TOXICOLOGICAL, BIOLOGICAL AND BIOCHEMICAL IMPACTS OF TWO PLANT EXTRACTS AGAINST *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

The study aims to evaluate the toxicity and biological activity of *Nigella sativa* and Coumarin extracts on the cotton leaf worm, *Spodoptera littoralis*. Data indicated that mortality percentage of 2nd instar larvae gradually increased with increasing the concentration used of the two tested plant extracts. The mortality percent were ranged from 6 to -25, 11-49 and 18-65% after 3, 5 and 7 days of treatment with *Nigella sativa*, respectively, while the mortality percentages ranged from 11-38, 19-58 and 25-74% after 3, 5 and 7 days of treatment with Coumarin, respectively . After 48 hrs Coumarin gave the lowest consumed leaf area (cm^2) treated 48 hrs than Nigella (as antifeedant effect). Taking into account larval survival after treatment by Nigella or Coumarin. It was observed that the larval feeding on food treated by either Nigella or Coumarin affected the larval duration, and had a significant latent effect on the pupal duration. Larval treatment by Coumarin treated larvae had shorter than Nigella. Results indicated that, *Nigella sativa* revealed reduction in invertase activity, but Coumarin had no effect on invertase . In contrast, Coumarin caused significant increase in the activity of trehalase, while *Nigella sativa* did not cause change on trehalase. Both plants extracts resulted in significant increase in amylase activity of larvae treated of *S. littoralis* larvae compared to untreated one. Also, Coumarin and Nigella caused significant reduction of total protein.

Keywords: *Spodoptera littorals*, *Nigella sativa,* Coumarin – toxicological, biological, antifeedant, biochemical effects.

INTRODUCTION

The cotton leaf worm *Spodoptera littorals* (Boisd.) (Noctuidae : Lepidoptera) is one of the most notorious and destructive phytophagous insect pests in Egypt, not only to cotton but also to other crops and vegetables (Kandil *et. al.,* 2003). It control program is based mainly on use of insecticides, which created some problems such as insecticides-resistance, environmental pollution and hazard to natural enemies and beneficial insects (Nada, 1990). Hence, it recent approaches now focus upon the use of environmentally safe compounds as pest control agents. Insecticidal activity of

many plants against several insect pests has been investigated (Carlini and Grossi-de-Sá 2002). Many different countries search for using the naturally products which might be less dangerous pesticides can be applied effectively in habitats (Brumo *et. al.,* 2003, Sadek, 2003). The use of plants as insecticides was commonly used in tropical countries before the advent of synthetic insecticides. The application of substances with insecticidal action extracted from plants has some advantages when compared with synthetic substances: they are easily degradable, that is, they do not remain in the environment and leave no residues in food products (Vendramim & Castiglioni 2000 and Antonia *et. al.,* 2010). The scientist reported that plants are considered as one of the richest sources that can be used as pest control agents (Nakatani *et. al.,* 2001 & Schmidt and Assembe, 2002). In Egypt, many trials have been done to discover insecticidal activity of different plants extracts against many insects (Farag, 2002, Sadek, 2003 and Sayed *et. al.,* 2011). Also recently, plant extracts have more attention in controlling many pests that are non-toxic to man and animals, possess distinct toxicity and lead to antifeedant activity and inhibition growth of some pests. Some botanical extracts and essential oils have been reported for their toxic effects against this insect pest (Emara and Ryan, 1997, Tripathi *et. al.,* 2000b). *Nigella sativa* L. is one of these species, which is naturally distributed in different parts of the country. In addition, it is extensively cultivated in various regions of Iran (Mozaffarian, 1998). NS seed (NSS) are often used as a spice but are also used extensively in the traditional medicine of many countries (Meddah *et. al.,* 2009). NSS has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. (Tariq, 2008).

Coumarin is a phytochemical (benzopyrone), a toxin found in many plants, notably in high concentration in the tonka bean, vanilla grass, strawberries, Coumarin can occur either free or combined with the sugar glucose (coumarin glycoside). Coumarins are naturally occurring polyphenolics distributed widely in plants, fungi, and bacteria and have found applications for centuries in traditional medicine (Kumar *et. al.,* 2008).The biosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclization of cinnamic acid. Coumarin has appetite suppressing properties, suggesting its widespread occurrence in plants, especially grasses, is because of its effect of reducing the impact of grazing animal (Laposata *et. al.,* 2007).

Synthesis of coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus. They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, dispersed fluorescent and laser dyes, insecticides and in optical brighteners (Fulchand *et. al.,* 2008).

The changes in the biochemical especially the carbohydrate hydrolyzing enzymes such as trehalase, invertase, amylase and protein content have an important role in biological and physiological activities of insects. (Khedr, 2002).

This work was designed to achieve the following purposes:

1- Evaluation of the toxicological and biological activities of two plant extracts against the *S. littoralis* larvae and their latent effect on the different development stages under laboratory conditions.
2- Antifeedant effect of two plant extract on larval *S. littoralis*
3- Evaluation of the aspects biochemical content trehalase, invertase, amylase and total protein of *Nigella sativa* and Coumarin extracts on the laboratory strain of *S. littoralis*.

MATERIALS AND METHODS

Mass rearing of the cotton leafworm, *Spodoptera littoralis* (Boisd.)

The stock culture of susceptible *Spodoptera littoralis* was reared on castor leaves (*Ricinus communis*) for several generations at 27±1°C and 70±5% RH. Egg masses were placed on castor leaves in glass jars.. Then, the emerged adults were supplied with a piece of cotton wetted with 10% sugar solution and branches of Tafla (*Nerium oleander*) as a suitable site for ovipositon. Newly laid egg masses were collected daily into the rearing jars according to El Defrawi *et. al.,* (1964).

The tested plant extracts: *Nigella sativa* used in this study was obtained from seeds production of *Nigella sativa* plants. Seeds (samples of 100 g) were extracted by addition of 20 ml of emulsifier polyethylene glycol 600 diluted in xylene to 80 ml crude plant extracts.

Coumarin: Chicory water-soluble extract (chicory extract) isolated from chicory plant, *Chichorium intybus*, dried chicory root were used as the starting material and ground into powder using a Micro mill. Distilled water (chicory root powder: distilled water, 1:5 wt/v) was added and mixed for 50 min. Then, the soluble fraction was filtered by squeezing the mixture using a cotton cloth, and the solution was centrifuged at 9000 x g for 20 min to further exclude the insoluble fraction according to the methods of Uchiyama, (1975).

Plant extracts concentrations

Five concentrations of two plant extracts Nigella and Coumarin were tested (2.5, 5.0, 7.5, 10 and 15 ml /l) compared to control.

Bioassay studies: Castor bean leaves were dipped for 30 seconds in each concentration for each plant extract (Nigella, Coumarin) and left for air dryness before

feeding the 2nd instar larvae . Three replicates of twenty larvae/each were made for each concentration. Also similar replicates and number of larvae were fed on untreated castor bean leaves to serve as check.

Mortality counts were recorded after 3, 5 and 7 days and mortality percentages were corrected for natural mortality according to Abbott´s formula (1925), to estimate the LC_{50}, LC_{90} values and slope, the corrected mortality percentages were subjected to Probity analysis according to the method of Finney, (1971).

$$\textbf{Corrected mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality \%}} \times 100$$

Antifeedent tests

For evaluation the antifedant effect of plant extracts, the 2nd instars larvae of *S. littoralis* were used. The larvae were starved for about 4 hours before treatment and divided into 3 replicates (20 larvae for each replicate), each larva kept in a Petri-dish. Discs of 20 cm^2 area of castor leaves were dipped in each concentration for each plant extract and allowed to dry. Only one disc was offered to each tested larva and untreated discs were introduced to each larva as control. The eaten area was estimated after 48 hours by planymeter. The percentage of feeding reduction over control was the factor used for determining the presence of feeding deterrent effect. The antifeedant activity was evaluated on the basis of the feeding ratio of the treated and untreated leaf discs. The antifeedant activity was calculated according Saleh *et. al.,* (1986) formula.

$$\textbf{Antifeeding activity} = \left(1 - \frac{\text{\% of eaten area in treatment}}{\text{\% of eaten area in control}}\right) \times 100$$

Effect of plant extracts on biological aspects of *S. littoralis*

The 2nd instar larvae of *S. littoralis* were starved for 4 hrs before feeding on castor treated leave discs of castor with the two plant extracts. Castor leave were dipped in various concentrations for 5 seconds, then the leaves were left for air dryness. Three replicates were used for each concentration while another group of larvae were fed on untreated leaves kept as a control. Daily records were taken for the percentages of larval and pupal duration, pupation percentage and pupal

malformation. The overall latent effect on reproduction was expressed as percent sterility and calculated according to Toppozada *et al.* (1966).

Determination of total soluble protein

Colorimetric determination of total protein in total homogenate *S. littoralis* larvae was carried based out as described by (Gornall *et. al.,* 1949). The principle of this method based on the presence of an alkaline cupric sulfate, the protein produce a violet purple color, the intensity of which is proportional to their concentration. Briefly, a volume of 0.2 ml of larval homogenate was added to 5 ml of Biuret reagent and incubated for 30 min at 20-25°C. The absorbance of the sample against a blank Biuret reagent was measured at wave length of 546 nm.

Determination of carbohydrate hydrolyzing enzymes

The methods used to determine the digestion of trehalose, starch and sucrose by trehalase, amylase and invesrtase enzymes respectively, The free aldehydic group of glucose formed after trehalose, starch and sucrose digestion were determined using 3, 5 dinitrosalicylic acid reagent. The trehalase reaction mixture consisted of 0.2 ml of 3% trehalose (substrate), 0.2 ml phosphate buffer (pH 5.4) and 0.2 ml larval homogenate. The invertase reaction mixture consisted of 0.2 ml of 4% sucrose (substrate), 0.1 ml phosphate buffer and 0.2 ml of larval homogenate. The amaylase reaction mixture consisted of 0.2 ml of 2% starch, 0.160 ml phosphate buffer and 0.2 ml of larval homogenate. The dinitrosalicylic acid reagent was prepared by dissolving one gram of 3, 5-dinitrosalicylic acid in 20 ml of 2 N NaOH and 50 ml of distilled water with the aid of a magnetic stirrer. Potassium sodium tartar ate (30 gm) was added, and magnetic stirring was continued until a clear solution was obtained. Distilled water then added to bring the final volume to 100 ml. All test tubes were incubated at 37°C for exactly 60 min, 0.8 ml of 3, 5 dinitrosalcylic acid reagent were then added. The reaction mixture was heated 5 min at 100°C in a boiling water bath followed by immediate cooling in an ice bath. The optical density of the produced colour is measured at 550 nm using spectrophotometer. The enzymatic activity was expressed as mg glucose released/g body weight/min.

RESULTS AND DISCUSSION

Relative toxicity of two plant extracts on the *S. littoralis*

Tabulated data indicate that the potency of the *Nigella sativa* and Coumarin was varied tremendously due to the nature of compound and the used concentrations. Generally, data proved that at any of the tested compounds, the higher concentration gave the higher mortality .

Comparing the toxicity of *Nigella sativa* and Coumarin against larval of *S. littoralis*. The evaluation of the two plant extracts efficiency was compared as follows:

1. Insecticidal activities

1.1. Mortality percentages

Data in table (1) show that mortality percentages increasing with increasing time elapsed of treatment. In Nigella mortality percentages of S. *littoralis* larvae ranged from 6 to 25, 11 to 49 and 18 to 65% after 3, 5 and 7 days of treatment, respectively. While it was ranged from 11-38, 19-58 and 25-74% after 3, 5 and 7 days of treatment in Coumarin extract, respectively.

El-Shewy, (2009) found that cumulated mortality percentage increased with increasing both the time elapsed after treatment and the tested concentration of these compounds. So, the larval mortality after application of the 4th instar larvae with lannate, protecto, coumarin and azadirachtin ranged between (48.3-70.0%), (20.0-50.0%), (25.0-60.0%) and (21.6-55.0%), respectively.

Table 1. Cumulated mortality percentages of *S. littoralis* 2nd instar larvae after application with *Nigella sativa* and Coumarin under laboratory conditions using leaf dipping technique.

Conc. (ml/L.)	Mortality percentages after					
	3	5	7	3	5	7 days
	Nigella			Coumarin		
2.5	6	11	18	11	19	25
5	9	15	23	16	22	31
7.5	15	22	42	20	31	46
10	17	38	56	25	41	60
15	25	49	65	38	58	74

1.2. LC_{50} and LC_{90} values

The required toxic values, i.e. LC_{50} and $LC_{90's}$ represented in Table (2) Coumarin was more effective than Nigella , while LC_{50} values were 7.51 and 9.406 ml/l for Coumarin and Nigella after 7 days of application , respectively

It is appearing that slop values of toxicity lines is lower than 2 This represents high homogeneity of treated larvae whereas the slop is stripper.

For *Nigella sativa* slope values was 1.85 after 7 days of treatment, while it was 1.76 after 7 days of coumarin. Abd ELatif, (2009) studied the effect of Nigella and Arugula oils on *S. littoralis* (with two different extract methods) against the fourth instars larvae. One day after feeding, data generally showed that all treatments reflected significantly lower effects than those recorded after 7 days, and the highest concentration (10ml/l) caused the highest percentage of mortality for all treatments.

Table 2. LC values and slopes of (*Nigella sativa* and Coumarin) on the 2nd larval instars of *Spodoptera littoralis* after 7 days.

Plant extract	LC values after 7 days						
	LC_{50}	95% Fiducial limits		LC_{90}	95% Fiducial limits		Slope
		Lower	Upper		Lower	Upper	
Nigella sativa	9.406	8.037	11.035	72.855	40.403	134.37	1.85 ± 5.65
Coumarin	7.5127	6.585	9.061	40.332	39.156	145.06	1.67 ± 5.2

2. Effect of Nigella and Coumarin as antifeedant on *S. littoralis*

In this trial only the 2nd larval instars of *S. littoralis* were used to establish the presence of antifeedant properties in two plant extracts. Data in Table (3) indicated that coumarin gave the lowest consumed leaf area (cm^2) treated 48 hrs than Nigella. The two tested plant extracts gave significantly lower consumed area than control. The mean percentages of eaten area were 11.43 and 7.89 obtained by Coumarin and Nigella, respectively comparing with control which reared mean percentages of eaten area to (23.48%). In addition, data in Table (3) clearly indicate the important role of tested compounds used in determining antifeedant activities. Coumarin showed the highest antifeeding activity mean (66.23 cm^2) followed by Nigella (52.30 cm^2), Data in Table (3) also showed increasing consumed area in cm^2 by decreasing the concentration of each tested plant extract. The obtained results indicate appositive relationship between the concentration of tested plant extracts and antifeedant activities. The highest used concentration (15ml/l) of Coumarin and Nigella gave the greatest antifeedant activity (90.5 and 80.5 cm^2), respectively. On the other hand, the lowest concentrations (2.5ml/l) of Coumarin and Nigella gave the least antifeedant activity values 16.8 and 31.4, respectively.

These results agree with the results of El-Shewy, (2009), tested 4 different compounds with regard to antifeedant effects against 4th instar larvae of the cotton leafworm larvae. Data indicated that the plant extract of coumarin and azadirachtin were recorded as the highest antifeedant activity while insecticides lannate and protecto gave the lowest antifeedant activity. Also, Mansour, (1981) found that Coumarin at higher concentration reduced the food consumption, acted as an antifeedant, completely inhibiting larval feeding, while lower concentrations at the compound decreased the amount of food digested and ingested.

Table 3. Effect of different concentrations of Nigella and Coumarin as antifeedant on the 2nd larval instars of *S. littoralis*.

Plant extract	Conc. ml/l	Consumed area in cm^2 after 48 hrs	Eaten area%	Antifeedant activity
Nigella	**15**	1.87±0.06	4.29	80.5
	10	2.28±0.16	7.31	76.2
	7.5	4.56±0.03	11.51	52.4
	5.0	6.17±0.10	14.85	35.6
	2.5	7.97±0.13	19.18	16.8
Mean	**-**	**4.57**	**11.43**	**52.30**
Coumarin	**15**	0.91±0.01	2.87	90.5
	10	1.99±0.35	4.58	79.2
	7.5	2.77±0.12	7.12	71.1
	5.0	3.91±0.03	9.74	59.2
	2.5	6.57±0.04	15.14	31.4
Mean	**-**	**3.23**	**7.89**	**66.28**
Control	**-**	**9.58**	**23.48**	**-**

3. Effect of *Nigella sativa* and Coumarin on some biological characteristics of the 2nd instars larval of *S. littoralis*

The latent effects of the two plant extracts on the biological characteristics of *S. littoralis* are shown in table (4) and (5). Data in table (4) indicate that there were slight differences in larval and pupal duration in Nigella and Coumarin between treated and untreated larvae. Larval duration in Nigella treated larvae ranged between 13.54 and 13.99 days, while it was ranged between 11.54 and 14.03 days in Coumarin treatment.

Table 4. Effect of two plant extracts on larval and pupal durations and pupal weight after feeding *S. littoralis* larvae on treated castor bean leaves at 27°C.

Conc. "ml/L."	Larval duration (days SE)		Pupal duration (days SE)		Average of pupal weight (mg/pupa)	
	Nigella	Coumarin	Nigella	Coumarin	Nigella	Coumarin
15	13.99	14.03	12.21	12.18	275	251
10	13.86	13.97	12.34	12.26	297	267
7.5	13.80	13.79	12.47	12.37	309	298
5	13.71	11.64	12.61	12.59	311	301
2.5	13.54	11.35	12.85	12.74	329	315
Control	13.12	13.08	13.03	13.19	327	329

From all treatments, the heaviest pupae were those of the control (327 and 329 mg/pupa). On the contrary, the lightest pupae were those obtained after larval treatments by the highest concentration of either of Nigella or Coumarin. It could be observed that a negative relationship between the weight and the concentration, as the weight of obtained pupae was found to be decreased as the used concentration was increased. The average weight of pupae after Nigella larval treatments ranged from 275-329 mg/pupae opposed to 327 mg/control pupa, when larvae were treated with Coumarin the corresponding mean weights of pupa banged between 251-315 mg/ pupae opposed to 329 mg/control pupa. ata recorded in Table (4), showed that the 2nd instars larvae of *S. littoralis* feeding, on food treated by Nigella or Coumarin affected the larval duration. In all treatments, the larval and pupal duration was slightly affected by treatment of the larvae with Nigella or Coumarin, the larval period extended from 13.99 to 13.54 days opposed to 13.12 days in case of the control larvae. Results in Table (4) confirmed that feeding the larvae of *S. littoralis* on castor leaves treated by different concentrations of Nigella or Coumarin had latent effect on the durations of subsequent pupae. Larval treatment by *Nigella sativa* caused significant shortening in the pupal period (12.21, 12.34, 12.47, 12.61, and 12.85 days after treatment with 15, 10, 7.5, 5 and 2.5%, respectively) than control (13.03 days). While, when treated different concentrations with Coumarin caused more significant shortening in the pupal period (12.18, 12.26, 12.37, 12.59, and 12.74 days after treatment with the same above concentrations) than control (13.19 days) .

Among the surviving larvae after treatment by *Nigella sativa* or Coumarin, some of these larvae showed different rates of deformities as shown in Table(5) and Figure (1).

Table 5. Effect of two plant extracts after treatments *S. littoralis* larval treatment under 27°C.

Plant extract	Conc. "ml/L."	Percentages of				
		Malformed larvae	% pupation	Malformed pupae	% Emergence	Malformed adults
Nigella	**15**	13.21	33.55	15.23	31.28	17.34
	10	9.97	41.59	10.28	38.79	14.75
	7.5	7.22	55.47	7.95	42.98	11.23
	5	6.97	78.91	5.12	51.69	8.67
	2.5	5.33	90.57	3.86	55.47	6.89
Coumarin	**15**	17.87	25.79	19.87	22.14	19.87
	10	14.23	39.78	15.12	30.57	15.11
	7.5	10.87	50.17	9.44	35.69	13.21
	5	7.42	71.58	7.86	40.19	9.97
	2.5	6.88	88.78	5.87	45.72	7.11

Percentages of malformed larvae ranged from 5.33-13.21% when the 2nd instar of *S. littoralis* larvae were treated by Nigella, while ranged between 6.88-17.87% occurred among the treated larvae by different concentrations of Coumarin Table(5).

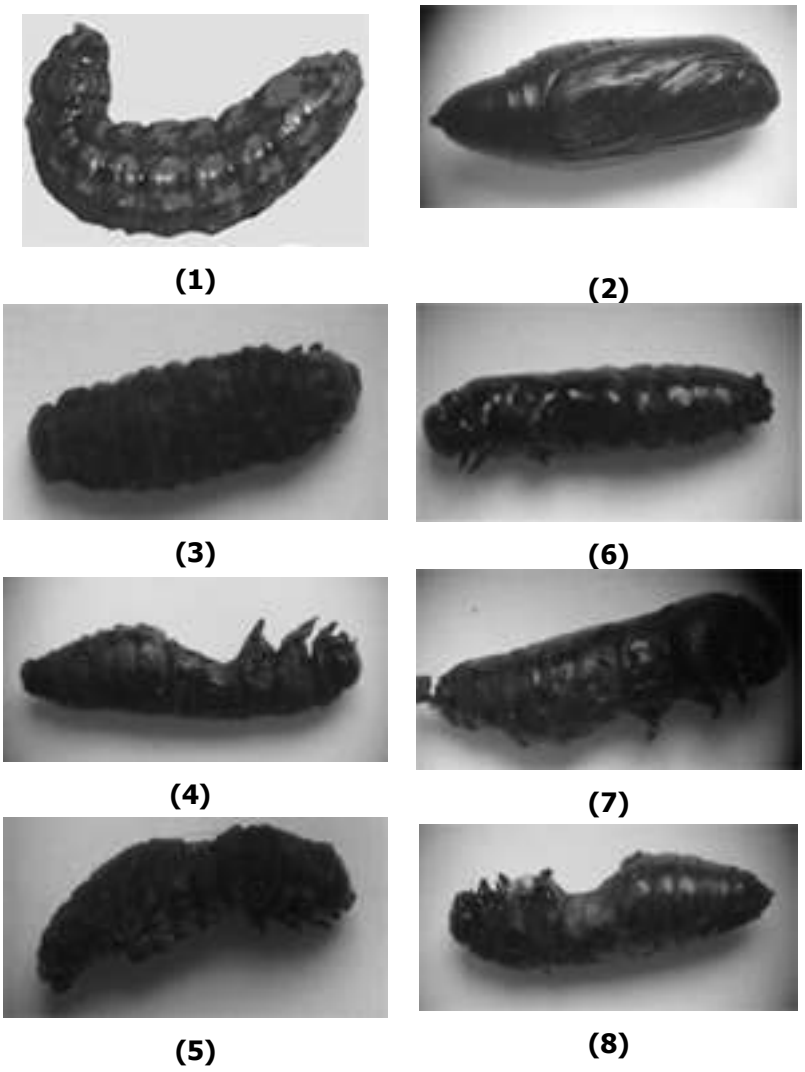

Fig. 1. Normal larvae (1) & Normal pupae (2) and Malformed larvae (3,4,5) & Malformed pupae(6,7,8) of *S. littoralis* treated with Nigella extract.

At the time of pupation, another kind of malformation was observed when the larvae could not develop to normal pupae, and the result was larval-pupal intermediates. Percentages of malformed pupae ranged from 3.86-15.32% when larvae were treated by it appear the pupal duration was less short in treated larvae than untreated in Nigella and Coumarin while ranged between 5.87-19.87% occurred among the treated larvae by different concentrations of Coumarin Table(5).

As shown in Table (5), the percentages of malformed adults ranged from 6.89-17.34% for Nigella treatments, and from 7.11-19.87% for Coumarin treatments to the larval of *S. littoralis*. The effect of plant extracts Data in Table (5) indicated that the percentages of pupation ranged from 33.55-90.57% for Nigella treatments, and from 25.79-881.78% for Coumarin treated *S. littoralis*, larvae table (5) .The emergence percentage recorded 55.47 and 45.72% at the higher concentration (15ml/l) and 31.28 and 22.14% at the lower concentration (2.5ml/l) when treated larvae with Nigella or Coumarin, respectively.

Effect of the tested compounds on some biochemical aspects of the cotton leaf worm larvae in the laboratory

In these tests of *S. littoralis* treated with the LC_{50} of each compound for biochemical assays to evaluate total protein, and the carbohydrate hydrolyzing enzymes (trehalase, invertase, and amylase).

4. Effect of *Nigella sativa* and Coumarin on carbohydrate hydrolyzing enzymes and total soluble protein of *S. littoralis*

4.1. Carbohydrate hydrolyzing enzymes

4.1.1. Invertase

Data in Table (6) showed that, Nigella gave reduction activity of invertase of treated 2nd instars larval of *S. littoralis* than in control. The mean values of invertase activities in the supernatant of the homogenate larvae reached to 1.56 mg/g b.w when larvae treated with (LC_{50}) of by Cumarin compared with 1.58 mg/g b.w. in control. On the other hand, Coumarin did not gave reduction activity of invertase (1.58 mg/g b.w.).

Table 6. Effect of *Nigella sativa* and Coumarin on the activities of invertase, trehalase, amylase enzymes and total protein of larvae of *S. littoralis* .

Treatments	Activity (mg/g of body weight)			
	Invertase	Trehalase	Amylase	Total soluble protein
Control	1.58	0.72	0.25	2.31
Coumarin	1.56	0.81	0.31	0.59
Nigella	1.43	0.71	0.33	0.49

4.1.2. Trehalase

Data in Table (6) indicated that Coumarin caused increase in the activity of trehalase of larvae of *S. littoralis* than control. The mean values of trehalase activities in the supernatant of the homogenated larvae reached 0.81 mg /g b.w. when larvae treated (at LC_{50}). Compared with 0.72 mg/g b.w. in control on the contrary, Nigella did not cause increase in the activity of trehalase of larvae (0.71 mg/g b.w.)

4.1.3. Amylase

Data in Table (6) indicated that Coumarin and Nigella caused significant increase in the activity of amylase of larvae of compared to control. The mean values of amylase activities in the supernatant of the homogenated larvae reached to 0.31 and 0.33 mg/g b.w. when larvae were treated at LC_{50}, compared with 0.25 mg/g b.w. in control.

Such results are in accordance with those obtained by Mohamady, (2000), who investigated the effect of treatment of the 4th instars larvae of *S. littoralis* with the LC_{25} and LC_{50} of fenvalerate on the activity of amylase enzymes at different time intervals (24, 48 and 72 hrs). The results indicated that, there was great reduction in the activity of amylase after treatment. On the other hand, Khedr, (2002) found an increase in the activity of trehalase enzyme of *S. littoralis* (2nd & 4th instar larvae) after treatment with Biorepel.

4.1.4. Concentration of total soluble protein

Data in Table (6) showed that, Coumarin and *Nigella sativa* caused significant reduce of total protein of total protein of the treated larvae than control. The other tested compounds. The values of total protein in the supernatant of the homogenate larvae reached to 0.59 and 0.49 mg/g b.w. when larvae treated with LC_{50} of the tested coumatin and Nigella, respectively, compared with 2.31 mg/g b.w. in control.

Mohamady, (2000) investigated the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC_{25} and LC_{50} of fenvalerate on the total protein at different time intervals (24, 48 and 72 hrs). The results indicated that there was high reduction in the level of total protein due to the treatment.

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التأثيرات التوكسيكولوجيه والبيولوجيه والبيوكيميائيه لإثنين من المستخلصات النباتيه ضد دوده ورق القطن

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استهدفت الدراسه تقييم السميه والتأثيرات البيولوجية والبيوكيميائيه لإثنين من المستخلصات النباتية (حبة البركة والكيومارين) ضد العمر الثانى لدودة ورق القطن وقد تمت معامله اليرقات بتركيزات تتراوح من 2,5 الى 15مللى/لتر لكل من المستخلصين وتم تحديد التركيز النصف مميت لمستخلص حبه البركه والكيومارين بعد 7 ايام. وتم تسجيل نسب الموت بعد المعامله بعد 3، 5، 7 أيام . وأوضحت النتائج أن نسبة الموت تراوحت بين 6–25، 11–49، 18–65% بعد 3و5 و7 أيام من المعاملة بمستخلص حبة البركة في حين تراوحت بين 11–38، 19–58، 25–74% من المعاملة بمستخلص الكيومارين علي الترتيب. كما وجد أن النسب المئوية لموت اليرقات المعاملة تزداد كلما زاد تركيز مستخلصي حبة البركة والكيومارين. كما أن مستخلص حبة البركة أعطي تأثير كمانع للتغذية أعلي من تأثير الكيومارين عند حساب الجزء المتأكل من الأوراق بعد المعاملة بالمستخلصات النباتية. كما أظهرت النتائج تأثير للمستخلصات النباتية المختبرة علي إحداث نسبه من التشوهات لليرقات وعدم وصولها لطور العذراء ونتج عن ذلك ظهور مرحلة وسطية بين اليرقات والعذاري كذلك حدوث تشوهات للعذاري. كما أشارت النتائج إلي إطالة فترة العمر اليرقي بعد المعاملة بمستخلص حبة البركة والكيومارين حيث سببت زيادة في فترة العمر اليرقي علي الجانب الآخر سببت المعاملة بالكيومارين الي قلة فترة العذاري مقارنة بالمعاملة بمستخلص حبة البركة. كما أوضحت النتائج قلة وزن العذاري بعد المعاملة بكل من مستخلصي حبة البركة والكيومارين. وتشير النتائج إلي انخفاض النشاط الإنزيمي لإنزيم Invertase لليرقات التي تتم معاملتها بالمستخلص النباتي كيومارين بينما لم يحدث انخفاض في النشاط الإنزيمي عند المعاملة بالمستخلص النباتي حبة البركة مقارنة بالكنترول عند معاملة العمر اليرقي الرابع لدودة ورق القطن بالتركيز النصفي للمستخلصات النباتية. علي الجانب الآخر, أوضحت النتائج إلي زيادة معنوية في النشاط الإنزيمي لإنزيم trehalase لليرقات التي تتم معاملتها بالمستخلص النباتي كيومارين بينما لم يحدث زيادة في النشاط الإنزيمي عند المعاملة بالمستخلص النباتي حبة البركة مقارنة بالكنترول.

بينما، بينت النتائج إلي زيادة في النشاط الإنزيمي لإنزيم amylase لليرقات التي تتم معاملتها بالمستخلصين النباتيين مقارنة بالكنترول عند معاملة العمر اليرقي الرابع لدودة ورق القطن بالتركيز النصفي للمستخلصات النباتية. وأخيرا، أوضحت النتائج إلي إنخفاض في البروتين الكلي لليرقات التي تتم معاملتها بالمستخلصين النباتيين مقارنة بالكنترول عند معاملة العمر اليرقي الرابع لدودة ورق القطن بالتركيز النصفي للمستخلصات النباتية.