BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348–0521, ISSN (E): 2454–4728
Vol. 9, Issue 1, Jun 2021, 99-104

BIOCHEMICAL ANALYSIS IN COCONUT TREE PEST *ORYCTES RHINOCEROS* INFECTED WITH*STEINERNEMA – XENORHABDUS* COMPLEX

ROKINIDEVI S[1], CHITRA P[2]& SUJATHA K[3]

[1]Research Scholar, Government Arts College, Coimbatore, India

[2]Assistant Professor, Avinahilingam Deemed University, Coimbatore, India

[3]Assistant Professor, Government Arts College, Coimbatore, India

ABSTRACT

Entomopathogenic nematodes may infect and kill pest insects. They do so by a free-living infective juvenile, which actively searches for soil-borne insect pests. They are insect-specific, safe and contribute to the natural regulation of many populations of pest insects. In this study, the survey of entomopathogenic nematode was conducted in Coimbatore district and the isolation of EPN- *Steinernema* spp. is used to kill their coconut tree pest*oryctes rhinoceros* beetle.So this study aims to evaluate the pathogenic potency or biopesticidal efficiency of the biochemical components in the infected pest which can be the indicator of the mode of action of the biopesticide. The physiological changes in *oryctes rhinoceros* curb thatresulted in entomopathogenic nematode infection have been investigated. It was observed that a drastic decline in total protein, total carbohydrates, total lipids and total amino acids occurred in infected *oryctes rhinoceros*. The biochemical contents were estimated at 24, 48 and 72 hours after infection of *Steinernema* spp. to the *oryctes rhinoceros*grub with respect to the untreated larvae.

KEYWORDS: Entomopathogenic nematodes, Steinernema, Oryctes rhinoceros& Xenorhabdus

INTRODUCTION

Safety and environmental insecticide issues surrounding the use of chemical insecticides has led to an emphasis on developing alternative control measures such as entomopathogens and their products. Entomopathogenic nematodes are effective biopesticide which can be incorporated in IPM programs because they are considered non-toxic to humans, relatively specific to their target pests and can be applied with standard pesticide equipment. Entomopathogenic nematodes have proven to be the most effective as biological control organisms. Entomopathogenic nematodes have been released extensively in crop fields with negligible effects on non-target insects and are regarded as exceptionally safe to the environment. Entomopathogenic nematodes (EPN), which are the most commonly considered biopesticides, play a major role in integrated pest management or biological control of the pests. The term Entomopathogenic has its Greek origin ‘Entomon’ referring to insects and pathogenic refers to disease causing. As the name suggests, the entomopathogenic nematodes infects only the insects. They are inhabitants of soil, lethal insect parasite belonging to the phylum Nematode, commonly known as round worms.

The EPNs are widely distributed and are easily available in agricultural lands, forests or even in deserts where plants are found. They survive within the body of their hosts and are known as endoparasite and also they are capable of

infecting different stages of the hosts, including the larval and pupal stages of moths, beetles, butterflies and also adults of grasshoppers and crickets. EPNs can be easily mass cultured and no specialized equipment is required for their application as they are compatible with standard agrochemical equipment that includes numerous sprayers and irrigation systems. Among various nematodes that were studied, two major genera namely Steinernematidae and Heterorhabitidae received utmost attention as they possess many characters of effective biological control agents and can be used as conservational, classical and augmentative bio-control agent.

MATERIALS AND METHODS

Collection and Isolation

The rearing of greater wax moth *Galleria mellonella*on artificial diet in the laboratory is for multiplication of entomopathogenic nematodes.The soil samples are collected from, Elur, Kaniyur,Annur in the agricultural land. Entomopathogenic nematodes were recovered from soil sample using the insects baiting methods as described by Bedding and Akhurst, (1975).The dead larvae were isolated and thoroughly rinsed in 0.01% formalin and placed in White's trap (Kaya and Stock, 1997).The culture flasks with nematodes were maintained at 25^0C.

Biochemical Analysis

The coconut pest *Oryctes orhinoceros* beetle (Coleoptera: Dynastinae) are infected with *Steinernema* sp.,The biochemical component Protein (Lowry *et al.,* 1951), Carbohydrates (Roe, 1995), lipids (Folch method 1957) and amino acid(Rutherford 1978) were analysed in the 24h, 48h and 72 hours both Whole larvae and Haemolymph.

RESULTS AND DISCUSSIONS

The beneficial bio control agent (EPNs) has opened a new way to study their mode of action through the biochemical changes in pests. So this study aims to evaluate the pathogenic potency or biopesticidal efficiency of the biochemical components in the infected pest which can be the indicator of the mode of action of the biopesticide. The physiological changes in *oryctes rhinoceros* curb thatresulted in entomopathogenic nematode infection have been investigated. It was observed that a drastic decline in total protein, total carbohydrates, total lipids and total amino acids occurred in infected *oryctes rhinoceros.*

The biochemical contents were estimated at 24, 48 and 72 hours after infection of *Steinernema* spp. to the *oryctes rhinoceros* grub with respect to the untreated larvae. In the untreated pests, the protein, carbohydrates, lipid and amino acids content were higher compared to *Steinernema* spp., infected pests at 24, 48 and 72 hours. The biochemical changes in *Parasarcophaga aegyptiaea* and *Argas persicus* haemolymph infected with EPNs were studied by Hanan *et al.,* (2009).

Protein content in the oryctes rhinoceros

The protein content in the *oryctes rhinoceros* infected by *Steinernema* sp., at 24, 48 and 72 hrs in all the three isolates are gradually decreased when compared to the untreated both whole larvae and haemolymph. The untreated *Oryctes rhinoceros* both whole larva and haemolymph showed the highest protein content in 24, 48, 72 hours namely, 4.82 mg/100mg, 4.80mg/100mg, 4.51mg/100mg and 1.30 mg/100mg, 1.25 mg/100mg, 1.10 mg/100mg respectively, when compared to *Steinernema* spp., infected larvae. *Steinernema* sp. of Kaniyur region had the lowest protein content both whole larva and haemolymph compared to other *Steinernema* spp. infected *Oryctes rhinoceros*. Proteins function as the building blocks, structural organizers, enzymes of functional regulators, and therefore could have been utilized by the

nematode bacterial complexes. The infected larvae protein may be demolished and those proteins might have been utilized by for the formation of new nematode bacterial developments.At 48 hrs a significant reduction of proteins was observed in the infected larvae, indicating that the infected juveniles have started emerging out and would have used the proteins for their growth and multiplication.

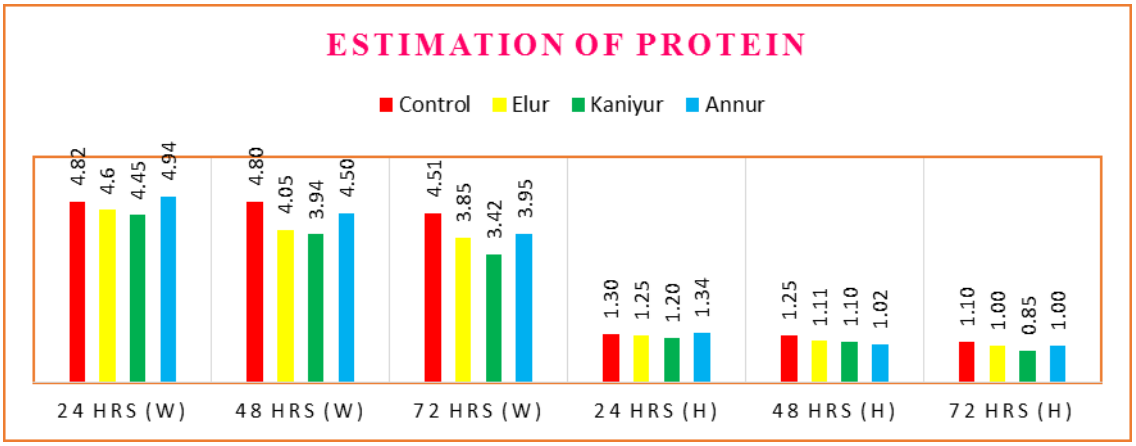

Figure: 1: Estimation of Carbohydrate (mg/100mg) in *Oryctes rhinoceros* infected with *Steinernema* spp.

Abdel-Razek *et al.,* (2004), mentioned that the total protein in the haemolymph of red palm weevil infected EPNs showed a 5.3-fold decrease after treatment.*S. carpocapsae - X. nematophilus* and 2.5-fold decrease after treatment with *H. bacteriophora - P. luminescens* compared with theuntreated.Tamilselvi (2018) reported that in four lepidopteran larval pests, *H. armigera, L. orbonalis, S. litura* and *G. mellonella*, the protein content decreased significantly in the infected larvae when compared to control larvae.In 48 hours of infection *G. mellonella* showed a 70% reduced protein content compared to others with moderate reduction.

Carbohydrate content in the Oryctes rhinoceros

The carbohydrate content in *O. rhinoceros* infected by *Steinernema* spp. at 24, 48 and 72 hours decreased gradually in all the three isolates from Ellur, Kaniyur and Annur when compared to the untreated. The untreated*O. rhinoceros* whole larva showed highest carbohydrate content in the 24, 48 and 72 hours namely, 2.90 mg/100mg, 2.50 mg/100mg, 2.45 mg/100mg and 2.32 mg/100mg, 2.30 mg/100mg, 2.15 mg/100mg respectively, when compared to *Steinernema* spp., infected larvae.In 24 hours haemolymph showed untreated carbohydrate content was similar in *Steinernema* spp., infected of Kaniyur area. In the Whole Larvae infected with *Steinernema* spp. of Kaniyur region showed the lowest carbohydrate content in 24, 48 and 72 hours.

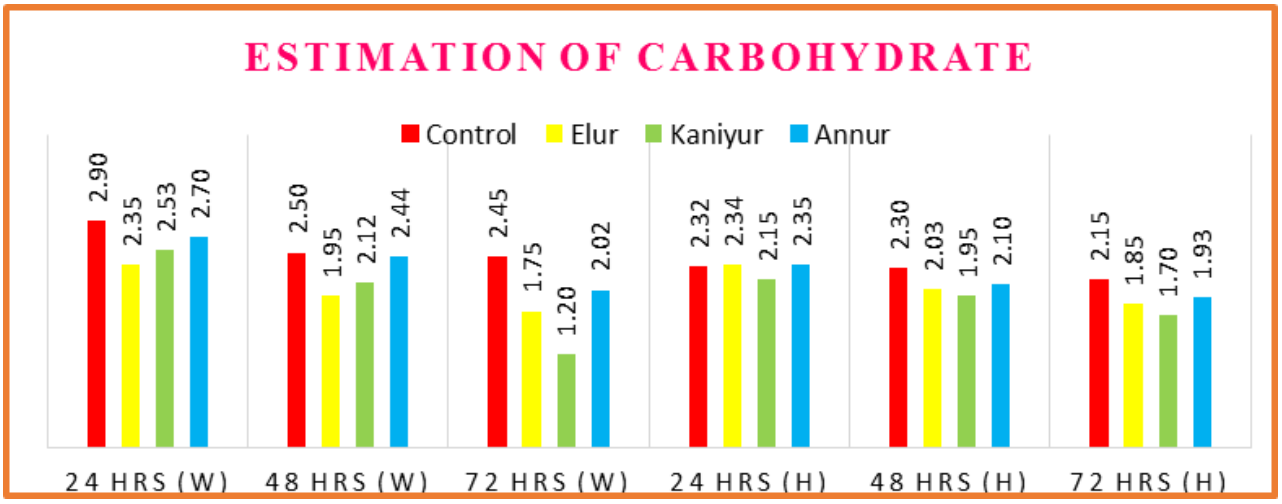

Figure: 2: Estimation of Carbohydrate (mg/100mg) in *Oryctes rhinoceros* infected with *Steinernema* spp.

Abdel-Razek *et al.*, (2004) reported that entomopathogenic infected with red palm weevil showed lower carbohydrate level the in the haemolymph compared with the untreated. While the body fat of untreated larva showed higher content that after treatments. This may be due to utilization of stored carbohydrates in protein synthesis. Tamilselvi (2018) reported that entomopathogenic infected *H. armigera* and *L. orbonalis* larvae in carbohydrate content would have been used as nutrient source for the survival of nematodes and its symbiotic bacteria. The nematode bacterial complex had utilized the carbohydrate content as an energy source.

Lipid content in the *Oryctes rhinoceros*

The lipid content in *O. rhinoceros*larvae infected with *Steinernema* spp., at 24, 48 and 72 hrs were in all the three isolates, namely Ellur, Kaniyur and Annur, were gradually decreased when compared to the untreated. The untreated *O. rhinoceros*showed highest lipid content in 24, 48 and 72 hours namely, 5.22 mg/100mg, 5.13 mg/100mg, 4.45 mg/100mg and 2.32 mg/100mg, 2.20 mg/100mg, 2.10mg/100mg respectively, when compared to *Steinernema* spp., infected larvae. *Steinernema* spp. of Ellur and Kaniyur region showed the lowest lipid at 48 and 72 hours, when compared to Annur *O. rhinoceros*infected with *Steinernema* spp.

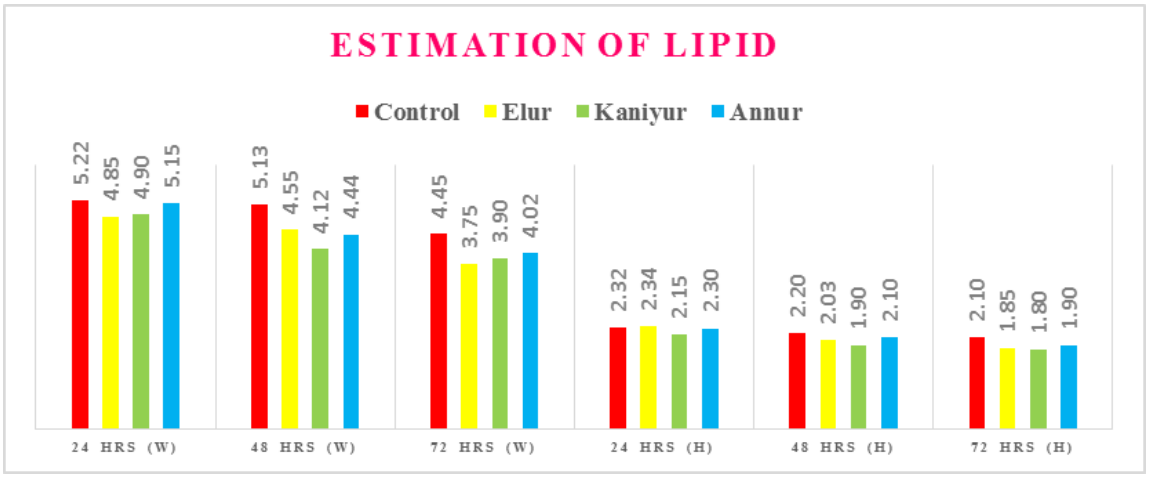

Figure: 3: Estimation of Lipid (mg/100mg) in *Oryctes rhinoceros* infected with *Steinernema* spp.

Thompson and Barlow (1983) reported that, an extreme depression of glyceride synthesis would allow the parasite to use its host fat after partial digestive hydrolysis and its own fatty acids for rapid triglyceride synthesis, thereby minimizing the energy cost of fat synthesis. A similar process would have taken place in the present study. The decline in lipid content of parasitized larvae *Anagata uchniella* was due to the endoparasitic action on host chemical reaction (Hanou *et al.*, 2009).

Amino acid content in the *Oryctes rhinoceros:*

The amino acid content in *O. rhinoceros* infected by *Steinernema* sp., isolated from the three sites gradually decreased at 24, 48 and 72 hours when compared to the untreated. The untreated larva of *O. rhinoceros*larva showed highest amino acid content in 24, 48 and 72 hours namely, 3.53 mg/100mg, 3.43 mg/100mg, 3.02 mg/100mg and 2.10 mg/100mg, 1.94 mg/100mg, 1.82 mg/100mg respectively, when compared to *Steinernema* spp., infected larvae. *Steinernema* spp. infected showed that Kaniyur region had lowest amino acid content compared to other *Steinernema* spp., infected *O. rhinoceros.*

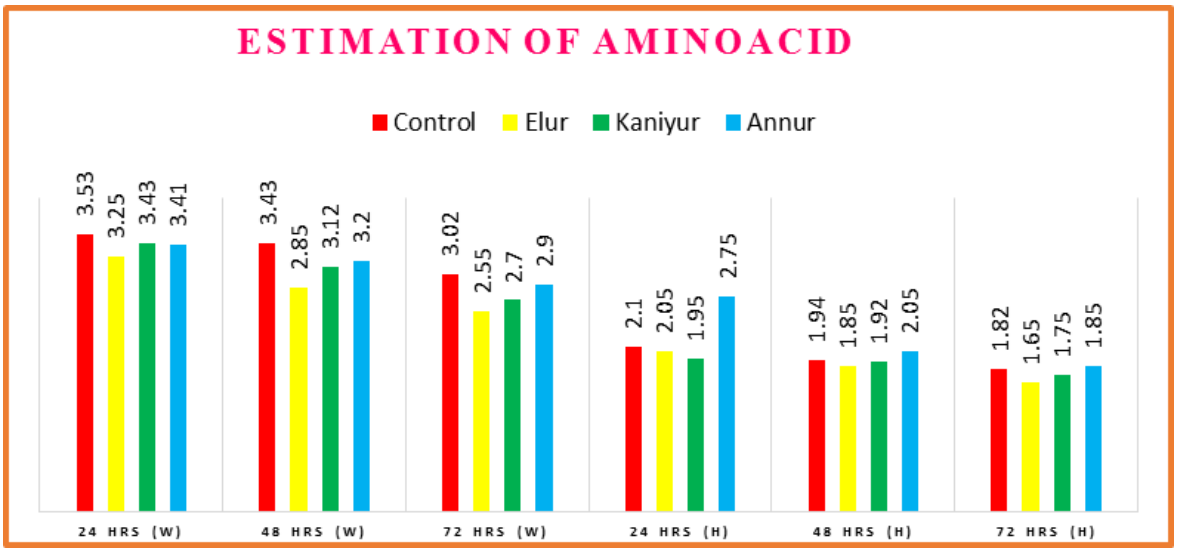

Figure: 4: Estimation of Amino acid (mg/100mg) in *Oryctes rhinoceros* infected with *Steinernema* spp.

Schmidt and Platzer (1980) reported that, the reduction in the haemolymph amino acids replenished by increased host proteolysis, decreased excretion or transport into the fat body and reduced protein synthesis.Abdel-Razek *et al.* (2004) reported that in *Steinernema* infected red palm weevil, the total amino acid compounds showed an obvious decrease by 62.58 and 65.67% in the haemolymph of larvae infected with each of these nematode-bacterial complexes, respectively, when compared with the untreated larvae. Some amino acids increased and others showed a decrease after infection.

SUMMARY AND CONCLUSIONS

Biochemical components studied showed less amount of Protein, Carbohydrate, Lipid and Amino acid in all the three *Steinernema* spp. infected larvae of *Oryctes rhinoceros* whole larva and hemolymphat 24, 48 and 72 hours of infection when compared to the untreatedlarvae. This proved it to be the energy source for the growth and development of Entomopathogenic nematodes *Xenorhabdus* symbionts.

REFERENCES

1. **Abdel-Razek AS (2004).** Pathogenic effects of *Xenorhabdus nematophilus* and *Photorhabdus luminescens*

against pupae of the diamondback moth, *Plutellaxylosella. Anz Shadlingskunde.***76:** 108-111.

2. **Folch J. (1956).**A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*
3. **Hanan A., El-Sadawy (2009).** Biochemical Changes in *Parasarcophaga. Aegyptiaca.*and *Argas (persicargas) persicus* Haemolymph Infected with Entomopathogenic Nematode. *Nature and Science*. **7(6):** 70-81
4. **Kaya H. K. and Stock S P. (1997)**. Techniques in insect nematology. Pp. 281–324.
5. **Lowry O. H., Rosembrough N. J., Farr A. L., Randall R. J. (1951)**. Protein measurement with the Folin's phenol reagent. *J. Biol. Chem.* **193**:265-275.
6. **Roe, J. H. (1955).** The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry, 212*, 335–343.
7. **Rutherford T. A., Webster J. M. (1978).**Some effects of *Mermis nigrescens* on the haemolymph of *Schistoerca gregaria.* Canad J. Zool. 56:339-347.
8. **Schmidt SP and Platzer EG (1980).** An investigation of possible Romanomermis culicivorax proteins in the haemolymph of Culex pipiens, *j. inver. Pathol.***30:** 149-158.
9. **Tamilselvi (2018).** Screening of indigenous entomopathogenic nematodes in various agroecosystems for their biopesticidal potency and their molecular characterization**.**PhD Thesis. Govt. Arts College. Zoology Dept. Library.Dissertation submitted for M.Sc. to Bharathiar University.
10. **Thompson, S. N., and Barlow J. S. (1983).**Metabolic determination and regulation of fatty acid composition in parasitic Hymenoptera and other animals. In: Metabolic Aspects of lipid nutrition in Insects. West view press, Boulder, Colorado.