375
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STUDIES ON NEEM SEED KERNEL EXTRACTS AS OVIPOSITION DETERRENTS AND OVICIDAL COMPOUNDS FOR THE COTTON LEAF WORM MOTHS, *Spodoptera littoralis* (Boisd.)

[32]

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ABSTRACT

Mated female moth of the cotton leafworm, *S. littoralis* laid a total number of 1564.22 eggs during its whole life. In a free choice test, treating *N. oleander* leaves (as an oviposition site) with an aqueous extracts of neem seed kernel solutions extracted by different organic solvents reduced the number of deposited eggs on it. The lowest number of eggs was recorded when methanol was used for extraction, followed by chloroform, and the highest was, however, obtained after using benzene, butanol and ethyl acetate. The number of deposited eggs decreased as the concentration of any tested neem extract increased to reach the minimum at 0.2%. This means that the most deterred neem seed kernel extract is that which extracted with methanol at the concentration of 0.2%. Hatchability percentage of *S. littoralis* eggs greatly affected by the type of organic solvents used for neem extraction and the concentration of each extract on treated *N. oleander* leaves. The lowest hatchability was recorded after using methanol, followed by chloroform. On the other hand, an increase in neem seed kernel extract concentration caused an obvious decrease in egg hatchability.

INTRODUCTION

It is well known that neem derivatives have a variety of effects including antifeedant, oviposition deterrent, growth regulatory and toxic activities against a wide variety of pests which include agricultural, stored products and household pests, in addition to vectors of animal and human diseases. Neem derivatives are primarily considered as toxicants to larvae or nymphs of phytophagous insects and hence show considerable selectivity toward natural enemies and biological control agents such as parasitoids and predators (**Awad *et al* 1998**). The same authors added that owing to the effectiveness of neem derivatives against pests, biodegradation, considerable selectivity, harmlessness to beneficial and nontarget organisms, these derivatives have potential for use in integrated pest management. **Deka *et al* (1999)** suggested that chloroform, methanol and petroleum ether extracts from *Melia azadirach* showed antifeedant and oviposition deterrent activity to tea mosquito bug. Margosan-O (an insecticide formulated from extracts of neem tree, *Azadirachta indica* seed kernels) besides being toxic, also has feeding, oviposition-deterrent, and growth-inhibitory effects on certain insects **(Scott and Kaushik, 1998)**. In India, neem products including seed kernel suspension, oil emulsion, seed cake and bark extract were used in order to control insect pests on tobacco plants. Antifeedant and oviposition deterrent effects were also noticed. It is possible to use neem extracts against the noctuid, *Spodoptera litura* **(Joshi *et al* 1987).**

The present work aimed at studying the ovipositional deterrency and ovicidal effect of neem seed kernel aqueous solutions extracted with different types of organic solvents against the cotton leaf worm moth, *Spodoptera littoralis* (Boisd.).

MATERIALS AND METHODS

Rearing technique

The egg masses of the cotton leaf worm, *S. littoralis* were obtained from cotton fields in Shalakan, kalyobia Governorate (20 km. north Cairo),

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transferred to the laboratory and reared in glass containers as previously described by **Gomaa (2006)** which were provided daily with sufficient amounts of clean and fresh castor oil leaves, *Ricinus communis* (L.) as larval food. This occurred for two successive generations at 24± 2°C and 72± 5% R.H. to ensure complete adaptation. The produced egg masses were placed separately in glass containers of 2 kg capacity each, covered with muslin material and held by a rubber band. Observation was made daily. Soon after hatching, newly hatched larvae of the same age were transferred to a new glass container in which saw dust was placed at the base to absorb excess humidity. Every day, the larval faeces and dried castor oil leaves were removed. The population density of larvae in each 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. The newly emerged moths were used in a free choice test.

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 one of the following organic solvents: methanol, benzene, chloroform, ethyl acetate or butanol by using soxhlet extractor. The obtained extracts were evaporated in a rotary evaporator under vacuum until dryness. The residues were 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 concentration for each neem seed kernel extract. The aqueous solutions were labeled and kept in refrigerator. Just before starting the experiment, different concentrations from each extract were made, i.e., 0.025, 0.05, 0.1, 0.15 and 0.2% to study their ovipositional deterrency, and 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% to study their ovicidal effects.

Experiments of ovipositional deterrency

Immediately after eclusion, one male and one female moths were placed for mating and egg deposition in a wooden breeding cage (40 cm in width, 40 cm in depth and 60 cm in height), having two separate branches of Tafla, *Nerium oleander* (L), each having at least three leaves as an ovipiosition site. Leaves of the first branch were treated by dipping them for about 5 seconds in one concentration of any of the tested extracts and dried at laboratory conditions. The second branch was left untreated. Each branch was placed in small glass vial (5 cm in height and 3 cm in diameter) and both were situated in the cage in free choice test 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. This technique was replicated 5 times for each concentration of any tested neem seed kernel extract.

Experiments of ovicidal action

Another set of experiments similar to that of ovipositionnal deterrency was conducted using lower concentrations of neem seed kernel extracts ranging between 0.0015 and 0.025% to study their ovicidal action in cages in a free choice test. The deposited eggs laid on treated *N. oleander* leaves were counted and left in a Petri desh for hatching. The newly hatched larvae were counted and the hatchability percentages were calculated. This technique was replicated 5 times. The obtained data in the present work were statistically analyzed.

RESULTS

1- Number of deposited eggs on treated leaves

As shown in **Table (1)** mated female moth of the cotton leafworm, *S. littoralis* laid a total number of 1564.22 eggs during its whole life span, ranging between 1921.88 and 1991.26 eggs/ female.

In a free choice test, treating *N. oleander* leaves (as an oviposition site) with an aqueous extracts of neem seed kernel solutions extracted by different organic solvents reduced the number of deposited eggs on the treated leaves and, on the other hand, increased the number of eggs laid on the untreated leaves. The lowest number of eggs (351.48 eggs/ female) was recorded when methanol was used for extraction, fcllowed by chloroform (412.83 eggs/ female). The highest number of deposited eggs was, however, obtained after using benzene and butanol (695.17 and 705.13 eggs/ female, respectively) as well as ethyl acetate (750.19 eggs/ female). The difference between these means proved to be statistically highly significant and the L.S.D. value (22.69 eggs) emphasizes the obtained results. By applying this

Table 1. Total number of eggs laid by mated *S. littoralis* female moth on untreated *N. oleander* leaves and those treated with five concentrations of neem seed kernel extracts using five organic solvents in free choice test (Means of 5 replicates ± S.E.)

Solvents	Conc. (%)	No. of eggs/ female on *N. oleander* leaves		
		Treated	Untreated	Total
Methanol	0.025	659.78±22.15	1270.25±31.81	1930.03±37.72
	0.05	504.02±16.75	1438.01±30.62	1942.03±39.38
	0.10	222.56±12.18	1746.44±25.23	1969.00±33.47
	0.15	64.19± 9.34	1825.55±34.57	1889.74±40.15
	0.20	6.86± 4.46	1940.51±32.66	1947.37±36.89
	Mean	**351.48±19.56 d**	**1644.15±36.27 a**	**1935.63±35.94**
Benzene	0.025	824.66±29.18	1134.68±38.35	1959.34±42.35
	0.05	769.16.±26.64	1169.84±40.19	1939.00±45.28
	0.10	699.32±25.28	1302.58±32.53	2001.90±36.77
	0.15	624.11±31.66	1286.66±34.98	1910.77±41.02
	0.20	558.62±33.35	1407.83±42.08	1966.45±44.16
	Mean	**695.17±30.61 b**	**1260.32±37.68 c**	**1955.49±41.05**
Chloroform	0.025	743.39±28.63	1191.02±34.11	1934.41±40.17
	0.05	591.35±21.30	1298.57±31.36	1889.92±38.52
	0.10	483.86±16.12	1437.59±29.45	1921.45±36.80
	0.15	206.24±15.24	1755.23±30.06	1961.47±41.45
	0.20	39.29± 6.02	1862.84±33.52	1902.13±39.68
	Mean	**412.83±18.88 c**	**1509.05±32.51 b**	**1921.88±41.14**
Ethyl acetate	0.025	888.35±32.16	1124.02±37.10	2013.18±41.57
	0.05	811.22±33.54	1152.56±34.92	1963.78±39.43
	0.10	755.78±30.52	1200.05±35.27	1955.83±40.58
	0.15	702.05±34.46	1274.42±32.88	1976.47±37.51
	0.20	593.57±29.89	1358.81±36.21	1952.38±41.22
	Mean	**750.19±30.59 a**	**1221.97±37.43 d**	**1972.33±40.28**
Butanol	0.025	845.45±29.99	1165.43±34.08	2010.88±42.83
	0.05	784.79±31.84	1221.75±31.57	2005.04±39.42
	0.10	704.21±30.42	1280.36±37.84	1984.57±40.37
	0.15	625.13±38.15	1347.32±29.99	1972.45±38.11
	0.20	566.09±28.97	1447.28±31.31	1983.37±36.68
	Mean	**705.13±31.12 b**	**1292.43±37.88 c**	**1991.26±39.96**
F. between solvents		29.73**	30.56**	1.09-
L.S.D. at 0.05		22.69	37.08	
F. between concentrations		17.14**	28.05**	2.04-
L.S.D. at 0.05		19.87	25.74	

-, Insignificant **, Significant at 0.01 level of probability

value, the mean number of deposited eggs could be arranged into the following descending orders as being affected by the type of organic solvent used for neem seed kernel extraction:

First group : Ethyl acetate (750.19 eggs/ female).
Second group: Butanol and benzene (705.13 and 695.17 eggs/ female, respectively)..
Third group : Chloroform (412.83 eggs/ female).
Fourth group : Methanol (351.48 eggs/ female)

The data clearly show that methanolic extract of neem seed kernel efficiently deterred the mated *S. littoralis* female moth more than those extracted with other tested organic solvents.

Irrespective of the type of organic solvent used, the concentration of neem seed kernel extract greatly deterred the mated female moths to lay their eggs on the treated surface of *N. oleander* leaves. As shown in **Table (2)** the number of deposited eggs decreased as the concentration of extract increased to reach the minimum after using 0.2% neem extract. Means of 792.33, 692.11, 573.11, 444.34 and 352.89 were calculated for the number of eggs laid by mated female moth, representing the oviposition percentages of 40.23, 35.53, 29.14, 22.88 and 18.08 on *N. oleander* leaves treated with 0.025, 0.05, 0.1, 0.15 and 0.2%, respectively. The opposite was, however, true for the number of eggs laid by female moths on the existed untreated leaves in the same cages in a free choice test.

2- oviposition percentages on treated leaves

The data given in **Table (3)** clearly show that irrespective of the concentration of neem seed kernel extract, the highest oviposition percentage (39.19%) was laid on leaves treated with aqueous solutions of ethyl acetate extracts, followed by those deposited on leaves treated with benzene and butanol extracts (36.98 and 34.12%, respectively). The lowest percentage (18.02%) was, however, recorded on leaves treated with methanolic extracts of neem seed kernels, followed by chloroform extracts (24.18%).

The oviposition percentage decreased as the concentration of neem kernel extracts increased to reach the minimum on leaves treated with the highest concentration used in the present work. This was common irrespective of the type of organic solvents used in the present work. Means of 41.28, 36.91, 31.12, 25.31 and 17.87% were recorded for the oviposition percentages of *S. littoralis* female moths laid on *N. oleander* leaves treated with neem seed kernel extracts at the concentrations of 0.025, 0.05, 0.1, 0.15 and 0.2%, respectively.

From the fore-mentioned results, it could be concluded that the most deterred neem seed kernel extract is that which extracted with methanol at the concentration of 0.2%. In this case, 3.58% only of the deposited eggs were laid on treated *N. oleander* leaves and the others were laid on untreated leaves in a free choice test.

3- Hatchability of deposited eggs on treated leaves

Preliminary results showed highly toxicity of neem seed kernel extracts to *S. littoralis* eggs laid on treated *N. oleander* leaves at the low concentration (0.025%). Therefore, lower concentrations (0.0015, 0.0031, 0.0062 and 0.0125%) were prepared and used to study their effects on the viability of deposited eggs. The data given in **Table (4)** clearly show that hatchability of eggs laid on untreated leaves averaged 98.65%. As shown in table 4, the hatchability percentage greatly affected by the type of organic solvents used for extraction of neem seed kernels and the concentration of each extract on treated *N. oleander* leaves.

Irrespective of the concentration of the extract, the lowest hatchability (17.02%) was recorded after using methanol, followed by chloroform (22.60%), and the highest (45.20%) was, however, obtained after using ethyl acetate. An intermediate hatchability (35.88 and 35.36%) was recorded after using benzene and butanol, respectively. This means that the most toxic neem seed kernel solution for *S. littoralis* embryos was that which extracted with methanol, followed by chloroform. On the other hand, egg hatchability was found to be concentration dependent, i.e., an increase in neem seed kernel extract concentration caused an obvious decrease in egg hatchability. Mean percentages of 45.96, 38.34, 31.44, 23.88 and 16.44 were recorded for egg hatchability after treating the oviposition site with 0.0015, 0.0031, 0.0062, 0.0125 and 0.025% of neem seed kernel extracts irrespective of the solvent type.

DISCUSSION

According to **Kumari *et al* (1998),** powders of neem seed kernels and neem leaves at 25 g/kg groundnut pods were evaluated against the groundnut bruchid, *Caryedon serratus* (Olivier);

Table 2. Mean number of eggs laid by mated female moth of *S, littoralis* on *N. oleander* leaves treated with different concentrations of neem extracts irrespective of the type of organic solvents used.*

Concenrations (%)	No. of eggs/ female laid on			Oviposition on treated leaves (%)
	Treated leaves	Untreated leaves	Total	
0.025	792.33	1177.08	1969,41	40.23
0.05	692.11	1256.15	1947.95	35.53
0.10	573.11	1393.40	1966.51	29.14
0.15	444.34	1497.84	1942.18	22.88
0.20	3.52.89	1603.45	1956.34	18.04
Mean	**570.96**	**1385.58**	**1956.48**	**29.18**

*) The data are derived from those recorded in Table (I)

Table 3. Oviposition percentages (OP%*) of *S. littoralis* mated female moths on *N. oleander* leaves treated with five concentrations of neem seed kernel extracts using five organic solvents in free choice test (Means of 5 replicates ± S.E).

Conc. (%)	Organic solvents					Mean
	Methanol	Benzene	Chloroform	Ethyl acetate	Butanol	
0.025	35.67±	42.82±	39.51±	45.06±	43.35±	**41.28±**
	5.15	4.87	4.84	4.79	3.78	**4.08**
0.05	28.20±	40.63±	33.08±	42.11±	40.52±	**36.91±**
	4.11	4.05	4.05	4.44	3.28	**3.53**
0.10	14.81±	36.74±	27.52±	39.70±	36.81±	**31.12±**
	2.85	3.41	5.82	3.92	2.79	**2.87**
0.15	7.86±	34.31±	14.17±	36.85±	33.38±	**25.31±**
	0.57	4.15	2.27	3.41	3.07	**3.04**
0.20	3.58±	30.40±	6.63±	32.22±	16.54±	**17.87±**
	0.11	3097	0.61	3.17	2.85	**3.15**
Mean	**18.02±**	**36.98±**	**24.18±**	**39.19±**	**34.12±**	**30.50±**
	3.64	**4.56**	**3.98**	**4.05**	**3.75**	**3.92**

*) OP % = $\frac{\text{Number of eggs on treated leaves}}{\text{Total number of deposited eggs**}} \times 100$

**) Total number of deposited eggs = Number of eggs on treated leaves + number of eggs on untreated leaves

Table 4. Hatchability percentages of *S. littoralis* eggs treated with different concentrations of neem seed kernel extracts using five organic solvents (Means of five replicates, each contained 100 eggs ± S.E.).

Conc. (%)	Organic solvents					Mean
	Methanol	Benzene	Chloroform	Ethyl acetate	Butanol	
0.0015	32.20±	51.60±	38.20±	57.80±	50.00±	**45.96±**
	3.13	3.59	2.22	3.54	3.75	**3.49**
0.0031	24.50±	43.20±	30.00±	51.20±	42.80±	**38.34±**
	2.55	3.11	2.75	3.07	3.99	**3.07**
0.0062	18.80±	36.40±	22.60±	45.00±	34.40±	**31.44±**
	2.79	2.53	1.85	3.11	3.51	**2.73**
0.0125	7.20±	30.20±	13.40±	39.60±	29.00±	**23.88±**
	0.82	3.87	0.98	3.40	2.42	**2.99**
0.025	2.40±	18.00±	8.80±	32.40±	20.60±	**16.44±**
	0.31	1.59	0.51	2.84	1.84	**1.01**
Mean	**17.02±**	**35.88±**	**22.60±**	**45.20±**	**35.36±**	**31.21±**
	3.04	**3.45**	**3.79**	**3.96**	**3.04**	**2.68**

a serious pest of groundnut pods and kernels by. These plant powders had an oviposition deterrent activity with different degrees but lost effectiveness gradually after less than one month.

The present data revealed that mated *S. littoralis* female moth laid a total number of 1564.22 eggs during its whole life span. In a free choice test, treating *N. oleander* leaves with an aqueous extracts of neem seed kernel solutions extracted by different organic solvents reduced the number of deposited eggs. The lowest number of eggs was recorded when methanol was used for extraction. This is in accordance with **Chen *et al* 1996)** who stated that using botanical extracts, oviposition by the yponomeutid diamond back moth, *Plutella xylostella* was deterred by extracts from fruits of *Melia azadarach*. They added that oviposition was significantly reduced by 49.6, 86.6 and 93.5% in free choice tests and 46.2. 72.1 and 80.2% in no choice tests at concentrations of 0.5, 2.0 and 4.0%, respectively. According to **Patil and Goud (2003)**, 0.5% *Azadirachta indica* recorded maximum reduction in egg laying of the mated female moth of the same insect species, both under no choice (50.33%) and free choice (62.43%) conditions. Moreover, **Meena-Thakur *et al* (1998)** reported that the methanol extract of *Melia azedarach* drupes produced the lowest number of eggs laid by the single mated female of *Henocepilachna (Epilachna) vigintioctopunctata* (24.80), whereas methanol extract of *M. azedarach* leaves produced the highest number of eggs (34.93). They added that 6% *M. azedarach* drupe extract completely inhibited egg laying whereas, *M. azedarach* leaf extracts caused an 92.66% reduction in egg laying. In India, methanol and chloroform extracts of neem seeds were very effective against the oviposition of *Helicoverpa armigera* female moth. Methanol extract at the concentrations ranging between 0.1 – 0.2%, chloroform extract up to 0.2% and butanol extract at 0.2% strongly inhibited oviposition **(Bajapai and Sehgal, 2003). Jenkins *et al* (2003)** found that neem kernel extract reduced infestation and effectively deterred oviposition with the cowpea weevil, *Callosobruchus maculates* (F.) even after the extract had been stored at high temperature. Contradicting resuls were given by **Naumann and Isman (1995)**, who reported that application of different concentrations of oil free neem seed extracts to cabbage plants did not deter oviposition by individuals of three species of noctuid moths; *Trichoplusia ni, Peridroma soucia* and *Spodoptera litura*.

In the present study, hatchability percentage of *S. littoralis* eggs greatly affected by the type of organic solvents used for neem extraction and the concentration of each extract on treated *N. oleander* leaves. The lowest hatchability was recorded after using methanol. On the other hand, egg hatchability was found to be concentration de-

pendent, i.e., an increase in neem seed kernel extract concentration caused an obvious decrease in egg hatchability. According to **Bhanukiran and Panwar (2000),** neem Azal at 1ml/l (0.005% Azadirachtin) and Multiplex at 4 ml/l (0.00012% Azadirachtin) gave significantly higher mortality of *Chilo partellus* (Swinhoe) eggs. **Jenkins *et al* (2003)** found that the number of neem kernel-treated eggs of *C.s maculates* (F.) that survive to become adults was significantly reduced.

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دراسات على مستخلصات بذور النيم كمركبات طاردة لوضع البيض ومبيدات بيض لفراشات دودة ورق القطن

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الموجـــــز

تضع أنثى فراشة دودة ورق القطن الملقحة في المتوسط ١٥٦٤,٢٢ بيضة طوال فترة وضع البيض . في مجموعة اختبارات ذات الاختيارات الحرة ، وجد أن معاملـــة أوراق التفلـــة (كمكان لوضع البيض) بالمحاليـــل المائيـــة لبـــذور النيم المستخلصة ببعض المذيبات العضوية تخفض من عدد البيض الموضوع على هـــذه الأوراق ، وكان أقـــل عـــدد بيض أمكن تسجيله عند استخـــدام الميثانول كمذيب عضوي يليه الكلوروفورم ، بينما سجل أعلى عـــدد بيض موضوع عند استخدام البنزين يليه البيوتانول وخلات الإيثايل .

وقد وجد أيضا أن عدد البيض الموضوع ينخفض عند زيـــادة تركيز المستخلص ليصل إلى أقل عدد على أوراق نبات التفلة المعاملة بأعلى تركيز (٢%). ومن نتائج التجارب المتحصل عليها وجد أن النسبة المئوية لفقس البيض الموضوع على أوراق التفلة المعاملة بمستخلصات النيم قد تأثرت بدرجة ملحوظة بنوع المذيب المستخـــدم في الاستخـــلاص وتركيـــز هـــذا المستخلص ؛ فقـــد لوحظ أن أقـــل نسبـــة فقس عند استخدام الميثانول يليه الكلوروفورم، وأن زيادة تركيز المستخلص قد سبب انخفاض ملحوظ في نسبة فقس البيض.

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