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USING THE BOTANICAL EXTRACT OF WORMWOOD, *ARTEMISIA JUDAICA* L. AS BIOCIDE AGAINST PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS)

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ABSTRACT: *The leaf extract of Artemisia judaica collected from Nuweiba Desert region, South Sinai, Egypt, was evaluated for its toxicity aspects on pink bollworm, Pectinophora gossypiella. The obtained results indicated that the pupae were the most tolerant stage, while the adults were the most sensitive. The active stages (adults and non-diapausing larvae) of insects are more susceptible than the pupal stage. LC_{50} values were determined for the different stages which could be arranged in an ascending manner starting by 3.70 ppm for adult stage passing through 6.31 and 7.95 ppm for larval and pupal stages, respectively. The number of deposited eggs/ female were decreased by increasing the concentrations of the tested extract of A. judaica on males and females, furthermore, the same trend was occurred in egg hatchability. On contrast, the deficiency in fecundity and the percent of sterility were increased by increasing the extract concentrations.*

Key words: *Plant extracts, toxicity aspects, cotton boll worm , bio-insecticides*

INTRODUCTION

The pink bollworm is one of the most destructive pests of cotton. Early in the spring the newly-emerged larvae penetrate into buds and flowers, while later in the season they attack the bolls as they become available. A single larva can destroy several buds and flowers as well as 2-3 bolls, eating the seeds and spoiling the lint. Total damage may reach 20-50% of the yield In the Middle East. (CGA, 1998).

The use of pesticides has resulted in toxicity to non-target organisms, development of resistance by pests, resurgence and outbreak of new pests, and harmful effects on the environment affecting the sustainability of ecosystems (Jayasankar and Jesudasan, 2005). Therefore, a method of controlling the pest with the use of a botanical insecticide that could affect the population of this pest with minimal effect on the environment is welcomed by scientists. *Artemisia* sp. is an aromatic annual herb which is a member of family Asteracea (Compositae), grown on Sinai Peninsula of Egypt (Samir *et al.*, 2008). The species *A. annua*is considered as an important medicinal plant. Several compounds have been isolated from this species including flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids, and sesquiterpenoids (Haghighian *et al.*, 2008; Brisibe *et al.*, 2009). The extracts and essential oils have demonstrated antimicrobial and insecticidal activity (Pavela., 2006; Mahmoodi *et al.*, 2014). The plant essential oils have various insecticidal activity such as contact toxicity (Asawalam *et al.*, 2006; Ogendo *et al.*, 2008), repellence (Kéita *et al.*, 2001 and Rozman *et al.*, 2007), fumigant toxicity (Rajendran and Muralidharan, 2005 and Shojaaddini *et al.*, 2008), and antifeedant effects (Saxena *et al.,* 1992). Several studies have reported insecticidal effects of *Artemisia* sp. extract containing

growth retardation, antifeedant, and larvicidal effects (Shekari *et al.*, 2008; Hasheminia *et al.*, 2011). (Khosravi *et al.*, 2010) observed that *Artemisia* sp. extract affected the nutritional indices and also showed antifeedant activities on *Glyphodes pyloalis* Walker. Anshul *et al.* (2013, 2014, and 2015) showed that methanolic extract of powdered *A. judaica* leaves adversely affect *H. armigera*. The essential oil of *Artemisia judaica* has been demonstrated to possess insecticidal activity against several insects, such as *Callosobruchus maculatus* (Fab.) and *Sitophilus oryzae* L. (Aggarwal *et al.,* 2001; Abd-Elhady., 2012).

The present study deals with the toxicological and biological effects of leaf extract of *Artemisia judaica* on pink bollworm, *Pectinophora gossypiella*.

MATERIALS AND METHODS

1- Origin and maintenance of insect culture:

The mass rearing of the pink bollworm larvae occurred on the kidney bean diet described by (Abdel-Hafez *et al.*, 1982). The artificial diet consists of 215 g dried kidney beans boiled in water, 32 g dried active yeast, 3 g ascorbic acid, 1.5 g methyl-p-hydroxy benzoate, 1.5 g sorbic acid and 12 g agar, and 150 ml. water. The kidney bean diet was placed in glass tubes (2 x 7.5 cm) at rate of 4 g diet/ tube then about 7 neonate larvae were placed into each tube using fine hair brush and capped by cotton wool. All tubes were kept at 27±1°C and 70±5 % R.H.

As the larvae completed their development (about 14 days) the full-grown larvae found their way to the cotton wool to pupate. The newly emerged adult moths were sexed and kept in a glass chimney. To avoid stress effects of crowding, the male and female moths were distributed at the rate of 10-males: 15-females/ chimney. The upper and lower surface of each chimney was covered with muslin secured by rubber bands. Moths were fed on sucrose solution by providing each cage with soaked cotton wool. The moths normally deposit eggs mostly on the upper muslin cover and lightly on the lower surface. The eggs were incubated in clean glass jars and placed in an incubator at the same conditions suitable for rearing the larvae. The newly hatched larvae were transferred to the kidney bean diet as described above. The original colony of the insect pest was supplied from the Plant Protection Research Institute, Agriculture Research Centre. Mass rearing was carried out in the laboratory of the Economic Entomology Unit, Plant Protection Department, Desert Research Center.

2-Plant material:

Leaf samples of *Artemisia judaica* were collected from three different regions (Elmejrh, Alhubaiqah and Ras Saada) in Nuweiba Desert region, South Sinai, Egypt on September, November 2016.

3-Preparation of plant extract:

Twenty grams of the powdered plant leaves was packed in a containers of glass separately and socked in 200 ml of ethyl alcohol 95% as solvent and shook for 15 minutes for 7 days using an electric shaker. The alcoholic extract was collected and then evaporated under vacuum at a temperature not exceeding than 30 °C in a rotary evaporator. The residues after evaporation was used as a standard stock to prepare concentrations for biological and toxicological tests (Yazdani *et al.*, 2014). The concentrations used for treatments of pink bollworm were 100, 200, 400, and 800 ppm.

4-Toxicity Test

4-1. On larval Stages

Equal weights of artificial media mixed with various concentrations of *Artemisia judaica* extract were prepared in the way mentioned before, and provide as food to starve newly hatched larvae of PBW. Food in solvent only was offered as a control, for each concentration. Ten replicates each of 10 larvae were tested. Numbers of alive and dead larvae were recorded daily till pupation. (Le Ora, 1987)

4-2. On Pupal Stages

Pupal stage was evaluated using the contact method in a sandy soil. Ten grams of clean sand was placed in 250 ml glass jars and treated with different concentrations of plant extract. The sand was stirred continuously for 1 minute to ensure the even spread of the extract over the surface. The solvent was allowed to evaporate for 10 minutes. Twenty individuals of each test stage were placed in the jars and then covered with a thin layer of the treated sand. In the control group, the sand was treated only with the solvent. Each jar was covered with nylon mesh held in place with rubber bands. Ten replicates each of 10 pupae were tested. Mortality percentage was recorded 24 hours later. The experiments were observed until the emergence of the adults to assess total inhibition of metamorphosis and adult malformations.

4-3 On Adult Stages:

Newly emerged moths were sexed and kept in 1 L glass jar, 5 pairs / jar. The upper surface of each jar was covered with muslin secured by rubber bands. The muslin was attached to the under surface of the glass jar screw caps. The caps were screwed tightly on the jars (Pangnakorn, 2009). Each jar was provided with a piece of cotton soaked in sugar solution treated with various concentrations of *Artemisia judaica* extract (100, 200, 400, and 800 ppm), and used as food for moths. For the control group treated only with sugar solution, the piece of cotton was replaced daily. Insect mortality was recorded after 24, 48 and 72 hours of treatment in separated experiments. Each experiment was replicated ten times and the insecticidal activity of the plant extract was expressed as mean mortality percentages of adult insects.

4-4. On eggs

The toxicity of *Artemisia judaica* extract to eggs was examined as contact bioassays. Adult insects (males and females) were collected from the stock culture after emergence and put together in glass jars covered with muslin (5 cm diameter) for oviposition. Muslin-egg batches of one day-old were collected and numbered in order to test the toxicity of plant extract. The first group of eggs was dipped in different concentrations of the extract, and water was used only for control group. After drying for 20 minutes, egg batches were inserted in Petri dishes and subsequently covered. In order to test the toxicity of plant extract, Hatchability percentages were recorded after 3 days.

5- Statistical Analysis:

Mortalities were corrected for the natural mortality according to (Abbot, 1925).

The corrected mortality % = (Observed % - Control %) x 100 / (100 - Control %)

Concentration / mortality regression lines were drawn on probit logarithmic graph according to the method developed by (Finney, 1971).

The LC_{50} and LC_{90} values were calculated according to probane program.

Females hatchability was calculated according to (Zidan *et al.*, 1998) as follows:

Sterility = No. of hatched eggs (control) – treated] x 100/ Control

% Hatchability = [No. of hatched eggs / No. of deposited eggs] x 100

Standard deviation was calculated according to (Bliss and Stevens, 1937) as follow:

Standard deviation (S) =

$$S=\sqrt{\frac{\Sigma(X-M)^2}{n-1}}$$

X=individual treatment M = mean of all treatments n = number of treatments Variance = S2

RESULTS AND DISCUSSION

I- Toxicity Tests:

1- On larval Stage

Feeding of starved newly hatched larvae of PBW on artificial media mixed with various concentrations of *Artemisia judaica* extract (Table 1) revealed adverse effects on the total percent of larval mortality, which was concentration dependent. Whereas the total larval mortality recorded 6 % in control trials, data in exposure revealed 30 to 62% of larval mortality at 100 and 800 ppm, respectively.

2- On Pupal Stage

Exposure of *P. gossypiella* pupae to various concentrations of extract (Table 1) revealed adverse effects on the total percent of mortality, which was concentration dependent. Whereas the total mortality recorded 6 % in control trials, data in exposure revealed 14 to 58% of pupal mortality at 100 and 800 ppm, respectively.

3- On Adult Stage

Exposure of newly emerged *P. gossypiella* adults to different plant extract concentrations (Table 1) increased total percent mortality. Such increase was concentration dependent. Whereas the total mortality recorded 5% in control trials, the total mortality increased from 34, 40, to76% at 100, 200 and 800 ppm, respectively. Comparable trend was recorded with the pupa and larvae (Table 1).

Table (1): Percentages of larval, pupal, and adult mortality of *P. gossypiella* at different concentrations of *Artemisia judaica* extract

Conc. ppm	Larval mortality %		Pupal mortality %		Adult mortality %	
	Observation	Corrected	Observation	Corrected	Observation	Corrected
0	6	0	6	0	5	0
100	30	24.8	14	8.2	34	28.8
200	36	30.8	26	20.6	40	35
400	48	43.4	42	37	58	53.6
800	62	57.8	58	53.6	76	72.2
L.S.D. 5%	3.04		2.66		1.83	

Lamiri *et al. (*2001) studied the effect of *A. judaica* extract for its toxicity against *P. gossypiella* stages, and found that the most effective botanical extracted would be those offering a broad spectrum of activity against various life stages of the pest. The control agent should reduce the insect population at all stages, and it should decrease the incidence of the pest. Mohamed & Abdelgaleil (2008) in a contact toxicity assay, revealed that the oils of *Mentha microphylla* and *Artemisia Judaica* were the most potent against *Sitophilus oryzae*. AS for *Tribolium castaneum*, the oils of *M. microphylla*, *Eucalyptus camaldulensis* and *A. Judaica* showed the highest activity among the test oils and the *M. microphylla* recorded the most potent. Furthermore, Rajendran and Sriranjini (2008) conducted fumigant toxicity tests with essential oils of plants (Apiaceae, Lamiaceae, Lauraceae and Myrtaceae) and their components (cyanohydrins, monoterpenoids, sulphur compounds, thiocyanates and others) and found that these oils have largely focused on beetle pests such as *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Sitophilus zeamais* but little or no attention has been paid towards moths such as *Corcyra cephalonica* and *Sitotroga cerealella*. Adults were generally susceptible, whereas, eggs were either tolerant or highly susceptible depending on insect species and the type of essential oil or component.

From the obtained results it could be noticed that the pupa was the most tolerant stage and that the adult was the most sensitive one., the active stages (adults and non-diapausing larvae) of insects are more susceptible than the sedentary stages (eggs and pupae), owing to differences in their respiratory rates.

LC_{50} and LC_{90} of *Artemisia judaica* extract:

All bio assays were carried out using larval, pupal, and adult stages of *Pectinophora gossypiella*. The LC_{50} values of the tested potent extract were computed from the data obtained on the percentage of larval mortality at each of the tested concentration through probit analyses within 95% confidence limits (Fig. 1). The data illustrated in (Table 2) determined the LC_{50} values for the different stages which could be arranged in an ascending manner starting by 3.70 ppm for adult stage passing through 6.31 ppm and 7.95 ppm for larval and pupal stages, respectively.

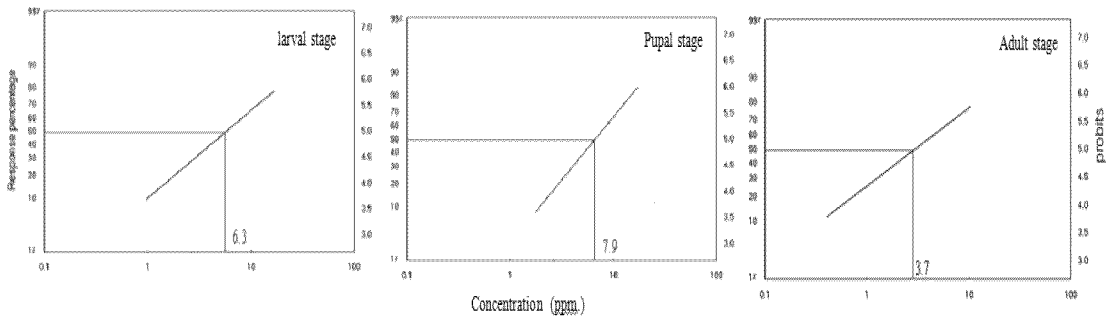

Fig. (1): Concentration / mortality regression lines for *P. gossypiella* larvae, pupal and adult stages treated with *Artemisia judaica* extract.

Table (2): LC_{50} and LC_{90} values (ppm) of potent *Artemisia judaica* extract against different stages of *P. gossypiella.*

P. gossypiella stages	LC_{50}	LC_{90}
Larval stage	6.31 (4.68 -10.18)	42.57 (24.69 -110.35)
Pupal stage	7.95 (6.38 -10.89)	48.00 (28.18 -116.73)
Adult stage	3.70 (2.96 - 4.67)	33.21 (19.43 -85.09)

Previous studied showed similar opinion to the present study where (Kordali *et al.,* 2006) found that *Artemisia* sp. exhibited obvious mortality against *Sitophilus granarius L.* Another research Li *et al. (*2000), also listed the extract of *A. annua*, which showed highly antifeedant action against *Aphis gossypii*, *T. urticae* and on the data obtained show that the various extracts of *A. judaica* were the most toxic against adult than immature stages. In addition, El-Sharabasy (2010), recorded that the LC_{50} values at 72 hr exposure to different extracts of *A. judaica* on adult females and immature stage of *T. urticae* and *P. persimilis*.

The obtained data show that, the extract of *A. judaica* were the most toxic against adult females than immature stages.

II- Effect of *Artemisia judaica* extract on the female reproductive capacity of *Pectinophora gossypiella*:

Treatment of males and females with different concentrations of *A. judaica* caused an obvious decrease in the number of deposited eggs from about 193 to 165 eggs, at 100 to 800 respectively, compared with about 205.9 deposited eggs in the control females, i.e. the number of deposited eggs decreased by increasing the tested concentrations (Table 3).

Accordingly, the percent deficiency in fecundity increased from 6.26 to19.86 % at the same concentration. Data revealed a gradual diminish in the number of hatched eggs from 179.2 to 168 to 144 to 132.3 eggs treated with 100, 200, 400 and 800 ppm, respectively, while the hatched eggs in control females recorded 195.7 eggs, i.e. the number of hatched eggs was indirectly proportional to the tested concentrations. also, A gradual reduction in the percent of egg hatchability from 92.84 to 80.18%, while untreated adults were recorded 95.04%. The percent of sterility recorded 8.43, 14.15, 26.49 and 32.39% at the concentrations 100, 200,400 and 800 ppm, respectively.

The results showed a significant reduction in the number of both deposited and hatched eggs. This reduction was negatively correlated with the different concentrations of *A. judaica*, According to (Semple, 1992), in the ovicidal bioassay, the test oil exhibited weak fumigant toxicity and strong contact activity against egg hatchability. Ability of the monoterpenoid vapours, especially those of terpinen- 4-ol and 1, 8-cineole, to reduce fecundity and hatchability of the laid eggs, recalls analogous properties of IGRs. It is likely that oil apours diffused into eggs and thereby affected the physiological and biochemical processes associated with embryonic development (Raja *et al.,* 2001).

Table (3): Effect of *Artemisia judaica* extract on the female reproductive capacity of *P. gossypiella*.

Conc. ppm	No. of deposited eggs	%Deficiency in fecundity	No. of hatched eggs	% of Sterility	% of Hatchability
0	205.9	0	195.7	0	95.04
100	193	6.26	179.2	8.43	92.84
200	189.3	8.062	168	14.15	88.74
400	172.2	16.36	144	26.49	83.62
800	165	19.86	132.3	32.39	80.18
L.S.D.5%	18.11		16.65		

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إستخدام مستخلص نبات الشيح الخليلي (البعيثران) كمبيد حيوي ضد دودة اللوز القرنفليه

ايمان ابراهيم امام

وحدة الحشرات الاقتصاديه- قسم وقاية النبات- مركز بحوث الصحراء

الملخص العربى

أجريت هذه الدراسة بغرض تقييم سمية نبات الشيح الخليلي (البعيثران) والتي تم جمعه من صحراء نوبيع بجنوب سيناء في مصر، علي أطوار دودة اللوز القرنفليه.

ولقد اوضحت الدراسه ان طور العذراء اكتر الاطوار احتمالا لتأثير المستخلص النباتي عن الاطوار البالغه واليرقات التي كانت اكثر حساسيه له، فقد سجلت التركيزات قاتلة النصف (LC_{50}) ٣,٧ جزء في المليون للاطوار البالغه بينما كانت ٦,٣١ و ٧,٩٥ جزء في المليون لليرقات والعذاري على التوالى . وأثرت معاملة الافراد البالغه (الاناث والذكور) بالمستخلص النباتي علي عدد البيض الذى تضعه الإناث حيث تم نقص البيض بزيادة تركيزات المستخلص. وقد حدث هذا أيضا في نسبة فقس البيض وفي نسبة العقم في الإناث، حيث نقص الأول وأزداد الثاني بزيادة التركيز.

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