biological control agents of SPW and their effect on different sweet potato cultivars, under field conditions. Plant resistance has also been viewed as an efficient, cost-effective and environmentally-safe form of pest control methods of SPW under field conditions. Four different cultivars were planted and six biocontrol treatment were applied after a month. Overall, both the cadaver and Barricade® gel formulations of *H. bacteriophora* SGI 245 were more effective in reducing the plant damage caused by SPW than the Barricade® gel formulation of *S. tophus* ROOI 352. Overall, the cultivar Monate suffered less insect damage (0.103) than the cultivars Blesbok and Bophelo, and it was the best yielding cultivar. A combination of Treatment F of *H.bacteriophora* and the sweet potato cultivar Monate can be recommended for further field testing.

Declaration

I, **Sinethemba Zulu**, declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, pictures, graphs or information, unless acknowledged as being sourced from other persons.

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Sinethemba Zulu (Candidate)……  ……

Dr. T. Ramakuwela (Supervisor) ……

Prof. M.D. Laing (Co-supervisor) [redacted]

Acknowledgements

Thank you to the God Almighty. I can do all things through Christ who gives me strength.

I would like to express my sincere appreciation to:

Dr Tshima Ramakuwela (immediate supervisor at ARC-SGI) for giving me a chance, guiding, supporting, being patient with me and proof-reading my entire dissertation.

Professor Mark D. Laing (supervisor, University of KwaZulu-Natal) for his insightful comments and encouragements. Thank you for all the time you spent reviewing my work and for paying for my university fees.

Nicolene Thiebaut, ARC-Biometry Unit, for assistance with statistical analysis.

Dr Letodi Luki Mathulwe for reviewing my chapters and her wise advice.

Motlotleng Thipe, for assisting me with planting and harvesting.

Extension officers and farmers that I worked with, in my trials.

My sister, Nobubele Zulu, for her support and prayers throughout my studies.

Contents

Dissertation Introduction

Sweet potato (*Ipomoea batatas*) is dicotyledonous and belongs to a family of Convolvulaceae. Sweet potato is an annual crop that was cultivated originally in tropical South America, where is has been cultivated for more than 5000 years (Bioweb, 2023). It has been ranked as the 5th most essential food globally (Ezin *et al*., 2018). This crop is grown in more than 100 different developing countries, mainly in tropical and subtropical regions (Kyereko *et al.,* 2019). China is the large producer of sweet potato annually, (Kyereko *et al.,* 2019). South Africa annually produces around 60,000 tons of sweet potato, which is a lower production than most other countries in sub-Saharan Africa (Laurie *et al*., 2017). Sweet potato is amongst popular crops produced by small scale farmers in South Africa (Laurie *et al.,* 2017). The sweet potato market in South Africa is very different and noticeable as there are few commercial producers of the crop. The market is dominated by informal producers, who are small-holder farmers. Small holder farmers easily include sweet potato in their cropping programme because it is easy to cultivate, and is versatile and hardy (Laurie *et al.,* 2017).

Sweet potato is valuable to human health as it provides beneficial components nutrients such as starch, cyanine, vitamin A and dietary fiber. Both the stem and the leaves are important as they contain nutritional components in higher concentrations than any other commercial vegetables (Ezin *et al*., 2018). Leaves are rich in zinc, vitamin B, β-carotene, iron, protein and calcium carotene, vitamin B2, vitamin C and vitamin E. Stems are an excellent source of carbohydrates, sugar, vitamin C, β-carotene, iron and several other minerals (Ezin *et al*., 2018). According to Ezin *et al.* (2018), sweet potato storage roots are richer in proteins than cassava and yam storage roots. Due to these superior qualities of sweet potato, it has potential to reduce hunger and malnutrition, specifically in low-income households in developing countries (Hue and Low, 2015), ensuring food and nutritional security.

Since 1995, the Agricultural Research Council-VEGETABLE, INDUSTRIAL AND MEDICINAL PLANTS (ARC-VOP) breeding programme has been focusing on high yields, sweet taste, good root system/ quality, high dry mass and adaptation. β-carotene became the major focus in 2003 in their breeding programme. Five new cultivars were released in the last 15 years namely, Ndou, Monate, Impilo, Bophelo and Mvuvhelo. However, there has been little research focused on breeding for cultivars that are pest and disease resistant. Sweet potato breeding for pest resistance is challenging. Conventional breeding is more effective when combined with nonconventional techniques such as genetic engineering, mutation breeding and biotechnology. These have played a significant role in developing new sweet potato cultivars

that are high yielding and resistant to sweet potato virus disease (SPVD). Regardless of the potential of genetic engineering in crop improvement, its application has not been politically acceptable in developing countries (https://www.arc.agric.za/arc-vopi/Pages/Plant%20Breeding/Sweet-Potatoes.aspx).

The main challenge faced by farmers is low yields, which results from using old landraces, little or no fertilization or irrigation, and the impact of pests and diseases (Laurie and Magoro, 2008). In the selection of variety, farmers focus on traits such as a sweet taste, dry texture and good yields. Breeding for these traits was initiated in 1992 in South Africa (Laurie and Magoro, 2008). In a study by Laurie *et al*. (2017), the cultivar Ndou performed the best for yields and good taste from the 22 lines that were screened.

Despite the economic importance of sweet potato; there are four key pests known to attack sweet potato in Africa, namely, sweet potato weevils (SPW), sweet potato hawk moth, white grubs and African bollworm (Hue and Low, 2015). Sweet potato weevils are widely distributed in tropical regions, and farmers struggle to manage them. There are four main species of SPW affecting sweet potato, namely, *Euscepes postfasciatus* (Fairmaire), *Cylas puncticollis* (Boheman), *Cylas formicarius* (Fabricius) and *Cylas brunneus* (Fabricius). All four are classified in Coleoptera: Apionidae (Kyereko *et al.*, 2019). Sweet potato weevils cause serious damage throughout their life cycle, from eggs to adult, affecting all parts of the sweet potato plant. When eggs are laid, female SPW create egg-laying punctures in the roots by excavating cavities. The eggs are laid underneath the surface of the roots and enclosed with a dark colored excrement from the female adults, resulting in unsightly punctures. The appearance of the roots and market price of sweet potato is reduced significantly, resulting in major economic losses (Hue and Low, 2015). According to studies conducted in Central America, Africa and Asia; production losses often reach 60-100% (Beyene *et al*., 2015). Hue and Low (2017) noted that SPW caused losses of up to 73% in Uganda, depending on the planting period and 15–20% in Tanzania. In other areas in Africa, losses have been shown to reach 100% (Hue and Low, 2015).

Chemical insecticides are widely used to manage and control SPW; however, they do not adequately control SPW populations (Kagimbo, 2017). Chemical insecticides are often ineffective, expensive, unreliable, and have environmental and safety risks. Biological methods have been identified as having the potential to be used as a safer alternative that may be more effective than pesticides, in some cases. Biological methods require minimal physical input, are usually cost-effective and offer the potential for long-lasting control of insect pests (Lawrence,

2017). The damage caused by the *Cylas* spp. can also be lowered using "Integrated Pest Management" (IPM). Integrated pest management is defined as the use of several methods to reduce pest numbers and damages. IPM employs: (i) cultural practices (crop rotation, field sanitation, correct planting season, proper plant spacing, site selection, companion cropping, salt water management). (ii) Mechanical control (hand picking, screening). (iii) Biological approaches (bacterial, viruses, nematodes, pheromones and plant extracts such as pepper and garlic) (iv) Chemical control [chemicals are only used when pest are present or crop damage is visible (Lawrence, 2017)].

Nematodes are unsegmented roundworms that belong to the phylum Nematoda, within the super-phylum Ecdysozoa, alongside arthropods and other organisms that build and shed their cuticle. Entomopathogenic nematodes (EPN) have gained a lot of interest for their potential to provide pest management, because more than 200 insect species have been found to be susceptible to EPNs (Hazir *et al*., 2003). They have parasitic properties, being able to kill host insect by means of symbiotic bacteria carried in their alimentary canal (Lalramliana and Kumar 2010). As the restrictions on the use of chemicals and mounting problem of resistance increases, biological methods such as EPNs can be an alternative. Entomopathogenic nematodes have shown the ability to kill *Cylas* spp. (Kagimbo, 2017). Entomopathogenic nematodes are safe, inexpensive to mass produce *in-vitro* with fast reproduction rates and possess high virulence; EPN kill most hosts in less than 48 hours, and reduce pest feeding before mortality occurs (Kagimbo, 2017). They also kill a wide range of insect hosts (Shapiro-Ilan and Goolsby, 2021). EPNs can be easily applied by high and low input methods such as high-pressure sprayers and pouring nematodes suspension onto plants, respectively (Shapiro-Ilan and Goolsby, 2021). Entomopathogenic nematodes have many ideal attributes as biological control agents, such as a wide host range, ease of large scale production *in vivo* or *in vitro*, rapid host mortality, long term efficacy, active host-seeking ability, ease of application to crops, compatibility with many chemical pesticides, and their safety to users and the environment (Kagimbo, 2017).

Problem statement

Sweet potato is an essential food in over 50 countries because of its nutritional and economic value. *Cylas* sppare a major destructive pest causing drastic yield decline and resulting in a decrease in income. Chemicals are widely used in controlling *Cylas* spp. However, they are

largely ineffective because the SPW larvae feed inside the sweet potato root. Entomopathogenic nematodes are non-polluting, environmentally safe and are known to be effective against cryptic pests. Infective juveniles can be used in combination with most pesticides (Ubaub *et al*., 2019). They are capable of infecting hosts either actively or passively in cryptic habitats and they have proven superior to chemicals in controlling *Cylas* spp. (Ubaub *et al.*, 2019). Entomopathogenic nematodes can access insect pests that feed on roots such as SPW because they are soil-dwellers, are persistent, and they can remain active for up to four months in the soil (Ubaub *et al.*, 2019). With the increase in restrictions on the use of chemicals and the growth of pesticide resistance, there is a need to use biological methods as EPNs. Specifically, EPNs possess many attributes that suggest that they could be an ideal biocontrol agent to control SPW (Kagimbo, 2017).

Aim

To explore potential of EPNs for the biological control of a SPW, *Cylas formicarius* (*Cylas* spp.).

Hypothesis

Indigenous EPNs from the genera *Steinernema* and *Heterorhabditis* have the potential to control *Cylas formicarius,* for sustainable sweet potato production.

Objectives

i. Study the diversity, incidence and damage severity caused to sweet potato by SPW in fields and in storage in South Africa.

ii. Formulate EPN isolates in Barricade® gel, an organic water soluble polymer and in larvae of *Galleria mellonella*. Run field trials to test the efficacy of EPNs against SPW under field conditions as compared to resistant varieties, and chemical pesticide.

The referencing system used in the chapters of this thesis is based on the Harvard system of referencing (De Montfort University), and follows the specific style used in the journal *Florida Entomologist* (Florida Entomological Society).

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is the dominant thesis format adopted by the University of KwaZulu-Natal because it facilitates the publishing of research from a thesis far more easily than the older monograph form of a thesis. As such, there is some unavoidable repetition of references and some introductory information between chapters.

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Chapter 1

Literature Review

1.1. Sweet Potato as a crop

Sweet potato (*Ipomoea batatas*) is root crop that is grown in numerous countries, mainly in developing countries (Baimey *et al.,* 2017). Sweet potato is dicotyledonous and belongs to the family of *Convolvulaceae*. It is grown largely for human food in African countries and produced primarily for animal feed in China (Sang-Soo, 2019). Its storage roots contain nutritional components that are vital for human health such as carbohydrates, polyphenols, anthocyanins and dietary fiber (Laurie *et al.,* 2017; Sang-Soo, 2019).

Sweet potato stems and leaves also have a nutritional value, often at higher concentrations than in many other commercial vegetables. Leaves are cooked as a vegetable in many parts of the world as they are rich in vitamin B, ß-carotene, iron, calcium, zinc and protein (Osundahunsi *et al*., 2003).

Sweet potato storage roots vary in color, including orange, white and purple. Orange-fleshed varieties are particularly rich in B-carotene. Irrespective of their color, all sweet potato varieties are low in protein quantity and quality. Orange-fleshed varieties are found naturally, while others have been developed through conventional breeding. Some varieties have been improved through modern biotechnology. Nutritional value differs according to fertilization, harvesting period and variety (Ezin *et al*., 2018).

1.2. Sweet potato weevils

Sweet potato weevil (SPW) is the common name for *Cylas formicarius* (Fabricius). It is distributed throughout the world. Its main host is sweet potato (*Ipomoea batatas*), and many species of morning glory (Hue and Low, 2015). Damage to the sweet potato roots is done by the adult weevils and larvae, whereas the leaves, vine, storage roots and buds are damaged by adults feeding on them. Most of the damage done to storage roots is caused by larvae tunneling through the stems and storage roots. Chemicals called terpenes are produced in the storage roots as a defensive response to SPW tunneling, resulting in the flesh having an unpleasant taste (Hue and Low, 2015).

Female SPW lay eggs either separately in the base of the vines, or crawl through cracks in the soil to lay eggs in the storage roots. The eggs are white, oval, and about 0.5 mm long. They are wrapped in a shield of grey faecal matter. With a lifespan of about 4 months, hundreds of eggs

are laid by each female weevil. The larvae are legless, white, with a brown head and a reddish-brown gut. At maturity, the larvae are up to 8 mm long. Pupae are creamy white, and are up to 6 mm long. This stage lasts about a week. After coming out of the pupa, the adult weevil remains in the tunnel for about a day before cutting through the flesh of the storage root to the outside (Hue and Low, 2015).

Adult weevils are small and ant-like insects. They are 5-7 mm long, with a smooth and slender body and snout. Their head and rear are metallic blue black, and their middle, legs and antennae are red. The full lifecycle takes just over a month. Adults can fly for up to 1,000 m and can also crawl. Males are more active flyers than females. Long-distance dispersal is through the movement of infested sweet potato storage roots and vines (Hue and Low, 2015).

1.3. Detection and identification

The adult weevils feed on the leaves, vines and storage roots, however they cause relatively little damage to the storage roots. They tend to make small shallow pits as they feed. Root damage may be caused by oviposition in the roots, which are covered with fecal matter. Infested roots have black spots on the skin where eggs have been laid and can easily be noticed at harvest. Feeding punctures of adults on the root surface is also a sign of damaged roots. There may also be visible tunneling and feeding on damaged roots by larvae. Larval feeding at the base of the stem causes the vine to swell and eventually die. Larval feeding creates irregular galleries in the inner part of the root. Leaf damage can be identified by holes caused by adult feeding on the epidermis, especially when the roots are not yet formed. In instances of severe infestation, adult feeding on stems during the early stages can cause sweet potato vines to be completely consumed, resulting in failure of the plant to grow (Kagimbo, 2017).

1.4. Impact

Sweet potato weevils are the most serious pest in all major sweet potato-producing countries in Africa, with reports of losses ranging from 5% to more than 80%. The longer the crop remains in the ground (unharvested), the greater are the losses (Hue and Low, 2015). The two primary factors that contribute to yield losses caused by SPW are soil and weather conditions. Yield losses may reach 100% during extended dry seasons. Cultivation of sweet potato in light sandy soils under conditions of low rainfall will have a high likelihood of heavy infestations (Hue and Low, 2015). Red-fleshed, low dry matter varieties are more susceptible to infestation than other

cultivars. According to studies conducted in Central America, Africa and Asia, production losses often reach 60-100% (Beyene, 2015). Hue and Low (2015) found that losses could reach 73% in Uganda, depending on the planting period and 15–20% in Tanzania. In other areas in the continent, losses have been shown to reach 100% (Hue and Low, 2015).

1.5. Control practices/ management

Integrated pest management (IPM) is one of the tool that is used to manage SPW. Integrated pest management is defined as a use of several methods to reduce pest numbers and damages (Suresh *et al*., 2018). Integrated pest management include the following: (i) cultural practices (crop rotation, field sanitation, correct planting season, proper plant spacing, site selection, companion cropping, salt water management). (ii) Mechanical control (hand picking, screening). (iii) Biological approaches (bacterial, viruses, nematodes, pheromones and plant extracts such as pepper and garlic) (iv) Chemical control [chemicals are only used when pest are present or crop damage is visible (Suresh *et al*., 2018).

1.6. Cultural practices/ control

Early harvesting is the most common method used by farmers to reduce root damage, combined with field sanitation, mulching, re-hilling, and use of clean planting material, crop rotation of at least two years, can significantly reduce weevil damage. Traps for male adult weevils can be used to monitor pest populations in the field (Suresh *et al*., 2018).

1.7. Chemical control

Chemical insecticides are widely used to manage and control SPW; however, they do not adequately control weevil population (Kagimbo *et al*., 2017). Chemical insecticides are often ineffective, expensive, unreliable, and have environmental and safety risks. These are some of the World Health Organization class II insecticides approved and recommended, which are less toxic; namely permethrin, dimethoate, and cypermethrin. They can be used for treatment of vines at planting and early in the growing season, at 1 and 2 months after planting. Insecticides applied during late growing season, after storage root formation, is not very effective (Suresh *et al*., 2018).

Chemicals tend to be expensive to small holder farmers. The following recommendations are for commercial growers, the signs of weevil damage would affect acceptability and price of

sweet potato vine cuttings are treated with insecticides to kill weevils and prevent infestation in new plantings. Insecticides are better to use than blanket sprays, particularly if combined with suitable sanitation and the other measures listed under cultural control. Crops should be inspected regularly, at least once a week, the base of the vines should be checked for damage and holes (Suresh *et al*., 2018).

Male pheromone can also be used to monitor weevil populations. If damage occurs at the crown area of the vines, insecticide (e.g., bifenthrin or fipronil) can be sprayed routinely every 3-4 weeks (Suresh *et al*., 2018).

1.8. Biological control

There are several biological methods that has gain a lot of scientific interest because of the advantages they have over widely used insecticides. Namely; fungi, bacterial, viruses, nematodes, pheromones and plant extracts such as pepper and garlic. *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (white muscardine fungus), is produced in large quantities and can used intensively for the control of SPW. Sprays of the fungus have largely replaced the use of chemical insecticides. This method has not been highly used in Africa to control *Cylas puncticollis* (Boheman) 1883 because of the unfavorable conditions for the fungal pathogen at the time (dry season) during infestation and damage. *Beauveria bassiana* requires moist environments for dissemination (Abd-Elgawad, 2016).

1.9. Entomopathogenic nematodes as a biocontrol agent

Entomopathogenic nematodes (EPNs) are biological control agents that can be used to control a diversity of economically important insect pests (Vicente-Díez, *et al.,* 2021). Nematodes possess advantages over the widely used chemicals, which are; non-polluting, environmentally safe and acceptable (Shapiro-Ilan, *et al*., 2016). They can be applied with conventional equipment and they are compatible with most pesticides (Shapiro-Ilan, *et al.,* 2016). They find their host either actively or passively in soil and cryptic habitats. Research evidence proves that they are superior to chemicals in controlling the target insects. The EPNs, *Heterorhabditis* and *Steinernema,* together with their symbionts bacteria, *Photorhabdus* and *Xenorhabdus*, respectively, are obligate and lethal parasites of insects (Shapiro-Ilan, *et al*., 2016). Entomopathogenic nematodes have a great access to insect that feed on roots like SPW as they

are soil dwellers. They are persistent, they can remain active for up to four months (Capinera, 2018).

The two genus (*Heterorhabditis*, *Steinernema*) have gain a lot of attention and considered promising because of their ability to seek host target, environmental friendly and safe to non-targeted organisms, easy to commercially produce *in vivo* or *in vitro*, rapid host mortality and has long term efficacy (Shapiro-Ilan, 2016).

1.10. EPN mass production

The first attempt to produce nematodes was in Japan, against Japanese beetles, this however failed because of less knowledge and understanding between the relationship between the EPNs and the symbiotic bacteria (Hatting *et al.*, 2019). In 1966, the relation between *Steinernema* and *Xenorhabdus*was revealed by Poinar and Thomas. Again in 1976 Poinar discovered the relationship between *Hecterorhabtidis* with symbiotic bacteria, *Photorhabdus*. These discoveries were a huge breakthrough to other researchers which eventually led to the mass production of EPN commercially. Entomopathogenic nematodes can be mass produced using *in vivo* (baiting technique on insect), insect serve as bioreactors; and commercially by *in vitro* production; artificial solid or liquid media used. Both methods have advantages and disadvantages in terms of production costs, technical expertise, quality and economic scale (Shapiro-Ilan, 2016).

In vivo is a low technology method with low start-up costs that involves the production of EPNs by using live insects, which are highly susceptible and easy to grow and easily available. The insects used under this method are the larvae of the greater wax moth, *Galleria mellonella* (Linnaeus) 1758, which are reared in the laboratory. *Galleria mellonella* is preferred, due to its high susceptibility, easy availability and high yield of IJs (Shapiro-Ilan, 2016).

The *in vitro* method involves four steps: (i) preparation of solid medium; (ii) inoculation with bacteria; (iii) inoculation with IJs; and (iv) harvesting of IJs (Shapiro-Ilan, 2016).

1.11. EPN Formulation

Formulation of EPN is very vital and the main aim of this is to achieve long shelf life and appropriate application technology. This can be achieved by maximum survival of IJs in a formulation for a longer period before their application in the field. Nematode mortality may vary from 70 to 100% if storage life of the formulated product has expired (Shapiro-Ilan, 2014).

There are different formulations: (i) encapsulation of EPNs with calcium alginate based on immobilizing the nematode came in 1985 by Kaya and Nelson. This success eventually directed to the development of a commercial nematode products, such as (ii) Alginate and flowable gel formulations to trap nematodes physically in order to reduce their movement. (iii) EPNs can also be formulated in water-dispersable granules, (iv) nematode wool, (v) gels, (vi) vermiculite, (vii) clay peat and (viii) sponges (Shapiro-Ilan, 2014). The shelf life of nematodes in a formulated product can be improved by reducing nematode activity and metabolism through physical trapping, metabolic inhibition, cold storage, or by the induction of anhydrobiosis (Shapiro-Ilan, 2014). The shelf life can also be improved by genetic selection approaches, leading to an improved persistence of these beneficial organisms in the soil. Kagimu *et al.* (2019) conducted a research in formulation of South African EPNs using alginate beads and diatomaceous earth, where he concluded that the alginate beads successfully retained most of the IJs and can be stored for a longer time.

1.12. Cadaver application method

This method involves the culture and distribution of IJs in their infected host cadavers (Del Valle *et al.,* 2008). Entomopathogenic nematodes infected host insect cadavers are placed at the target site. The progeny IJs emerge from the cadaver, parasitize the target insect pests and as a result cause their suppression (Shapiro-Ilan, 2014).

1.13. Barricade® gel formulation

Entomopathogenic nematodes survival rely on abiotic and biotic factors in the environment (Nimkingrat *et al.,* 2013). Entomopathogenic nematodes are well adapted to underground environments, but less adapted to above-ground conditions. Above ground, EPNs are subjected to UV light and heat. EPN survival is poor once water had been lost from the substrate. Sunlight can dramatically decrease *Steinernema* spp.'s pathogenicity and survival (Askary, 2010). Additionally, EPNs have low tolerance to drying (Nimkingrat *et al.,* 2013). Consequently, when applied above-ground, EPNs require protection against drying.

Barricade® gel liquid concentration is made from absorbent polymers. The protective covering provided by Barricade gel can last for up to 24 hours, depending on weather conditions (temperature, wind and humidity). Mist up the coating with additional water spray can extend

the protection times (Shapiro-Ilan *et al.,* 2015). Barricade® gel is effective in improving EPN control of insects (Shapiro-Ilan *et al.,* 2015). Entomopathogenic nematodes applied with Barricade solution are protected from drying and from harmful UV radiation (Shapiro-Ilan *et al*., 2010). Shapiro-Ilan *et al*. (2010) showed that *Steinernema carpocapsae* (Weiser, 1955) applied with 2-4% Barricade® gel increased efficacy against *S. pictipes* with 0-30% survival. The EPN without Barricade® gel treatment does not affect larvae survival. Barricade® gel provides moisture on the exposed tree surface and temporarily helped nematode deposition, survival, and host invasion (Shapiro-Ilan *et al*., 2010). The study conducted by Shapiro-Ilan *et al.* (2016) confirmed that when applied with 2% Barricade, *S. carpocapsae* lowered populations of *S. pictipes* more than when applied with just water.

Barricade® gel is not toxic to IJs (Shapiro-Ilan *et al.,* 2016). Survival of the IJs is not different from a water control. The ability of EPNs to infect and kill a host after exposure to the sun was greater (50-80%) for 1 and 2% Barricade gel solutions than only water (Shapiro-Ilan *et al*., 2010). Barricade® gel permitted the EPNs to stick to the leaf more efficiently than when applied with only water (Dito *et al.*, 2016).

Barricade gel has showed potential as a protectant that can increase the biological control potential of EPNs against above-ground pests.

1.14. EPN Application options

The application of EPNs mainly for the control of larval or pupal stages of insect pests in the soil, plant surface (Askary, 2010). Application of EPNs is done by the equipment of other control agents; however, in order to achieve a cost-effective control, techniques required for their application should be optimized (Askary, 2010). Most of agriculture equipments, such as mist blowers, pressurized sprayers, electrostatic sprayers or aerial sprays can be used in the application of EPNs (Askary, 2010). During application; volume, nozzle type, agitation, pressure, recycling time, system environmental conditions and spray distribution pattern should be taken to consideration (Askary, 2010). During the liquidation process, the tank mixture should be stirred in order to make it homogenized for better application of EPNs.

1.15. Commercialization of EPNs

Entomopathogenic nematodes are one of the groups of biological controls that has been successfully been proven to work with high percentage of efficacy in laboratory and field trials.

There are a lot of factors contributing to the successful commercialization of EPNs globally namely; advances mass-production and formulation technology (Shapiro-Ilan, 2015), their ability to control many soil-dwelling insect pests (Birhan *et al.,* 2020), the ease of application using conventional liquid application equipment and systems (Birhan *et al.,* 2020) and the effect of reducing harmful chemical pesticide applications.

In the early 1980s, EPNs were initially commercialized and a semi-artificial medium for their production was developed. There has been research and development to improve the production technology, formulation and storage of EPNs in the past decades till today (Lewis *et al.,* 2015) Production of EPNs in a large scale have been achieved using liquid culture in bioreactors, this was a huge breakthrough in EPN commercialization industry (Hiltpold, 2015). Several formulations were also developed while developing the large-scale liquid production. The other factors that contribute in a rapid commercialization of EPNs are; storage, application, storage and availability of EPNs. (Lewis *et al*., 2015).

Some of the reasons why EPNs are successful as effective biological control agents (BCAs) is their ease of application using conventional liquid application equipment (Dolinski, 2015). The application technologies have been used in numerous planting systems on soil and above-ground insect (Shapiro-Ilan *et al.,* 2012). The spraying technology differs from small watering cans for home garden use to aerial application over large fields to conventional spraying equipment (Shapiro-Ilan *et al.,* 2012). EPNs can be applied using different irrigation systems in open fields and greenhouse (Birhan *et al.,* 2020). Infected host cadavers can also be used to apply EPNs (Dolinski *et al*., 2015). Improvement to the application systems of EPNs, coupled with the need to reduce harmful chemical pesticides, has contributed to the efficient transfer from EPNs are used in a variety of crop (Birhan *et al.,* 2020). The following are nematodes that are currently widely commercialized, *Steinernema* species [*Steinernema carpocapsae* (Weiser, 1955), *Steinernema feltiae* (Stanuszek, 1974), *Steinernema glaseri* (Steiner, 1929), *Steinernema riobrave* (Cabanillas, Poinar and Raulston, 1994) and *Steinernema scapterisci* (Nguyen and Smart, 1990*), S. kraussei* (Steiner, 1923), *Steinernema kushidai* (Mamiya, 1988) *and Steinernema scarabaei* (Alluaud, 1902)] and *Heterorhabditis* species *[Heterorhabditis bacteriophora* (Poinar, 1976) and *H. megidis* (Poinar, Jackson and Klein 1987), *Heterorhabditis indica* (Poinar, Karunakar and David, 1992), *Heterorhabditis marelatus* (Liu and Berry, 1996), *Heterorhabditis downesi* (Stock, Griffin and Burnell, 2002)] (Birhan *et al.,*

2020*). Steinernema carpocapsae, S. feltiae, S. kraussei, S. glaseri, S. riobrave, S. scapterisci, H. bacteriophora* and *H. megidis* are currently the most commonly produced and successfully applied nematodes as they are easily produced in liquid culture (Lacey *et al.*, 2015).

Currently, three of the largest producers of EPNs are the previous Becker Underwood (now bought by BASF), based in the USA, e-nema in Germany and Koppert Biological Systems in the Netherlands (Birhan *et al.,* 2020). The distribution and production levels of EPNs have increased dramatically over the years. The market value per annum in 2006 in Europe and the USA has been estimated at $6 and $8.25 million, respectively. Sale of EPNs in the Netherlands alone in 2008 was estimated at about €1 million for the control of greenhouse pests (Birhan *et al.,* 2020). The current markets are mostly in the regions of production, developing countries represent growing markets for companies producing EPNs. More research is being done in developing countries like South Africa, however there has been no commercial company established successfully. From the year 2000 to 2010, a 20-fold growth of the global market of biopesticides was estimated (Abate *et al.,* 2017) and, this growth is likely to continue (Hatting *et al.,* 2019). Nxitywa and Malan 2021 published a review in the formulation of Entomopathogenic Nematodes for the Control of Key Pests of Grapevine. The focus was on the different types of formulations required for storage and ease of transport, together with the application formulation for above-ground pests and the factors affecting them. The quality assessment, storage and handling of formulated EPNs are also discussed.

1.16. Regulation of EPNs (SA vs. other countries)

The management of pests using EPNs is increasing worldwide (Brown, 2014). More are under investigation for future release (Ehlers, 2011). The increase in the use of nematodes due to the advantages they have compare to chemicals which are environmentally friendly, safe to human health and have minimal non-target effects compared to chemical pesticides. Nevertheless, concerns were raised in the 1980s concerning the potential non-target effects of nematodes which lead to an increase in regulations (Bailey *et al*., 2010). The biggest issue was grouping nematodes as invertebrate bio-control agents, microbial bio-control agents, plant extracts (Ehlers, 2011). EPNs can be treated in two groups: (i) macrobials that include predatory insects and mites, insects that parasitise other insects (parasitoids) or nematodes, and (ii) microbial agents that include bacteria, viruses, fungi and protozoa (Ehlers, 2011). Bailey *et al.* (2010) grouped BCAs in three categories: (i) predatory insects and mites; (ii) parasitoids, which are insects with a free-living adult stage and a larval stage that is parasitic on another insect; and

(iii) parasites, microbial pathogens and antagonists, such as nematodes, fungi, bacteria, viruses and protozoa which contradict with what was reported by Ehlers, 2011. Bailey *et al*. (2010) further grouped the first two as 'macrobial' and bacteria, viruses and fungi as 'microbial' control agents, but EPNs were grouped as 'BCAs placed between the macrobial and microbial control agents' (Birhan *et al.,* 2020). The grouping of EPNs as microbial or macrobial biocontrol agents is influenced by whether their symbiotic association with bacteria is considered or not (Birhan *et al.,* 2020). EPNs are grouped under microbial bio-control agents together with bacteria, fungi and viruses (Birhan *et al.,* 2020).

In South Africa, hundreds of invertebrate pests infest agricultural crops (Hatting *et al.,* 2019). In order to decrease their impact, more than 500 pesticides actually allowed by the Act 36 of 1947 under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies continue to pose a risk on humans, animals and the environment (Hatting *et al.,* 2019). Since late 1970s the risk awareness has been increasing, leading to the restriction of a number of insecticides namely monocrotophos, chlorpyrifos, endosulfan and aldicarb. Methyl bromide have been totally restricted by the South African government (Hatting *et al.,* 2019). "National Bioeconomy Strategy" was established by the Department of Science and Technology (DST) now called Department of Science and Innovation (DSI), in 2013 to put more focus on the use of alternatives biological pest control agents (Hatting *et al.,* 2019). An increasing in regulations on chemical pesticides, it creates pressure on the market and industries to supply the newly discovered markets from the west (Brain *et al.,* 2015).

There has been a huge shift from the agricultural industries, moving towards using biological pesticides. Bio-pesticide companies now being purchased by these multinational agriculture chemical companies and this as drastically increased the biopesticide market (Malan *et al.,* 2011)

The research currently done is important because of strict regulations preventing the import of exotic organisms in the amendment of Act 18 of 1989 under the Agricultural Pest Act, No, 36 1947 (Hatting *et al*., 2019). South African research on EPNs is mostly focusing on prevalent South African strains against key insect pests such as *Cydia pomonella* (Lepidoptera: Tortricidae), *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) and *Eldana saccharina* (Lepidoptera: Pyralidae) naming the few (Hatting *et al.,* 2019).

There are no restrictions in place in South Africa for the use of indigenous EPNs. EPNs are not included in the list of prohibited alien species, on National Environmental Management

Biodiversity Act of 2004; published by the department of environmental affairs and tourism, (government gazette, 3 April 2009). In developed countries such as New Zealand, Australia and USA, have regulations in place on the use of EPNs. Entomopathogenic nematodes in USA were exempted from regulation by the Environmental Protection Agency (Ehlers, 2011) until legislation was introduced for the regulation of exotic nematodes in 1996 (Ehlers, 2011). Regulations differs significantly between countries. Countries like Spain, Denmark, Finland, Italy, France, Portugal Greece, do not have any requirements on the use of EPNs. In some of the countries such as Austria, Belgium, Czech Republic, Hungary, Ireland, the Netherlands, Norway, Poland, Sweden, Switzerland and UK required regulations (Ehlers, 2011). In these countries, a project called regulation of BCAs in Europe (REBECA Policy Support Action) has been established, targeting at improving regulation of BCAs in Europe (Ehlers, 2011).

1.17. Latest developments in EPN research and product development SA

There has been a number of research done in South Africa on EPNs, however more research is being undertaken for application of indigenous EPNs on variety of crops grown in SA. Entomopathogenic nematodes were first used in the late 1800s in SA (Hatting *et al.,* 2019). Report on EPNs were first recorded in the Eastern Cape Province in early 1950s; they were collected in a maize field from the black maize beetle, *Heteronychus sanctae-helenae* (Coleoptera: Scarabaeidae) (Hatting *et al.,* 2019). There are a number of institutions in South Africa that have done and are still conducting research on EPNs, namely; Agricultural Research Council, universities, South African Sugar Research Institute, School of Agriculture and Environmental Sciences, Citrus Research International, Illovo Sugar Ltd, Bayer Crop Science, Potato South Africa, Pepsico International and the universities (www.sanematodes.com). There's also a society called Nematological Society of Southern Africa which aim to advance the science of nematology in Southern Africa, fundamentally and application. The society includes countries like South Africa, Malawi, Swaziland Namibia, Mozambique, Lesotho, Zimbabwe and Botswana. The society holds symposia regularly to for the exchange of information on the research in all phases of the subjects done in these different African countries (www.sanematodes.com). A lot of work has been done in South Africa, mostly under laboratory environment, Vernon *et al.* (2021), conducted a study in controlling false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) using several entomopathogenic nematode (EPNs) and entomopathogenic fungus (EPFs) species, that were

identified from soil samples collected from orchards and vineyards throughout the Western Cape province in South Africa.

Katumanyane *et al.* (2023), also conducted a study in "Susceptibility of white grubs from forestry and sugarcane plantations in South Africa to entomopathogenic nematodes". He concluded that; the dissection of insect cadavers revealed possible limiting factors for low susceptibility, due to the nematodes and their associated symbiotic bacteria's inability to infect the insect haemocoel, with no EPNs being found inside some of the cadavers.

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Chapter 2

The occurrence and distribution of sweet potato weevils, North West, Limpopo and Gauteng in South Africa.

Abstract

Sweet potato *Ipomoea batatas* (L) is one of the important crops worldwide because of its high yields and nutritional value. Sweet potato weevil (SPW) is the major pest affecting sweet potato, leading to a massive yield loss annually. The aim was to **s**tudy the diversity, incidence and damage severity caused to sweet potato by SPW in the field and in storage in three provinces, and to evaluate the knowledge of small scale farmers about SPW. A survey was conducted in Gauteng, North West and Limpopo provinces. A majority of the farmers (96%) planted sweet potato for income generation, 3% farm for home consumption and 1% farm for employment. The high levels of unemployment have resulted in small scale farmers needing to generate income to survive. The total of 64% farmers had knowledge of SPW, 28% had no knowledge and 8% were not sure. The farmers were affected by SPW (79%), rats (12%), porcupine (8%) and other pests (1%). Farmers were affected by SPW throughout the year Farmers that agreed that SPW had a negative impact on the production were 34%, 28% were not sure and 14% disagreed that it had any effect. Only 6% farmers estimated that they lost 50% of their yield to SPW, 67% estimated that they lost 25% of their yield annually, and 27% of the farmers lost 12.5% of their yield. All the three provinces were affected by SPW. Whilst the majority of farmers were aware of SPW damage, they lacked knowledge of effective control methods.

Keywords: Integrated Pest Management, SPW, sweet potato, yield lost.

2.1. Introduction

Sweet potato, *Ipomoea batatas* (L) is an important root crop grown worldwide. It is the fifth most important food crop in developing countries (Gapasin *et al*., 2019). In Sub-Saharan Africa (SSA), it is grown on about 2.1 million ha, providing 9.9 million tons of storage root (Beyene *et al*., 2015). It is the second most important root crop after cassava and a key crop after maize and rice. Three African countries, namely Nigeria, Uganda, and Tanzania, are among the top 10 largest producers of sweet potato in the world. Ethiopia is one of the largest sweet potatoes producing country in east Africa and the Southern Nations Nationalities and Peoples' Region (SNNPR) is the major sweet potato producing region in the country (Kagimbo *et al*, 2017). In South Africa, it is cultivated as an annual crop over an area of about 3 000 ha (Sang-Soo, 2019). The major production areas in South Africa are Limpopo, Mpumalanga, North West Provinces, and parts of KwaZulu-Natal and Western Cape, but it is grown by small-holder and resource-poor farmers in virtually all provinces. Nearly 41 insect pests are known to attack sweet potato in South Africa (Jackson *et al.,* 2012). Sweet potato weevils (SPW) are amongst the four key pests affecting sweet potato in South Africa (Kagimbo *et al,* 2015). It affects sweet potato stems and roots in the field and in the storage.

Three species have been identified in Africa: *Cylas puncticollis* (Boheman) 1883, *Cylas formicaries (*Fabricius) 1798, and *Cylas brunneus* (Olivier)1790). The three species have a similar life history, making all of them difficult targets for conventional pest control measures because of their cryptic environment (Kagimbo *et al*., 2017). *Cylas puncticollis* only occur in Africa, having been recorded from Ethiopia, Guinea, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, Sudan, Tanzania, Burundi, Cameroon, Chad and Congo (Laurie *et al*., 2015). *Cylas formicarius* is pan-tropical, being distributed from West Africa, through to Southern Africa, East Africa, Madakascar, Seychelles, India, Mauritius, Bangladesh, Sri Lanka, South-east Asia, China, Philippines, Indonesia, USA, West Indies, Mexico, northern South America and several other locations around the world (Kagimbo *et al*., 2017).

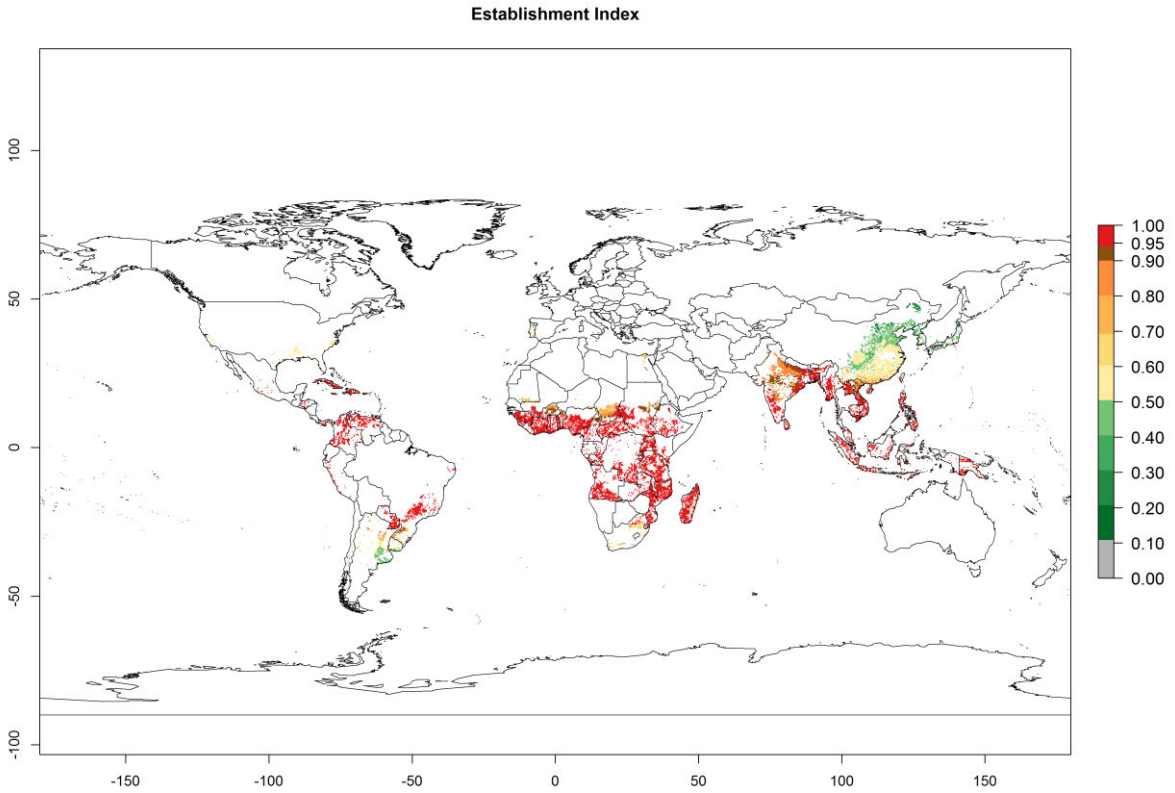

Figure 1: Global maps for the activity index (AI) of sweet potato weevils in the year 2000 (Hue *et al*., 2015).

Sweet potato weevils populations occur across tropical regions of Africa, Central and South America, and South and Southeast Asia. The predictions of changes for 2050 scenario shows a slight increase in the potential growth of SPW population in most of the tropical and subtropical countries where sweet potato is grown. In Africa, SPW occurs naturally in some countries in West (Nigeria, Ghana, Ivory Coast, Sierra Leone, and Togo) and East (Uganda, Kenya, Rwanda, Burundi) Africa. Suitable temperature conditions are present for the establishment in other countries such as Tanzania, Ethiopia, South Sudan, DR Congo, Central Africa Republic, Chad, Niger, Mozambique, Congo, Zambia, Angola, Madagascar, Cameroon, Liberia, Benin, Burkina Faso, Mauritania, Gabon, Malawi, Sudan, Guinea, and Madagascar. A slight range expansion might be possible to East and some parts of Central Africa (Ethiopia) as well as to Southern Africa (Malawi, Angola, Zambia, northern South Africa, and Madagascar (Hue *et al*., 2015).

Infestations by SPW vary with seasons. In the tropics, they are more frequent on sweet potato growing during the dry season. In sub-tropical and warm temperate regions, the cold temperature late in the season markedly limits the egg laying ability of the adult female (Beyene *et al*., 2015). At temperature between 21^0 C and 15.5^0C, eggs laying is low, below 15.5 egg laying stops completely. Temperatures at or below freezing can kill the adult in seven days, the larva in 15 days, and the pupae in 21 days (Schafleitner *et al.,* 2010). A complete life cycle requires one to two months, but only 35–40 days during the summer months. The generations are unpredictable. In the USA, the number of generations occurring annually is usually eight in Louisiana and about five in Texas. Adult weevils do not undergo a period of diapause in the winter, but search for shelter and remain inactive until the weather is favorable. All stages can be found throughout the year if suitable host material is available (Capinera, 2008).

In African countries, it is reported that the losses from SPW ranges from 5-80%. The longer the crop remains in the ground unharvested, the greater the losses (Hue *et al.,* 2015). The factors that contribute greatly on the yield impact of SPW are the soil and weather conditions. Yield losses are high under dry conditions, and can reach 100%, especially during extended dry seasons. Light sandy soils and low rainfall increase the chances of heavy infestations of SPW (Hue *et al.,* 2015). Research evidence confirms that red-fleshed, low dry matter varieties are more susceptible to weevil infestation. According to studies conducted in Central America, Africa and Asia; production losses often reach 60-100% (Beyene *et al*., 2015). Findings of a study done by Hue *et al*. (2017) concluded that losses were recorded to be 73% in Uganda, depending on the planting period and 15–20% in Tanzania. In other areas in the continent, losses have been shown to reach up to 100% (Hue *et al.,* 2015).

Few studies have been done to discover appropriate management options for SPW (Beyene *et al*., 2015). Studies have been done on the association between rooting characteristics and tuber infestation in Ethiopia, Uganda and Kenya. Additional studies surveyed the likelihood of using pheromones to trap weevils to levels that do not cause economic yield losses (Kagimbo *et al.,* 2017). Most of these studies did not consider concrete measures to control the pest. Unfortunately, no varieties have so far displayed reasonable levels of resistance to this pest; investigations to produce transgenic varieties resistant to the pest seems to be a realistic option for consideration, if political barriers to GMO crops can be removed (Kagimbo *et al.,* 2018).

Chemical insecticides are widely used to manage and control SPW; however, they do not adequately control weevil populations (Kagimbo *et al*., 2017). Chemical insecticides are often expensive, unreliable, and have environmental and safety risks. These are some of the World Health Organization class II insecticides approved and recommended, which are less toxic; namely permethrin, dimethoate, and cypermethrin (World Health Organization, 2022). Cultural control methods are also used to control SPW such as sanitation, mulching and the destruction of alternative hosts. Biological methods such as entomopathogenic nematodes (EPNs) has also gained research interest as they have ability to control pests. In a study by Fanou *et al*. (2019) it was concluded that the EPNs tested were effective and significantly reduced the emergence of adult insects in Benin.

Studies have been conducted in other countries on occurrence and distribution of SPW. However, there have been no studies in SA on the occurrence and distribution of SPW. The study aimed to have a clear view on the occurrence and distribution of SPW in SA and this was achieved by interviewing farmers in three different provinces.

2.2. Materials and Methods

2.2.1. Description of the study areas

The farmers' survey was conducted in three provinces: Gauteng, Limpopo and North West.

Figure 2: A map showing three provinces were the study was conducted.

2.2.2. Data collection and analysis

The sampling techniques that were used are purposive and census sampling techniques. Data was primarily collected using semi-structured questionnaire. Interviews were conducted with individual farmers to collect data.

Data was analyzed using The GLM Procedure, t Tests (Least Significant Difference Test), Analysis of variance (ANOVA) and the Frequency (FREQ) procedure (chi- Square test) (Lee *et al.,* 2013).

2.3. Results

In all 3 provinces, above 90% of the farmers are planting for income generation, North West with 100%. Most of the farmers in North West, Limpopo and Gauteng are aware of what SPW are, with 71%, 63% AND 58%, respectively. Most farmers agreed that SPW have negative impact on their production, North West with 29%, Limpopo and Gauteng with 33% each. More than 83% of the farmers in all the three provinces reported to have pest problems. More farmers experience pest damage above ground and SPW is the most prevalent pest that affects farmer with 67%, 90%, and 80% in North West, Limpopo, and Gauteng respectively.

Farmers used different methods of pest management, 48% of farmers in North West and 23% Limpopo used chilies and dishwashing liquid. Gauteng farmers (11 %) use sunlight to control weevils. Farmers in Limpopo and Gauteng lost 25% of the yield at harvest at harvest with

79% and 85% respectively. North West losses up to half of the yield at harvest. At storage, three of the provinces (North West, Limpopo and Gauteng) 25% of the yield at harvest lost 38%, 63% 85% respectively (Table 1).

2.3.1. Table 1: Farmers' survey results on the impact of sweet potato weevils in three provinces in South Africa (f = 2.64).

Questions	Response	Province		
		North West	Limpopo	Gauteng
Reason for sweet potato production	Income generation	100 a	94 b	93 b
	Home consumption	0 a	6 b	4 b
	Employment	0 b	0 b	2 b
Do you know what a SPW is?	Do not know	0a	2a	22 a
	No	29b	35 b	20 a
	Yes	71 b	63 b	58 b
SPW has a negative impact on produces production	Strongly agree	0a	6b	7b
	Agree	29b	33b	33b
	Not sure	14 a	37a	33b
	Disagree	19b	4a	18b
	Strongly disagree	38a	19b	9a
Do you experience insect damage on sweet potato crops?	No	0a	4b	17a
	Yes	100a	96b	83a
Where are pests of sweet potato causing damage?	Above ground	33a	4a	28b
	Below ground	67b	92a	61a
	Missing data	0a	4b	11a
Most problematic pest	SPW	67a	90a	80b
	Rats	14b	8b	15b
	Porcupines	19a	0a	4b
	Other	0b	2b	0b
Pest control methods	None	24a	50b	69a
	Sanitation	0b	4a	0b
	Mulching	0a	10a	0a

	Early harvesting	**0b**	**0b**	**0**
	Flooding	**0b**	**2b**	**2a**
	Clean planting material	**0a**	**0a**	**3b**
	Resistant cultivars	**0b**	**0b**	**0b**
	Catch and kill	**14a**	**0b**	**4b**
	Cypermethrin	**5b**	**0b**	**0a**
	Sunlight soap	**9b**	**6b**	**11b**
	Chilies and dishwashing liquid	**48**	**23b**	**7b**
	Bicarbonate of soda	**0b**	**0b**	**2b**
	Normal chemicals	**0b**	**0b**	**2b**
	Methomex adama	**0b**	**2b**	**0b**
	Hypermetric	**0b**	**0b**	**0b**
Specify other options	**None**	**5a**	**52a**	**17a**
	Catch and kill	**19a**	**1a**	**2a**
	Cypermethrin	**5a**	**0b**	**0b**
	Sunlight soap	**10b**	**6b**	**13b**
	Chilies and dishwashing liquid	**28a**	**23b**	**7a**
	Bicarbonate of Soda	**0b**	**0b**	**2b**
	Normal Chemicals	**0b**	**0b**	**2b**
	Methomex adama	**0b**	**3a**	**0b**
	Hypermetric	**0b**	**3a**	**0b**
	Missing data	**13b**	**13a**	**57a**
Estimated yield loss at harvest	**25%**	**38a**	**79a**	**85a**
	37.5%	**57a**	**19a**	**4a**
	50%	**5b**	**2a**	**11a**
Estimated yield loss in storage	**25%**	**38a**	**63b**	**85a**
	37.5%	**52a**	**16a**	**4a**
	50%	**10b**	**21a**	**11b**

2.3.2. The average farmers' survey responses in the three provinces

Most farmers planted sweet potato to generate an income (96%), 3% planted for home consumption and only 1% planted it for employment. Most farmers knew of SPW (64%), whereas 28% did not know of SPW and 8% were not sure if they knew what SPW are. Most farmers agreed that they had experienced damage to their crops caused by SPW (32%), 28 were not sure, 14% disagreed, 4% strongly agreed and 22% strongly disagree. Most farmers had experienced pest problems (93%), whereas 7% said that they had not had any pest problems on sweet potato. Most farmers experience pest damage above ground with 73%, and the most problematic pest was SPW (79%) (Table 2).

Most farmers did not use any pest management methods (49%), and 26% used a homemade mixture of chilies and dishwashing liquid. At harvest, 67% of the farmers lost 25% of the yield, 27% of the farmers lost yield at 37.5% of the yield and only 6% farmers lost 25% of the yield. At storage, 62% of the farmers lost 25% of the yield, 24% yield lost at 37.5% of the yield and only 14% farmers lost 25% of the yield (Table 2)

Table 2: The average farmers' survey results on the impact of sweet potato weevils in three provinces in South Africa (Pr >= ChiSq <.0001)

Questions	**Response**	**%**
Reason for sweet potato production? (Chi-Square = 176.7800)	**Income generation**	**96**
	Home consumption	**3**
	Employment	**1**
Do you know what a SPW is? (Chi-Square = 48.3200)	**Do not know**	**6**
	No	**28**
	Yes	**64**
SPW has a negative impact on produces production (Chi-Square = 25.2000)	**Do not know**	**0**
	Strongly agree	**4**
	Agree	**32**
	Not sure	**28**
	Disagree	**14**

	Strongly disagree	**22**
Pest control methods (Chi-Square = 280.7426)	**None**	**49**
	Sanitation	**1**
	Mulching	**3**
	Early harvesting	**0**
	Flooding	**1**
	Clean planting material	**1**
	Resistant cultivars	**0**
	Catch and kill	**6**
	Sypermethrin	**2**
	Sunlight soap	**9**
	Chillies and dish washing liquid	**26**
	Bicarbonate of soda	**1**
	Chemicals	**1**
	MethomaxAdama	**1**
	Hypermetric	**0**
Specify Other options (Chi-Square = 123.2000)	**None**	**25**
	Catch and kill	**7**
	Sypermethrin	**2**
	Sunlight	**10**
	Chillies and dish washing liquid	**19**
	Bicarbonate of soda	**1**
	Chemicals	**1**
	MethomaxAdam	**1**
	Hypermetric	**1**
	Missing data	**33**
Do you experience insect damage on sweet potato crops? (Chi-Square = 73.9600)	**No**	**7**
	Yes	**93**
Where are pests of sweet potato causing damage? (Chi-Square = 75.1400)	**Above ground**	**22**
	Below ground	**73**
	Missing data	**5**
Most problematic pest	**SPW**	**79**

(Chi-Square = 158.0000)	**Rats**	**12**
	Porcupines	**8**
	Other	**1**
Estimated yield loss at harvest (Chi-Square = 57.6200)	**25%**	**67**
	37.5%	**27**
	50%	**6**
Estimated yield loss at storage (Chi-Square = 38.4800)	**25%**	**62**
	37.5%	**24**
	50%	**14**

2.4. Discussion

Majority of farmers in these three provinces plant sweet potato to generate income. The main reason for this is because of unemployment, hunger and malnutrition in the country. South African Child Gauge 2020 report states that: "Malnutrition hurts millions of South Africa's children now, just as it did 20 years ago" (https://www.arc.agric.za/arc-vopi/Pages/Plant%20Breeding/Sweet-Potatoes.aspx).

Most people are substituting starch with sweet potato, which makes this crop attractive to the market. Sweet potato has vital nutrients such as of carbohydrates and has a low glycaemic index (GI). Orange-fleshed types are rich in beta-carotene, a pro-vitamin A carotenoid that is converted to vitamin A by the human body (Laurie et al., 2015).

Severe acute malnutrition (SAM) remains a serious fundamental cause of child deaths in South Africa, accounting for 1/4 child in-hospital deaths. Sweet potato has been reported as good potential source of phenolic compounds and antioxidants (Gavender 2020).

More than 50% of the farmers from all provinces are aware of SPW. North West having the highest number of farmers who know SPW with 71%. This clearly indicate that in South Africa, there is a SPW problem.

Among the major pests, the SPW (*C. puncticollis* and *C. brunneus*) are the main constraints affecting sweet potato production in Africa. According to (Kagimbo *et al.*, 2017), weevils were the most important constraint to sweet potato production in most zones of Africa, especially in Tanzania. However, no recent studies exist on damage and yield loss caused by SPW in South Africa, farmers' perceptions regarding SPW, and farmers' preferred sweet potato varieties; hence the main reason why this study was conducted.

Limpopo and Gauteng both have high percentage of famers who agreed that SPW have negative impact in productivity. In North West, farmers have strongly disagreed, this may be because of less knowledge about SPW damage and the market standards.

Most commercial farmers use integrated pest management methods, with chemicals being highly used. Small scale farmers also use IPM. In Limpopo and Gauteng, a high number of farmers, don't use any methods to control SPW. Farmers in North West use chilies and dish washing liquid method to control weevils and the reason to this, it's because these two are easily accessible and cheap.

Farmers in all three provinces; North West, Limpopo and Gauteng have indicated that they have pest problem, specifically weevils. The pests are highly experienced below ground, this is because the main interest is in storage roots and are subjected below ground. Leaves are also used for consumption, 33% of the farmers in North West stipulated they also experience pest damage above ground.

According to most studies such as Kagimbo *et al*. (2018) states that "One of the greatest threats to sweet potato production is the SPW. The weevil attacks all parts of the plant; adults feed directly on vines, and larvae tunnel into the roots, causing extensive damage both in field and storage. Often causing losses of 60% – 100% during periods of drought".

According to our survey in North West, Limpopo and Gauteng, the pest that they most experience is SPW.

Sweet potato weevil is a major source of economic loss in developing countries. In each province, the estimated yield lost at harvest and storage is very important (Kagimbo *et al*., 2018).

At harvest, all three provinces experience an estimated yield loss that was above 60% at 25% of the yield. At storage; Limpopo and Gauteng had the high percentage of estimated yield loss at 25% of the yield with 63% and 85% respectively, with statistical significance between the two. North West experience the highest yield loss of quarter half of the yield.

The first question on the graph states "the reason for producing sweet potatoes". The farmers in all provinces produces sweet potato to generate income, 3% produce sweet potato for home consumption and 1% for employment. There are a lot of reasons for this; a high rate of unemployment in South Africa, most people or rather young people are moving towards farming and creating employment for themselves and generate income (Grasswitz, 2019).

Sweet potato weevil the pest that mostly affect sweet potato and cause a massive yield decline. Most farmers that were interviewed in our survey from the three provinces, said to have

knowledge about SPW. A small number of farmers said they don't know what SPW are. The survey was done to establish the knowledge of farmer about SPW.

Sweet potato weevils because 80% production lost in commercial farmers if not treated properly (Ezin *et al.,* 2018). Small scale farmer interviewed agreed that SPW have negative impact on their production were 32% and 28% are not sure, this is because most of these farmers do not keep a record of how much they make from each harvest and how much they lose. They also do not compare how much they make in every harvest.

Integrated pest management is used by farmers to control SPW (Kagimbo *et al.,* 2017). Farmers were interviewed on the methods they used in controlling SPW. Most of famers indicated that they do not use any method. These are small scale famers, most of them they do not afford pesticides to treat SPW and they normally sell on the informal market which does not follow certain standards. A shortage of essential horticultural knowledge in small scale farmers is most likely the cause of farmers not using any method of pest control (Grasswitz, 2019).

Sweet potato weevils can affect sweet potato both above (on the leave and stem) and below ground (storage roots). Farmers were then asked where they experience sweet potato weevil's damage. Most farmers indicated that they experience more damage below ground, on the storage roots.

More farmers confirmed that they have pest problems. Only a small number of farmers, said not to experience pests may be because they do not know how to identify pest damage (Grasswitz 2019).

The citrus industry published information on AgNet media confirming that most farmer experience below ground damage because it is easy to control above ground pest by spraying of insecticides frequently and it's easy to identify above damage than below ground (https://citrusindustry.net/2018/01/03/root-weevil-management-ground/).

Most farmer's interviewed said to be affected by SPW. Sweet potato weevils are the most important pest of sweet potato in most sub-Saharan Africa; it a cause of a massive yield lost annually as a result of an unmarketable and bitter storage roots (https://teca.apps.fao.org/teca/en/technologies/6936).

2.5. Conclusion

Sweet potato is planted by most of the small scale farmers because of its nutritional value. It can be planted throughout the year which make it attractive to farmers. Sweet potato is the major pest affecting sweet potato in the three provinces. Storage roots are more affected since it is not easy to identify and treat below ground pests compare to above ground.

The survey indicated that most of the small-scale farmers are aware of sweet potato weevil damage, however, they lack knowledge of control measures. Most farmers normally do not use any weevil control method. This lead to a 25% yield lost annually.

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Chapter 3

Use of entomopathogenic nematodes for the management of the artificially infested sweet potato under field conditions

Abstract

Entomopathogenic nematodes (EPNs) have potential as biocontrol agents of economically important pests. This study was conducted to explore the potential of EPNs for the biological control of the SPW, *Cylas puncticollis* (Boheman) 1883, in an artificially infested field using larvae-infested sweet potato pieces placed in perforated containers. A total of six treatments were applied in the field, namely; Treatment A control, Treatment B chemical insecticide, Treatment C *Heterorhabditis bacteriophora* Formulation 1 with 2% Barricade® gel, Treatment D *H. bacteriophora* Formulation 2 (cadavers), Treatment E *Steinernema tophus* Formulation 1 with 2% Barricade® gel, Treatment F *S. tophus* Formulation 2 (cadavers) and an untreated control. After two weeks, larvae, pupae and adult SPW numbers were counted to determine the SPW populations. *Heterorhabditis bacteriophora* (SGI 245) (Treatment F, cadaver formulation) was the most effective treatment with 0% live larvae and 36% dead larvae. Larvae, pupae and adult weevils were evaluated, Treatment F obtained 0% for live larvae, pupae, weevils (LPW) and 48% for LPW. This short-term field trial demonstrated a successful reduction of all life stages of SPW by *H. bacteriophora* SGI 245. Further trials of this EPN for the control of SPW are needed throughout the crop production cycle.

Keywords: Artificial infestation, Barricade gel, Biological control cadaver formulation, field trials, *Heterorhabditis bacteriophora*, *Steinernema tophus.*

3.1. Introduction

The sweet potato weevil (SPW), *Cylas puncticollis* (Boheman 1883) is a major destructive pest of sweet potato, causing substantial yield losses and economic losses (Kagimbo *et al*., 2017). Roots damaged by SPW fetch a reduced market price, resulting in major economic losses (Hue *et al.,* 2015). In studies conducted in Central America, Africa and Asia, production losses often reach 60-100% (Beyene *et al*., 2015). Hue *et al*. (2015) found that losses of up to 73% occurred in Uganda, and 15–20% in Tanzania, and up to 100 losses in other parts of Africa (Hue *et al.*, 2015).

The life cycle of the SPW takes about a month in summer. All stages may be found throughout the year in a suitable environment. In Stage 1 eggs are laid in small cavities inside sweet potato storage roots. The eggs take 3-10 days to hatch. In Stage 2, the larval stage; larvae develop from Day 10-25 before pupating. In Stage 3, the pupal stage, typically lasts for 7-10 days (from day 25-35). In Stage 4, adult weevils emerge from the pupal cocoons after 35 days, depending on the environmental conditions (Devi *et al*., 2014).

Adult weevils feed on the leaves, vines and storage roots, but they do little damage to the storage roots, making shallow pits as they feed. Feeding on roots results in the presence of fecal matter layering oviposition holes in the root. Infested roots have black spots on the skin where eggs have been laid and can be identified at harvest. Larvae cause the most damage by tunneling and feeding on storage roots. Larvae feeding creates irregular galleries in the inner part of the root. Larval feeding at the stem base can cause the vine to swell and eventually to die. Leaf damage can be identified by holes caused by adult feeding on the epidermis, especially when the roots are not yet formed. In instances of severe infestation, adult feeding on stems during the early stages of crop growth may consume the emerging sweet potato vines, causing the plants to die (Kagimbo *et al.*, 2017).

Integrated Pest Management (IPM) is one of the tools that is used to manage SPW. IPM is defined as the use of several methods to reduce pest numbers and damage (Heu *et al*., 2015). IPM includes the following: (i) cultural practices (crop rotation, field sanitation, correct planting season, proper plant spacing, site selection, companion cropping, salt water management); (ii) mechanical control (hand picking, screening); (iii) biological approaches (beneficial insects, bacteria, fungi, viruses, nematodes, pheromones and plant extracts such as pepper and garlic); and (iv) chemical control (chemicals are only used when pests are present or crop damage is visible) (Lacey *et al*., 2012).

Insecticides are widely used to control the SPW. However, they are ineffective against larvae feeding inside the sweet potato root. Moreover, there is widespread opposition to the use of insecticides on food crops, and because of the environmental impact of insecticides.

Entomopathogenic nematodes (EPN) have been used as biocontrol agents against a number of insects (Kagimbo *et al.,* 2017). EPNs are roundworms that are obligate parasites of insects (Kagimbo *et al.,* 2017). EPNs are found worldwide in the soil (Nouh, 2021). Two genera, *Steinernema* and *Heterorhabditis,* have been studied extensively because of their ability to invade and kill host insects (Malan *et al.,* 2006). The two genera are associated with symbiotic bacteria, namely *Xenorhabdus* and *Photorhabdus,* respectively.

Juvenile EPNs (juveniles, IJs) gain entrance into host insects through the natural openings of the host and directly through the cuticle. Symbiotic bacteria are then released into the hemocoel, where they create a suitable and conducive environment for reproduction and multiplication of nematodes. This leads to the death of the host insect. The IJs then exit the host cadaver, carrying the symbiotic bacteria in their intestines, in search of new hosts (Ramakrishnan *et al.,* 2022).

EPNs are safe, inexpensive to mass produce *in-vitro,* have fast reproduction rates, and are effective against target pests. EPN kill most hosts in less than 48 hours, reducing crop damage (Kagimbo *et al.,* 2017). The aim of the study was to explore the efficacy of artificially inoculated EPNs against SPW.

3.2. Materials and Methods

3.2.1. Isolates

The two isolates used, *Heterorhabditis bacteriophora* (Poinar, 1976) (SGI 245) and *Steinernema tophus* (Cimen *et al*., 2014) (ROOI 352), were initially isolated from soil samples from a soybean field in Standerton, Mpumalanga Province, South Africa (26° 56' 37.7556" S 29° 13' 13.638" E) and from soil samples from a rooibos field at Clanwilliam, Cape Province, South Africa (32°10′43″S 18°53′28″E), respectively. An insect baiting technique was used to isolate these nematodes (Ramakuwela *et al.* 2018).

The number of IJs were adjusted to 200 ml. 1ml of IJs suspension was distributed evenly on a 9 cm filter paper in the lid of a 15 mm petri-dish. A total of Ten *Galleria* larvae were added in the petri-dish, covered with a lid and stored in a zip-block plastic. This was then incubated at 25^0C for 3 days. Infective Juveniles were harvested by a method of white trapping described Orozco *et al.* (2014). Cadavers were placed in the 15 mm petri-dish with a filter paper. The 15mm petri-dish containing the cadavers was placed into the larger Petri dish and add water to the larger dish. IJs were allowed to emerge (8 to 14 days). IJs were washed three times (Kaya and Stock, 1997). They were then stored in an aqueous suspension at 10^0C, and were used within two weeks after harvesting.

3.2.2. Trial site

The study was conducted at Meyerton, Gauteng province, South Africa (26032 ′39.0″S 27059 ′57.8″E).

3.2.3. Sweet potato weevil field trial: layout and trial design

A randomized complete blocks design was used for the trial. EPNs and cultivars were main effects. The EPNs had 2 factors: (a) the species (*H. bacteriophora (*SGI 245) and *S. tophus* (ROOI 352); and (b) the application method. Three (3) plants per treatment were planted with a 2 m spacing between treatments and rows. The borders between the plants were 3 m (Table 1).

Table 1. Field trial layout. One colour-coded block represents one treatment with three plants.

BLOCK 1

B4	**E3**	**A1**	**F4**	**D2**	**C3**
A2	**C4**	**B3**	**D1**	**F3**	**E2**

F2	A3	E1	B2	C1	D4
E4	B1	D3	C2	A4	F1

BLOCK 2

A4	C2	B1	D1	E3	F4
F1	E2	D3	C1	B4	A3
C3	B2	A2	E1	D4	F2
B3	A1	C4	F3	E4	D2

BLOCK 3

B2	E4	F1	D4	A2	C3
C4	D2	A3	B4	E3	F2
F3	E2	D1	C1	B3	A1
A4	B1	C2	D3	E1	F4

3.2.4. Treatment applications and artificial infestation

The selected cultivars were previously bred by the Agricultural Research Council (ARC) (Vegetable, Industrial and Medicinal Plants Division), namely, the cultivars Blesbok, Ndou, Monate and Bophelo. Sweet potato cuttings of these cultivars were planted in December 2020.

Six treatments were applied to the plants for the control of sweet potato weevils. These included Treatment A - Control, B - Chemical pesticide (Lirifos 480 EC), C – *S. tophus*, Formulation 1 (Barricade® gel, 3000 IJs / ml), D - *S. tophus*, Formulation 2 (one cadaver), E - *H. bacteriophora*, Formulation 1 (Barricade® gel, 3000 IJs / ml) and F - *H. bacteriophora*, Formulation 2 (cadaver). Treatments were applied one-month post-planting as per the trial plan described above.

In order to artificially inoculate the plots with the larvae of SPW were reared under laboratory conditions and then introduced into each plot (Fig 1).

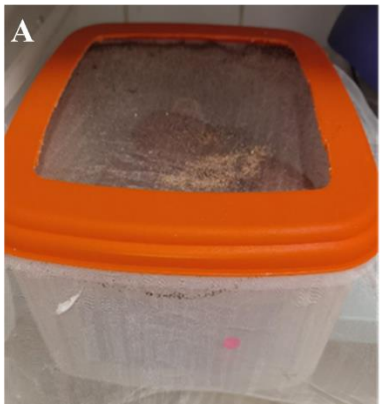


Figure 1: A- A container used to rear sweet potato weevils. **B -** Sweet potato roots infected with sweet potato weevils).

Plastic containers (500 ml) were prepared by cutting holes in the top and the bottom of each container. The holes were then covered with a netting (105 nanometre), in order to allow the IJs or the pesticide, to move into the containers from the surrounding plots, to kill the weevils in the containers. Infested sweet potato roots were then diced into cubes weighing 15 g each, with roughly forty (40) weevil larvae in each cube (Fig. 2).

Figure 2: Cubes of sweet potato storage roots (15 g) infested with sweet potato weevils.

Soil from the site in each treatment was filled half-way up in each container, then a cube of infested sweet potato root was placed inside, and the container was filled with soil (Fig.3).

Figure 3: A - Container with infested sweet potato cube and soil at the bottom. **B –** A plastic container filled with soil and an infected sweet potato cube inside.

One container was then buried 20 mm below ground in each treatment plot (Fig.4).

Figure 4: Burying the containers with infected sweet potato cubes 2 cm below ground.

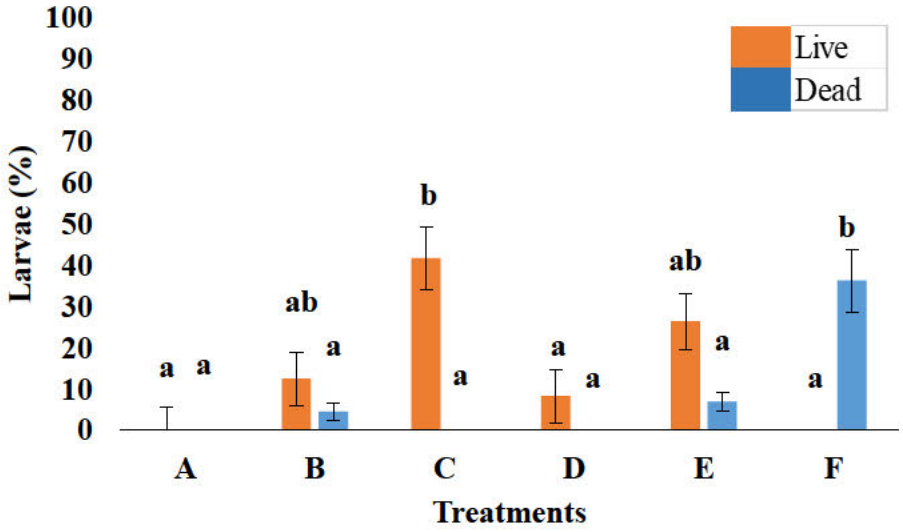
Container samples were collected after two weeks of treatments application. The number of alive and dead larvae, pupa and adult weevils were counted in each container in the laboratory to determine mortality and weevil population. The corrected value was calculated using Abbotts Correction formula (Fleming *et al.*, 1985).

3.2.5. Statistical analysis

The data were analysed using the analysis of variance (ANOVA) using statistical analysis Software (SAS). The standardized residuals were normally distributed (Shapiro–Wilks test) and therefore the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 5% level of significance (DeLucca *et al*., 1990).

3.3. Results

Treatment F obtained 0% for live larvae and 36% for dead larvae. Treatment E obtained 26% and 6% for live and dead larvae respectively. Treatment D and C obtained 8%, 42% for live larvae and both obtained 0% for dead larvae. Treatment B obtained 12% and 5% for live and dead larvae (Fig. 5).



A = Control

B = Chemical pesticide

C = *S. tophus*, Formulation 1 (Barricade® gel)

D = *S. tophus,* Formulation 2 (cadaver)

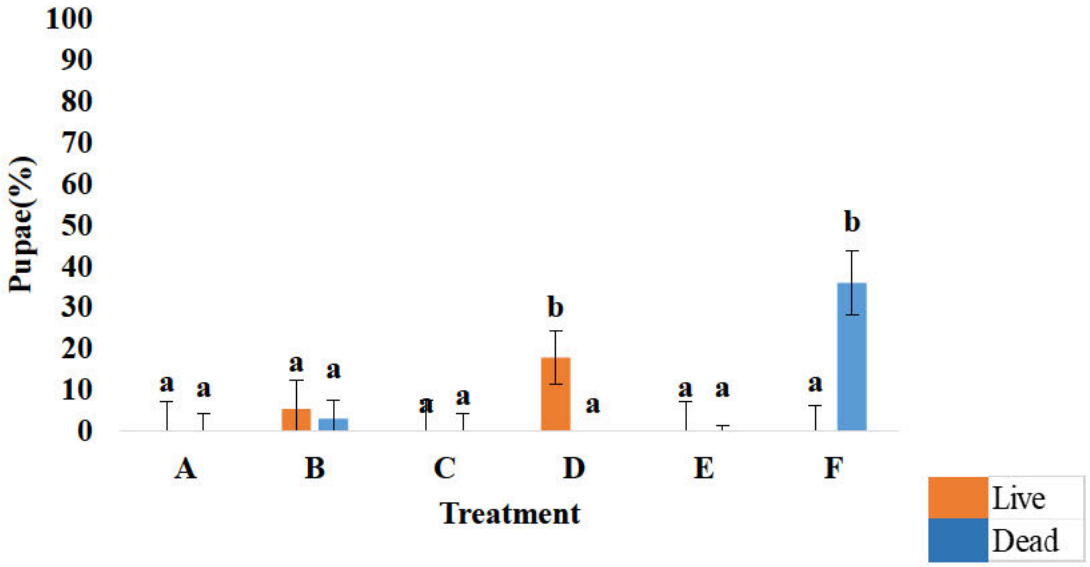
E = *H. bacteriophora,* Formulation 1 (Barricade® gel)

F = *H. bacteriophora,* Formulation 2 (cadaver)

Figure 5: Graph showing number of larvae after five treatments [LSD (p=0.05) = 24, 369; (f=2,05; df=5)]; [LSD (p=0.05) = 34,602; (f=2,05; df=5)].

Treatment F obtained 0% for live pupae and 36% for dead pupae. Treatment E obtained 0% and 0% for live and dead pupae respectively. Treatment D and C obtained 17%, 0% for live

pupae and both obtained 0% for dead pupae. Treatment B obtained 5% and 3% for live and dead pupae (Fig. 6).



A = Control

B = Chemical pesticide

C = *S. tophus*, Formulation 1 (Barricade® gel)

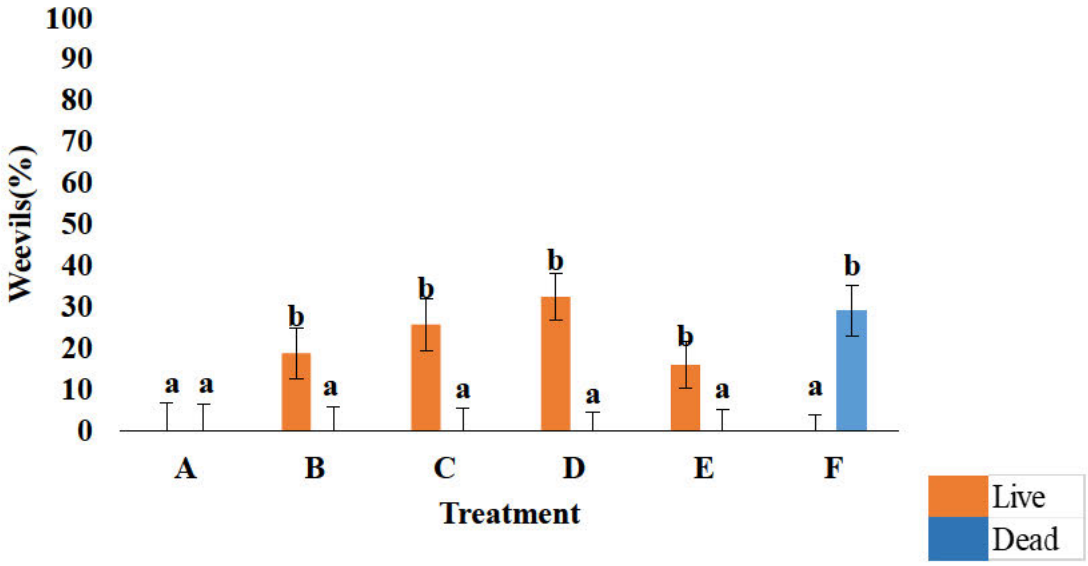
D = *S. tophus,* Formulation 2 (cadaver)

E = *H. bacteriophora,* Formulation 1 (Barricade® gel)

F = *H. bacteriophora,* Formulation 2 (cadaver)

Figure 6: Number of live and dead pupae after five treatments [LSD (p=0.05) = 18,432, (f=2,70; df=5)] ; [LSD (p=0.05) = 34,602, (f=2,70; df=5)].

Treatment F obtained 0% for live weevils and 29% for weevils. Treatment E obtained 0% and 15% for live and dead weevils respectively. Treatment D and C obtained 32%, 26% for live weevils and both obtained 0% for dead weevils. Treatment B obtained 19% and 0% for live and dead weevils (Fig. 7).



A = Control

B = Chemical pesticide

C = *S. tophus*, Formulation 1 (Barricade® gel)

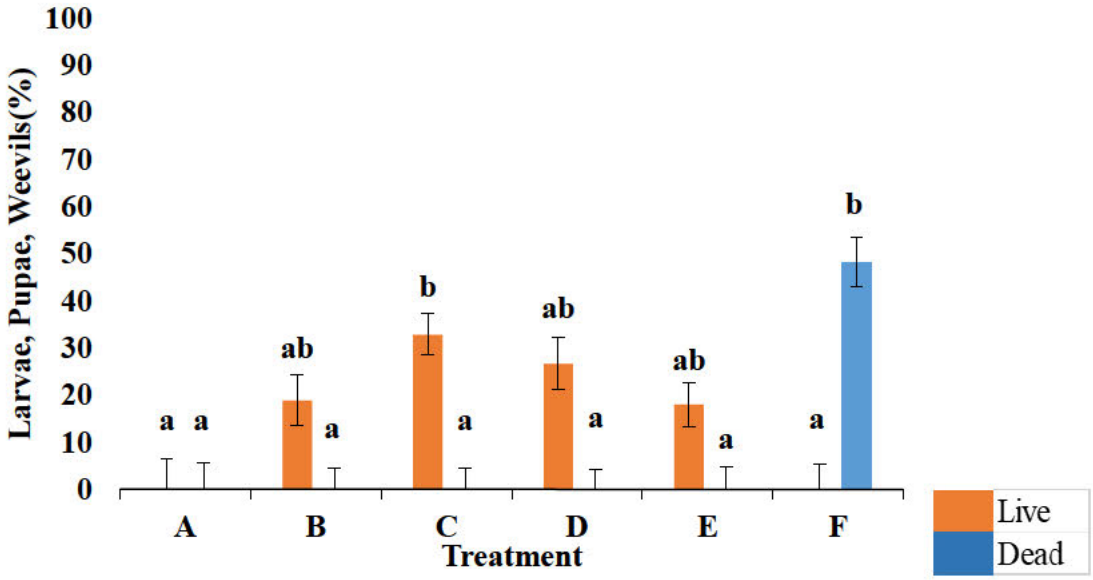
D = *S. tophus,* Formulation 2 (cadaver)

E = *H. bacteriophora,* Formulation 1 (Barricade® gel)

F = *H. bacteriophora,* Formulation 2 (cadaver)

Figure 7: Number of adult weevils after five treatments [LSD (p=0.05) = 31,508; (f=3,68; df=5)] ; [LSD (p=0.05) = 32,931; (f=3,68; df=5)].

Treatment F obtained 0% for live LPW and 48% for LPW. Treatment E obtained 0% and 18% for live and dead LPW respectively. Treatment D and C obtained 27%, 33% for live LPW and both obtained 0% for dead LPW. Treatment B obtained 19% and 0% for live and dead LPW (Fig. 8).



A = Control

B = Chemical pesticide

C = *S. tophus*, Formulation 1 (Barricade® gel)

D = *S. tophus,* Formulation 2 (cadaver)

E = *H. bacteriophora,* Formulation 1 (Barricade® gel)

F = *H. bacteriophora,* Formulation 2 (cadaver)

Figure 8: Total number of larvae, pupae and weevils after five treatments [LSD (p=0.05) = 27,111; (f=4,68; df=5)]; [LSD (p=0.05) = 30,039; (f=4,68; df=5)].

3.4. Discussion

EPNs have the potential to control SPW (Lalramliana *et al.*, 2010). Artificial infestation of weevils was essential in this study in order to ensure that there was even population distribution of weevils in the field of study. *Heterorhabditis bacteriophora* (SGI 245) and *Steinernema tophus* (ROOI 352) were evaluated with two different formulations, namely Barricade® gel and cadaver formulations. The effect of the six treatments against weevils were compared and the number of live and dead SPW were counted at all development stages of the SPW. *Heterorhabditis bacteriophora* SGI 245 (Treatment F) was more effective against the larval stage. *Heterorhabditis bacteriophora* SGI 245 was more effective than other treatments. *Heterorhabditis bacteriophora* SGI 245 (Treatment F) cadaver formulation had the highest percentage of dead larvae. Treatment C and D (*S. tophus*) both cadaver and barricade formulation were non effective. Shehata *et al.* (2021) conducted a study that is in agreement with these findings, were it was concluded that *H. bacteriophora* (Hb-EG strain), can be used as a potential biocontrol in strawberry fields.

Chemicals are widely used in controlling weevils and are very effective (Lacey *et al.*, 2012). It was to compare the EPN formulations with the chemical. The cadaver formulation of *H. bacteriophora* SGI 245 (treatment F) was consistently the most effective treatment with the highest number of dead pupae. *Steinernema tophus* (treatment C barricade and D cadaver) were the least effective. The chemical (B), *S. tophus* ROOI 352 (treatment D) obtained the highest number of live pupae; these treatments were less effective compare to *H. bacteriophora* SGI 245 (treatment F) which obtained 0% of live pupae. *Heterorhabditis bacteriophora* SGI 245 (treatment F) was able to crawl or move vertically in to the soil and kill the pupae inside the sweet potato roots (Wennemann *et al.*, 2004).

Portillo-Aguilar *et al.* (1999) concluded that in a sandy loam soil, *H. bacteriophora* moved at least 18 cm within 4 d of soil inoculation across all bulk densities tested, whereas *S. carpocapsae* moved only 9 cm at thelower densities and <9 cm at the highest soil density. *S. glaseri* showed intermediate levels of movement. These results agreed with our study, in which *H. bacteriophora* SGI 245 (Treatment F) was able to move faster in soil and was more effective against pupae inside the storage roots below the ground than *S. tophus.* This can be attributed to the difference in mobility and size of the two species. *Heterorhabditis bacteriophora* can easily crawl and attach larvae in cryptic environments, inside the sweet potato roots because of its cruiser mobility and smaller size (Portillo-Aguilar *et al.*, 1999), in contrast to *S. tophus*. *Heterorhabditis* species have a vertical-lateral tooth and a dorsal tooth that allows them to easily

penetrate to the cuticle and inside the roots (Poinar Jr, 2016). *Heterorhabditis bacteriophora* SGI 245 (Treatment F) was more effective with than *S. tophus* (Treatment C with D) and the chemical (Treatment B) and there was a statistical significance between *H. bacteriophora* SGI 245 (Treatment F) and the other 5 treatments.

Heterorhabditis bacteriophora was consistently more effective than the other treatments at weevil stage. Treatment B (chemical) was less effective than *S. tophus* ROOI 352 formulations, barricade and cadaver (Treatment C and D). *Steinernema tophus* ROOI 352 has shown to be less effective in controlling sweet potato weevils compare to *H. bacteriophora* SGI 245. *Heterorhabditis bacteriophora* SGI 245 (Treatment F) was more effective in controlling weevils. *Steinernema tophus* ROOI 352 (treatment C and D) was less effective compare to *H. bacteriophora* SGI 245 (Treatment F). Although *H. bacteriophora* SGI 245 (Treatment F) successfully control sweet potato weevils, infected cadavers have not been widely used because they desiccate, stick together or rupture during production and storage (Ansari et al., 2008). A study by (Shapiro-Ilan *et al*. 2001) addressed these problems by formulating the cadaver with different materials, that can protect them against rupture and sticking together. The study concluded that "Two formulations (starch-clay and flour combination) enabled cadavers to be partially desiccated without affecting reproduction. Non-formulated cadavers exhibited reduced reproduction upon desiccation. Four-day-old cadavers were more amenable to desiccation than 8-day-old cadavers. Formulated cadavers were more resistant to rupturing and sticking together during agitation than non-formulated cadavers."

Heterorhabditis bacteriophora effectively control SPW in all three developmental stages. Acharya *et al.,* (2020) conducted a study on the susceptibility of various developmental stages of the fall armyworm, *Spodoptera frugiperda* (Smith) **(**Lepidoptera, Noctuidae), to EPNs. They concluded that *S. frugiperda* was highly susceptible at the larval and pupal stages to various EPNs, including *Steinernema carpocapsae* (Weiser, 1955), *Heterorhabditis indica* (Poinar, Karunakar and David, 1992). These EPNs, therefore, could be used as biological control agents to sustainably manage the overlapping generations of *S. frugiperda* in the environment. This study was in agreement with our findings; EPNs have potential to control SPW in all development stages.

The results have evidently shown that *H. bacteriophora* SGI 245 (Treatment F) cadaver formulation was more effective in control SPW compared to *S. tophus* ROOI 352 (treatment C and D) and the chemical (Treatment B). These findings were in agreement with study conducted

by Gulzar *et al.* (2020) which concluded that nematodes exhibited higher survival and reproduction in the cadaver treatment than in the aqueous treatment. There are additional advantages when using the cadaver approach for biocontrol applications, EPNs existing in natural populations may have broader environmental tolerance than those applied via aqueous suspension. The study of Gulzar *et al.* (2020) was in agreement with this study where *H. bacteriophora* SGI 245 Treatment F, which is the cadaver formulation, was more effective against SPW compared to the chemical and both *S. tophus* Barricade® gel and cadaver treatments.

3.5. Conclusion

Heterorhabditis bacteriophora SGI 245 has shown to have the ability to control SPW in all development stages. It can be used as a biocontrol substituting chemicals, however further research is required for control of SPW throughout the crop production cycle.

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Chapter 4

Formulation of entomopathogenic nematodes in Barricade® gel and potassium polyacrylate hydrogel

Abstract

Entomopathogenic nematodes (EPNs) are of current research interest because of their ability to kill insect pests as biocontrol agents. However, EPNs are sensitive to ultra violet (UV) light, high temperatures and desiccation. The aim of the study was to analyse the survival and efficacy of two strains of EPNs, produced either *in vivo* or *in vitro,* and formulated in either Barricade® gel or potassium polyacrylate hydrogel formulations (PPH). EPNs were produced *in vivo* using *Galleria mellonella*, greater wax moth, and *in vitro* using an artificial medium containing ground, desiccated larvae of *Musca domestica*, the housefly. Infective juveniless of the three selected strains of EPNs were suspended in 2% gel formulations of Barricade® gel or PPH by mixing 98 ml of water containing IJs (1000 IJs / ml) with 2 g of Barricade® gel or PPH. The formulations were stored in 2 ml Eppendorf tubes held at 15°C. The survival of the IJs was then tested at two week intervals. The efficacy of formulated IJs was tested by infecting 10 mealworms with IJs (1000 IJs / ml), incubated for 72 hours at 25ºC, and counting the number of dead mealworms at two weeks intervals. On Day one, the survival of the EPNs of both isolates, both formulations, and the controls were 100%. After two weeks, the controls had 0% IJs survival for all the isolates produced *in vivo* and *in vitro*. The three isolates in both the Barricade® gel and PPH formulations had a 0% IJs survival after eight weeks. On Day One, the mortality of mealworms was 100% for all the formulations. Both formulations of the three isolates caused 0% mortality of mealworms after eight weeks. Use of Barricade® gel and PPH in the formulating three isolates of PENs enhanced their survival for six weeks. The *in vitro* produced EPNs had a higher level of survival than the *in vivo* produced EPNs. However, the *in vivo* production resulted in EPNs that were more effective at killing mealworms than the *in vitro* produced EPNs.

Keywords:Heterorhabditidae, *in vivo* and *in vitro* production, shelf life, Steinernematidae.

4.1. Introduction

Entomopathogenic nematodes (EPNs) are roundworms that are obligate parasite of insects (Ascar *et al*., 2022). EPNs are found worldwide in the soil (Nouh, 2021). Isolates of two families, *Steinernematidae* and *Heterorhabditidae,* are of interest as potential biocontrol agents because of their ability to attack and kill a wide range of insects within 48 hours. This is achieved by a symbiotic interaction between the EPNs and symbiotic intestinal bacteria of genera *Xenorhabdus* and *Photorhabdus* of *Steinernematidae* and *Heterorhabditidae,* respectively. The non-feeding infective juveniles (IJs) have the ability to track and infect hosts. Infection occurs when IJs gain entrance through the natural openings of the host and through the cuticle of some species. Inside the host (target insect), symbiotic bacteria are then released into the hemocoel, where the bacteria reproduce and release pathogenic factors such as secondary metabolites, toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds, killing the target insect (Yooyangket *et al*., 2018). It creates a suitable and conducive environment for reproduction and multiplication of nematodes. This leads to the quick death of the host. Upon nutrient depletion, the next generation of IJs exit the host cadaver, carrying the symbiotic bacteria in their intestine, in search of new hosts (Ramakrishnan *et al.,* 2022).

Although EPNs are proven to be effective in controlling insects, they are fragile organisms, and there are some environmental conditions that negatively affect their survival, reproduction and efficacy. These includes high temperatures, UV light and desiccation (Acar *et al*., 2022).

Therefore, there is a need to develop nematode formulations that tolerate a range of environmental conditions. Recently Acar *et al.* (2022) conducted research to assess a number of products that can enhance the survival of EPNs such as chemicals that block or absorb UV radiation, and which slow desiccation, including P-amino benzoic acid (PABA), octyl methoxycinnamate (OMC), Congo red, titanium dioxide, and zinc. PABA and OMC are widely used in human sunscreens (Acar *et al*., 2022). Despite these chemicals showing potential, other alternatives are needed that are cost-effective and environmentally friendly. These may include the use of Barricade® gel and hydrogels such potassium polyacrylate hydrogel (PPH).

Barricade® gel is a trademarked product made from absorbent polymers and is largely sold as a fire protectant. Barricade® gel has shown potential in protecting nematodes from desiccation and UV light (Acar *et al*., 2022). Barricade® gel improves EPNs efficacy and longevity, and is non-toxic to the environment and easy to apply.

Superabsorbent hydrogels have been widely used in the agricultural sector for over 40 years. This has been conducted to ensure and enhance water availability for plants, by increasing the water holding properties of soil, especially sandy soils, reducing irrigation frequencies, and minimizing compaction, soil salinization and water run-off (Dhiman *et al.,* 2020). Potassium polyacrylate e can be used to inhibit is considered to be environmentally friendly and has the ability to save water, fertilizer, man power, improves nutrient usage, and improves soil conditions (Dhiman *et al.,* 2020). The aim of this study was to assess the effect of Barricade® gel and PPH formulations on the survival and efficacy of EPNs under laboratory conditions.

4.2. Materials and methods

4.2.1. Isolates

The three isolates of EPNs used, *Heterorhabditis bacteriophora* Poinar 1976 (SGI 245), *Steinernema tophus* (ROOI 352) and *Steinernema innovationi* Çimen *et al.*, 2014. These were initially isolated from soybean soil samples in Standerton, Mpumalanga province, South Africa (26° 56' 37.7556" S 29° 13' 13.638" E), from rooibos tea soil sample in Clanwilliam, Cape province, South Africa (32°10′43″S 18°53′28″E) and from rooibos tea soil sample in Clanwilliam, Cape Province, South Africa (32°10′43″S 18°53′28″E), respectively. An insect baiting technique was used in isolating these nematodes according to Ramakuwela *et al.* (2018).

The number of IJs were adjusted to 200 IJs / ml. One ml of IJs suspension was distributed evenly on a 9 cm filter paper in a lid of a 15 mm petri-dish. Ten larvae of *Galleria mellonella* L. (the greater wax moth, Lepidoptera: Phyralidae) were added in the petri-dish, covered with a lid and stored in a zip-block plastic. The Petri-dishes were then incubated at ± 25°C for three days. Cadavers were placed on a White trap, on a 15 mm petri-dish. After eight to 14 days IJs were harvested and washed three times (Kaya and Stock, 1997). The collected IJs were then stored in an aqueous suspension in horizontal culture flasks at 10°C, and were used within two weeks after harvesting.

4.2.2. In vivo and in vitro IJs production.

Entomopathogenic nematodes were produced *in vivo* using the protocol described by Ramakuwela *et al.* (2018) with minor modifications. Ten *G. mellonella* larvae were placed in a Petri-dish on top of the filter paper and inoculated using a concentration of 3 000 IJs ml^{-1}. For each isolates, ten Petri-dishes were prepared and incubated at ± 25 °C for 48 hr in the dark to ensure infection. Infected cadavers were individually transferred into modified White traps for

further incubation until IJs emerged. The IJs were then washed three times with autoclaved distilled water and used immediately in formulations.

The *in vitro* production of EPNs was achieved by means of a medium, based on pureed larvae of *Musca domestica* L., the common housefly (Diptera: Muscidae). The flies were reared at the Agricultural Research Council Small Grains research laboratory on a diet containing 2 kg bran, 300 g Nespray® milk powder, 6 g sodium benzoate, 20 g brewer's yeast, and 3 L lukewarm water. Five grams of *M. domestica* larvae were pureed using a blender, 0.15 g canola oil was added and the mixture was absorbed in sponge cubes. The media was then placed inside 250 ml conical flasks, closed with a cotton wool, covered with a foil and an elastic band to secure the foil before autoclaving at 121°C for 15 min. The media was allowed to cool down, and was inoculated with symbiotic bacteria and IJs at a concentration of 100 IJs ml^{-1}, and incubated at 25 °C for four weeks (Ramakuwela *et al*., 2018).

4.2.3. EPNs survival in different formulations and subsequent infectivity

Both *in vivo* and *in vitro* produced IJs were washed with sterile distilled water three times, to eliminate dead IJs by allowing the live IJs to settle at the bottom of a 50 ml centrifuge tubes. Infective juveniles suspensions were adjusted to 100 000 IJs / ml for each isolate, which creates a highly concentrated paste. Barricade® gel and PPH formulations were prepared using nematode paste of each EPNs species and each production method. A total of 80 ml of IJs suspension from each isolate, from both *in vivo* and *in vitro* were transferred to a 150 ml beaker. Thereafter, 2% of the gel (Barricade® gel /PPH) formulation was prepared by mixing 2 ml of Barricade® gel / PPH, 2 g of Barricade® gel /PPH and 98 ml IJs suspension in a 150 ml glass beaker. The 2 ml of the formulations were transferred into 2 ml Eppendorf tubes using a syringe, placed on a rack and covered with a foil. The formulations were incubated at ±14 ℃. The survival of the formulated IJs was assessed by counting the live and dead IJs under a stereomicroscope, in two weeks intervals. Three repetitions were prepared for each set of the formulation. Three 2 ml Eppendorf tubes were taken out every two weeks. Each 2 ml formulation was re-suspended and diluted with 20 ml of distilled water in a Petri dish. The first 50 IJs were counted as live or dead IJs in order to get a percentage survival using the nematode counting slide**.** The IJs were then adjusted to a concentration of 1 000 live IJs / ml and ten larvae of *Tenebrio molitor* L. (mealworm, Coleoptera: Tenebrionidae) per plate were infected to assess the infectivity of the surviving nematodes from the formulations. Plates were then incubated at 25 ̊C for 48 hrs. Mortality of mealworms was assessed after 48 hrs. The control treatments were

prepared using only the two EPNs suspensions (100 000 IJs / ml) without the gels. The same protocol for storage, assessment of survival and infectivity was followed as described above. The experiments were repeated three times using different nematode batches.

4.2.4. Statistical analysis

Collected data was analysed using the analysis of variance (ANOVA) on Genstat or SAS version. The standardized residuals were normally distributed (Shapiro–Wilks test), therefore the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 5% level of significance (Ramakuwela *et al*., 2020) and the area under the disease progress curve (AUDPC) was also used to compare the data (Jeger *et al.*, 2001).

4.3. Results

4.3.1. Entomopathogenic nematodes survival in gel formulations.

On Day One, there was 100% survival of *in vivo* produced EPNs in the three isolates and the control treatments. After two weeks, the *in vivo* produced EPNs survival decreased for each species, *H. bacteriophora* diminishing to 59%, *S. tophus* to 76%) and *S. innovationi* to 68%. The EPNs survival continued to decrease to 0% at eight weeks for all three isolates formulated in Barricade® gel. After two weeks 0% of the control treatments survived. In contrast, after two weeks, the survival of the *in vitro* produced EPNs formulated in Barricade® gel decreased: *Heterorhabditis bacteriophora* to 64%, *S. tophus* to 62% and *S. innovationi* to 58%. The survival of EPNs continued to decrease, ending at to 0% after eight weeks (Table 1).

The EPNs formulated in PPH had 100% survival of *in vivo* EPNs for three isolates and the control treatments on Day One. After two weeks, the survival of the *in vivo* EPNs decreased: *Heterorhabditis bacteriophora* SGI 245 to 59%, *S. tophus* to 76% and *S. innovationi* to 68%. The EPNs survival continued to decrease up to 0% after eight weeks for the three EPNs formulated in PPH. Control treatments survived for only less than two weeks (Table 1).

The survival of EPNs produced *in vitro* and formulated in PPH was tracked at two weeks intervals. On Day One, 100% of the EPNs were viable for all three isolates. At eight weeks, the survival of *in vitro* grown EPNs in PPH decreased: *H. bacteriophora* SGI 245 to 10%, *S. tophus* to 2% and *S. innovationi* to 1%. None of the controls grown *in vitro* EPNs survived after two weeks. There was a significant difference in the survival of the three EPNs in either formulation compared with the controls (Table 1).

Table 4.1: Survival counts of EPNs produced *in vivo* and *in vitro* and formulated in either Barricade® gel (B) or Potassium polyacrylate hydrogel (PPH) with significance letters (LSD (P<.0001) = 20.64).

Strain	**Formulation**	**Day 1**	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
SGI 245	*In vivo* B	100 r	59 lkjih	56 hi	37 e	13 d
Heterorhabditis bacteriophora	*In vitro* B	100 r	64 ponmlk	65 ponmlk	67 pon	8 dcb
	In vivo PPH	100 r	59 lkjih	55 ponml	37 e	13 d
	In vitro PPH	100 r	61 nmlkji	63 ponmlkj	68 po	10 dc
	In vivo control	100 r	0 a			
	In vitro control	100 r	0 a			
ROOI 352	*In vivo* B	100 r	76 q	60 nmlkji	47 fg	4 ba
Steinernema tophus	*In vitro* B	100 r	62 onmlkji	57 jih	66 ponm	0 a
	In vivo PPH	100 r	76 q	60 ijklmn	47 gf	4 ba
	In vitro PPH	100 r	62 onmlkji	63 ponmlkj	66 ponm	2 ba

	In vivo control	100 r	0 a			
	In vitro control	100 r	0 a			
SGI 35 ***Steinernema innovationi***	*In vivo* B	100 r	68 po	53 gh	47 f	0 a
	In vitro B	100 r	58 kijh	63 ponmlkj	68 po	0 a
	In vivo PPH	100 r	68 po	53 ba	47 f	0 a
	In vitro PPH	100 r	60 mlkji	69 p	69 b	1 a
	In vivo control	100 r	0 a			
	In vitro control	100 r	0 a			

The figures for the area under the survival curve of in vivo produced EPNs in Barricade® gel were 1535 for *H. bacteriophora* SGI 245, 1666 for *S. innovationi* SGI 35 and 1535 for *S. tophus* ROOI 352. The in vitro produced EPNs in Barricade® gel with the areas 1778 for *H. bacteriophora* SGI 245, 1671 for S. tophus and 1675 for *S. innovationi* SGI 35 (Fig.1).

With the PPH formulations of in vivo produced EPNs, the areas under the survival curve were 1535 for *H. bacteriophora* SGI 245, 1666 for *S. tophus* ROOI 352 and 1545 for *S. innovationi.* The PPH formulated in vitro produced EPNs had areas under the survival curve for *H. bacteriophora* SGI 245, *S. tophus* ROOI 352 and *S. innovationi* SGI 35 of 1764, 1694 and 1694, respectively(Fig.1).

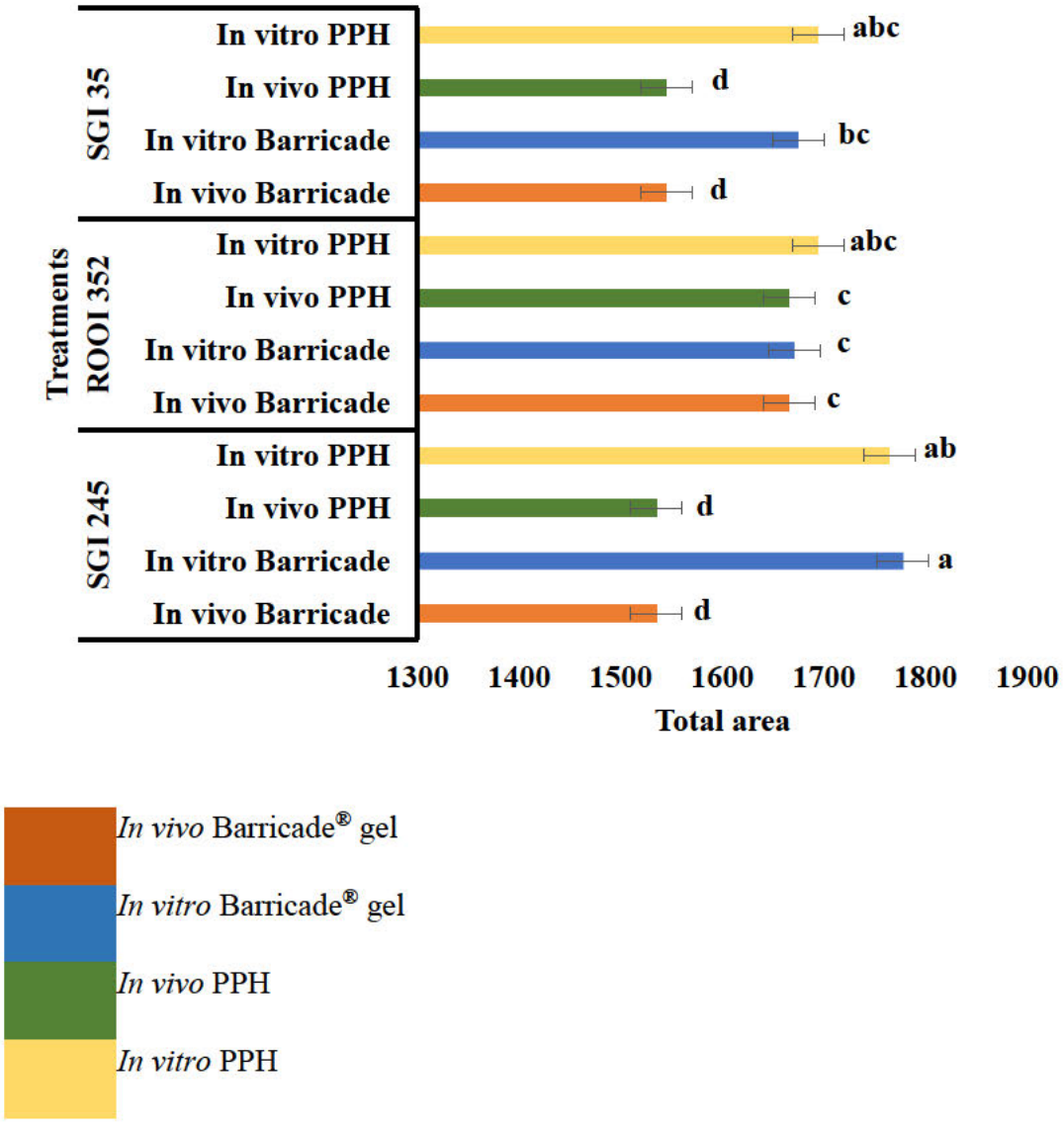

Figure 1: Histogram of the area under the curve for the survival of three EPNs (*Heterorhabditis bacteriophora*, *Steinernema tophus*, *Steinernema innovation)* in two formulations over an eight weeks period.

4.3.2. Efficacy of EPNs

The efficacy of the three control treatments was 0% mortality after two weeks. With the EPNs produced in vivo and in vivo, and formulated in Barricade® gel or PPH, efficacy diminished from Day One. After eight weeks, the three isolates caused 0% mortality for both production methods and formulations. (Table 2)

Table 2: Table showing the efficacy of EPNs produced in vivo in a Barricade® gel formulation or Potassium polyacrylate hydrogel (PPH) with significance letters (LSD (p= 0,05) = 6,1742).

Strain	**Formulation**	**Day 1**	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
SGI 245 ***Heterorhabditis bacteriophora***	*In vivo* B	100 a	90 bc	93 ab	70 fg	57 ij
	In vitro B	100 a	83 dc	77 def	70 fg	50 j
	In vivo PPH	100 a	90 bc	90 bc	70 fg	53 ij
	In vitro PPH	100 a	90 bc	80 de	73 efg	5 ij
	In vivo control	100 a	0 k			
	In vitro control	100 a	0 k			
ROOI 352 ***Steinernema tophus***	*In vivo* B	100 a	97 ab	100 a	83 dc	90 bc
	In vitro B	100 a	97 ab	73 efg	67 gh	
	In vivo PPH	100 a	100 a	100 a	90 ab	93 ab

	In vitro PPH	100 a	100 a	80 de	67 gh	93 ab
	In vivo control	100 a	0 k			
	In vitro control	100 a	0 k			
SGI 35 ***Steinernema innovationi***	*In vivo* B	100 a	93 ab	90 bc	80 de	
	In vitro B	100 a	80 de	77 def	57 ij	
	In vivo PPH	100 a	93 ab	90 bc	80 de	
	In vitro PPH	100 a	80 de	80 de	60 ih	93 ab
	In vivo control	100 a	0 k			
	In vitro control	100 a	0 k			

The Barricade® gel formulation with *in vivo* produced EPNs for the three isolates, *H. bacteriophora* SGI 245, *S. tophus* ROOI 352 and *S. innovationi* SGI 35 had area under the mealworm mortality curve figures of 569, 588 and 504, respectively. With *in vitro* produced EPNs formulated in Barricade® gel, the areas for *H. bacteriophora* SGI 245, *S. tophus* and *S. innovationi* SGI 35 were 509, 541and 462, respectively (Fig.2).

With the PPH formulation of *in vivo* produced EPNs the areas under the mortality curve for the three isolate *H. bacteriophora* SGI 245, *S. tophus* ROOI 352 and *S. innovationi* SGI 35 were 574, 565 and 495, respectively. With the *in vitro* produced EPNs formulated in PPH, *H. bacteriophora* SGI 245, *S. tophus* ROOI 352 and *S. innovationi* SGI 35 had areas under the mortality curve of 509, 546 and 485, respectively (Fig.2).

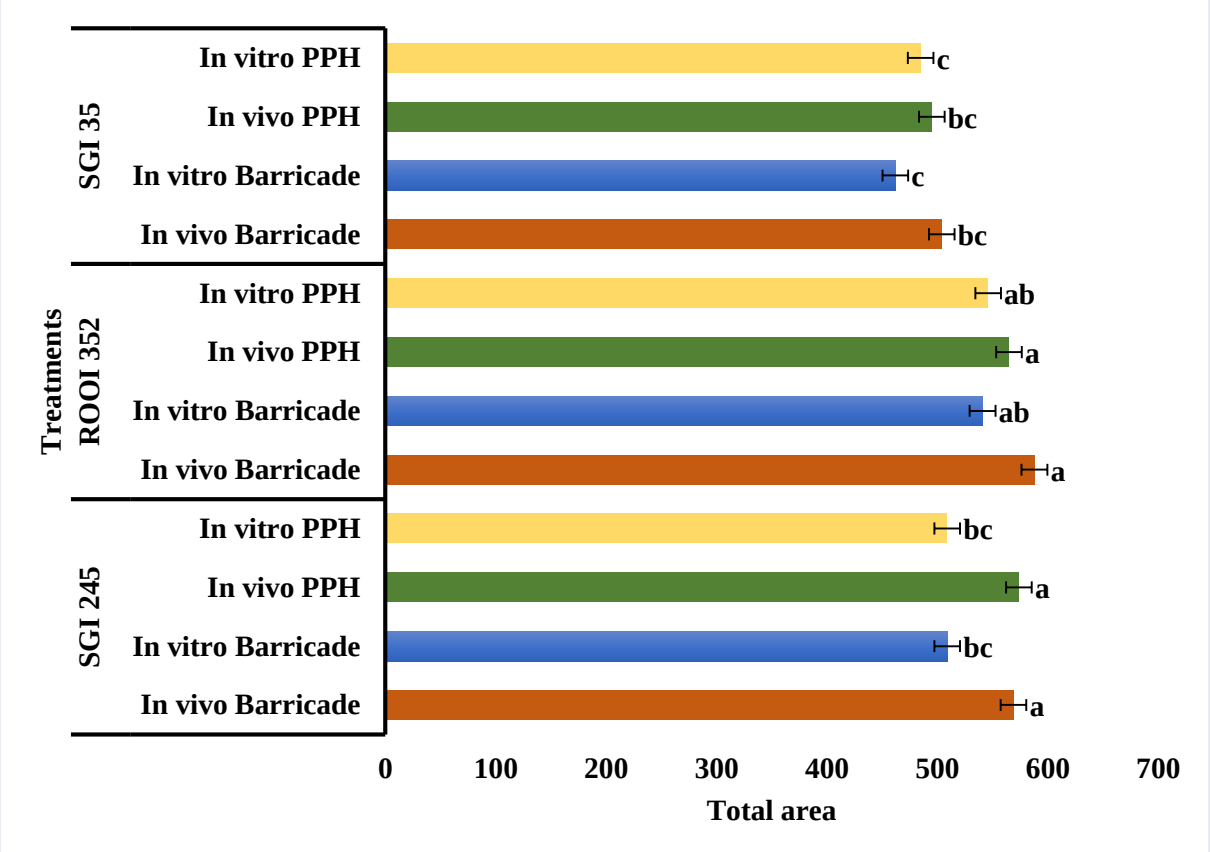

Figure 2: Area under the curve for mortality of mealworms infected with three EPNs, produced *in vivo* or *in vitro,* with two formulations, in Barricade® gel or PPH The cumulative area is from mealworm mortality data over eight weeks, and reflects the cumulative efficacy of the EPN strain, production method and formulation.

4.4. Discussion

On Day one, the survival counts of all the EPNs in the Barricade® gel and PPH formulations and in the control formulation were 100%. After two weeks all of the control formulated EPNs were dead. However, after 2 weeks, more than 50% of the formulated EPNs survived. This confirmed that both formulations enhanced the survival of the three EPNs, produced both *in vivo* and *in vitro*. Acar *et al.* (2022) conducted a study on enhancing the biological control potential of *Steinernema feltiae* Filipjev formulated for protection from desiccation and UV radiation. The study concluded that the survival of *S. feltiae* can be enhanced by application with a desiccant protectant such as Barricade® gel. These results are in agreement with the results obtained in this study. After eight weeks, the survival of EPNs, with both the Barricade® gel and PPH formulations, were below 20% for *H. bacteriophora* (SGI 245) and *S. tophus* (ROOI 352), and 0% for *S. innovationi* (SGI 35). However, at 6 weeks, the survival levels were roughly 40-60% for all three EPNs and both formulations. The results are also in agreement with the study conducted by (Abate *et al.*, 2019), where it was concluded that nematode survival was highest in a gel medium, followed by a gel-soil mixture, and lowest in soil. Kagimu *et al* (2017) developed formulation of South African EPNs using alginate beads and diatomaceous earth; where the alginate beads successfully retained most of the IJs and can be stored for a longer time.

The survival of EPNs produced *in vivo* and *in vitro* were similar after two weeks. However, at four and six weeks, the EPNs produced *in vitro* had a higher level of survival for two of the species. At eight weeks, there was a similar level, low of survival for EPNS produces in both ways.

There was little difference in the ability of Barricade® gel and PPH to protect the EPNs, and levels of survival were similar for the same species produced over the eight weeks period.

The use of the area under the curve for mortality of mealworms infected with three EPNs produced *in vivo* or *in vitro* and with two formulations calculated from the accumulated mortality data over eight weeks provided very similar data. The best performing EPN was ROOI352, given that all of the products were in the top performing group (letters “a” or “ab”). SGI245 performed well, especially the two *in vivo* formulations, which also fell in to the top

performing group (letter “a”). SGI35 was the worst performing EPN, with the all four products falling into the worst performing category (letter “c”). The *in vitro* production method was less effective than the *in vivo* production method for all three EPN strains. However, the *in vitro* method is a scaleable technology that allows for the large-scale production of EPNs, which the *in vivo* production method does not. There were no significant differences between the two formulations, Barricade® gel and PPH for similar strains and production methods. However, the PPH formulation is more accessible and cheaper than Barricade® gel. Hence, the product with the most commercial potential would be most potential would be the EPN strain ROOI352 produced *in vitro* and formulated in PPH.

Glazer *et al*. (2008) conducted a study on the field efficacy of EPNs against the beetle *Maladera matrida* Brenske (Coleoptera: Scarabaeidae). Their findings were in agreement with our findings that EPNs could still be effective after 78 days.

4.5. Conclusion

EPNs were able to survive up to six weeks in both the Barricade® gel and PPH formulations. In contrast, the control treatments survived for less than two weeks. The *in vitro* production method was slightly less effective than the *in vivo* production method for all three EPN strains, but it is a more commercially viable production method. The two gel formulations were equally effective. This favours PPH formulations as is readily available and cheaper than is a cheaper than Barricade® gel. Overall, the best commercial product would be the EPN strain ROOI352 produced *in vitro* and formulated in PPH.

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Chapter 5.

Persistence of entomopathogenic nematode formulations in five sweet potato fields

Abstract

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae, coupled with their symbiotic bacteria, are utilised for the biological control of a wide range of agricultural insect pests. Persistence of EPNs in the field is affected by a number of abiotic factors such as UV light, fluctuation in temperature and desiccation. The aim of this study was to assess the persistence of EPNs under field conditions, applied in two different formulations (cadaver formulation and Barricade® gel formulation). Persistence was assessed after one and two months post treatment application by baiting soil with the greater wax moth larvae (*Galleria mellonella*) and observing the insect mortality. *Heterorhabditis bacteriophora* (SGI 245) cadaver formulation showed better survival in all the five sites, followed by *H. bacteriophora* Barricade® gel formulation. Persistence declined drastically two months post treatment from a maximum 100% to 0%. The findings suggest that EPNs post application survival in the field can be enhanced by both cadaver and Barricade® gel formulations.

Keywords: Barricade® gel, biological control, desiccation, entomopathogenic nematodes, formulation, persistence,

5.1 Introduction

Entomopathogenic nematodes (EPNs) from the order Rhabditida (Heterorhabditidae and Steinernematidae) are characterised by their pathogenic ability to kill insects with their symbiotic bacteria (Platt *et al*., 2019). The infective juveniles (IJs), which are the free-living, non-feeding survival stage of the EPNs, can easily be mass cultured, formulated and applied as biological control agents for use against insect pests (Kagimu *et al.*, 2017). Previous studies have shown that EPNs can be successfully used as a biocontrol agent against a number of insects. However, the application of EPNs commercially is affected by a number of environmental factors. As soil-adapted organisms, EPNs are poorly suited to above-ground environmental conditions, which often feature low relative humidity, extremes of temperature and exposure to ultraviolet (UV) radiation. UV light affects EPNs applied above ground, as both UV light and sunlight have been shown to affect the behaviour and pathogenicity of the EPNs (Kurtz *et al.*, 2007). This negatively impacts on the efficacy of EPNs (Platt *et al*., 2019). There have been a number of methods explored to ensure persistence of EPNs in the fields such as formulations, time and method of application (De Luca *et al*., 2015). Long term factors that determine EPN persistence includes host availability, population dynamics, alternative hosts, competition for hosts, in-host survival, suitable environmental conditions, and stable ecosystems such as pasture and alfalfa, which favour long-term persistence. EPNs populations can persist for years after application (Koppenhöfer *et al*., 2009).

Entomopathogenic nematodes are highly sensitive to changes in temperature, and are basically stored in aqueous solutions ranging in temperature from 4°C to 30°C. Most species are intolerant of temperatures higher than 35°C for longer than 30 min (Wright *et al.*, 2005). Comparatively high temperatures also reduce the solubility of oxygen in solution. Depriving EPNs of oxygen for prolonged periods of time results in their deactivation and subsequent death (Hatting, 2015). The commercialisation of EPNs depends on the shelf life, persistence post application. This is achieved EPNs formulation. Different EPN species also have different thermal niches within which they can infect and establish themselves within their respective hosts (Platt *et al*., 2019).

EPNs can survive for years in the soil. Their survival depends on a number of pre-application and post-application factors. The quality of EPNs is the major pre-application factor that is looked at when tracking and analysing the persistence of EPNs (Bayene *et al*., 2015). Entomopathogenic nematodes quality is influenced by the production method of EPNs, storage

after production, distribution to the growers or the field of application, storage on shelf-life and application method/apparatus (Griffin, 2015).

Persistence of EPNs in the field is regularly checked by baiting soil with insects and reporting the proportion of bait insects that are killed (Duncan *et al.*, 2013). The aim of this study was to assess the persistence of two EPN species formulated in protective formulations (Barricade gel and insect cadavers), under field conditions.

5.2. Materials and methods

5.2.1. Source of EPNs isolates and inoculum production

The two isolates used, *Heterorhabditis bacteriophora*, Poinar, 1976 (SGI 245) and *Steinernema tophus*, Cimen 2016 (ROOI 352) were initially isolated from soybean soil samples in South Africa, Mpumalanga province in Standerton (26° 56' 37.7556" S 29° 13' 13.638" E) and from a rooibos tea soil sample from Western Cape province in Clanwilliam (32°10′43″S 18°53′28″E), respectively. Insect baiting technique was used in isolating these EPNs according to Kaya and stock (1997). Briefly, soil samples were placed inside 500 ml containers. Five last instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus (Pyralidae: Lepidoptera), were placed inside each container, which were closed but with lids perforated with small holes to allow air circulation. Each tin was placed upside down. After 5 days, cadavers were transferred to White traps (Kaya and stock, 1997) followed by harvesting IJs and storage in water at 10°C.

Entomopathogenic nematodes were produced *in vivo* using the protocol described by Ramakuwela *et al.* (2018) with minor modifications. *Galleria mellonella* infected with EPNs isolates selected for the study. *Galleria mellonella* larvae were placed in a Petri-dish on top of the filter paper and inoculated using a concentration of 3 000 IJs ml^{-1}. For each isolates, ten Petri-dishes were prepared and incubated at ± 25 °C for 48 hr in the dark to ensure infection. Infected cadavers were individually transferred into modified White traps for further incubation until IJs emerged. The IJs were then washed three times with autoclaved distilled water and stored at 15ºC.

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5.2.2. Trial site and trial plan

The study was conducted in three provinces and at five different sites: Bela-Bela, Limpopo ($25^0 45'52.8'' 28^0 13'26.0''$E); Brits (GMMM), North West Province ($25^0 05'17.7''$S $27^0 46'42.7''$E);

Brits (Klipvoor), North West Province (25.0926° S, 27.7725°E); Bronkhorstspruit, Gauteng ($25^0$43 '19.4"S $28^0$43 '29.0"E); and Meyerton, Gauteng ($26^0$32 '39.0"S $27^0$59 '57.8"E).

A randomised complete block design was used, with two EPN species and two formulations. Each trial site had three blocks. Three plants were planted about 3-5 cm apart per treatment in a block with 2 m spacing between blocks (treatments) (Table 1).

Table 1: Trial plan

B4	C3	A3	D4
D1	A4	C2	B1
D2	D3	C1	A1
C4	B3	A2	B2

BLOCK 2

A2	B1	C3	D4
D1	C2	B3	A1
A3	C1	B4	D2
A4	D3	C4	B2

BLOCK 3

C4	D1	B4	A3
A4	B2	C3	D2
D3	C2	B1	A1
A2	B3	C1	D4

A - *Steinernema tophus*, Formulation 1 (Barricade® gel,)
B - *Steinernema tophus*, Formulation 2 (Cadaver)
C - *Heterorhabditis bacteriophora*, Formulation 1 (Barricade® gel)
D - *Heterorhabditis bacteriophora*, Formulation 2 (Cadaver)

5.2.3. Treatments application and soil sample collection

Both strains were formulated in Barricade gel and in cadavers and they were applied one month after planting as per trial plan above at the five sites. The Barricade® gel formulation was formulated by mixing 2ml of the gel and 98ml of IJs suspension that was adjusted to

3000IJs/ml to make 2% gel formulation. The Barricade® gel formulation was applied by spraying five ml per plant directly onto the plant canopy using a spray bottle.The cadevar formulation was formulated by infecting *G. mellonella* with the two selected EPN strains and applying them just before IJ emergence. The *G. mellonella* cadavers were applied by burying one cadaver per plant 5cm below ground.

The soil samples were collected from each treatment block, one scoop was collected from each treatment from block one to block three in one plastic bag for each treatment and stored in a cooler box for transportation to the lab for evaluation. Persistence of EPNs in the field was evaluated by mixing the representative sample and baiting 500g of soil from each representative sample using *G. mellonella* larvae (Kaya and Stock, 1997), incubating the samples at 25°C and recording the number of cadavers after five days (Duncan *et al.*, 2013). Baiting of the same soil sample was repeated after 10 and 15 days to deplete the IJs in each sample. A second set of soil samples was collected two months after treatment application, and persistence was evaluated as described for one month samples.

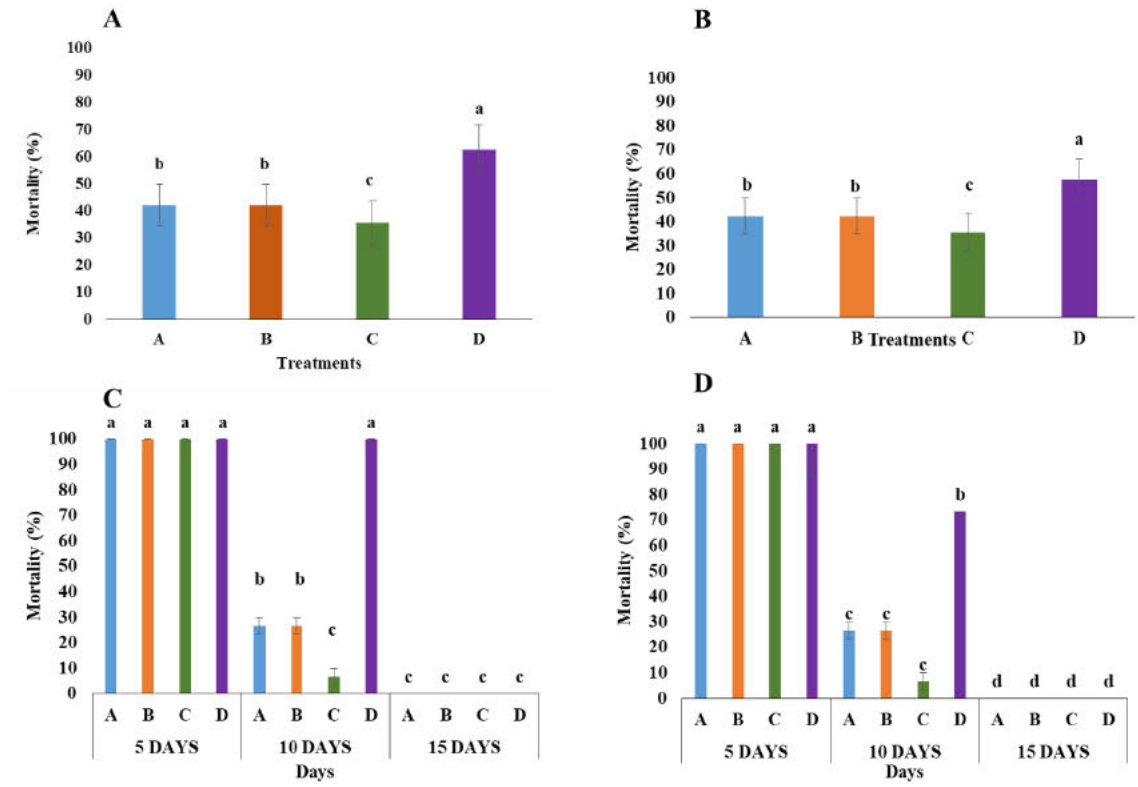
5.2.4. Statistical analysis

Collected data were analysed using analysis of variance (ANOVA) using Statistical Analysis Software (SAS). The standardized residuals were normally distributed (Shapiro–Wilks test) and therefore the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 0.5% level of significance (DeLucca *et al*., 1990).

5.3. Results

BelaBela (Limpopo) trial site: The *Heterorhabditis bacteriophora,* (SGI 245), cadaver formulation (Treatment D) gave highest mortality level of 63% and 58% one and two months post application, respectively (Figure 1). The mortality of the same species, *H. bacteriophora* (SGI 245) in Barricade® gel (Treatment C) was 36% at both one and two months sampling. *Steinernema tophus* (ROOI 352) cadaver formulation, (Treatment B) caused an overall mortality of 42% in both one and two months sampling and the same species in Barricade® gel (Treatment A) caused 42% mortality of *G. mellonela,* at both one month and two months sampling. There was a statistical significance ($f = 0.55$; $df = 85.57071$; $p = 0.7007$) between Treatment D and C at both one and two months sampling, with the cadaver formulation (Treatment D) outperforming the gel formulation (Treatment C) (Fig.1).

At day five baiting of both one and two months samples, all the treatments obtained 100% mortality. On one month samples, mortality decreased for treatment A, B, C to 27% (Aand B),and 7% for Treatment C after 10 days while Treatment D (*H. bacteriophora*, cadaver) obtained 100%. After 15 days, all the treatments obtained 0% mortality (Figure 1 C). For two months samples (Figure 1 D), The same trend was observed with 100% mortality after 5 days, decline after ten days with Treatment D being significantly different ($f = 12.45$; $df = 166.2080$; $p = 0.0001$) than all other treatments and no mortality for all treatments after 15 days (Fig.1).

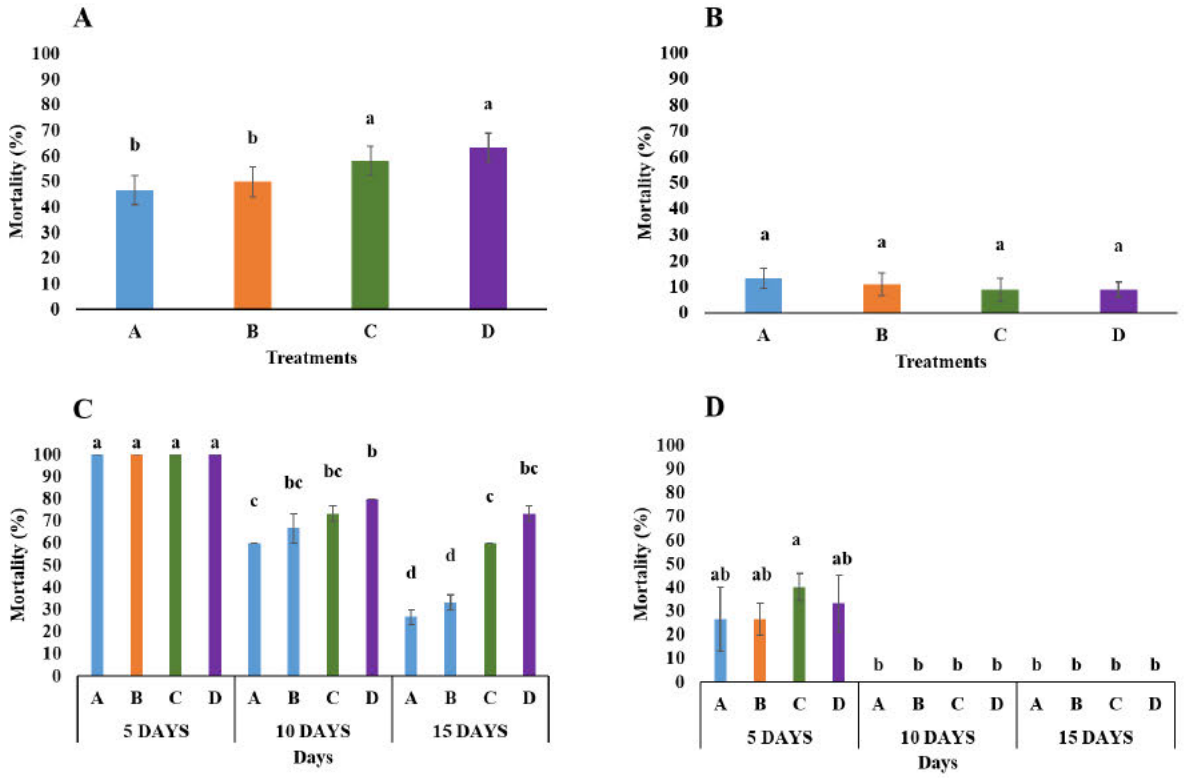


A - *Steinernema tophus*, Formulation 1 (Barricade® gel,)
B - *Steinernema tophus*, Formulation 2 (Cadaver)
C - *Heterorhabditis bacteriophora*, Formulation 1 (Barricade® gel)
D - *Heterorhabditis bacteriophora*, Formulation 2 (Cadaver)

Figure 1: Persistence of infective juveniles as reflected by mortality of *Galleria mellonella* in baited soil samples from treated plots in BelaBela (Limpopo) trial site. **A**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, one month post treatment application (**B**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, two month post treatment application; **C**-Mortality of *G. mellonella* after baiting one month sample successively after 5, 10 and 15 days; **D**- Mortality of *G. mellonella* after baiting and traping one month each sample three successive times successively after 5, 10 and 15 days). Bars with the same lowercase letter indicate no significant difference.

GMMM (Brits North West) trial site: At one month, Treatment C and D (*H. bacteriophora*) gave better mortality (63%) (f = 4.90, CV = 14.95872), p = 0.0245) than Treatment A and B (the *S. tophus* formulations) (Figure 2A). Overall mortality for two months samples declined to less than 20% for all formulation (Figure 2B). After 10 days, mortality decreased from 100% for treatment A, B, C and D to 60%, 67% , 73% and 80% respectivly Although there was decline

in mortality after 15 days for one months samples, Treatment C and D were significantly greater than Treatment A and B (f = 10.46, CV = 14.95872, p = 0.0245). At two month sampling, all treatments showed decline in mortality with 0% mortality after 10 and 15 days baiting (Figure 2D). There was no statistical difference in all the treatments after two months sampling (f = 0.10, CV = 189.4737, p = 0.0245) (Fig.2).



A - *Steinernema tophus*, Formulation 1 (Barricade® gel,)
B - *Steinernema tophus*, Formulation 2 (Cadaver)
C - *Heterorhabditis bacteriophora*, Formulation 1 (Barricade® gel)
D - *Heterorhabditis bacteriophora*, Formulation 2 (Cadaver)

Figure 2: Persistence of infective juveniles as reflected by mortality of *Galleria mellonella* in baited soil samples from treated plots in GMMM (Brits North West) trial site. **A-** Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, one month post treatment application (**B-** Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, two month post treatment application; **C**-Mortality of *G. mellonella* after baiting one month sample successively after 5, 10 and 15 days; **D-** Mortality of *G. mellonella* after baiting and traping one month each sample three

successive times successively after 5, 10 and 15 days). Bars with the same lowercase letter indicate no significant difference (**Figure 2)**.

Klipvoor (North West) trial site: *Heterorhabditis bacteriophora* SGI 245 cadaver formulation,(Treatment D) survived at a level of 50% and 6% in Sample one and two respectively. *Heterorhabditis bacteriophora* (SGI 245) in Barricade® gel (Treatment C) survived at a level of 45% and 8% in Sample one and Sample two respectively. There was no significant difference (f = 3.21, CV = 15.29527, p = 0.0007) between the two formulations of *H. bacteriophora* SGI 245 at one and two month sampling. *Steinernema tophus* (ROOI 352) cadaver and gel formulation, Treatment A and B caused an overall mortality of 42% in one month sampling and there was no mortality at two months sampling. There was no statistical significance between Treatment C and D in both one and two month sampling and survival was low (<10%) for all treatments (f = 3.21, CV = 15.29527, p = 0.0007) (Fig.3).

At day five baiting of one month samples, all the treatments obtained 100% mortality. At day ten, mortality decreased for treatment A, B, C and D to 26%, 33% , 60% and 67% respectivly with Treatment C and D causing higher mortality than Treatment A and B. At day 15, treatment A, B, C and D obtained 0%, 6%, 20% and 33%%. Trweatment D performed better than all other treatments (Fig.3).

On two months smples; At day five, mortality for treatment A, B, C and D to 0%, 0% , 20% and 13% respectivly with no significant difference. At day ten, all the treatment (A, B, C and D) obtained 0% mortality (f = 1.04, CV = 282.9259, p = 0.0007) (Fig.3).

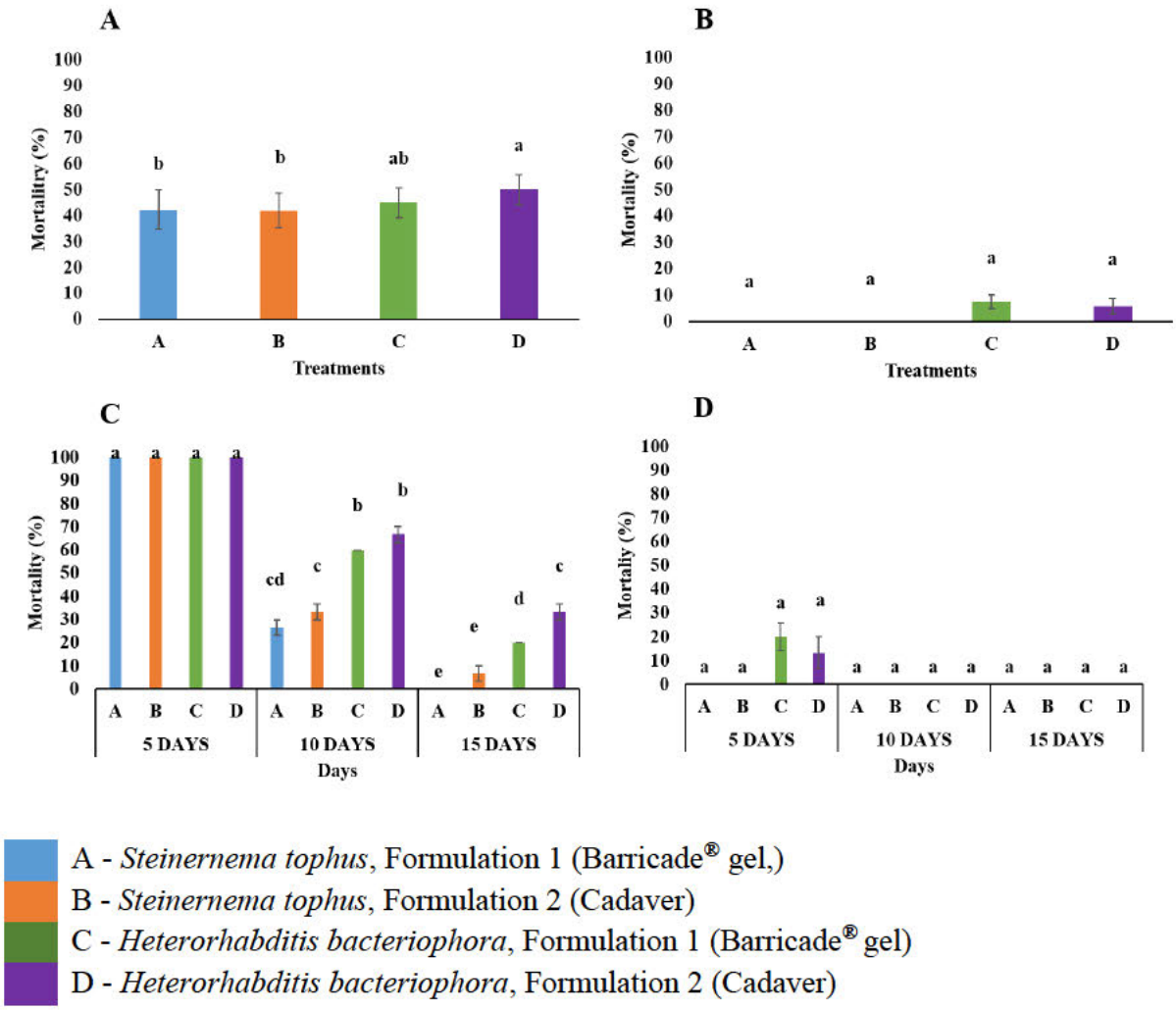


Figure 3: Persistence of infective juveniles as reflected by mortality of *Galleria mellonella* in baited soil samples from treated plots in Klipvoor (North West) trial site. **A-** Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, one month post treatment application (**B-** Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, two month post treatment application; **C**-Mortality of *G. mellonella* after baiting one month sample successively after 5, 10 and 15 days; **D-** Mortality of *G. mellonella* after baiting and traping one month each sample three successive times successively after 5, 10 and 15 days). Bars with the same lowercase letter indicate no significant difference.

Bronkhorstspruit (Gauteng) trial site: Results were similar to the Bronkhorspruit trial with Treatment C and D causing overall mortality (58% and 57%) than A and |B at one month sampling (45% and 47%), followed by decline A, B, C and D(0%, 9%, 3% and 13%) with no

significant difference ((f = 5.07, 204.3345, CV = 18.44090, p = 0.0001) between all treatments (Fig.4).

At, five days baiting of one month samples, all the treatments obtained 100% mortality. At day ten, mortality decreased for treatment A, B, C and D to 53%, 60% , 87% and 93% respectivly nd Treatment C and D were higher than A and B with no statitical difference. At day 15, treatment A, B, C and D obtained 13%, 13%, 27% and 33%. There was no statistical difference between treatment D and C on one month samples (Fig.4).

For two months samples, mortality for treatment A, B, C and D to 0%, 20% , 7% and 33% respectivly at five days baiting without significant differences (f = 1.25, 204.3345, CV = 18.44090, p = 0.0001). At day ten, all the treatment (A, B, C and D) obtained 0% (Fig.4).

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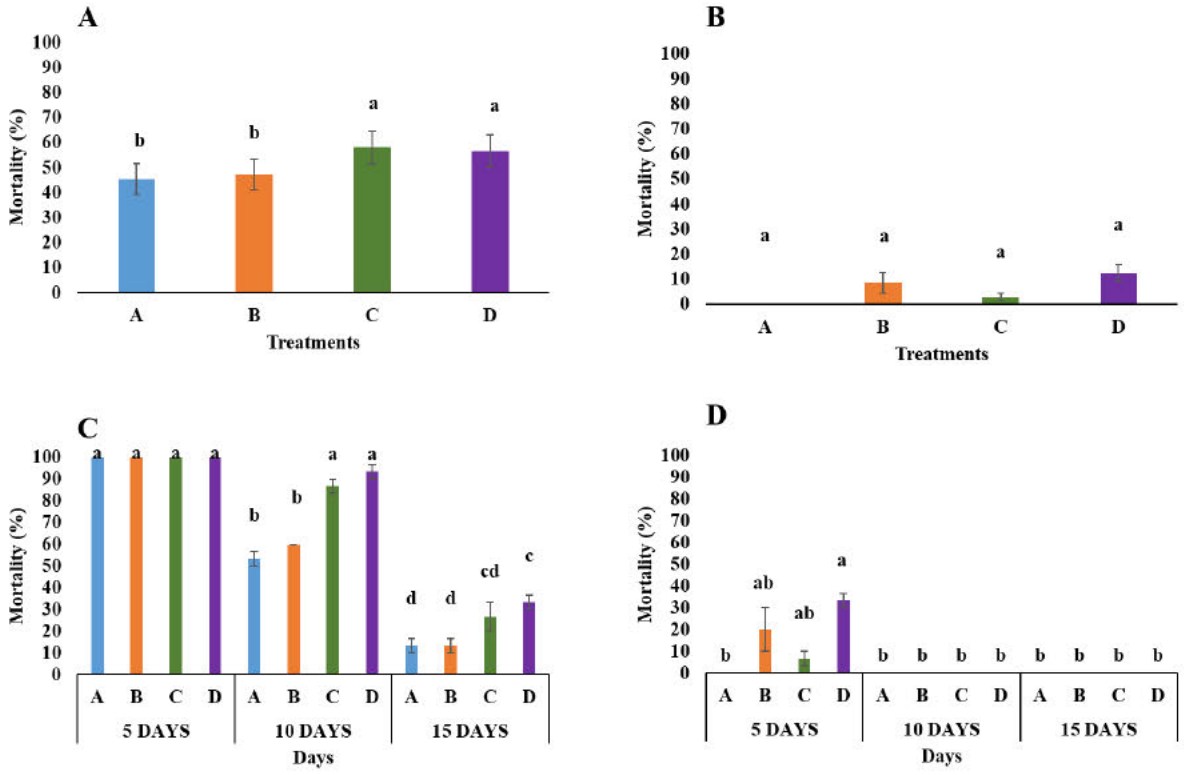

A - *Steinernema tophus*, Formulation 1 (Barricade® gel,)
B - *Steinernema tophus*, Formulation 2 (Cadaver)
C - *Heterorhabditis bacteriophora*, Formulation 1 (Barricade® gel)
D - *Heterorhabditis bacteriophora*, Formulation 2 (Cadaver)

Figure 4: Persistence of infective juveniles as reflected by mortality of *Galleria mellonella* in baited soil samples from treated plots in Bronkhorstspruit (Gauteng). trial site. **A**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, one month post treatment application (**B**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, two month post treatment application; **C**-Mortality of *G. mellonella* after baiting one month sample successively after 5, 10 and 15 days; **D**- Mortality of *G. mellonella* after baiting and traping one month each sample three successive times successively after 5, 10 and 15 days). Bars with the same lowercase letter indicate no significant difference.

Meyerton (Gauteng) trial site: *Heterorhabditis bacteriophora* SGI 245 (Treatment D) caused mortality of 53% and 13% in Sample one and two respectively. *Heterorhabditis bacteriophora* (SGI 245) in Barricade® gel (Treatment C) caused 48% and 10% mortality at one and two month sampling, respectively. *Steinernema tophus* (ROOI 352) cadaver formulation, Treatment B caused an overall mortality of 42% in one month sampling and 13% in two months sampling. *Steinernema tophus* (ROOI 352) in Barricade® gel (Treatment A) caused 38% mortality of *G. mellonella*, at one month and 10% in two months sampling. There was no statistical significance (f= 12.47, CV = 13.78881, p = 0.0001) between Treatment D and C in both one and two month sampling and there was no statistical difference (f = 0.13, CV = 151.6040, p = 0.0001) in all the treatments after two months sampling (Fig.5).

At day five baiting of one month samples, all the treatments obtained 100% mortality. At day ten, mortality decreased for treatment A, B, C and D to 60%, 47% , 47% and 60% respectivly. At day 15, treatment A, B, C and D obtained 0%, 0%, 7% and 7% with no significant differences (Fig.5).

For two months smples; At day five, mortality for treatment A, B, C and D to 27%, 33% , 27% and 40% respectivly. At day ten, all the treatment (A, B, C and D) obtained 0% (Fig.5).

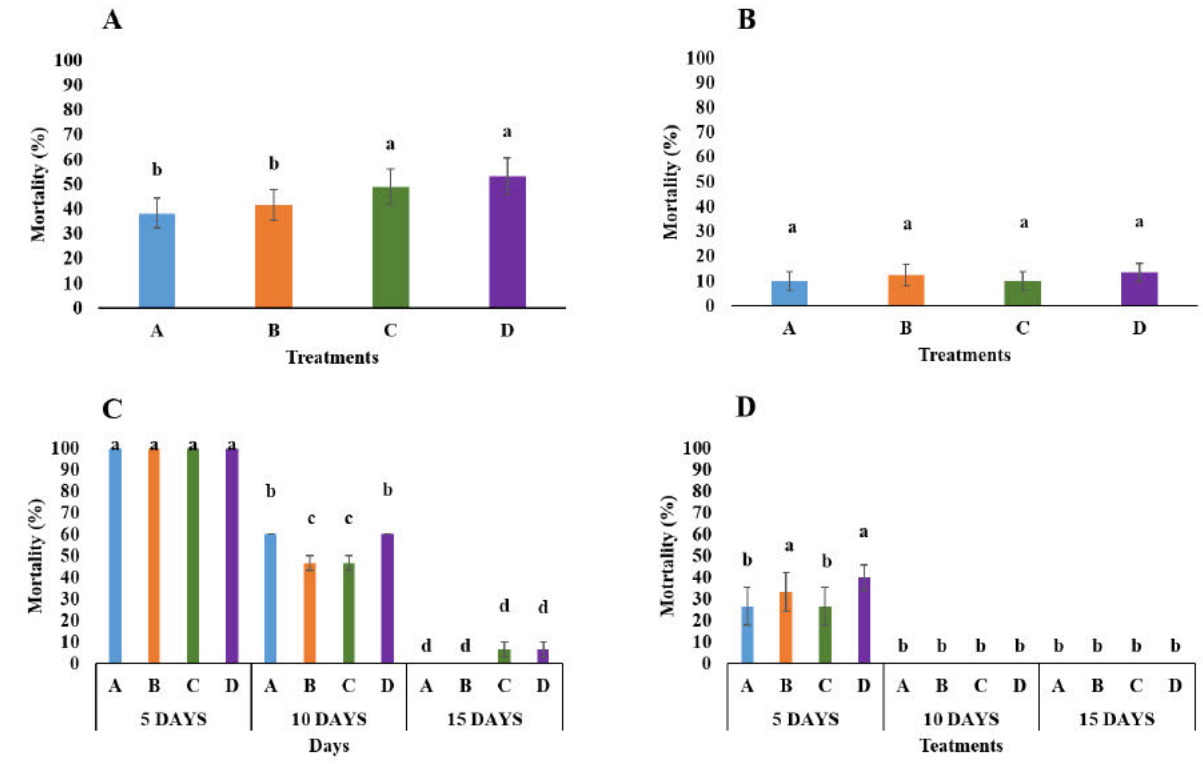

Figure 5: Persistence of infective juveniles as reflected by mortality of *Galleria mellonella* in baited soil samples from treated plots in Meyerton (Gauteng) trial site. **A**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, one month post treatment application (**B**- Overall cummulative mortality of *G. mellonella* formulations after three repeated soil baiting in 15 days, two month post treatment application; **C**-Mortality of *G. mellonella* after baiting one month sample successively after 5, 10 and 15 days; **D**-Mortality of *G. mellonella* after baiting and traping one month each sample three successive times successively after 5, 10 and 15 days). Bars with the same lowercase letter indicate no significant difference.

5.4. Discussion

There was variation in persistence of IJs of different species in different formulations. Long-term persistence of EPNs depends to a large extent on their ability to find and reproduce in suitable hosts (Shapiro-Ilan *et al.*, 2006). The probability of recycling and potential for

prolonged insect control depends on how long the EPNs can survive without a host (Shapiro-Ilan *et al*., 2006).

Different EPNs species vary in their susceptibility to UV light and temperature. Gaugler *et al.* (1992) found that *Steinernema carpocapsae* (Weiser) 1955 IJs were rendered completely inactive after 10 min of moderate UV exposure, whereas *H. bacteriophora* was significantly affected after only 4 minutes, indicating that the susceptibility to UV light varies across species. Contrary to the current study, IJ survival/persistence was recorded for at least one moth. However, *S. tophus* was less persistent than the *H. bacteriophora*. There was a higher percentage of EPNs persistence at one month sampling after treatment application than at the sampling at two months after treatment application. Persistence declined drastically two months post treatments application. This may have been as a result of UV light, high temperatures and a low number of host insects for reproduction. *Heterorhabditis bacteriophora* (SGI 245) in cadaver formulation survived better, possibly because it was applied five cm below the ground, hence the EPNs were less affected by UV light and high above ground temperature. However, there was no statistical significance between cadaver and the Barricade® gel formulation in most of the field trials for this species which may suggest that the Barricade® gel was protective. Barricade® gel Retardant forms a unique thermal protective gel-coating that stops fires from burning. Barricade® gel was found to improve the efficacy of *Steinernema carpocapsae* for control of the lesser peach tree borer, *Synanthedon pictipes* (Grote and Robinson 1868) lesser peachtree borer. It was also found to improve longevity and efficacy of *S. carpocapsae* (Shapiro-Ilan *et al.,* 2010). However, it does not kill pests and it is not deleterious to *S. carpocapsae* (Shapiro-Ilan *et al.*, 2016).

Gaugler *et al*. (1978) observed the effects of short UV radiation and natural sunlight on *S. carpocapsae,* in terms of their interaction with *G. mellonella* larvae. They concluded that exposure of IJs to short-term UV radiation for 7 minutes cause reduced pathogenicity and increased larval survival time post-infection. Exposure to direct sunlight also reduced their pathogenicity by as much as 95% after 60 minutes. This study present strong results in which good *G. mellonella* mortality (> 50%) was obtained one month post treatment application and in few instances, two months post application.

Soil type also plays a role in the persistence of EPNs, however it is not significant according to the literature review (Pilz *et al*., 2014). Koppenhöfer *et al*., 2006 conducted a study in "Effect of soil type on infectivity and persistence of the EPNs *Steinernema scarabaei* (Stock 2003),

Steinernema glaseri (Glaser 1932), *Heterorhabditis zealandica* (Poinar, 1990), and *Heterorhabditis bacteriophora*", it was concluded that EPN species performance varies with soil types and the effect of different soil parameters have to be considered carefully, such as soil texture, pH, and organic matter. Koppenhöfer *et al*. (2006) also concluded that *H. bacteriophora* has relatively poor longevity. These results contradict our study as we found *H. bacteriophora* to be more persistent in all five field trial sites in both cadaver and Barricade® gel formulations as compared to *S. tophus*. However, Shields *et al.* (1999) concluded in his study that, this strain exhibit substantial persistence and infectivity for 700 days or more in the field. The percentage of *G. mellonella* mortality (indicating persistence) at GMMM, Bronkhorstspruit, Klipvoor and Meyerton declined drastically from one month sampling to two months sampling. However, in Bela Bela there was a slight decline after one and two months sampling. This may be because the soil in Bela Bela was mostly clay and it was moist due to frequent rainfall and proper irrigation.

Treatment D (*H. bacteriophora* cadaver formulation), was able to obtain high percentage of mortality after 10 and 15 days however treatment C (*H. bacteriophora* Barricade® gel performed relatively well as treatment D after one month sampling and the persistence decreased after two months sampling. Each trial side had different climatically conditions. Treatment D (*H. bacteriophora* cadaver formulation was persistent in all five different climatic conditions of the trial sites.

The treatments were also monitored and analysed over time in days. Sample after one month of treatment application showed high percentage of persistence. Treatment C and D were able to be persistence up to ten to 15 days. After two months sampling, treatment A and B did not survive longer the 10 days in most field sites. Treatment D was more persistence, treatment C obtained the second highest persistence percentage and there was no statistical significance between treatment C and D in most field trials.

5.5. Conclusion

EPNs have the ability survive for more than two months after application however, novel application methods have to be developed to retard the desiccation of foliar-applied EPNs, ranging from the post-application spraying of a Barricade® gel and burying cadaver infected with EPNs. *Heterorhabditis bacteriophora* was more persistent, two months post treatment

application at all five sites. The mortality percentage was very high after one month sampling and declined drastically after two months sampling. The results propose that EPNs post application survival in the field can be enhanced by both cadaver and Barricade® gel formulations. Further research to be conducted for persistence of different species in different climate and soil conditions.

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Chapter 6

Entomopathogenic nematodes and plant resistance for the control of sweet potato weevils under field conditions

Abstract

Sweet potato (*Ipomoea batatas*) is one of the most widely cultivated root and tuber crops worldwide. The SPW (*Cylas puncticollis*) Fabricius is one of the most important insect pest of sweet potatoes. The control of *C. puncticollis* has proven to be a challenge due to the cryptic nature of the larvae and night activity of the adults. In Africa, control of *C. puncticollis* relies primarily on the use of synthetic chemical insecticides. However, the insect has developed some level of resistance against a wide range of chemical insecticides. Entomopathogenic nematodes (EPN), which are cosmopolitan soil-borne entomopathogens, have gained interest as potential biological control agents of various economically important insect pests. The main aim of this study was to evaluate the potential of EPN isolates from the families *Heterorhabditidae* and *Steinernematidae* as biological control agents of *C. puncticollis* and its effect on different sweet potato cultivars, under field conditions. Plant resistance has also been viewed as an efficient, cost-effective and environmentally-safe form of pest control methods of the SPW under field conditions. Four different cultivars were planted and six biocontrol treatment were applied after a month. Overall, both the cadaver and Barricade® gel formulations of *Heterorhabditis bacteriophora* Poinar SGI 245 were more effective in reducing the plant damage caused by *C. puncticollis* compared to the Barricade® formulation of *Steinernema* sp., and *Steinernema tophus* (0.114, 0.080). Overall, the cultivar Monate suffered less insect damage (0.103) than the cultivars Blesbok and Bophelo, and it was the best yielding cultivar (1.389). Treatment F of *Heterorhabditis bacteriophora* and Monati were recommended for this study.

Keywords: Barricade gel, Biocontrol agent, *Cylas puncticollis*, formulation, *Heterorhabditis sp, Ipomoea batatas, Steinernema sp.*

6.1. Introduction

Sweet potato (*Ipomoea batatas*) is a dicotyledonous root crop that belongs to the Convolvulaceae family. The crop is grown in numerous countries, mainly in developing countries (Baimey *et al.,* 2017). In African countries, it is largely grown for human consumption, whereas in China, it is produced primarily for animal feed (Sang-Soo, 2019). The crop contains functional components that are vital for human health, such as polyphenols, anthocyanins and dietary fiber (Sang-Soo, 2019).

Sweet potato roots vary in color: there are orange-fleshed, white and purple-fleshed roots. The color variation is dependent on the nutrients each type of cultivar contains. Orange-fleshed type is particularly rich in B-carotene and provitamin A carotenoid (Kidasi *et al*., 2021). Irrespective of their color, all sweet potato cultivars are low in protein quantity and quality. Orange-fleshed varieties are found naturally, while others have been developed through conventional breeding. Some varieties have been improved through modern biotechnology. Nutritional value differs according to harvesting period and variety (Ezin *et al*., 2018).

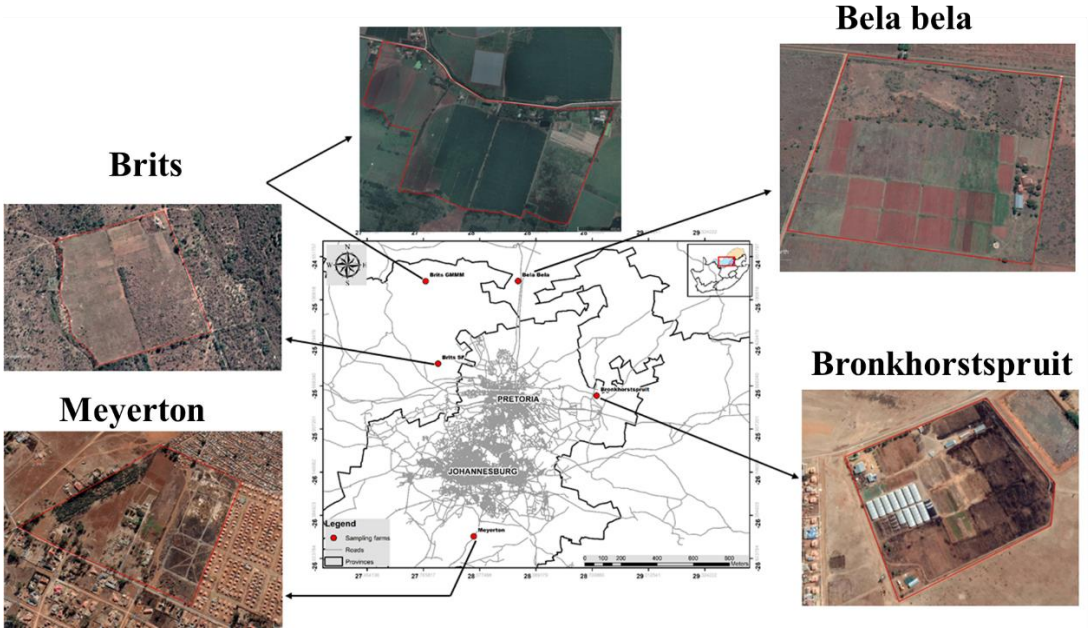
Cylas puncticollis Fabricius (Coleoptera: Curcilionidae), commonly known as SPW, is an important polyphagous insect pest affecting sweet potato production, globally (Hue *et al.*, 2015). Both the larval and adult stages of the insect cause damage to sweet potato roots, whereas damage to the leaves, vine, storage roots and buds are mostly due to feeding by the adults. The greatest economic damage is caused by larval instars, commonly known as grubs, tunneling in the stems and storage roots (Hue *et al.,* 2015).

Sweet potato weevil is said to be the most serious pest in all major sweet potato-producing countries in Africa, with reports of losses ranging from 5% to more than 80% (https://www.arc.agric.za/arc-vopi/Pages/Plant%20Breeding/Sweet-Potatoes.aspx). The longer the crop remains in the ground unharvested, the greater are the losses (Hue *et al.,* 2015). The factors that contribute to the losses that they cause are soil and weather conditions. Light sandy soils and low rainfall increase the chances of heavy infestations (Hue *et al.,* 2015). Yield losses can reach 100%, especially during extended dry seasons. Research evidence confirms that red-fleshed, low dry matter varieties are more susceptible to infestation than other varieties (Kagimbo *et al.,* 2018). According to studies conducted in Central America, Africa and Asia; production losses often reach 60-100% (Beyene *et al*., 2015). Hue *et al*. (2015) also concluded

that losses were up to 73% in Uganda, depending on the planting period and 15–20% in Tanzania. In other areas in the continent, losses have been shown to reach up to 100% (Hue *et al.*, 2015). The main aim of this study was to evaluate the potential of EPNs from the families Heterorhabditidae and Steinernematidae as biological control agents of *C. puncticollis*, under field conditions. Plant resistance was also assessed as efficient, cost-effective and environmentally-safe pest control methods of the SPW under field conditions.

6.2. Materials and Methods

6.2.1. Trial localities



Study areas, Bronkhorstpruit Gauteng ($25^0$43'19.4"S $28^0$43'29.0"E), Bela-Bela Limpopo ($25^0$45'52.8"$28^0$13'26.0"E), Brits North West ($25^0$05'17.7"S $27^0$46'42.7"E), Brits North West (25.0926° S, 27.7725° E) and Meyerton Gauteng ($26^0$32'39.0"S $27^0$59'57.8"E) (**Figure 1)**.

6.2.2. Trial plan

A randomized complete blocks design was used in designing the trial plan. Entomopathogenic nematodes and sweet potato cultivars were the two main effects. The EPNs had two factors within: the species (*Heterorhabditis bacteriophora* (SGI 245) and *Steinernema tophus* (ROOI 352); and the application method. Three plants per treatment were planted with 2 m spacing between treatments and rows. The borders between the plants were 2 - 3 m.

Table 1: Field trial layout. One colour-coded block represents one treatment with three plants.

BLOCK 1

B4	E3	A1	F4	D2	C3
A2	C4	B3	D1	F3	E2
F2	A3	E1	B2	C1	D4
E4	B1	D3	C2	A4	F1

BLOCK 2

A4	C2	B1	D1	E3	F4
F1	E2	D3	C1	B4	A3
C3	B2	A2	E1	D4	F2
B3	A1	C4	F3	E4	D2

BLOCK 3

B2	E4	F1	D4	A2	C3
C4	D2	A3	B4	E3	F2
F3	E2	D1	C1	B3	A1
A4	B1	C2	D3	E1	F4

6.2.3. Planting and treatment

Planting was done during December 2021. Four sweet potato cultivars, Blesbok, Ndou, Monati and Bophelo, were planted. Six treatments were applied during the field trials: a chemical pesticide (Lirifos 480 EC), *H. bacteriophora* Formulation 1 with 2% Barricade® gel, *H. bacteriophora* Formulation 2 (production in a cadaver), *S. tophus* Formulation 1 with 2% Barricade® gel, *S tophus* Formulation 2 (production in a cadaver) and a control treatment was included in the experiments. Each liquid treatment was applied using a spray bottle. The infested cadavers were buried 2-5 cm below ground in the treated plot.

Table 2: Six treatments applied on the field trials.

Treatments	
A	Non-treated (control)
B	Chemical insecticide (Lirifos 480 EC)
C	*Steinernema tophus* Formulation 1 with 2% Barricade® gel
D	*S. tophus* Formulation 2 (cadaver)
E	*Heterorhabditis bacteriophora* Formulation 1 with 2% Barricade® gel
F	H. bacteriophora Formulation 2 (cadaver)

6.2.4. Statistical analysis

Collected data were analysed using analysis of variance (ANOVA) using Statistical Analysis Software (SAS). The standardized residuals were normally distributed (Shapiro–Wilks test) and therefore the means of the significant mortalities were separated using Fisher's Unprotected t-test (least significant difference – LSD) at the 0.5% level of significance (Ramakuwela *et al*., 2018).

6.3 Results

6.3.1. Bela-Bela

Mean tuber insect damage for different treatments. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 3).

Table 3: The effect of different treatments on sweet potato weevil damage.

	Sweet potato weevil damage			
Treatment	Rating (1-5)	Area Tuber %	No. of holes (Tubers)	No. of Storage roots
A	0.111 **b**	0.029 b	0.278 b	0.052 b
B	0.2778 ab	0.059 b	0.389 b	0.071 b
C	0.528 a	0.119 a	1.353 a	0.187 a
D	0.0833 b	0.016 b	0.111 b	0.019 b
E	0.056 b	0.013 b	0.167 b	0.023 b
F	0.111 b	0.029 b	0.361 b	0.058 b

Mean tuber insect damage per cultivar. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 4).

Table 4: Sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes (Tubers)	No. of Storage roots
Blesbok	0.148 b	0.038 b	0.370 b	0.061 b
Ndou	0.093 b	0.019 b	0.167 b	0.027 b
Monati	0.037 b	0.0311 b	0.204 b	0.037 b
Bophelo	0.148 a	0.089 a	1.031 a	0.148 a

Table 5: Total cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	1.018 a
Ndou	1.121 a
Monati	1.202 a
Bophelo	1.098 a

6.3.2. GMMM

Mean tuber insect damage for different treatments. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 6).

Table 6: The effect of different treatments on sweet potato weevil damage.

	Sweet potato weevil damage			
Treatment	Rating (1-5)	Area Tuber %	No. of holes (Tubers)	No. of Storage roots
A	0.15152 a	0.03804 a	0.2424 a	0.04855 a
B	0.03571 a	0.01075 a	0.0357 a	0.01075 a
C	0.06061 a	0.01446 a	0.1515 a	0.02358 a
D	0.06061 a	0.01446 a	0.1212 a	0.02118 a
E	0.09091 a	0.02358 a	0.2121 a	0.03943 a
F	0.09091 a	0.01824 a	0.3636 a	0.03376 a

Table 7: Sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes (Tubers)	No. of Storage roots
Blesbok	0.10417 ab	0.02248 ab	0.3542 a	0.03942 ab
Ndou	0.00000 b	0.00000 b	0.0000 a	0.00000 b
Monati	0.04167 ab	0.00994 b	0.0625 a	0.01254 b
Bophelo	0.18750 a	0.04863 a	0.3542 a	0.06877 a

Mean yield per cultivar. Means with the same lowercase letter (in the same column) no significant difference, based on an unprotected Fisher's LSD (α=0.05) (Table 8).

Table 8: Total cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	0.2138 b

Ndou	1.0502 a
Monati	1.3542 a
Bophelo	0.6074 b

6.3.3. Klipvoor

Mean tuber insect damage per cultivar. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 8).

Table 8: Sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes	No. of Storage roots
Blesbok	0 a	0 a	0 a	0 a
Ndou	0 a	0 a	0 a	0 a
Monati	0 a	0 a	0 a	0 a
Bophelo	0 a	0 a	0 a	0 a

Table 9: Total cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	0.3333 a
Ndou	0.4918 a
Monati	0.5145 a
Bophelo	0.5978 a

6.3.4. Meyerton

Mean tuber insect damage for different treatments. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 10).

Table 10: The effect of different treatments on sweet potato weevil damage.

	Sweet potato weevil damage			
Treatment	Rating (1-5)	Area Tuber %	No. of holes	No. of Storage roots
A	0.2879 b	0.06743 b	1.2121 b	0.12975 b
B	0.2609 b	0.06949 b	0.9750 b	0.13404 b
C	0.3636 ab	0.09016 ab	1.4242 b	0.16723 ab
D	0.6735 a	0.15551 a	3.2647 a	0.32594 a
E	0.1875 b	0.04863 b	0.5000 b	0.07895 b
F	0.3229 ab	0.07477 ab	1.3714 b	0.14978 b

Table 11: Sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes	No. of Storage roots
Blesbok	0.6341 a	0.14997 a	2.4634 a	0.27787 a
Ndou	0.3606 ab	0.08292 ab	1.6538 ab	0.16809 ab
Monati	0.1623 b	0.04046 b	0.4189 b	0.06925 b
Bophelo	0.3151 b	0.08075 b	1.5849 ab	0.17238 a

Mean yield per cultivar. Means with the same lowercase letter (in the same column) no significant difference, based on an unprotected Fisher's LSD (α=0.05) (Table 12).

Table 12: Total cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	0.7565 b
Ndou	1.1864 a

Monati	1.3116 a
Bophelo	0.9342 b

6.3.5. Bronkhorstspruit

Mean tuber insect damage for different treatments. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 13).

Table 13: The effect of different treatments on sweet potato weevil damage.

	Sweet potato weevil damage			
Treatment	Rating (1-5)	Area Tuber %	No. of holes	No. of Storage roots
A	0.2593 a	0.05471 a	0.2963 ab	0.05764 a
B	0.0800 ab	0.01908 ab	0.1200 ab	0.02408 a
C	0.1935 ab	0.04452 ab	0.3871 c	0.06207 a
D	0.0333 ab	0.01003 b	0.1333 ab	0.02330 a
E	0.0000 b	0.00000 b	0.0000 b	0.00000 a
F	0.0000 b	0.00000 b	0.0000	0.00000

Mean tuber insect damage per cultivar. Means with the same lowercase letter (in the same column) indicate no significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 14).

Table 14: Sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes	No. of Storage roots
Blesbok	0.0588 a	0.01771 a	0.0588 a	0.01771 a
Ndou	0.0000 a	0.00000 a	0.0000 a	0.00000 a
Monati	0.1000 a	0.02511 a	0.2400 a	0.04158 a
Bophelo	0.1961 a	0.04077 a	0.2745 a	0.04709 a

Mean yield per cultivar. Monati had the highest yield with 2.1962, followed by Ndou, Blesbok and Bophelo with 1.7118, 1.2647 and 1.2486, respectively. Means with the same lowercase letter (in the same column), based on an unprotected Fisher's LSD (α=0.05) (Table 15).

Table 15: Total cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	1.2647 b
Ndou	1.7118 ab
Monati	2.1962 a
Bophelo	1.2486 b

6.3.2. The overall mean of the sweet potato damage on treatments, cultivars and total yield.

Mean tuber insect damage for different treatments. Means with the same lowercase letter (in the same column) indicate significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 16).

Table 16. The overall effect of different treatments on sweet potato weevil damage.

	Sweet potato weevil damage			
Treatment	Rating (1-5)	Area Tuber %	No. of holes (Tubers)	No. of Storage roots
A	0.168 ab	0.750 ab	0.434 abc	0.357 ab
B	0.158 ab	1.100 ab	0.364 abc	0.424 ab
C	0.273 a	0.839 a	0.788 a	0.357 ab
D	0.198 ab	1.006 ab	0.843 ab	0.521 a
E	0.080 b	0.405 b	0.210 c	0.254 b
F	0.114 b	0.687 b	0.456 bc	0.353 ab

Mean tuber insect damage per cultivar. Means with the same lowercase letter (in the same column) indicate significant difference, based on an unprotected Fisher's LSD (α=0.05). 1-5 scale, where 1 is minimal damage, and 5 is maximal damage (Table 17).

Table 17. Overall sweet potato weevil damage on different cultivars.

	Sweet potato weevil damage			
Cultivar	Rating (1-5)	Area Storage roots %	No. of holes (Tubers)	No. of Storage roots
Blesbok	0.221 a	1.144 a	0.768 a	0.236 bc
Ndou	0.102 b	0.543 b	0.406 b	0.080 b
Monati	0.103 b	0.331 b	0.210 b	0.209 c
Bophelo	0.248 a	1.245 a	0.745 a	0.616 a

Table 18. Overall of cultivar yield.

Cultivar	Yield/Plant (kg)
Blesbok	0.685 d
Ndou	1.173 b
Monati	1.389 a
Bophelo	0.934 c

6.3.3. Total sweet potato weevil damage

Bronkhorstspruit had the highest SPW damage, followed by Klepvoor, Meyerton, GMMM and BelaBela with 45%, 40%, 35%, 30% and 30% respectively (Table 19).

Table 19: Total sweet potato weevil damage per trial site (p =0.01).

Site names	Sweet potato weevil damage (%)
Meyerton	35 ab
Bronkhorstspruit	45 b
GMMM (Brits)	30 a
Klepvoor (Brits)	40 b
Bela Bela	30 a

6.2. Discussion

Sweet potato weevil is considered to be the most serious pest of sweet potato. Yield losses range from 5% to 97% in areas where the weevil occurs (Kagimbo *et al.,* 2018). The current study assessed the effect of four biocontrol treatments and an insecticide treatment on SPW damage at five sites, and the effect of SPW on different cultivars. Treatments at each farm performed differently due to different climate, moisture content, soil type and weevil infestation levels. *Heterorhabditis bacteriophora* was effective to four sites (Bela Bela, Klipvoor, Meyerton and Bronkhorstspruit) with the mean ratings of 0.111, 0.00, 0,1875 and 0.00, respectively. The treatment also was more effective than the control and the insecticide. The soil moisture on these farms was very low and temperatures were high. *Steinernema tophus* was more effective on the GMMM (Brits) farm site resulting in a mean rating of 0.6061. This may have been caused by a higher soil moisture content and a large plant canopy, ensuring that EPNs were less affected by heat, UV- light and dryness. Treatment F *Heterorhabditis bacteriophora* with the lowest rating of 0.00 was more effective than other treatment as its use resulted in less SPW damage in all the field trial sites. However, there was no statistical differences between the EPN treatments at most sites.

The relationship between vine damage or weevil density, and tuber damage is important (Ddumba, 2018). The weevil density and weevil damage are directly proportional to each other, as the results above display.

Weevil damage was also assessed in different cultivars. The cultivar Monati showed less damage by SPW. These results contradict with results published by Hlerema (2021) who found the cultivar Bophelo to be superior because it could be stored for 12 days longer in hessian bags than the other cultivars. There was statistical significance between Bophelo and the three other cultivars, Blesbok, Ndou and Monati in weevil susceptibility.

The yields of different cultivars were assessed at the five sites. In Klipvoor, cultivar Bophelo yielded 0.5978 kg more than the other cultivars. In Bela Bela, GMMM (Britz), Meyerton and Bronkhorstspruit, the cultivar Monati had the highest yield, producing 1.202, 1.3542, 1.3116, 2.1962 kg per plant, respectively. However, there was no differences in yield with the other three cultivars, Bophelo, Ndoe and Blesbok. The cultivar Monati was the preferred cultivar in terms of yield (1.389kg / plant) and was less affected by weevils (rating, 0.103).

However, farmers have their own preferred varieties based on high yield, drought tolerance, resistance to diseases and pests and taste. The most important farmer preferred, culinary-quality traits of sweet potato are dry matter content, reduced cooking time, taste, and low fibre content. In a study conducted in Uganda by Mwanga *et al*. (2012), farmers considered agronomic traits, such as resistance to pests and diseases, drought resistance, and high yield, as the main criteria for selecting good sweet potato varieties for production and further indicated that farmers in Uganda preferred these traits because they favoured sequential harvesting.

The total SPW damage was compared between the five trial sites; Bela Bela and GMMM obtained the lowest SPW damage with both 30%, Meyerton, Klipvoor obtained 35%, 40% SPW damage respectively. Bronkhorstspruit obtained the highest SPW damage with 45%. These results indicate that the treatments were effective as the damage was less the 50% in all the trial sites.

6.3. Conclusion

Treatment E and F of *H. bacteriophora* were the most effective treatment against SPW. The cultivars Bophelo and Monati were less affected by sweet potato weevils, however Monati had more yield than the other cultivars. The total SPW damage in the trial sites were all below 50%. Treatment F of *H. bacteriophora* and Monati were recommended for this study.

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Chapter 7

Dissertation Overview

Entomopathogenic nematodes (EPNs) are obligate insect parasitic nematodes that have a symbiotic relationship with bacterial endophytes living in their gut. All life stages of the nematode, except for the free-living third-stage infective juvenile (IJs), are found inside insect hosts (Malan and Ferreira, 2017). Nematodes from the genera *Steinernema* and *Heterorhabditis* are of research interest because of their ability to be kill insect pests, and their potential for mass-production as biological control agents (Poinar and Grewal, 2012). Factors that affect the application and success of EPNs as biological control agents in the field include abiotic factors such as temperature, relative humidity and UV radiation. Other factors such as the formulation, shelf life, target insects, type of crops, usage directions, technical support, cost and markets, also influence their usage for management of pests in agroecosystems (Cruz- Martinez *et al*. 2017). The formulation of EPNs is one of the most important factors and has focused on increasing the shelf life and EPNs efficacy in the field since the late 1970's. An optimal formulation should show high and consistent quality, easy handling and transport, high effectiveness and ease of application in the field. Formulations include aqueous suspensions, synthetic sponges, gels, clays, powders, polymers, granules and EPN infected cadavers. One of the species of sweet potato weevils (SPW), *Cylas formicarius* Fabricius, has been shown to be susceptible to infection by EPNs. The threat of SPW is increasing with climate change (Johnson and Gurr, 2016), which is important in Africa as it is a critical food crop and SPW is found in several African countries (Kotchofa *et al*., 2019). If left uncontrolled, this pest causes a significant damage to sweet potatoes, with up to 98% yield losses, under field conditions (Kyereko *et al.*, 2019). Many factors affect the interaction of sweet potato and SPW, including cultivar, maturity date, root depth, shape, arrangement, attraction and plant canopy, ultimately impacting on sweet potato yields (Stathers *et al*., 2003).

In the current study, a farmers' survey was conducted on SPW, its occurrence and distribution in three provinces, Limpopo, Gauteng and North West, and the knowledge of small scale farmers of this pest. The key finds were that:

- The farmer's surveys showed that most of the farmers planted sweet potato for income generation purposes. The high levels of unemployment have resulted in many families growing sweet potato to generate income to survive. About 64% of the farmers had some knowledge of SPW. The crop was attacked by SPW throughout the year. 34% of

the farmers agreed that SPW had a negative impact on sweet potato production, whereas 28% were not sure and 14% did not think that SPW caused any crop loss. Farmers estimated that they lost 25% of their yield annually were 67%, 27% of the farmers estimated losses of 12.5% and 6% estimated losses of 50% of the yield. In all three of the provinces the sweet potato crop was affected by weevils, most of the farmers were aware of weevil damage, however, none of the farmers knew how to manage the pest effectively.

A field trial on the control of SPW using EPNs was conducted. The persistence of EPNs under field conditions was also evaluated. Lastly, the comparison of EPNs formulations in Barricade® gel and potassium polyacrylate hydrogel (PPH) was assessed. The key outcomes were:

- The three EPNs isolates were evaluated in two formulations, a Barricade® gel and a PPH formulation. Survival of IJs was 0% after eight weeks, however, the survival of all three EPNs was around 50% after six weeks. The efficacy of the formulations was also assessed on mealworm larvae (*Tenebrio molitor*). On Day one, the mortality of mealworms were 100% for all the formulations. After two weeks the mortality obtained for the control treatments was 0%. After eight weeks the mortality of mealworms was 0% for all the three isolate formulations. However, The Barricade® gel and PPH were able to enhance survival of EPNs by six weeks. The *in vitro* produced EPNs had a highest percentage of survival than the *in vivo* produced EPNs. However, the *in vivo* produced EPNs were more effective than *in vitro* produced EPNs. This finding was confirmed in that the *in vivo* produced EPNs had the highest cumulative mortality score (area under the morality curve) than the *in vitro* produced EPNs
- Persistence of the EPNs in the field trials was tracked. The persistence declined drastically two months after treatment, compared to samples taken after one month. However, some EPNs survived for more than two months after application with both the cadaver and barricade gel formulations.

The potential of EPNs to control SPW was evaluated on three sweet potato cultivars, under field conditions. The cadaver and Barricade® gel formulations of *Heterorhabditis bacteriophora* Poinar 1976 SGI 245 were more effective in reducing the plant damage caused by SPW than the Barricade® gel formulation of *Steinernema* sp. and *Steinernema tophus*. The cultivar Monate suffered less SPW damage than the cultivars Blesbok and Bophelo, and it was

the best yielding cultivar. Treatment F of *H. bacteriophora* and Monate can be recommended for further field trials. These should lead to commercialization EPNs for the control of SPW, especially when combined with resistant sweet potato cultivars.

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