Academic Journal of Entomology 13 (2): 50-56, 2020
ISSN 1995-8994

DOI: 10.5829/idosi.aje.2020.50.56

The Toxic Effect of Some Plant Extracts on the Larvae of *Spodoptera lttoralis* (BOISD.) (Lepidoptera: Noctuidae)

Inas M.Y. Mostafa

Plant Protection Research Institute, Agriculture Research Center Dokki, Giza, Egypt

Abstract: *Spodoptera littoralis* (Boisd) is very destructive pest for all crops. Many efforts are made to manage this pest. The effect of four plant extracts (vinca, ak, neem and chinaberry) were evaluated against 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). The data proved positive correlation between mortality rates of 2nd instar larvae of *S. littoralis* and concentration of each extract (vinca, ak, chinaberry and neem). Also, the results indicated that, ak was the most effective plant extract to larvae of *S. littoralis* with LC_{50}: 866.24 and LC_{90}: 12738.04 ppm, while LC_{50} of neem, vinca and chinaberry extracts were 2658.98, 3297.28 and 3939.20 ppm, respectively; and LC_{90}: 13814.33, 35274.92 and 41583.94 ppm, respectively. Also, the results indicated that total carbohydrate, proteins and total lipids suffered considerable reduction in the treated 2nd instar larvae of *S. littoralis* due to the treatment with various plant extracts (vinca, ak, chinaberry and neem) but protein content increased when larvae treated with ak extract.

Key words: *Spodoptera littoralis* · Ak · Vinca · Chinaberry · Neem

INTRODUCTION

Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is one of the key pests that causes great damage to cotton plants as well as other field and vegetable crops. The widespread and continuously increasing use of different types of nonselective pesticides in cotton fields in Egypt and elsewhere disturb the biological balance and cause outbreaks of insect and mite pests [1].

Catharanthusroseus (Madagasker periwinkle) formerly known as *Vincarosea* Linn. Grows throughout India and is found in wet and sandy land. It has great medicinal value and extensive work has been done on the medicinal uses of this plant [2]. The active compound in ak is calotropin, which is cardiac glycoside. These cardiac glycosides have a great role to plants; they protect them from pests, insects and microorganisms because they are antiseptic. Also, these cardiac glycosides used for human as anticancer [3].

Neem formulations have significant reproductive inhibition on rice hoppers [4]. The compounds present in the neem oil are reported as strong anti feedants and growth inhibitors against lepidopteran larvae [5]. Deota and Upadhyay [6] reported that azadirachtin, the active ingredient of *A. indica* against *S. litura* showed toxicity and antifeedancy.

Increasing problems of synthetic chemical insecticides encourage scientists to exchange them with botanical extracts which were safety [7].

Carbohydrates contribute to the structure and functions of all insect tissues [8]. Proteins are essential to the process of cell deviation and control many reactions in the cellular metabolism [9]. Lipids are essential structural components of the cell membrane and cuticle, they provide a rich source of metabolic energy for periods of sustained energy demand and they include important hormones and pheromones [10].

The objective of this researchwas to assess the effect of some plant extracts on the 2nd instar larvae of cotton leaf worm, *Spodoptera littoralis*and detection of carbohydrate, protein and lipid contents in the body of larvae.

MATERIALS AND METHODS

Tested Insect Rearing: A laboratory strain of cotton leaf worm, *S. littoralis* (maintained on above 30 generations)

Corresponding Author: Inas M.Y. Mostafa, Plant Protection Research Institute, Agriculture Research Center Dokki, Giza, Egypt

was initiated from freshly collected egg-masses supplied from the division of cotton leaf worm of Plant Protection Research Institute (PPRI), Dokki, Egypt. Larval stages were reared on castor leaves, which were provided daily, in laboratory under constant conditions of 27±2°C, photoperiod of 14 h light and 10 h dark and 65±5% R.H. The adults were kept separately and mated on the third day of emergence in clean jars (4 lb.). Adults were fed on 10% honey solution, fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying.

Preparation of Plant Samples and Extraction: Leaves of vinca, ak and chinaberry plants were left to dry at room temperature for one month then they were grinded into fine powder. Also, the seeds of neem were grinded into fine powder in an electric mill. Powder of each plant was soaked in a mixture of hexane, acetone and ethanol solvents of equal proportion (1:1:1) in a flask for about one week.

Finally, the flasks were shaked in a shaker and their contents were filtered. The solvents were evaporated under reduced pressure; the crude extracts were weighted and kept in deep freezer until use.

Preparing the Stock Solution of the Tested Plant Extracts: Convenient stock concentrations of each extract were prepared on basis of the tested plant weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for each plant extract were used to draw the LC-P Lines. Three replicates were used for each concentration.

Methods of Application: Under laboratory conditions, 2nd instar larvae were used to determine the toxicity action of the plant extracts, ak, vinca, neem and chinaberry. 5 cm^2 of castor leaf discs were cut and dipped into the treatments for 20 seconds, then left for air dryness, 10 larvae for each replicate were released to each leaf disc placed. Four concentrations and three replicates were used to estimate each concentration-mortality line. The same number of leaf discs per treatment was dipped into dis. water as an untreated check. Before and after treatment, larvae were maintained under laboratory conditions (constant temperature 25 ±2°C and 70± 5 % R.H. After 24 h of treatment the mortality was recorded and the data were corrected relatively to control mortality [11]. LC_{50} values were determined using probit analysis statistical method of Finney, [12]. LC_{50} index was determined by the following equation according to Sun [13].

$$\text{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} X100$$

Biochemical Effects: For this purpose, treated larvae of the highest concentrations were used to determine the total carbohydrate, protein and lipid content.

Determination of the Total Carbohydrate Content: Total carbohydrate content was determined as glycogen by the anthrone method that was described by Seifter [14].

Reagents:

- Potassium hydroxide 30%.
- Ethyl alcohol 95%.
- Anthrone reagent 0.2 %: 0.4g of anthrone was dissolved in 200 ml analarsulphuric acid.
- Glucose standard: 20 mg glucose was dissolved in 10 ml distilled water. A series of concentrations were prepared by dilutions 40-200 mg.

Procedure:

- In a dry clean Pyrex centrifuge tube containing 3 ml of 30% KOH, a weighted sample of homogenate larvae was added. The mixture was digested for 20-30 min. in boiling water bath.
- After cooling, 3ml of 95% ethyl alcohol were added. The mixture was reheated for 5 min. at 50± 5°C.
- After centrifugation at 3000 rpm for 15 min. the supernatant was discarded.
- The precipitate was responded in 1 ml. distilled water and 3ml. of ethyl alcohol, then incubated for 5 min. at 50°C, recentrifuged and the supernatant was also discarded.
- The dried pellet precipitate was dissolved in 50 ml water.
- One ml. of glycogen extract was mixed, under cooling, with 5 ml antherone reagent and then heated for 10 min. in a boiling water bath. The developed colour was measured at 620 nm.

Calculation: Mg glycogen in aliquot = O.D. of test sample/ O.D. of standardX Concentration of standard/ 1.11.

Determination of the Total Protein Content: Total protein in the whole insect homogenate was determined by the Biuret method Wooten [15].

Reagents

Biuret Reagent: Nine gm of sodium potassium tartarate were dissolved in 500 ml of 0.2 N- sodium hydraride, 3 gm of copper sulphate were stirred. Five gm of potassium iodide were then mixed and the volume was completed to one liter with 0.2 N- sodium hydroxide.

Protein Standard: 0.5 gm borines serum albumin (BSA) was dissolved in 100 ml of distilled water.

Procedure:

- 0.2 ml of larvae homogenate extract was mixed with 0.2 ml of distilled water.
- Five ml of biuret reagent were added and kept at 37° C in a water bath for 10 minutes.
- The sample was left to cool up to room temperature, then the optical density (O.D.) at 450nm was measured.

Calculation: The total protein concentration of the sample was calculated according to the following equation.

$$\text{Total protein} = \frac{\text{Reading of test} - \text{Reading of blank}}{\text{Reading of standard} - \text{Reading of blank}} X100$$

Determination of the Total Lipid Content: Total lipid content was determined according to Knight [16].

Reagent:

- Concentrated sulphuric acid.
- Concentrated phosphoric acid.
- Vanillin 0.6% (W/V).

This was prepared by dissolving 0.6 gm Vanillin in 8-10 ml of absolute ethanol.

- Phosphovanillin reagent.

200 ml of 0.6% Vanillin solution were added to 800ml concentrated phosphoric acid with constant stirring and stored in a brown bottle at room temperature.

Standard Solution: 10, 20, 30, 40, 50, 60, 80 and 100 mg cholesterol / 1 ml ethanol.

Procedure:

- 0.1 ml of larvae homogenate extract was mixed with 0.1 ml of distilled water.
- 0.2 ml of concentrated H_2SO_4 was added, mixed and placed for 5 min. in a boiling water bath.
- After cooling, 10 ml of phosphovanillin solution were added.
- The sample was left to cool up to room temperature, then the (optical density) O.D. at 540 nm was measured.

Calculation: The total lipid concentrate of the sample was calculated according to the following equation:

Total lipid = Reading of test / Reading of standard X conc. of standard

Design of the experiments and statistical analysis of the obtained data were made according to Le Clerg [17]. Duncan's new multiple range tests was used for testing the differences between treatments [17].

RESULTS AND DISCUSSION

Effect of Some Plant Extracts Against 2nd Larval in Star of Cotton Leaf Worm, *Spodoptera littoralis* (Boisd): Data showed a positive correlation between mortality rates of 2nd instar larvae of *S. littoralis* and concentration of each extract (vinca, ak, chinaberry and neem) , whereas increasing of concentration for each extract caused increasing mortality rates. Calculated data after 1 day, 3 days and 7 days and in most cases, mortality rates were high in the 7th day.

The results in Table (1) revealed that the plant extracts of ak and neem had the highest larval mortality reached 90 & 86.67%, respectively at 10.000 ppm. While the tested plant extracts (vinca and chinaberry) had the same total larval mortality 73.33% at the same concentration 10000 ppm. Abd- Allah [18] recorded similar results when the extracts ak, neem, vinca and chinaberry were examined on *Pierisrapae* larvae and ak extract was the most effective one.

Determination of LC_{50} of Some Plant Extracts Against 2nd Instar Larvae of the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd): Data in (Table 2) and (Fig. 1) showed the efficiency of the plant extracts to larvae of *S. littoralis*. Results indicated that, ak was the most effective plant extracts against 2nd instar larvae of *S. littoralis* with

Table 1: Corrected mortality % of 2nd instar larvae of *S. littoralis* treated with some plant extracts under laboratory conditions 27±2C°and 65±5%RH

		Mortality after treatment. %			
Treatments	Conc. ppm.	One day	Three days	Seven days	Total Mortality%
Vinca leaves extract	1000	---------	13.33	13.33	26.67
	5000	---------	6.67	50.00	56.67
	10000	6.67	13.33	53.33	73.33
	15000	6.67	20.00	53.33	80.00
Ak leaves extract	500	----------	6.67	33.33	40.00
	1000	----------	26.67	26.67	53.33
	5000	6.67	26.67	50.00	76.67
	10000	3.33	16.67	70.00	90.00
Chinaberry leaves extract	1000	----------	13.33	13.33	26.67
	5000	----------	13.33	30.00	43.33
	10000	----------	3.33	70.00	73.33
	15000	----------	13.33	66.67	80.00
Neem seeds extract	1125	----------	6.67	20.00	26.67
	2500	----------	20.00	26.67	46.67
	5000	10.00	30.00	26.67	66.67
	10000	6.67	20.00	60.00	86.67

Table 2: Efficiency of some plant extracts against 2nd instar larvae of *S. littoralis*

Treatment	Conc.	Corrected mortality %	Lc_{50}	Lc_{90}	Slope +S.D.	Toxicity Index	Lc_{90}/ Lc_{50}	R	P
Vinca	1000	26.67	3297.28	35274.92	1.25± 0.153	32.83	10.70	0.998	0.865
	5000	56.67							
	10000	73.33							
	15000	80.00							
Ak	500	40.00	866.24	12738.04	1.098± 0.136	100	14.70	0.991	0.982
	1000	53.33							
	5000	76.67							
	10000	90.00							
Chinaberry	1000	26.67	3939.20	41583.94	1.252± 0.154	27.48	10.56	0.953	0.019
	5000	43.33							
	10000	73.33							
	15000	80.00							
Neem	1125	26.67	2658.98	13814.33	1.791± 0.202	40.71	5.20	0.996	0.715
	2500	46.67							
	5000	66.67							
	10000	86.67							

R: Regression P: Probability

LC_{50}: 866.24 and LC_{90}: 12738.04 ppm, while LC_{50} of neem, vinca and chinaberry extracts were 2658.98, 3297.28 and 3939.20 ppm, respectively, LC_{90}: 13814.33, 35274.92 and 41583.94 ppm, respectively.

Slope values indicated that, neem extract had a highest value 1.791 while vinca, chinaberry had the same slope value: 1.252 and ak had 1.098, respectively. Data also showed that the toxicity index (Ti = 100) 100% for ak extract, while it recorded 40.71, 32.83&27.48 % for neem, vinca and chinaberry, respectively.

The LC_{90}/ LC_{50} confirm the value of this criterion recorded 14.70, 10.70, 10.56 and 5.20 for ak, vinca, chinaberry and neem, respectively. Thus, the highest slope value or the lowest ratio LC_{90}/ LC_{50} means the steepest toxicity line.

The results indicated that, ak was the most toxic plant extracts to larvae of *S. littoralis* followed by neem, vinca and chinaberry. Bakavathiappan [19] proved that ak was the most effective extract against 3rd instar larvae of *S. littoralis*. El-Hefny [20] also, proved the effectiveness of ak extract against the greater wax moth, *Galleria mellonella*.

These results, agreement with Meisner [21] who investigated vinca leaves extract that had significant effect on larvae of *Spodoptera littoralis*. Chaudhry [22] reported that ak, neem and chinaberry extracts had significant mortality against *Plecopterareflexa*. Also, Meadow [23] reported that neem extract had significant effects on larvae of *Pierisrapae* L.

The obtained results in contrast with results of Khan and Siddiqui [24] who observed that neem seeds and chinaberry leaves were effective against larvae of *Pierisbrassicae* than whole plant of ak extract.

Chemical Analysis of the Treated Larvae of *Spodoptera littoralis* (Boisd): The effect of the plant extracts on total carbohydrate, total proteins and total lipids percentages was presented in Table (3).

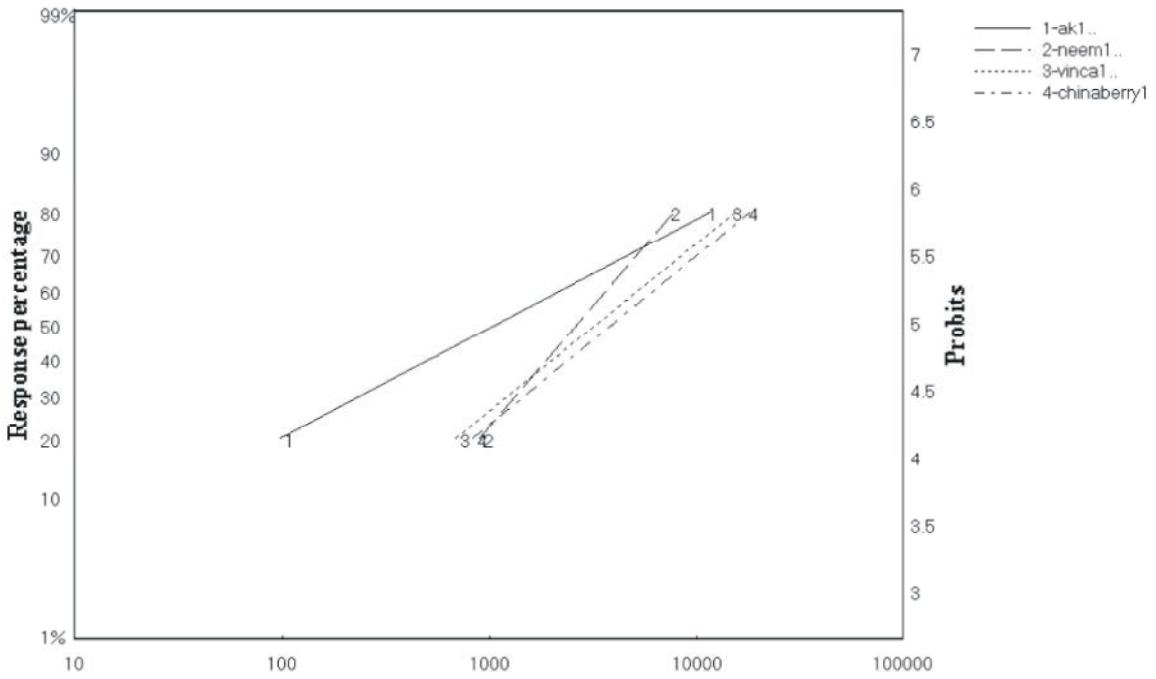

Fig. 1: LC-P line some plant extracts against 2nd instar larvae of *S. littoralis*

Table 3: Effect of some plant extracts on components of the body of 2nd instar larvae of *Spodoptera littoralis* (Boisd):

Treatments	Total carbohydrate (mg.)%	Total proteins (mg.)%	Total lipids (mg.)%
Vinca	69.00 c	118.00 b	31.66 c
Chinaberry	151.00 b	183.66 a	43.66 c
Neem	167.00 a	155.66 a	69.33 b
Ak	173.13 a	213.00 a	68.00 b
Control	174.43 a	187.66 a	81.66 a

Table 4: The correlation between phytochemical components of the body of 2nd instar larvae of *Spodoptera littoralis* (Boisd) treated with some plant extracts and mortality

Treatments	Mortality mean %	Total Carbohydrate (mg.)% (r)	Total Proteins (mg.)% (r)	Total lipids (mg.)% (r)
Vinca	59.17	69.00 (0.965)	118 (0.998)	31.66 (0.982)
Chinaberry	55.83	151 (0.953)	183.66 (0.528)	43.66 (0.944)
Neem	56.67	167 (0.977)	155.66 (0.977)	69.33 (0.99)
Ak	65	173.13 (0.989)	213 (0.714)	68 (0.922)
Control	----------	174.43	187.66	81.66

(r) = Correlation

Total Carbohydrate: The carbohydrate was not affected by all plant extracts for all treatments except the plant extract vinca decreased the carbohydrate content compared with control, 69.00 and 174.43 mg. % respectively.

Total Proteins: The proteins were not affected by the plant extracts for chinaberry compared with control, 183.66 and 187.66 mg. % respectively. While the plant extracts, vinca and neem were decreased the proteins compared with control, 118, 155.66 and 187.66 mg. % respectively. Also the plant extract ak was increased the proteins compared with control, 213 and 187.66 mg. %, respectively.

Total Lipids: Data in Table (3) cleared that the total lipids decreased by all treatments, 31.66 , 43.66 , 68 and 69.33 mg. % with plant extracts vinca, chinaberry, ak and neem, respectively, compared with control 81.66 mg %. The correlation between phytochemical components of the body of larvae of *Spodoptera littoralis* (Boisd) treated with some plant extracts and mortality:

Data represented in Table (4) and showed the total carbohydrate, total proteins, total lipids and mortalitymean percentages.

The simple correlation analysis showed that there was a positive correlation between the total carbohydrate, total proteins and total lipids contents in the body of larvae of *Spodoptera littoralis* and mortality mean percentages of the four plant extracts; vinca, chinaberry, ak and neem.

On the other hand, the total carbohydrate was significant with vinca and chinaberry and high significant with neem and ak extracts. While total proteins were highly significant with vinca and neem extracts. The total proteins were significant with ak and non significant with chinaberry extracts. The total lipids were highly significant with vinca and neem extracts but significant with chinaberry and ak extracts.

The results indicated that total carbohydrate, proteins and total lipids suffered considerable reduction in the treated 2ndinstar larvae of *S.littoralis*due to the treatment with various plant extracts (vinca, ak, chinaberry and neem) but protein content increased when larvae treated with ak extract, compared with control.

In this connection, our results could be supported by the work of Taha [25] which recorded a highly significant reduction in the glycogen content of the 4th nymphal instar of *Spodoptera littoralis* previously treated with *V. rosea*acetone extract. Moreover, they observed a significant decrease of the nymphal total lipid and protein contents.

Our results agreed with Zhang and Chiu [26] who found that the activities of proteinases in the larval midgut of *Pierisrapae* L., decreased as the result of treatment of larvae with toosendanin (botanical material from the bark of chinaberry).

Also, our results agreed with Abu El- Ghar [27] who showed that, the treatment of 6th instar larvae of *Agrotisipsilon* with extracts of *Meliaazedarach* and *Vincarosea* resulted in considerable reduction in the total protein, lipids and carbohydrates. Schmidt [28] proved that protein content in the haemolymph of *Spodoptera littoralis* and *A. ipsilon*was decreased significantly due to larval treatment with *M. azedarach* extract.

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