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CONSUMPTION AND UTILIZATION OF CASTOR BEAN LEAVES TREATED WITH NEEM SEED KERNEL EXTRACTS BY THE COTTON LEAF WORM LARVAE, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Larva of cotton leaf worm, *Spodoptera littoralis* ate and digested an average of 13.79 and 10.82g, respectively of untreated castor bean leaves during its whole sixth instar. These amounts significantly reduced when larvae were fed on leaves treated with different concentrations of neem seed kernel methanolic extract. The reduction was concentration dependent, i.e., an increase in neem extract concentration caused an obvious decrease in the amount of ingested or digested leaves. The consumption and digestion indices are always negative indicating that treated castor bean leaves are not preferred by *S. littoralis* larvae for feeding. These indices decreased as the concentrations of neem extract increased**.** Approximate digestibility in *S. littoralis* sixth instar larvae averaged 78.50%. This percentage insignificantly fluctuated between 77.71 and 81.08% when larvae were fed on castor bean leaves treated with different concentrations of neem extract. Efficiencies of conversion of ingested and digested food to body substances (ECI and ECD) are an overall indicators for ability of *S. littoralis* larvae to utilize ingested and digested food for growth and food storage in the insect. Such values depend upon the food digestibility, its nutritional values and the ingestion rates. ECI and ECD in larvae fed on untreated leaves reached 22.25 and 28.25%, respectively. These values decreased after feeding the larvae on treated leaves. The decrease was gradually occurred as the concentration of neem extract increased to reach the minimum value at the highest concentration (0.025%).

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INTRODUCTION

Neem derivatives have a variety of effects against a wide variety of insect pests including antifeedant and feeding deterrenc, growth regulation inhibitor, toxicity and sterility effects **(Raguraman and Singh, 1999)**. Such derivatives are primarily considered as toxicants to larvae or nymphs of phytophagous insects at required concentrations and hence show considerable selectivity toward natural enemies of insect pests such as parasitoids and predators (**Awad *et al* 1998**). Therefore, these derivatives have potential for use in integrated pest management.

The feeding deterrent activity of Neem Azal-S and Margosan-O (a pesticidal products of neem seeds) was found to be significant against the adults of *Liriomyza trifolii* (Burg.), particularly at high concentrations **(Dimatry *et al* 1995)**. **Deka *et al* (1999)** suggested that methanol extract from *Melia azadirach* showed antifeedant activity to tea mosquito bug *Helopeltis theivora*. Margosan-O, besides being toxic, also has feeding, and growth-inhibitory effects on certain insects **(Scott and Kaushik, 1998)**.

Recently, **Gomaa, (2009)** studied the effects of different concentrations of neem seed kernel aqueous extracts using different types of organic solvents on oviposition deterrency of mated *Spodoptera littoralis* (Boisd.) female moths in free choice test and the ovicidal action of such extracts on the deposited eggs. He found that the methanol extract is considered as the most active solution as oviposition deterrent and ovicidal compound. The same findings were given by **Ayyangar and Rao, (1990)** on *Spodoptera litura* (Fabr.) and **Dilawari *et al* (1994)** on diamondback moth, *Plutella xylostella.*

The present work aimed at studying the consumption and utilization of castor bean leaves treated with differe4nt concentrations of neem seed kernel aqueous solutions extracted with methanol by the last instar cotton leaf worm larvae, *Spodoptera littoralis* (Boisd.).

MATERIALS AND METHODS

Rearing technique

The cotton leaf worm, *S. littoralis* was obtained from cotton fields in Shalakan, kalyobia Governorate in form of egg masses, transferred to the laboratory and reared in glass containers as previously described by **Gomaa, (2006).** Soon after hatching, larvae were provided daily with sufficient amounts of clean and fresh castor oil leaves, *Ricinus communis* (L.) as food, kept at laboratory conditions of 25± 3ºC and 70± 4% R.H. for several generations to ensure complete adaptation. The population density of larvae in each 2 kg capacity container decreased as the larval instar progressed (20 larvae for the first and second instars, 10 individuals for the third and fourth instars, and 5 larvae for the fifth and sixth instars) to avoid over crowding. Upon pupation, pupae of the same age were collected, sexed and observed daily till moth emergence. Couple of male and female moths was transferred to a wooden breeding cage supplied with a branch of *Nerium oleander* (Tafla), which was fixed in small glass vial (5 cm in height and 3 cm in diameter) and both were situated in the cage for egg deposition. A piece of cotton wool soaked in a 20% sugar solution was immersed as a wick in a small glass vial and put in the cage as a source of nutrient for moths.

Preparation of the extracts

Neem seed kernels were dried under laboratory conditions, ground into fine powder and 50 g were extracted with 100 ml of methanol by using soxhlet extractor. The obtained extract was evaporated in a rotary evaporator under vacuum until dryness. The residue was weighed and dissolved in the same amount of distilled water after adding few drops of Tween-80 as emulsifier to obtain water emulsions of known neem seed kernel extract concentration as previously described by **Gomaa, (2009)**. The aqueous solution was kept in refrigerator. Just before starting the experiment, the following concentrations were made: 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% to study their effects on consumption and utilization of food by the sixth instar larvae.

Consumption and utilization of castor bean leaves by larvae

Castor bean leaves were collected daily, cleaned, treated by dipping them for about 5 seconds in one of the tested concentration of the stored neem seed kernel extract and dried at laboratory conditions. Another group of castor bean leaves was left untreated as control.

Newly moulted sixth instar *S. littoralis* larvae were offered sufficient amounts of known weights of fresh treated castor bean leaves daily. To calculate the daily natural loss of water from the leaves, similar weights of leaves were kept in a separate container without larvae to obtain the daily evaporation rate of leaves (ER) under the same laboratory conditions as follows:

ER = Weight of fresh leaves(g)/ Weight of the same leaves in the following day (g)

The remaining dry leaves in the containers having larvae were collected daily, weighed and their weights were corrected to be fresh using the following formula:

Weight of fresh remnants (g) = Weight of dry remnants (g) x ER

The fecal pellets were sifted daily, weighed and recorded. The experimental larvae were also weighed daily.

To estimate the mean weights of fresh castor bean leaves either treated with different concentrations of neem seed kernel extract or untreated, which were consumed and assimilated by each larva during the whole sixth instar, the following equations suggested by **Waldbauer, (1964&1968)** and modified by **El-Shaarawy and Gomaa, (1975)** were used:

Weight of fresh ingested leaves (g) (I) = weight of fresh leaves offered to larvae (g) – weight of fresh remnants (g)

Approximate weight of fresh digested leaves (g) (D) = I (g) – weight of fecal pellets (g)

Approximate digestibility (%) (AD)= (D / I) x 100

Efficiency of conversion of ingested food to body substances (ECI) (%) = {Increase in weight of larva during the Instar (g) (S) / I } x 100

Efficiency of conversion of digested food to body substances (ECD) (%) = (S / D) x 100

The consumption and digestion indices (CI and DI) for each concentration of the neem seed kernel extract were calculated according to the following formula suggested by **Udayagiri and Mason (1997)** with slight modification:

CI = (It –Ic) / (It + Ic)
DI = (Dt – Dc) / (Dt + Dc)

Where,

It, weight of ingested treated leaves (g)
Ic, weight of ingested untreated leaves (g)
Dt, weight of digested treated leaves (g)
Dc, weight of digested untreated leaves (g)

Each index value takes a positive or negative figure indicating castor bean leaves that preferred by *S. littoralis* larvae for feeding (positive value) and those that are not preferred (negative value).

The obtained data were statistically analyzed and the "F" values as well as the L.S.D. at 0.05 level of probability were calculated.

RESULTS

Results of a previous work **(Gomaa, 2009)** showed highly toxicity of neem seed kernel extracts to *S. littoralis* at 0.025%. Therefore, lower concentrations (0.0015, 0.0031, 0.0062 and 0.0125 and 0.025%) were used to study their effects on the viability of deposited eggs. The same concentrations were chosen in the present work to study their effects on rates of consumption and utilization of treated castor bean leaves by the last instar *S. littoralis* larva.

1- Weight of consumed castor bean leaves

As shown in **Table (1)** *S. littoralis* larva ate an average of 13.79g untreated castor bean leaves during its whole sixth instar. This amount significantly reduced (7.983 g/larva) when larvae were fed on treated leaves with different concentrations of neem seed kernel extract. The reduction in this case was concentration dependent, i.e., an increase in neem extract concentration caused an obvious decrease in the amount of ingested leaves. Means of 10.15, 8.90, 7.42, 7.04 and 6.41g castor bean leaves treated with 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% neem extract were ingested by the sixth instar larva, representing the rates of food repellency of 26.36, 35.46, 46.12, 48.91 and 53.51%, respectively.

The difference between the mean weights of consumed food by single *S. littoralis* larva as being affected by the concentrations of neem extract proved to be statistically highly significant. By applying the L.S.D. value (1.215 g), the amounts of consumed leaves could be arranged into the following four descending groups:

First group : Untreated leaves (13.79g)
Second group : Leaves treated with 0.0015% neem extract (10.15g)
Third group : Leaves treated with 0.0031% neem extract (8.90g)
Fourth group : Leaves treated with 0.0062, 0.0125 and 0.025% neem extract (7.42, 7.04 and 6.41g, respectively).

Values of the consumption indices are always negative indicating that treated castor bean leaves are not preferred by *S. littoralis* larvae for feeding. These indices decreased as the concentrations of neem seed kernel extract increased, being -0.1518, -0.2155, -0.3005, -0.3285 and -0.3653 at the concentrations of 0.0015, 0.0031, 0.0062, 0.0125 and 0.035%, respectively.

2- Approximate weight of digested castor bean leaves

As in case of the quantity of consumed food, the same trend could be applied for the approximate mean weight of digested castor bean leaves treated with different concentrations of neem extract in the the larval midgut.

The data given in **Table (2)** clearly show that *S. littoralis* larva normally digested and utilized 10.92 g of untreated castor bean leaves during the whole sixth instar. As a general, larvae that fed on treated leaves digested less amount (6.33g leaves/ larva) of ingested food. The decrease in food digestion, in this case, was concentration dependent. Means of 8.17, 7.00, 6.01, 5.47 and 5.09g were digested in the larval gut when castor bean leaves were treated with 0.0015, 0.0031, 0.0062, o.o125 and 0.025% neem seed kernel extract, respectively.

Highly significant difference was noticed between the mean weights of digested food by single larva as being affected by the concentrations of neem extract. By applying the L.S.D. value (0.924g), the amounts of digested leaves could be arranged into the following four descending groups:

Table 1. Weight of castor bean leaves consumed by the sixth instar *S. littoralis* larva after treating them with different concentrations of aqueous solution of neem seed kernel methanolic extract (Means of 10 individuals ± S.E.)

Concentration of extract (%)	Weight of consumed castor bean leaves (g/ larva)	Food repellency (%)	Consumption Index (CI)
0 (Control)	**13.786±0.542a**	**-**	**-**
0.0015	10.152±0.347b	26.36±2.22c	-0.1518
0.0031	8.898±0.331c	35.46±2.95b	-0.2155
0.0062	7.415±0.295d	46.21±3.17a	-0.3005
0.0125	7.043±0.303d	48.91±3.22a	-0.3285
0.0250	6.409±0.274d	53.51±4.05a	-0.3653
Mean for treated leaves	**7.983±0.328**	**42.09±3.64**	**-0.2666**
F between concentrations	18.64**	24.25**	
L.S.D. at 0.05	1.215	8.011	

Table 2. Approximate weight of castor bean leaves digested by the sixth instar *S. littoralis* larva after treating them with different concentrations of aqueous solution of neem seed kernel methanolic extract (Means of 10 individuals ± S.E.)

Concentration of extract (%)	Weight of ingested castor bean leaves (g/ larva)	Food repellency (%)	Digestion Index (DI)
0 (Control)	**10.822±0.438a.**	**-**	**-**
0.0015	8.167±0.402b	24.53±.1.99c	-0.1398
0.0031	6.996±0.359c	35.35±2.27b	-0.2147
0.0062	6.012±0.327d	44.45±1.87a	-0.2857
0.0125	5.473±0.321d	49.43±3.01a	-0.3283
0.0250	5.094±0.305d	52.93±2.84a	-0.3599
Mean for treated leaves	**6.330±0.412**	**41.33±2.62**	**-0.2619**
F between concentrations	16.83**	19.45**	
L.S.D. at 0.05	0.924	8.76	

First group : untreated leaves (10.82g)
Second group: Leaves treated with 0.0015% neem extract (8.17g)
Third group : Leaves treated with 0.0031% neem extract (7.00g)
Fourth group : Leaves treated with 0.0062, 0.0125 and 0.025% neem extract (6.01, 5.47 and 5.09g, respectively).

As shown in **Table (2)**, the digestion indices are negative indicating that the rate of digestion of treated castor bean leaves by *S. littoralis* larvae are always lower than feeding on untreated leaves. These indices decreased as the concentrations of neem seed kernel extract increased, being -0.1398, -0.2147, -0.2857, -0.3283 and -0.3599 at the concentrations of 0.0015, 0.0031, 0.0062, 0.0125 and 0.035%, respectively.

3- Approximate digestibility

The data given in **Table (3)** clearly show that approximate digestibility in *S. littoralis* sixth instar larvae fed on untreated castor bean leaves averaged 78.50%. This percentage fluctuated between 77.71 and 81.08% when larvae were fed on leaves treated with different concentrations of neem seed kernel extract. The difference between these mean percentages proved to be statistically insignificant. This means that the amount of secreted digestive enzymes was positively correlated with the amount of ingested either treated or untreated food and, thus no apparent difference was noticed between the digestibility values.

4- Efficiency of conversion of ingested food to body substance (ECI)

ECI is an overall indicator for ability of *S. littoralis* larvae to utilize ingested food for growth and food storage in the insect. Such value depends upon the food digestibility, its nutritional value and the ingestion rate.

As shown in **Table (3)** ECI in larvae fed on untreated leaves reached 22.25%. This value decreased (17.24%) after feeding the larvae on treated leaves. The decrease was gradually occurred as the concentration of neem seed kernel extract increased to reach the minimum value at the highest concentration (0.025%). Means of 21.03, 20.61, 19.87, 13.16and 11.56% were recorded for ECI in larvae fed on castor bean leaves treated with 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% neem seed kernel extract, respectively. The difference between these means proved to be statistically highly significant. By applying the L.S.D. value (1.18), these means could be arranged in to the following four descending groups:

First group : larvae fed on untreated leaves (22.50%).
second group : larvae fed on leaves treated with 0.0015, 0.0030 and 0.0062% neem extract (21.03, 20.61 and 19.87%, respectively).
Third group : larvae fed on leaves treated with 0.0125% neem extract (13.16%)
Fourth group : larvae fed on leaves treated with 0.025% neem extract (11.56%).

5- Efficiency of conversion of digested food to body substance (ECD)

ECD is an indicator for ability of *S. littoralis* larvae to utilize digested food for growth and food storage in the insect. The data recorded in **Table (3)** show that, ECD in larvae supplied with untreated leaves reached 28.25%. This value decreased (21.42%) when larvae were fed on treated leaves. Gradual decrease occurred as the concentration of neem extract increased to reach the minimum value at 0.025%. Means of 26.14, 25.69, 25.50, 16.94and 14.55% were obtained for ECD in larvae fed on castor bean leaves treated with 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% neem extract, respectively. The difference between these means proved to be statistically highly significant. By applying the L.S.D. value (2.01), these means could be arranged into the following four descending groups:

First group : larvae fed on untreated leaves (28.25%).
second group : larvae fed on leaves treated with 0.0015, 0.0030 and 0.0062% neem extract (26.14, 25.69 and 25.50%, respectively).
Third group : larvae fed on leaves treated with 0.0125% neem extract (16.94%).
Fourth group: larvae fed on leaves treated with 0.025% neem extract (14.55%).

DISCUSSION

In a free choice test, **Gomaa, (2009)** found that treating Tefla leaves, *Nerium oleander*, as an oviposition site, with an aqueous extracts of neem seed kernel solutions extracted by different organic

Table 3. Approximate digestibility (AD) of castor bean leaves and efficiency of conversion of ingested (ECI) and digested food (ECD) to body substances in the sixth instar *S. littoralis* larva after treating the leaves with different concentrations of aqueous solution of neem seed kernel methanolic extract (Means of 10 individuals ± S.E.)

Concentration of extract (%)	Approximate digestibility (AD) (%)	ECI (%)	ECD (%)
0 (Control)	**78.50±4.46**	**22.25±0.62a**	**28.25±0.59a**
0.0015	80.44±4.94	21.03±0.49b	26.14±0.51b
0.0031	78.62±4.26	20.61±0.51b	25.69±0.46b
0.0062	81.08±3.83	19.87±0.41b	25.50±0.41b
0.0125	77.71±4.02	13.16±0.38c	16.94±0.33c
0.0250	79.48±3.95	11.56±0.37d	14.55±0.35d
Mean for treated leaves	**79.46±4.28**	**17.24±0.48**	**21.42±0.44**
F between concentrations	1.81-	10.95**	12.02**
L.S.D. at 0.05	-	1.18	2.01

solvents reduced the number of deposited eggs laid by *Spodoptera littoralis* mated female moth, especially when methanol was used for extraction. The same findings were given by **Meena-Thakur *et al* (1998)** and **Patil and Goud, (2003**) on the yponomeutid diamond back moth, *Plutella xylostella* and by **Bajapai and Sehgal, (2003)** on *Helicoverpa armigera*. Therefore, different aqueous concentrations of methanolic extract of neem seed kernel was used in the present study.

It is well known that the food ingested and digested by insects must fulfill their nutritional requirement for normal growth and development. In the present work, *S. littoralis* larva ate an average of 13.79g and digested about 10.92 g of untreated castor bean leaves during its whole sixth instar. These amounts significantly reduced when larvae were fed on treated leaves with different concentrations of neem extract. The reduction was concentration dependent, i.e., an increase in neem extract concentration caused an obvious decrease in the amounts of ingested and digested leaves. The efficiencies of conversion of ingested and digested food to body substances in insects (ECI and ECD) are an overall indicators for insect abilities to utilize ingested and digested food for growth. In the present work, ECI and ECD in larvae fed on untreated leaves reached 22.25 and 28.25%, respectively. These values decreased after feeding the larvae on treated leaves. The decrease was gradually occurred as the concentration of neem seed kernel extract increased to reach the minimum value at the highest concentration (0.025%). Both ECI and ECD depend upon the food digestibility as well as its nutritional value and the ingestion rate **(Waldbauer 1964 and Bailey 1976)**. If a type of food can be converted to biomass with a high value of conversion of ingested food (ECI), then a larva of any insect species may consume smaller amounts of food to reach its ideal weight **(Slansky and Scriber, 1985)**. Relative consumption rate of an insect varies according to the physiological properties of the food. AD is inversely correlated with ECI since the last instar larvae of any insect species consumed the food in an indiscriminate manner, including leaf nurvures. Thus, a smaller amount of food is utilized for energy and a large part of it is incorporated into body tissue increasing ECI **(Panizzi and Parra, 1991)**.

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