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Efficacy of Heterorhabditis species against Sweet Potato Weevil (*Cylas formicarius* Fabr.) in tropical islands: a case study of Visayas, Philippines

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ABSTRACT

Sweet potato weevil (*Cylas formicarius* Fabr.) is a serious threat to sweet potato production in the world. This insect pest can cause damage as high as 97% yield reduction when not managed. The use of biological control agent like entomopathogenic nematodes (EPNs) can be an alternative to the use of synthetic chemicals to manage this insect pest. The aim of this study was to determine the pathogenicity of the four EPN isolates from the Visayas islands in the Philippines in controlling the sweet potato weevil in–vitro and in greenhouse pot trials. All four isolates were initially identified under the Heterorhabditis genera based on the coloration of the cadavers. Of these four, three isolates, namely UBD, AVD, and CP were identified as *H. indica* and HO1 as *H. baujardi*. All of these four (4) EPN isolates were equally promising for the control of both larvae and prepupae. Among the four isolates, UBD at 300EPN/ml recorded the highest mortality on larvae (99.4%) and prepupae (88%), highest percent infection on larvae (95%) and prepupae (83.4%), and fastest to achieved LC_{50} which was 2 days after application on larvae while 4 days on prepupae. At higher concentration, LC_{50} were achieved faster than in lower concentration across isolates. A lower percent mortality was recorded on prepupae compared to larvae. In the greenhouse experiment, tubers inoculated with UBD isolate at 300EPN/ml obtained the least damage with no apparent weevil feeding damage on the tuber and no adult exit holes. The four isolates were found effective in controlling sweet potato weevil under tropical island situation in Visayas, Philippines.

Keywords: Entomopathogenic Nematodes, Biological Control Agents, Sweet Potato, Sweet potato weevil

INTRODUCTION

The Sweet potato weevil (*Cylas formicarius* Fabr.) is by far the most destructive pest of the sweet potato plant. The degree of infestation varies from region to region but weevils nevertheless, cause severe damage to plantations (Hue and Low, 2015). It is a destructive pest both in the field and in storage (Mullen et al., 1980). Reports indicated that losses in sweet potato production due to this pest ranged from 5-97% especially in areas where the weevil occurs (Capinera, 2018). In the Philippines, sweet potato yield was reduced by 50% due to C. formicarius infestation (Gapasin, 1998) but it could reached up to 100% infestation inside the tuberous roots (Department of Agriculture, 2012). There is a positive relationship between vine damage or weevil density, and tuber damage (Capinera, 2018). Sweet potato weevils generally cause serious damage to all parts of sweet potato plant throughout their life cycle, from egg to adult. When laying eggs, female weevils excavate cavities and create egg-laying punctures in the roots. The eggs are laid below the surface of the roots and covered with dark colour excrement from the female adults (Capinera, 2001). The insect infest both the fleshy roots and stems by tunneling into them and tainting them with disagreeable odor and bitter taste that render them unfit for human and animal consumption (Amalin and Vasquez, 1993). In addition, larvae tunneling inside the tubers indirectly facilitates the entry of soil-borne pathogens, which can cause further damage from secondary infections by fungi and bacteria (Onwueme and Charles, 1994). Since most of the infestations generally occur below the soil level, these problems can go undetected until harvesting season arrives or when there is a heavy infestation then damage manifested by yellowing of the vines become apparent (Hue and Low, 2015; Capinera, 2018).

The use of pheromone trap and selected approved pesticides were implemented as part of the IPM strategies in the US as well as information dissemination about planting strategies to minimize infestation and the introduction of sweet potato cultivar with higher resistance to weevils such as “Regal” (Maynard et al., 1999). However, the use of chemicals to control the weevil is seldom practiced especially by small farmers because they find them too costly or inaccessible to growers (Kyereko et al., 2019) including in the Philippines. Sweet potato weevil management in the country is manage by burning infested vines and roots after harvest, removal of weevil-infested debris in the field, use of healthy cuttings preferably terminal cuttings since tender portions of vines are rarely deposited with eggs of weevil as planting materials, practice hilling-up, proper cultivation and crop rotation, and use pheronome traps when available (Department of Agriculture, 2012). Likewise, chemicals are hazardous to the environment and non-target organisms. Thus, there is a need for cheaper and safer control methods for the sweet potato weevil. The use of biological control using entomopathogenic nematodes (EPNs) is one alternative. Nematodes are non-polluting and thus, environmentally safe and acceptable. Infective juveniles can be applied with conventional equipment and they are compatible with

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most pesticides (Rovesti and Deseo, 1990). They find their host either actively or passively and in cryptic habitats and sometimes in soil, and they have proven superior to chemicals in controlling the target insects (Gaugler, 1981). The EPNs Heterorhabditis and Steinernema together with their symbionts bacteria Photorhabdus and Xenorhabdus, respectively, are obligate and lethal parasites of insects (Burnell and Stock, 2000). Being soil-dwellers, EPNs have a good access to insect pests that feed on roots like sweet potato weevil. Several strains of Steinernema carpocapsae and Heterorhabditis bacteriophora are persistent, remaining active for up to four months (Capinera, 2018). Both Heterorhabditis spp. and

Steinernema spp. are considered as promising biological control agents of important insect pests due to their ability to seek target hosts, kill hosts rapidly, being safe to non-target organisms and having no threat to the environment as compared to chemical insecticides (Vashisth et al., 2013). In the Philippines, there is no record yet on the presence and the possible use of EPN isolates for the management of the sweet potato weevil. Recently, there were four isolates of Heterorhabditis spp. collected from the islands of Visayas. The third stage infective juveniles (IJs) of Heterorhabditis gains entry to the insect larva through natural openings such as mouth, anus and spiracles as well as by abrading the intersegmental membranes of the insect using a dorsal tooth. Once the IJs have gained entry to the haemocoel of the insect, the bacterial cells of the symbionts Photorhabdus are released. The bacterium releases toxins and exoenzymes which kills the insect host within 24-48 hours (Burnell and Stock, 2000).

In this study, four Heterorhabditis spp. collected from three provinces in the Visayas islands namely AVD and UBD from Negros Oriental, CP from Leyte and HO1 from Cebu were tested to determine their pathogenicity against the larvae and prepupae of sweet potato weevils in in vitro and to determine their efficacy in controlling the sweet potato weevil in potted plants under greenhouse condition.

MATERIALS AND METHODS

Mass rearing of sweet potato weevil

Sweet potato weevils were reared following the procedure by Cockerham (1954) with some modifications. Initial population of the sweet potato weevils collected from the field were confined in rearing cages and provided with sweet potato tubers as oviposition substrate. They were allowed to lay eggs on the tubers. Exposed tubers were removed from the rearing cage and examined for eggs. Tubers with eggs were placed in another rearing cage to allow the eggs to hatch, develop and emerge. Moist cottonwool was placed inside the cage to prevent the tubers from drying immediately. Newly emerged larvae were transferred in another rearing cage and regularly supplied with fresh tubers until the desired life stage for testing which is between 53 to 93 days after emergence were obtained. Third instar larvae and prepupae were used in the study. All test insects were reared inside an incubator with temperature set to 25-270C all throughout the rearing process.

Culture of entomopathogenic nematodes isolates

Four Heterorhabditis spp. collected from the different sampling sites in the Visayas islands were maintained and mass produced at the Nematology Laboratory in the Department of Pest Management, Visayas State University. EPNs were mass produced using the last instar larvae of the lesser wax moth, *Achroia grisella* Fabr.,. In a 50 mm petri plate lined with filter paper, 1 ul of EPN suspension was added to the petri plate and ten (10) last instar larvae of A. grisella were placed on the filter paper. After covering the plates, they were kept in an incubator at 250C for optimum infection for 5 days. Nematodes were harvested regularly from a modified White Trap (Kaya and Stock, 1997). All isolates were stored in a tissue culture flask with distilled water and maintained at 50C. EPN in the suspension was checked and infection was done regularly to maintain the inoculum.

Laboratory experiment

The four EPN isolates were previously identified as Heterorhabditis spp. based on the characteristics of the cadavers infected by the nematodes and species were confirmed using its morphological characteristics through morphometrics analysis. The three isolates namely CP, AVD and UBD were identified as *H. indica* while isolate HO1 was *H. baujardi*. An invitro assay was carried out to determine the effectiveness of the four EPN isolates. The following concentrations were used: 100EPN/ml, 200EPN/ml, and 300EPN/ml. Freshly harvested third stage infective juveniles of the nematodes suspended in aqueous medium were used.

In a 90 mm petri dish lined with filter paper, each nematode concentration was inoculated on fifty (50) larvae and prepupae of sweet potato weevil using a hand held micro-sprayer bottle. Petri plates with sweet potato weevil larvae and prepupae without EPN were used as control. Mortality was monitored and recorded regularly. All cadavers were collected and placed on a modified White Trap (Kaya and Stock, 1997) to confirm EPN infection as the cause of mortality. Infective juveniles emerged from the cadavers were harvested and stored in tissue culture flask with distilled water and maintained at 50C.

Greenhouse experiment

In this experiment, the three most promising EPN isolates based on the in-vitro assay namely; UBD, CP,

and HO1 with the concentration of 300EPN/ml were used. Enough 'VSP30' sweet potato variety were planted in pots for the greenhouse evaluation. Sterilized potting soil was used in the experiment. The pots were placed on an elevated bed lined with plastic sheet to avoid contamination. One sweet potato vine per pot was planted in 2-inch deep hole in the soil. Pots were positioned directly towards the sunlight. To create a cooling off effect and maintain the temperature inside the greenhouse to 23-260C throughout the duration of the experiment water was regularly sprayed along the sides of the greenhouse structure. Sweet potatoes were grown up to 105 days before harvesting.

Application of treatments and data collection

Two months after planting, ten sweet potato weevil adults (2 males and 8 females) were introduced in each pot and confined by covering the pots with nylon tulle to ensure infestation and avoid the escape of the weevils. Thereafter, UBD, CP and HO1 isolates were inoculated by pouring the 300 EPN/ml aqueous suspension directly to the potting soil of each pot. Three (3) replications were made each treatment and control plants treated with synthetic chemical (Cypermethrin at 10ml/100L of water) and water alone were provided.

At harvest, each individual roots and stems were inspected for weevil damage and rated on a scale from 1 to 6 after Jansson et al. (1990), as follows: 1 – no weevil feeding damage or adult exit holes; 2 – up to 25% of the root surface area covered with weevil feeding punctures but no adult exit holes; 3 – from 26 to 50% of the root surface area covered with weevil punctures but no adult exit holes; 4 – greater than 50% of the root surface area covered with weevil feeding punctures or 1-3 adult exit holes (or both); 5 – 4 to 5 adult exit holes; 6 – greater than 6 exit holes per root. Average weight and number of sweet potato tubers harvested per treatment were recorded.

Statistical analysis

Insect mortality was corrected according to the control treatment values using Abbott's formula. LC50 was calculated for each EPN isolate using probit analysis. All the data obtained were analyzed by analysis of variance (ANOVA) using SPSS (Version 17.0) statistical software package and Least Significant Difference (LSD) test ($P<0.05$) was used to separate means values.

RESULTS

All EPN isolates were able to kill third instar larvae of sweet potato weevil at all concentrations in-vitro (Table 1). The results show that at higher concentration, percent mortality was also higher. At 100 EPN/ml, mortality was ranged from 90-97.4%, while at 200EPN/ml it ranged from 91.4-98.6%. The highest mortality (99.4%) was recorded when 300EPN/ml UBD isolate was applied. UBD isolate produced a consistently high mortality rate across concentration however, the results were statistically comparable with the three other isolates at 100EPN/ml and 200EPN/ml. While at 300EPN/ml concentration, CP isolate has recorded the lowest mortality (90%) which was significantly different with the other three isolates. Among the four isolates, increased in concentration will not improve the pathogenicity of CP isolate. The same trend was observed in the number of days to LC50. At higher concentration, larvae were killed faster than at lower concentration. At 100EPN/ml, it takes 3-4 days before mortality was recorded while at 200EPN/ml, it only took 3 days across isolates. At 300EPN/ml concentration, LC50 was achieved within 2 days through inoculation larvae with AVD and UBD isolates, while 3 days after inoculation with CP and HO1 isolates. In terms of % infection, there is no significant difference across isolates at 100EPN/ml and 200EPN/ml concentrations however, at 300EPN/ml concentration UBD attained the highest percent infection (95.3%) which was comparable with AVD (94%) and HO1 (92.7%) while CP obtained the lowest at 85.3% infection.

Table 1. Percent mortality, infection and LC_{50} on larvae of Sweet Potato Weevil treated with different EPN isolates under laboratory conditions

Treatments	% Mortality with different concentration						% Infection with different concentration		
	100/ml	No. of days to LC_{50}	200/ml	No. of days to LC_{50}	300/ml	No. of days to LC_{50}	100/ml	200/ml	300/ml
Control	24.0^{b}	*	24.0^{b}	*	24.0^{c}	*	0.0^{b}	0.0^{b}	0^{c}
AVD	95.4^{a}	3	96.6^{a}	3	98.6^{ab}	2	90.6^{a}	92.7^{a}	94.0^{ab}
UBD	97.4^{a}	3	98.6^{a}	3	99.4^{a}	2	90.0^{a}	92.0^{a}	95.3^{a}
CP	90.0^{a}	4	91.4^{a}	3	90.0^{c}	3	85.3^{a}	96.0^{a}	85.3^{c}
HO1	94.6^{a}	4	96.6^{a}	3	96.6^{ab}	3	88.7^{a}	91.3^{a}	92.7^{ab}

*50% mortality was not reached.
Mean values followed by different uppercase letters in the same column are statistically different according to LSD test ($P \leq 0.05$)

Percent mortality was reduced in all isolates across concentrations on prepupae of sweet potato weevil (Table 2). At lower concentration (100EPN/ml) percent mortality ranges only at 60.6% (CP isolate) to 84.6% (UBD isolate), the same trend was observed at 200EPN/ml and 300EPN/ ml wherein percent mortality was ranging from 72-83.4% and 72.6-88%, respectively. Among isolates, UBD has consistently recorded the highest mortality which was comparable with AVD isolate across concentration and with H01 at 200EPN/ml and 300EPN/ml concentration. It took 4 to 6 days after inoculation to obtain LC50. UBD isolate attained LC50 4 days after which was the earliest at 300EPN/ml. Likewise, UBD isolate obtained the highest percent infection (78.7-83.4%) across concentration which is comparable with AVD isolate across concentration and HO1 at 200EPN/ml (77.3%) and 300EPN/ml (75.3%) concentration while CP isolate recorded the lowest percent infection in all concentration ranging from 56.7-69.4%.

Table 2. Percent mortality, infection and LC_{50} on prepupae of Sweet Potato Weevil treated with different EPN isolates under laboratory conditions

Treatments	% Mortality with different concentration						% Infection with different concentration		
	100/ml	No. of days to LC_{50}	200/ml	No. of days to LC_{50}	300/ml	No. of days to LC_{50}	100/ml	200/ml	300/ml
Control	10.0^{c}	*	10.0^{c}	*	. 10.0^{c}	*	0^{c}	0^{c}	0^{c}
AVD	80.6^{a}	5	80.0^{ab}	5	78.0^{ab}	5	75.3^{a}	77.3^{b}	75.3^{ab}
UBD	84.6^{a}	5	83.4^{a}	5	88.0^{a}	4	78.7^{a}	81.3^{ab}	83.4^{a}
CP	60.6^{b}	6	72.0^{b}	5	72.6^{b}	5	56.7^{b}	63.4^{b}	69.4^{b}
HO1	66.0^{b}	6	80.0^{ab}	5	76.6^{ab}	5	62.6^{b}	76.0^{ab}	73.4^{ab}

*50% mortality was not reached.
Mean values followed by different uppercase letters in the same column are statistically different according to LSD test ($P \leq 0.05$)

Moreover, a trial on pot at the greenhouse experiment was conducted using the three most promising EPN isolates based on the laboratory experiment namely; UBD, CP, and HO1 with the concentration of 300EPN/ml. The said concentrations were used anticipating the effect of environmental factors on the survival of the EPN isolates. There was no significant difference for the weight of tubers and number of tubers across treatments on harvested tubers planted in pots under greenhouse conditions (Table 3). The harvested tubers average weight ranged from 255-309 g and average number of tubers ranged from 5-6per treatment. There was a significant difference between treatments on the damage in the harvested tubers by the sweet potato weevil. Tubers inoculated with UBD isolate had the least damage (1.20) which was comparable with tubers treated with chemical (1.46). Tubers inoculated with the other two EPN isolates showed comparable results with damage ratings of 1.6 (HO1) and 1.73 (CP) respectively.

Table 3. Average weight, number of tubers and damage rating of Sweet potato weevils on harvested tubers planted on pots under greenhouse condition

Treatments	Weight of Tubers ^{ns}	Number of Tubers ^{ns}	Damage in the Tubers*
Control	285.43	5.06	3.0^{a}
Chemical (10ml/100L)	309.5	6.06	1.46^{bc}
UBD (300EPN/ml)	255.06	5.93	1.20^{c}
HO1(300EPN/ml)	295.03	5.6	1.6^{b}
CP (300EPN/ml)	272.36	5.66	1.73^{ab}

* This is based on the rating use to assess the damage of Sweet potato weevils on the harvested crops.
Mean values followed by different uppercase letters in the same column are statistically different according to LSD test ($P \leq 0.05$)

DISCUSSION

The use of entomopathogenic nematodes, Heterorhaditis spp., against sweet potato weevil has been recorded to be effective (Capinera, 2018; Korada et. al., 2010). The efficacy of the EPN isolates on the different life stages were affected by the morphological structure of the sweet potato weevil. Percent mortality and infection was higher in larval stage compared to prepupal stage.

In the study conducted by Rezael et. at (2015), pathogenicity of different EPN species was affected by the life stages of the test insects. Heterorhabditid nematodes penetrate the soil and tubers, killing weevil larvae (Capinera, 2018) while they are more pathogenic to pre-pupae than to adults (Korada et. al., 2010).

The susceptibility of larvae to the EPN isolates could be attributed to its soft cuticle in addition to the natural openings such as spiracles and oral openings making penetration and entry by the EPN easier than in prepupae with the prepupal case and its inactivity particularly on feeding that restricts the entry of the EPN isolates. The prepupae are easily distinguished from the actively feeding last instar larvae by the cessation of feeding and the shortening and clearing of the body. The larval body changed from a reddish or purplish cast to a clear, deep yellow when entering the prepupal stage (Sherman and Tamashiro, 1954). Infective juvenile entomopathogenic nematodes penetrate the body cavity of larvae via the mouth or breathing pores by using a tooth-like structure that pierce into the host soft intersegmental membranes (Bedding and Molyneux, 1982). Once inside, the Heterorhabditid nematodes start releasing pathogenic bacteria, Photorhabdus spp. into the larvae body cavity, where the bacteria will reproduce rapidly and cause larvae death within two days (Adams and Nguyen, 2002). The same findings were reported by Nderitu et al. (2009) wherein mortality rate was also higher in larvae than in prepupae.

In this study, H. indica (UDB isolate) proved to be effective than synthetic insecticide (Cypermethrin at 10ml/100L). This finding conforms with the observation of Capinera (2018) that in some cases, nematodes are more effective than insecticides at reducing damage. It can be noted that in terms of the average weight of tubers and average number of tubers harvested, tubers treated with EPN isolates were as good as those were treated with chemicals, thus, the result implied that EPN isolates can be used as alternative to synthetic chemicals in controlling sweet potato weevils. In the field, the susceptibility of larval stage to EPN is expected to even increase since the weevil will spend up to three-four weeks at this stage undergoing three instars which is longer than the prepupal stage which will only take 7 to 10 days (Capinera, 2018). This larval stage of sweet potato weevil is susceptible to infection by EPN larvae. Infective juvenile is the only free-living stage and can survive in soil for several months until susceptible insects are encountered (Gozel and Gozel, 2016) thus once EPN population is already established in the field, there may not be a need for a multiple application of EPN. Long-term persistence in the field was a result of recycling in host insects (Susurluk and Ehlers, 2007). Naturally-occurring EPN populations in the soil, is much more economical and safer to the environment compared to the application of synthetic insecticides which pose environmental threats, are expensive and require multiple application for managing sweet potato weevil.

CONCLUSION

The four isolates caused mortality of the sweet potato weevil larvae and pre-pupae in the laboratory. In pot experiment, all three isolates tested reduced the damage of the sweet potato weevil in the tubers and UBD isolates (*H. indica*) was found to be comparable with the chemical treatment. UBD isolates collected from the province of Negros Oriental was found to be the most effective among the EPN isolates tested in controlling sweet potato weevils both in in vitro and in vivo. To further confirm its efficacy, field experiment is recommended before it can be recommended for use as biological control agent to sweet potato farmers. Further experiments using other insect pests of major crops is recommended to optimize the utility of these isolates in other tropical islands.

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