EVALUATION OF PETROLEUM-ETHER EXTRACT OF ZANZALAKHT TREES (Z- SEED OIL) ON PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) [LEPIDOPTERA: GELECHIIDAE]

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Abstract

Laboratory studies were carried out to determine the effect of seed extract of (Zanzalakht Seeds) [Azadirachtin (AZA)], *Melia azadiracht* on certain biological aspects of *Pectinophora gossypiella* (Saund.) larvae. Newly hatched larvae were exposed to treated diet with three concentrations of AZA i.e.1, 2 and 3%.

Azadirachtin adversely affected larval mortality in a dose and time dependent where the cumulative larval mortality showed 22.50, 22.50 and 25.50 % after three days, respectively at 1, 2 and 3 % conc.; respectively. Also AZA influences the biological aspects of the pest included, larval and pupal duration and weight, pupation percentage, adult emergence percentages, oviposition periods and female fertility, fecundity and longevity.

INTRODUCTION

The cotton bollworm *Pectinophora gossypiella* is one of the most important pest causes extensive losses of cotton bolls in Egypt. Traditional chemical insecticides are commonly used for controlling this pest, but certain problems are caused by the use of insecticides, such as undesirable chemical resistance and disturbance of the natural biological balance. The possibility of controlling this pest by causing larval mortality, disturbance in the pest growth as well as fecundity and development, without being harmful to human health would provide a safe and selective method for this pest control.

One of the most promising insecticidal alternatives is Azadirachtin, triterpenoid, a seed extract of the Indian tree, *Azadirachta indica*, it possess antifeedants and insecticidal activity against wide range of insects belonging to several insect species including Lepidoptera (Koul, 1987 and Bamby *et al* .1989).

The action of these compounds is a mostly similar in that time lag between ingestion and death .However, little is known about their action on growth and food utilization and their antifeedant characteristics.

Therefore, the present work was carried out to study the effect of zanzalakt Seed extract {Azadirachtin] on *Pectinophora gossypiella*, via feeding newly hatched larvae on artificial diet treated with different concentrations of AZA extract and recording the direct and latent biological effects on the parents and first generation.

MATERIALS AND METHODS

1-Procedure

To evaluate the biological effects of Z-seed oil on pink bollworm, *Pectinophora gossypiella* (PBW), three concentrations (1, 2 and 3 %) were prepared by adding water plus Triton x100 (10 drops/100 ml water) to 1, 2 and 3 ml of Z- Seed oil to complete ten ml for each..

Artificial diet was prepared according to Abd El- Hafez *et al.* (1982). Five grams of artificial diet was added in Petri- dishes of 10cm diameters .The surface of artificial diets were treated with one ml of different Z-Seed oil concentrations using syringe. Petri –dishes was shacked to provide a good distribution of the aqueous concentrations over the artificial diet then left to air dryness. Twenty newly hatched larvae were transferred to every treated Petri – dish using fine brush. Petri- dishes were covered by fine paper and glass. Four replicates were prepared for each concentration .Control was treated with water plus triton x100 only.

Twenty four hour after exposure and feeding, the dead and alive larvae were counted. Alive larvae were transferred to glass tube of (2 x7.5 cm) containing about five grams of untreated artificial diet which were examined after 1 and 2 days for counting dead and alive larvae .The accumulative larval mortality were calculated for the first, second and third day of the bioassay and the alive larvae were left to complete their life cycle under 26°c ±1 and 85 ± 5 % R.H.. Larvae were examined daily till pupation, certain biological aspects included larval and pupal duration as well as weight of 4th instar larvae and pupae, then the pupation percentages were estimated. After pupation, pupae were transferred individually into clean vials and incubated till moth emergence. The adult emergence percentages, of females were determined. The emerged moths were caged in pairs (male and female) in a glass Jar (quarter litter) covered with muslin secured by rubber bands. Moths were fed on sucrose solution 10 % by providing each cage with soaked cotton wool and changed by new one every 48 hours, and the cages were provided by paper for egg laying. Four replicates for each concentration were used. Each Jar was examined daily to count the number of eggs deposited per female and date of adult death was reduced. Hatchability was determined (100 eggs) in each replicate. Hatched eggs were taken and incubated under the previously mentioned optimum condition and the date of hatching was recorded. From these records, several biological aspects such as oviposition periods, females' fecundity, longevity of adult males and females, female emergence percentages, hatchability percentage and incubation period were estimated.

2- The Plant extraction

The fresh fruits were collected throughout the growing season (May-July) from Zagazig - Sharkia governorate .The seeds of Zanzalakht tree were used as source of our plant extract.

One kg of Z- fruits was soaked in water for two days, filtered and rubbed manually to remove the fleshy part. The shells obtainer was dried far from sun rays then were crushed by corn grinding mill. The seed obtained from one kg fruits were weighed and grounded by a manually grind. One hundred g of seeds –powders was introduced into a large Erlenmeyer Flasks and added to 220 ml of the hydrocarbon solvent (Petroleum - ether). This procedure was replicated two times. After 1-day, the flask was shaken for 30 min. in a shaker, the seed suspensions were filtered, and the obtained filtrate was put under room temperature to evaporate the solvent by volatility. The residue (Z-seed oil) was emulsified in water at different concentrations.

Extract method Show that solved 100 gram of seed powder, in 220 ml of petroleum- ether gave 18 ml pure Z-seed oil (AZA).

Statistical analysis

All obtained resulted were statistically analyzed according to the analysis of variance. The proper "F" value was calculated as described by Fisher (1950) and Duncan's multiple range tests (1955).

RESULTS AND DISCUSSION

A- Acute effect of different concentrations of Z-seed oil against New hatched larvae of PBW.

Data in Table (1) indicated that , the rate of mortality of newly hatched larvae of pink bollworm was varied considrably based on time after exposure and the concentration used .The advanement the time after exposure ,the higher the rate of mortality and vice versa .The cncentration used had a slight effect in this respect.

These results coincide with Rashad *et al.* (1991) they studied the effect of crude extract of the *Melia azedarach* (Meliaceae) leaves at different concentrations against the cotton bollworms *E. insulana* and *P. gossypiella* They revealed that the highest concentrations increased the mortality of newly hatched larvae of the two species, prolonged larval and pupal durations and reduced moth emergence.

Table 1. Accumulative mortalities of newly hatched larvae of pink bollworm, at three days after feeding on artificial diet treated with different concentrations of Z- Seed oil.

Concentrations %	accumulative mortality after		
	1st day	2nd day	3rd day
1	6.25	16.25	22.50
2	8.75	17.50	22.50
3	8.75	18.75	25.50
control	0.00	0.00	0.00

Table 2. Effect of different concentrations of Z- seed oil on larval and pupal stages of the pink bollworm, *P. gossypiella.*

Concentrations %	Larval period (days)	Larval weight (gram)	Pupal weight (gram)	Pupation percentages	Pupal period/(days)	Adult emergence percentages	Rate of female emergence (%)
1	14.64	0.0634b	0.0495ab	65.75	10.27a	61.25ab	53.41
2	14.32	0.0580b	0.0429b	56.67	10.09a	47.50bc	52.53
3	13.97	0.0564b	0.0463ab	49.17	10.00a	45.63c	55.61
Control	14.84	0.0718a	0.0509a	67.06	8.91b	63.04a	56.84
F. test	N.S.	**	*	NS	**	*	N.S.
$LSD_{0.05}$		0.0079	0.0070		0.7483	14.5813	

N.S. = non significant *= significant **highly significant at 0.05% probability

Table 3. Effect of different concentrations of Z- seed oil on adult stage of the pink bollworm, *P. gossypiella.*

Concentrations %	Oviposition periods/female/day			Adult longevity in days		Number of laid eggs/ female	Incubation period in days	Hatchbility eggs %
	Pre-Oviposition Period /days	Oviposition period /days	Post-oviposition Period /days	Female	Male			
1	1.95ab	16.90a	2.03a	20.88a	14.16 a	119.51a	2.14b	46.82b
2	4.31a	13.11ab	1.13ab	18.54b	12.93 a	108.43a	3.88a	52.55b
3	1.50b	0.50c	0.25 b	10.09b	9.87 b	2.66b	2.75ab	0.00c
Control	3.00ab	13.71b	1.35 a b	18.06b	14.09a	169.42a	2.50ab	80.10a
F. Test	*	**	*	**	*	**	*	**
$LSD_{0.05}$	2.4109	4.1349	1.6002	1.3921	2.9941	82.9974	1.6044	15.0571

N.S.= non significant *= significant **highly significant at 0.05% probability

B-Latent effect

Latent effect of Z-seed oil in this study was expressed as some biological aspects including, larval duration, weight of 4th instar larvae, pupation percentage, pupal weight, pupal duration, percent of adult emergence, percent of female emergence, oviposition periods of emerged females, longevity of both males and females, incubation periods of egg and hatchability.

1-Duration of larval stage

Data summarized in Table (2) revealed that the used concentration of Z-Seed oil lead to gradual shortening in larval duration, but it has insignificant affect at 5% probability. The averages of larval duration were 14.64, 14.32 and 13.97 days at 1, 2 and 3% concentrations, respectively compared with untreated individuals (14.84 days). In this respect Abdel Rhman *et al.* (2002) evaluated the effect of NeemAzal formulation (T, F and/S) *on p. gossypi*ella and showed different grades of deformities during pupal development and adult emergence. Our results Coincide with Hegab (2002) who indicated that the percent of eggs hatching was significantly affected with tested insecticides and their concentrations with exception of those respecting profenofos which proved to be insignificant.

2- Weight of the 4th instar larvae

Data presented in Table (2) showed that, the influence of different concentrations of Z-seed oil, on the weight of the 4th instar larvae proved to be highly significant. The larval weight gave 0.0634, 0.0580 and 0.0564 gram with 1, 2 and 3 % concentrations, respectively, comparing with 0.0718 gram in case of the untreated check. Increasing Z- Seed oil concentrations induced gradual decrease in larval weight.

3-Pupal weight

Table (2) indicated that, the influence of different concentrations of Z-seed oil on pupal weight proved to significantly reduced at 1, 2 and 3 % concentrations, respectively. The PBW larvae fed on artificial diet treated with 2% of Z-Seed oil recorded (0.0429 gram) comparing with 0.0509 gram in control.All concentrations of Z seed oil decreased significantly pupal weight comparing with the control.

4-Pupal duration

Data in Table (2) proved that all tested conc. prolonged significantly the pupal duration of pink bollworm showing 10.27, 10.09 and 10.00 days with 1, 2 and 3 % conc., respectively .However the untreated check revealed 8.90 days.

5-_Percent of pupation

Analysis of variance of the data given in Table (2) indicated that all tested concentrations insignificantly reduced the pupation percentage. The highest average

pupation percentage was 65.75% recorded with 1% concentration, whereas the lowest i.e. 49.17% was obtained with 3% conc. compared. 67.06% which were recorded with check experiment. Increasing the concentrations of Z- seed oil induced gradually reduce in pupation percentage.

6- Percent of adult emergence

Data in Table (2) indicated that, concentration of 3% caused a significant decrease in adult emergence (45.63%), while 2% concentration exhibited a significant decrease (47.50%). On the other, hand 1% concentration proved insignificant decrease of the adult emergence (61.25%) comparing with untreated check (63.04%). Increasing concentrations caused gradual decrease of adult emergence.

7- Female moth's emergence

Data presented in Table (2) indicated that, all tested concentrations insignificantly reduced the female emergence. The highest mean of the female emergence (56.84%) was recorded in untreated check. Whereas the lowest one (52.53%) was recorded in the concentration of 2 %.

8- Oviposition periods of emerged females

1- Pre- oviposition periods

Data in Table (3) showed that, Z- seed oil extract , had highly effect on pre-oviposition period at 3 % concentration .The lowest period 1.50 day was noticed at 3% concentration, while the highest female pre-oviposition period (4.31 days) was recorded at 2 % concentration of Z- seed oil , compared with 3.00 days for untreated check.

2-Oviposition periods

As shown in Table (3) the effect of AZA on oviposition period at 3 % conc. was very high, comparing with untreated check (13.71days), while that resulted from 1 and 2% conc were 16.75 and 13.11 days respectively.

3- Post oviposition period

Data in Table (3) indicated that, the post oviposition periods for *P. gossypiella* female were 2.03 and 1.13 days when 1 and 2% concentration were tested, while the highest effect was noticed at 3 % concentration, comparing with untreated check that exhibit 1.35 days. Increasing the concentration decreasing gradually post oviposition period.

9-Adult longevity

A- Female longevity

Table (3) showed that concentration of 1% caused significant prolongation in female longevity (20.88days), while of 3 % concentration exhibited significant shortening in female longevity (7.17days), comparing with (18.06days) in untreated one. On the other hand concentration of 2 % gave (18.55 days) .

B –Male longevity

Data in Table (3) indicated .that, the concentration of 3 % only lead to significant shortening in male longevity (9.87days) comparing with (14.09 days) in untreated check, while male longevity recorded 14.16 and 12.93 days at 1 % and 2% concentration ,respectively . Increasing the concentration induced gradual shortening in male longevity.

10-Female Fecundity

Statistical analysis in Table (3) indicated that all concentration used induced reduction in the number of eggs laid / female comparing with untreated check, but the reduction was highly significant only with the concentration of 3 %, while the other concentration caused insignificant reductions.

The number of deposited eggs /female were 119.51, 108.43 and 2.66 eggs for 1, 2 and 3% concentrations, respectively as compared with 169.42 eggs in the untreated check Generally increasing the concentrations induced reduction in the female fecundity.

The present results agree with those obtained by Rashad, *et al.* (1993) who studied the efficacy of IGR compounds, S-31183, pyriproxyfen and precocene I & II on newly hatched larvae of pink bollworm, *Pectinophora gossypiella* (Saunders) and the spiny bollworm, *Earias insulana* (Boisd.) which used at different concentrations Results showed that metamorphosis and development of the treated insects were greatly altered. Using JHA prolonged the larval and pupal durations, caused precocious metamorphosis for the pupae and adults, and also reduced moth emergence and fecundity. In addition, AJH (precocene I & II) revealed sterilant action on the adult females so these new compounds appear as promising agents in controlling bollworms without producing undesired effects on the agro-ecosystem. .Shalaby (1996) indicated that, the survivors of PBW and SBW were affected by Z-Seed oil treatments. These effects appeared later on fecundity and fertility. Z- Seed oil produced significant progressive reduction in fecundity and fertility of both PBW and SPW except at the two lowest doses (1:2 and 1:3 concentrations). Idraw (2003) studied the effect of compound NeemAzal T/S on various stages of parasite, *Bracon brevicornis* Wesm using the larvae of *Pectinophora gossypiella* .Result indicated that at highest percentage of

NeemAzal 150 ppm caused no eggs were laid by female parasitoid. and by comparing the other forms of the *Melia azidarach* L.preparation where the expulsion rate of the mature fruit extract of *M. azidarach* L. Was higher where the average eggs laying was 19.66 at the concentration of 0.5% where it was 24.33 with green leaves extract at the same concentration.

11-Incubation period

The results in Table (3) showed that concentrations induced reduction in incubation period of laid eggs. This reduction was significant at 1 and 2 % concentrations (2.14 and 3.88 days) comparing with 2.50 days in untreated check. On the other hand the concentration of 3 % caused no-significant reduction (2.75 days) comparing with untreated check.

12 - Hatchability of eggs

As shown in Table (3) all tested concentrations reduced the egg hatchability, this reduction was significant at concentrations of 1 and 2 % that causing 46.82 and 52.55 % comparing with 80.10 % in untreated check. On the other hand the concentration of 3 % exhibited highly significant reduction in the egg hatchability comparing with untreated check.

The obtained result are in agreement with El-Sayed (2001) who found that NeemAzal-T and NeemAza!T/S caused significant reduction in the viability of eggs deposited by spiny bollworm females developed from treated neonate larvae. This reduction was increased sharply by increasing the concentrations. NA-T affected the viability of the deposit eggs by female moths developed from treated neonate in high level than NA-T/S.

Recently Amer (2004) showed that the percent of the hatching eggs was significantly reduced by Chinmix and Spintor, whereas the influence of Biorepel had insignificant Effect. Increasing the concentration of all tested compound induced gradual decrease in the percentages of hatching eggs.

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تقييم مستخلص بذور نبات الزنزلخت المستخلصه بواسطة البترولييم- إثير على دودة اللوز القرنفلية

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أجريت تجربه معمليه لتقييم كفاءة بذور نبات الزنزلخت المستخلص بواسطة البتـر ولـيم- إثير تحت ظروف المعمل عند درجات حرارة ورطوبة ثابتة علي يرقات حديثة الفقس لدودة اللـوز القرنفلية حيث قدرت النسبة المؤيه لموت اليرقات بعد ٣ يوم من المعاملة ومعدل تكوين العـذارى وخروج الفراشات ، كذالك دراسة التأثير المتأخر للمركب على أطوار الحشرة المختلفة وعلى نسـبة وضع البيض والفقس للإناث الناتجة من اليرقات المعاملة حديثة الفقس . ولقد أوضحت النتائج حدوث نسبة موت تصل ٢٢,٥٠، ٢٢,٥٠ و٢٥,٥٠٪ بعد المعاملة بثلاث أيام عند تركيزات ١و٢و٣ % علي التوالي من المستخلص، كما أوضحت النتائج أن لهذا المستخلص تأثير على جميع الصـفات البيولوجيه المدروسة حيث انه كلما ذاد تركيز المستخلص انخفض كل من فترة العمر اليرقي ووزن كل من طوري اليرقات و العذراء والنسبة المؤية للتعذير ونسبه خروج الفراشات الكاملة وكـان لـه تأثير معنوي على فترات وضع البيض ،وكان للمستخلص تأثير معنوي علي طـول فتـرة حيـاة الحشرات الكاملة (الذكور والإناث) وكذلك كان له تأثير معنوي علـى االكفـاءه التناسـلية لإنـاث الفراشات والنسبة المؤية لفقس البيض الموضوع بواسطة الفراشات الناتجة مـن اليرقـات المعاملـة بالمستخلص . لذا يمكن اقتراح استخدام مستخلص بذور نبات الزنزلخت ضمن برنامج المكافحـه المتكاملة ضد ديدان اللوز في حقول القطن.