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Biocontrol potential of *Heterorhabditis indica* against the maggot of *Bactrocera cucurbitae* (Diptera:Tephritidae)

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ABSTRACT: Studies conducted to determine the pathogenic efficiency and bio-control potential of the entomopathogenic nematode *Heterorhabditis indica* against melon fruit fly *Bactrocera cucurbitae* showed dose-dependent and time dependant effect. The 24 h LD_{50} value of *H. indica* was 50.28 and that at 48h was considerably reduced (38.17). Highest mortality was observed @ 160 IJs/ maggot in the bioassay. In field trials also, *H indica* successfully reduced pest population. A higher mortality of 65% was realized when the parasite was used @ 400,000 in 100 ml of aqua suspension. The present study confirms the susceptibility of *B. cucurbitae* against *H. indica* under laboratory and field conditions. © 2017 Association for Advancement of Entomology

KEYWORDS: *Bactrocera cucurbitae, Heterorhabditis indica,*cucurbits, infective juveniles, biological control.

Fruit flies are the most damaging insect pests of Cucurbits. There are about 325 species of fruit flies occurring in the Indian subcontinent of which 205 are from India alone (Kapoor, 2005). *Bactrocera cucurbitae* and *B. ciliatus* are the two most common fruit flies in India (David and Kumaraswami, 1996; Singh and Sachan, 2010). The melon fruit fly, *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) is a very serious pest found in temperate, tropical and subtropical regions including Kerala, infesting cucurbit vegetables and the crop loss varies between 30-100% (Dhillon *et al*., 2005; Kapoor, 1993).

Rhabditid nematodes of the families Steinernematidae and Heterorhabditidae are entomopathogenic nematodes that are pathogenic to a wide range of agriculturally important pests and successfully used as alternatives to chemical insecticides (Gaugler and Kaya, 1990; Forst and Clarke, 2002). Biocontrol efficiency of *H. indica* against various lepidopteran pests have been studied extensively for green house and nursery crops (Jagdale, 2013; Lacey and Georgis, 2012). The IJs of *H. indica* harbour symbiotic gut bacteria, *Photorhabdus luminescence* (Boemare, 2002) which is lethal to a wide range of insect hosts. There is a dearth of information on the effective control of the melon fruit fly *B. cucurbitae* using entomopathogenic nematodes. The present study is an attempt to fill this gap and assess the susceptibility of *B. cucurbitae* to *H. indica* .

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Starter cultures of *H. indica* and the greater wax moth, *Galleria mellonella* were procured from the Central Plantation Crops Research Institute (CPCRI), Kayamkulam, Alleppy, Kerala. *Heterorhabditis indica* was cultured *in vivo* using the last instar larvae of *G. mellonella* (Woodring and Kaya, 1988) at room temperature (28°-32°C). Emerging IJs were harvested through modified White trap method (White, 1927) within 24h and stored in open Petri dishes as aqua suspension. The nematode suspension was aerated using filler and the water level was maintained to aid survival during storage. Different concentrations were prepared by serial dilution of the suspension and counting of nematodes was done using a micro pipette (Vertex) and a stereo microscope (Labomed CZM 2). *Galleria mellonella* culture was maintained using three types of culture chambers- mating, rearing and pupal (sterilized 4- liter plastic cages) in the laboratory and the larvae were fed with an artificial diet (Singh, 1994). Fully grown last instar larvae were used for EPN culture.

The duration of the study was for two years (September 2014 to August 2016) and the experiment was conducted in a model field of 4 cents located in the campus of Fatima Mata National College, Kollam, Kerala (8° 53' 35.56" N and 76° 36' 50.9" E). Host plants chosen for the study were *Momordica charantia,* the bitter gourd and *Trichosanthes cucumerina,* the snake gourd. Seeds of known varieties (Preethi and Kaumudi) were purchased from the Regional Agriculture Research Station (RARS), Vellayani, Thiruvananthapuram, Kerala. Ripe yellowish–orange infested fruits were collected from the field and incubated in the laboratory in clean, dry and sterilized 3-liter plastic bottles for rearing *B. cucurbitae*. Mouth of the bottles were covered using band aid cloth and tightened with rubber bands. The last instar maggot- the only larval instar that came out of the fruits were transferred to separate rearing bottle and a quarter of its bottom area was provided with soil (20gm) for pupation. Emerging adults were transferred to fresh bottles containing fruit pieces. To avoid starvation during early hours of emergence, adults were provided with honey placed on cotton balls. Fruits infected with eggs were placed in rearing bottles till the emergence of last instar maggots.

Late third instar maggots of *B. cucurbitae* were used for the laboratory bioassay employing filter paper exposure method of Woodring and Kaya (1988). Petri plates of 10 cm diameter were floored with Whatman No.1 filter paper and in each plate, 1 ml of the EPN suspension was dispensed equally onto the filter paper and 10 maggots were introduced. Sixteen treatments ranging from 100 IJs/ml (10 IJs/ instar) and progressing in multiples of 100 were prepared for the laboratory bioassay. Each treatment with ten replicates and a control (11 sets) containing 10 maggots in each set were studied. Observations were made at 24h and 48h post inoculation. Field trials were carried out in randomized block design in soil and the field was randomly plotted into 20×20×5 cm size soil plots. Five treatments including a control, with six replications each were carried out. Each replication was with 3 plots. Doses @ 100 000 IJs, 200 000 IJs, 300 000 IJs and 400 000 IJs in 100 ml aqua suspension were prepared by serial dilution using well water. Control plots were treated with 100 ml well water. EPN suspension was applied using a hand sprayer 10-12 hours before maggots fell on the ground. Four days after nematode application, the soil was collected and carefully observed for pupae. Numerical density of the infected and uninfected pupae were recorded. Laboratory bioassay data of dosage mortality relationship was subjected to Probit analysis (Finney, 1971) using the software SPSS version 16 to determine 24h and 48h LD_{50} and LD_{90} values. Percentage of infection in the field was subjected to analysis of variance (ANOVA) and significance was calculated at 5% level.

Inoculation of *H. indica* revealed successful infection. Purple colour of third instar maggots of *B. cucurbitae* signalled its susceptibility to the nematode. Mortality rate was directly proportional to numerical density of the nematode and 100% mortality was observed within 48h @ 160 IJs/ instar. Infected maggots became inactive and died within 24 to 48h. Early cadavers appeared bright purple in color (Fig.1) and later turned dark brown.

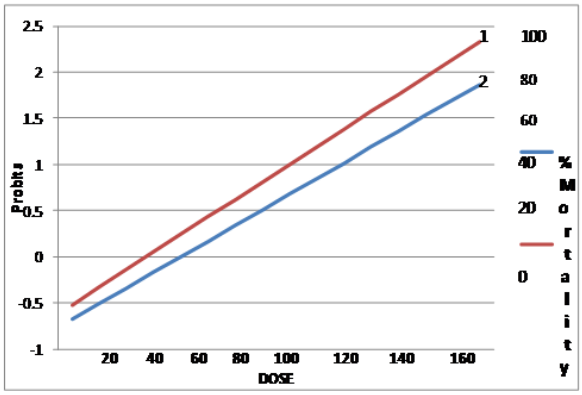
Fig. 1 Maggots - Before and after inoculation

Fig. 2 White trapped cadavers of maggots

Cadavers subjected to White's trap (Fig.2) resulted in emergence of EPNs and the cause of infection was confirmed. Newly emerged nematodes collected and preserved as aqua suspension in double distilled water always lived more than 5 days.

Mean percentage mortality of the third instar maggots of *B. cucurbitae* at different concentrations of *H. indica* is given in Fig.3. A significant decrease in the LD_{50} and LD_{90} values (38.17 and 107.04 respectively) was found at 48h than that at 24h (50.28 and 125.95 respectively) showing that mortality increased with exposure time. The Chi Square values, regression equation, 24h and 48h LD_{50} and LD_{90} values and the fiducial limits are presented in Table 1. More number of individuals (100) and treatments (16) were employed in the present study and the data obtained was highly significant. It was also observed that the doses applied were highly effective and relevant for the bio-control process. Median lethal dosage was high at 24h (50.28) and low at 48h (38.17), indicating a significantly higher mortality at 48h ($P< 0.05$). This revealed a higher susceptibility of *B. cucurbitae* at 48h exposure to *H. indica* and the virulence exhibited by the nematode increased with exposure time. Thus mortality was dose and time dependant.

Studies on the pathogenecity of *H. indica* in the field (Fig. 4) against the late third instar maggot of



1 = 48h, 2 = 24h

Fig. 3 Log Probit Curve of mean percentage mortality of III instar maggot of *B. cucurbitae* at different concentrations of *H. indica*

B. cucurbitae before pupation resulted in an increase in mortality with increasing dose. As the third instar maggot pupated soon after it entered the soil, only infected pupae could be observed in the soil after field trials (Fig. 5). The mortality of the maggots was found to be the same as the number of infected pupae. The data recorded 4days after treatment (DAT) revealed that the infection of the pupae varied from 12.73 (T_2) to 65.36% (T_5). Considerable increase in mortality was observed from T_2 (d) to T_3 (c) and T_4 (b) to T_5 (a) than that between T_3 and T_4. T_5 (a) was significantly more effective than the other treatments and recorded a maximum of 65.36 % infection to the pupae. Infection and mortality was in the order $T_5>T_4>T_3>T_2$ (Table 2).

Fig.4 Experimental field

Fig.5 Infected pupae in the field

Table 1. Probit analysis of dosage mortality relationship of III instar maggot of *B. cucurbitae* by *H. indica*

Time	Heterogeneity		Regression Equation (Y)= a+bX	LD_{50}	LD_{90}	Fiducial Limit	
	Chi-Square	Df				Lower	Upper
24 h..	5.47	14	0.017 X - 0.852	50.28	125.95	45.27	54.82
48 h..	1.96	14	0.019 X - 0.710	38.17	107.04	32.90	42.83

Chi-Square Table Value ($P < 0.05$) = 23.68.

Table 2. Evaluation of *H. indica* against the third instar maggot of *B. cucurbitae* (4 DAT) in the field

Treatments	Dose (IJs/100ml)	% Mortality(4 DAT)*	Range
T_1	0	1.11 (1.125) ** e	0 - 3.57
T_2	100, 000	12.73 (3.609) d	9.0 - 17.6
T_3	200, 000	32.83 (5.734) c	25.0 - 48.2
T_4	300, 000	46.93 (6.866) b	38.0 - 58.3
T_5	400,000	65.36 (8.108) a	57.8 - 73.9
CD at 5% - 0.717			

* DAT – Days after treatment.: ** Figures in parenthesis are square root transformed values.

Laboratory bioassay studies of Supekar and Mohite (2013) and Maneesakorn *et al.* (2010) revealed the efficacy of *H. indica* against *Pappillia japonica* and *Holotrichia serrata* Fab. respectively. Divya *et al.* (2010), Sankar (2009) and Garcia *et al.* (2008) employed higher doses of *H. indica* than the present study to obtain maximum mortality. Third instar larvae of the diamond black moth (DBM) of cabbage, *Plutella xylostella* showed 96% mortality when infected by *H. indica* within 72h of application. The proportion of DBM mortality increased with increased exposure time (Nyasani *et al.,* 2008). In our study also mortality increased when exposure time increased from 24h to 48h and mortality was proportional to dose and time. Though the final instar maggots of *B. cucurbitae* were small in size, high dose of IJs was required for higher mortality. This can be attributed to the shape of the body (elongated) and dynamic movements (folding and jumping) of the maggots.

Green house pot culture experiments conducted by Bharati and Mohite (2014) revealed that application of 450 IJs ml^{-1} of *H. indica* were very effective in controlling second instar grub of *Leucopholis lepidophora* (Blanchard) and recorded 45.33 to 87.60 per cent grub mortality at 15 DAT. Cotton plants, *Gossypium herbaceum* sprayed with *H. indica* at a dose of 1000 IJs ml^{-1} against various larval instars of *Helicoverpa armigera* and *Spodoptera litura* under green house study revealed increased percentage of mortality with increasing age of larva and duration of exposure (Divya *et al.*, 2010). Results of the current investigation also showed a similar trend. The present study confirms the possibility of *H. indica* as an effective biocontrol agent of *B. cucurbitae*.

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