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Biochemical changes in *Holotrichia repetita* Sharp (Coleoptera: Melolonthidae) and *Galleria mellonella* L. (Lepidoptera: Pyralidae) due to the biopesticide *Steinernema* sp.

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ABSTRACT: In the present study, *Steinernema*- sp. were isolated from Tirupur soil sample and tested for its antagonistic potential against larvae of *Galleria mellonella* and *Holotrichia repetita* in laboratory condition. 100 per cent mortality was observed after 24 and 48 hours for *G. mellonella* and *H. repetita* respectively. Further biochemical estimations *viz.,* protein, carbohydrates and lipids were carried out after 24 and 48 h of application. The results showed that there was a significant decrease in all biochemical parameters of parasitized larvae compared to control. *Steinernema* sp. can be incorporated in IPM program for the control of *G. mellonella* and *H. repetita.*

KEY WORDS: EPN, *Steinernema* sp, protein, carbohydrate, lipid

INTRODUCTION

The objective of biocontrol is to maximize the efficiency of the natural enemy complex in suppressing pests and ultimately enhancing crop yield in agriculture (Denno *et al.,* 2008). The Entomopathogenic nematodes (EPN) consist of genus *Steinernema* and *Heterorhabdus* (Nematoda: Rhabditidae) are symbiotically associated with entomopathogenic bacteria *Xenorhabdus* sp., (Thomas and Poinar, 1979) and *Photorhabdus* sp., (Boemare *et al*., 1993) respectively. These EPNs have been successfully used as biological control agents of various insect pests (Shahina and Mehreen, 2015). Once infective juveniles (IJs) of EPNs entered into host body by natural openings like mouth, anus and spiracle then it releases symbiotic bacteria from the intestine to body cavity of the host body (Kaya and Gaugler, 1993). The bacteria will logarithmically replicate in haemolymph (Hussien and Hanan, 2008) and act as a primary agent for killing the host usually by haemocoel infection such as septicemia (Forst *et al.,* 1997) within 24 to 48 hr. The greater wax moth, *Galleria mellonella* Linnaeus, (Lepidoptera: Pyralidae) is widely distributed throughout the world in temperate, tropical and subtropical beekeeping regions and major economically destructive pest of the honeybee, *Apis mellifera* Linnaeus, and *Apis cerana* Fabricius (Kwadha *et al*., 2017). *G. mellonella* damage only during their larval stages. It obtained nutrients from honey, pollen, wax, castoff pupal skins and other impurities found in

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the beeswax (Mohamed, 2014). *Holotrichia repetita* Sharp (Coleoptera: Melolonthidae), severely damages tuber vegetables such as potato, groundnut and sugarcane in all over India (TNAU, 2018). Adult beetles are 16.0–22.0 mm, dark bronzy green, legs greenish black, and abdomen deep red color (Chandel *et al*., 2015). *H. repetita* grubs are 'C' shaped with orange head. It feeds on roots and tubers. Adults were emerged as soon as temperature starts rising. Adults feeds on foliage during night and damage is more during autumn (TNAU, 2018). Both *G. mellonella* and *H. repetita* considered as a major destructive pest in agriculture. So, the present study was carried out to control *G. mellonella* and *H. repetita* through EPN and their biochemical parameters were studied.

MATERIALS AND METHODS

The larvae of greater wax moth *Galleria mellonella* were used for baiting the nematodes (Bedding and Akhurst, 1975). The larvae were cultured and maintained in a large container at room temperature on an artificial diet. 250 gram of soil samples were collected at a depth of 15 - 20cm from different agricultural location in and around Coimbatore and Tirupur Districts, Tamilnadu. Collected soil samples were brought to laboratory and stored at room temperature for further study.

Entomopathogenic nematodes were recovered from soil sample using standard method as described by Bedding and Akhurst (1975). Five fifth instar larvae of *G. mellonella* were placed in 100ml plastic containers which contained 50 grams of collected moisture soil. 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 method (Kaya and Stock, 1997) until the emergence of third-stage infective juveniles of nematodes in another two to three days. EPN genes were identified based on the dead cadaver (parasitized) of *G. mellonella*. Further conform to genes of EPNs, a loopful of haemolymph from parasitized larvae were streaked on NBTA medium (Akhrust, 1980). The plates were incubated at 28^0C for 24 hours. Bacterial colony color noted and morphological studies such as Gram's staining and Motility test were done.

After isolation of nematodes, their pathogenic potential was tested against *G. mellonella* and *H. repetita* at laboratory condition. *H. repetita* larvae were collected from Nilgiri Hills and reared in the laboratory. Five last instar larvae of *G. mellonella and H. repetita* were taken in a sterilized Petridish and *Steinernema* sp. (IJs) was gently applied over the larvae by the help of small camel hair brush then maintained at room temperature with untreated controls were identical to the treatment except that no IJs were added. The larval mortality was observed for 24 and 48 hours and the duration for the death of the larvae was noted. Further biochemical analyses were carried out between parasitized larvae and control (Untreated) for 24 and 48 hrs of after infection. Protein estimation was done by Lowry's method (Lowry *et al*., 1951). Carbohydrates estimation was carried out by Roe's method (Roe, 1995) and Lipids estimation by Folch's Method (Folch, 1957).

Obtained data were subjected to multi-factorial ANOVA by using SPSS v16.0 software. Results with $p<0.05$ was considered as a statistically significant.

RESULTS AND DISCUSSION

Total 10 soil samples were collected from different agro ecosystems in and around Coimbatore and Tirupur, Tamilnadu. Among 10 soil samples only one soil sample tested positive for the presence of EPN. Both biotic factors (vegetation and host availability) and abiotic factors (temperature, soil type, depth, and moisture) are responsible for the presence of EPN in soil (Molyneux, 1985). The infected cadaver of *G. mellonella* was black in color (Woodering and kaya 1988), these shows that the isolated EPN, in this study belongs to *Steinernema* Genus (kaya and Nelson, 1985). Further to conform EPNs genus, a loop of haemolymph were streaked on NBTA medium, color of bacterial colony was maroon which shows the isolated symbiotic bacteria was *Xenorhabdus* sp. (Akhrust, 1980). Morphological studies show that isolated bacteria was gram negative, motile and showed no bioluminescence. So, in this present study isolated EPNs were *Steinernema* sp. with its symbiont *Xenorhabdus* sp.

The isolated *Steinernema* sp. was checked for their pathogenic potential against *G. mellonella* and *H. repetita*. Larval mortality occurred after 24 hours and 48 hours in laboratory condition at room temperature which shows that the isolated EPNs are effective against *G. mellonella* and *H. repetita* respectively. Larval mortality occurred via an abundance of tissue damage in parasitized larvae because of actin cytoskeletons rearrangements and induced apoptosis in both haemocytes and epithelial tissues due to activity of Mcf toxins which was produced by symbiotic bacteria (Daborn *et al.,* 2002). Members of Enterobacteriaceae such as *Photorhabdus*, *Xenorhabdus*, *Serratia*, and *Yersinia* sp. produce insecticidal toxins (neurotoxins, digestive toxins and cytotoxins) with oral toxicity similar to that of Bt toxins (Castagnola and Stock, 2014).

The protein content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 4.82mg/100mg and 5.73 mg/100mg respectively, in control were 11.88 mg/100mg and 13.41 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 2.78 mg/100mg and 4.18 mg/100mg respectively, in control were 7.53 mg/100mg and 9.25 mg/100mg respectively (Fig. 1).

The carbohydrate content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 16.36mg/100mg and 12.73 mg/100mg respectively, in control were 28.03mg/100mg and 25.03 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 13.21mg/100mg and 10.53 mg/100mg respectively, in control were 24.16mg/100mg and 22.73 mg/100mg respectively (Fig. 1).

The lipid content in the *Steinernema* sp. infected *G. mellonella* and *H. repetita* were 10.23mg/100 mg and 15.4mg/100mg respectively, in control were

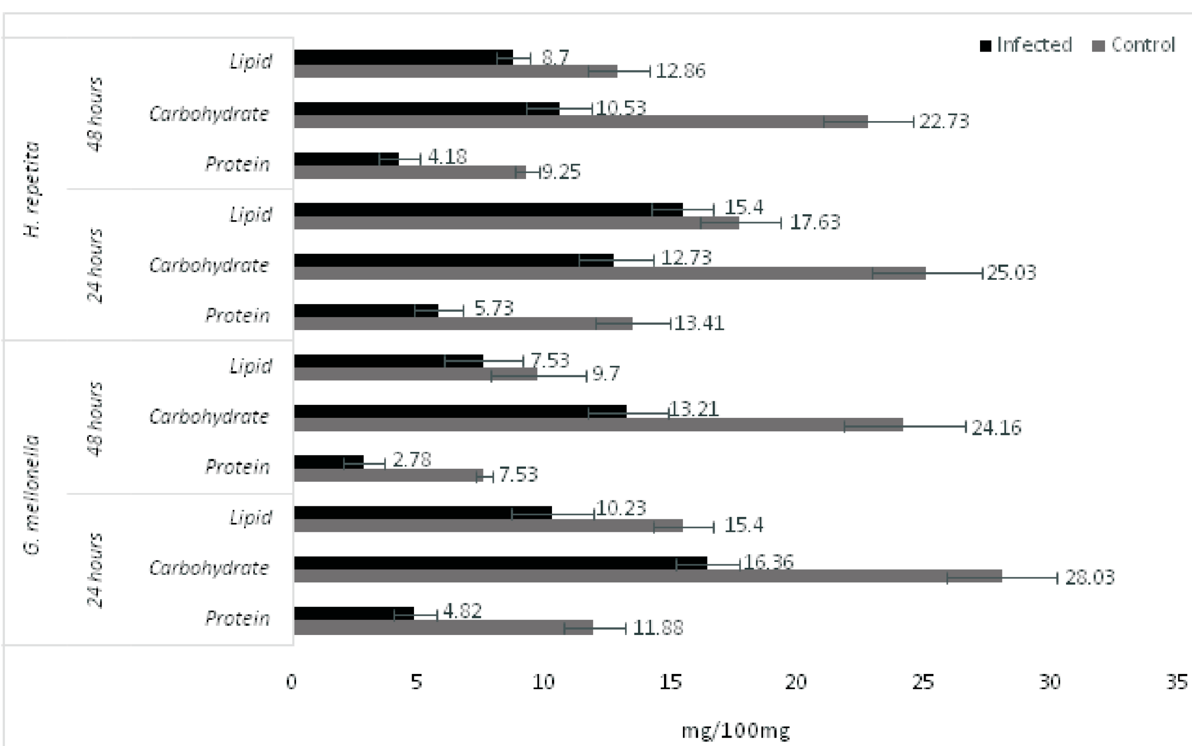

Fig. 1. Estimation of protein, carbohydrate and lipid (mg/10mg) in *G. mellonella* and *H. repetita* infected by *Steinernema* sp. after 24 and 48 h of application (Mean ± SD, n=3)

15.4mg/100mg and 17.63 mg/100mg respectively at 24 hours. After 48 hours, infected *G. mellonella* and *H. repetita* were 7.53mg/100mg and 8.7 mg/100mg respectively, in control were 9.7mg/100mg and 12.86 mg/100mg respectively (Fig. 1).

Biochemical estimations *viz.,* protein, carbohydrate and lipid showed highly significant (df=2, F=27.17, $p<0.01$) reduction in infected larvae of *G. mellonella* and *H. repetita* when compared with control groups. Significance difference (df=1, F=5.48, $p<0.05$) of biochemicals compounds was noted in between 24 and 48 hours of after estimation. Between *G. mellonella* and *H. repetita,* biochemical reduction was not a significant at $p>0.05$. Control larvae of both *G. mellonella* and *H. repetita* having a high biochemical content of carbohydrate followed by protein and lipid. After the 24 h and 48 h of application, in both *G. mellonella* and *H. repetita*, biochemical reduction percentage was high in protein content (54% to 63%) follwed by carbohydrate (41% to 53%) and lipid content (12% to 33%) (Fig. 2). Eventhough carbohydrate is present as a high quantity in non paeasitized larvae, but symbiotic bacteria relase more proteolytic enzymes and proeolytic toxins towards to host proteins. EPNs and its symbiotic bacteria first targets proteins because proteins might be play an important role for its growth, reproduction and development. It might be reason for high reduction percentage of protein in parasitized larvae. Jaroz (1996) observed that low level of antibiotics are present in cadaver of *G. mellonella* larvae when infected with *S. carpocapsae*. It might be reason that *G. mellonella* more susceptible to EPNs.

Gotz *et al.* (1980) recorded that even EPNs also secretes and release toxic substances against the host. Both *S. carpocapsae* and *H. bacteriophora* release protease secretions which destroys the antibacterial factors of *G. mellonella* larvae. Hanan, (2009) has observed biochemical changes in *Sarcophaga aegyptiaea* and *Argaspersicus* haemolymph infected with EPNs which shows

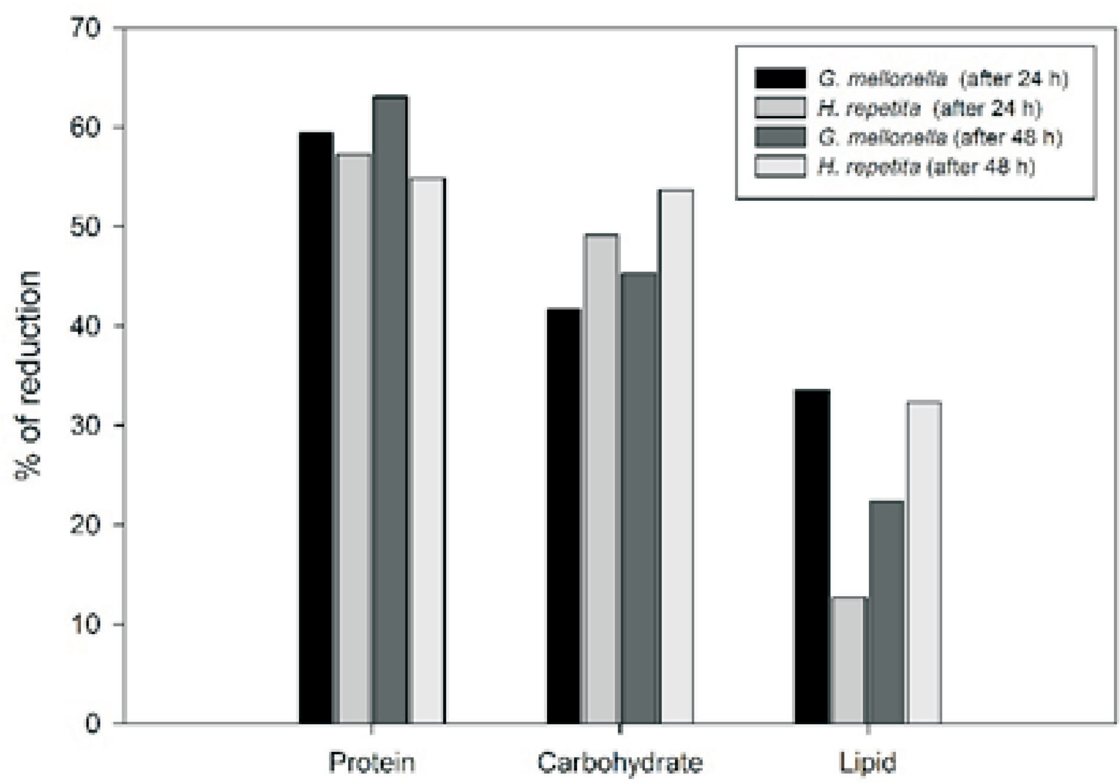

Fig 2. Reduction percentage of biochemicals in *G. mellonella* and *H. repetita* due to infestation of EPNs at 24 and 48 h of after application.

EPNs hydrolysis the host proteins by secreting proteolytic enzymes into haemocoel of host body. Amino acid transport, hormones regulating excretion process and host endocrine balance was disturbed by the EPNs. They were responsible for reduced protein content in haemolymph. The depletion of glycogen and lipid contents in parasitized larvae might be due to utilization of these reserves for energy generation as a result of Cry1Ac-HacCPV- induced stress. Similarly, a study by Santhana bharathi *et al.,* (2016) showed significant decrease in protein and carbohydrate content of *Steinernema* infected *H. armigera* larvae may be due to energy loss for immune reaction against infection. *Steinernema* sp. secretes lipase enzyme into host haemolymph. Lipase has insecticidal toxic activity which would have degraded the lipid content and suppressed the immunity of the pest *H. armigera* and *L. orbonalis* (Chitra *et al.,* 2016). *Helicoverpa armigera* (Santhana bharathi *et al.,* 2016), *Spodoptera litura* (Sindhu, 2016), *Leucinodes orbonalis* (Sujatha, 2017) showed decreased biochemical content in *Xenorhabdus* sp. infected larvae when compared to non-infected larvae (control) due to the utilization of carbohydrate and protein resources by nematode bacterial complex for their growth, multiplication and reproduction (Sindhu, 2016). Txp40 protein and *xpt* gene has been identified in both *Photorhabdus* sp. and *Xenorhabdus* sp. and this is involved in causing damage to the insect midgut and the fat body in dipteran and lepidopteran insects (Castagnola and Stock, 2014), may be true in the study also since the greater wax moth *Galleria mellonella* is also an lepidopteran.

The present study paves way for the control of pests like *G. mellonella* and *H. repetita* through the bio pesticide *Steinernema* sp., isolated from Tirupur soil sample and it is efficient bio pesticide as this does not affect non-target organisms and is environmentally friendly, which can be incorporated in IPM programs.

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