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Potential Enhancement of Nuclear Polyhedrosis Virus by Azadirachtin and its Effects on the Food Utilization, Development and Mortality of *Helicoverpa armigera* Hubner

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ABSTRACT. *The potential enhancement of nuclear polyhedrosis virus (NPV) by azadirachtin and its impact on Helicoverpa armigera Hubner was evaluated in the laboratory. The time to kill 50% of fourth instar larvae of H. armigera was significantly reduced to 96 h when azadirachtin (AZA; 1.0 ppm) was combined with virus (15×10^3 PIB ml^{-1}). The larval duration was extended to 23 days and oviposition period and fecundity were reduced to 3.5 days and 217 eggs, respectively, at 1×10^3 PIB ml^{-1} NPV + 0.1 ppm AZA treatment. The combined treatment of NPV and azadirachtin significantly reduced the consumption index to 0.79 mg mg^{-1} day^{-1} and efficiency of conversion of ingested food to 5.07% and digested food to 9.8%. The combination of NPV and azadirachtin would certainly play a significant role in integrated pest management (IPM) of H. armigera.*

INTRODUCTION

The American bollworm, *Helicoverpa armigera* Hubner, or gram caterpillar is a major polyphagous pest in Asia, causing serious damages to agricultural, ornamental and horticultural crops (Chari *et al.*, 1990). Despite the voluminous work on the control of bollworms on cotton, still it remains an unabated problem. Chemical pesticides are not always effective and improper use have created several complications in agro–ecosystems such as development of resistance to insecticides, pest resurgence and environmental contamination. These pressures in the agricultural industry are making alternative control agents more attractive as supplements to, or replacements for, synthetic pesticides in integrated pest management (IPM).

Nuclear polyhedrosis virus (NPV) has shown potential as control agents of lepidopteran pests due to their high pathogenicity, restricted host range and safety to man and other beneficial organisms. The NPV can

provide control of larval insect populations which is comparable with, or superior to, that provided by a synthetic insecticide (Cowgill and Bhagwat, 1996). Baculoviruses have been used for biological control purposes for two decades (Huber, 1986), but like most biological insecticides, they are slow to act. Larval mortality often does not occur until 3–4 days after NPV exposure. The larvae continue to feed throughout this period even though the virus ultimately reduces the larval population density. Increase in the potential of NPV for the control of Gypsy moth has been carried out using certain adjuvants namely, optical brighteners (Shapiro and Vaughn, 1995), neem seed extract (Shapiro *et al.*, 1994) and azadirachtin (Cook *et al.*, 1996).

Indian neem tree, *Azadirachta indica* A. Juss., has been demonstrated to have antifeedant, insecticidal and insect growth regulatory activity (Saxena, 1989). Earlier reports have noted the biological activity of neem extracts against *H. armigera* (Senthil Kumar and Murugan, 1995; Murugan *et al.*, 1995, 1997a). Although there are several potential insecticidal compounds in neem seed extract, the principle active ingredient in most formulations is the triterpene azadirachtin (Lee *et al.*, 1991). Azadirachtin (AZA) affects insect feeding, growth, moulting and reproduction (Mordue (Luntz) and Blackwell, 1993; Murugan *et al.*, 1998).

Neem products can be mixed with other biopesticides, microbials or with synergists, to increase their efficacy (Schmutterer, 1990; Senthil Kumar, 1998). A synergistic interaction of neem seed kernel extract, *Vitex negundo* leaf extract and NPV against *H. armigera* has been reported by Murugan *et al.* (1997b).

In the present study, the potential enhancement of the effect of NPV by azadirachtin on the mortality, food utilization and development of *H. armigera* was evaluated.

MATERIALS AND METHODS

Helicoverpa armigera Hubner larvae were collected from the cotton fields in and around Coimbatore, India, and were cultured in the laboratory and fed with *Gossypium hirsutum* leaves *ad libitum*. Nuclear polyhedrosis virus (NPV) was inoculated in the laboratory–reared one day old IVth instar of *H. armigera* larvae with a viral suspension of 1×10^8 PIB ml^{-1}. The viral suspension was mixed in the artificial diet, without formalin, and fed to the larvae. The infected cadavers were collected and thoroughly macerated with distilled water for purification of virus by differential centrifugation. The viral

suspension was filtered with muslin cloth and the filtrate was centrifuged at 300–500 rpm for 5 minutes. The supernatant containing the virus was further centrifuged at 3000 rpm for 2–3 minutes. The final supernatant was discarded and the sediment containing the viral bodies was stirred with distilled water. The concentration of viral suspension was determined by counting in a Neubayer Haemo–cytometer. For experiments, viral suspensions ranging from 10^2–10^4 PIB ml^{-1} was prepared by diluting with distilled water and stored in deep freezer (Senthil Kumar, 1998). Authentic samples of azadirachtin (AZA) was used to prepare the desired concentrations.

The fresh cotton leaves were coated with NPV (5×10^3, 1×10^4, 15×10^3 PIB ml^{-1}) and AZA (0.05, 0.1, 1.0 ppm) concentrations and air dried. The control leaves were treated with distilled water alone. The newly moulted fourth instar larvae (30 larvae per concentration, five replicates) were fed with treated and untreated leaves after a 3 h starvation period. After 96 h (4 days), the larvae were transferred to fresh untreated cotton leaves and maintained until they moulted into adults or died. Total number of normal adults that survived were recorded. The larvae were observed for mortality and morphological changes associated with growth disrupting effects. Mortality was recorded in hours after the treatment of NPV and AZA. Results were corrected for control mortality using Abbott's (1925) formula.

For assessment of the duration of post–embryonic development, freshly laid eggs were separated and observations were made based on duration of each larval stage, oviposition period, adult longevity and fecundity of treated *H. armigera*. Food consumption, growth rates and post ingestive food utilization efficiencies (all based on dry weight) such as consumption index (CI), relative growth rate (RGR), approximate digestibility (AD), efficiency of conversion of ingested (ECI) and digested (ECD) food were calculated by gravimetric technique (Waldbauer, 1968; Slansky and Scriber, 1985; Murugan and Ancy George, 1992). Statistical analysis was done by using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Figure 1 illustrates the mortality in hours of IV instar *H. armigera* larva after the treatment with NPV and AZA. Mortality was observed in a dose dependent manner and was higher from the second day onwards after NPV and AZA treatment. The combined treatments of NPV and AZA showed higher percentage of mortality than the individual treatments.

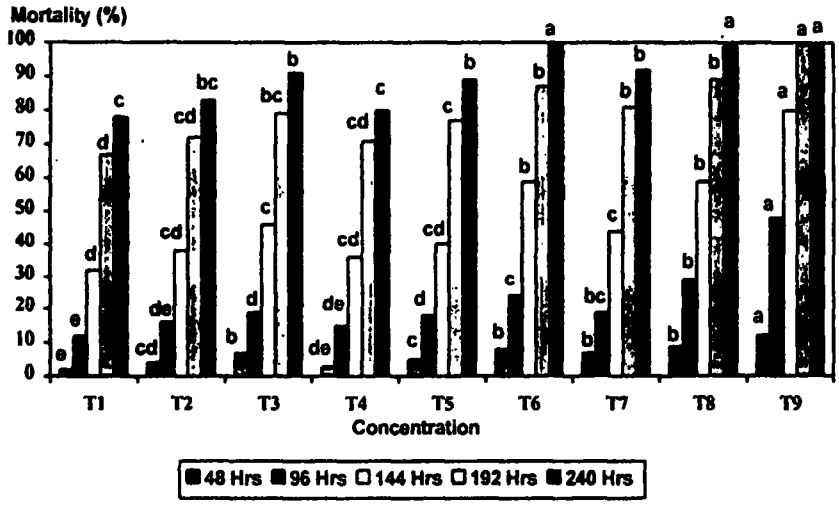

Figure 1. Time dependent mortality (%) of IVth instar *H. armigera* after treatment with NPV and AZA.

[Note: T1–NPV (5×10^3 PIB ml^{-1}); T2–NPV (1×10^4 PIB ml^{-1}); T3–NPV (15×10^3 PIB ml^{-1}); T4–AZA (0.05 ppm); T5–AZA (0.1 ppm); T6–AZA (1.0 ppm); T7–NPV (5×10^3 PIB ml^{-1}) + AZA (0.05 ppm); T8–NPV (1×10^4 PIB ml^{-1}) + AZA (0.1 ppm); T9–NPV (15×10^3 PIB ml^{-1}) + AZA (1.0 ppm). Within each treatment, bars bearing the same letter are not significantly different by the DMRT at p=0.05].

The mortality in NPV treatment at 15×10^3 PIB ml^{-1} was 19% at 96 h (4 days) and 91% at 240 h (10 days), whereas in AZA treatment the insect mortality at 1 ppm was 24% was at 96 h and 100% at 240 h. The mortality under the combined treatment of NPV+AZA at 15×10^3 PIB ml^{-1} + 1.0 ppm increased from 48% at 96 h to 99% at 192 h.

The NPV treatment at high doses resulted in adult mortality and deformities. Growth and development in *Lymantria dispar* larvae were affected by NPV (Shapiro *et al.*, 1994). In the present study, consumption of NPV and AZA resulted in retarded growth and development of *H. armigera*. When combined with AZA, NPV increased the percentage of larval mortality in *H. armigera* and decreased the time taken to kill larvae, thus proving the enhancement of NPV activity by AZA. . Similar results were reported by Shapiro *et al.* (1994) using whole neem extract against gypsy moth.

The total larval duration of *H. armigera* in the control treatment was 14.8 days. The NPV treatment at 1×10^3 PIB ml^{-1} enhanced the larval period to 16.7 days by increasing the duration of every instar (Table 1). Azadirachtin treatment at 0.1 ppm increased the larval duration to 21.6 days by increasing the moulting period of every instar in a dose-dependent manner. The combined treatment of NPV and AZA at 1×10^3 PIB ml^{-1} NPV + 0.1 ppm AZA significantly increased the total larval duration and pupal period to 23.0 and 11.1 days, respectively when compared to the individual treatments and the control.

Table 1. Larval and Pupal duration of *H. armigera* after the treatment with NPV and AZA.

Treatment	Larval Duration (Days)						Pupal Duration (Days)
	I	II	III	IV	V	VI	
Control	1.5^{e}	2.0^{e}	2.6^{e}	3.2^{c}	3.5^{d}	2.0^{e}	9.7^{d}
NPV (PIB ml^{-1})							
1×10^2	1.6^{e}	2.2^{e}	2.6^{e}	3.1^{c}	3.6^{d}	2.0^{e}	9.7^{d}
5×10^2	1.8^{bc}	2.4^{bc}	2.7^{de}	3.3^{bc}	3.6^{d}	2.0^{e}	9.9^{cd}
1×10^3	2.0^{bc}	2.5^{bc}	3.0^{cde}	3.4^{bc}	3.7^{cd}	2.1^{de}	10.0^{cd}
AZA (ppm)							
0.01	1.7^{e}	2.2^{e}	2.7^{de}	3.2^{c}	3.7^{cd}	2.1^{de}	9.9^{cd}
0.05	2.1^{bc}	2.6^{bc}	3.1^{bcde}	3.9^{ab}	4.3^{abc}	2.7^{bcd}	10.2^{bcd}
0.10	2.4^{ab}	3.0^{ab}	3.7^{abc}	4.3^{a}	4.8^{a}	3.4^{a}	10.3^{bc}
NPV+AZA (PIB ml^{-1}) (ppm)							
1×10^2+0.01	1.9^{bc}	2.6^{bc}	3.4^{bcd}	3.2^{c}	4.1^{bcd}	2.3^{cde}	10.4^{bc}
5×10^2+0.05	2.5^{ab}	2.9^{ab}	3.7^{abc}	3.8^{ab}	4.6^{ab}	2.8^{abc}	10.8^{ab}
1×10^3+0.1	3.0^{a}	3.4^{a}	4.3^{a}	4.2^{a}	4.9^{a}	3.2^{ab}	11.1^{a}

NPV : nuclear polyhedrosis virus, AZA : azadirachtin. Within a column means followed by the same letter are not significantly different by the DMRT at p=0.05.

The oviposition period and adult longevity were decreased to 3.5 days and 4.5 days (female), respectively by the treatment 1×10^3 PIB ml^{-1} NPV + 0.1 ppm AZA when compared to the 6.5 and 9 days, respectively, observed in the control treatment (Table 2). Insects in the control treatment laid 780 eggs, but in the combined treatment (1×10^3 PIB ml^{-1} + 0.1 ppm AZA) laid only 217 eggs.

Table 2. Oviposition period, adult longevity and fecundity of *H. armigera* after treatment with NPV and AZA.

Treatment	Oviposition period (days)	Adult Longevity (days)		Fecundity (No. of eggs/female)
		Male	Female	
Control	6.5^{a}	7.5^{a}	9.0^{a}	780^{a}
NPV (PIB ml^{-1})				
1 x 10^2	6.5^{a}	7.4^{a}	9.0^{a}	701^{b}
5 x 10^2	6.1^{ab}	7.1^{ab}	8.6^{ab}	639^{c}
1 x 10^3	5.7^{b}	6.5^{bc}	8.0^{b}	554^{d}
AZA (ppm)				
0.01	6.0^{ab}	7.1^{ab}	8.6^{ab}	562^{d}
0.05	5.1^{c}	6.0^{cd}	6.7^{c}	421^{e}
0.10	4.4^{de}	4.8^{d}	5.6^{d}	226^{g}
NPV+AZA (PIB ml^{-1}) (ppm)				
1 x 10^2+0.01	4.9^{cd}	6.0^{cd}	6.7^{c}	408^{e}
5 x 10^2+0.05	4.1^{ef}	4.9^{cd}	5.2^{d}	289^{f}
1 x 10^3+0.1	3.5^{f}	4.0^{e}	4.5^{e}	217^{g}

NPV : nuclear polyhedrosis virus, AZA : azadirachtin. Within a column means followed by the same letter are not significantly different by the DMRT at p=0.05.

The adults of *H. armigera* treated with NPV showed reduced fecundity. Reduced fecundity due to a viral disease has been observed by Rothman and Myers (1994). Sublethal effects are the most probable cause of reduced reproductive potential. The extended developmental time may be the result of diversion of host energy from metabolism and growth to combat or support the pathogen. Azadirachtin treatment disrupted the normal process of feeding of *H. armigera*. Mortality of AZA treated *H. armigera* larvae was closely linked with poor feeding and concomitant lack of growth due to post–ingestive toxic effects. Mordue *et al.* (1986) showed that azadirachtin would

Table 3. Nutritional indices of IVth instar of *H. armigera* after treatment with NPV and AZA.

Treatment	CI (mg mg^{-1} day^{-1})	RGR (mg mg^{-1} day^{-1})	AD (%)	ECI (%)	ECD (%)
Control	1.78^{a}	0.250^{a}	43.9^{e}	14.09^{a}	32.1^{a}
NPV (PIB ml^{-1})					
1 x 10^{2}	1.63^{ab}	0.211^{b}	44.2^{de}	12.99^{ab}	29.4^{ab}
5 x 10^{2}	1.55^{abc}	0.185^{bc}	44.8^{de}	11.96^{bc}	26.7^{bc}
1 x 10^{3}	1.48^{bcd}	0.151^{d}	46.1^{cd}	10.23^{cd}	22.2^{cd}
AZA (ppm)					
0.01	1.52^{abc}	0.170^{cd}	48.1^{bc}	11.20^{bc}	23.3^{c}
0.05	1.36^{cd}	0.126^{e}	49.4^{ab}	9.28^{cd}	18.8^{de}
0.10	1.19^{de}	0.088^{f}	51.3^{a}	7.43^{ef}	14.5^{ef}
NPV+AZA (PIB ml^{-1}) (ppm)					
1 x 10^{2}+0.01	1.33^{cde}	0.115^{e}	50.2^{ab}	8.68^{de}	17.3^{e}
5 x 10^{2}+0.05	1.08^{e}	0.068^{f}	51.1^{a}	6.38^{ef}	12.5^{f}
1 x 10^{3}+0.1	0.79^{fh}	0.040^{g}	51.8^{a}	5.07^{f}	9.8^{f}

NPV : nuclear polyhedrosis virus, AZA : azadirachtin, CI = Consumption index; RGR = relative growth rate; AD = approximate digestibility; ECI = conversion efficiency of ingested food; ECD = conversion efficiency of digested food. Within a column means followed by the same letter are not significantly different by the DMRT at p=0.05.

retard insect growth due to apparent blockage of ecdysteroid release. In the present study, the AZA treatment showed a low food consumption index, low relative growth rate (RGR), less efficiency in converting the ingested and digested food into biomass resulting in reduced growth. Reduction in RGR of treated *H. armigera* indicates the physiological impact of AZA. The decrease in consumption index (CI) may be due to the impact of AZA on chemoreceptors. The reduction in the efficiency of ingested (ECI) and digested (ECD) food could be due to a diversion of energy from production of biomass into detoxification (Koul and Isman, 1991). The increased approximate digestibility (AD) may be due to the extended duration of food accumulation in the gut resulting in increased exposure to digestive enzymes (Barnby and Klocke, 1987).

Delays in moulting observed in the present study, resulting in prolonged developmental period in AZA–treated insects may be due to antiprothoracicotropic effect as described by Barnby *et al.* (1989). Azadirachtin extends the larval duration, thereby increasing the incubation period for viral infection. Minimal feeding during advanced stage of viral infection, extended larval life and failure to pupate after NPV infection may be due to the maintenance of high level of circulating JH (Subrahmanyam and Ramakrishnan, 1981). Therefore, the physiology of host endocrine system may be interfered due to virus infection resulting in abnormal metamorphosis.

Azadirachtin treatment inhibit or alter the mid–gut chitinous peritrophic membrane, that may serve as a barrier for virus invasion, creating gaps and making it easier for NPV penetration. Over all mortality observed in *H. armigera* may be accounted for the combined effects of partial or total starvation, toxicity and either absence or abnormality of moults. The combined treatment of NPV and AZA further caused a synergistic interactive and deleterious effects on mortality, feeding, moulting and reproduction of *H. armigera.*

CONCLUSIONS

The impact of azadirachtin (AZA) on the feeding and growth regulating activity suggests the toxic effect and insecticidal property of AZA against *H. armigera.* At decreased dose levels of AZA along with NPV resulted in an early kill and higher mortality of larvae than the individual treatments. Further, the decreased food intake, extended larval duration and reduced adult longevity and egg output clearly indicate that the interactive role of azadirachtin and virus has brought out such insecticidal action. Hence, a

formulation of azadirachtin (AZA) + nuclear polyhedrosis virus (NPV) could be an effective technique for the management of *H. armigera*.

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