EFFECTS OF THE NUCLEOPOLYHEDROVIRUS (*SPLI* MNPV), AND AZADIRACHTIN ON NUTRITIONAL PHYSIOLOGY AND ENZYME ACTIVITIES OF *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA; NOCTUIDAE).

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INTRODUCTION

Management of the cotton leaf worm using synthetic chemicals has failed because of insecticide resistance, pest resurgence, and environmental pollution (Mckinely *et al.*, 1989).

Nucleopolyhedroviruses (NPVs), members of the family Baculoviridae, have received considerable attention as potential microbial insecticides, and some of the NPVs have successfully been used for the control of insect pests in agriculture. They have no disadvantages as control agents for insect pests (Lacey *et al.*, 2001; Moscardi, 1999).

Botanical insecticides are naturally occurring chemicals extracted from plants. The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae), is a promising source of botanical insecticides. Due to their relative selectivity, neem products can be recommended for many integrated pest management programs (Biswas *et al.*, 2002). It is generally believed that bioactivity of neem is due to the azadirachtin (AZA) (complex limonoids) content (Butterwoth and Morgan, 1971). AZA is known to have adverse effects on more than 400 insect species. The antifeedant effects of AZA are known for many insects (D'Ambrosio and Guerriero, 2002; Saxena *et al.*, 1984).Combining insecticidal viruses with insecticides for improved pest control is of considerable interest in pest control (Murugan *et al.*, 1999). The use of AZA as an additive to the *Spodoptera littoralis* NPV has been already reported by Abd El-Aziz, (2004).

The consumption and conversion efficiency were highly correlated with the gut enzyme activity of the host insect (Huang *et al.*, 2004; Senthil Nathan, 2000).

The present work focuses on the study of the effect of azadirachitin and nucleopolyhedrovirus on nutritional indices and activities of gut enzymes (Lactate dehydrogenase "LDH", amylase, lipase and protease) in the cotton leaf worm,

Spodoptera littoralis.

MATERIAL AND METHODS

Laboratory culture of *Spodoptera littoralis*

S. littoralis egg masses were obtained from Insecticide Center, Faculty of Agriculture, Cairo University. Larvae were reared in the laboratory on castor leaves at 27°C 2°C & 65-70% relative humidity, with 12: 12 light: dark cycle. After pupation, emerging adult moths were transferred to cages (1 male 2 females per each cage) and fed on a 10% sucrose solution. For egg laying, cages were covered with muslin cloth. Eggs were surface sterilized in 10% formaldehyde solution for 1-3 min. (Hughes and Wood, 1981).

Nucleopolyhedrosis virus

Spodoptera littoralis nucleopolyhedrovirus (*Spli* MNPV) isolated from *S. littoralis* was used. This virus was selected based on its pathogenicity, causing 25% mortality to fourth instar *S. littoralis* (Abd El-Aziz, 2004).

Azadirachtin

Azadirachtin (purity > 96%, M.wt. 730,72 received from Carl Roth Gmbh + CO. 76185 Karlsrue) was dissolved in acetone and different concentrations were prepared by dilution with acetone. The concentration which causes 25% mortality to 4th instar *S littoralis* was selected (Abd El-Aziz, 2004).

Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption, and feces produced. Consumption, growth rates and post-ingestive food utilization efficiencies were calculated (Waldbauer, 1964 & 1968). Consumption index (CI) = E/TA; relative growth rate (RGR)= P/TA; approximate digestibility (AD) = 100 (E-F) / E; efficiency of conversion of ingested food (ECI) = 100 P/E; efficiency of conversion of digested food (ECD) = 100P/ (E-F), where A is the mean weight of the insect during the duration of experimental period (T), E is the weight of food eaten, F is the weight of feces, and P is the weight gained by the insect.

Preparation of enzyme extract

Two-day-old fourth instars of treated *S. littoralis* (treated with NPV and AZA separately or in combination) were used to quantify the enzymes activity. The

method used to prepare the enzyme extract was that of Applebaum *et al.* (1961) and Applebaum (1964).

Enzyme assay

Amylase activity was estimated as described by Bernfeld (1955), protease and lipase as Teo *et al.* (1990). Lactate dehydrogenase (LDH) was assayed using the method of Senthil Nathan (2006). Absorbance of amylase, protease, lipase and LDH were read at 530, 590, 435 and 440 nm, respectively using a Schimadzu UV/ visible spectrophotometer against a control blank.

Statistical analysis

The effective concentration was calculated using probit analysis (Finney, 1971) and values were expressed as the mean of 3 replicates with standard error. Data from nutritional indices, enzyme activities, weight and feeding deterrent were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test (P □ 0.05) (SAS Institute, 2001).

RESULTS AND DISCUSSION

Food consumption, Digestion and utilization

The indices of consumption, digestion, and utilization by the fourth instar larvae on treated castor leaves are shown in table (1). It was observed that the larval duration of NPV-infected larvae was at least 3-4 days longer than the untreated larvae. Also, food consumption was higher in fourth instar *S. littoralis* infected with NPV, which do not reflect on either weight gain or consumption index. Subrahmanyam and Ramakrishnan (1981) reported similar results for *S. litura*. In contrast, the consumption of soybean foliage was reduced in NPV- infected *Pseudoplusia includens* and *Anticarsia gemmatalis* (Beach and Todd , 1988).

Table (1) shows that RGR, ECI and ECD were significantly decreased in NPV-infected larvae. Similar results were obtained for *Lymantria dispar* larvae infected with NPV (Shieppard and Shapiro, 1994).

Treatment with AZA revealed a noticeable decrease in larval weight, consumption, RGR, ECI and ECD of *S. littoralis* larvae (Table 1). The present data are in agreement with those obtained for *Helicoverpa armigera* larvae treated with neem limonoids (Murugan *et al.*, 1998). These reductions in dietary utilization and

growth were suggested as a result of behavioral and physiological effects (Venzon *et al.*, 2004).

Table (1) depicts that the feeding (CI) and growth (RGR) of fourth instar *S. littoralis* are affected more by the combined treatment of NPV and AZA than single treatments. Consistent results were also obtained by Senthil Nathan and Kalaivani (2005) who reported that RGR and CI of the fourth instar *S. liura* larvae remained significant at lower levels than control.

Treatment of *S. littoralis* larvae with NPV plus AZA resulted in decrease of ECI, ECD and weight gain (Fig. 1). ECD was decreased from 46.9 % in control to 42.9 % with NPV treatment and was further reduced to 25.1 % in combined NPV and AZA treatment. Also, larval weight gain was reduced to reach 22.4% in relation to control.

The present data revealed that the approximate digestibility (AD), in all treated cases, was insegnificantly increased. Similar results in different insects were documented by several authors (Beach and Todd, 1988; Koul and Isman, 1991; Murugan *et al.*, 1998 & 1999; Senthil Nathan and Kalaivani, 2005).

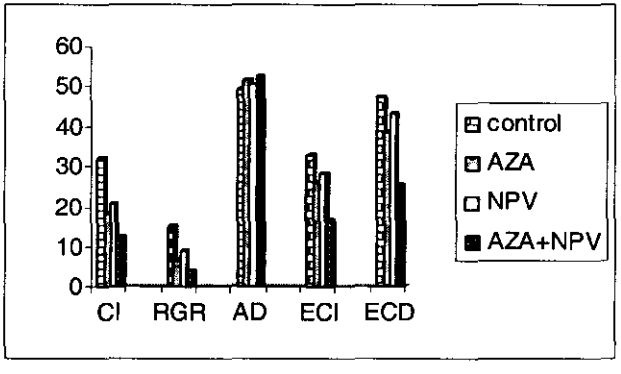

Fig. (1): Nutritional indices of fourth instar larvae *S. littoralis* after treatment with AZA and NPV separately or in combination.

Midgut enzyme activity of *S. littoralis*

Activities of amylase, protease, lipase and lactate dehydrogenase (LDH) activities in the gut of the control and treated fourth instar *S. littoralis* are shown in table (2). The present data revealed that AZA treatment significantly suppressed the amylase, protease, lipase and LDH activities to 52, 24, 36 and 39 %, respectively (Fig.2). Consistent results were also obtained by Senthil Nathan (2006) who

reported that LDH activity of *Cnaphalocrocis medinalis* showed maximum reduction after treatment with 2% *Melia azedarach* extrtact.

Results show that AZA has an inhibitory effect on the activities of gut enzymes and may affect the gut physiology of *S. littoralis* larvae. The results obtained are in consistence with those detected by Senthil Nathan *et al.* (2004) who reported that inhibition of the enzyme activities could be involved in the effects of AZA on the food processing in *S. litura*. Similarly, Martinez and Van-Emden (2001) reported that the decrease of gut enzyme activities was due to antifeedant activity of AZA.

On the other hand, the present data revealed that neither protease nor lipase activities of *S. littoralis* fourth instar were affected by the viral infection (Fig. 2). The maximal suppression of gut enzyme activity was obtained in the treatment of S. *littoralis* larvae with AZA plus NPV. LDH, amylase, protease and lipase activities were 41.5, 36.2, 56.9 and 63.5% of control, respectively.

In the present study, treatment with AZA apparently interacts synergistically with NPV infection in cotton leaf worm, resulting in reduced growth, which in turn affected normal larval development. AZA treatment might have a direct effect on the insect's gut lining to allow easier pathogen penetration (Murugan *et al.*, 1999).

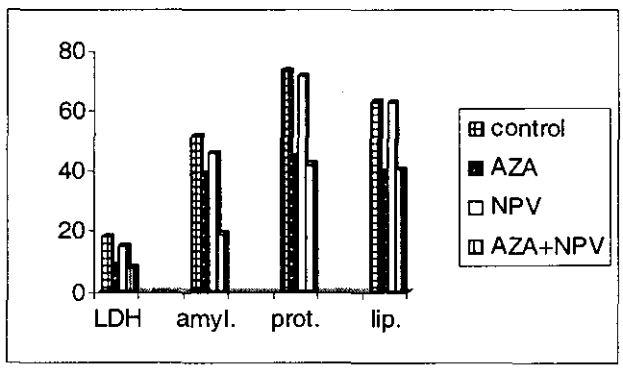

Fig. (2): Enzyme activities of fourth instar larvae of *S. littoralis* after treatment with AZA and NPV.

In conclusion, AZA has been found to potentiate the effect of NPV and it influences the larval growth, weight, food consumption, digestion, and efficiency of food conversion. In other words, the combination of NPV and AZA confers an advantage for pest management especially of a polyphagous pest like *S. littoralis*.

TABLE (I)

Consumption indices (CI), relative growth rate (RGR) approximate digestibility (AD), efficiency conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) of 4th instar larvae of *S. littoralis* after treatment with NPV and AZA.

Treatment	Weight gain (mg)	CI (mg/day)	RGR (mg/day)	AD (%)	ECI (%)	ECD (%)
Control	440.8±1.25 [a]	31.6±3.61 [a]	15.05±2.30 [a]	48.9±5.11 [a]	32.7±1.9 [a]	46.9±5.90 [a]
AZA	169.5±3.09 [b]	18.1±1.72 [b]	6.39±1.03 [c]	51.03±6.8 [a]	25.5±3.6 [b]	38.3±4.21 [b]
NPV	278.7±1.76 [c]	20.53±1.36 [b]	9.00±1.40 [c]	50.00±7.33 [a]	28.1±4.01 [b]	42.93±5.4 [b]
AZA + NPV	98.7±2.65 [d]	12.00±1.5 [c]	3.93±0.51 [d]	52.1±5.9 [a]	16.09±3.0 [c]	25.1±3.2 [c]

Means (±SE) followed by a same letter don't significantly differ (Tukey test, P≤0.05).

TABLE (II)

Enzyme activities of fourth instar larvae of *S. littoralis* after treatment with AZA and NPV.

Treatment	LDH*	Amylase **	Protease **	Lipase**
Control	18.11±2.42 [a]	51.71±4.20 [a]	73.60±3.99 [a]	63.10±1.93 [a]
AZA	8.64±1.12 [b]	39.08±6.12 [b]	44.80±1.36 [b]	39.90±3.01 [b]
NPV	14.70±1.99 [c]	46.11±3.23 [a]	71.39±2.50 [a]	62.81±1.05 [a]
NPV + AZA	7.53±2.64 [d]	18.77±2.04 [c]	41.90±2.04 [b]	40.10±2.11 [b]

Means standard error (SE) followed by the same letters within a column indicate no significant difference (P≤0.05) in a Tukey test.

*mlu /gm wt / min.

**In Mnoles / mgwt / h^{-1}

SUMMARY

Laboratory assays were done to evaluate the effect of azadirachtin (AZA) and a nucleopolyhedrovirus, separately or in combination, on nutritional indices and gut enzymes lactate dehydrogenase, amylase, lipase and protease of the Egyptian cotton leaf worm, *Spodoptera littoralis*.

Food consumption, digestion, relative growth rate, efficiency of conversion of ingested food and efficiency of conversion of digested food values declined significantly. On the other hand, approximate digestibility slightly increased insignificantly.

As compared to control, maximal suppression of gut enzyme activity was investigated in the combined treatment with AZA and NPV. Reduction of LDH, amylase, protease, and lipase activities were 58, 63, 43, and 36 %, respectively.

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