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Relative toxicity of neem to natural enemies associated with the chickpea ecosystem: a case study

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Abstract. Neem products are often perceived as harmless to natural enemies, pollinators and other non-target organisms. For this reason, several integrated pest management (IPM) programmes have adopted neem as one of the prime components. This study revealed toxic effects of neem on soil-inhabiting and aerial natural enemies in chickpea to an extent of 41 and 29% population reduction, respectively, compared with 63 and 51% when using a conventional insecticide (endosulfan). Neem also affected the parasitization of *Helicoverpa armigera* (Hübner) larvae by *Campoletis chlorideae* Uchida up to 20%. The natural enemy population started building up from the vegetative phase and reached their peak during the reproductive phase, and there was a gradual decline from pod formation to pre-harvest phases of the crop. Adapting the currently used IPM system in chickpea using neem during the vegetative phase, followed by an application of *Helicoverpa* nuclear polyhedrosis virus (HNPV) at flowering and need-based application(s) of chitin inhibitors like novaluron or flufenoxuron instead of endosulfan during pod formation would strongly augment natural enemy populations. This paper discusses the relative toxicity of neem and other IPM components on soil-inhabiting and aerial natural enemies in the chickpea ecosystem.

Key words: natural enemies, neem, endosulfan, novaluron, flufenoxuron, chitin inhibitors, chickpea

Introduction

In recent years, several botanical insecticides have shown encouraging results in the management of insect pests. Among them neem *Azadirachta indica* Arch. (Meliaceae), appears to be promising for use in integrated pest management (IPM) programmes and provides broad-spectrum control on more than 200 species of insect pests (Ascher, 1993). In India alone, neem seed kernel extracts have been evaluated against 106 species of insect pests. Several workers have reported repellent, antifeedant, growth inhibition and oviposition suppression effects of neem against a large number of insects.

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Neem and neem products have shown spectacular results in several pest management programmes across the globe resulting in substantial reductions in toxic insecticidal usage (Singh and Kataria, 1991).

Chickpea *Cicer arietinum* L. (Fabaceae), is one of the most important pulse crops, which is grown on more than 8 million ha worldwide of which *c.* 70% are in India (FAO, 2001). Besides being a very rich source of protein, it also maintains soil fertility through biological nitrogen fixation. Chickpea is affected by various biotic and abiotic stresses. Among various biotic stresses, the pod borer *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), the cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) and the aphid *Aphis craccivora* Koch (Homoptera: Aphididae) are recognized as the most important in field and the bruchid *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Bruchidae) in storage (Ranga Rao and Shanower, 1999). Insect pests cause an average of 25–30% crop losses in chickpea annually which resulted in *c.* US$ 330 million loss globally (Ranga Rao and Shanower, 1999). Under epidemic situations, complete crop loss is not uncommon in spite of intensive plant protection strategies. Since a high level of host–plant resistance to *H. armigera* in chickpea is not available, the development of IPM strategies is of paramount importance for the management of this insect pest (Ranga Rao *et al.*, 2002).

Neem seed kernel extracts have a number of biological properties affecting insects and possess low mammalian toxicity (Schmutterer, 1990). Several authors have reported no deleterious effects of neem products on generalist predators such as spiders, syrphids and coccinellids (Wu, 1986; Fernandez *et al.*, 1992; Parmar, 1993; Markandeya and Diwakar, 1999). Lal (1990) and Jayaraj (1992) suggested an IPM approach in pulses by integrating different components such as resistant cultivars, cultural, physical, biological and chemical control options to minimize the often negative impact of insecticides on the natural enemies and the environment. However, little is known on possible negative effects of neem on beneficial organisms. Hence, the primary objective of the present study was to evaluate the effect of different IPM options such as neem, the *Helicoverpa* nuclear polyhedrosis virus (HNPV) and endosulfan as a conventional pesticide on soil-inhabiting and aerial natural enemies in the chickpea ecosystem.

Materials and methods

The basic concept of IPM is the containment of a pest below economically damaging levels using a combination of all feasible control measures. An IPM approach will also reduce the negative impact of insecticides on beneficial insects like natural enemies of crop pests and the environment. The IPM components developed and implemented in chickpea during this study were as follows:

- seed treatment with fungicides (bavistin (1 g/kg), thiram, diathane M 45 (2–3 g/kg)) before sowing;
- the use of the wilt *Fusarium oxysporum* f. sp. *ciceri* tolerant chickpea variety ICCC 37;
- installation of bird perches after crop establishment (15–20/ha);
- application of 5% neem seed kernel extract at flower initiation (neem seed kernel powder (12.5 kg/ha));
- application of HNPV at 250 larval equivalent (LE)/ha at peak oviposition of *H. armigera* and repetition of the same after 15 days, if the oviposition persisted and
- need-based application of an insecticide if the above-recommended measures did not contain pest population below the economic damage levels (two second/third instar larvae/plant).

To study the relative efficacy of various IPM components, the effects of neem product (AZA 1500 ppm), 1750 ml/ha, HNPV at 250 LE/ha (1 LE $= 6 \times 10^9$ POB/ml) and endosulfan 0.07% (35 EC, 2 ml/l) on soil-inhabiting and aerial natural enemies and larval parasitoids were investigated at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India during the 1998–2000 and 2003/2004 post-rainy seasons (October–February) on a chickpea crop. The crop (cv. ICCC 37 'Kranthi') was sown with 60×15 cm row and plant spacing in a plot measuring 24×12 m for each treatment. The experiment was conducted with five treatments, i.e. neem, HNPV, endosulfan, IPM (combination of the above three) and a control (water spray), with four replications using a randomized block design (RBD). The plots with individual interventions (neem, HNPV and endosulfan) were treated at 20, 35, 50, 65 and 80 days after sowing (DAS) corresponding to the vegetative, flowering, pod formation, pod maturation and pre-harvest stages of the crop, respectively. The IPM plots were treated at 20 and 65 DAS with neem, 35 and 80 DAS with HNPV and 50 DAS with endosulfan. The seeds were treated in all treatments but bird perches were only used in the IPM treatment. In the 2003/2004 experiment, seven treatments, i.e. neem fruit extract (obtained from neem fruit powder, 12.5 kg/ha), neem oil (1 ml of 1500 ppm/l), HNPV (250 LE/ha), endosulfan 35% EC 0.07% (2 ml/l), novaluron 10% EC 0.01% (1 ml/l), flufenoxuron 10% DC 0.01% (1 ml/l) and a control were used with four replications using an RBD to additionally study the relative toxicity of the two chitin inhibitors (novaluron and flufenoxuron) against natural enemies and to compare them with the other IPM components.

Sampling of soil-inhabiting natural enemies

To monitor soil-inhabiting natural enemies, three pitfall traps/plot (a plastic container measuring 13 cm in height and 10 cm in diameter) were buried in the soil with their opening in line with the soil surface. These traps were placed at 22 DAS and filled with water to half capacity. One millilitre of formaldehyde was added to the water to kill the trapped insects and a small quantity (5 ml) of liquid soap was added to prevent evaporation of the fluid. Observations on soil-inhabiting natural enemies, such as ants (Formicidae), ground beetles (Carabidae), crickets (Gryllidae), earwigs (Forficulidae) and spiders (Arachnidae), were taken at 10-day intervals until the crop attained maturity.

Sampling of aerial (canopy-dwelling) natural enemies

To monitor the activity of various aerial natural enemies in the crop canopy of the different treatments, a D-Vac® (Model AV520, D-VAC Co., Riverside, USA) suction trap was used at 22, 54, 76 and 99 DAS during the 1998/1999 season. The D-Vac® was operated in the net plot (243 m^2) excluding the 0.5 m border on all sides of the plots for a 1-min duration. The insects caught in the crop canopy were brought to the laboratory for sorting and identification of the different groups of parasitoids and predators (Ichneumonidae, Braconidae, Syrphidae, Trichogrammatidae, Tachinidae, Formicidae and spiders (Arachnidae)).

Sampling for larval parasitization

To assess the percentage of larval parasitization of *H. armigera*, 100 larvae were collected at random from each treatment at the vegetative (25–30 DAS) and reproductive phases (55–75 DAS) of the crop during the 1998/1999 and 1999/2000 seasons and kept individually in glass tubes. The larvae were fed with soaked chickpea seeds and the feed was changed on alternate days. Larvae were observed daily until parasitoid/host adult emergence. The total number of parasitized larvae was recorded and the percentage of parasitization was calculated. During the 2003/2004 trial, the larval parasitization was assessed at 26 and 56 DAS. The emerged parasitoids were identified with the help of the reference collection at ICRISAT, Patancheru.

Statistical analysis

To differentiate the effects of various treatments on natural enemies, the data were subjected to ANOVA. The test of significance was assessed using the critical difference obtained at 5% level.

Results

Effect of IPM interventions on soil-inhabiting natural enemies

During 1998–2000 post-rainy seasons, the population of soil-inhabiting natural enemies started building up at the vegetative phase (302/trap) and attained their peak during the flowering phase (455/trap), followed by a gradual decline during pod formation and pre-harvest phases. Plots treated with endosulfan had significantly lower populations (107.7/trap) than the control (302.3/trap), which translated into a 64% reduction. Plots treated with neem had 199.5/trap with 34% reduction against control and plots treated with HNPV showed minimum disturbance to natural enemies with 267.1/trap, which was on a par with the control. IPM plots that had received neem as first spray revealed similar effect as individual neem treatment. In the subsequent crop development stages, flowering, pod formation and pre-harvest phases, there was a similar trend in the reduction of natural enemies in the endosulfan treatment followed by neem and HNPV. However, the overall effect of various treatments clearly brought out the relative toxicity of neem among various IPM interventions on soil-inhabiting natural enemies in the chickpea ecosystem (Table 1).

Effect of IPM interventions on aerial natural enemies in the chickpea canopy

Using a D-Vac® during 1998/1999, the impact of various IPM options on aerial natural enemies was assessed (Table 2). At 22 DAS, fewer natural enemies in plots treated with endosulfan compared with plots treated with neem, HNPV, IPM and the control were recorded. Similarly at 54, 76 and 99 DAS (corresponding to flowering, pod formation and pre-harvest phases, respectively), significant reduction of natural enemies in the endosulfan-treated plots was observed, while the populations in neem- and HNPV-treated plots were on a par with the control. The overall effects of endosulfan, neem and HNPV resulted in reduction in population of aerial natural enemies, compared with the control (Table 2).

Effect of IPM interventions on larval parasitoids of H. armigera

During the study period, larval parasitization of *H. armigera* was mainly due to *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae). Apart from

Table 1. Effect of various IPM treatments on soil-inhabiting natural enemies present in chickpea during 1998–2000

Treatment	Mean no. of soil-inhabiting natural enemies/trap				Mean reduction over control (%)
	Vegetative	Flowering	Pod formation	Pre-harvest	
Neem 0.006% (AZA 1500 ppm)	199.5	234.6	101.4	64.6	41.4
HNPV (250 LE/ha)	267.1	404.6	124.9	57.9	21.7
Endosulfan 35% EC (0.07%)	107.7	162.0	60.3	36.6	63.1
IPM	230.8	262.9	93.2	79.5	34.9
Control	302.3	455.4	178.3	87.7	–
Mean	221.5	303.9	111.6	65.3	–
SE ±	44.80	29.27	26.45	15.82	–
CD ($P = 0.05$)	90.50	60.54	54.50	32.28	–

C. chlorideae, the other larval–pupal parasitoid, *Carcelia illota* Curron (Diptera: Tachinidae) was recorded only in control plots; however, its incidence never exceeded 2%. During the vegetative phase in 1998/1999 (25 DAS), at 4 days after the first spray, plots treated with endosulfan showed reduction in parasitization by *C. chlorideae* (Table 3). In the other treatments (neem, HNPV and IPM), there was no significant reduction in percentage of parasitization compared with the control. At the reproductive phase (56 DAS), 6 days after the third spray, no significant difference between treatments in terms of larval parasitization was found. Yet, at this time, the level of parasitization was low (4.5–7.3%).

At the vegetative phase in 1999/2000 (36 DAS), at 4 days after the first spray, we observed a significant reduction in larval parasitization by *C. chlorideae* in plots treated with endosulfan, followed by neem and IPM. In the latter, neem followed by HNPV were applied. The level of parasitism in all the treatments was on a par with each other and differed significantly from the control. The overall effects of endosulfan, neem, HNPV and the IPM treatments resulted in reduction in larval parasitization by *C. chlorideae*, compared with the control (Table 3).

In subsequent studies during the 2003/2004 post-rainy season, at the vegetative phase (26 DAS), higher levels of larval parasitism were observed in plots treated with HNPV, which was on a par with the untreated control, followed by neem oil and neem fruit extracts. In the endosulfan plots compared with all other treatments, the significant lowest parasitization by *C. chlorideae* was recorded. A similar trend was noticed during the reproductive phase with significant reduction in natural enemy population in endosulfan-treated plots followed by the chitin inhibitors, neem and HNPV treatments. The overall effect of these treatments compared with the control was highest in endosulfan-treated plots and revealed the lowest reduction of parasitism in the HNPV treatment (Table 4).

Discussion

One of the main reasons for failure of pest control using synthetic pesticides is the destruction of

Table 2. Effect of various IPM treatments on aerial natural enemies present at different crop growth phases in chickpea canopy during 1998/1999

Treatment	No. of aerial natural enemies/sample				Mean reduction over control (%)
	22 DAS	54 DAS	76 DAS	99 DAS	
Neem 0.006% (AZA 1500 ppm)	50.0	20.8	25.3	17.5	29.49
HNPV (250 LE/ha)	69.7	21.5	28.8	17.5	14.69
Endosulfan 35% EC (0.07%)	39.5	9.8	18.0	9.5	52.36
IPM	51.0	11.4	27.3	18.0	33.18
Control	87.1	23.8	32.3	18.0	–
Mean	59.5	17.5	26.3	16.1	–
SE ±	9.36	2.13	1.54	1.16	–
CD ($P = 0.05$)	20.52	4.53	3.26	2.48	–

Sample size: D-Vac® was operated for 1 min in the net plot.
DAS, days after sowing.

Table 3. Effect of various IPM treatments on *Helicoverpa* larval parasitization by *Campoletis chlorideae* in chickpea during 1998–2000

Treatments	Parasitization by *Campoletis chlorideae* (%)				Mean reduction over control (%)
	1998/1999		1999/2000		
	Vegetative (4 DAT)	Reproductive (6 DAT)	Vegetative (4 DAT)	Reproductive (6 DAT)	
Neem 0.006% (AZA 1500 ppm)	9.0	5.3	3.3	8.8	20.2
HNPV (250 LE/ha)	9.3	5.5	4.0	9.0	16.0
Endosulfan 35% EC (0.07%)	8.0	4.5	3.0	8.5	27.5
IPM	9.0	5.3	3.5	8.5	20.5
Control	11.0	7.3	5.3	9.5	–
Mean	9.3	5.6	3.8	8.9	–
SE ±	1.04	1.28	1.04	1.38	–
CD ($P = 0.05$)	2.21	NS	2.21	NS	–

DAT, days after treatment.

natural enemies present in the agroecosystem, which often leads to pest resurgence. Hence, it is necessary to develop and incorporate appropriate plant protection options that are less harmful to natural enemies. Among the various IPM options tested during this study in chickpea, endosulfan proved to be the most detrimental on soil-inhabiting and aerial natural enemies, followed by neem and HNPV. Several authors had reported no deleterious effects of neem products on generalist predators such as spiders, syrphids and coccinellids (Wu, 1986; Fernandez *et al.*, 1992; Parmar, 1993; Markandeya and Diwakar, 1999). Though Banken and Stark (1997, 1998) suggested neem as a safe option in IPM they reported several abnormalities in the development of coccinellids when exposed to aphids fed on neem-treated foliage. Similar observations on the detrimental effects of neem and conventional insecticides on natural enemies were reported by Pfrimmer (1964), Luff (1987), Krishnamurthy (1995) and Srinivasan and Sundara Babu (2000). The present study emphasized the deleterious effects of neem on natural enemies in the chickpea crop. This study also revealed fluctuating population levels of natural enemies at different phases of the crop development, that may apply to other crops as well, which is mainly due to the prevailing crop phenology and environmental conditions.

The investigations on larval parasitization of *H. armigera* revealed *C. chlorideae* as the major larval and *C. illota* as the only larval–pupal parasitoid, though the latter occurred at very low incidence. The significant reduction in natural enemy activity in IPM treatments was mainly due to the application of endosulfan during the pod formation stage of the crop.

In the present study, HNPV and neem had fewer deleterious effects on the larval parasitoid

Table 4. Effect of various IPM treatments on larval parasitization by *Campoletis chlorideae* in chickpea during 2003/2004

Treatments	Parasitization (%)		Mean reduction over control (%)
	Vegetative (3 DAT)	Reproductive (5 DAT)	
Neem fruit extract at 15 kg/ha	4.7	5.0	17.09
Neem oil at 1 ml of 1500 ppm/l	5.0	5.3	11.96
HNPV at 250 LE/ha	5.7	5.7	2.56
Endosulfan 35% EC at 0.07%	2.0	2.7	59.82
Novaluron 10% EC at 0.01%	3.0	3.7	42.73
Flufenoxuron 10% DC at 0.01%	3.3	4.0	37.60
Control	5.7	6.0	–
Mean	4.2	4.6	–
SE ±	0.38	0.42	–
CD ($P = 0.05$)	0.82	0.91	–

DAT, days after treatment.

C. chlorideae than on soil-inhabiting and more generalist aerial natural enemies. Yet, the overall level of parasitization of pod borer larvae was low and seldom exceeded 11%. This low level of *C. chlorideae* incidence could be due to the previous continuous use of synthetic pesticides for management of pod borer in chickpea in the study area, which corroborates earlier findings by Odak (1982) on the reduction of *C. chlorideae* in several chickpea cultivating districts in Madhya Pradesh, India. The present study also indicated less harmful effects of chitin inhibitors on larval parasitization compared with endosulfan. Hence, chitin inhibitors can be considered as an effective alternative to conventional chemicals in an IPM context. Several other studies also found chitin inhibitors to be a highly useful component in different IPM programmes (Olsazk *et al.*, 1994; Lavchieva Nacheva and Shishiniova, 2000; Ishaaya *et al.*, 2002).

Conclusions

The present study clearly revealed the population dynamics of soil-inhabiting natural enemies and their potential in suppressing pod borers.

The investigations showed natural enemy peak activity occurring during the vegetative and flowering phases with a gradual decline in the subsequent phases of chickpea crop development. Thus, we recommend a neem treatment during the vegetative phase, followed by the application of HNPV at flowering and a need-based application of a selective chemical pesticide like the tested chitin inhibitors during the pod formation phase as this will lead to a considerable augmentation of the natural enemy fauna in the chickpea ecosystem.

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