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Research Article

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BIOPESTICIDAL EFFECT OF STEINERNEMA SP. ON LEPIDOPTERAN PEST *HELICOVERPA ARMIGERA* AND BIOCHEMICAL PARAMETER ANALYSIS

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ABSTRACT

The entomopathogenic nematodes (*Steinernema* and *Heterorhabditis)* are used effectively as Bio-pesticide against various pests. These soil dwelling nematodes can be recovered from various soil samples. In this present study EPN, recovered from Perur soil sample, was found to be *Steinernema*, and effectively killed the *Helicoverpa armigera* (Lepidopteran pest) within 48 hours of infection. The biochemical parameters (protein, carbohydrate and lipid amount) in EPN infected larvae showed a significant decrease compared to normal larvae.

Keywords: Bio-pesticide, *Steninernema,* Biochemical parameters, *Galleria Mellonella, Xenorhabdus sp.*

INTRODUCTION

Biological control is defined as the action of natural enemies (arthropod, predators, insect parasitoids and microbial pathogens) that maintain a host population at levels lower than what would occur in the absence of those enemies. The entomopathogenic nematodes come under the family *Steinernematidae* and *Heterorhabditidae.* They are associated with mutualistic bacteria in the genus *Xenorhadbus* for *Steninernema* and *Photorhabdus* for *Heterorhabditis* (Kaya and Nelson,1985; Kaya and Gaugler; 1993, Kaya and Stock, 1997). Thus, it is the nematode and bacterium complex that work together act as a biological control unit to kill an insect host. The mutualistic bacterium propagates and produces substances that rapidly kill the host and protect the cadaver from colonization by other microorganisms. The nematode initiates its development, feeding on the bacterial cells and host tissues that have been metabolized by the bacterium and has 1-3 generations, depending on host size. As food resource in the host cadaver are depleted, a new generation of infective juveniles are produced and emerges from the host cadaver into the soil to search for new hosts. This is exactly why EPNs are employed as bio-pesticide, in the present study aimed to control the Lepidoptern pest of economically important plants like tomato, chickpea, cotton, tobacco, cabbage, cauliflower, caster and banana.

MATERIALS AND METHODS

Rearing of *Galleria mellonella*

The larvae of greater wax moth *Galleria Mellonella* (Lepidoptera: *Galleridae*) were used for baiting the nematodes. The larvae were reared in 1,500 ml containers at 32^0C on an artificial diet in the laboratory. They reached the last instar stage between 5 to 6 weeks. They were collected and used for baiting the nematode in the study. Some larvae left in the containers itself for pupation.

Collection of soil samples

A total of five samples were collected from different locations in and around Coimbatore (Kumuttipathi, Perur, Thirupur, Valayar, and Valavadi). From all locations 250 gm of soil sample were collected at a depth of 15 cm from the surface and transferred into clean polyethene bags and were brought to the laboratory and stored at 25°C for future study.

Isolation and identification of Nematode

Entomopathogenic nematode was recovered from soil samples using the insect baiting method as described by Bedding and Akhurst (1975). Insect baits fifth (last) instar larvae of *G. mellonella* were placed in 200 ml plastic container which contained 75 gm of collected soil, moisture with the water. Each collected soil from different areas was kept in separate containers. Every day the soil samples were observed for the infection of nematode, by noticing the death of Galleria larvae. The dead larvae were isolated and thoroughly rinsed in 0.01% formalin placed in white`s trap (Kaya and Stock, 1997) until the emergence of third-stage infective juveniles of nematodes in another two or three days.

The emerging nematodes were pooled from each sample and stored in culture flasks (T-flasks) by changing

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the formalin once in a week. These nematodes were identified and used to infect fresh larvae of insect's pests, for mass propagation of nematodes, for identification and establishment of culture (T-flasks). The culture flasks with nematodes were maintained at 25℃.

Culturing of Symbiotic Bacteria

Nematodes act as vector by carrying the bacterium and infect the larvae. They enter through the natural opening like mouth, anus and spiracles. It was multiplied within the larvae by using body parts of the larvae as nutrient source. The symbiotic bacteria in the nematodes also multiply along with nematodes. They lead to the death of the larvae by septicemia. The dead larvae were sterilized with 70% ethanol by immersing the larvae in ethanol for 3 times and air-dried. The larvae harbored the symbiotic bacteria, so to isolate the bacteria a loopful of haemolymph was streaked on Nutrient agar plate containing 0.004% triphenyl tetrazolium chloride and 0.025% Bromothymol blue (NBTA). The plates were incubated at 28^0 C for 24 hrs. The two forms phase I and phase II were differentiated based on the color of the culture. Only primary from bacteria were used in the study.

Morphological studies

Following tests was carried out for identification of the isolates.

Gram's staining: A thin smear of the bacteria colony was made on the glass slide and heat fixed .Then it was flooded with crystal violet solution and allowed to remain 1 minute. It was washed with water and flooded with grams iodine solution and left for one minute. It was then drained and decolorized with 95% ethanol followed by running water. The slide was counter stained with saffranin for 30-60 seconds excess stains were washed with water and air dried. The stained smear was observed under low power and turned to oil immersion objective and the results were recorded.

Motility test: Liquid broth (LB) containing 0.5% agar, 60 g *ampicillin* per ml, 0.0025% bromothymol blue was used to observe swarming motility. The plates were dried at room temperature. *Xenorhabdus* isolates grown in nutrient broth placed on the LB agar plates. The plates were incubated at 28℃ and observed for swarming motility (Volgi *et al*., 1998).

Pathogenicity of *Steinernema* on *Helicoverpa armigera* larva

H. armigera larva were collected from chickpea field at Perur, and brought to the laboratory. The native EPNs (S*teinernema*) isolated from soil sample (Perur) was sprayed (50 nematodes/5 larva) (10:1) on *H. armigera* larvae and the time of death duration was noticed. Then the biochemical parameters (carbohydrates, protein and lipids) were tested in the haemolymph and cuticle of *H. armigera* larvae at different intervals.

Estimation of biochemical parameters

Protein: The protein content of *H. armigera* (haemolymph and cuticle) was determined by the method of Lowry, *et al*, (1951) using and bovine serum albumin as the standard. 100 mg of sample was homogenized in 2 ml of 5% trichloro acetic acid and centrifuged. The precipitate was dissolved in 1% sodium hydroxide solution and used for the estimated of protein. The protein present in the sample reacts with Folin phenol reagent and produces a blue color by the reduction of phosphomolybdic phospotungstic components biuret reaction. The colour developed was measured at 530 nm using photoelectric colorimeter. The results were expressed in mg protein /ml**.**

Carbohydrate: The Carbohydrate content of *H. armigera* (haemolymph and cuticle) was homogenized in 20 ml of 55 tricholoro acetic acid and centrifuged. The supernatant was collected and used for estimation of Carbohydrate. The supernatant was taken in test tubes and evaporated keeping the test tubes in a boiling water bath. After complete evaporation, 4 ml of anthrone reagent was added and heated in a boiling water bath for exactly 15 minutes. After 15 minutes the mixture was cooled in running tap water. The red colour developed was read at 630 nm using a photoelectric colorimeter.

Lipid: The lipid content of *H. armigera* (haemolymph and cuticle) was estimated by semi micro determination method (Knight *et al.,* 1972) of the homogenate was prepared in cold choloroform –n – butyl alcohol mixture. The filtrate was collected in test tubes and evaporated using desiccator. After complete removal of the solvent, the tubes were taken out and 3.0 ml of 2% potassium dichromate in 98% sulphuric acid was added. The tubes were kept in a boiling water bath for 15 minutes and then cooled in ice –water. 4.5 ml of distilled water was added and the tubes were cooled again in running tap water. The intensity of coloured developed was measured at 590 nm using a photoelectric.

Biochemical parameters were calculated using the formula:

$$\frac{\text{Absorbance of sample}}{\text{Concentration of standard x Absorbance of Standard}} = \text{mg/ml.}$$

RESULTS

Soil samples were collected from 5 different areas, (Kumuttipathi, Perur, Thirupur, Valayar, Valavadi) and were infected with *H. armigera*. Out of them only one sample alone (Perur) harbored Entomopathogenic nematodes, the colour of the cuticle of dead Galleria larva was black. This indicated the genus of EPN to be *Steinernema*. According to Kaya and Gaugler (1993), if the colour of cuticle of dead Galleria larva is black then the EPN trapped by that larva is *Steinernema* and if the colour of dead Galleria larva is brick red then EPN trapped by that larva is *Heterorhabditis*. Similarly, with the available literature, the symbiotic bacterium of *Stinernema* is *Xenorhabdus* which was confirmed by the color of culture in NBTA media. Blue-Green color of bacteria confirmed

the *Xenorhabdus sp*. The EPN from Perur sample was taken for further research in which the bio pesticides (The isolated EPN) were sprayed to 5th instar larva of *Helicoverpa armigera*. The death was observed in 2 days (48 hrs) in *H. armigera*. The same Perur EPN was used to infect the larvae of *H. armigera* and was tested for carbohydrates, lipids and protein in both control (uninfected) and infected *H. armigera* (Figure 1). In control the amount of estimated protein was 1.67 mg/ml and infected was 0.96 mg/ml. In control the amount of carbohydrates was 0.91 mg/ml and infected was 0.36 mg/ml. The lipid level in control and infected *Helicoverpa* larvae was 0.61 mg/ml and 0.16 mg/ml respectively.

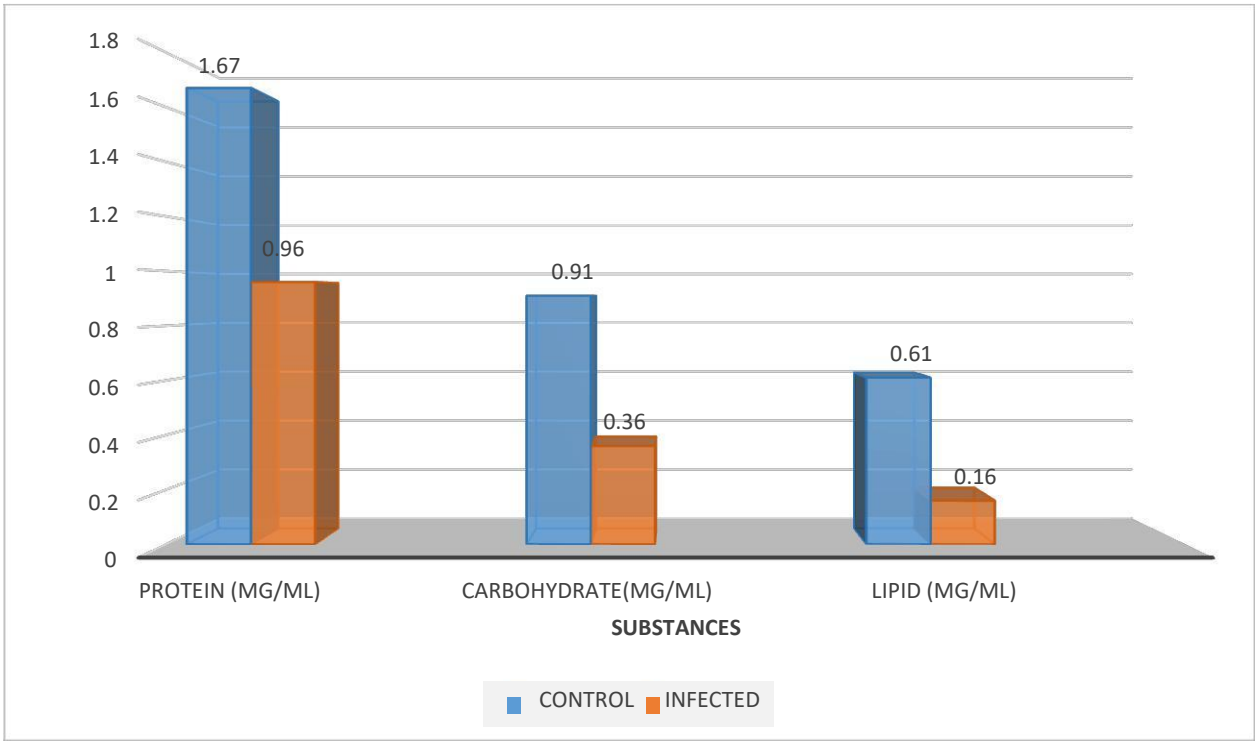

Figure 1. Biochemical estimation of EPN infected larvae of *Helicoverpa armigera.*

DISCUSSION

In the present study, soil samples collected from the 5 different places of Coimbatore (Kumittipathi, Perur, Valayar, Thirupur, Valavadi) out of which one sample alone (Perur) was found to harbour entomopathogenic nematodes. The EPNs identified in that sample belong to the genus *Steinernema* which has the symbiont of bacteria belonging to the genus *Xenorhabdus.* The occurrence of Entomopathogenic nematodes in soil depends on various factors, both biotic and abiotic. A variety of environmental factors including the effect of temperature, soil type, depth, moisture, presence and type of vegetation, and proximity of host insects affect the distance and rate of vertical and horizontal movement of infective juveniles (Molyneux, 1985).

The *Galleria mellonella* infected with entomopathogenic nematode isolate of Perur sample appeared black in colour which was on par with the report of Kaya and Nelson (1985). Therefore, the isolate was proved to be *Steinernema* genus. Further, to confirm the genus, the haemolymph of *Galleria mellonella* infected with entomopathogenic nematodes was streaked in NBTA media. The sample showed green colour colonies. This shows that the entomopathogenic nematodes belong to the genus Steinernema (Peel *et al.*, 1991).

The bacteria were analysed for morphological studies and proved to be Gram negative, motile and rod shaped as earlier reported by Akhurst and Boemaer (1988). He reported that only primary forms of the bacterial symbiont were motile and rod shaped, this is true in this study also which shows that it belonged to *Xenorhabdus* sp. Therefore, the entomopathogenic nematodes collected in the present study belongs to *Steinernema* sp.

Entomopathogenic nematodes (EPN) represent an important spectrum of biopesticides. They have been used to control insect pests of high value crops (the viability higher when used in larger scale) in sustainable agricultural systems. Several species of Entomopathogenic nematodes in the two families *Steinernematidae* (*Steinernema*) and *Heterorhabditidae* (*Heterorhabditis*) are being produced commercially and used as biological control agents against many soil insect pest and insects in cryptic habitats in many parts of the world (Seluck *et al.*, 2003).

In the present study, it was observed that the cotton boll worm *H. armigera*, infected with *Steinernema* sp. was killed within 48 hrs after infection under the laboratory conditions. The biochemical parameters (protein, carbohydrate and lipid amount) in EPN infected larvae showed a significant decrease compared to normal larvae.

This significant decrease in protein and carbohydrate content of *Steinernema* infected *H. armigera* larvae may be due to energy loss for immune reaction against infection and must be due to the utilization of carbohydrate and protein resources by nematode bacterial complex. *S. glaseri* is initially encapsulated by larvae of Japanese beetle, *Popillia japonica*, but it escapes from the capsule and successfully infests its host because the nematodes has surface coat proteins that suppress the host immune response and destroy the haemocytes.

Further the available literature on pathogenicity of *Steinernema* on *H. armigera* revealed as follows. *Steinernema abbasi* sp. n. from the Sultanate of Oman could be used as a biological control agent in high temperature situations particularly in the Middle East (Elawad *et al*., 1996).

CONCLUSION

Steinernema and *Xenorhabdus* symbionts of Perur soil sample can be exploited as a bio pesticide in agriculture for the control of pest like *H. armigera*, which cause economic damages to economically important crops.

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