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## **Feeding deterrent and growth inhibitory properties of *Neotorularia aculeolata* against the cotton leafworm, *Spodoptera littoralis* (Boisd.)**

**Ibrahim Gaaboub<sup>1</sup>, Safaa Halawaa\*, Ahmed F El –Aswad<sup>2</sup> and  
Enas M. Khamis<sup>3</sup>**

**<sup>1</sup>Department of Plant Protection, Faculty of Agriculture Moshtohor, Benha Univ. <sup>2</sup>Pesticide Chemistry Department, Faculty of Agriculture Alexandria Univ. <sup>3</sup>Desert research center**

### **Abstract**

The research has been conducted to evaluate the activity of the botanical extracts from *Neotorularia aculeolata* against *Spodoptera littoralis* (Biosd.). Five solvents differ in their polarity benzene; ethyl acetate; chloroform; ethanol and water were used to extract the plant material. The following points were studied: (a) Evaluation the toxicity of different extracts against *S. littoralis* larvae (b) effect of plant extracts on the development of *S. littoralis* (c) evaluation of the antifeedant activity of plant extracts against 4<sup>th</sup> instar larvae of *S. littoralis*. Results showed that the 10% concentration of *N. aculeolata* exhibited strong antifeedant activity. On the other hand ethanol and benzene extracts caused the highest mortality percentage and antifeedant activity, respectively. The type and concentration of extracts by water, ethanol, chloroform and ethyl acetate affected on the sex ratio and development of *S. littoralis*. In general, the biological activity of *N. aculeolata* extracts was solvent type-and concentration-dependent.

**Key words:** *Spodoptera littoralis*, *Neotorularia aculeolata*, Brassicaceae, oviposition deterrence, Antifeedant activity, glucosinolates.

### **Introduction**

Secondary plant substances are defensive substances that inhibit food intake in the majority of plant-feeding insects, except for some specialized species, which may exploit these chemicals with a limited taxonomic occurrence as sign stimuli enhancing acceptance (Fraenkel, 1959 and Schoonhoven, 1972). Relatively few studies have addressed rejection as mechanism of host-plant specificity in a systematic way (Renwick *et al.*, 1992 and Nishida, 1995). (Jermy, 1958 and 1966) clearly demonstrated that rejection of non-hosts by various insects is due to the presence of feeding inhibitors (feeding deterrents) (Gaaboub and Halawaa, 2003). Plants offer a huge diversity of secondary

metabolites to herbivorous. In this diversity taxonomic patterns are discernible: a chemically distinct group of substances often occurs in one or a few related plant families only. Some other categories of secondary metabolites, however, have a wide distribution among unrelated plant families, notably many phenolics and flavonoids (Meiners and Hilker, 2000).

The number of instances in which particular taxon-specific secondary metabolites act as feeding or oviposition stimulants to monophagous or oligophagous species has grown considerably since (Verschaffelt, 1910) days. Examples of feeding or oviposition activity governed by secondary plant substances in number of food specialists belonging to different orders (Honda *et al.*, 1995)

Family Brassicaceae (*Cruciferae*) is known to be rich of medicinal and edible plants, which are characterized by the presence of oils, flavonoids (unusual structures) and glucosinolates or their aglucones (isothiocyanates) (Verschaffelt 1910, Van Loon *et al.*, 1992 and Huang and Renwick, 1994). The aqueous extracts and total glucosinolates of plant showed a potent effect on cotton leaf worm (*S. littoralis*). Pet. Ether extract of plant showed an ovicidal activity on *S. littoralis*. Plants adversely affected all the developmental aspects of the pest (Jermy and Szentesi, 1978, Feeny *et al.*, 1988 and Gaaboub and Halawa, 2003).

The use of synthetic pesticides for plant protection against agriculture pests during the last 50 years created many problems. Namely, pest resistance, environmental pollution and disturbance in natural balance. To overcome this problem many researchers all over the world are looking to discover new safe types of pest control agents. Many plant products possess properties that give the same effect of pesticides and are known to be used in pest management strategy. The plant extracts obtained in this study are among those compounds under investigation as potential biopesticides.

## Material and Methods

### Preparation of the plant extracts

The plant parts were dried for 2-3 weeks at room temperature and ground in an electric mill into fine powder. The ground plant materials 200g were soaked in the different solvents of increasing polarity as follows: [Benzene, chloroform, ethyl acetate, ethanol and water respectively] in a large flask for 72 hrs. The flask was then shaken for 30 min in a shaker and its contents were filtered. The solvents were evaporated under vacuum pressure using a rotary evaporator. Samples were kept in refrigerator (-4 C°). Concentrations of 10,5 and 2.5 % (w/v) were used for testing their insecticidal activities against *S. littoralis*.

### Tested Insect:

Laboratory strains of the cotton leaf worm *S. littoralis* (Biosd.) were used in these studies. The culture of *S. littoralis* originated from eggs obtained from laboratory strain established in the Department of Plant Protection, Faculty of Agriculture Moshtohor, Zagazig University. These eggs were kept in glass jar covered with gauze under laboratory condition of  $27 \pm 1$  C°. and  $65 \pm 5$  % RH. When the egg became dark enough in color fresh castor leaves were introduced daily into the jars as dietary medium for the hatched larvae. This procedure continued till the second larval instar. The 3rd instar larvae were individually isolated in glass jars capacity 1 kg the rate of 15 larvae to avoid cannibalism. The jars were cleaned daily, The number of larvae was limited to 10 larvae per jar, when larva reached the last instar then transferred to a clean jar, provided with moistened sawdust 4 cm thick and allowed to pupate. To perpetuate the stock culture of this insect, full-grown pupae of both sexes were transferred to cylindrical cages covered with gauze for oviposition. The adults were reared by using 10% sugar solution.

### Bioassay tests for *S. Littoralis*

- 1- Antifeedant activity
- 2- Biological effects
- 3- Toxicity effects

#### 1-Bioassay for feeding deterrence of plant extracts

Tests for feeding deterrence were carried out using fourth instar larvae of a laboratory culture of *S. littoralis*. The larvae were starved for 6 hours before treatment and divided into 3 replicates (10 larva for each replicate). Discs of 39.12-mm<sup>2</sup> area of castor leaves were impregnated with the extract under investigation and allowed to dry. Only one disc was offered to each tested larva. Untreated discs were introduced to larvae as blank control. Discs impregnated with the same solvents allowed drying then offered to test insects as control with solvents. The eaten area was estimated after 24 hours by planimeter.

The percentage of feeding reduction over control was the factor used for determining the presence of feeding deterrent effect. The antifeeding activity of the plant extracts was evaluated on the basis of the feeding ratio of the treated and untreated leaf discs. The antifeeding activity was calculated by using the formula of (Saleh *et al.* (1986) as follow

$$\text{Antifeeding activity} = 1 - \frac{\% \text{ of. Eaten area in treatment}}{\% \text{ of Eaten. Area in control}} \times 100$$

## 2- Biological effects:

All treated larvae were left for 24 hrs. and supplied with fresh castor leaves and kept under observation until the emergence of adults. Adults were supplied daily with 10% sugar solution. Mortality counts during the larval, pupal and adult stages were recorded. Abnormal pupation and the percentage of malformed adults were recorded for each treatment. Control tests were carried out by the use of solvents only alongside untreated blanks.

## 3- Toxicity effects:

In order to study the toxicity of the concerned plant extracts preliminary screening tests were carried out to the plant extracts with five different solvents at three concentration levels of 10, 5 and 2.5 % w/v). Three replicates (10 larvae each) were used for each concentration. Insect larvae were kept under controlled temperature  $27 \pm 1$  C°. Percentage of total mortality was recorded after the end of the larvae stage. Percentage mortalities were corrected according to Abbots formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{observed mortality \%} - \text{control mortality \%}}{100 - \text{control mortality \%}} \times 100$$

## RESULTS AND DISCUSSION

### 1- Antifeedant activity against *S. littoralis*

In this trial only 4<sup>th</sup> instar larvae of *S. littoralis* were used to establish the presence of antifeeding properties in these plant extracts.

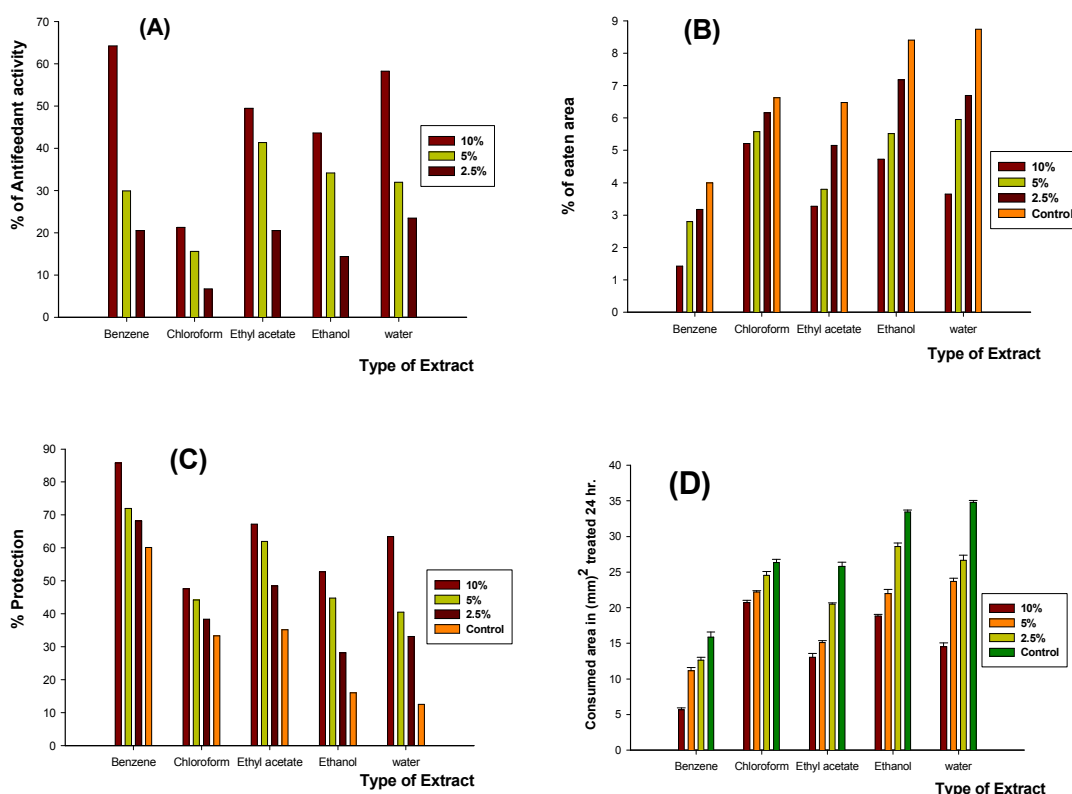
#### 1.1- *N. aculeolata* leaves extracts

Results in Table (1) & Fig. (1) indicated that benzene extracts possess antifeedant activity and this activity increases with increasing the concentration of the extracted materials. The greatest antifeedant activity 64.28 % were obtained with using of 10 % concentration, followed by water, ethyl acetate, ethyl alcohol and chloroform at the same concentration. Corresponding percentages of antifeeding activity were 58.23, 49.5, 43.64 and 21.3 %, respectively Fig. 1(A&B).

On the other hand, the lowest effect was recorded with 2.5 % chloroform giving 6.7 % antifeeding activity. Data in table (1) showed that benzene was the best solvent system in extracting bioactive components of *N. aculeolata* which caused the highest protection to the plant 85.74 %) Fig. 1(C&D).

**Table (1)** Effect of *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] crude extract concentrations on antifeedant activity of 4<sup>th</sup> instar larvae of *S. littoralis*, protection % and consumed area of the treated caster leaves with different polarity solvents.

Solvents	% Conc.	Consumed area in (mm) <sup>2</sup> treated 24 hr.	% of eaten area	% protection	Antifeedant activity
<b><i>Benzene</i></b>	10	5.68 ± 0.14	1.43	85.74	64.28
	5	11.14 ± 0.48	2.80	72.01	29.94
	2.5	12.64 ± 0.42	3.18	68.23	20.50
<b>Control</b>	-----	15.88 ± 0.70	4.00	60.1	-----
<b>Chloroform</b>	10	± 0.30	5.21	47.94	21.3
	5	22.22 ± 0.16	5.58	44.18	15.6
	2.5	24.54 ± 0.55	6.16	38.35	6.70
<b>Control</b>	---	26.35 ± 0.43	6.62	33.35	----
<b>Ethyl acetate</b>	10	13.03 ± 0.54	3.27	67.25	49.50
	5	15.12 ± 0.24	3.80	62.01	41.40
	2.5	20.51 ± 0.17	5.15	48.47	20.50
<b>Control</b>	---	25.80 ± 0.58	6.48	35.17	----
<b>Ethanol</b>	10	18.82 ± 0.25	4.73	52.71	43.64
	5	21.99 ± 0.59	5.52	44.76	34.17
	2.5	28.59 ± 0.49	7.18	28.17	14.41
<b>Control E</b>	---	33.42 ± 0.28	8.40	16.03	-----
<b>Water</b>	10	14.54 ± 0.53	3.65	63.48	58.23
	5	23.68 ± 0.47	5.95	40.49	31.94
	2.5	26.63 ± 0.75	6.69	33.08	23.47
<b>Control</b>	----	34.80 ± 0.23	8.74	12.55	-----



**Fig. (1)** Effect of *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] crude extract concentrations on (A) antifeedant activity of 4<sup>th</sup> instar larvae of *S. littoralis*, (B) %of eaten area, (C) % protection and (D) consumed area of the treated castor leaves with different polarity solvents.

As shown in table (2), the different solvents varied widely in their content of toxic and undesirable materials for *S. littoralis* depends on the degree of their polarity, Benzene and chloroform were rich in terpenes and comarins and poor in glucosiolates, flavonoids and tannins. The richness of benzene and chloroform in the former two compounds was the reason of the high protection percentage with less consumed area in the plant leaves with less mortality percentage Fig. (3). On the other hand, decreasing the protection percentage and the consumed area of treated leaves and increasing mortality percentages were accompanied with ethanol and ethyl acetate extracts. This may be the existence of glucosiolates in high level, which may encourage the *S. littoralis* to consume plant material. It is worthy to note that, there are some biocompounds in the above mentioned solvents encourage the insects to eat plants as it contained, for instance glucosiolates, but they increased the mortality because of the presence of other compounds, i.e., glucosiolates and tannins Fig. (2) & table (3).

Mustard oil glucosides (glucosinolates), which are taxonomically characteristic for cruciferous plants, are decisive factors for plant acceptance by caterpillars of the cabbage white butterflies *Pieris brassicae* and *P. rapae* (Renwick *et al.*, 1992). The chemosensory basis of this behaviour was revealed only much later by the discovery of taste cells on the maxilla of the caterpillars that are specifically sensitive to these glucosides (Verschaffelt, 1910; Feeny, *et al.*, 1988 and Gaaboub, *et al.*, 2005).

The role of terpenoids contained in essential oils of Apiaceae in host-plant acceptance of black swallowtail (*Papilio polyxenes*) caterpillars, specialized feeders on this plant family. (Dethier, 1941; Fraenkel, 1959 and Meiners and Hilker, 2000). The role of deterrents, secondary plant substances inhibiting feeding or oviposition (Jermy and Szentesi, 1978) and advocated the view that host-plant selection is mainly based on avoidance of deterrents present in non-hosts (Jermy, 1958 and 1966).

### **Insecticidal activity studies:**

The insecticidal activity of different extracts benzene, chloroform, ethyl acetate, ethanol and water as well as the total glucosinolates of *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] were studied. The aqueous extracts and total glucosinolates of *N. aculeolata* showed potent effects on cotton leaf worm (*Spodoptera littoralis*). The extract of *N. aculeolata* showed an ovicidal activity on *S. littoralis*. Plants adversely affected all the developmental aspects of the pest (Table 2&3). Family *Cruciferae* is known to be rich of medicinal and edible plants, which are characterized by the presence of oils, flavonoids (unusual structures) and glucosinolates or their aglucones (isothiocyanates) (Bernays, *et al.*, 1991). The results showed that the aqueous fractas and the total glucosinolates of *N. aculeolata* (Family *Cruciferae*) were remarkably toxic against *S. littoralis* larvae and this is in agreement with that reported by El-Gengahi, *et al* (1996). These results agree with that reported by Khan and Shahjahan (1998). When the total glucosinolates of *C. annua* and *F. aegyptia* were applied, the antifeeding activity showed that the percentage of starvation was found to be 78.1% and 70.8 1% respectively. These results were coincided with that reported by El-Gengahi *et al*, (1996) who found a significant reduction in the food consumed and a considerable decrease in the body weight gained by the larvae of *S. littoralis* and *Agrotis ipsilon* offered castor bean leaves treated with different plant extracts.

## **2- Biological effects of plant extracts on 4<sup>th</sup> instar larvae of *S. littoralis***

Extracts were studied to investigate the biological effects, which may occur after exposure of 4<sup>th</sup> larval instar to these extracts.

### A.2.1- Effect of *N. aculeolata* extract on insect's development: -

Table (2) showed the amounts of coumarins, flavonoids, tannins and glucosinolates. The flavonoids of both plants present either in the free or in the glycosidic form were studied. The flavonoids were obtained from the alcoholic extract 70% of the defatted material of both plants by using the conventional methods *i.e.* by treating the concentrated alcoholic extract with hot distilled water followed by successive extraction with organic solvents (chloroform, ethyl acetate and ethanol). Polyphagous species also may be stimulated by the presence of flavonoids in their food has been documented as a feeding stimulant for both the desert locust (*Schistocerca americana*) (Bernays, *et al.*, 1991) and *Helicoverpa virescens* caterpillars (Schoonhoven, 1967 and 1972). Data in Table (3) & Fig. (2) revealed that development of larvae after the exposure to *N. aculeolata* extract with the different five solvents (benzene, chloroform, ethyl acetate, ethyl alcohol and water). The highest percentage of dead larvae was recorded at 10 % concentration of ethyl alcohol extract (30.79 %). However, the lowest number of dead larvae was recorded with benzene at 2.5 %, which gave only 1.31-% Fig. (2).

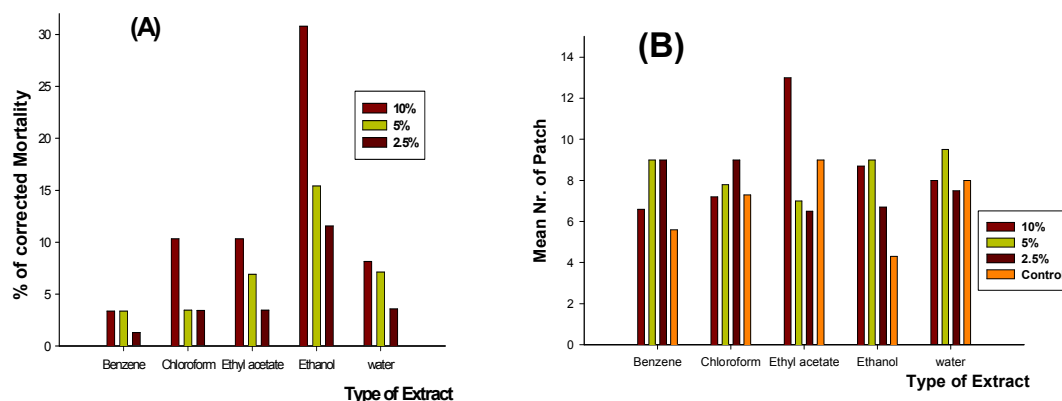
**Table (2)** Visible (+ve) and non- visible (-ve) phytochemicals of *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] plant extracted with different solvents.

	Benzene	Chloroform	Ethyl acetate	Ethyl alcohol	H <sub>2</sub> O
Flavonoids	- ve	+ ve	++ ve	+++ ve	++ ve
Tannins	- ve	++ve	+++ ve	+++ ve	+++ ve
Glycosides &/or carbohyd.	- ve	+ ve	++ ve	++ ve	+++ ve
Terpenes	+++ ve	+++ve	+ ve	- ve	- ve
Alkaloids	- ve	- ve	- ve	- ve	- ve
Chloride & sulfates	- ve	- ve	- ve	- ve	- ve
Resins	+ ve	++ve	+++ ve	- ve	- ve
Saponins	- ve	- ve	-ve	+ ve	++ ve
Anthraquinons	- ve	- ve	- ve	- ve	- ve
Comarins	+++ve	++ ve	- ve	+ve	- ve
Cardiac glycosides	- ve	- ve	- ve	- ve	- ve
Glucosinolates	- ve	+ ve	++ ve	+++ ve	+++ ve



**Table (3)** Biological activity of *S. littoralis* as affected by *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] crude extract concentration.

Solvents	% Conc .	Larval stage		Pupae stage			Adult stage		
		% correcte d Mortality	Duratio n day	% Mortal.	% Malforme d.	Duratio n day	% malf orme d	Sex Ratio F. M.	
Benzene	10	3.38	13.2	0.00	26.66	14.16	0.00	11	11
	5	3.38	13.2	6.66	3.33	14.75	7.14	14	11
	2.5	1.31	12.7	10.00	16.66	14.75	10.17	14	7
Control B		0.00	12.6	0.00	6.8	17.64	3.45	15	14
Chloroform	10	10.34	12.3	0.00	7.69	15.75	0.00	11	12
	5	3.45	13	0.00	7.14	15.31	4.00	8	17
	2.5	3.44	13.5	0.00	20.69	14.64	0.00	10	13
Control		0.00	13	0.00	3.57	14.83	0.00	17	16
Ethyl acetate	10	10.34	13	15.38	7.69	15.4	0.00	12	12
	5	6.90	13.5	3.70	3.70	16.25	0.00	11	14
	2.5	3.45	13.5	7.14	3.57	16.50	0.00	8	17
Control		0.00	13.5	0.00	0.00	17.33	0.00	10	13
Ethanol	10	30.79	17	0.00	5.88	10.03	6.25	10	7
	5	15.42	16	9.09	4.67	13.5	10.53	9	12
	2.5	11.57	16	0.00	13.04	12.28	0.00	8	12
Control E		0.00	16.5	0.00	3.85	11.31	0.00	11	14
Water	10	8.14	14	0.00	6.66	12.00	0.00	12	11
	5	7.14	15.25	0.00	10.00	13.50	0.00	9	13
	2.5	3.57	16	6.66	10.00	13.76	3.57	11	12
Control		0.00	16	0.00	0.00	14.64	0.00	16	12



**Fig. (2)** Effect of *N. aculeolata* [Family Brassicaceae (*Cruciferae*)] crude extract concentrations on (A) % Mortality of 4<sup>th</sup> instar larvae of *S. littoralis* and (B) mean number of patch eggs fed on the treated castor leaves with different polarity solvents.

The study of the developmental effects showed that *N. aculeolata* (Family *Cruciferae*) tested plant adversely affected all developmental aspects of the pest (table 3). These results were in agreement with El-Gengahi, *et al.* (1996) who found that both *Diplotaxis acris* and *D. hara* (family *Cruciferae*) had developmental inhibition against *S. littoralis*.

The type and concentration of water, ethanol, chloroform and ethyl acetate extracts affected the sex ratio. The male ratio was generally greater than female one but with benzene extract the female ratio was greater than male one. The type of chemical compounds such as flavonoids, coumarin, glucosinolate, tannins, alkaloids, saponins, cardiac glycosides and anthraquinones play one of the important role in this ratio.

The female/male sex ratio was higher on Brassica species than on non-Brassicaceae. *P. xylostella* infestation and percentage parasitism in the field in Michigan were higher on broccoli than on the other Brassica crops, but the female/male ratio of *D. insulare* was not significantly different (Idris and Grafius, 1993).

On the other side data in table (3) showed that the highest number of abnormal pupae resulted after exposure of 4<sup>th</sup> instar larvae to benzene and chloroform extracts giving between 26.66 and 20.69 % for all used concentration. The low concentrations of terpenes and coumarins showed positive effect on plant growth. However, the high concentration of such materials induced a toxic effect to insect, while low concentration acted as the juvenile hormone (JH) (Halawa, 2003).

An intriguing example is the cabbage root fly, *Delia radicum*, for which glucosinolates act as taxon-specific oviposition stimulants (Roessingh, *et al.*, 1992a and 1992b; Staedler, 1978 and Staedler and Roessingh, 1991). These were assumed to be the prime phytochemicals on which host-plant specificity in this species was based.

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## خواص مستخلصات نبات نيوتورولاريا أكيولاتا كمانعات تغذية ومثبطات لنمو لدودة ورق القطن

إبراهيم عبد الله جعوب<sup>1</sup> و صفاء محمود حلاوة<sup>1</sup> و أحمد فرحات الأسود<sup>2</sup> و إيناس محمد خميس<sup>3</sup>

1- قسم وقاية النبات ،كلية الزراعة بمشتر، جامعة الزقازيق.

2- قسم كيمياء المبيدات ، كلية الزراعة ، جامعة الإسكندرية

3- مركز بحوث الصحراء

### الملخص العربي

يهدف هذا البحث تقييم الكفاءة البيولوجية لبعض مستخلصات نبات نيوتورولاريا أكيولاتا (*Neotorularia aculeolata*) ضد دودة ورق القطن. استخلص هذا النبات بخمسة أنواع من المذيبات مختلفة القطبية وهما البترين والكلوروفورم والايثايل أسيتات وكحول الايثايل والماء . وقد تم دراسة النقاط التالية (1) تقييم سمية المستخلصات على العمر الرابع لدودة ورق القطن (2) تأثير المستخلصات على تطور وغزو دودة ورق القطن (3) تقييم كفاءة المستخلصات كمانعات تغذية على العمر الرابع لدودة ورق القطن . أظهرت النتائج أن تركيز 10% من كل المستخلصات أعطى أعلى تأثير كمانع للتغذية على العمر الرابع لدودة ورق القطن . سبب مستخلص كحول الايثايل أعلى نسبة موت وسبب مستخلص البترين أعلى تأثير كمانع للتغذية. ونوع المستخلص وتركيزه تؤثر على النسبة الجنسية وتطور دودة ورق القطن. وعموماً يمكن القول أن التأثير البيولوجي لمستخلصات نبات نيوتورولاريا أكيولاتا يعتمد على كل من نوع المذيب وكذلك التركيز المستخدم.