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# EFFECT OF DIFFERENT CONCENTRATIONS OF EPNS AND MALATHION ON SUGARCANE WHITE GRUBS, Heteronychus licas (Coleoptera: Scarabaeidae) MORTALITY

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## ABSTRACT

Sugarcane, Saccharum officinarum L. is one of the major commercial crops grown in Eswatini. However, pest and diseases are pivotal to the decrease in yield output in the country. Sugarcane white grubs (SWG), Heteronychus licas Klug (Coleoptera: Scarabaeidae) are amongst the important pests of sugarcane in the country. Entomopathogenic nematodes (EPNs) were found to be effective in controlling both soil borne as well as above ground insect pests. The current study determined the effect of different concentrations of Malathion and EPNs on the mortality of SWG. SWG larvae were dipped in different concentrations (0, 0.625, 1.25, and 2.5 g/L) of malathion and (0, 200, 400, and 600 IJ/insect) EPNs. SWGs were also exposed to the same concentrations in soil bioassays. Results obtained in the bioassays showed that Steinernema yirgalemense at 600 IJs/insect (85.4%) gave the highest mortality which was not significantly different from S. yirgalemense at 400 IJs/insect (72.9%). The Malathion concentrations gave 0, 79.2, 83.3 and 89.6% mortality for 0, 0.625, 1.25 and 2.5g/L of Malathion, respectively. Results obtained in the soil bioassays showed that Steinernema yirgalemense, at 600 IJs/insect (80.5%), provided higher ($p < 0.05$) mortality of the H. licas larvae which was not significantly different from S. yirgalemense at 400 IJs/insect (70.2%). The Malathion concentrations gave 0, 83.3, 85.4 and 87.5% mortality for 0, 0.625, 1.25 and 2.5g/L of Malathion, respectively. The results indicated that both EPN and Malathion were tested were effective against H. licas larvae. Steinernema yirgalemense at 400 IJs/insect and Malathion at 0.625g/L can effectively H. licas larvae but large-scale field trials are recommended to demonstrate the potential use of the biocontrol agent within integrated pest management programs.

**Keywords:** Bioassay, Entomopathogenic nematodes, Malathion, Steinernema yirgalemense, Sugarcane, Sugarcane white grubs.

## 1. INTRODUCTION

Sugarcane, *Saccharum officinarum* (Poaceae: Poales) is one of the largest cash crops grown and exported in bulk quantities in Eswatini, with approximately 730 000 tonnes is marketed (Eswatini Sugar Association, 2017). Sugarcane is cultivated in 2.4 million hectares resulting in 70,5 tonnes/ha being produced throughout the world where it is produced (FAO, 2011). The sugar industry is the backbone of the Kingdom of Eswatini contributing annual revenue exceeding E1440 million which is about 18% of the country's GPD. Area under sugarcane production in Eswatini was approximately 62000 ha in the year 2018-2019 with a combined annual sugar production capacity in excess of 800 000 tonnes (Sisuka, 2019).

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**Table 1: The Production of Sugarcane and Cane Yields in Eswatini**

| Marketing year | Cane crushed (MT) | Area Planted (Ha) | Area harvested (Ha) | Cane Yield (MT/Ha) |
|---|---|---|---|---|
| 2013/14 | 5,591,830 | 58,979 | 55,478 | 101 |
| 2014/15 | 5,639,193 | 59,586 | 56,438 | 100 |
| 2015/16 | 5,836,553 | 59,924 | 57,685 | 101 |
| 2016/17 | 4,973,571 | 61,073 | 56,420 | 88 |
| 2017/18 | 5,405,000 | 62,000 | 57,700 | 94 |
| 2018/19 | 6,097,214 | 65,000 | 60.000 | 102 |
| 2019/20 | 6,500,000 | 70,000 | 63,000 | 103 |

Source: USDA Foreign Agricultural Service, 2019

Sugarcane is a perennial grass widely grown for its juice from which sugar is processed (Peterson *et al*., 2012). Most of the world's sugar is grown in subtropical and tropical areas (Daniels *et al*., 1993). The plant is also grown for biofuel production, especially in Brazil, where the cane is used to produce ethyl alcohol (ethanol) (Schubert, 2006). Sugarcane is propagated primarily by planting of seed cane which have 2 or 3 buds (Takeo, 2019). The seed cane is planted in well worked fields whereby mechanical planters open furrows, fertilize, drop seed cane and cover it with the soil. Under favourable conditions each bud germinates and produces a primary shoot (James, 2004). Root bands adjacent to each bud give rise to a large number of roots, and each shoot develops its own root system. Tillering at the base of the plant takes place and each original seed cane develops into a number of growing canes, forming a stool, where a plant crop is obtained (Takeo, 2019).

In order to secure a good crop, weeds in the cane fields must be controlled until the cane stools develop a good canopy, which checks weed growth. Chemical herbicides are widely used to control weeds in sugarcane fields (Brown, 1951). The sugarcane plant is subject to many diseases (such as red rot, *Colletotrichum falcatum* Went; leaf scald, *Xanthomonas albilineans* (Ashby) Dowson; smut, *Ustilago scitaminea* Syd; mosaic virus, *Potyvirus* spp. and wilt, *Fusarium sacchari* (Butler) etc.).

Insect pests, among other factors, are considered as one of the main problems that reduce average productivity per unit area. The most important insect pests of sugarcane are: yellow sugarcane aphid, *Sipha flava* (Forbes); sugarcane thrips, *Fulmekiola serrata* (Kobus); white grub, *Lepidiota stigma*; sugarcane white grubs, *Heteronychus licas*; maize stalkborer, *Busseola fusca*; spotted stem borer, *Chilo partellus*; sugarcane stem borer, *Saccharum officinarum* L and pink Stem Borer, *Sesamia calamistis* that bore into and feed on the different parts of the plant (Crop Protection Compendium, 2012).

Control measures include biological control by parasites or predators, chemical control by insecticides, as well as the introduction of resistant varieties (Nussley and Hentz, 2002b).

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Sugarcane white grubs (SWG) are usually infected with entomopathogens which are disease causing organisms that kill their hosts and debilitate their future generations (Singh, 1991). Important entomopathogens that infect white grubs include fungi, bacteria, nematodes and viruses. Infected insects are unable to feed, remain stunted, lose their body colour and become paralysed (Singh, 1991). Entomopathogens exert a controlling effect on SWG by means of their invasive properties, toxins, enzymes and other substances. The entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) received more attention than other groups of microbial organisms with potential use for management of SWG (Yadava and Sharma, 1995).

EPNs are a diverse group of invertebrates found as parasites or free living forms in soils, freshwater, and marine environments. They are from families Steinernrmatidae and Heterorhabiditidae and are potential bio control agents (Gaugler, 1999). EPNs are more effective than most bio control agents because they kill pests within 24-48 hours after penetrating into the insect host with the help of their symbiotic bacteria. Entomopathogenic fungi are also important bio control agents because they have a broad host range, route of pathogenicity and its ability to control sap sucking insects (Butt, 2002; Qazi and Khachatouurians, 2005; Thomas and Read, 2007; Fan et al., 2007)

Synthetic chemical insecticides used for pest management has vast negative impact like insecticide resistance to pests, food hazards, destruction of natural enemies as well as contamination of underground water. Therefore, introduction of alternative insect pest control measures like integrating chemical control with different concentrations of bio pesticides would help reduce SWGs infestation, thus maximizing yields. Integrated control is successful when sound economic thresholds have been established, sampling methods available as well as when the selective insecticides are available. They will reduce the risk of damage, increase the production of sugarcane and kill the pest in an effective manner which reduces the damage to sugarcane caused by the SWG. The study determined the effect of different concentrations of EPNs and Malathion on SWG mortality in laboratory and soil bioassays

## 2. MATERIALS AND METHODS

### 2.1 Insects

*Heteronychus licas* third-instar larvae were collected from infested sugarcane fields around Mhlume, kept in Perspex boxes measuring 15×20cm and transported to the Entomology laboratory, Faculty of Agriculture, University of Eswatini. They were placed individually in soil containing perennial ryegrass, *Lolium perenne* L., seeds and stored for 1-12 weeks at room temperature. Before use in experiments, 3rd instars were kept at room temperature for 2-4 days; only healthy larvae were assayed.

### 2.2 Source of nematodes

*Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams 2004 was originally sourced from the EPN collection of the Nematology Laboratory, Department of Conservation Ecology and Entomology, Stellenbosch University, South Africa. Infective juviniles mass rearing and harvesting procedures were carried out as outlined by methods presented by Kaya and Stock (1997), using last instars of the greater wax moth, *Galleria mellonella* (L.), and stored in water at room temperature (±25°C). Infective juviniles were harvested from White traps within the first week of emergence, stored horizontally using 500-ml vented culture flasks, and

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used within one month after harvesting. The culture flasks containing the infective juviniles were shaken daily to increase the amount of aeration and the survival of the infective juviniles during storage.

**Table 2. Characteristics of the *Steinernema* species used in the study.**

| Species Name | Strain | Habitat | Locality | GenBank Accession Number | Length of IJ (µ m) | Body Width of IJ (µ m) | Reference |
|---|---|---|---|---|---|---|---|
| *S. yirgalemense* | 157-C | Citrus orchard | Friedenheim, Mpumalanga | EU625295 | 685 (570–740) | 29 (24–33) | Malan et al., 2011 |

### 2.3 Laboratory bioassay

Virulence experiments were conducted in 12-well bioassay plates (flat-bottom, Nunce, Cat. No.144530, Thermo Fisher Scientific [Pty] Ltd, Gauteng, Johannesburg, South Africa). The 12-well bioassay protocol was used to test the potential of *S. yirgalemense* to infect *H. licas* larvae under optimal laboratory conditions. Wells were lined with a circular piece of filter paper, to secure an even distribution throughout the well. Each well with a *H. licas* larva (n = 60), was inoculated with 200, 400, 600 IJ/50µl water, while the control received distilled water only (Navon and Ascher, 2000). One insect was added to each well, and the trays were closed with the lid, placed in a closed plastic container lined with moistened tissue paper, and left in a growth chamber at 25°C for 48 hours. Five trays with 12 wells were used (*n* = 60). Mortality by nematode infection was confirmed after 48 h, by dissection under a stereo microscope to check for the presence of *S. yirgalemense* juveniles. The procedure was repeated on a separate date, with a different batch of nematodes.

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### 2.4 Soil bioassay

#### 2.4.1 Susceptibility of *H. licas* larvae to *S. yirgalemense* in soil bioassay

Centrifuge tubes measuring 1.5 cm × 15 cm in diameter were filled with sieved moist river sand (10:100 v/v, river sand to water) that was frozen overnight (Yu et al. 2008). *Heteronychus licas* larvae were placed at the bottom of each of the 30 sand-filled centrifugal tubes. *Steinernema yirgalemense* at 200, 400, 600 IJ/50µl water, for each treatment (with 10 replicates per treatment), were added by means of pipetting onto a 13-mm-diameter filter paper disc, turned upside down and placed on top of the sand in each tube to prevent the nematodes from being washed into the soil. The control treatments received water only. All the tubes were closed with the lid to prevent excess water loss. The mortality caused by the infection of *H. licas* was confirmed by dissection after 48 h had elapsed. The experiment was conducted on two separate test dates, with freshly prepared inoculum.

#### 2.4.2 Susceptibility of *H. licas* larvae to malathion in soil bioassay

Centrifuge tubes measuring 1.5 cm × 15 cm in diameter were used for the soil bioassay. The tubes containing a *H. licas* larva were filled with soil with sieved moist soil (10:100 v/v, soil to water). For each treatment (with 10 replicates per treatment), Malathion at O.625, 1.25 and 2.5(g/L) were added by means of pipetting onto the soil filled centrifuge tubes. The control treatments received water only. All the tubes were closed with the lid to prevent excess water loss. The mortality caused on *H. licas* confirmed after 48 h had elapsed. The experiment was conducted on two separate test dates, with freshly prepared insecticide.

## 3. DATA ANALYSIS

Virulence assays were analysed using analysis of variance (ANOVA); if the F value was significant ($p < 0.05$), the means were differentiated by LSD MEANS (SAS Institute, 1985). The mortality data were corrected for the corresponding control mortality, using the formula: CM (%) = {(T–C)/(100–C)}×100, where CM is the corrected mortality, T is the percent mortality in treated insects, and C is the percent mortality in untreated insects (Abbott, 1925). If no significant interactions were found to occur between the main effect of the test dates and the treatments concerned, data obtained from the two test dates was pooled and analysed, using a two-way ANOVA.

## 4. RESULTS

### 4.1 Susceptibility of *H. licas* larvae to *S. yirgalemense*

Analysis using a one-way ANOVA showed no significant effect ($F(3, 36) = 26.34$, $p < 0.001$) of the treatment on percentage mortality between the different treatment dates. The mortality of *H. licas* larvae in the laboratory trial ranged from 0 to 85.4% after inoculation with 0, and 600 IJs/insect over a period of 2 days, with a significant effect ($F(3, 28) = 17.50$, $p < 0.001$) of the treatment on the percentage mortality. *Steinernema yirgalemense*, at 600 IJs/insect (85.4%), provided significantly higher ($p < 0.05$) mortality of the *H. licas* larvae, than *S. yirgalemense* at 200 IJs/insect and the control (Figure 1). *Steinernema yirgalemense* at 600 IJs/insect was not significantly different from *S. yirgalemense* at 400 IJs/insect (72.9%). *Steinernema yirgalemense*, at 200 IJs/insect (37.5%), provided significantly different ($p < 0.05$) mortality of the *H. licas* larvae to all the other three concentrations (Figure 1).

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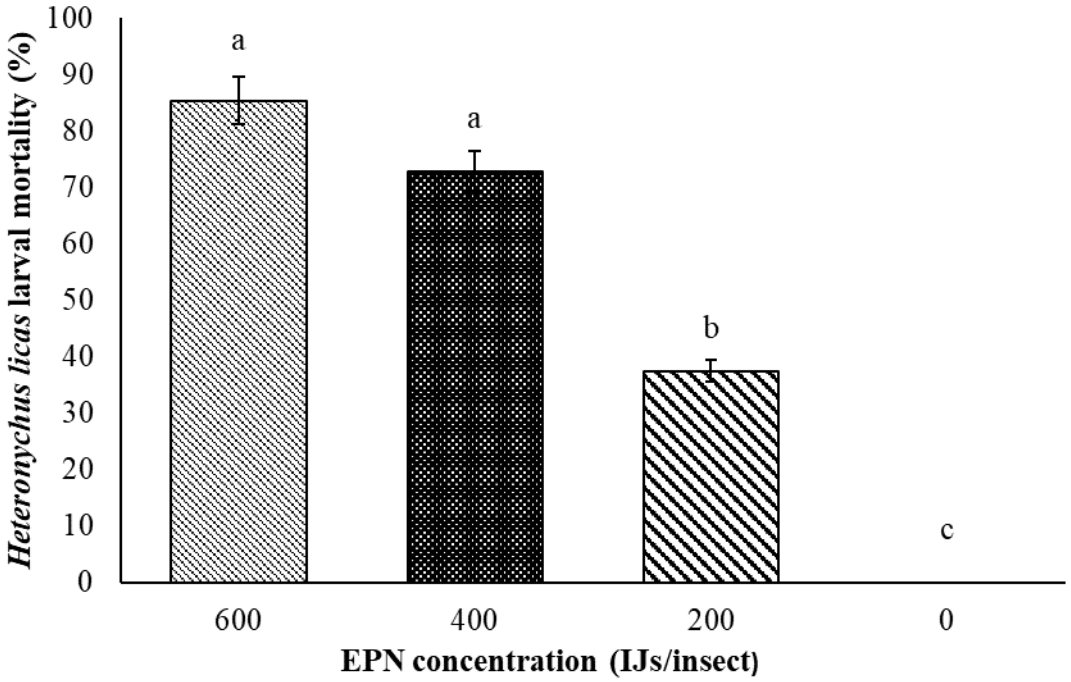


**Figure 1:** Mean percentage mortality (95% confidence level) of *H. licas* larvae inoculated with 0, 200, 400, and 600 IJs/insect of *Steinernema yirgalemense* (one-way ANOVA: $F(3, 28) = 17.50$, $p < 0.001$) in a laboratory trial. Different letters above the vertical bars indicate significant differences ($p < 0.05$).

### 4.2 Susceptibility of *H. licas* larvae to Malathion

Analysis using a one-way ANOVA showed no significant effect ($F(3, 36) = 26.34$, $p < 0.001$) of the treatment on percentage mortality between the different treatment dates. The mortality of *H. licas* larvae in the laboratory trial ranged from 0 to 89.6% after inoculation with 0, and 2.5 g/L of malathion, with significant effect ($F(3, 28) = 17.50$, $p < 0.001$) of the treatment on the percentage mortality. The malathion concentrations gave 0, 79.2, 83.3 and 89.6% mortality for 0, 0.625, 1.25 and 2.5g/L of malathion, respectively (Figure 2). All the three malathion concentrations resulted in *H. licas* larval mortality that was not significantly different ($p < 0.05$) from each other (Figure 2).

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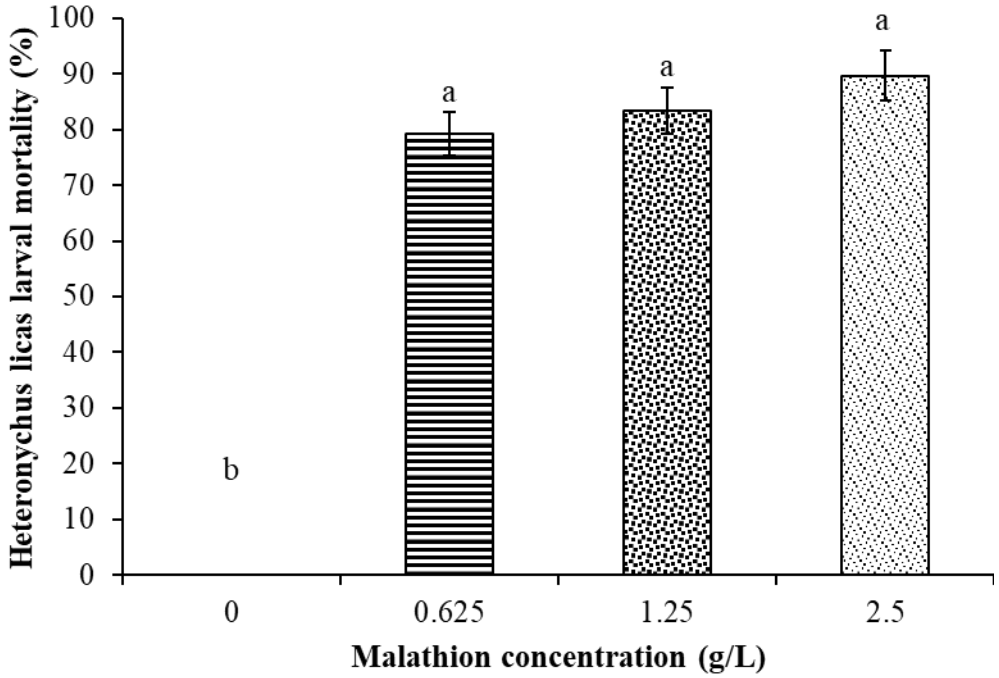


**Figure 2:** Mean percentage mortality (95% confidence level) of *H. licas* larvae inoculated with 0, 0.625, 1.25 and 2.5g/L of malathion (one-way ANOVA: $F(3, 28) = 17.50$, $p < 0.001$) in a laboratory trial. Different letters above the vertical bars indicate significant differences ($p < 0.05$).

### 4.3 Susceptibility of *H. licas* larvae to *S. yirgalemense* in soil bioassay

Analysis using a one-way ANOVA showed no significant effect ($F(3, 36) = 26.34$, $p < 0.001$) of the treatment on percentage mortality between the different treatment dates. The mortality of *H. licas* larvae in the soil bioassy ranged from 0 to 80.5% after inoculation with 0 and 600 IJs/insect over a period of 2 days, with significant effect ($F(3, 28) = 17.50$, $p < 0.001$) of the treatment on the percentage mortality. *Steinernema yirgalemense*, at 600 IJs/insect (80.5%), provided significantly higher ($p < 0.05$) mortality of the *H. licas* larvae, than *S. yirgalemense* at 200 IJs/insect and the control (Figure 3). *Steinernema yirgalemense* at 600 IJs/insect was not significantly different from *S. yirgalemense* at 400 IJs/insect (70.2%). *Steinernema yirgalemense*, at 200 IJs/insect (33.5%), provided significantly different ($p < 0.05$) mortality of the *H. licas* larvae to all the other three concentrations (Figure 3).

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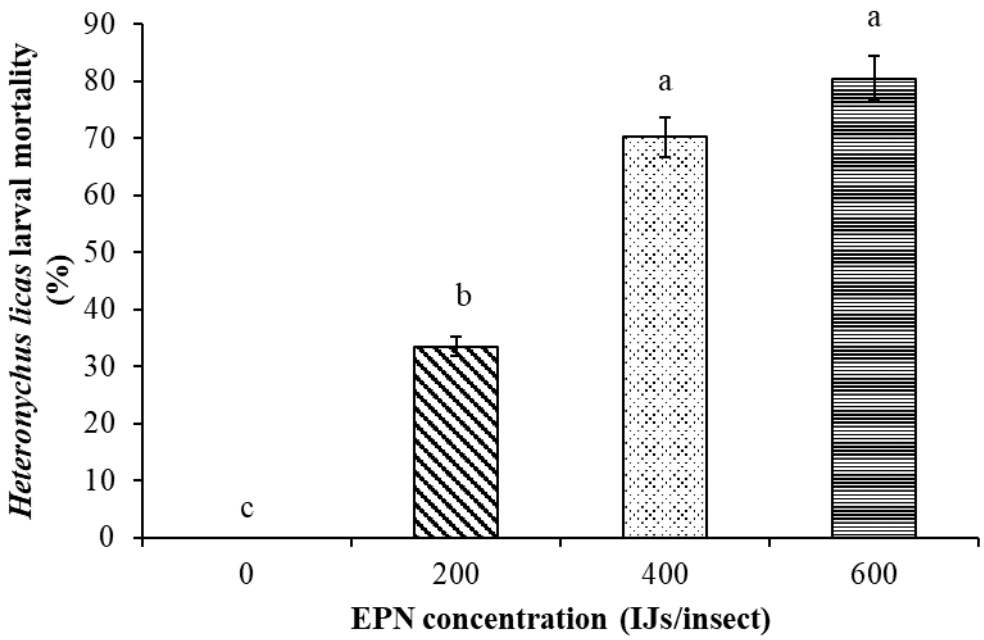


**Figure 3:** Mean percentage mortality (95% confidence level) of *H. licas* larvae inoculated with 0, 200, 400 and 600 IJs/insect of *Steinernema yirgalemense* (one-way ANOVA: $F(3, 28) = 17.50$, $p < 0.001$) in a in soil bioassay. Different letters above the vertical bars indicate significant differences ($p < 0.05$).

### 4.4 Susceptibility of *H. licas* larvae to Malathion in soil bioassay

Analysis using a one-way ANOVA showed no significant effect ($F(3, 36) = 26.34$, $p < 0.001$) of the treatment on percentage mortality between the different treatment dates. The mortality of *H. licas* larvae in the soil bioassay ranged from 0 to 87.5% after inoculation with 0, and 2.5 g/L of malathion, with significant effect ($F(3, 28) = 17.50$, $p < 0.001$) of the treatment on the percentage mortality. The malathion concentrations gave 0, 83.3, 85.4 and 87.5% mortality for 0, 0.625, 1.25 and 2.5g/L of malathion, respectively (Figure 4). All the three malathion concentrations resulted in *H. licas* larval mortality that was not significantly different ($p < 0.05$) from each other (Figure 4).

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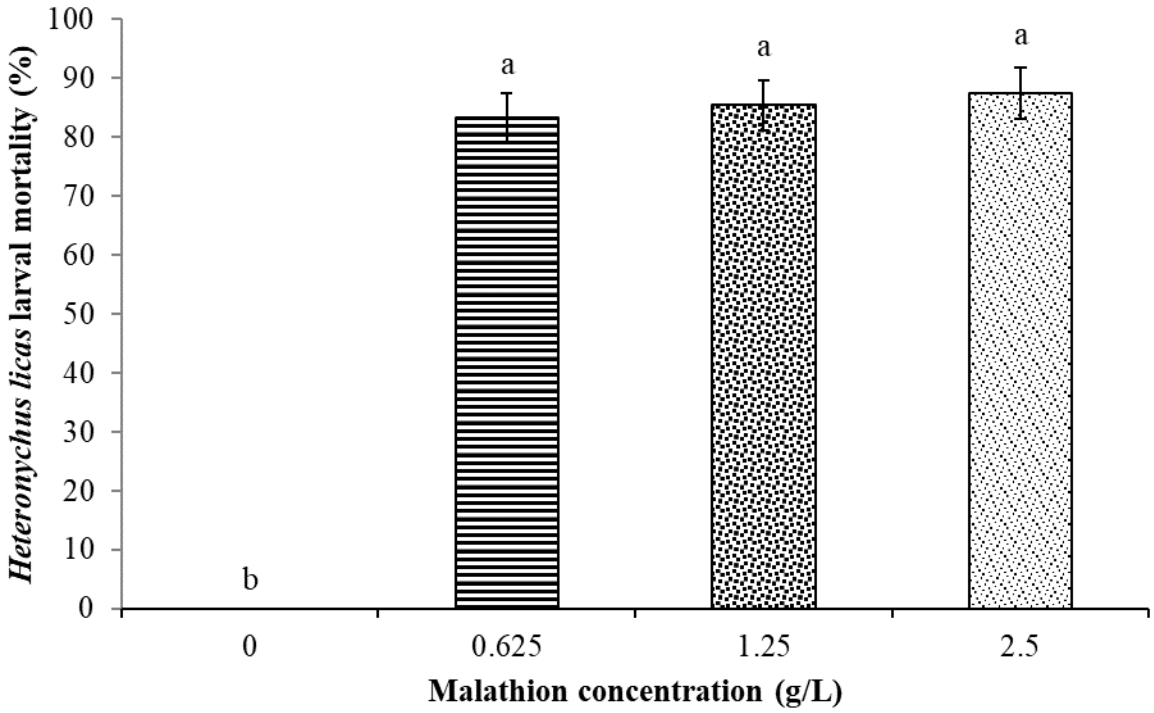


**Figure 4:** Mean percentage mortality (95% confidence level) of *H. licas* larvae inoculated with 0, 0.625, 1.25 and 2.5g/L of malathion (one-way ANOVA: $F(3, 28) = 17.50$, $p < 0.001$) in a soil bioassay. Different letters above the vertical bars indicate significant differences ($p < 0.05$).

## 5. DISCUSSIONS

In the current study, the effect of commercially available Malathion and an EPN were tested on the mortality of sugarcane white grubs larvae. The results from the study indicated that both Malathion and the EPN were able to kill sugarcane white grubs larvae. The results are in line with the findings of Khrishnaswam et al., (2019), who found out that the mortality of *Holotricha serrata* F *(*Melolonthidae: Coleoptera) was influenced by the concentrations of Chloropyriphos, and through his findings, he reported that the mean mortality of *H. serrata* increased with increasing the concentrations. The author further reported that a mean mortality of 17.3 was recorded at a concentration of 1.5g/L, which is consistent with the percentage of 89.58% on the lab bioassay experiment at an almost similar concentration which was 2.5g/L.

From the results obtained, it is evident that the percentage mortality of SWG decreased with decreasing concentrations of Malathion in both the lab and soil bioassays. As the concentration of Malathion increased from 0.625g/L to 2.5g/L, the mortality also increased from 79.17% to 89.58% on the lab bioassay experiment and from 83.33% to 85.42% on the soil bioassay experiment. These findings also correspond with the findings by Carnegie (1998) who reported an increase in mortality of SWG larvae with an increase in concentration of Malathion.

The mortality in the lab bioassays trays was slightly higher than that in these soil bioassays experiment. This means that SWG larvae in the lab bioassays trays were more susceptible to the

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Malathion than those in soil bioassays. This was because those in the lab bioassays trays experiments were dipped in the Malathion directly for about a minute at different concentrations, whilst those in the soil bioassays were not dipped directly but different concentrations of Malathion were sprayed on to the soil with the SWG larvae underneath. In the current study, the mortality was relative to the concentration of Malathion. A low mortality was recorded at relatively low concentrations and short exposure time in both experiments of lab and soil bioasssays and increased mortality with increasing concentrations.

With EPNs results of this study shows that attempt at biological control of SWG using parasitic nematodes in the lab were effective with a high mortality rate being recorded. This experiment gave results on the mortality effects of entomopathogenic nematodes on sugar cane white grubs. In the screening of nematodes a high mortality was observed on the species used (*S. yirgalemense*) because these species can easily penetrate through the natural openings of SWG and is able to search for the insect through the carbon dioxide emitted by the insect.

The use of different concentrations also influenced their infectiousness on SWG larvae. This means that a positive relationship was obtained between the number of infectious juveniles and the mortality of SWG larvae. It was prevailed that at lower concentrations, highly significant differences were found between the different concentrations of EPNs, however, at higher concentrations (600IJ/insect) after 48 hours. This means that applying more IJs/ insect does not result in the highest mortality of SWG. This is because the release of the symbiotic bacteria by the EPN, does not have an impact on the mortality. Ferreira and Mulan (2013), also found a positive relationship between the IJ concentration and the mortality of the banded fruit weevil larvae after exposure to *H. zealandica* for 4 days.

The control of SWG at different concentrations resulted in a mortality of 0% to 89% after inoculation with the EPN species used in the study, using different concentrations of 200 IJs, 400 IJs and 600 IJs. However, the mortality was then determined after 48 hours of sugarcane white grubs exposure to nematodes in the laboratory. Dlamini et al (2018) showed that, in the case of banded fruit weevil, *Phlyctinus callosus* (Coleoptera: Curculionidae) mortality was found to range from 24% to 95%, using double concentrations (200IJs) of nematodes whose mortality was also determined after 48 hours exposure to nematodes in the laboratory. Although higher concentrations were used in the present study because the sugarcane white grub is a bigger insect pest than banded fruit weevil and because of its defence mechanisms. It is known that white grubs have adopted a variety of behavioural traits which help them avoid infection from EPNs, which include grooming, rolling up into ball and movement away from nematodes (Koppernhofer *et al.*, 2000).

Virulence of EPNs at higher concentrations is enhanced by higher quantities of the symbiotic bacteria in the haemocel. The concentrations of applied EPNs species influenced their infectiousness on SWG. This effect was due to an increasing infectiousness at concentration 400IJ/ insect larvae, whilst 0 and 200 IJ/insects gave a low mortality rate. The best concentration is 400IJs /insect to control SWG larvae. These results from this study are in agreement with an observation by Le Vieux and Mulan (2013) who reported that *S. yirgalemense* was found to be

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highly virulent against the female vine mealybug, *Planococcus ficus* at 200IJs / insects which is almost similar from the results of this study.

## 6. CONCLUSIONS AND RECOMENDATIONS

The study revealed that the commercially available chemical (Malathion) and an EPN had the potential to cause mortality on SWG larvae. The overall efficacy of all the different concentrations of Malathion was better on the SWG lab bioassays compared to the soil bioassays. The concentration, 0.625g/L, of Malathion was found to be the most efficient in controlling SWG after 12 hours. This was because the mortality at 0.625g/L of Malathion gave mortality not significantly different from the other concentrations. With the EPNs, the concentration 400IJs /insect was found to be more efficient in the control of SWG. Based on the result of the experiment, EPNs and Malathion can be used for the control of sugarcane white grub larvae.

Further investigations need to be carried out in order to determine the effect of nematodes and Malathion on the mortality of SWG in open fields trials or greenhouses where all other environmental conditions are present. The study only determined the mortality effect of the EPN and Malathion in soil and lab bioassays alone. Further studies can be conducted using EPNs with EPFs as well as the combination with the chemical (Malathion) control in the sugar industry.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: DBE. Performed the experiments: MM and DBE. Analyzed the data: MM and DBE. Contributed reagents/materials/analysis tools: DBE. Wrote the paper: MM and DBE.

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