THE EFFECT OF HOST PLANTS ON SUSCEPTIBILITY OF THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) TO INSECTICIDAL TREATMENT

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Abstract

The effect of different host plants on the susceptibility of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae) larvae to insecticides was investigated. Larvae were reared from egg hatching till fifth larval instar on one of four plant species including the usual laboratory diet (castor bean leaves), grapevine, cabbage and garden rocket leaves. Feeding on garden rocket decreased significantly LC_{50} of chloropyrifos than the larvae fed on the castor bean leaves, but increased that of the larvae fed on the cabbage and grapevine. LC_{50} of cypermethrin was decreased significantly for the larvae reared on all plants as compared to the usual laboratory leaves. The rapidity (feeding for 10 days) by which host plant altered the susceptibility of larvae adds to the significance of such changes. Significant variable was observed titres in detoxication enzyme activities, alkaline phosphatase, general esterases and glutathione S-transferase, but each host plant had its own effect on the tested enzymes. It was suggested that one can not designate a lethal concentration of an insecticide in bioassay tests without specifying the host plant fed by larvae where the magnitude of tolerance or resistance depends on complex interacting factors including the nutritional ecology of the pest, demonstrating the importance of non genetic factors (diet) in expressing such resistance. One possibility could be the complex plant chemistry which might alters the expression of the enzyme responsible for detoxifying the pesticide.

INTRODUCTION

The potential of a pest population for development resistance to pesticides necessitated performance of laboratory studies or bioassay tests for evaluating the effectiveness of insecticides. The cotton leafworm, *Spodoptera littoralis,* as a major lepidopteran pest, undergone intensive bioassay measures every year for determining median lethal concentration for an insecticide (LC_{50}), resistance ratio, homogenity of the tested strain and other measures which help in evaluating the success of an insecticide in field use.

In order to minimize factors which may cause erroneous results, standard methods for rearing and toxicological evaluation were cited. Reports interested in studying the effect of variability of larval age, weight, insecticide solutions, number of

treatments, history of susceptible strain.....etc. on bioassay tests (e.g. El-Defrawi *et al.,* 1964, Ahmed *et al.,* 1978-1979).

Although cotton leafworm is a polyphagous pest and exposed to several host plants, caterpillars are usually reared, at laboratory, on castor bean leaves which are available daily all the year round and proved to be satisfactory diet. One important factor which might alters bioassay values is the larval host plant itself.

Host plants can modify the susceptibility of herbivorus arthropods to pesticides. Aphid species collected from different host plants showed different susceptibilities to primicarb (Furk *et al.,* 1980). Similar observations have been reported in two spider mite (Yang *et al.,* 2001) and lepidoptera (Dominguez-Gil and Mcpherson, 2000). Although host plants effects on susceptibility have been documented in cotton leafworm (Khalil, 1965, Abou El-Ghar *et al.,* 1972, Makady, 1986, Rizk *et al.,* 1988-1989), information is limited about the rapidity of interaction between larvae and its host plants, and the cause of such phenomenon besides its effect on pest management.

The purpose of the present work was three fold : (1) To determine any changes in susceptibility to insecticide between *S. littoralis* larvae reared on the usual laboratory diet (castor bean leaves) and larvae reared on other three different hosts i.e. cabbage or garden rocket or grapevine leaves. (2) If there were changes in susceptibility, the situation was merely related to host plant nutritional value? It is hyposisthyzed that either host plant affects susceptibility via altering activity of detoxification enzymes as a poor nutrient source, or due to the presence of plant chemicals as allelochemicals which might affect activity of enzymes such as glutathione S-transferase, esterases and alkaline phosphatase. (3) Another point was whether host plants effect on susceptibility was a rapid response or due to selection over successive generations.

MATERIALS AND METHODS

Chemicals

Reduced glutathione (GHS), α-naphthyl acetate, β-naphthyl acetate, 1-chloro 2,4-dinitrobenzene, disodium phenyl phosphate, bovine serum albumin and commasie brilliant blue G-250 were purchased from Sigma (Sigma Chemical Co.). 4-aminoantipyrine was purchased from Aldrich Chemical Co. The rest of chemicals were of high quality and purchased from commercial local companies.

Host plants

Four different host plants were chosen (Table 1). They are among many plant species known to attack by cotton leafworm in Egypt. They were collected daily from Menoufia province cultivars and the leaves were introduced to caterpillars as fresh ones.

Table 1. Plant species used in rearing and bioassay tests for *Spodoptera littoralis* larvae.

Common name	Scientific name
Cabbage	*Brassica oleracea*
Grapvine	*Vitis vinifera*
Castor bean	*Ricinus communis*
Garden rocket	*Eruca sativa*

Insecticides

To evaluate susceptibility to insecticides for larvae reared on different host plants, two insecticides were used. One an organophosphorus insecticide, chloropyrifos, and the other was a pryrethroide, cypermethrin. They were used as emulsifiable concentrate (E.C.). Their concentrations were 3.6 and 48% for cypermethrin and chloropyrifos, respectively.

Insects

Newly hatched larvae of S. littoralis were provided from a colony of a laboratory breeding strain, and reared under laboratory conditions (25±2°C, 70±5 R.H.). They were divided into four groups each of which was fed by one of the tested host plants. Each group contained at least 500 larvae (50 larvae/jar). The leaves were replaced daily by fresh ones. Larvae were daily weighed to determine the effect of host plants on their growth. Pesticide bioassays and enzyme determinations were performed after larvae had fed on the host plant for 10 days (mid-fifth larval instar).

Bioassay

To determine the effect of feeding on certain host plants against the susceptibility to insecticides, a series of concentrations for chloropyrifos and cypermethrin were prepared. Freshly collected host plants leaves were dipped for 30 seconds in each concentration then left for one hour to dry. Fifth-instar larvae were

confined with tested leaves in glass jars covered with muslin cloth for 24 h. Control larvae were fed on water-tested leaves. Five replicates each of which had 10 larvae were tested for each concentration. The mortality percentages were recorded and corrected according to Abbott formula (Abbott, 1925). To estimate LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to the method of Finney (1952).

Enzyme assays

Detoxification enzyme activities were measured in fifth larval instar after rearing from egg hatching on four different plant species. Assays were conducted on general esterases, glutathione S-transferase (GST) and alkaline phosphatase (alkpase), which are among the common detoxification enzymes that metabolize pesticides in arthropods.

The larvae were well homogenized in distilled water (5 larvae/5 ml). The homogenation was done at 8000 r.p.m. for 15 min to remove cellular and mitochondrial debris. The supernatant was frozen and stored at -10°C in a deep freezer until used for enzyme assays. All experiments contained 3-5 replicates (enzyme preparations) per treatment, and enzyme incubations were conducted in triplicate.

Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to the method of Van Asperen (1962) using α and β-naphthyl acetate as substrates, respectively. Alkpase was determined according to the method described by Powel and Smith (1954) using disodium phenyl phosphate as substrate. GST was assayed following the method of Habig et al. (1974), where 1-chloro 2,4-dintrobenzene conjugates with reduced glutathione due to enzyme catalysis. The amount of glutathione conjugate formed was calculated using extinction coefficient of 9.6 mM-1 Cm-1, while quantification of the esterases production was based on a standard curve prepared using α and β-naphthol. Alkpase activity was compared to standard enzyme. Enzyme activities were corrected for protein concentration, which was determined by the method of Bradford (1976), using bovine serum albumin as the standard. The absorbance of all reactions was recorded on spectronic 1201 uv/visible spectrophotometer (Milton, Roy Co., USA).

Statistics

Results from LC_{50}'s, weight of larvae and enzyme assays were analyzed by one-way analysis of variance (ANOVA) using Costat statistical software. When the ANOVA F statistic was significant ($P< 0.05$), means by the Duncan's multiple range test were compared.

RESULTS AND DISCUSSION

Castor bean plant is a suitable host for cotton leafworm caterpillars and its leaves are often used for laboratory rearing, and in experiments deal with determination of potency of insecticides or bioassay tests. During evaluation of insecticides effectiveness, in the present work, it was found that LC_{50} of an insecticide significantly varied according to host plant that larvae fed including laboratory diet or castor bean leaves (Table, 2). Feeding on garden rocket leaves significantly lowered LC_{50} for the organophosphorus insecticide, chloropyrifos, than feeding on castor bean leaves (LC_{50} was 6.56 and 14.4 ppm, respectively) LC_{50} was increased when larvae fed on cabbage and grapevine leaves (LC_{50} was 20.9 and 25.66 ppm, respectively). The situation was different for cypermethrin. LC_{50} was significantly decreased for larvae fed grapevine, garden rocket and cabbage leaves as compared to those fed on castor bean leaves. Caterpillars fed on grapevine were the most susceptible to cypermethrin followed by larvae consumed garden rocket and cabbage leaves.

The results clearly demonstrate that bioassay tests obtained from a laboratory strain reared on certain host plant might differ from that in the field where rearing occur on different host plants such as cotton...etc.

Earlier studies (Khalil, 1965, Abou El-ghar *et al.,* 1972) pointed out to the effect of host plant on susceptibility of cotton leafworm to insecticides. Makady (1986) recommended the use of the same dose of pyrethroids to control cotton leafworm on clover and cabbage, due to the susceptibility insignificant difference of the larvae reared on both crops.

The present paper, emphasizes the importance of nutritional ecology of a pest in expressing the potency of insecticides, like the importance of other factors listed by Busvine (1957) such as species, strain, age, sex, temperature.... etc. Also, the rapidity by which host plant can alters the susceptibility of larvae, adds to the significance of such observation. Although, the tested strain was a laboratory strain, reared over generations on castor bean leaves before the start of the present experiments, rearing of larvae from egg hatching to fifth-larval instar (10 days) on different host plants induced valuable changes in susceptibility. Most of studies were conducted after insects had been fed on the host plant for several generations e.g. Rizk *et al.* (1988-1989). They reared cotton leafworm on host plants for 3 successive generations before testing the effect on larval susceptibility, without reporting the cause of such selection. Presumably to ensure significant changes to occur. However, Yang *et al.* (2001) found changes in susceptibility to pesticides after mites feeding on cucumber, maize, or new Lima plants for only 24h.

Table 2. Effect of host plant on susceptibility of fifth-instar of *Spodoptera littoralis* to insecticides.

Insecticide	Host plant	LC_{50} ppm	Ratio of susceptibility
Chlorpyrifos	Castor bean	14.40 ± 1.50^{c}	1.000
	Cabbage	20.90 ± 2.57^{b}	0.688
	Grapvine	25.66 ± 2.08^{a}	0.561
	Garden rocket	6.56 ± 1.00^{d}	2.195
Ceypermethrin	Castor bean	52.00 ± 5.28^{a}	1.000
	Cabbage	43.46 ± 3.10^{b}	1.196
	Grapvine	26.00 ± 4.00^{c}	2.000
	Garden rocket	29.32 ± 3.04^{c}	1.773

- Within a column, means bearing different subscripts are significantly different ($P< 0.05$)
- Ratio of susceptibility was calculated by dividing LC_{50} from larvae fed on castor bean leaves by that of larvae on cabbage or grapevine or garden rocket.
- Data are present as mean±SD.

In an attempt to explain such difference in susceptibility to insecticides, and due to enhanced metabolism by enzymes has been implicated as a major mechanism of resistance to insecticides, a number of detoxifying enzymes were examined. It is clear from Table 3 that host plant could significantly modify the titre of detoxication enzymes. Larvae fed on grapevine had highest alkpase activity. Phosphatases are commonly the major metabolic route of organophosphorus insecticides (O'Brein, 1967). This might explain when such larvae showed the highest $LC_{50}$of chloropyrifos. Larvae fed on castor bean leaves had highest β-esterases and GST activities associated with highest LC_{50} value for cypermethrin. Esterases can play significant role in the detoxification of OP and pyrethroids pesticides. The lowest level of the tested enzymes present in larvae fed garden rocket. They were also the most susceptible larvae. Host plant that increased all of the detoxication enzymes was cabbage leaves. It is interest to note that those larvae showed, relatively, low susceptibility to cypermethrin and chloropyrifos.

Effect of host plant on detoxication enzymes had been studied (e.g. Rizk *et al.,* 1988-1989, Yang *et al.,* 2001, Dominguez-Gil and McPherson, 2000). Many studies failed to correlate between toxicity and enzyme activity in different host plants. In the this respect one can't construct LC_{50}'s of a certain insecticide against certain enzyme activity of larvae fed on different host plants. It might gives false correlation value, due to the variable effects of those different plants on the pest. Dominguez-Gil and McPherson (2000) studied the effect of different host

plants on detoxication enzymes of *Platynota idaeusalis* (Lepidoptera). They found that toxicity and enzyme activity are not clearly linked, reinforcing the complex relationship of the insect with the chemistry of its plant. This means that each host plant has its own specific effect on

Table 3. Host plant effect on detoxication enzyme activities of fifth-instar larvae of *Spodoptera littoralis.*

Host plant	Alkapase$X10^3$ (U/mg protein)	α-esterases (μg α-naphthol/ min/mg protein)	β-esterases (μg β -naphthol /min/mg protein)	GST (μ mol substartae conjugated/ min/mg protein)
Castor bean	16.10 ± 0.84^c	7.11 ± 0.24^c	34.00 ± 1.18^a	1.05 ± 0.05^a
Cabbage	45.83 ± 3.01^b	24.3 ± 1.52^a	33.60 ± 1.99^a	0.814 ± 0.103^b
Grapvine	97.00 ± 4.35^a	14.92 ± 1.11^b	15.27 ± 0.63^b	0.267 ± 0.06^c
Garden rocket	11.47 ± 0.50^c	8.93 ± 0.30^c	13.73 ± 0.639^b	0.277 ± 0.01^c

- Data are present as mean±SD.
- For columns, means bearing different subscripts are significantly different ($P< 0.05$).
- I unit (U) of alkpase corresponds to the amount of enzyme which hydrolyze I μ mol 4-nitrophenyl phosphate per minute at pH 9.8 and 37°C.

larvae, which might affects an enzyme system which in turn might not affected by another host plant.

The observed changes, either decrease or increase, in the titre of detoxication enzymes in the present study, cannot be explained from changes of nutrient requirements point of view, since larvae reared on garden rocket weighed 212 mg/larva (Table, 4) and had the lowest level of the enzymes tested (Table, 3), while the larvae fed on cabbage and grapevine weighed 116 and 127 mg/larva, respectively, showed high significant activity in alkpase and esterases, i.e. the nutritional value of host plant, which could affect growth and enzymes was not critical. Lindroth *et al.*

(1991) reared larvae of the gypsy moth, *Lymantria dispar* on protein-, mineral-and vitamin-deficient diet. They observed few significant effects and no clear-cut patterns of response of detoxication enzymes to nutrient limitation.

On searching about the cause of altering detoxication enzyme activities, it was assumed that the presence of plant allelochemicals was primarily responsible for such variation in the cotton leafworm, *S. littoralis.* A reasonable number of studies suggest this view, Hunter *et al.* (1994) determined that an apple allelochemical, phloridzin, influenced detoxication activities of larval of *P. idaeusalis.* Phloridzin decreased GST activity, inhibited esterase and aniline hydroxylation of the susceptible larvae, but induced higher esterase activity in resistant strain. Dominguez-Gil and McPherson (2000) related such modification in detoxication enzyme activities to the changes in susceptibility of *Platynota idaeusalis* (Walker) to pesticides.

Table 4. Host plant effect on fifth-instar larval weight of *Spodoptera littoralis.*

Host plant	Castor bean	Cabbage	Grapvine	Garden rocket
Larval weight (mg/larva)	263 ± 9.2^{a}	116 ± 4.3^{d}	127 ± 2.0^{c}	212 ± 4.0^{b}

- Data are present as mean±SD.
- Within row, means bearing different subscripts are significantly different ($P< 0.05$)

The data suggest that one can not designate a lethal dose or concentration of an insecticide in bioassay tests without specifying the host plant fed by larvae where the magnitude of tolerance or resistance depends on complex interacting factors including the nutritional ecology of pest. Although resistance to insecticides is a preadaptive character, i.e. genetically determined, this study clearly demonstrates the importance of non-genetic factors (diet) to express such tolerance. One possibility could be the complex plant chemistry which might changes the expression of the enzyme responsible for detoxifying the pesticide.

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تأثير العوائل النباتية علي حساسية دودة ورق القطن للمعاملة بالمبيدات

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تم تربية يرقات دودة ورق القطن علي أربعة أنواع مختلفة من النباتات وهي اوراق الكرنب، العنب ، الجرجير، الخروع وذلك لغرض معرفة تأثير نوع النبات علي حساسية اليرقات للمبيدات متضمنا عمل مقارنة بين اليرقات المغذاه علي ورق الخروع المعتاد استخدامه في التربية المعملية والنباتات الأخري.

وقد وجد أن نوع النبات يؤثر بصورة معنوية علي مقدار التركيز النصفي القاتل للمبيد حيث بلغت مع مبيد الكلوربيروفوس ١٤,٤، ٢٠,٩، ٢٥,٦٦، ٦,٥٦ جزء في المليون وذلك لليرقات المغذاة علي ورق الخروع ، الكرنب، العنب، الجرجير علي التوالي. بينما كانت التر كيز النصفي القاتل لمبيد السيبرمثرين ٥٢، ٤٣,٤٦، ٢٦، ٢٩,٣٢ جزء في المليون لنفس النباتات علي التوالي. مما يدل علي أن بعض نتائج التقييم الحيوي مثل التركيز النصفي القاتل المتحصل عليه في المعمل عند التربية علي ورق الخروع قد لايطابق الحال في الحقل حيث يوجد أنواع مختلفة من العوائل تتغذي عليها اليرقات. ومما يلفت النظر أن التغير في الحساسية للمبيدات كان سريعا حيث تغذت اليرقات علي هذه النباتات لمدة عشرة أيام فقط إبتداء من فقس البيض وحتي العمر اليرقي الخامس.

كما وجد أيضا أن هذا التغيير مصاحب بتغير في نشاط الأنزيمات المسئولة عن هدم المبيدات مثل الإستيريزات والفوسفاتيز القلوي وأنزيم الجلوتاثيون ترانسفيريز وكان لكل نبات تأثيره الخاص علي نوعية من الإنزيمات. وقد رجح أن المواد الكيميائية الموجودة في النباتات هي المسئولة عن هذه التغييرات.

وبصفة عامة فإن هذه الدراسة تؤكد أهمية العوامل البيئية للآفة ومن ضمنها النبات الذي تتغذي عليه الآفة لأظهار صفة وراثية ألا وهي الحساسية للمبيدات.