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Host plants shifting affects the biology and biochemistry of *spodoptera littoralis* (boisd.) (lepidoptera: noctuidae)

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ABSTRACT

The effect of shifting *Spodoptera littoralis* larvae from a host plant to another pointed out that pupal weight, growth index, female longevity and fecundity were affected. Host plants considered were okra, tomato and castor oil plants. Larvae were reared for three successive generations under laboratory conditions (25-30˚C, 70%R.H. and 12D:12L) on each of the three chosen plants, and then shifted to each of the other two hosts.

Larvae reared continuously on castor oil leaves, okra and tomato leaves then shifted to either one of the other plants induced a highly significant decrease in the mean pupal weight. Shifting from the favourable host (castor oil leaves) to either okra or tomato leaves, highly significantly decrease the growth rate and the mean number of eggs deposited by the female. Shifting from castor oil leaves to either okra or tomato induced insignificant increase in the female longevity. Phytochemical analysis showed that, castor oil leaves, possess a high value of total carbohydrates, total proteins and nitrogen meanwhile the phenolic compounds were present at a low value level. Biochemical analysis showed that both essential and non essential amino acid were higher in the hemolymph of the last larval instars of *S. littoralis* reared on castor oil leaves. A noticeable decrement was detected in the amino acid level of larvae fed on okra leaves followed by more decrement in larvae fed on tomato leaves.

Key words: *Spodopter littoralis* –Host plants – Fecundity – Hemolymph – Amino acid - Phytochemical analysis

INTRODUCTIOU

Most insects have qualitatively similar nutritional requirements since the basic chemical composition of their tissues and their metabolic processes are generally similar. Most of these requirements are normally met by the diet. Some chemicals can only be obtained in the diet, they are essential. Others may be synthesized by the insect from dietary components (Chapman, 2002).

The cotton leaf worm, *Spodoptera littoralis* Boisd. is a well polyphagous herbivore and is regarded as one of the most important agricultural pest in the Middle East. It is a very harmful potential pest of many crops including cotton, alfalfa, peanut, potato, lettuce, celery, pepper and tomato. Consequently, several authors namely (Badr, 1982; Rizk *et al*., 1988; Mohamed, 2003) contributed to the biology of *S. littoralis* and the effect of different host plants on its development and reproduction capacity.

The present work aims to study the effect of shifting from a host plant to another on the female pupal weight, longevity, growth index and fecundity; and to study the changes in free amino acids composition of hemolymph larvae in accordance with the leaf composition of the different host plants used.

MATERIAL AND METHODS

Stock colony:

The cotton leaf worm, *Spodoptera littoralis* Boisd. (*S.littoralis*) colony has been maintained in the laboratories of the Zoology Department, Faculty of Science, Zagazig University, under room conditions of 25-30˚C, 70% R.H., and photoperiod 12D: 12L. Ten larvae were kept in glass jar (15x9x20cm) to avoid the production of undersized individuals. The larvae were fed on castor oil leaves (*Ricinus communis*) for three successive generations. Fresh clean leaves were supplied daily. The glass jars were thoroughly cleaned every day. They were washed in formalin and soap and placed for few hours in oven in order to avoid any possible contamination with viral infection (fatal polyhydrosis). Prepupae were removed and placed in wooden cages (70x90x50cm) with wire gauze sides (2mm. meshes). The floor of these cages was covered with a layer of fine autoclaved wood dust which was always kept moist with water, as relatively high humidity is essential for the formation of the cocoons. Emerging adults were fed on 10%sugar solution and offered fresh twig of *Nerium oleander* carrying about 10 leaves to serve as ovipositional site. Deposited egg batches were transferred to a glass jar (15x9x20cm), provided with fresh clean castor oil leaves and secured with tightly muslin caps and they were left to hatch and so on.

Biological studies:

The experimental food plants were castor oil leaves (*Ricinus communis*), tomato leaves (*Lycopersicon esculentum*), and okra leaves (*Hibiscus esculentus*).
The egg batches obtained from females were kept in glass jars secured with tightly muslin caps. Three groups of 50 newly hatched larvae of *S.littoralis* (each) considered as control were offered fresh clean castor oil, okra and tomato leaves in a glass jar (15x9x20cm) continuously. Newly hatched larvae, from each host plant were reared for three successive generations under constant conditions of 25-30˚C and 70%R.H. Starting from the fourth generation, 5th instar larvae from each group were shifted to each of the other two host plants on which they were reared for another two generations. The effects of host plants on the female pupal weight, female adult longevity, growth index and fecundity for each host plant in every generation were calculated.

Phytochemical composition of the different host plants:

Plant leaves of castor oil, okra and tomato were dried in the oven, and prepared for spectrophotometer analysis. Total carbohydrates were measured according to Dubois *et al.* (1951) and Naguib (1964). Total protein were determined according to the method of Bradford (1976).Total nitrogen was determined according to Russell (1944). Phenolic compounds were extracted from dried leaves according to the method of Jindal and Singh (1975).

Amino acid composition of *S.littoralis* larvae:

Hemolymph of the last larval instars reared continuously on castor oil, okra and tomato leaves, was collected (after cutting a proleg, on ice) in apropylene microcentrifuge tube containing few crystals of phenylthiourea to prevent melanization. The hemolymph was centrifuged at 3000r.p.m. for 5min. at 4˚C to remove hemocytes. All the hemolymph samples were kept at -20˚C until amino acid analysis was carried out. The samples were hydrolysed in sealed, evacuated ampoules in an oven at 110˚C for 16h. The extraction and analysis were performed according to the methods described by Rashad and Abdel Zaher (2008).

RESULTS

The effect of shifting the larvae from a host plant to another, on the female pupal weight, female adult longevity, growth index and fecundity was presented in Table (1).

Table (1): Effect of shifting the larvae from a host plant to another on the female pupal weight, female longevity, growth index and fecundity of *Spodoptera littoralis*

Host plants	Generation	Female pupal weight (mg) Mean ± S.E.	Female longevity (days) Mean ±S.E	Growth index Mean ± S.E	Fecundity (No.eggs/female) Mean ± S.E
Castor oil leaves continuously	F1 F2	370.87±17.82 356.45±15.42	5.63 ± 0.5 5.44 ±0.5	3.09 ±0.05 3.07 ±0.18	1326.27±81.36 1266.37±73.64
Okra leaves after castor oil leaves	F1 F2	359.65±43.04* 322.22±41.35**	6.84±0.66 6.82±0.62	2.44±0.14** 2.73±0.138*	1081.08±72.19** 1019.11±64.23**
Tomato leaves after castor oil leaves	F1 F2	310.01±20** 319.75±23.66**	7.61±0.57 7.21±0.61	1.76±0.07** 1.97±0.11**	869.31±45.39** 926.44±47.21**
L.S.D. (0.05-0.01)	F1 F2	(11.292-15.321) (11.451-15.135)	(2.41-3.18) (2.31-3.06)	(0.39-0.51) (0.58-0.77)	(26.95-22.56) (24.81-32.79)
Okra leaves continuously	F1 F2	249.13±14.78 238.85±15.87	6.51±0.453 6.21±0.55	2.35±0.09 2.46±0.05	978.30±64.72 830.75±42.83
Castor oil leaves after okra leaves	F1 F2	208.26±19.13** 249.78±16.88**	6.46±0.50 6.01±0.44	2.47±0.16** 2.71±0.06**	1011.76±69.41** 1197.73±72.33**
Tomato leaves after okra leaves	F1 F2	191.73±6.52** 205.06±5.78**	9.13±0.80* 8.51±0.49	1.42±0.06** 1.45±0.12**	739.76±51.15** 906.53±39.18**
L.S.D. (0.05-0.01)	F1 F2	(5.725-7.567) (5.460-7.216)	(2.39-3.15) (1.96-2.59)	(0.44-0.58) (0.38-0.50)	(20.64-24.65) (21.20-28.03)
Tomato leaves continuously	F1 F2	229.13±15.21 261.42±14.75	7.95±0.62 7.83±0.46	1.41±0.12 1.47±0.15	712.85±39.92 866.47±44.83
Castor oil leaves after tomato leaves	F1 F2	214.39±14.34** 234.51±13.04**	6.49±0.70 6.48±0.37	2.01±0.25 2.12±0.36	894.24±63.94** 1017.21±72.13**
Okra leaves after tomato leaves	F1 F2	203.73±16.52** 199.11±12.33**	8.51±0.73 7.41±0.61	1.68±0.24 1.93±0.24	758.28±55.31** 737.79±49.44**
L.S.D. (0.05-0.01)	F1 F2	(6.09-5.103) (5.307-7.015)	(2.70-3.57) (1.94-2.57)	(0.83-1.10) (1.05 -1.38)	(21.38-17.90) (22.47-29.70)

I- Shifting from castor oil leaves to okra and tomato leaves

Shifting the larvae from castor oil leaves to okra leaves decreased significantly ($P<0.05$) and highly significantly ($P<0.01$) the mean **female pupal weight**, during the F1 and F2 generation, respectively. On the other hand, shifting the larvae from castor oil leaves to tomato leaves showed a highly significant decrease ($P<0.01$) in the female pupal weight during F1 and F2 generations. Statistical analysis between F1 and F2 showed a highly significant ($P<0.01$) increase in female pupal weight of larvae fed castor oil leaves continuously. When larvae were shifted to okra leaves a highly significant decrease in the female pupal weight of F2 compared to F1 was observed. However, no significant increase ($P>0.05$) in the female pupal weight was observed when larvae were shifted to tomato leaves in F1 and F2.

Shifting the larvae continuously reared on castor oil leaves to okra and tomato leaves, insignificantly increase ($P>0.05$) the **female adult longevity** during F1 and F2. Comparison between the two generations showed that female adult longevity resulting from larvae continuously reared on castor oil leaves or shifted to okra or to tomato leaves, were insignificantly increase during F1 (P>0.05) as compared to those of F2 generation.

The mean **adult growth index** showed a highly significant decrease (P < 0.01) when larvae were shifted from castor oil leaves to okra and tomato leaves during F1. However, it decreased significantly (P<0.05) and highly significantly (P<0.01) during F2. Statistically, no significant differences (P>0.05) were observed between the two generations when larvae were reared on castor oil leaves continuously or when shifted to okra or tomato leaves.

A highly significant increase (P<0.01) was observed in the mean **number of eggs laid per female** resulting from larvae continuously reared on castor oil leaves than for larvae shifted to okra and tomato leaves during the F1 and F2 generations. Moreover a highly significant decrease (P < 0.01) was observed in the mean number of eggs laid by females from F1 and F2 generation despite all the diets offered to the larvae.

II- Shifting from okra leaves to castor oil and tomato leaves

Results revealed a highly significant decrease (P<0.01) in the mean **female pupal weight** when larvae were reared continuously on okra leaves during F1 and F2 as well as when shifted to castor oil and tomato leaves during F1. However, a significant increase (P<0.05) was noticed in F2 when larvae were shifted to castor oil leaves although a highly significant decrease (P < 0.01) in the F2 was recorded when shifted to tomato leaves. Comparison between the two generations in each host plant, revealed a significant decrease (P<0.05) in the female pupal weight for larvae offered okra leaves continuously during the F1 as compared to F2, and a highly significant increase (P<0.01) when larvae shifted to castor oil and tomato leaves during F1 as compared to F2.

The **female adult longevity** showed insignificant decrease (P>0.05) in the F1 and F2 when larvae were shifted from okra leaves to castor oil leaves**.** However, a significant (P < 0.05) and insignificant increase (P>0.05) was observed in the F1 and F2 generations, respectively when the larvae were shifted to tomato leaves.

When larvae were reared on okra leaves continuously and were shifted to castor oil leaves, insignificant decrease (P > 0.05) in the female adult longevity during F1 and F2 was noticed, meanwhile when larvae were shifted from okra leaves to tomato leaves a highly significant decrease (P < 0.01) in the female adult longevity was observed.

Results indicated that a highly significantly increase (P<0.01) in the mean **growth index** when larvae were shifted to castor oil leaves in F1 and F2, meanwhile a highly significant decrease (P < 0.01) was recorded when larvae were shifted to tomato leaves in the two generations. Statistical analysis between F1 and F2 revealed insignificant differences (P>0.05) in the growth index regarding all the food offered to the larvae.

A highly significant increase and decrease (P < 0.01) were noticed in **the mean number of eggs laid by females** when shifting larvae from okra leaves to castor oil leaves, during F1 and F2 respectively and when shifting larvae from okra leaves to tomato leaves. Statistically a highly significant decrease (P<0.01) was observed between F1 and F2 as larvae fed continuously on okra leaves, while a highly significant increase (P<0.01) was observed between the two generations when larvae were shifted to castor oil and tomato leaves, respectively.

III- Shifting from tomato leaves to castor oil and okra leaves

The mean **female pupal weight** highly significantly decrease (P<0.01) during F1 and F2 when larvae were shifted to castor oil and okra leaves after tomato leaves. On the other hand, a high significant difference (P<0.01) was noticed between F1 and F2 in the female pupal weights as larvae fed on the three mentioned hosts.

The **female longevity** decreased insignificantly (P>0.05) when larvae were reared on tomato leaves continuously and where shifted to castor oil leaves during F1 and F2. However larvae shifted to okra leaves showed insignificant increase during F1 and insignificant decrease during F2.

When larvae were reared on tomato leaves continuously and shifted to castor oil leaves no significant difference (P>0.05) was recorded between the two generations, however, a significant difference (P<0.05) was observed between the two generations when they were shifted to okra leaves.

The **growth index** of adult *S.littoralis* showed insignificant increase (P>0.05) as larvae reared on tomato leaves and then shifted to castor oil and okra leaves. Also, no significant difference (P>0.05) was noticed between the two generations as larvae were reared on all types of food.

The mean **number of eggs laid by female** *S. littoralis* shifted on castor oil leaves increased highly significantly (P<0.01) during F1 and F2. When larvae shifted to okra leaves, the mean number of eggs laid by the female highly significantly increased (P<0.01) during F1 and highly significantly decreased (P<0.01) during F2. Statistical analysis revealed a high significant difference between the two generations.

Phytochemical composition of the different host plants:

Chemical composition of the castor oil, okra and tomato leaves revealed a significant decrease (P<0.05) in the total carbohydrates in okra leaves and a high significant decrease (P<0.01) in tomato leaves as compared to castor oil leaves (Table 2). A high level of total proteins and nitrogen in castor oil leaves, and a low level were detected in tomato leaves. Also, results in Table (2) revealed a high level of phenolic compounds in tomato leaves as compared to okra leaves and castor oil leaves.

Table (2): The mean total carbohydrates, total proteins, nitrogen and phenoilc concentrations of the castor oil, okra and tomato leaves.

Host plant leaves	Total carbohydrates(g/100g) Mean ± S.E.	Total proteins(g/100g) Mean ± S.E	Nitrogen (g/100g) Mean ± S.E.	Phenolic (g/100g) Mean ± S.E.
Castor oil	28.450 ± 0.298	16.439 ± 0.197	3.131± 0.081	1.501± 0.055
Okra	26.011±0.273*	14.112 ± 0.168*	2.688 ± 0.055	2.962 ± 0.108*
Tomato	23.831± 0.246**	10.382 ± 0.099**	1.978 ± 0.023*	4.710 ± 0.157**
L.S.D. (0.05-0.01)	(2.406 - 3.213)	(2.173 – 3.772)	(0.871 – 1.21)	(1.216 – 2.316)

Amino acid compositions:

The amino acid levels in the last instar larval hemolymph of *S. littoralis* fed as larvae on castor oil leaves, okra leaves and tomato leaves continuously, were depicted in Table (3). Results revealed that β- aminoisobutyric was the most predominant amino acids in castor oil leaves, okra leaves and tomato leaves followed by tyrosine in castor oil leaves only. On the other hand, the least amino acid levels were methionine in castor oil leaves, taurine in okra leaves and cystathionine and phenylalanine in tomato leaves.

The essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) together with the non-essential amino acids (aspartic, glutamic, glycine, serine and tyrosine) showed a decrease level as the larval diets changed from castor oil leaves to okra and tomato leaves.

Table (3): Amino acid compositions of the hemolymph of *Spodoptera littoralis* larvae reared on three host plant leaves.

Amino acids	Concentration µg / ml		
	Castor oil leaves	Okra leaves	Tomato leaves
Arginine	92.84	5.52	6.91
Histidine	125.34	64.19	37.11
Isoleucine	44.79	19.36	13.15
Leucine	58.39	23.89	13.10
Lysine	113.53	47.72	-
Methionine	14.35	5.35	5.50
Phenylalanine	27.56	5.82	2.29
Threonine	50.46	34.49	12.61
Valine	45.86	17.50	7.67
Alanine	21.26	7.58	-
Aspartic acid	25.94	9.30	6.32
Cystine	46.69	-	-
Glutamic acid	73.35	26.63	16.80
Proline	125.57	-	-
Glycine	60.30	21.97	17.29
Serine	183.92	28.11	19.66
Tyrosine	343.60	22.07	14.49
α-aminoadipic acid	183.63	23.00	13.44
β-aminoisobutyric	347.71	183.42	62.19
γ-aminobutyric	48.04	49.94	3.86
Ornithine	24.60	5.04	-
Cystathionine	-	12.70	1.30
Taurine	-	4.23	-
Phosphoserine	31.72	21.32	9.33
Total pool	2089.45	639.17	265.91

Also, the amino acids α- aminoadipic, β- aminoisobutyric, γ-aminobutyric and phosphoserine highly decreased as the larval diet changed from castor oil leaves to okra and tomato leaves. Whereas, alanine and ornithine were not detected as larvae fed on tomato leaves.

Hemolymph amino acid of larvae fed on castor oil leaves was characterized by the presence of proline and cystine in moderate level and by the absence of cystathionine and taurine.

DISCUSSION

Data presented in Table (1) emphasize that the development and fecundity of *S. littoralis* was affected by shifting from a host plant to another. Shifting *S. littoralis* larvae previously maintained for 3 generations on castor oil, okra and tomato leaves to either okra or tomato leaves induce a highly significant decrease in the mean female pupal weight, growth index and number of eggs deposited by female. However a prolongation of the female adult longevity was observed when shifting from castor oil leaves to either okra or tomato leaves. These results were in agreement with the findings of Badr *et al*. (1983) for *Spodoptera littoralis* shifted from cotton leaves (favourable host) to castor oil and clover leaves; Mohamed (2003) for *S. littoralis* reared on castor oil, cotton, clover, lettuce and broad bean leaves.

Insects, like all living organisms require energy and nutrient to survive grow and reproduce. Table (2) revealed during this study that castor oil leaves was the best host for *S. littoralis* larvae, this might be due to the high levels of total carbohydrates, total protein and nitrogen and the low level of phenolic compounds recorded in castor oil leaves. Similar results were reported by Mohamed (2003) for *S.littoralis* larvae reared continuously on castor oil, cotton, clover, lettuce and broad bean, by Berlinger (1984) who stated that carbohydrates form a large part of the diet of many insects, although that they are a common source of energy, not always essential, are usually necessary for normal growth. During the present study, *S.littoralis* offered tomato leaves either continuously or after shifting to castor oil, and okra leaves caused a high significant decrease in female pupal weight, growth index and fecundity. This might be due to the poor nutritional quality in the tomato leaves, poor content of carbohydrates, proteins and nitrogen as revealed by the phytochemical analysis (Table 2) (Slansky and Scriber, 1985); or the ability of tomato to manufacture proteinase inhibitors once stimulated by this chewing insect, resulting in an inhibition of insect's digestion of tomato leaves protein (Steinberg *et al*., 1993).

High levels of both essential and non essential amino acids in the hemolymph of the last larval instar of *S. littoralis* were obtained when the larvae fed on castor oil leaves. However, a noticeable decrement when larvae shifting from castor to okra leaves. Moreover, more decrement was observed as larvae fed on tomato leaves. This decrease in the concentration of essential amino acids when larvae were fed okra and tomato might be due to a deficiency in the metabolism of the amino acids (Abdel-Meguid *et al*., 2001). The hemolymph of larvae fed tomato leaves is characterized by the absence of lysine which is an essential amino acid, also alanine, cystine, proline and ornithine. Meanwhile okra leaves was characterized by the absence of cystine and proline (Table 3), the latter was described as an important source of energy.

The amino acids were usually present in the diet as proteins, and the value of any ingested protein to an insect depends on its amino acids content and the ability of the insect to digest it. Table (3) showed that proteins contain 20 different amino acids of which 10 of these are essential in the diet; absence of any one prevents growth. Although the other 10 amino acids are not essential, but they are necessary for optimal growth. Their synthesis lead to conversion from essential amino acids resulting in energy consuming (Chapman, 2002).

β-aminoisobutyric acid and serine have very high level in the hemolymph of larvae fed castor oil leaves, where the former is obtained by the degradation of thymine and serine is synthesized from 3-phosphoglycerate which is oxidized and transaminated to phosphoserine and then hydrolyzed to serine. Serine is the precursor of glycine and cystine (Stryer, 1988). Chapman demonstrated γ-aminobutyric acid and glutamate are neurotransmitters. Moreover, histidine which is an essential amino acid linked to the pathway of purine synthesis, while threonine was considered as one of the end products of succinyl coenzyme A catabolism.

Biochemical analysis of *S.littoralis* larvae fed on different host plants continuously revealed the presence of proline only in the hemolymph of larvae reared on castor oil this might explain the high mean number of eggs laid by the resulting female as proline increase the egg laying capacity. Similar observations were stated by Hrassnigg *et al*. (2003) for *Apis mellifera* queens as proline accounts for more than 50% of the total content of free amino acids in egg-laying capacity.

Hemolymph of larvae fed okra leaves revealed the presence of taurine in low level. This result was confirmed by Bodnaryk (1981), who detected taurine in the flight muscles of moth larvae of *Mamestra configurata*. Whitton *et al. (*1987) found taurine in the flight muscles of *Schistocerca americana gregaria*, *Blatella orientalis* and *Tenebrio molitor*. They stated that stress due to picrotoxin poisoning caused the release of taurine from the muscles into the hemolymph.

In conclusion, the present results revealed that the reproductive capacity of *S. littoralis* was influenced by shifting the larvae from a host plant to another. Thus, castor oil leaves might be the most favourable host for *S. littoralis* larvae followed by okra leaves while tomato leaves was the less favourable one due to the differences in the leaf nutritional quality as mentioned during phytochemical analysis and the high level of amino acid in the hemolymph of larvae reared on castor oil leaves.

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ARABIC SUMMARY

تبديل العوائل النباتية و تأثيره على بيولوجية وبيوكيميائية حشرة سبودوبترا ليتوراليس

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تم فى هذه الدراسة إستخدام ثلاثة عوائل لتربية دودة ورق القطن تحت الظروف المعملية و هى أوراق زيت الخروع و البامية و الطماطم لمدة ثلاثة أجيال متتابعة ثم تبديلها من عائل إلى العائلين الأخرين. وقد أظهرت الدراسة أن تبديل يرقات *سبودوبترا ليتوراليس* من عائل إلى أخر قد أثر على وزن العذارى و دليل النمو وعمر الاناث الناتجة و خصوبتها. و قد أدت تربية اليرقات تربية مستمرة على أوراق زيت الخروع و البامية و الطماطم ثم تبديلهم من عائل إلى العائلين الأخرين إلى نقصان معنوى عالى فى متوسط أوزان العذارى الناتجة عن هذا التغيير. بينما أدى تبديل اليرقات المرباة على أوراق زيت الخروع المفضلة ليرقات دودة القطن إلى كل من أوراق البامية و أوراق الطماطم إلى نقصان معنوى عالى فى دليل النمو و الخصوبة للأناث و إلى زيادة غير معنوية في عمر الأناث الناتجة عن هذا التبديل.

و قد أظهرت التحليلات الكيميائية لأوراق النباتات المستخدمة (الفيتو كيميا ئية) أن أوراق زيت الخروع تتميز بمحتوى عالى من الكربوهيدرات و البروتينات و النيتروجين و محتوى منخفض من المركبات الفينولية. كذلك أظهرت التحليلات البيوكيميائية للعمر اليرقى الاخير، أن الأحماض الأمينية الأساسية وغيرالاساسية قد وجدت بنسب عالية فى دم اليرقات المرباه على أوراق زيت الخروع بينما وجدت بنسب أقل فى دم اليرقات المرباه على أوراق البامية ثم المرباه على أوراق الطماطم.