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The efficacy of *Xenorhabdus* sp. in the quantitative determination of protein, carbohydrates and lipids content in larvae of *Helicoverpa armigera* (cotton boll worm) and *Lucinodes orbonalis* (shoot borer)

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Abstract

The biochemical components namely protein, carbohydrates and lipids were analyzed. The symbiont of *Steinernema* sp. munnar samples *Xenorhabdus* sp. was cultured in liquid broth and 1 loop of inoculum was taken and infected to five larvae of *Helicoverpa armigera* and *Lucinodes orbonalis*. The death was observed in 24 hours after infection and Protein, Carbohydrates and Lipids were estimated. In *Helicoverpa armigera* the protein content of control showed 3.12 g/dl, infected were 2.18 g/dl. Carbohydrate in control was 42 mg/100mg, infected were 23 mg/100mg. Lipid in control was showed 18.75 mg/100mg, infected were 6.25 mg/100mg. *Lucinodes orbonalis* protein content of control showed 3.93 g/dl, infected was 0.77 g/dl. Carbohydrates in control were 27 mg/100mg, infected were 18 mg/100mg. Lipid in control was showed 12.5 mg/100mg, infected were 6.25 mg/100mg. This low content of protein, carbohydrates and lipid in infected may be used by the *Steinernema* sp. for its growth, development and reproduction and the pathogens have also destroyed the hosts for *Helicoverpa armigera* and *Lucinodes orbonalis* immune system for their development.

Keywords: *Steinernema* sp., *Xenorhabdus* sp., *Helicoverpa armigera, Lucinodes orbonalis.*

Introduction

Entomopathogenic nematodes occur naturally in soil environments and locate their host in response to carbon dioxide, vibration and other chemical cues (Kaya and Gaugler, 1993). *Steinernema* sp. effectively used as biological insecticides in pest management programs (Grewal *et al*., 2005). Entomopathogenic nematodes fit nicely into integrated pest management or IPM programs because of their symbiont which is virulent t the pests.

In the non-infestant-stage nematode living in the soil, *Xenorhabdus* sp. are carried in a specialized region of the intestine, termed the receptacle. At the third-stage of development, the infective juvenile (IJs) invade the hemocoel of susceptible insect hosts. The bacteria are released in the insect hemocoel, where they overcome the insect's defence systems and produce numerous virulence factors such as hemolysin and cytotoxin. They participate in suppressing insect immunity and killing the host. The bacteria proliferate to high levels in the insect cadaver and produce diverse antimicrobial compounds that suppress the growth of antagonistic microorganisms. *Xenorhabdus* sp. also secretes an array of exo-enzymes that stimulate macromolecular degradation, the products of which, together with the bacteria themselves, are thought to provide a nutrient base for nematode growth and reproduction. When

nematode numbers become high and nutrients become limiting in the insect cadaver, nematode progeny re-associate with bacteria and differentiate into colonized, non-feeding IJs that emerge into the soil to forage for new hosts.

The symbiotic association is essential for the survival of both nematode and its symbiotic bacteria (Poinar, 1979). This bacterium has great potential as a biological control agent for noxious insects in cryptic environment. *Xenorhabdus* sp. occur in two forms, termed phase I and II. These forms are distinguished *in vitro* by the colony types produced on nutrient agar supplemented with bromothymol blue and triphenyltetra zolium chloride (NBTA) (Akhrust, 1980). Phase I bacteria can be isolated from the intestine of infective stage nematodes, but phase II occurs almost exclusively in *in vitro* cultures and rarely in IJ's phase I bacteria differs from Phase II forms the type of secondary metabolites that they produce. Some of these metabolites have antibiotic properties. As well, phase I forms are pathogenic to insects. These metabolites help create an environment in the insect cadaver that is optimal for nematode reproduction. It is unclear as to the function of the phase II bacteria. However, there is some evidence that phase II are capable of surviving better in nutrient limiting condition.

On cotton, two to three larvae on a plant can destroy all the bolls within 15 days. On corn, the larvae consume grains. On tomatoes, larvae invade fruits, preventing fruit development and causing the fruit to fall (CABI, 2007). Young larvae (second and third instar) can cause up to 65% loss to cotton yields (Ting, 1986). In pigeon pea, an important grain legume in south asia, east Africa, and Latin America, this single pest causes yield losses of up to 100% in some years and locations, and worldwide losses to pigeon pea of more than $300 million per year (Thomas *et al*., 1997). Management of *Helicoverpa sp*. in the past has relied heavily on the use of insecticides, and this has led to resistance problems in cotton (Fitt, 1994). Resistance to pyrethroids amongst *H. armigera* is a serious problem (McCaffrey *et al*., 1989; Trowell *et al*., 1993).

The eggfruit and shoot-borer, *Leucinodes orbonalis* (Crambidae), is a major exotic pest of garden egg, *Solanum gilo*, causing enormous shoot and fruit damage especially in Southeast Asia (Thapa, 2010). As a result of its feeding inside fruit, the fruits become unmarketable and yield losses up to 90 percent. It also reduces the content of Vitamin C in fruit up to 80 percent (Sharma, 2002). The developmental stages consist of an egg, five larval instars, pupa and adult. *Leucinodes orbonalis* (Crambidae) is the most damaging pest of *Solanum gilo* (Solanum aethiopicum), *S. melongena* and other related vegetables. It is regarded as the most obnoxious and destructive pest of the egg-plant, *S. melongena* in all Asian countries contributing more than 80% loss in marketable yield. *L. orbonalis* is active throughout and is a major constraint to the production of eggplant world-wide.

Materials and Methods

Isolation of Bacterial Symbiont

Nematode acts as a vector by carrying the bacterium and infects the larvae. They enter through the natural opening like mouth, anus and spiracles. It multiplies 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 after 24 hours sterilized. The larvae harbour the symbiotic bacteria, so to isolate the bacteria a loopful of haemolymph were streaked on nutrient agar plate containing 0.004% 2,3,5 Triphenyl tetrazolium chloride and 0.025% Bromothymol blue (NBTA). The plates were incubated at 28^0C for 24 hours. The two forms phase I and II were differentiated based on the colour of the culture. Only primary form bacteria were used in the study. From the plate, single colony is isolated by inoculation loop and is left into Liquid broth (LB) for multiplication by shaking the conical flask frequently by keeping it at 37ºC or in a shaker. The conical flask is left overnight at 37ºCfor culturing bacteria and is used for further studies.

Pathogenicity

Five larvae of *Helicoverpa armigera* was transferred to a sterilized petri dish and a loop full of bacteria *Xenorhabdus nematophila* was gently applied over the taken larvae. The petri dish along with the larvae was sealed tightly using a cellophane tape. It was maintained at room temperature. The larvae death was observed for 24 hours and the duration for the death of the larvae was noted. A similar procedure was repeated for *Leucinodes orbonalis.*

Bio-Chemical studies

Estimation of protein

The protein content of *Helicoverpa armigera* and *Leucinodes orbonalis* larvae were determined by the method of Lowry, *et al*. (1951) using Bovine serum

albumin as the Standard .50mg 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 estimation of protein. The protein present in the sample reacts with Folin ciocalteus reagent and produces a blue colour by the reduction of phosphomolybdic phosphotungstic by biuret reaction. The colour developed was measured at 530nm using the Protein Analyser. The results were expressed in g protein/dl.

Estimation of Carbohydrate

The Carbohydrate content of *Helicoverpa armigera* and *Leucinodes orbonalis* larvae was homogenized in 20ml of 55 trichloro 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, 4ml 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 colorimeter. Carbohydrates level was calculated using the formula: Absorbance of test /Standard x concentration of standard and expressed in mg/100mg.

Estimation of Lipid

The lipid content of *Helicoverpa armigera* and *Leucinodes orbonalis* larvae were estimated by semi micro determination method of Pande *et al*., (1961). The homogenate was prepared in cold chloroform –n – butyl alcohol mixture. The filtrate was collected in test tubes and evaporated using desicator. 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 colour developed was measured at 590 nm using a colorimeter. The lipid level of the sample was calculated using formula: Unknown /standard x400 and value were expressed is mg/ml.

Results

The study carried out in Munnar soil sample. The bacteria were isolated from the EPN infected *Galleria* larvae of Munnar soil sample and cultured in Liquid Broth (LB) and used for further analysis. The bacteria were gram negative, motile and rod shaped.

The pathogenicity under lab conditions were performed for both *Helicoverpa armigera* and *Leucinodes orbonalis*. A loop of bacteria *Xenorhabdus* was inoculated for 5 larvae/petridish respectively, the inoculum approximately had minimum 1000 bacteria/loop. The death of the *H. armigera* took place 11 hours after infection whereas *L. orbonalis* took 13 hours for death. The protein content in the *Xenorhabdus* bacteria infected *H. armigera* was 0.77g/dl and that of the control was 3.93g/dl and the protein content of infected *L. orbonalis* was 2.25g/dl and the control was 3.17g/dl (Figure 1).

The carbohydrate content in the infected *H. armigera* was 23 mg/100mg and in control was 42mg/100mg. Also, the carbohydrate content in infected *L. orbonalis* was 18mg/100mg and that in control was 27mg/100mg (Figure 2). The lipid content in the bacteria infected *H. armigera* was 10mg/100mg and in control was 18.75mg/100mg. the lipid content of infected *L. orbonalis* was 6.25mg/100mg and that of the control was 12.5mg/100mg (Figure 3).

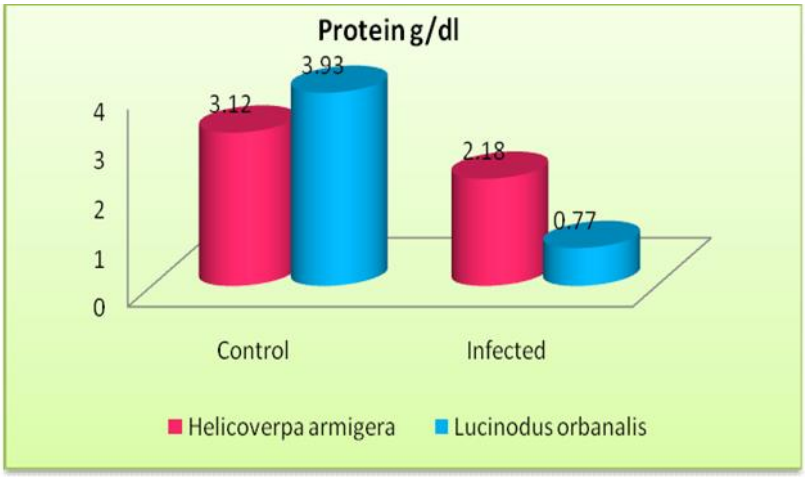

Figure 1. Quantitative determination of protein in the *Helicoverpa armigera* and *Lucinodes orbonalis* infected with *Xenorhabdus* sp.

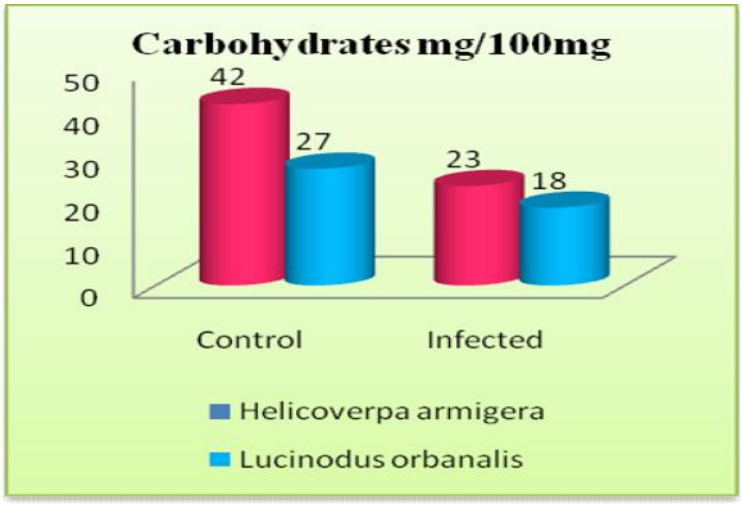

Figure 2. Quantitative determination of carbohydrates in the *Helicoverpa armigera* and *Lucinodes orbonalis* infected with *Xenorhabdus* sp.

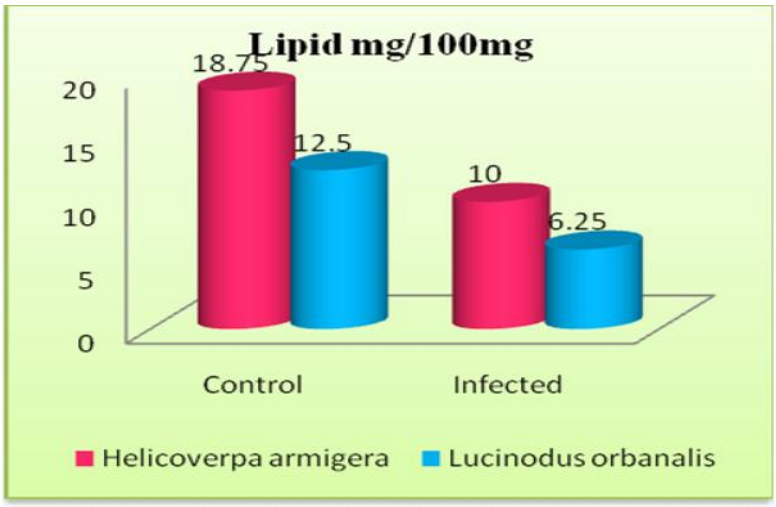

Figure 3. Quantitative determination of lipid in the *Helicoverpa armigera* and *Lucinodes orbonalis* infected with *Xenorhabdus* sp.

Discussion

The symbiotic bacteria *Xenorhabdus* sp. of *Steinernema* sp. was isolated and cultured in Liquid broth (LB) was used for further studies. Protein were estimated in the *H. armigera* and *L. orbonalis* larvae healthier and infected with the Entomopathogenic bacteria *Xenorhabdus* sp. symbiont of *Steinernema* sp. isolated from Munnar. The infected larvae had less quantity of protein compared to its control, this may be due to utilization of the host protein by the *Xenorhabdus* for its development and reproduction.

Proteins extracted from *Xenorahabdus* show damage to insect haemocytes, which is consistent with observations that the bacteria manage to evade the cellular immune response of the insect (Au *et al*., 2004). In general, *Xenorhabdus* bacteria and their toxins cause damage to the insect midgut. Study reported that *Xenorhabdus* bacteria disrupt the fat body, silk glands, hypoderma and epithelia (Jarosz, 1991). Txp 40 is a widely occurring and highly conserved toxin in *Xenorhabdus* bacteria. Txp40 is important toxins with broad insecticidal activity of these bacteria and is a significant component of the extensive array of toxins that the bacteria and nematodes use to destroy their insect hosts.

Protein metabolism in the host fat body may be altered by nutritional stress or endocrine manipulation brought by parasitism. Protein metabolism, transport of amino acids and excretion are all regulated hormonally and there by disturb the host endocrine balance by parasite could be responsible for reduced protein content in the host haemolymph.

Xenorhabdus disable the immune system and the disappearance of the haemolymph sample and hydrolyses the host protein when released into the host body. The result of Sadaway and Sanna (2009), who stated that, the losses of soluble protein from the host haemolymph during parasitism may be explained the parasite secretion of proteolytic enzymes into the haemocoel of the insect and hydrolyse the host protein. The proteins are secreted during the initial phase of nematode growth more abundantly by high than low virulent strains (Simoes *et al*., 2000). The characterization of toxin proteins produced by *S. carpocapsae* was just partially done and no homologies were established with other toxic molecules. Even crude extracts from different nematode species cause distinct responses from insects, neither the mode of action nor the susceptibility of insects to these molecules is known.

X. nematophila PAII digested some proteins of the insect haemolymph, the hydrolysis of these proteins may provide nutritional factors to the associated nematode necessary for its complete development and reproduction inside the infected insect cadaver. The inability of *X. nematophila* PAII to degrade collagen, the primary component of the nematode's cuticle, would argue for the absence of collagenase activity and the involvement of such activities in the destruction of the collagenous matrix of the nematode's cuticle could turn out. *X. nematophila* PAII is substrate specific and it is involved in degradation of insect tissues for providing nutrients to the associated nematode, which is unable to grow on insects without a previous bioconversion of the insect cadavers by symbiotic bacteria. This may be the reason in the present study also for the decrease in the amount of protein and lipid in *H. armigera* and *L. orbonalis*. (Ding *et al*. 2005).

H. armigera has a highly developed, functional, post-injestive nutrient balancing system responsible for its survival and polyphagy. Larvae adjust the release of amylases in response to the level of carbohydrate intake. The total protein content in the haemolymph showed huge decrease after treatment with *Xenorhabdus nematophilus* when compared with control. Carbohydrate content is decreased by 40 to 60% in the present study. The entomophilic nematodes and *Steinernema* sp. destroyed the host immune system and changed the energy content in the haemolymph in the *Helicoverpa armigera* and *Leucinodes orbonalis* host as reported by Ding *et al*. (2005). So the larvae *H. armigera* and *L. orbonalis* would have used the carbohydrate as nutrient balance for its survival and polyphagy. So the carbohydrate content has decreased in the *Xenorhabdus* sp. infected larvae as said above may be true in this study also. The drop in the total lipid after treatment considered with an increase in their amount in body fat in the other hand the increase in total lipid content of body fat of treatment red palm weevil larvae may be done to conversion of some proteins to fats (Abdel *et al*., 2004). As reported the host *Helicoverpa armigera* and *Lucinodes orbonalis*. immune system would have been destroyed in this study, which has led to the decrease in protein, carbohydrates and lipid content of the host infected with the *Xenorhabdus* sp. symbiont of *Steinernema* sp.

References

Abdelnasser, S.S. Ibrahim., M.A. Nefisa., E.I. Shayeb and S. Sohair and Mabrouk, 2004. Isolation and Identification of Alkaline Protease Producing Alkaliphilic Bacteria from an Egyptian Soda Lake. *J of Applied Sci. Research.* 3(**11**): 1363-1368

Akhurst, R. J. 1975. Morphological and functional dimorphism in Xenorhabdus spp., bacteria symbiotically associated with the insect pathogenic nematodes Neoaplectana and Heterorhabditis. *Journal of General Microbiology* **121**:303–309.

Akhurst, R.J. 1996. From then to now- A brief review of entomopathogenic nematodes and their symbiotic bacteria. 2nd Intl. Sym.on Entomopathogenic Nematodes & Their Symbiotic Bacteria. pp: 3-8.

Au, C., P. Dean, S. E. Reynolds, and R. H. ffrench-Constant. 2004. Effect of the insect pathogenic bacterium *Photorhabdus* on insect phagocytes. Cell. Microbiol. **6**:89-95.

Firempong, S. and M. Zalucki. 1990. Host plant selection by Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae); role of certain plant attributes. *Australian Journal of Zoology* **37**: 675-683.

Grewal, P.S, Ehlers, R.U, Shapiro-Ilan, D.I. **2005**. Nematodes as Biocontrol Agents. CABI, New York.

Hardwick, D. F, 1965. The corn earworm complex. Memoirs of the Entomological Society of Canada, 40: 1-247.

Kaya, H.K, Gaugler, R. 1993. Entomopathogenic nematodes. Annual Review of Entomology **38**: 181-206.

King, A. B. S. 1994. Heliothis/Helicoverpa (Lepidoptera: Noctuidae) In: G. A. Matthews & J. P. Tunstall (eds), Insect Pests of Cotton.

Wallingford, UK: CAB International, Wallingford, 39-106.

Lowry, O.H., Rosembrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin's phenol reagent. *J. Biol. Chem*. **193**:265-275.

McCaffery, A. R., A. B. S. King, A. J. Walker, and H. El-Nayir. 1989. Resistance to synthetic pyrethroids in the bollworm, Heliothis armigera from Andhra Pradesh, India. *Pesticide Science* **27**: 65-76

Thomas, C. S., M. Yoshida, and J. Peter. 1997. Survival, growth, fecundity, and behavior of Helicoverpa armigera (Lepidoptera:Nocutidae) on pigeonpea and two wild Cajanus species. *Journal of Economic Entomology* 90(**3**): 837-841.

Ting, Y. C. 1986. An analysis on population fluctuation and damage characterization of cotton bollworm in cotton areas of north China. Acta Entomologica Sinica **29**: 272-282.

Tripathi, S. and R, Singh. 1991. Population dynamics of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae). Insect Science Applications **12**: 367-374.

Trowell, S. C., G. A. Lang, and K. A. Garsia. 1993. A Heliothis identification kit, In: S. A. Corey, D. J. Dall and W. M. Milne (eds.) Pest Control and Sustainable Agriculture. CSIRO Publishing, Collingwood, Australia. pp. 176-179.

Woodring, J.L. and H.K. Kaya, 1988. *Steinernematid* and *Heterorhabditid* Nematodes: A Handbook of Techniques. Southern Cooperative Series Bulletin. **331**. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas. 30p.

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