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Changes in Protein Content of *Spodoptera litura* Larvae Infected with *Steinernema – Xenorhabdus* Symbiont Isolated from Marudhamalai Region of Western Ghats

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Abstract

The polyphagous devastating pest *Spodoptera litura* was infected in with the bioinsecticide *Steinernema – Xenorhabdus* symbiont and the larvae were used to analyse the protein content in both infected and control larvae. The EPN – Entomopathogenic nematodes were isolated from Westernghat region of Marudhamalai area and used in this study. The infected larvae after 24 hours were taken along with non infected control S. litura with three replication revealed that the infected had less protein content compared to control. This may be due to the utilization of the protein by the *Steinernema – Xenorhabdus* symbiont for their growth, development and reproduction.

Keywords: Protein, *Spodoptera litura*, *Steinernema, Xenorhabdus*

1. Introduction

Beneficial nematodes naturally occur in soil and are used to control soil pest insects and whenever the infected larvae or grubs are present like all of our products; it will not actively search for the insect larva. Once inside the larva the nematodes excrete specific bacteria from its digestive tract before it starts to feed. The bacteria multiply very rapid and convert the host tissue into products that the nematodes take up and use for food. The nematodes actively search for the insect larva. With high reproductive potential and the ability to migrate long distances as adults. These factors contribute to the role of *Spodoptera litura* as a major pest of many agricultural crops throughout its geographical range.

In India, it has attained a major pest status on agricultural crops such as cotton, groundnut and cauliflower. In order to avoid resistance problems, as well as to address environmental concerns regarding chemical pesticide use, it is important to identify and develop suitable alternate control strategies. In this context, pathogens may be key biological control agents against major agricultural pests because they are eco-friendly and may be manipulated to effectively control insect pests [1]. So, in this study *Steinernema – Xenorhabdus* strains have been commercially important genera, producing oral insecticidal toxins for use as bio control agent. So the present study is to estimate protein in *Spodoptera litura* infected with *Steinernema* sp. which is a polyphagous devastating pest of many crops.

2. Materials Method

2.1 Rearing of *Galleria mellonella*

The larvae of greater wax moth *Galleria mellonella* L. (Lepidoptera: Galleridae) were used for baiting the nematodes. The larvae were reared in 1,500 ml containers at

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32°C on an artificial diet. The lids of the containers had small holes for aeration. Periodically the larvae were transferred to another container with fresh diet, on weekly basis. They reached the last instars stage between 5 to 6 weeks. They were collected and used for the study. Some larvae were left in the containers itself for pupation. When it reached the adult stage, they were placed on a container having wax coated stencil paper, in which the females lay eggs. The eggs were washed in 0.01% formalin 3 times and washed once with distilled water and dried. The eggs were then placed in a butter paper over fresh diet in a container for rearing. The eggs hatch within 3–4 days and the procedure was repeated.

2.2 Collection of Sample

A total of two soil sample were collected from different locations in and around Westernghats of Maruthamalai. From all location 25gm of soil samples were collected at a depth of 15cm from the surface of soil and transferred into clean polyethene bags and were brought to the laboratory and stored at 25°C for future study.

2.3 Isolation of Nematode and Propagation

Entomopathogenic nematodes were recovered from soil samples using the insect baiting methods as described by Akhurst and Bedding [2] and insect baits (five last – instar larvae) of *Galleria mellonella* were placed in 100 ml plastic containers which contain 25gm of collected soil moistured with water. Each collected soil from different areas was kept in separate containers. These containers were covered with respiration for air throughout the baiting period. Larvae were checked for infection every day and the dead ones were removed and live larvae were placed in the containers. The dead larvae were isolated and thoroughly rinsed in 0.01% formalin and placed in white's trap [3] until the emergence of third-stage infective juveniles of nematodes in another two to three days.

The emerging nematodes were pooled from each sample and stored in culture flask (T-flask) by changing the formalin once in a week. These nematodes were used to infect fresh larvae of *Galleria mellonella* for mass propagation of nematodes for identification and establishment of culture (T-flask). The culture flasks with nematodes were maintained at 25°C. The bacterial symbiont was confirmed as *Xenorhabdus.* These *Steinernema – Xenorhabdus* symbiont were used as bioinsecticide for further study.

2.4 Culturing of *Spodoptera litura*

Spodoptera litura was collected from cowpea field at Kambainallur in Dharmapuri district and brought to lab and cultured in lab with castor leaves in plastic containers. The container was covered with muslin cloth for aeration. Each day the larvae were fed with fresh castor leaves and the container were cleaned for faecal matter. When the larvae went for pupation after VI instars, the pupas were transferred to another clean container with sterilized sand. When adults emerged, they were again transferred to another container and were fed with sugar syrup dipped in cotton and fresh clean muslin cloth was used to close the container so that after mating they laid eggs in the cloth. The cloth was removed every day to check for the eggs and if laid, they were washed three times with 0.1% formalin and then with distilled water two times. This egg cloth was placed in a clean container with fresh clean castor leaves for the newly hatched larvae to feed. They hatch from the egg and come down for feed and the life-cycle is again started freshly and the culture is maintained for further study.

2.5 Estimation of Protein

The protein content of *Spodoptera litura* was determined by the method of Lowryet al. [4] using bovine serum albumin as the standard. 100 mg of sample the bioinsecticide *Steinernema – Xenorhabdus* symbiont infected *Spodoptera liture* larvae and control larvae were homogenized in 2 ml of 5% trichloro acetic acid and centrifuged for twenty minutes in 10000 RPM. The precipitate was dissolved in 1% sodium hydroxide solution and used for the estimation of protein. The protein present in the sample reacts with Folin phenol reagent and produces a blue colour by the reduction of phosphomolybdic phospotungstic components biuret reaction. The colour developed was measured at 530nm using photoelectric colorimeter. The results were expressed in mg protein /ml.

3. Results

The soil samples were collected from seven location of Marudhamalai. Only one sample harboured entomopathogenic nematode as the soil baited with *Galleria mellonella* larva died within 24-48 hrs. The *Galleria mellonella* larvae were cultured in laboratory in artificial

diet since it is the host for culturing the Entomopathogenic nematodes. The dead larva was brown to black in colour. The bacteria streaked in NBTA media showed blue to green colour colonies. The protein was analysed in *Spodoptera litura* infected with *Steinernema* sp. of Marudhamalai sample and non infected *Spodoptera litura*. The amount of protein showed 111.30 and 64.88 mg/ml respectively in control and infected. After 24 hrs, compared to control the infected showed less amount of protein content (Table 1 and Figure1).

Table 1. Estimation of protein in the larvae of Spodoptera litura infected with *Steinernema* sp.

S. No	Control	Infected
1.	108.92	66.07
2.	116.07	64.28
3.	108.92	64.28
MEAN	111.30	64.88
S.D	4.128	1.033

control soil-borne insect pests. Entomopathogenic nematodes. The isolated EPN belonged to *Steinernema* species since the cadaver of *Galleria mellonella* was brown to black in color, Woodring and Kaya [5]. The bacteria isolated showed blue to green color colonies and no bioluminescence was observed so the bacteria belonged to genus *Xenorhabdus* sp., so the EPN isolated in the present study was *Steinernema – Xenorhabdus* symbiont.

Protein was estimated in the larvae of *Spodoptera litura* healthier and infected with the Entomopathogenic nematodes of *Steinernema* sp. isolated from Marudhamalai. The infected, larvae had less quantity of protein compared to control, this may be due to the utilization of host protein by both *Steinernema – Xenorhabdus* complex for their development and reproduction, as earlier reported by Magda et al. [6].

Xenorhabdus when released into the host body cavity they disable the immune system and the disappearance of haemolymph sample and occur hydrolyzes of the host protein. This result Hanan et al. [7], who stated that, the losses of soluble protein from the host haemolymph

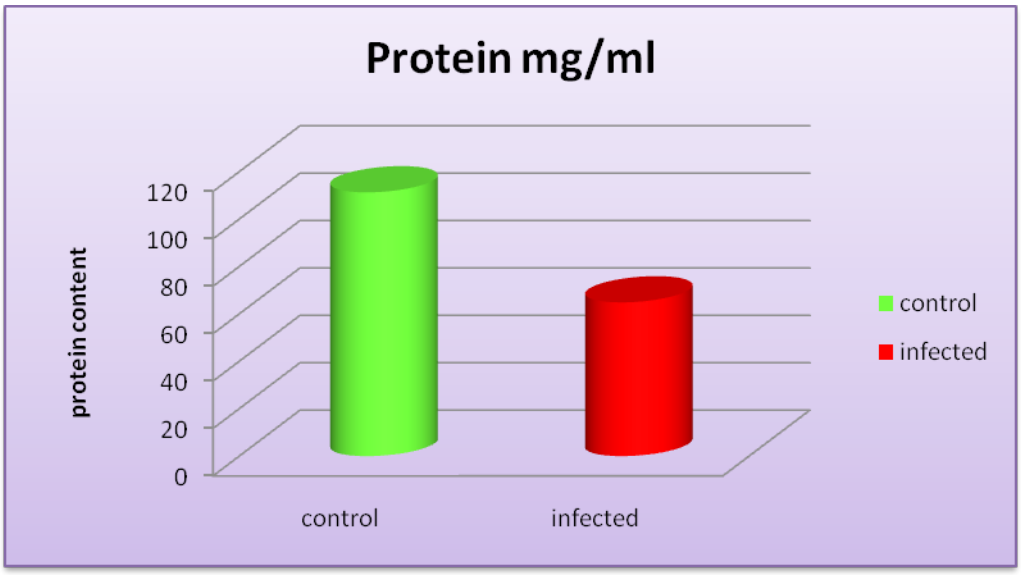

Figure 1. Estimation of protein in the larvae of *Spodoptera litura* infected with *Steinernema* sp.

4. Discussion

Entomopathogenic nematodes represent one of the important parts of the spectrum of bio control agents. They are used to control insect pests in high-value crops and potentially they could be used in integrated pest management, organic farming and sustainable agriculture systems to

during parasitism may be explained by the parasite secretion of proteolytic enzymes into the haemocoel of the insect and hydrolyze the host proteins. There was reduction in total protein content of the haemolymph of *Schistocerca gregaria* during the course of infection with the Entomopathogenic fungus, *Mertarhizium varacridium*.

So from this study the *Steinernema* sp. isolated from Marudhamalai is an efficient biopesticide which can be used to control the pest - *Spodoptera litura* and can also be used against other Lepidopterans and can also be incorporated in IPM to save economically important crops.

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