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Bio-efficacy of *Heterorhabditis indica* Poinar in combination with *Helicoverpa armigera* polyhedrosis virus against *Helicoverpa armigera* (Hübner)

A. PRABHURAJ, B. V. PATIL, SHIVALEELA and K. S. GIRISH
Department of Entomology, College of Agriculture
University of Agricultural Sciences, Raichur 584 101, Karnataka, India
E-mail: prabhusha@rediffmail.com

ABSTRACT: **The bio-efficacy of *Heterorhabditis indica* Poinar in combination with *Helicoverpa armigera* nuclear polyhedrosis virus (*Ha*NPV) against *Helicoverpa armigera* (Hübner) was evaluated during 2002 in the laboratory. Results revealed that combination of *H. indica* and *Ha*NPV at all concentrations recorded highest mortality (100%) and were on par with alone inoculation of *H. indica* treatment, but significantly superior over *Ha*NPV alone against third instar larva. Fourth instar larva suffered cent per cent mortality when inoculated with *H. indica* plus *Ha*NPV at all concentrations after 48 h compared to *H. indica* alone (@ 200 IJs/ larva) which recorded 75 per cent mortality. The results indicate the synergistic effect when both the bioagents were combined against fourth instar larva.**

KEY WORDS: Entomopathogenic nematode, *Ha*NPV, *Helicoverpa armigera*, *Heterorhabditis indica*, infective juvenile

INTRODUCTION

Helicoverpa armigera (Hübner) is a serious pest of legumes, cotton and vegetables in the Indian subcontinent and South East Asia (Reed and Pawar, 1982; King, 1994). Widespread appearance of resistance to chemical insecticides, including the widely used pyrethroids in the late 1980s caused increased losses and has made control by chemicals increasingly unreliable and expensive (Armes *et al.*, 1992). This has stimulated efforts to develop alternative control methodologies including the use of entomopathogens for the control of this pest.

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae have considerable potential to control insect pests. These nematodes have received considerable attention as bioinsecticides because of their broad host range, high virulence, easy mass production, storage and application (Gaugler and Kaya, 1990).

Research has shown that *Ha*NPV can be used successfully on several crops (Jayaraj *et al.*, 1987; Pawar *et al.*, 1990). Combined effect of entomopathogenic nematodes and virus has been studied by several workers. Kaya and Brayton (1978) have studied the interactive effect of steinernematids and granulosis virus on armyworm. Similarly, Karunakar *et al.* (2002) studied the interactive effect of two species of *Steinernema* and *Heterorhabditis indica Poinar* and granulosis virus of *Chilo infuscutellus* Snellen and *Chilo sacchariphagus indicus* (Kapur). However, no

study was conducted on the interactive effect of *H. indica* and *Ha*NPV on *H. armigera*, hence, the study was undertaken to examine the nature of interaction between two pathogens.

MATERIALS AND METHODS

Source of nematode and virus

A new isolate of *H. indica* (RCR) was collected from naturally infected grape flea beetle grubs, *Scelodonta strigicollis* M. from Horticulture garden of College of Agriculture, Raichur, Karnataka, India and its identity was confirmed by Dr. Poinar G. O, visiting Professor, Oregon State University, USA. The nematode was maintained on larvae of greater wax moth, *Galleria mellonella* Linn. in the laboratory for further studies. The virus, *Ha*NPV was obtained from the biocontrol unit of Regional Agricultural Research Station, Raichur and stored at 7°C until used.

Source of *H. armigera* larvae

Larvae of *H. armigera* collected from the field were reared in the laboratory on the soaked chickpea seeds. Moths obtained were released in a pot containing 15–20 days old chickpea seedlings for oviposition and covered with nylon net to prevent the adult escape. Hatched larvae were maintained on the chickpea seedlings up to second instar and later shifted individually into a plastic container (25ml capacity). Soaked chickpea seeds were provided daily as food for the larvae till pupation.

Determination of LC_{50} for the nematode

A laboratory bioassay was conducted against third and fourth instar larvae of *H. armigera* to find out the nematode concentration to kill 50 per cent of the test insects. Larvae were placed individually in plastic vials (25ml capacity) internally lined with 3X3 cm filter paper. Required concentration of nematode suspension was prepared through serial dilution method and with the help of micropipette, 0.5 ml of desired nematode load *viz.*, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 infective juveniles (IJs) per larva was applied to the filter paper separately. Ten vials containing 10 larvae formed one replication. Each treatment was replicated four times. Thus 40 larvae were used per treatment. Control included application of distilled water only. Observation on larval mortality was recorded after 12 h of inoculation. The concentration mortality response (LC_{50}) was computed using MLP software developed by Central Research Institute for Dryland Agriculture (CRIDA), India.

Interaction between nematode and virus

To study the interactive effect of *H. indica* and *Ha*NPV, one concentration of *H. indica* (150 IJs/larva and 200 IJs/larva for third and fourth instars, respectively) based on LC_{50} experiment, one concentration of *Ha*NPV and five concentrations of *H. indica* plus *Ha*NPV and one control were tested against third and fourth instar larvae, separately. The treatments detail is given in the Table 1.

Third instar larvae of *H. armigera* having equal weight were released into a plastic vial (25ml capacity) lined with a layer of filter paper individually. Nematode suspension of 0.5ml containing 150 IJs was spread on the filter paper evenly. Required concentration of *Ha*NPV was prepared following serial dilution method and with the help of micropipette 10μl of virus suspension was spread on overnight soaked chickpea seeds and shade dried. Treated seeds were placed in the plastic vials individually and closed with the cap. Small holes were provided on the plastic caps for aeration.

After 24 hours of treatment imposition, filter paper was removed and fresh seeds without virus were given for feeding. Thus each treatment was replicated four times with 10 larvae in each replication. In control, filter paper was treated with distilled water only and chickpea seeds without virus were given as food.

Table 1. Treatment details

Sl. no.	Treatment	Dosage	
		Third instar larva	Fourth instar larva
1.	*H. indica*	150 IJs	200 IJs
2.	*Ha*NPV	$3x10^3$ PIB	$3x10^3$ PIB
3.	*H. indica* + *Ha*NPV	150 IJs + $3x10^3$ PIB	200 IJs + $3x10^3$ PIB
4.	*H. indica* + *Ha*NPV	150 IJs + $1.5x10^3$ PIB	200 IJs + $1.5x10^3$ PIB
5.	*H. indica* + *Ha*NPV	150 IJs + $0.75x10^3$ PIB	200 IJs + $0.75x10^3$ PIB
6.	*H. indica* + *Ha*NPV	150 IJs+$0.375x10^3$ PIB	200 IJs+$0.375x10^3$ PIB
7.	*H. indica* + *Ha*NPV	150 IJs+$0.1875x10^3$PIB	200 IJs + $0.1875x10^3$PIB
8.	Untreated control	Distilled water	Distilled water

Similar procedure was followed for fourth instar larvae of *H. armigera* except that the larvae received *H. indica* @ 200 IJs/larva.

Observation on larval mortality was recorded at 12, 24, 36 and 48 hours after treatment imposition. The data so obtained were converted to per cent mortality using 'arcsine transformation' and subjected to factorial CRD with factor A as combination and factor B as the larval mortality at different hours after treatment imposition.

RESULTS AND DISCUSSION

LC_{50} of *H. indica*

The effective lethal concentrations estimated to cause 50 per cent mortality (LC_{50}) was 145 IJs/larva with slope and fiducial limit (95%) of 1.05 and 105–172 nematodes, respectively after 12h for third instar larva. Similarly, for fourth instar it was 196 IJs/larva with slope and fiducial limit (95%) of 2.14 and 165–239 nematodes, respectively. This dosage was used in combination studies with *Ha*NPV.

Combine effect of *H. indica* and *Ha*NPV

Highest mortality of 95 per cent in third instar larvae was obtained when exposed to the combinations of *H. indica* + *Ha*NPV @ $0.75x10^3$ PIB/larva and *H. indica* + *Ha*NPV @ $0.1875x10^3$ PIB/larva at 36 hours after treatment. Mortality recoded in other combination treatments was on par. However, *Ha*NPV @ $3x10^3$ PIB/larva alone recorded only five per cent mortality. Untreated control recorded zero per cent larval mortality. However, after 48 hours of infection all the treatments except *Ha*NPV alone (10%) and untreated control (0%) recorded 100 per cent larval mortality (Table 2). It is quite evident form the above result that there is no synergistic effect between nematode and *Ha*NPV against third instar larva. The mortality obtained was purely due to nematodes alone. This might be due to the fact that, larvae being very small were quickly killed by nematodes before *Ha*NPV could act upon it.

Table 2. Combine effect of *H. indica* and *Ha*NPV against third instar larvae of *H. armigera*

Sl. no.	Treatment/dose	Mortality at different hours (%)				Mean
		12	24	36	48	
1.	*H. indica* 150 IJs (48.16)^{DEF}	55.00 (60.86)^{BCD}	70.00 (76.72)^{AB}	90.00 (90.00)^{A}	100.00 (68.93)^{A}	78.75
2.	*Ha*NPV 3x10^3 PIB (0.00)^{K}	0.00 (0.00)^{K}	0.00 (6.64)^{JK}	5.00 (13.28)^{IJK}	10.00 (4.98)^{C}	3.75
3.	*H. indica* 150 IJs + *Ha*NPV3x10^3 PIB	25.00 (26.25)^{GHIJ}	45.00 (42.11)^{DEFG}	85.00 (70.08)^{ABC}	100.00 (90.00)^{A}	63.75 (57.11)^{B}
4.	*H. indica* 150 IJs + *Ha*NPV 1.5x10^3 PIB	35.00 (36.06)^{EFGH}	55.00 (47.88)^{DEF}	90.00 (76.72)^{AB}	100.00 (90.00)^{A}	70.00 (62.66)^{B}
5.	*H. indica* 150 IJs + *Ha*NPV 0.75x10^3 PIB	25.00 (26.25)^{GHIJ}	65.00 (53.93)^{CDE}	95.00 (83.36)^{A}	100.00 (90.00)^{A}	71.25 (64.25)^{B}
6.	*H. indica* 150 IJs + *Ha*NPV 0.375x10^3 PIB	25.00 (26.25)^{GHIJ}	70.00 (57.10)^{BCD}	85.00 (70.08)^{ABC}	100.00 (90.00)^{A}	70.00 (61.72)^{B}
7.	*H. indica* 150 IJs + *Ha*NPV 0.1875x10^{3}PIB	15.00 (19.92)^{HIJ}	50.00 (45.00)^{DEFG}	95.00 (83.36)^{A}	100.00 (90.00)^{A}	65.00 (59.57)^{B}
8.	Untreated control	0.00 (0.00)^{K}	0.00 (0.00)^{K}	0.00 (0.00)^{K}	0.00 (0.00)	0.00 (0.00)^{C}

Figures in the parentheses are 'arcsine' values.

	Factor A (Combination)	Factor B (Hours)	Interaction (A x B)
SEM ±	2.28	1.61	4.57
CD (P=0.01)	6.34	4.48	12.69

Fourth instar larva suffered cent per cent mortality when exposed to *H. indica* + *Ha*NPV @ 3x10^3 PIB/larva, *H. indica* + *Ha*NPV @ 1.5x10^3 PIB/larva and *H. indica* + *Ha*NPV @ 0.1875x10^3 PIB/larva, however they were on par with other combinations but significantly superior over alone treatments. Once again *Ha*NPV alone @ 3x10^3 PIB/larva failed to bring any mortality in the larvae and was on par with untreated control (Table 3).

Infectives of *H. indica* when combination with *Ha*NPV has a synergistic effect leading to highest mortality in short period. This was evident in fourth instar larva. Whereas, even though integration of these two bioagents resulted in highest mortality in third instar, it was not due to synergistic effect but by nematode alone. However, no antagonistic effect was observed against third instars larvae by these two bioagents.

Thus the above study clearly indicates that *H. indica* has good compatibility with *Ha*NPV resulting in higher mortality in fourth instar compared to either of the bioagents when used alone. Hence, there is a scope for integration of these two bioagents for the better management of *H. armigera*.

Table 3. Combine effect of *H. indica* and *Ha*NPV against fourth instar larvae of *H. armigera*

Sl. No.	Treatment/dose	Mortality at different hours (%)				Mean
		12	24	36	48	
1.	*H. indica* 150 IJs	20.00 (23.08)^{GH}	35.00 (32.02)^{DEFG}	55.00 (48.16)^{BCD}	75.00 (60.27)^{BC}	78.75 (68.93)^{B}
2.	*Ha*NPV 3x10^3 PIB	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{C}
3.	*H. indica* 150 IJs + *Ha*NPV 3x10^3 PIB	30.00 (32.89)^{DEFGH}	75.00 (63.74)^{B}	100.00 (90.00)^{A}	100.00 (90.00)^{A}	76.25 (69.16)^{A}
4.	*H. indica* 150 IJs + *Ha*NPV 1.5x10^3 PIB	25.00 (29.72)^{EFGH}	55.00 (47.88)^{DEF}	100.00 (90.00)^{AB}	100.00 (90.00)^{A}	67.50 (62.02)^{A}
5.	*H. indica* 150 IJs + *Ha*NPV 0.75x10^3 PIB	25.00 (29.72)^{EFGH}	50.00 (45.00)^{BCDE}	95.00 (83.36)^{A}	100.00 (90.00)^{A}	71.25 (64.25)^{B}
6.	*H. indica* 150 IJs + *Ha*NPV 0.375x10^3 PIB	25.00 (29.72)^{EFGH}	45.00 (42.11)^{DEFG}	95.00 (83.36)^{A}	100.00 (90.00)^{A}	66.25 (60.43)^{A}
7.	*H. indica* 150 IJs + *Ha*NPV 0.1875x10^{3}PIB	25.00 (29.72)^{EFGH}	60.00 (51.05)^{BCD}	100.00 (90.00)^{A}	100.00 (90.00)^{A}	71.25 (64.25)^{A}
8.	Untreated control	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{I}	0.00 (0.00)^{C}

Figures in the parentheses are 'arcsine' values.

	Factor A (Combination)	Factor B (Hours)	Interaction (A x B)
SEM ±	2.15	1.52	4.30
CD (P=0.01)	5.96	4.21	11.92

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