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Integration of *Heterorhabditis indica* with other biorationals for managing chickpea pod borer, *Helicoverpa armigera* (Hüb.)

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Short Title: Integration of *H. indica* with biorationales against chickpea pod borer.

ABSTRACT: A successful management strategy was developed against chickpea pod borer, *Helecoverpa armigera* (Hüb) by integrating locally isolated entomopathogenic nematode, *Heterorhabditis indica* (RCR) with other entomopathogens like *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) and *Bacillus thuringiensis* (Bt) and botanicals. Preliminary laboratory studies were conducted, to standardize the optimum dosage of nematodes required for field application, to evaluate the compatibility of nematode with entomopathogens and botanicals and their combinations. The optimum dosage of infective juveniles was standardized to third (LC_{50} of 145 IJs/larva) and fourth (LC_{50} of 195 IJs/larva) instars based on the concentration mortality response. Persistence study on chickpea foliage in field condition indicated that, infective juveniles along with 0.1 % glycerol survived better (80%) compared to other anti-dessicants. In compatibility studies, though *H. indica* was compatible with other entomopathogens, but was susceptible to higher concentrations of aqueous leaf extracts of some selective botanicals. A series of laboratory bioassay was carried out to select best combinations of *H. indica* with other entomopathogens and botanicals against third and fourth instar *H. armigera* and these were tested in field for two consecutive years. Two year field evaluation indicated that, sequential application of *H. indica* + *Prosopis juliflora* (1 lakh IJs/l + 10%) at 50 and 75 days after sowing was superior with highest larval reduction (23.47%), minimum pod damage (11.27%) and maximum seed yield (19.24 q/h).

KEY WORDS: *Heterorhabditis indica*, *Helicoverpa armigera*, management, chickpea, botanicals, *Ha*NPV, entomopathogens, mortality.

INTRODUCTION

Chickpea, an important pulse crop of India occupies an area of 7.6 mha with average productivity of 0.9q ha^{-1} (Anonymous, 2000). In Karnataka it is grown in an area of 4.79 lakh hectares with a production of 2.81 lakh tones and productivity of 618 kg ha^{-1} (Anonymous, 2004). The pod borer, *Helicoverpa armigera* (Hübner) is a major pest causing reduction in yield ranging from 40-50 per cent (Rai *et al.*, 2003). Wide spread appearance of resistance to chemical insecticides including widely used pyrethroids in late 1980's caused an increase in losses due to this pest and has made control by chemical increasingly unreliable and expensive.

Entomopathogenic nematodes (EPNs) in families Steinernematidae and Heterorhabditidae have considerable potential to control several insect pests (Gaugler and Kaya, 1999). A native species, *Heterorhabitis indica* (Poinar *et al.*, 1992) from India has great potential in controlling several crop pests including *H. armigera* (Karunakar *et al.*, 2002). It has also been shown that the performance of EPNs can be enhanced by integrating with other entomopathogens and botanicals (Choo *et al.*, 1998). Hence, laboratory and field studies were undertaken to develop bio-intensive management strategy against *H. armigera* by integrating locally isolated *H. indica* (RCR) strain with selective entomopathogens and botanicals in chickpea ecosystem during 2002-2005.

MATERIALS AND METHODS

Source of entomopathogens

Culture of *H. indica* was isolated from naturally infected grape flea beetle, *Sceledonta strigicollis* M. from Agriculture College, Raichur and was maintained on *Galleria mellonella* L.

Helicoverpa armigera NPV (HaNPV) was obtained from bio-control unit of Regional Agricultural Research Station, Raichur. The source of Bt was DipeL R of Sumitomo Chemicals Pvt. Ltd. India having 17,600 IU/mg.

Source of larva

The culture of *H. armigera* obtained from infested chickpea was maintained on soaked chickpea seeds individually. Moths were made to oviposit on 15-20 days old chickpea seedlings grown in a pot and neonate larvae were reared on seedlings before shifting to plastic vials.

Preparation of plant extract

Freshly plucked (100g) leaves of *Prosopis juliflora* L., *Pongamia pinnata* (L.) Pierre and *Vitex nigundo* L. were ground separately using pestle and mortar. Leaf pulp was tied in a muslin cloth and dipped in 100 ml distilled water for 6-8 hours. Later pulp was squeezed along with muslin cloth to extract leaf content. The solution thus obtained served as stock solution and diluted to desired concentration.

Preparation of neem seed kernel extract (NSKE)

Fifty grams of neem seeds were deshelled, ground and soaked in one liter of water overnight. The next day the content of cloth was drained by squeezing. The solution obtained was served as stock solution. Neem oil was obtained from commercial mill having 15,000 ppm of azadirachtin.

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 (25 ml capacity) internally lined with 3 x 3 cm filter paper. Required concentration of nematode suspensions were prepared through serial dilution method and with the help of micropipette, 0.5 ml of desired nematode suspension 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. Control included application of distilled water only. Observation on larval mortality was recorded at 12, 24 and 48 hours after inoculation. The concentration mortality response (LC_{50}) was computed using MLP software 'DESIGN' developed by CRIDA, India.

Nematode persistence on chickpea foliage

The experiment was conducted under field condition (12-26°C with 50-60% RH) on 25 days old chickpea crop. Aqueous solution of *H. indica* at a concentration of 600 Ijs ml^{-1} was sprayed on chickpea foliage using hydraulic sprayers during evening hours. Each treatment block consisting of 75 plants received 750 ml of spray solution. The treatment included the use of various anti-desscants *viz.*, glycerol, paraffin wax, and Triton X-100 at 0.1% and castor oil, palm oil and sunflower oil at 1% along with *H. indica*. Sodium bicarbonate (0.5%) was added in all the treatments to nullify the acidic pH prevailed on chickpea foliage due to malic acid except in control (*H. indica* alone). Immediately

after application five leaflets were taken from each nematode sprayed plant which constituted one replication. Thus, totally 15 leaflets were taken separately from three plants. Leaflets plucked from each plant were dipped in 100 ml water in a plastic container and shaken thoroughly to ensure that all the nematodes were removed into the water. The nematodes thus collected were observed under microscope to record the survivability. Observations ware taken on 2, 4, 6 and 8 hours after spray. Data presented as percentage were normalized using 'arc sin' transformation and was subjected to ANOVA test.

Compatibility of nematode with botanicals

Six concentrations of *P. juliflora*, *P. pinnata* and *V. nigundo* leaf extracts (10, 5, 4, 3, 2, 1%) were prepared separately and 25 ml of stock solution of each concentration was taken in conical flasks and 10,000 ± 50 IJs were released in each flask. Control consisted of nematode with distilled water only. For NSKE 5, 2.5, 2.0, 1.5, 1.0 and 0.5% and neem oil 2, 1, 0.75, 0.5, 0.25 and 0.1% concentrations were used. Each treatment was replicated thrice. Microscopic observation on juvenile mortality was recorded after 48 hours of exposure.

Combination study with entomopathogens

EPN and HaNPV

Third instar larvae of equal weight were released into plastic vials (25 ml capacity) individually lined with a layer of filter paper. Nematode suspension of 0.5 ml containing 150 IJs was spread on the filter paper. With the help of micropipette desired concentration of HaNPV (3, 1.5, 0.75, 0.375 and 0.1875 PIB x 10^3/larva) prepared through serial dilution was spread on soaked chickpea seeds and fed to larvae. After 24h filter paper was removed and fresh seeds without virus were given for feeding. Similar procedure was followed for fourth instar except that larvae required *H. indica* @ 200 IJs/larva. Control included application of distilled water alone. Larval mortality was recorded after 48 h. The data obtained were converted to per cent mortality using 'arc sin' transformation and subjected to analysis of variance.

EPN and Bt

Third and fourth instar larvae were exposed to various treatments *viz.*, *H. indica* alone, Bt alone (@ 0.264 IU/mg), *H. indica* + Bt @ 100 + 0.264, 50 + 0.264, 75 + 0.132 and 75 + 0.066 IJs/larva + IU/mg. Control included application of distilled water only. Treatments were replicated four times with ten larva in each replication. Larval mortality was recorded after 48 h and converted to per cent mortality using 'arc sin' transformation and subjected to one way analysis to test the level of significance.

EPN and botanicals

Third instar larvae were released into plastic vials (25 ml capacity) lined with a layer of filter paper. In first treatment nematode suspension (150 IJs) was evenly spread on filter paper, whereas, in second, third and fourth treatments leaf extracts alone (10, 5 and 2.5%) was used. In fifth, sixth and seventh treatments larvae were first exposed to leaf extracts (10, 5 and 2.5%) followed by nematodes (50 IJs) after 24 h. In eighth treatment 1% leaf extract and 50 IJs larva^{-1} were applied simultaneously. In another set, *H. indica* alone, NSKE alone (5%) and sequential application of NSKE (5, 2.5 and 1%) followed by nematode (50 IJs), simultaneous application of NSKE (1 and 0.5%) and nematode (50 IJs larva^{-1}) were imposed. In third set, neem oil alone (2 and 1%) and simultaneous application of neem oil (0.5 and 0.1%) and nematode (50 IJs larva^{-1}) were imposed. Each treatment was replicated four times with 10 larvae in each replication. After 24 h of treatment imposition (48 h in case of sequential treatments) filter paper was removed and fresh seeds were given for feeding. Similar procedure was followed for fourth instar except that larvae received nematode @ 200 IJs larva^{-1} in first treatment only. Observation on larval mortality was recorded at 48 h. Data obtained were converted to per cent mortality by 'arc sin' transformation and subjected to analysis of variance.

Field evaluation

In first year study, totally 22 combination

treatments which performed superior in laboratory study were tested in the field. The trial was conducted during 2003-04 in RCBD in a plot size of 12 m^2. Each treatment was replicated thrice. Glycerol (0.1%) was added as an anti-dessicant in all treatments except chemical and untreated plots to enhance the nematode survival. Similarly, sodium bicarbonate (0.5%) as buffer and a sweetener (0.1%) as phagostimulant was added to all treatments. Based on Economic Threshold Level (ETL), two sprays (50 and 75 days after sowing) were undertaken. Observations on larval population were recorded from three rows of 1 m length in each plot one day before spraying and subsequently 2, 4 and 7 days after spraying. Data obtained from two sprays was pooled, after converting into per cent larval reduction and subjected to analysis of variance. At the time of harvesting damaged as well as healthy pods were counted and per cent pod damage was computed. Seed yield per plot was recorded and subjected to ANOVA.

In the second year of experiment during 2004-05, best treatments from previous field study were evaluated in larger area. Treatments included the combination of *H. indica* with Bt, *P. juliflora* and *P. pinnata* and *H. indica* alone and insecticidal spray. Each treatment was replicated four times with each replication having a plot size of 200m^2. Glycerol (0.1%), sodium bicarbonate (0.5%) and a sweetener (0.1%) were added as anti-dessicant, buffer and phagostimulant, respectively in all treatments. Two sprays were given at 50 and 75 days after sowing depending on ETL. Observations on larval mortality, pod damage and yield were taken similar to that of first field trial. Cost of plant protection and additional income over untreated check were calculated.

RESULTS AND DISCUSSION

Determination of LC_{50} for the nematode

The effective lethal concentration estimated to cause 50 per cent mortality (LC_{50}) was 145 IJs $larva^{-1}$ with slope and fiducial limit (95%) of 1.05 and 105-172 nematodes, respectively after 12 h for third instar larva. Similarly, for fourth instar it was 196 IJs $larva^{-1}$ with a slope and fiducial limit (95%) of 2.14 and 165–239 nematodes, respectively (Table 1).

Nematode persistence on chickpea foliage

Survivability of *H. indica* was significantly higher when mixed with glycerol (0.1%) recording 81.2% after 2h of application. This was followed by Triton X-100 (0.1%) and paraffin liquid (0.1%) with 62.6 and 50.6% survivability. Whereas, nematode with castor, palm and sunflower oil recorded less than 20% survivability. However, after 4h of application, nematode survivability got reduced substantially with only 24.9% in nematode with glycerol which is still significantly superior over other treatments. Thus, among the anti-dessicants, glycerol (0.1%) performed better over other synthetic anti-dessicants, whereas, natural oils completely failed to protect the nematodes (Fig. 1). Similar opinion was expressed by Welch and Briand (1961). However, Mason *et al.* (1999) recorded 100% mortality of *Plutella xylostella* when *Heterororhabditis* sp. was sprayed along with Triton X-100 (2%) on cabbage. This increased efficacy of nematodes might be due to use of higher concentration used compared to present study. Based on the present study, glycerol at 0.1% was used as anti-dessicant for field evaluation.

Compatibility of nematode with botanicals

Higher concentrations of tested botanicals were most detrimental to infectives. Aqueous leaf extracts of *P. juliflora, P. pinnata* and *V. nigundo* at 10%, NSKE at 5% and neem oil at 2% caused 90% mortality (Fig. 2). However, as the concentration of botanicals decreased, the survivability increased registering lowest mortality of 20% in all leaf extracts at 1% and 40% in case of NSKE (Fig. 3) and neem oil at 0.5 and 0.1%, respectively (Fig. 4). Nematicidal property of neem has been well established (Colin and Pussemier. 1992).

Combination study with entomopathogens

With HaNPV

Infectives of *H. indica* in combination with HaNPV has a synergistic effect leading to increased

Table 1. Concentration mortality of *H. indica* against third and fourth instar larva of *H. armigera* at different hours of exposure

Hour	D.F	÷ 2	Regression equation	LC_{50}	Slope	Fiducial limit (99%)
Third instar						
12	39	3.611	Y= 2.71 + 1.05 X	145.05	1.05	105 - 172
36	39	22.51	Y = 3.81 + 0.99 X	15.65	0.99	11 - 31
48	39	26.21	Y = 3.45 + 1.64 X	8.79	1.64	7 - 12
Fourth instar						
12	39	7.88	Y = 0.07 + 2.14 X	196.01	2.14	165 - 239
36	39	14.55	Y = -1.74 + 3.8 X	58.90	3.8	35 - 78
48	39	8.73	Y = 0.43 + 2.81 X	419	2.81	28 - 56

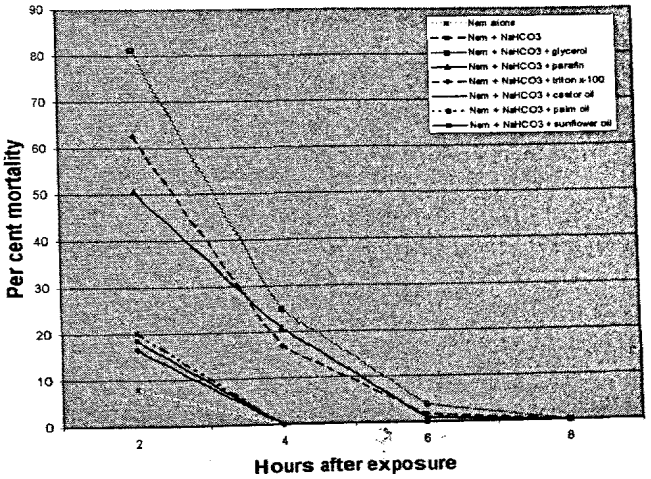

Fig. 1. Persistence of *H. Indica* with antidessicants on chickpea foliage

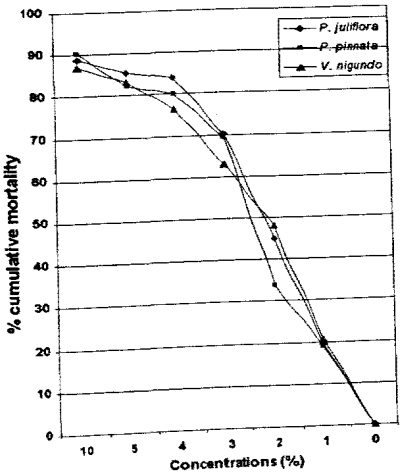

Fig. 2. Mortality of *H. Indica* in different concentration of plant extracts

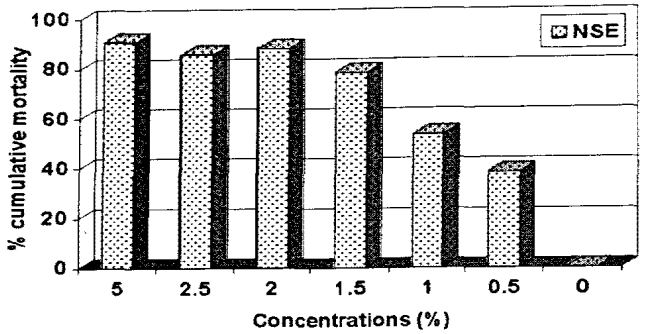

Fig. 3. Mortality of *H. indica* in different concentrations of NSKE

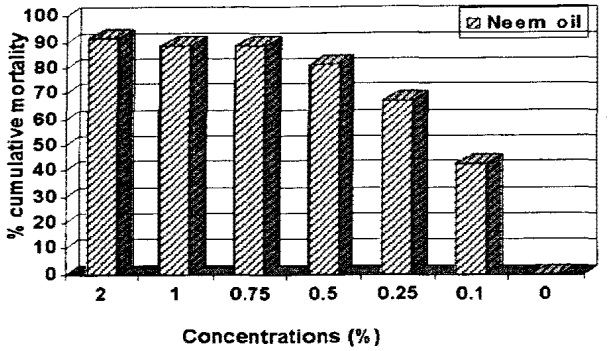

Fig. 4. Mortality of *H. indica* in different concentrations of neem oil

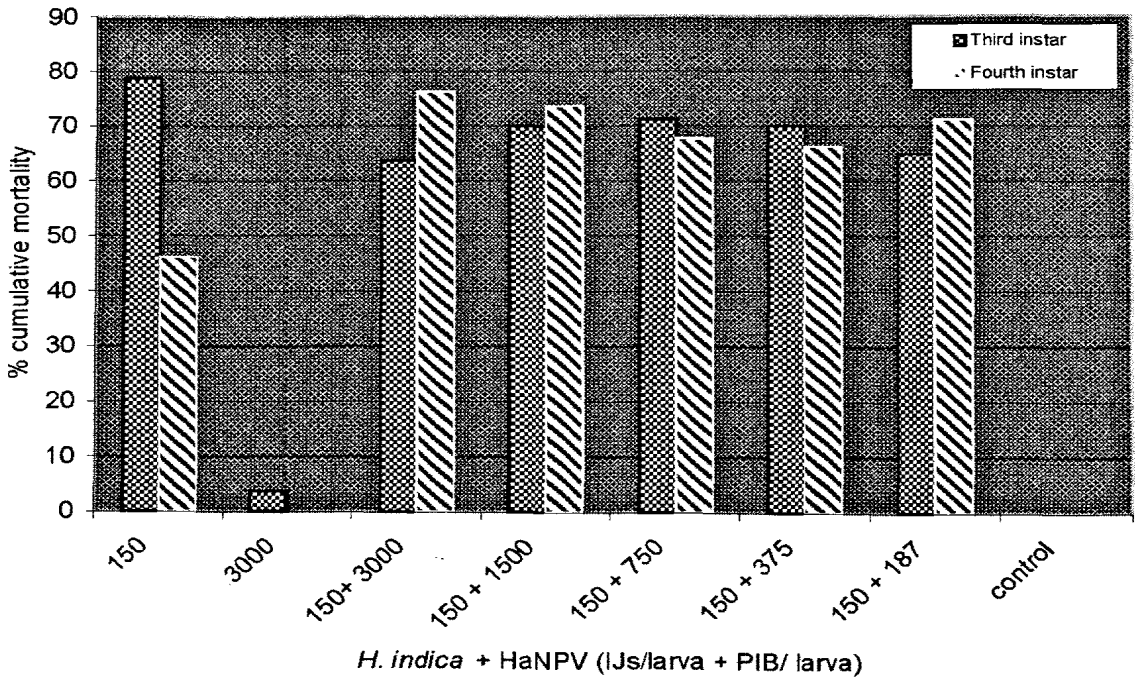

Fig. 5. Synergistic effect of *H. indica* + HaNPV on *H. armigera*

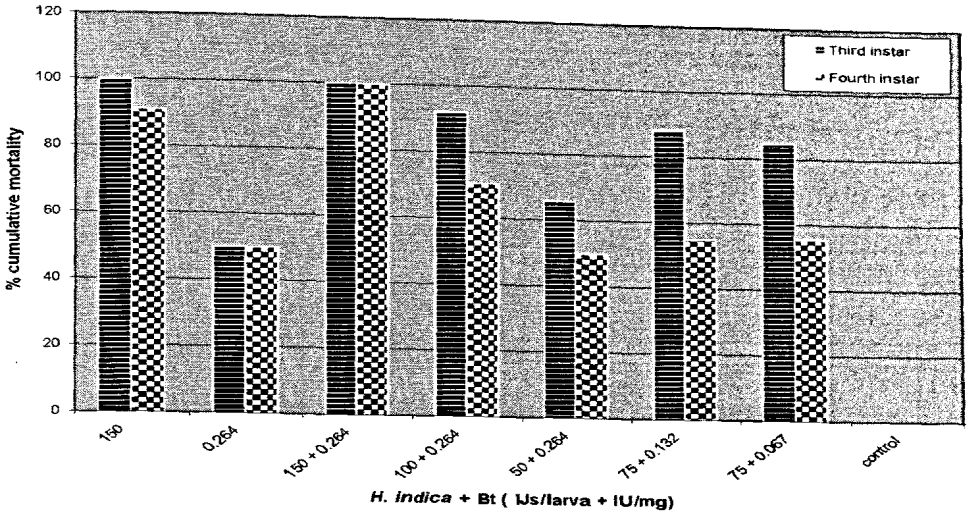

Fig. 6. Efect of combination of *H.indica* + Bt on *H. armigera*

Table 2. Cumulative mortality of *H. armigera* due to combination of *H. indica* and *P. juliflora*

Treatment details	Third instar		Fourth instar	
	24	48	24	48
H. indica (@ 150 for 3rd and 200 IJs for 4th instar larva)	65.85 (54.24)	100 (90)	28.8 (32.46)	82.4 (65.18)
P. juliflora (10 %)	0.00 (0.00)	0.34 (3.32)	0.00 (0.00)	1.40 (6.64)
P. juliflora (5 %)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P. juliflora (2.5%)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P. juliflora + *H. indica* @ 10% + 50 IJs/larva (Sequential)	41.60 (40.17)	100 (90)	13.45 (21.51)	63.7 (52.94)
P. juliflora + *H. indica* @ 5% + 50 IJs/larva (Sequential)	47.80 (43.72)	94.7 (76.72)	6.60 (14.86)	47.30 (43.42)
P. juliflora + *H. indica* @ 2.5% + 50 IJs/larva (Sequential)	38.80 (38.52)	94.7 (76.72)	19.40 (26.12)	47.30 (43.42)
P. juliflora + *H. indica* @ 1% + 50 IJs/larva (Simultaneous)	26.10 (30.72)	97.30 (80.40)	3.00 (9.96)	47.50 (43.56)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S. Em ±	4.14	1.59	3.17	3.13
CD (5%)	12.10	4.66	9.26	9.13
CD (1%)	16.40	6.31	12.55	12.37
CV	6.1	6.9	4.44	12.08

Figures in the parenthesis are 'arc sin' values

Table 3. Cumulative mortality of *H. armigera* due to combination of *H. indica* and *P. pinnata*

Treatment details	Third instar		Fourth instar	
	24	48	24	48
H. indica (@ 150 for 3rd and 200 IJs for 4th instar larva)	61.20 (51.49)	100 (90)	21.40 (27.56)	90.30 (71.81)
P. pinnata (10 %)	0.00 (0.00)	1.30 (6.64)	0.34 (3.32)	0.34 (3.32)
P. pinnata (5 %)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.34 (3.32)
P. pinnata (2.5 %)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P. pinnata + *H. indica* @ 10% + 50 Ijs/larva (Sequential)	49.82 (44.86)	100 (90)	52.70 (46.58)	82.60 (65.33)
P. pinnata + *H. indica* @ 5% + 50 IJs/larva (Sequential)	42.20 (40.53)	100 (90)	27.00 (31.32)	90.30 (71.81)
P. pinnata + *H. indica* @ 2.5% + 50 IJs/larva (Sequential)	47.30 (43.42)	99.37 (85.10)	15.40 (23.11)	75.90 (60.57)
P. pinnata + *H. indica* @ 1% + 50 IJs/larva (Simultaneous)	13.45 (21.51)	65.60 (54.10)	11.40 (19.77)	70.50 (57.11)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S. Em ±	2.67	2.21	3.64	3.40
CD (5%)	7.80	6.45	10.62	9.94
CD (1%)	10.52	8.75	14.39	13.47
CV	13.85	9.58	13.22	18.40

Figures in the parenthesis are 'arc sin' values

Table 4. Cumulative mortality of *H. armigera* due to combination of *H. indica* and *V. nigundo*

Treatment details	Third instar		Fourth instar	
	24	48	24	48
H. indica (@ 150 for 3rd and 200 IJs for 4th instar larva)	78.10 (62.13)	100 (90)	98.80 (83.66)	84.8 (66.92)
V. nigundo (10 %)	0.00 (0.00)	1.30 (6.64)	0.00 (0.00)	0.00 (0.00)
V. nigundo (5 %)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
V. nigundo (2.5 %)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
V. nigundo + *H. indica* @ 10% + 50 IJs/larva (Sequential)	6.60 (14.87)	88.70 (70.39)	2.10 (8.23)	38.80 (38.52)
V. nigundo + *H. indica* @ 5% + 50 IJs/larva (Sequential)	4.00 (11.55)	82.60 (65.33)	9.40 (17.89)	38.9 (38.36)
V. nigundo + *H. indica* @ 2.5% + 50 IJs/larva (Sequential)	1.30 (6.64)	80.60 (63.88)	8.00 (16.45)	60.57 (40.10
V. nigundo + *H. indica* @ 1% + 50 IJs/larva (Simultaneous)	23.40 (28.99)	42.00 (39.81)	0.34 (3.32)	21.90 (27.90)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em±	3.15	5.41	2.88	3.02
CD (5%)	9.22	15.81	8.40	8.81
CD (1%)	12.49	21.41	11.38	11.94
CV	15.78	29.00	5.17	5.67

Figures in the parenthesis are 'arc sin' values

Table 5. Cumulative mortality of *H. armigera* due to combination of *H. indica* and NSE

Treatment details	Third instar		Fourth instar	
	24	48	24	48
H. indica (@ 150 for 3rd and 200 IJs for 4th instar larva)	62.00 (51.98)	100 (90)	33.70 (35.49)	93.45 (73.14)
Neem Seed Extract (NSE) 5%	0.34 (3.32)	9.70 (18.19)	1.30 (6.64)	1.30 (6.64)
NSE + *H. indica* @ 5% + 50 IJs/larva (Sequential)	12.90 (21.06)	85.40 (67.51)	9.60 (18.04)	30.40 (33.49)
NSE + *H. indica* @ 2.5% + 50 IJs/larva (Sequential)	16.60 (24.09)	87.20 (69.09)	9.60 (18.04)	35.80 (36.78)
NSE + *H. indica* @ 1% + 50 IJs/larva (Sequential)	11.10 (19.48)	99.67 (86.68)	11.10 (19.48)	35.80 (36.78)
NSE + *H. indica* @ 0.5% + 50 IJs/larva (Simultaneous)	28.80 (32.45)	86.70 (68.65)	26.10 (30.73)	50.30 (45.16)
NSE + *H. indica* @ 0.25% + 50 IJs/larva (Simultaneous)	24.30 (29.58)	98.00 (81.77)	2.05 (8.23)	58.30 (49.78)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S. Em ±	4.39	3.37	4.75	5.04
CD (5%)	12.9	9.92	13.97	14.82
CD (1%)	17.6	13.56	19.04	20.21
CV	8.62	11.21	5.66	28.44

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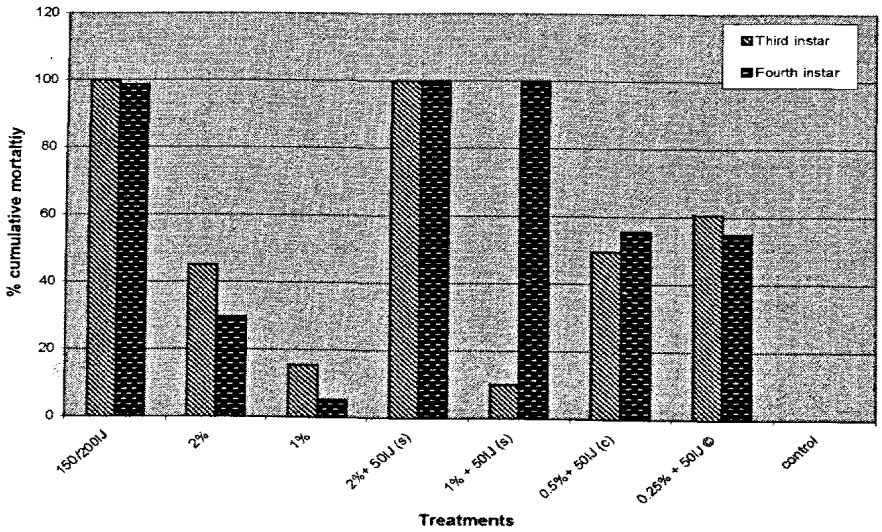

Fig. 11. Cumulative mortality of *H. armigera* due to combination of *H. Indica* and neem oil

Table 6. Effect of combination treatments on larval population, pod damage and seed yield of chickpea (2003-04)

Treatment	Dosage	Per cent larval reduction (average of two sprays)			Per cent pod damage		Seed yield (kg/ plot (12 m^2))
		2 DAS	4 DAS	7 DAS			
H. indica alone	3.0 lakhs/l.	2.58^{CD}	27.93^{E}	22.83^{IJ}	15.40	$(23.17)^{BC}$	1.48^{CD}
H. indica + *B. thuringiensis*	1.5 lakhs/l + 1.5 ml/l	2.45^{CD}	33.70^{D}	23.97^{GH}	13.20	$(21.38)^{FG}$	1.58^{BC}
H. indica + *B. thuringiensis*	1.5 lakhs/l + 0.75 ml/l	3.62^{A}	36.92^{C}	37.80^{C}	11.90	$(20.22)^{JK}$	1.73^{AB}
H. indica + *Helicoverpa* NPV	1.5 lakhs/l + $3x10^{9}$PIBs/l	1.22^{HI}	17.21^{L}	33.19^{D}	13.60	$(21.65)^{EF}$	1.54^{BC}
H. indica + *Helicoverpa* NPV	2.0 lakhs/l + $3x10^{9}$PIBs/l	2.54^{CD}	17.92^{KL}	26.46^{F}	13.10	$(21.26)^{GH}$	1.67^{AB}
H. indica + *N. rileyi*	1.5 lakhs/l + 0.75g/l	1.30^{HI}	12.33^{M}	9.80^{N}	17.60	$(24.78)^{A}$	1.71^{AB}
H. indica + *N. rileyi*	2.0 lakhs/l + 0.75g/l	2.32^{CD}	24.96^{FG}	20.73^{L}	15.80	$(23.45)^{BC}$	1.58^{BC}
H. indica + *M. anisopliae*	1.5 lakhs/l + 0.75g/l	1.88^{EF}	26.08^{F}	25.33^{FG}	12.10	$(20.39)^{IJ}$	1.54^{BC}
H. indica + *M. anisopliae*	2.0 lakhs/l + 0.75g/l	2.99^{AB}	22.63^{H}	14.61^{M}	14.90	$(22.71)^{CD}$	1.75^{AB}
H. indica + *B. bassiana*	1.5 lakhs/l + 0.75g/l	2.01^{EF}	17.61^{KL}	21.69^{JK}	15.20	$(22.91)^{CD}$	1.59^{BC}
H. indica + *B. bassiana*	2.0 lakhs/l + 0.75g/l	3.40^{AB}	19.82^{IJ}	23.26^{HI}	15.00	$(22.75)^{CD}$	1.57^{BC}
H. indica + *P. pinnata*	1.0 lakh/l + 2.5% (sequential)	2.83^{BC}	37.15^{B}	44.99^{B}	10.90	$(19.30)^{L}$	1.96^{A}
H. indica + *P. pinnata*	1.0 lakh/l + 1.0%	2.23^{DE}	22.94^{H}	22.94^{IJ}	14.70	$(22.57)^{DE}$	1.52^{BC}
H. indica + *V. nigundo*	1.0 lakh/l + 10% (sequential)	2.06^{EF}	20.85^{I}	26.49^{F}	14.60	$(22.46)^{DE}$	1.59^{BC}
H. indica + *V. nigundo*	1.0 lakh/l + 1.0%	1.35^{GH}	25.92^{F}	30.22^{E}	13.20	$(21.35)^{FG}$	1.69^{AB}
H. indica + *P. juliflora*	1.0 lakh/l + 10% (sequential)	2.56^{CD}	35.77^{C}	39.09^{C}	11.50	$(19.81)^{KL}$	1.83^{B}
H. indica + *P. juliflora*	1.0 lakh/l + 1.0%	1.03^{IJ}	28.30^{E}	29.64^{E}	15.90	$(23.52)^{BC}$	1.65^{AB}
H. indica + NSKE	1.0 lakh/l + 5.0% (sequential)	2.21^{DE}	24.17^{G}	20.87^{L}	16.70	$(24.16)^{AB}$	1.70^{AB}
H. indica + NSKE	1.0 lakh/l + 2.5%	1.68^{FG}	24.83^{FG}	24.00^{FG}	13.60	$(21.65)^{EF}$	1.69^{AB}

Table 6. Effect of combination treatments on larval population, pod damage and seed yield of chickpea (2003-04) ..... Contd.

Treatment	Dosage	Per cent larval reduction (average of two sprays)			Per cent pod damage		Seed yield (kg/ plot (12 m^2))
		2 DAS	4 DAS	7 DAS			
H. indica + NSKE	1.0 lakh/l + 2.5%	1.68^{FG}	24.83^{FG}	24.00^{FG}	13.60	$(21.65)^{EF}$	1.69^{AB}
H. indica + Neem oil	1.0 lakh/l + 2.5% (sequential)	2.37^{CD}	18.40^{KL}	21.17^{KL}	16.30	$(23.81)^{AB}$	1.63^{AB}
H. indica + Neem oil	1.0 lakh/l + 1%	2.53^{CD}	18.86^{JK}	30.58^{E}	12.40	$(20.62)^{HI}$	1.62^{AB}
Chlorpyriphos/ Quinalphos	0.04/0.05 %	3.31^{AB}	41.08^{A}	47.63^{A}	14.50	$(22.36)^{DE}$	1.82^{B}
Untreated check	——	0.77^{J}	3.71^{N}	7.39^{O}	17.80	$(21.11)^{A}$	1.32^{D}
C.V.		15.98	12.91	13.59		2.80	4.84
C.D. at 5%		0.59	1.16	1.55		1.01	0.13
S. Em±		0.20	0.41	0.55		0.356	0.03

Figures in the parenthesis are angular transformed values; DAS – Days after spray

Table 7. Effect of combination treatments on larval population, pod damage and seed yield of chickpea (2004-05)

Treatment	Dosage	Per cent larval reduction (average of two sprays)			Per cent pod damage	Seed yield (q/ha)
		2 DAS	4 DAS	7 DAS		
H. indica alone	3.0 lakh/l.	5.31 (13.31)	17.40 (24.65)	9.61 (18.05)	16.22 (23.73)	18.10
H. indica + *B. thuringiensis*	1.5 lakh/l + 0.75ml/l	6.40 (14.65)	20.63 (26.99)	12.51 (20.70)	14.81 (22.63)	16.40
H. indica + *P. pinnata*	1.0 lakh/l + 2.5% (sequential)	6.97 (15.34)	21.39 (27.56)	12.51 (20.70)	28.29 (32.14)	16.91
H. indica + *P. juliflora*	1.0 lakh/l + 10% (sequential)	9.10 (17.56)	23.47 (29.00)	12.31 (20.53)	11.27 (19.64)	19.24
Chlorpyriphos/ Quinalphos	0.04/0.05 %	25.22 (30.13)	26.49 (30.98)	14.14 (22.06)	17.19 (24.50)	19.43
Untreated check	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	27.25 (31.50)	13.85
S. Em ±		2.04	1.71	1.96	0.41	0.21
C.D. at 5%		6.15	5.17	5.91	1.24	0.64
C.V.		26.9	14.78	23.08	3.19	12.48

Figures in the parenthesis are angular transformed values; DAS – Days after spray

Table 8. Economics of integration of *H. indica* with other biopesticides in chickpea ecosystem.

Sl. No.	Treatment	Cost of treatment (including application charges) (Rs./ha)	Seed yield (Q/ha)	Gross income (Rs./ha)	Additional income over untreated check (Rs./ha)	Incremental cost benefit ratio (ICBR)
1	*H. indica* alone	1320	18.10	28960	6800	1:5.1
2	*H. indica* + *B. thuringiensis*	1220	16.40	26240	4080	1:3.34
3	*H. indica* + *P. pinnata*	1050	16.91	27056	4896	1:4.66
4	*H. indica* + *P. juliflora*	1130	19.24	30784	8624	1:7.63
5	Chlorpyriphos/ Quinalphos	770	19.43	31088	8928	1:11.60
6	Untreated check	-	13.85	22160	-	

Market price value of chickpea seed = Rs. 1600/quintal

mortality in short period. This was evident in fourth instar larva, whereas, even though the combination has resulted in high mortality in third instar it was not due to synergism but by nematode alone. This might be due to the quick action of the nematode compared to HaNPV. However, no antagonistic effect was observed against third instar (Fig. 5).

With Bt

The increased mortality in third (100%) and fourth (90%) instar in higher combination treatment (150 IJs + 0.264 IU/mg) over nematode and Bt alone treatment indicated the presence of synergism between the two bio-agents (Fig. 6). The results were in accordance with Koppenhoffer and Kaya, 1997 and Koppenhoffer *et al.*, 1999 though the target insects were different.

Combination study with botanicals

Highest mortality (100%) of third instar was noticed in sequential application of *P. juliflora* + *H. indica* (10% + 50 IJs larva^{-1}) and was on par with *H. indica* alone, whereas, same combination recorded 63.7% mortality as against 82.4% in nematode alone after 48h (Table 2). Thus, the above combination was found to be more lethal as it brought highest morality with sub-lethal nematode dose. Similar trend was noticed in *P. pinnata* wherein, sequential application of karanja at 10, 5 and 2.5% with nematode (50 IJs larva^{-1}) resulted in highest mortality (100, 100 and 99.37%, respectively) and were on par with nematode alone. Fourth instar recorded the highest mortality (90.3%) with nematode alone followed by *P. pinnata* +*H. indica* (at 10 and 5% with 50 IJs larva^{-1}) (Table 3). Sequential application of *V. nigundo* and *H. indica* at all the three concentrations caused higher mortality and were on par with nematode alone against third instar. However, lower mortality (38.8 and 38.9%) was recorded against fourth instar by sequential application (Table 4).

Sequential application of NSKE and *H. indica*

(1% + 50 IJs larva-1) and simultaneous application (0.25% + 50 IJs larva^{-1}) registered the highest mortality (99.67 and 98%) of third instar (Table 5). Similarly, sequential application of neem oil 2% + 50 IJs larva^{-1} was found most effective in comparison to other treatments (Fig. 7).

The above result in all the botanicals indicated the significant increase in the mortality in sequential application over simultaneous application. This might be due to the fact that application of botanicals predisposed larvae for nematode infection resulting in superior result.

Field evaluation

In first field trial, sequential application of *P. pinnata* + *H. indica, P. juliflora* + *H. indica* and simultaneous application of *H. indica* + Bt resulted the highest larval reduction (44.99, 39.09 and 37.8%, respectively), lower per cent pod damage (19.30, 19.81 and 20.22) with highest yield (1.96, 1.83 and 1.73 kg/plot) (Table 2). These treatments were on par with insecticidal spray. Hence, the above treatments were once again tested for second year along with sole treatment of *H. indica*.

Similar trend was observed in the second year also wherein, sequential application of *P. juliflora*+ *H. indica* recorded highest larval reduction (12.3%), minimum pod damage (11.3%) and maximum yield (19.2 q/ha) and was on par with insecticidal treatment. Sequential application of *P. pinnata* +*H. indica* was the next best treatment (Table 3). In terms of economics also, *P. juliflora* + *H. indica* and insecticidal spray recorded higher net returns of Rs. 8624/ha and Rs. 8928/ha, respectively (table 4). Thus, it was evident that sequential application of *P.juliflora* @ 10% + *H. indica* @ 1 lakh IJs ml^{-1} was effective in reducing the larval load, pod damage and increase the seed yield and additional return if not superior than chemical control. Considering the increase in the environmental pollution due to application of large quantity of insecticides, use of such eco-friendly bio-agents and botanicals can be encouraged as alternative methods for the management of *H. armigera* in chickpea ecosystem.

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