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Effect of *Tylophora asthmatica* Alkaloid on Growth and Development of Tobacco Caterpillar, *Spodoptera litura* F.

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Continuous exposure of freshly hatched *Spodoptera litura* F. larvae to diets containing 0.001% or higher concentrations of *Tylophora asthmatica* alkaloid extract resulted in larval mortality. Lower concentrations prolonged larval period and caused reduction in pupal weight. Older larvae kept on untreated diet upto pupation after 48 hr of initial feeding of alkaloid extract (0.01 and 0.001% concentration) showed considerable recovery from the growth retardation effect.

Key Words: *Tylophora asthmatica*, **Alkaloidal extract**, *Spodoptera litura*, **Growth and development**

Introduction

Acceptance or rejection of a plant species as food by polyphagous insects would largely depend on the presence or absence of chemicals which stimulate or inhibit feeding (Hsiao & Fraenkel 1968). Such feeding deterrents in plants may be of importance in protecting them against herbivorous insects (Hsiao 1969). Secondary metabolites belonging to the acetogenins, alkaloids, phenylpropanes, steroids, terpenoids and other class of compounds were shown to deter feeding of insects (Levinson 1976). A chloroform extractable substance from the leaves of chinaberry tree, *Melia azedarach* L. was shown to deter feeding, retard development and cause mortality in the larval stages of corn earworm, *Heliothis zea* and fall armyworm, *Spodoptera frugiperda* (McMillian et al. 1969). Inhibition of post-embryonic development, morphological aberrations and high mortality were also observed when *S. frugiperda* were either topically treated or kept on food treated with azadirachtin (Redfern et al. 1980). The total alkaloids isolated from *Tylophora asthmatica* plant were observed to show antifeedant activity against *Spodoptera litura* F. larvae (Verma et al. 1986). It was considered of interest to study the effect of continuous feeding of *T. asthmatica* total alkaloids on growth and development of this insect, which is reported in this communication.

Materials and Methods

Tylophora asthmatica alkaloid extract was obtained by the method described earlier (Mulchandani & Venkatachalam 1976). The tobacco caterpillars were grown on castor, *Ricinus communis* leaves at 28 ± 1°C and 65-75% RH. *Tylophora* alkaloid portion was dissolved in chloroform. Aliquots containing 3.0, 1.5, 0.3, 0.15 and 0.03 mg were further diluted with chloroform, added to requisite quantities of Bengal gram, *Cicer arietinum* L. powder to give 0.01, 0.005, 0.001, 0.0005 and 0.0001% alkaloid concentrations in the test diets respectively. The solvent was evaporated in a rotary evaporator under vacuum at 50°C. Agar was digested in 24.7 ml water and after cooling to 60°C, all other ingredients of the diet (Verma et al. 1986) were added, thoroughly mixed and allowed to gel at room temperature. Control diet was prepared in a similar manner except that the alkaloid extract was excluded. Experiments were carried out with two groups of larvae.

In the first group, five freshly hatched larvae were introduced into each beaker (50 ml). Twenty replicates were run for each test concentration. Treated food was provided *ad libitum* and was changed every 48 hr. A similar group of larvae was provided with untreated diet to serve as control. To avoid cannibolism which occurs in the older larvae on 8th day, the larvae were weighed and kept singly in separate beakers along with their respective diets.

In the second group, freshly hatched 5th instar larvae (50-60 mg) were allowed to feed on diets containing 0.00, 0.01 and 0.001% alkaloids. Some of these larvae were transferred to untreated diet after 48 hr feeding. These larvae were allowed to feed on normal diet until they formed pupae.

For determining the food consumption, the larvae were provided with preweighed food. After 24 hr the left over food was weighed again. The difference between these two weights formed the amount of food consumed over this period. Preweighed food kept in beakers under the same experimental conditions without any insect was weighed again after 24 hr and the weight loss due to water evaporation was computed. Using this data, daily larval food consumption was corrected for the loss of weight due to water evaporation (Waldbauer 1968)

Observations on larval mortality, pupation and adult emergence were recorded daily.

Results

When freshly hatched larvae were allowed to feed continuously on the treated diet, larval mortality progressively increased and gain in weight decreased with the increasing concentration of the alkaloid in the diet (table 1). At the two highest concentrations of 0.005 and 0.01%, 70.6 and 77.9% larvae died by the 4th day and 92.6 and 100% respectively died by the 8th day. Larvae feeding on 0.005% diet gained only 2.3 mg in weight as against 145.9 mg by the larvae feeding on untreated diet over the same period.

Effect of continued feeding of older larvae on treated diet on their development, pupation and adult emergence are presented in table 2. In the case of 0.001% alkaloid treatment, only 30% larvae pupated after an average period of 33 days compared with 13.9 days taken by the control larvae. There was progressive reduction in the pupal weight, with increase in the alkaloid concentration. As compared to 326.9 mg pupal weight in control, the insect fed on 0.001% alkaloid containing diet weighed only 204.9 mg. Similarly adult emergence in the treatment was considerably lower.

Table 1 *Development of* S. litura *F. larvae feeding on diet containing* Tylophora asthmatica *alkaloid*

Concentration (%)	Per cent mortality after 3 days	Per cent mortality after 7 days	Mean larval weight (mg) ± SE after 7 days
0.01	77.9	100.0	—
0.005	70.6	92.6	2.3 ± 0.66*
0.001	32.4	59.5	2.1 ± 0.19*
0.0005	13.0	18.8	15.1 ± 1.42*
0.0001	4.5	10.1	127.5 ± 11.13
Control	7.0	7.0	145.9 ± 12.23

* Values significantly different from control ($P>0.01$)

Table 2 *Effect of feeding diet containing* T asthmatica *alkaloid extract to older* S. litura *F.*

Concentration (%)	Per cent larvae pupated	Mean days to pupation ± SE	Mean pupal weight (mg) ± SE	Emergence (%)
0.001	30.0	33.8 ± 1.75*	204.0 ± 27.73*	66.7
0.0005	90.0	26.5 ± 0.33*	299.2 ± 19.40	72.2
0.0001	100.0	14.2 ± 0.18	344.1 ± 14.65	90.0
Control	100.0	13.9 ± 0.34	326.9 ± 12.65	90.0

* Values significantly different from control ($P>0.01$)

Fifth instar larvae transferred to normal diet after 48 hr initial exposure to 0.01 and 0.001% alkaloid-containing diet, showed considerable reduction in the duration of feeding period (table 3). The pupal weights were 371 and 373 mg respectively as against 416 mg weight in the control. In both the cases there was no difference in the number of larvae successfully completing development. However, when the larvae were allowed to feed continuously on 0.001% alkaloid containing diet, it caused considerable reduction in pupal weight, i.e. 200 mg in treated as against 416 mg in control.

Total quantity of food consumed by the older larvae, feeding on different concentrations of alkaloid-containing diet, is presented in table 4. Larvae feeding on 0.01% alkaloid-containing diet could consume only 26.45 mg food over a period of 4 days. These larvae died on 4th or 5th day following exposure to the alkaloid-containing diet. As against this, larvae transferred to normal diet after 48 hr initial exposure to 0.01% alkaloid-containing diet fed almost same quantity of food as that of larvae feeding on alkaloidless diet.

Discussion

Feeding of neonate *S. litura* larvae on diet containing extractable *T. asthmatica* alkaloids causes mortality and retardation of development. Larvae feeding on lower concentration of alkaloid-containing diet gained considerably less weight compared

Table 3 *Effect of transferring 50–60 mg* S. litura *larvae to untreated diet after 48 hr being fed on* T. asthmatica *alkaloid extract*

Concentration (%)	Duration of feeding	Per cent larvae pupated	Mean days to pupation ± S.E.	Mean pupal weight (mg) ± S.E.	Mean days to emergence	Emergence (%)
0.01	Continuous	0.0	–	–	–	–
0.01	48 hr	100.0	10.3 ± 0.15*	371.3 ± 17.67	7.7	90.0
0.001	Continuous	100.0	11.0 ± 0.24*	200.0 ± 25.31*	7.9	90.0
0.001	48 hr	100.0	8.5 ± 0.27*	373.1 ± 11.06	8.3	100.0
Control	Continuous	100.0	7.0 ± 0.15	416.7 ± 18.25	8.2	100.0

*Values significantly different from control ($P>0.01$)

Table 4 *Food consumption of* S. litura *larvae feeding on different concentrations of* T. asthmatica *alkaloid containing diet*

Concentration (%)	Duration of feeding	Mean days of larval feeding	Total food consumed (mg)
0.01	Continuous	4	26.45 ± 2.96*
0.01	48 hr	10	2947.10 ± 62.45
0.001	Continuous	11	3127.80 ± 85.36
0.001	48 hr	8	2996.60 ± 76.84
Control	Continuous	7	3018.52 ± 87.3

* Values significantly different from control (P>0.01)

to weight gain of the larvae feeding on normal diet. Alkaloid extract of *Tylophora asthmatica* was found to act as antifeedant to this insect (Verma et al. 1986). The larval mortality observed here could result from prolonged starvation imposed by the antifeedant alkaloids present in the diet. Isoboldine, an alkaloid isolated from *Cocculus trilobus* (Wada & Munakata 1968) and vinblastine from *Catharanthus roseus* (Meisner et al. 1981) have been shown to inhibit larval feeding, retard development and cause mortality of *S. litura* and *S. littoralis* larvae respectively. Similar results have been reported (Redfern et al. 1980) when freshly hatched *S. frugiperda* larvae were continuously exposed for 7 days to a diet containing 0.2 ppm of azadirachtin, a triterpenoid antifeedant isolated from Indian neem, *Azadirachta indica* A Juss. However, when these larvae were exposed for 48 hr or longer to diets treated with 0.5-10 ppm azadirachtin they were unable to overcome the growth retardation effect. In the present case, however, transfer of older *S. litura* larvae from alkaloid treated to untreated diet reversed the growth retardation effect. Since the transferred larvae consumed the same quantity of food as the larvae feeding continuously on untreated diet it is likely that the observed larval growth retardation effect is due only to antifeedant effect of this compound. It is difficult to conclude at this stage whether the ingested compounds are rapidly metabolised and/or eliminated in such insects. Further studies are in progress to understand the mechanism of action of the *Tylophora* alkaloids.

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