RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

INSECTICIDAL EFFICACY OF *Azadirachta indica*, *NUCLEOPOLYHEDROVIRUS* AND CHLORANTRANILIPROLE SINGLY OR COMBINED AGAINST FIELD POPULATIONS OF *Helicoverpa armigera* HÜBNER (LEPIDOPTERA: NOCTUIDAE)

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The development of resistance in cosmopolitan insect *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) forced the researchers for alternative control measures. In the present study, insecticidal efficacy of formulations of *Azadirachta indica*, a *Nucleopolyhedrovirus* (NPV), and new anthranilic diamide insecticide (chlorantraniliprole) formulations was determined against 2nd, through 5th larval instars of *H. armigera* collected from diverse geographical locations in the Punjab province, Pakistan. *Azadirachta indica* was applied at 5 μL L^{-1}; NPV at 2.1×10^5 polyhedral occlusion bodies (POB) mL^{-1} and chlorantraniliprole at 0.01 μL L^{-1}, either alone or in combinations with each other. The bioassays were conducted at 27 ± 1 °C and 65 ± 5% relative humidity. The mortality varied greatly among treatments, larval instars, and locations. The combinations of NPV with *A. indica* and chlorantraniliprole caused higher mortality, pupation and produced an additive effect compared to their application singly in all the tested populations. The population from Rawalpindi was always susceptible while the Gujranwala was the resistant. The results herein suggest that the effectiveness of NPV and *A. indica* can be improved by the presence of chlorantraniliprole against the larvae of *H. armigera*.

Key words: *Azadirachta indica*, NPV, chlorantraniliprole, additive, *Helicoverpa armigera*, populations.

Helicoverpa armigera Hübner (Lepidoptera: Noctuidae), a major polyphagous insect pest of many crops (Marzban *et al*., 2009), has a wide geographical range, mobility, migratory potential, facultative diapause, high fecundity and a great tendency to develop resistance against insecticides, key factors that determine its importance as insect pest (Fitt, 1989; Zalucki, 1991; Wakil *et al*., 2009a; 2009b; 2010). Excessive use of synthetic insecticides worldwide warrants environmental and human health concerns, and urges researchers to develop safer alternatives for eco-friendly pest management (Cherry *et al*., 1997). Insect resistance to synthetic insecticides (Ahmad *et al*., 2003; 2007) and development of awareness of their detrimental effects has prompted the introduction of integrated pest management programs (Nathan and Kalaivani, 2006). The promising alternatives of insecticides would be *Nucleopolyhedrovirus* (NPV), plant based products and new chemistry molecules which can be successfully included in the integrated pest management (IPM) program to lessen the resistance issues in the lepidopterous insects.

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Received: 26 June 2011.
Accepted: 27 December 2011.

Helicoverpa armigera, single nucleocapsid NPV (*Hear*NPV, also called *Ha*SNPV) was first isolated in 1975 in Hubei, China and has been used for over 25 yr against *H. armigera* (Zhang, 1994). The *Baculoviridae* including NPVs and granuloviruses (GV) with more than 600 viruses (Nathan and Kalaivani, 2006) have great potential for the control of *H. armigera* (Jayaraj, 1985; David, 2008). Occluded viruses (OV) initiate primary infections in midgut epithelial cells of susceptible hosts and Budded viruses (BV) spread from cell to cell in the larvae (Keddie *et al*., 1989; Washburn *et al*., 1995). The infected larvae become pale in color, which ultimately swell due to the deposition of OV's, climb upper parts of the host plants and ultimately die (Inceoglu *et al*., 2001; Nakai *et al*., 2002).

Over the last three decades, *Azadirachta indica* A. Juss (neem) has received attention all over the world (Stark and Walter, 1995; Nathan and Kalaivani, 2006). The major constituent of neem is azadirachtin (AZA), which affects the feeding, growth, molting, and reproduction of insects (Kumar *et al*., 2008), and may be combined with other bio-based insecticides (Koppenhöfer and Kaya, 2000). The effects of neem based pesticides have been studied under laboratory and field conditions (Gahukar, 1995; 1996), however, in Pakistan the efficacy of the neem has not been properly explored against *H. armigera* except few studies like Wakil *et al*. (2008).

The chlorantraniliprole (Rynaxypyr) is the first anthranilic diamide, a new class of insecticide (Lahm *et al*., 2007) that prevents the build-up of pest populations

if applied soon after pest outbreaks. It has very low mammalian toxicity, acts at relatively low application rates, promotes eco-friendly insect pests management and achieves excellent control of pest population resistant to other insecticides (Cordova *et al*., 2007). It activates the insect ryanodine receptors (RyRs), which causes uncontrolled release and depletion of internal Ca and prevents further muscle contraction. The death of insects occurs by the rapid cessation of feeding, lethargy, regurgitation and muscle paralysis (Cordova *et al*., 2007; Temple *et al*., 2009).

This research was aimed to evaluate mortality and pupation rate of *A. indica* and NPV in combination with chlorantraniliprole against 2nd, through 5th larval instars of *H. armigera* from several locations in the Punjab province, Pakistan.

MATERIALS AND METHODS

Insects rearing

Field populations of *H. armigera* were collected in six major tomato (*Solanum lycopersicum* L.) growing locations (Faisalabad, Gujranwala, Lahore, Rawalpindi, Sargodha, Sheikhupura) in Punjab, Pakistan. All the populations were reared in Integrated Pest Management (IPM) laboratory in the Department of Agricultural Entomology, University of Agriculture Faisalabad (Pakistan) at 25 ± 2 °C, 75% RH and 16:8 h photoperiod. Batches of 500 field collected larvae of each population were reared in plastic trays with 32 wells (6 cm diameter × 5.5 cm depth) with one larva each to avoid cannibalism, and provided with 5 mL of artificial diet (chickpea [*Cicer arietinum* L.] flour: 125 g; red kidney beans [*Phaseolus vulgaris* L.]: 125 g; canned tomotao paste: 25 g; agar: 17 g; ascorbic acid: 3 g; sorbic acid: 1 g; yeast: 40 g; methyl-4-hydroxybenzoate: 2 g; streptomycin: 1 g; vitamin mixture: 10 mL; distilled water: 1300 mL) (Wakil *et al*., 2011), which was renewed regularly till pupation. Twenty five pairs of unsexed newly emerged adults of *H. armigera* were placed in plastic jars (15 cm diameter × 19 cm depth) lined with coarse tissue paper to facilitate egg laying. Honey solution (10%) was provided in 5 mL test tube plugged with cotton, placed vertically on the top of each jar. Populations were reared for more than 10 generations in the laboratory before the bioassays, where L2 through L5 larval instars were used.

Test formulations

Azadirachta indica. A commercial product of neem *A. indica* (AgriLife, Hyderabad, India) was used at the rate of 5 μL L^{-1}.

Chlorantraniliprole. A novel insecticide from class anthranilic diamide Coragen 20% SC powered by Rynaxypyr (DuPon Private Limited, Pakistan) was used which contains chlorantraniliprole (20% w/v) and other ingredients (80% w/v). The formulation was applied by dissolving in distilled water at a concentration of 0.01 μL L^{-1} mixed with the diet, thoroughly mixed in electric shaker for 30 s for even distribution of insecticide.

***Nuclear Polyhedrosis Virus* (NPV).** The commercial formulation of NPV was provided by AgriLife, Hyderabad, India. To recover the purified virus, the second instar larvae of *H. armigera* were infected with a suspension of NPV by spraying and the larvae were allowed to feed on artificial diet (Wakil *et al*., 2011) for 7 d. Then the midguts of the infected cadavers were homogenized in deionized water, filtered through muslin cloth and centrifuged at 16 000 rpm for 45 min (Shapiro *et al*., 2005; Green *et al*., 2006). The purified virus was given washes three times in distilled water, and held in 0.1 mM NaOH at 5 °C. Polyhedral occlusion bodies (POB) in 1 mL suspension was recorded 10 times using haemocytometer (Cory and Myers, 2004). The formulation was applied (2.1 × 10^5 POB mL^{-1}) on both sides of surface sterilized tomato leaf discs and air dried.

Larval treatment

Pre-starved (24 h) larval instars (L2-L5) from all populations of *H. armigera* were kept in plastic vials (base radius 2.8 cm × height 7 cm) individually containing tomato leaf discs treated with NPV and *A. indica*, alone and in combination while the discs sprayed with distilled water only served as control. After 24 h the larvae from alone treatments were removed and shifted into new vials (base radius 2.8 cm × height 7 cm) each containing 1 cm^3 pieces from chlorantraniliprole treated and untreated (control) diet for next 24 h and then were shifted to normal artificial diet. For alone treatments (NPV and *A. indica* and chlorantraniliprole) the larvae were held in the vials separately for 24 h and directly transferred to the artificial diet.

Bioassay

The bioassay was conducted at 25 ± 2 °C, 75% RH and 16:8 h photoperiod, each treatment was repeated thrice independently using 20 larvae per replicate for each population (n = 360 for all populations). The mortality counts were made after every other day upto 12 d for all populations and larval instars (L2-L5). After removing the dead individuals the remaining larvae were kept till pupation. The larvae were prodded with the blunt needle and those unable to move in coordinated manner were considered as dead (Ma *et al*., 2008). The larvae infected with NPV showed the symptom of oozing of body contents, transparency and stretching of the body, *A. indica* application resulted in blackening and shrinkage of the larval body, however, the combined treatments resulted in the larval blackening and oozing of the body contents (Kumar *et al*., 2008).

Statistical analysis

Data were analyzed statistically by using three-way factorial analysis (Minitab, 2003) both for the mortality and pupation rate for six localities, four larval duration and seven treatments. The means of corrected percent mortality and pupation rate was separated and compared using Tukey-Kramer (HSD) (Sokal and Rohlf, 1995) test at 5% significance level. The type of interaction between different concentrations for the *H. armigera* mortality was worked out by using the equation CTF = $(o_c\text{-}o_e)/o_e \times 100$, where CTF is cotoxicity factor, o_c is observed percentage mortality resulted from the combined application and o_e, the expected percentage mortality, is the sum of percentage produced by each of the treatment used in the combination (Mansour *et al*., 1966). On the basis of this factor, any intermediate value (i.e. between -20 and +20) was considered additive (Marzban *et al*., 2009).

RESULTS

All main effects and the interaction between larvae and treatments were significant, however, other associated interactions at $P = 0.05$ were not significant both for mortality and pupation rate (Table 1) of *H. armigera* larvae. The mortality of larvae and pupation was greatly influenced by the origin of the population and the stage of the larvae as with the advancement of growth, the larvae became more resistant to the treatments. Significant differences were noted in the mortality and pupation rate of second instar larvae of *H. armigera* when exposed to chlorantraniliprole, NPV, and *A. indica* alone and in combinations. The additive effect on the mortality and pupation of *H. armigera* was exhibited by the combined treatments of chlorantraniliprole, NPV, and *A. indica*. The highest mortality was recorded in the treatment where NPV and chlorantraniliprole was applied in combination, with lowest pupation of *H. armigera*, in all the populations. The population from Gujranwala showed resistance with 96.10% mortality and the pupation was 0.13% while the population from Rawalpindi exhibited susceptibility towards the combined treatment with 100% mortality with no pupation (Table 2). However, in case of larvae treated with chlorantraniliprole alone, significantly more mortality was recorded in all the populations tested with maximum 63.23% and 33.10% pupation in Rawalpindi populationbut this was less effective in Gujranwala population with 51.23% mortality and 45.13% pupation.

The mortality in 3rd instar larvae of *H. armigera* was higher when exposed to the combined treatments of chlorantraniliprole, NPV, and *A. indica* with less pupation respectively in all the populations. The larval mortality was significantly higher by the combination of NPV with chlorantraniliprole than chlorantraniliprole alone. Also the interaction between the combined treatments was additive depending upon the origin of the population. The population of Gujranwala showed resistance with 91.11% mortality and 3.89% pupation while the population of Rawalpindi exhibited susceptibility towards the combined treatment with 100% mortality with no pupation (Table 3). However, in case of larvae treated with chlorantraniliprole alone, significantly more mortality was recorded in all the populations tested with maximum mortality of 60.40% and 35.28% pupation in Rawalpindi population but this was less effective in Gujranwala population (47.13% mortality and 49.43% pupation).

The same trend was observed in case of mortality in 4th instar larvae of *H. armigera* which was higher when exposed to the combined treatments of chlorantraniliprole, NPV, and *A. indica* with less pupation respectively in all the populations. The larval mortality was significantly increased by the combination of NPV with chlorantraniliprole over that in chlorantraniliprole alone. Similarly the interaction between the combined treatments was additive depending upon the origin of the population. The population of Gujranwala was more resistant with 58.07% mortality and the 36.56% pupation, whereas the population of Rawalpindi exhibited susceptibility towards the combined treatments with 77.83% mortality and 16.90% pupation (Table 4). However, in case of larvae treated with chlorantraniliprole alone, significantly higher mortality was recorded in all the populations tested with maximum 41.79% and 52.97% pupation in Rawalpindi population but this was less effective in Gujranwala population with 29.55% mortality and 65.08% pupation.

The additive effect was noted in 5th instar larvae by the combined treatments of chlorantraniliprole, NPV, and *A. indica* among all the populations tested and mortality was higher in the combined treatments than the

Table 1. Factorial analyis of localities, larval duration and treatments on the mortality and pupation of 2nd, 3rd, 4th and 5th larval instars of *Helicoverpa armigera* from six different localities treated with *Azadirachta indica*, *Nucleopolyhedrovirus* (NPV), and chlorantraniliprole.

		Mortality		Pupation	
Source	*df*	*F*	*P*	*F*	*P*
Localities	5	32.84	0.000	30.88	0.000
Larval duration	3	675.20	0.000	645.60	0.000
Treatments	6	1 732.13	0.000	1 826.97	0.000
Localities × larval duration	15	0.26	0.998	0.43	0.971
Localities × treatments	30	1.03	0.427	0.81	0.754
Larval duration × treatments	18	28.81	0.000	27.79	0.000
Localities × larval duration × treatments	90	0.20	1.000	0.34	1.000
Error	336	-	-	-	-
Total	503	-	-	-	-

individual treatments (Table 5). The decreasing order of susceptibility at NPV and chlorantraniliprole treatment was Rawalpindi population (mortality 67.81%, pupation 28.94%), Sargodha (65.64%, 31.06%), Lahore (62.29, 32.45), Faisalabad (60.41, 36.27), Sheikhupura (57.46, 39.29) and Gujranwala (54.40, 42.33).

DISCUSSION

Long term use of synthetic insecticides have culminated into serious health and environmental issues (Nathan and Kalaivani, 2006) which redirect the researchers to look for some safer alternatives and ecologically acceptable pesticides (Wood and Granados, 1991) with no or less residual effect and resistance development property, for the control of important insect pests. The results of the present study indicate that NPV, *A. indica*, and chlorantraniliprole can be used successfully against various larval instars of *H. armigera*, but their effectiveness may depend on several factors like the origin of the populations and the stages of the larvae. Decline in susceptibility of *H. virescens* to NPV, azadirachtin, and imidacloprid has been documented after second instar by Koppenhöfer and Kaya (2000). Kumar *et al*. (2008) supported the earlier notion about the susceptibility of the early instar larvae compared to the late instars as they ingest more treated leaf area during scrap feeding. However, in another study against *Spodoptera litura* F. (Murugan *et al*., 1999) the maximum mortality of larval stages was observed at highest concentrations of virus but Allen and Ignoffo (1969) noted that the susceptibility decreases with the age of larvae. These results are in confirmation to the present

Table 2. Mean mortality and pupation of 2nd instar larvae of *Helicoverpa armigera* from six different locations treated with *Azadirachta indica* (Ai: 5 µL L^{-1}), *Nucleopolyhedrovirus* (NPV: 2.1 × 10^5 POB mL^{-1}) and chlorantraniliprole (Ch: 0.01 µL L^{-1}) alone and in combination.

Localities	Treatments	Observed mortality (% ± SE)	Pupation (% ± SE)	Expected mortality	CTF	Interaction
Gujranwala	NPV+Ch	96.10 ± 0.69a	0.13 ± 0.13c	97.09	-1.01	Additive
	Ai+Ch	92.33 ± 3.73a	1.79 ± 0.91c	91.93	0.42	Additive
	Ai+NPV	87.54 ± 2.29a	8.82 ± 2.29c	86.56	1.13	Additive
	Ai	40.70 ± 3.91b	55.65 ± 3.91b			
	NPV	45.86 ± 4.72b	50.50 ± 4.72b			
	Ch	51.23 ± 2.94b	45.13 ± 2.94b			
	Control	1.21 ± 0.66c	97.18 ± 0.61a			
Sheikhupura	NPV+Ch	98.76 ± 0.68a	0.00 ± 0.00c	102.06	-3.24	Additive
	Ai+Ch	94.96 ± 1.30a	0.89 ± 0.63c	94.84	0.13	Additive
	Ai+NPV	91.80 ± 2.14a	4.56 ± 2.14c	91.65	0.16	Additive
	Ai	42.21 ± 3.97b	54.15 ± 3.97b			
	NPV	49.43 ± 3.60b	46.92 ± 3.60b			
	Ch	52.63 ± 1.91b	43.73 ± 1.91b			
	Control	1.93 ± 0.99c	96.16 ± 1.18a			
Faisalabad	NPV+Ch	100.0 ± 0.00a	0.00 ± 0.00c	109.76	-8.88	Additive
	Ai+Ch	96.33 ± 1.54a	0.00 ± 0.00c	102.94	-6.41	Additive
	Ai+NPV	93.29 ± 1.82a	2.47 ± 1.29c	99.79	-6.51	Additive
	Ai	46.49 ± 1.74b	49.87 ± 1.74b			
	NPV	53.30 ± 4.41b	43.06 ± 4.41b			
	Ch	56.46 ± 4.54b	39.90 ± 4.54b			
	Control	1.780 ± 1.05c	96.88 ± 1.18a			
Lahore	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00c	113.45	-11.45	Additive
	Ai+Ch	97.31 ± 1.62a	0.00 ± 0.00c	105.87	-8.08	Additive
	Ai+NPV	96.54 ± 1.90a	1.12 ± 0.68c	102.55	-5.86	Additive
	Ai	47.48 ± 3.52b	48.88 ± 3.52b			
	NPV	55.06 ± 4.31b	41.29 ± 4.31b			
	Ch	58.39 ± 5.34b	37.97 ± 5.34b			
	Control	2.32 ± 0.67c	95.14 ± 1.77a			
Sargodha	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00c	116.84	-14.41	Additive
	Ai+Ch	98.80 ± 0.70a	0.00 ± 0.00c	110.23	-10.37	Additive
	Ai+NPV	97.95 ± 1.59a	0.00 ± 0.00c	107.85	-9.17	Additive
	Ai	50.62 ± 4.66b	45.71 ± 4.66b			
	NPV	57.23 ± 4.57b	39.10 ± 4.57b			
	Ch	59.62 ± 4.56b	36.71 ± 4.56b			
	Control	1.96 ± 0.64c	95.78 ± 0.90a			
Rawalpindi	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00c	122.29	-18.22	Additive
	Ai+Ch	100.00 ± 0.00a	0.00 ± 0.00c	115.45	-13.38	Additive
	Ai+NPV	98.22 ± 1.15a	0.00 ± 0.00c	111.29	-11.74	Additive
	Ai	52.22 ± 6.08b	44.11 ± 6.08b			
	NPV	59.07 ± 10.20b	37.26 ± 10.20b			
	Ch	63.23 ± 6.80b	33.10 ± 6.80b			
	Control	2.96 ± 0.96c	94.29 ± 0.70a			

Percentage means within the locality followed by the same letter are not significantly different, Tukey-Kramer HSD test at $P < 0.05$. POB: Polyhedral occlusion bodies, CTF: Cotoxicity factor.

Table 3. Mean mortality and pupation of 3rd instar larvae of *Helicoverpa armigera* from six different locations treated with *Azadirachta indica* (Ai: 5 µL L^{-1}), *Nucleopolyhedrovirus* (NPV: 2.1 × 10^5 POB mL^{-1}) and chlorantraniliprole (Ch: 0.01 µL L^{-1}) alone and in combination.

Localities	Treatments	Observed mortality (% ± SE)	Pupation (% ± SE)	Expected mortality	CTF	Interaction
Gujranwala	NPV+Ch	91.11 ± 6.46a	3.89 ± 2.89c	90.01	1.21	Additive
	Ai+Ch	89.65 ± 4.94a	7.24 ± 4.68c	84.58	5.99	Additive
	Ai+NPV	79.40 ± 4.01a	17.16 ± 4.01c	80.33	-1.15	Additive
	Ai	37.45 ± 3.15b	59.11 ± 3.15b			
	NPV	42.88 ± 4.01b	54.62 ± 3.09b			
	Ch	47.13 ± 5.16b	49.43 ± 5.16b			
	Control	1.86 ± 0.68c	95.29 ± 1.98a			
Sheikhupura	NPV+Ch	95.87 ± 3.81a	2.79 ± 2.79c	94.98	0.93	Additive
	Ai+Ch	91.69 ± 4.22a	5.19 ± 4.12c	90.08	1.78	Additive
	Ai+NPV	82.26 ± 4.79a	14.47 ± 4.79c	82.86	-0.72	Additive
	Ai	38.98 ± 4.28b	58.40 ± 3.73b			
	NPV	43.87 ± 3.64b	53.68 ± 2.93b			
	Ch	51.10 ± 3.61b	45.63 ± 3.61b			
	Control	2.32 ± 0.55c	93.83 ± 2.15a			
Faisalabad	NPV+Ch	98.16 ± 0.98a	0.00 ± 0.00e	97.92	0.24	Additive
	Ai+Ch	94.48 ± 1.76ab	2.58 ± 1.38de	91.93	2.77	Additive
	Ai+NPV	84.74 ± 1.26b	11.92 ± 1.26d	84.24	0.60	Additive
	Ai	39.13 ± 4.32d	56.53 ± 3.97b			
	NPV	45.11 ± 3.24cd	52.33 ± 2.48bc			
	Ch	52.81 ± 3.80c	43.85 ± 3.80c			
	Control	2.26 ± 0.57e	94.58 ± 1.36a			
Lahore	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00c	101.05	-1.04	Additive
	Ai+Ch	97.75 ± 1.24a	0.33 ± 0.33c	95.94	1.89	Additive
	Ai+NPV	86.36 ± 4.29a	10.32 ± 4.29c	86.78	-0.49	Additive
	Ai	40.83 ± 4.56b	55.84 ± 4.56b			
	NPV	45.95 ± 5.04b	50.21 ± 4.60b			
	Ch	55.10 ± 3.56b	41.58 ± 3.56b			
	Control	1.73 ± 0.38c	95.63 ± 1.86a			
Sargodha	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00c	106.47	-6.08	Additive
	Ai+Ch	100.00 ± 0.00a	0.00 ± 0.00c	102.51	-2.45	Additive
	Ai+NPV	93.54 ± 5.14a	3.15 ± 5.14c	91.29	2.47	Additive
	Ai	43.66 ± 4.99b	53.02 ± 4.99b			
	NPV	47.62 ± 4.41b	49.07 ± 4.41b			
	Ch	58.85 ± 4.95b	37.84 ± 4.95b			
	Control	2.15 ± 1.25c	94.67 ± 0.95a			
Rawalpindi	NPV+Ch	100.00 ± 0.00a	0.00 ± 0.00d	110.20	-9.25	Additive
	Ai+Ch	100.00 ± 0.00a	0.00 ± 0.00d	107.01	-6.54	Additive
	Ai+NPV	95.41 ± 1.20a	1.55 ± 0.96d	96.40	-1.02	Additive
	Ai	46.60 ± 5.84b	50.07 ± 5.84b			
	NPV	49.79 ± 3.92b	46.89 ± 3.92bc			
	Ch	60.40 ± 2.48b	35.28 ± 2.26c			
	Control	2.38 ± 0.97c	93.58 ± 1.68a			

Percentage means within the locality followed by the same letter are not significantly different, Tukey-Kramer HSD test at $P < 0.05$. POB: Polyhedral occlusion bodies, CTF: Cotoxicity factor.

work as the age of the *H. armigera* larvae increased the mortality of the larvae decreased. The reason of this behavior is not yet explored properly except Nathan *et al*. (2005), who studied the effects of azadirachtin (AZA) and NPV on midgut enzyme activity in *Spodoptera litura* and noted that the gut enzyme activities were decreased by AZA and NPV individually and in combination. Upon feeding on diet of castor leaves treated with AZA and NPV in bioassays, gut enzyme-acid phosphatases, alkaline phosphatases, adenosine triphosphatases, and lactate dehydrogenase-activities decreased in *S. litura* early instar larvae. But still there is room for more research on the biochemical, molecular, and histopathological studies of the midgut necessary to understand the mechanism of decreased susceptibility of aging larvae.

Presently, the viral formulations (especially belonging to the family Baculoviridae) are one of the most promising biological insecticides (Lavina *et al*., 2001). *Nuclear Polyhedrosis Viruses* move from cell to cell and after its ingestion, resulted infected cells disintegrate which ultimately leads to the death of the insects. While, the neem has great antifeedant, insect growth regulatory and insecticidal effects, containing several compounds (Ramya and Jayakumararaj, 2009). The principal active ingredient in neem is AZA, which disrupt the action of molting hormone, reproduction of insects (Lee *et al*., 1991) and can be mixed with other biopesticides, microbials or with synergists (Koppenhöfer and Kaya, 2000). There are reports regarding the interaction of NPV and AZA against other insects with varying success. Shapiro *et al*. (1994) found that addition of neem extract with NPV against gypsy moth resulted in faster mortality, similarly, Nathan and Kalaivani (2006) evaluated NPV with AZA against *S. litura* and noted dose and larval instar

dependent growth retardation after treatment with NPV and AZA and also decrease in nutritional indices by two fold at low concentrations. Nathan *et al*. (2005) exhibited the synergistic effect of botanical insecticides and virus when combined in low doses. The above narrated results are in confirmation with our findings as combinations are more effective in reducing the populations of *H. armigera* which also showed the additive effect compared to alone treatments. Cook *et al*. (1996) also endorsed these findings when they treated second instar gypsy moth larvae with AZA and NPV that resulted in higher mortality compared with that caused by individual treatments so both AZA and virus in combination may give good foliage protection against gypsy moth larvae. The synergistic action of AZA and NPV was also observed by Nathan and Kalaivani (2005) against tobacco cutworm and this interaction was not dose dependent.

Our findings suggest that presence of chlorantraniliprole enhanced the insecticidal activity of NPV and *A. indica* as the present study is the first report on potential combination of all three agents against the larvae of various populations of *H. armigera*. Less mammalian toxicity, effectiveness at relatively low dose rates as compared to other insecticides (pyrethroids, organophosphates, and carbamates), longer residual properties, and wide range of activity of chlorantraniliprole against lepidopteran pests will make it as an excellent control option in an overall integrated pest management system (Anonymous, 2007). It demonstrated very good activity at relatively low rates against larvae and pupae evaluated in this study and insecticidal activity of chlorantraniliprole was at par with that reported by Lahm *et al*. (2007). In laboratory study, LC_{50}'s for chlorantraniliprole (0.1 μL L^{-1}) have been significantly lower when compared with other two standard insecticides

Table 4. Mean mortality and pupation of 4th instar larvae of *Helicoverpa armigera* from six different locations treated with *Azadirachta indica* (Ai: 5 μL L^{-1}), *Nucleopolyhedrovirus* (NPV: 2.1 × 10^5 POB mL^{-1}) and chlorantraniliprole (Ch: 0.01 μL L^{-1}) alone and in combination.

Localities	Treatments	Observed mortality (% ± SE)	Pupation (% ± SE)	Expected mortality	CTF	Interaction
Gujranwala	NPV+Ch	58.07 ± 2.94a	36.56 ± 2.94c	55.65	4.34	Additive
	Ai+Ch	56.89 ±2.89a	37.74 ± 2.89c	51.74	9.96	Additive
	Ai+NPV	49.04 ± 2.49a	45.59 ± 2.49c	48.29	1.56	Additive
	Ai	22.19 ± 3.05b	72.44 ± 3.05b			
	NPV	26.10 ± 1.90b	68.53 ± 1.90b			
	Ch	29.55 ± 4.31b	65.08 ± 4.31b			
	Control	2.63 ± 1.33c	94.02 ± 1.66a			
Sheikhupura	NPV+Ch	61.24 ± 3.56a	33.39 ± 3.56c	59.03	3.74	Additive
	Ai+Ch	58.39 ± 3.13a	36.24 ± 3.13c	54.98	6.20	Additive
	Ai+NPV	50.11 ± 2.74a	44.52 ± 2.74c	51.17	-2.08	Additive
	Ai	23.56 ± 3.82b	71.07 ± 3.82b			
	NPV	27.61 ± 1.86b	67.02 ± 1.86b			
	Ch	31.42 ± 4.79b	63.21 ± 4.79b			
	Control	2.44 ± 0.61c	94.80 ± 1.83a			
Faisalabad	NPV+Ch	65.24 ± 3.72a	29.43 ± 3.72c	62.45	4.45	Additive
	Ai+Ch	63.04 ± 3.89a	31.63 ± 3.89c	58.41	7.93	Additive
	Ai+NPV	55.05 ± 4.14a	39.62 ± 4.14c	54.09	1.78	Additive
	Ai	25.02 ± 4.22b	69.65 ± 4.22b			
	NPV	29.07 ± 1.53b	65.60 ± 1.53b			
	Ch	33.38 ± 6.05b	62.43 ± 5.25b			
	Control	2.55 ± 0.92c	94.76 ± 2.22a			
Lahore	NPV+Ch	69.28 ± 2.63a	25.45 ± 2.63c	66.49	4.19	Additive
	Ai+Ch	67.37 ± 1.88a	27.36 ± 1.88c	61.70	9.18	Additive
	Ai+NPV	58.10 ± 3.08a	36.63 ± 3.08c	58.07	0.05	Additive
	Ai	26.64 ± 3.57b	68.79 ± 3.09b			
	NPV	31.42 ± 3.95b	63.30 ± 3.95b			
	Ch	35.06 ± 4.92b	60.25 ± 4.50b			
	Control	2.73 ± 0.77c	93.76 ± 0.93a			
Sargodha	NPV+Ch	73.75 ± 4.01a	20.92 ± 4.01c	72.14	2.22	Additive
	Ai+Ch	71.57 ± 6.51a	23.10 ± 6.51c	67.51	6.01	Additive
	Ai+NPV	60.43 ± 4.56a	34.24 ± 4.56c	61.83	-2.25	Additive
	Ai	28.60 ± 3.85b	66.07 ± 3.85b			
	NPV	33.23 ± 2.57b	61.44 ± 2.57b			
	Ch	38.91 ± 4.99b	57.47 ± 4.08b			
	Control	2.15 ± 1.25c	95.06 ± 1.44a			
Rawalpindi	NPV+Ch	77.83 ± 4.98a	16.90 ± 4.98c	77.21	0.80	Additive
	Ai+Ch	74.10 ± 1.45a	20.63 ± 1.45c	72.72	1.90	Additive
	Ai+NPV	63.69 ± 3.77a	31.03 ± 3.77c	66.41	-4.09	Additive
	Ai	30.96 ± 4.80b	64.14 ± 5.36b			
	NPV	35.45 ± 4.05b	59.28 ± 4.05b			
	Ch	41.76 ± 6.43b	52.97 ± 6.43b			
	Control	1.93 ± 1.01c	95.38 ± 0.81a			

Percentage means within the locality followed by the same letter are not significantly different, Tukey-Kramer HSD test at $P < 0.05$. POB: Polyhedral occlusion bodies, CTF: Cotoxicity factor.

Table 5. Mean mortality and pupation of 5th instar larvae of *Helicoverpa armigera* from six different locations treated with *Azadirachta indica* (Ai: 5 μL L^{-1}), *Nucleopolyhedrovirus* (NPV: 2.1 × 10^5 POB mL^{-1}) and chlorantraniliprole (Ch: 0.01 μL L^{-1}) alone and in combination.

Localities	Treatments	Observed mortality (% ± SE)	Pupation (% ± SE)	Expected mortality	CTF	Interaction
Gujranwala	NPV+Ch	54.40 ± 3.57a	42.33 ± 3.57c	51.37	5.90	Additive
	Ai+Ch	51.26 ± 2.92a	45.47 ± 2.92c	48.64	5.38	Additive
	Ai+NPV	46.32 ± 1.21a	50.41 ± 1.21c	45.80	1.13	Additive
	Ai	21.53 ± 2.07b	75.19 ± 2.07b			
	NPV	24.26 ± 2.49b	72.47 ± 2.49b			
	Ch	27.11 ± 1.55b	69.62 ± 1.55b			
	Control	1.23 ± 0.66c	96.32 ± 0.82a			
Sheikhupura	NPV+Ch	57.46 ± 2.64a	39.29 ± 2.64c	55.18	4.13	Additive
	Ai+Ch	55.22 ± 4.10a	41.52 ± 4.10c	51.19	7.87	Additive
	Ai+NPV	50.10 ± 1.19a	46.65 ± 1.19c	48.89	2.48	Additive
	Ai	22.45 ± 2.11b	74.29 ± 2.11b			
	NPV	26.43 ± 2.90b	70.31 ± 2.90b			
	Ch	28.74 ± 1.04b	68.01 ± 1.04b			
	Control	1.93 ± 0.99c	95.46 ± 0.94a			
Faisalabad	NPV+Ch	60.41 ± 2.55a	36.27 ± 2.55c	58.69	2.92	Additive
	Ai+Ch	58.76 ± 0.82a	37.92 ± 0.82c	55.05	6.74	Additive
	Ai+NPV	52.59 ± 2.73a	44.09 ± 2.73c	53.36	-1.44	Additive
	Ai	24.86 ± 0.93b	72.83 ± 2.48b			
	NPV	28.50 ± 2.35b	68.17 ± 2.35b			
	Ch	30.19 ± 2.42b	66.49 ± 2.42b			
	Control	1.77 ± 1.05c	96.09 ± 1.23a			
Lahore	NPV+Ch	62.29 ± 4.52a	32.45 ± 2.65c	60.35	3.22	Additive
	Ai+Ch	61.38 ± 5.19a	34.33 ± 4.42c	56.31	9.01	Additive
	Ai+NPV	55.34 ± 3.79a	41.38 ± 3.79c	54.55	1.45	Additive
	Ai	25.26 ± 1.87b	71.46 ± 1.87b			
	NPV	29.29 ± 2.29b	67.43 ± 2.29b			
	Ch	31.06 ± 2.84b	64.29 ± 3.59b			
	Control	2.32 ± 0.67c	93.35 ± 1.06a			
Sargodha	NPV+Ch	65.64 ± 8.43a	31.06 ± 8.43c	64.60	1.61	Additive
	Ai+Ch	63.79 ± 3.27a	32.92 ± 3.27c	61.77	3.25	Additive
	Ai+NPV	59.26 ± 3.07a	38.44 ± 3.67c	58.05	2.07	Additive
	Ai	27.61 ± 3.14b	69.10 ± 3.14b			
	NPV	30.44 ± 2.43b	66.27 ± 2.43b			
	Ch	34.16 ± 2.46b	62.55 ± 2.46b			
	Control	1.96 ± 0.64c	94.94 ± 0.93a			
Rawalpindi	NPV+Ch	67.81 ± 3.53a	28.94 ± 3.53c	67.81	0.00	Additive
	Ai+Ch	65.40 ± 3.41a	31.35 ± 3.41c	65.33	0.10	Additive
	Ai+NPV	61.37 ± 4.13a	35.38 ± 4.13c	60.70	1.09	Additive
	Ai	29.11 ± 3.90b	67.64 ± 3.90b			
	NPV	31.59 ± 5.45b	65.16 ± 5.45b			
	Ch	36.22 ± 2.83b	60.53 ± 2.83b			
	Control	2.96 ± 0.96c	92.31 ± 1.16a			

Percentage means within the locality followed by the same letter are not significantly different, Tukey-Kramer HSD test at $P < 0.05$. POB: Polyhedral occlusion bodies, CTF: Cotoxicity factor.

indoxacarb (1.5 μL L^{-1}) and cypermethrin (13.5 μL L^{-1}) in an insecticide-treated diet assay on a laboratory colony of tobacco budworm (Anonymous, 2007).

The variation in the susceptibility levels among insecticides is likely to occur among populations originating from different parts of the world or even from the same region. Our study is the first in which variable effectiveness of NPV, *A. indica*, and chlorantraniliprole against *H. armigera* populations originating from different locations in the Punjab province (Pakistan) is documented. In the light of our findings, the population from Gujranwala appeared to be more tolerant to NPV and *A. indica* and chlorantraniliprole compared with the remaining populations collected from other localities. Resistance to different conventional insecticides has been observed in varying degree in lepidopteran insect populations (Ahmad *et al*., 2003; 2007), which might be attributed to the excessive applications of insecticides on different field crops grown in that particular localities.

CONCLUSIONS

Among various evaluated treatments, NPV+Ch proved to be the best one against all the populations of *H. armigera* further 2nd stage larvae were the most susceptible one. Present laboratory study suggests that, under specific experimental conditions, NPV and *A. indica* can be used with success in conjunction with chlorantraniliprole against various larval instars of *H. armigera*, thereby allowing a lower dose of their combination for the management of *H. armigera*. Findings of the present study earnestly suggest that the mortality demonstrated in the laboratory does not give the true picture of the mortality in the field and also the generalizations should

be avoided. More research is needed to have better idea on the interaction of chlorantraniliprole, NPV, and *A. indica* to control the *H. armigera* both in laboratory and field conditions. The data generated here comprises initial efforts in establishing baseline information about chlorantraniliprole that can be used as reference points for future integration with other safer microbial based management programs.

ACKNOWLEDGEMENTS

The study was financially supported by grant (N° 1185) from Higher Education Commission (HEC), Islamabad, Pakistan, under National Research Program for Universities.

Eficacia insecticida de *Azadirachta indica*, *Nucleopolihedrovirus* y clorantraniliprol solo y sus aplicaciones integradas contra poblaciones de campo de *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Se determinó la eficacia insecticida de formulaciones de *Azadirachta indica*, *Nucleopolihedrovirus* (VPN) y el nuevo insecticida diamida antranílico (clorantraniliprol) en contra de segundo, tercero, cuarto y quinto estadios larvales de *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) recogidos de diversas ubicaciones geográficas de la provincia de Punjab, Pakistán. *Azadirachta indica* se aplicó en dosis de 5 μL L^{-1}; VPN en dosis 2.1 × 10^5 POB mL^{-1} y clorantraniliprol fue 0,01 μL L^{-1} ya sea solos o en combinaciones. Los bioensayos se realizaron a 27 ± 1 °C y 65 ± 5% de humedad relativa. La mortalidad fue notablemente variada entre los tratamientos, estadios larvales y diversas poblaciones. Las combinaciones de VPN con *A. indica* y clorantraniliprol dio una mayor mortalidad, la fase de pupa y el efecto aditivo producido en comparación con su aplicación solo en todas las poblaciones de la prueba. La población de Rawalpindi fue siempre susceptible, mientras que Gujranwala fue resistente. Los resultados del presente trabajo sugieren que la eficacia de VPN y *A. indica* pueden ser beneficiados por la presencia de clorantraniliprol contra las larvas de *H. armigera*.

Palabras clave: Clorantraniliprol, VPN, aditivos.

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