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Article

Occurrence and Distribution of Entomopathogenic Nematodes in Sweet Potato Fields in the Philippines and Their Implication in the Biological Control of Sweet Potato Weevil

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Abstract: The sweet potato weevil (*Cyclas formicarius* Fabr.) remains a serious threat to sweet potato (*Ipomoea batatas* Poir.) production and is considered the most destructive pest of sweet potatoes in the field and storagein the Philippines. Chemical control of the weevil is seldom practiced by farmers because they find it too costly, it may increase the chance for pesticide resistance, and because of public concern of its effectson non-target organisms. The use of biological controls such as entomopathogenic nematodes (EPN) could offer an effective, economical, and environmentally-friendly alternative management of the weevil. This study determined the occurrence and distribution of entomopathogenic nematodes in selected sweet potato growing areas in the Philippines. Using soil from 13 sweet potato growing areas, EPNs were recovered using the insect baiting method. Morbid insect larvae were suspended in sterile water for 48 h, and the suspension was examined under a stereomicroscope for the presence of EPN. Out of 47 samples collected from the 13 sweet potato production areas, 39 (82%) were positive for the presence of EPNs. Preliminary identification of the EPNs through morphological characters showed that they belonged to Rhaditida: Heterorhabditidae and Steinernematidae. This is the first report on the occurrence of EPNs in sweet potato fields in the Philippines, and their distribution strongly supports the possibility of utilizing them in an IPM management approach as biological agents against the sweet potato weevil. Morphometric and molecular-based identification and pathogenicity studies are underway.

Keywords: *Ipomoea batatas*; *Cylasformicarius*; biological control agent; integrated pest management; IPM; Rhabditida; Heterorhabditidae; Steinernematidae

1. Introduction

The sweet potato weevil (*Cylasformicarius* Fabr.) is a serious threat to sweet potato production, not only locally in the Philippines but also globally. It is the most destructive pest of sweet potato in the field and in storage. Reports indicated that losses in sweet potato production due to this pest ranged from 5% to 97%, especially in areas where the weevil occurs [1]. The insect attacks both the fleshy roots and the stems by tunneling into them and tainting them with a disagreeable odor and a bitter taste which renders them unfit for human and animal consumption.

The use of chemicals to control the weevil is seldom practiced, especially by small farmers because they find them too costly. The chemicals are also considered hazardous to the environment and non-target organisms. Thus, there is a need for cheaper and safer control methods against the sweet potato weevil. The use of a 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 most pesticides [2]. They find their host either actively or passively, and, in cryptic habitats and sometimes in soil, they have proven superior to chemicals in controlling the target insects [3].

An excellent example of a situation in which a nematode may replace chemicals for control of an insect is the black vine weevil (*Otiorhynchussulcatus*) in cranberries. The use of chemical insecticides is restricted or has not provided adequate control of black vine weevil larvae. When *Heterorhabditis bacteriophora* "NC" strain was applied, it provided 70% control of the black vine weevil soon after treatment and was still providing the same level of control a year later [4].

Entomopathogenic nematodes seem to be organisms with the greatest potential for practical biological suppression of sweet potato weevil [1]. In the United States, studies have shown the superiority of some genera of EPNs for suppressing or reducing weevil populations. To date, no studies have been conducted utilizing EPNs against the sweet potato weevil in the Philippines. Thus, this study determined the occurrence and distribution of EPNs in selected sweet potato growing areas in the Philippines.

2. Experimental Section

2.1. Collection of Soil Samples

A survey was conducted in selected sweet potato growing areas of Visayas, Mindanao and Luzon, Philippines (Table 1). In each of the provinces/municipalities selected, specific areas/sites where sweet potato were grown were identified and sampling sites were established.

Soil samples from around the roots of sweet potato plants at each site were collected. From each sampling site, 10 core samples were taken, and 10 kg composite soil samples were derived. The soil samples were placed in plastic bags and transported to the laboratory. An aliquot of 300 g from each 10 kg sample was processed in the Nematology Laboratory of Visayas State University (VSU), Visca, Baybay City, and Leyte.

2.2. Rearing of Galleria mellonella

Larvae of *Galleria mellonella* were collected from honeybee hives infested with *G. mellonella*. Larvae were reared in the laboratory by feeding a nutrient diet [5]. The insects were allowed to develop into adults in a rearing box, and then were transferred to an ovipositor box to mate and oviposit the eggs. Eggs were collected and placed ina new, clean rearing box, wherein they were reared and maintained to serve as a source of insects for EPN baiting. The larvae were supplied regularly with fresh nutrient diet.

2.3. Nematode Extraction

Two methods of EPN extraction from the soil samples were employed. In the first method, a soil sample was placed in a large pail and mixed thoroughly. Then, water was added and it was mixed again. The mixture was sieved through a 120 μm mesh sieve, and then transferred to a 45 μm mesh sieve. The nematodes were collected from the 45 μm sieve in a beaker and transferred to an improvised Baerman funnel. The EPN suspension was kept in a test tube. In the second method, soil samples were placed in small plastic containers and healthy *G. mellonella* larvae were added to act as bait for the EPNs. After 48 h, dead larvae were recovered and examined under the microscope for the presence of EPN.

2.4. Killing, Staining and Mounting of Nematodes

Nematodes were teased out, killed and stained following standard procedures and techniques used for nematodes [6]. Representative of each EPN were then mounted on slides to facilitate identification.

2.5. Identification of Nematodes

Nematodes were identified up to genera using available keys and other references.

3. Results and Discussion

Results from 47 areas in 13 provinces showed the presence of EPNs, with most found in sweet potato growing areas in Negros Oriental, Visayas (Table 1). Their abundance in these sweet potato areas could be due to the soil type, moisture and temperature in the area at the time of sampling which could have favored the reproduction of EPNs in the soil. Soil from these areas were sandy or sandy loams. According to Molyneux [7], survival of juveniles was high in these types of soils. The unlimited host range and niche conditions may also have contributed to the high frequency of the nematodes [8].

Table 1. A list of the sweet potato growing areas in the Philippines that were sampled, and occurrence of entomopathogenic nematodes (EPNs) from the soil at each site.

Major Islands	Province	City/Barangay (Brgy)/Municipality	Occurrence of EPN [z]
Luzon	Benguet	Benguet State University (BSU)	+
	Laguna	IPB, UP Los Banos	+
	Nueva Ecija	Central Luzon State University (CLSU)	-
	Tarlac	Tarlac College Of Agriculture	-
Visayas	Biliran	DA, Naval	Steinernema
		Provincial scion grove and nursery, Sitio San Roque, Brgy. Larrazabal	-
		Sitio San Roque, Larrazabal,	+
	Cebu	Dakit, Barili	Steinernema
		Cebu Technological University (CTU), Barili Campus	-
		Cambinocot	+
	Eastern Samar	Lawaan	+
		Brgy. Dos, Giporlos	-
		Brgy. Cabong, Borongan	Steinernema
		Balangiga	+
		Brgy. San Pablo, Taft	+
	Leyte	Brgy. San Esteban, Burauen	Steinernema
		Brgy. Maliwaliw, Dagami	-
		Brgy. Tinocdugan, Leyte	Steinernema
		DA, Babatngon (SP)	-
		DA, Babatngon (Okra)	-
		Del Pilar, Dulag	-
		Fatima, Dulag	-
		Gakat, Baybay	-
		San Vicente, Dulag	Heterorhabditis
		VSU, Agromet	-
		Zone 11, Mayorga	Steinernema
		Sitio Madocao, Brgy. Damos, Leyte	+

Table 1. *Cont.*

Major Islands	Province	City/Barangay (Brgy)/Municipality	Occurrence of EPN [z]
Visayas	Negros Oriental	Brgy. Apolong, Valencia	Heterorhabditis
		Brgy. North Poblacion, Valencia	Heterorhabditis
		Brgy. Lepayo, Dauin	+
		Bacong	Heterorhabditis
		Brgy. MaayongTubig, Dauin	-
		Sito May-abo, Brgy. Maluwag, Zamboangita	-
		Zamboangita	+
	Western Samar	Brgy. Bachao, Basey	-
		Brgy. Mabuhay, Marabut	-
		Brgy. Osmena, Hinabangan	-
Mindanao	Agusan del Sur	Sta. Josefa	+
		Trento	-
	Compostela Valley	Mabini	Steinernema
		New Bataan	-
		Kapatagan, Laak	-
	South Cotabato	Sulit, Polomolok	Steinernema
		Sarabia, Koronadal	-
		Sitio Cabuling, Sarabia, Koronadal	-
		Crossing, Polkan, Polomolok (Peanut)	+
		Crossing, Polkan, Polomolok (SP)	-

[z] Genera, or - = absent, and + = possible EPNs/to be determined.

There were two genera of EPNs extracted from the samples, *Steinernema* (Figure 1) and *Heterorhabditis* (Figure 2). Based on morphology of males, females, and juveniles, the most prevalent EPN was *Steinernema*, in agreement with Molyneux that Heterorhabditidae do not survive as well as do Steinernematidae [7]. All members of the order Rhabditidae are bacteriophagous, with many of them forming phoretic association with insects. Through time, some of these nematodes may have evolved into insect pathogens [9]. This may explain the occurrence of EPNs in the different areas surveyed. In Visayas, 18 out of 33 areas were positive for EPNs with 6 identified as *Steinernema*, and 3 as *Heterorhabditis*. Nine are yet to be identified. In the 10 areas of Mindanao sampled, 2 were positive for *Steinernema* while 2 were not identified. Out of 4 areas in Luzon, 2 were positive for the occurrence of EPNs. However, the nematodes in these samples have yet to be identified.

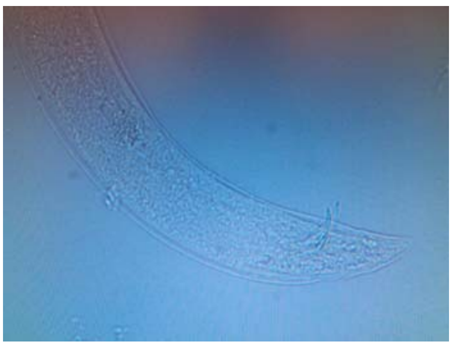

Figure 1. *Steinernema* sp. isolated from Sulit, Polomolok, and South Cotabato showing thespicule withoutthe bursa.

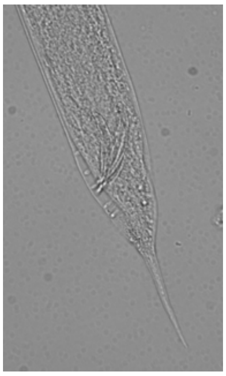

Figure 2. *Heterorhabditis* sp. isolated from Apolong, Valencia, Negros Oriental showing the spicule with bursa.

4. Conclusions

The results established the occurrence of *Heterorhabditis* and *Steinernema* EPNs in some sweet potato growing areas in the Philippines. This implies their abundance which is encouraging for their possible use as biological control agents against sweet potato weevil.

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Conflicts of Interest: The authors declare no conflict of interest.

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