BIOLOGICAL EFFICACY OF THREE COMMERCIAL FORMULATIONS OF PLANT EXTRACTS ON SPINY BOLLWORM *EARIAS INSULANA* (BOISD.) UNDER LABORATORY CONDITIONS

HEGAB, M. E. M. A. and S. F. ABD-EL ATTY

Plant Protection Res., Inst., ARC, Dokki, Giza, Egypt

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Abstract

Three commercial formulations derived from *Azadirachta indica* (Neem), *Citrullus colocynthis* (Hanzal) and *Thymus vulgaris* (Zaatar) were bioassayed individually against 1st instar larvae of spiny bollworm, *Earias insulana* (Boisd.) to compare their effect on the different biological aspects of the larval, pupal and adult stages. The data obtained indicated that the plant extracts tested with two concentrations (5 and 10 %) adversely affected the mortality of larval and pupal stage (57.33 & 50.67 %), (45.33 & 66.66 %) and (49.33 & 57.33 %), respectively comparing with (2.34 %) in control and significantly decreasing in pupation percentage and influenced on pupal mortality. In addition, proved that larval and pupal weight gains didn't significantly differ from that recorded at the concentrations. Also, results indicate that three tested extracts inhibition the percentages of adult emergence resulted from treated larvae in the two tested concentrations. Results also, caused significantly decreasing numbers of laid eggs. The respective numbers are (54.00 & 0.00 eggs / ♀); (71.66 & 28.00 eggs / ♀) and (22.66 & 84.67 eggs / ♀), respectively compared with (213.33 eggs / ♀) in control. Significant effects on the hatchability percentages of eggs were recorded as follows: (59.26 & 0.00 %), (75.51 & 51.79 %) and (87.72 & 66.62 %), respectively comparing with (90.18 %) recorded in control treatment.

Key word: Spiny bollworm, *Earias insulana*, *Azadirachta indica*, *Citrullus colocynthis*, *Thymus vulgaris*, laboratory, biological aspects, extracts.

INTRODUCTION

In Egypt, during the late cotton-season, cotton plants suffer from the infestation with the spiny bollworm, *Earias insulana* (Boisd.). Integrated Pest Management (IPM) is a sustainable approach which combines the use of biological, cultural, physicals and chemical tactics in a way that minimize economic, health and environmental risks. Several types of practices have been suggested for reducing pest populations and conserving natural enemies i. e. the use of natural products derived from bacteria, fungi, or plants and apply insecticides only when they are needed. Dhawan *et. al.* (1992) recorded that damage to cotton by *P. gossypiella*, *E. insulana* , *E. vittella* and *H. armigera* was significantly reduced by treating cotton by 3

formulations extracted from *Azadirachta indica* compared with non treated. In addition, El-Sayed (2001) found that the NeemAzal formulation had highly effect on different biological aspects of spiny bollworm. More recently, non-Azadirachtin extracts derived from different plant species have been proved to have insecticidal activity against different pest species. In addition, Sayed *et. al.* (2011) results showed that methylene chloride extract of *A. indica*, *C. colocynthis* are the most potent extracts against *S. littoralis* larvae. Sharaby *et. al.,* (2009) tested *Citrullus colocynthis* extract against potato tuber moth, *Phthorimaea operculella* caused the maximum inhibition of egg hatchability, followed by *Citrullus colocynthis* caused the highest depression in the deposited eggs, as they played a remarkable role as ovipositor deterrents. Al- Shnnaf (2002) reported that gossypol extract affected the developmental stages of pink bollworm *P. gossypiells.* (Mahesh and Vidya 2008) reported that *Citrullus colocynthis* (Linn.) is an important medicinal plant belonging to family Cucurbitaceae.

The aim of the present investigation is to determine insecticidal effects of three formulations of plant extracts: *A. indica*, *C. colocynthis* and *T. vulgaris* against newly hatched larvae of the spiny bollworm, *Earias insulana* lab. strain. Survivors were observed to detect latent effect on subsequent stages.

MATERIALS AND METHODS

Tested extracts

Three plant extracts commercial formulated were obtained from Agriculture company in Egypt

A. Neem, *Azadirachta indica A. juss*. (Azadirachtin, 40% EC), (application rate 1L./200L. water).
B. Hanzal, *Citrullus colocynthis*, (Colocynth, 40% EC), (application rate 1L./200L. water).
C. Zaatar*, Thymus vulgaris,* (Thymol, 40% EC), (application rate 1L./200L. water).

Experimental insect

Newly hatched larvae of spiny bollworm, (*Earias insulana* Boisd.) used in this study was obtained from laboratory colony of Bollworm Research Department, Plant Protection Research Institute, Agric. Res. Center (Sharkia Branch), reared for many generations away from any insecticides treatments and kept in incubator at 26 $\pm 1^0$C and 80 ± 5 % R.H. on artificial diet have been described by Rashad and Ammar (1985).

Procedure

The effects of Neem*, Azadirachta indica A. juss*); Hanzal, *Citrullus colocynthis* and Zaatar, *Thymus vulgaris* medicinal plant extracts on the spiny bollworm, *Earias insulana* newly hatched larvae. Stock solution of each tested formulation was achieved by adding 5 ml to 95 ml water (first concentration or 10 ml to 90 ml water (second concentration). Ten grams of semi artificial diet described by Rashad and Ammar (1985) was poured into conventional Petri-dish (7 cm diameter). One ml of the tested concentration /each extract was distributed on the upper surface of the poured diet using volume syringe by moving the dish gently in circles. The treated dishes were held uncapped for half an hour to allowed absorption of the diluted concentration in to the diet under room conditions. Twenty of newly hatched larvae of the spiny bollworm were transferred to the surface of treated diet surface using fine hair brush were covered by soft twilet paper below the glass cover and kept at 26 ±1ºC and 80 ± 5 % R. H. in an electrical incubator running. Similar number of larvae were transferred into diet treated with 1ml water was maintained under the conditions and considered the control treatment. Three replicates for each concentration /formulated extract as well as three for control were used. After 48 hrs of exposure and feeding, alive larvae from each replicate were transferred individually to glass tubes (2 x7.5 cm), each containing about two grams of untreated diet and covered with a piece of absorbent cotton and incubated under the above mentioned conditions. 48 hrs later all tubes were inspected for acute toxicity of Neem, Hanzal and Zaatar plant extracts. Latent effect of each concentration /tested formulation was determined by inspecting tubes daily. Percentage of larval mortality, larval duration, pupation percentage, pupal duration & moths emergence percentage were recorded. Newly emerged moths of SBW were paired into glass Jars (250 ml in size). The upper and lower opining of each Jar was covered with muslin cloth serving as oviposition site, and secured by rubber bands. Moths were fed on 10 % sucrose solution, using soaked cotton wool changed daily by new once. Three replicates/ conc./ formulation were used. Each replicate has 5 pairs of newly emerged moths. Each replicate was inspected daily to inspected daily to record the number of deposited eggs / female, oviposition periods, longevity of males and females and hatchability percentages.

Statistical analysis

The obtained data were statistically analyzed. The proper "F" and LSD value & Standard Error values was calculated as described by Fisher (1950) and Snedecor (1970) using Costat computer program Cohort Software. P.O. Box 1149, Berkeley CA 9471 (Costat program methods 1990).

RESULTS AND DISCUSSION

Table (1) summaries the effect of *Azadirachta indica, Citrullus colocynthis* and *Thymus vulgaris* formulated plant extracts used against newly hatched larvae of *E. insulana.* The mortality percentage of larvae and pupae of *E. insulana* and pupae survival of SBW neonate fed on treated diet with two concentrations of *Azadirachta indica, Citrullus colocynthis* & *Thymus vulgaris,* increases significantly comparing with untreated. The highest percentage of larval mortality was recorded at the 1st conc. of *A. indica* (48 %) while the lowest was recorded at the 2nd conc. of *A. indica* & 1st conc. of *Thymus vulgaris* (38.67 %) compared to 0.00 % mortality in control. On the other hand all the treatments caused significant reduction in pupation percentage compared with the untreated check (Table1). The mortality percentage of larvae and pupae ranged between (45.33-66.66 %) oppose to 2.34 % (Table1). In addition, all the experimental concentrations of the tested compounds caused significant reduction in SBW moth emergence. The percentage emergence of moths ranged between 33.34 % to 54.67 % while it reached 97.66 % in control. The least emergence was recorded at the 2nd conc. of *C. colocynthis*, while the highest was at the 1st conc. of *C. colocynthis* (Table1).

Table 1. Effect of three formulated plant extracts on larval mortality, pupation percentage, pupal mortality and adult emergence percentages of the spiny bollworm, *E. insulana* 1st instar larvae

Plant extracts	% Concentrations	Accumulated mortality % of larval & pupal stages	Pupation %	Adult emergence %
Azadirachta indica	5	57.33 ^{a}	52.00 ^{d}	42.67 ^{c}
	10	50.67 ^{a}	62.00 ^{b}	49.33 ^{b}
Citrullus colocynthis	5	45.33 ^{b}	48 .00 ^{d}	54.67 ^{b}
	10	66.66 ^{a}	60.00 ^{bc}	33.34 ^{d}
Thymus Vulgaris	5	49.33 ^{ab}	60.00 ^{bc}	50.67 ^{b}
	10	57.33 ^{a}	54.67 ^{cd}	42.67 ^{c}
Control		2.34 ^{c}	76.19 ^{a}	97.66 ^{a}
F test		**	**	**
LSD $_{0.05}$		11.60	7.34	6.00

Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately.

Effect of plant extracts on some biological aspects of *Earias insulana*:

1- Larval duration

Data presented in Table (2) showed that the two tested concentrations of *A. indica* & *T. vulgaris* caused significant decrease in larval period compared with untreated larvae, while there was no significant difference between the two concentrations of *C. colocynthis* and the control.

These results disagreement with those of El-Sayed (2001) who reported that tested concentrations of NemAzal -T /S caused highly significant increase in larval periods of SBW as compared with untreated. Also, (Hegab, 2008) reported that Z-seed oil of Zanzalacht extract (Azadirachtin) was slightly decreased larval duration of the spiny bollworm. (Sayed *et. al.*, 2011) indicated that *Citrullus colocynthis* are the most potent extract against *Spodoptera littoralis*, larval duration.

2-Pupal period

Results in Table (2) show that most tested concentrations didn't affect pupal period (days) than untreated control. Two exceptions were fond in 2nd conc. of *A. indica* where the pupal period were significantly reduced than control, and the 2nd conc. of *C. colocynthis* where the pupal period elongated significant than control. The present results are in agreement with (Hegab, 2008) who reported that the Z-seed oil of Zanzalacht extract caused shortening pupal duration of SBW at the highest and lowest concentration (1and 3%). Also, Sayed *et. al.*, 2011 reported that *Citrullus colocynthis* are the most potent extract against *Spodoptera littoralis*, pupal duration.

3-Weight of the 4th instars larvae & pupal weight

Table (2) show that all the tested concentrations did not affected the 4th instar larval weight and the pupal weight when newly hatched larvae of *E. insulana* fed on treated diet. These results are in contrast with Hegab (2008) who found that Z- seed oil of Zanzalacht extract (Azadirachtin) caused moderately decrease of the 4th instar larvae and severely decrease in pupal weight of SBW.

Table 2. Effect of the plant extracts on some biological aspects of the spiny bollworm, *Earias insulana* 1st instar larvae at constant conditions.

Treatments	% Concentrations	Mean± S.E.			
		Larval duration/days	Larval weight/g	Pupal period (days)	Pupal weight/g
Azadirachta indica	5	15.15±0.11	0.0728±0.004	11.89±0.33	0.0585±0.003
	10	15.55±0.09	0.0719±0.06	10.62±0.03	0.0604±0.01
Control		16.57±0.56	0.0767±0.004	11.24±0.19	0.0618±0.01
F. test		NS	NS	NS	NS
$LSD_{0.05}$		5.41	0.039	3.99	0.039
Citrullus colocynthis	5	15.24±0.12	0.0752±0.003	11.89±0.33	0.0569±0.001
	10	16.02±0.02	0.0732±0.083	12.46±0.28	0.0651±0.003
Control		16.57±0.56	0.0767±0.004	11.24±0.19	0.0618±0.01
F. test		NS	NS	NS	NS
$LSD_{0.05}$		3.99	0.039	4.00	0.039
Thymus Vulgaris	5	14.19±0.05	0.0795±0.006	11.85±0.38	0.0599±0.003
	10	14.48±0.29	0.0792±0.002	12.14±0.69	0.0266±0.20
Control		16.57±0.56	0.0767±0.004	11.24±0.19	0.0618±0.01
F. test		NS	NS	NS	NS
$LSD_{0.05}$		3.99	0.04	3.99	0.039

Data are the means ±SE of the three replicate of immature stages. Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately.

4-Adult stage

Results in Table (3) indicate that, the Neem and Hanzal tested extracts slightly reduced male longevity slight shortening in male longevity. Otherwise, Zaatar extract showed no significant effect on the spiny bollworm male moths longevity (23.00±0.00 & 17.66 ±3.93 days) in the two tested concentrations, respectively compared with untreated check. On the other hand Neem and Hanzal tested extracts caused significant decrease female longevity at the two tested concentrations, while Zaatar extract caused a moderately decrease compared with the check. Hegab (2008) found that Z-seed oil tested concentrations at 1, 2 and 3% caused a moderately decreased the spiny bollworm female longevity.

Table 3. Effect of the plant extracts on some biological aspects of the spiny bollworm, *Earias insulana* 1st instar larvae at constant conditions.

Treatments	% Concentrations	Mean ± SE								
		Adult longevity (days)		Oviposition periods (days)			Sex ratio %		Female fecundity	Hatchability %
		♂	♀	Pre Oviposition	Oviposition	Post Oviposition	♀	♂		
Azadirachta indica	5	11.33±0.33	16.00±1.53 [a]	3.00±0.00	5.00±2.08	8.00±0.58	52.73±10.14	47.27±10.14	54.00±10.40	59.26
	10	10.33±2.03	9.16±1.67 [b]	-	-	-	60.74±7.66	36.26±4.70	-	-
Control		13.37±5.95	17.34±1.83 [a]	4.06±0.74	6.12±0.94	8.67±0.75	45.09±1.33	53.83±1.34	213.33±9.29	90.18
F. test		NS	**	-	–	-	NS	NS	-	-
$LSD_{0.05}$		3.99	4.37				17.30	19.98		
Citrullus colocynthis	5	13.33±1.20	21.33±2.34[a]	2.33±1.33[b]	10.00±2.89	9.00±1.73	47.13±11.02 [b]	56.91±7.66[a]	71.66±46.19[b]	75.51[b]
	10	12.33±0.88	14.66±1.20[b]	1.00±0.00[b]	6.33±0.66	7.33±0.88	73.87±8.37 [a]	26.13±8.37[b]	28.00±14.44[b]	51.79[c]
Control		13.37±5.95	17.34±1.83[ab]	4.06±0.74[a]	6.12±0.94	8.67±0.75	45.09±1.33 [b]	53.83±1.34[a]	213.33±9.29[a]	90.18[a]
F. test		NS	*	**	NS	NS	**	*	*	**
$LSD_{0.05}$		3.99	3.99	1.63	4.76	4.75	14.13	22.80	46.62	22.80
Thymus Vulgaris	5	23.00±0.00 [a]	14.32±0.88 [b]	3.00±0.00	5.66±1.33	5.66±0.88	73.87±8.37 [a]	42.67±3.97 [b]	22.66±5.49[b]	87.72[b]
	10	17.66±3.93 [b]	18.99±4.51[a]	3.00±0.00	7.33±2.66	8.66±1.86	66.56±12.03 [a]	33.44±12.03 [c]	84.67±6.69[b]	66.62[b]
Control		13.37±5.951[c]	17.34±1.83 [ab]	4.06±0.74	6.12±0.94	8.67±0.75	45.09±1.33 [b]	53.83±1.34 [a]	213.33±9.29[a]	90.18 a
F. test		**	*	NS	NS	NS	**	**	*	*
$LSD_{0.05}$		3.99	3.52	2.83	4.76	4.76	7.74	4.75	103.25	73.06

Within the same column and source data followed by the same letter are not significantly different (P>0.05).

Pre-oviposition, oviposition and post- oviposition, the total number of deposited eggs (fecundity) and the total number of hatching larvae (fertility) for all the experimental concentrations of the 3 tested compounds in comparison to the untreated control were recorded in Table (3). It is clearly obvious that treating newly hatched larvae of SBW with the 1st concentration of Neem elongated the Pre-oviposition period, while the 1st& 2nd concentrations reduced the oviposition & post-oviposition periods with significantly high reduction in numbers of laid eggs and % of hatchability than untreated check (Table 3). In addition, the two tested concentrations of Hanzal and Zaatar showed non- significant fluctuations in pre-oviposition, oviposition & post-oviposition periods with high reduction in fecundity and fertility (Table 3). These results are agree with those of Hewady, 2002), Hegab (2008)& Sharaby (2009) who stated that some plant extract formulations have an extended (latent) effect on the reproduction capacity of the adults derived from the formulations treated larvae.

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تأثير ثلاثة مستحضرات تجارية لمستخلصات نباتية على الصفات البيولوجية لدودة اللوز الشوكية تحت الظروف المعملية

محمد السيد محمد على حجاب ، صلاح فريد عبد العاطى

معهد بحوث وقاية النباتات – فرع الشرقية – مركز البحوث الزراعية– دقى–جيزة– مصر

تم تقييم ثلاثة مستحضرات تجارية مشتقة من النيم ، الحنظل و الزعتر ضد العمر اليرقى الأول لدودة اللوز الشوكية لمقارنة تأثيراتهما على الصفات البيولوجية المختلفة لليرقات وكل من طورى العذراء والحشرة الكاملة. وقد خلصت النتائج إلى أن المستخلصات النباتية المختبرة بتركيزاتها (٥ و ١٠٪) تؤثر سلبا على نسبة الموت فى طورى اليرقات والعذارى وكانت كالتالى: (٥٧,٣٣ &٥٠,٦٧٪) ، (٤٥,٣٣ &٦٦,٦٦٪) و (٤٩,٣٣ &٥٧,٣٣ ٪) موت لكل من النيم والحنظل والزعتر على الترتيب مقارنة بالكنترول (٢,٣٤ %) ،كما أدت المستخلصات النباتية المختبرة الى حدوث خفض معنوى فى نسبة التعذير وكانت كالتالى: (٥٢,٠٠ &٦٢,٠٠ %) و (٤٨,٠٠ %٦٠,٠٠ %) و (٦٠,٠٠ & ٥٤,٦٧ %) على الترتيب مقارنة بالكنترول (٧٩,١٩ %) . وبالإضافة إلى ذلك ، ثبت أن وزن اليرقات والعذارى لم يختلف معنويا عن المقارنة. أيضا، تشير النتائج إلى أن المستخلصات الثلاثة المختبرة ثبطت النسبه المئوية لخروج الحشرات الكاملة الناتجة من معاملة اليرقات بالتركيزات المختبرة حيث سجلت (٤٢,٦٧ & ٤٩,٣٣ %) و(٥٤,٦٧ &٣٣,٣٤ %) و (٥٠,٦٧ & ٤٢,٦٧ %) مقارنة بالكنترول (٩٧,٦٦ %) على الترتيب. أيضا النتائج اوضحت وجود خفض معنوى فى عدد البيض الموضوع وكانت كالتالى: (٥٤,٠٠ & ٠,٠٠ بيضه / ♀) ، (٧١,٦٦ &٢٨,٠٠ بيضه / ♀) و(٢٢,٦٦ ٨٤,٦٧ بيضه / ♀) على الترتيب مقارنة بـ (٢١٣،٣٣ بيضة / ♀) في المقارنه. وسجلت النتائج وجود تأثير معنوى على نسب فقس البيض وكانت كالتالي: (٥٩,٢٦ & ٠,٠٠٪) ، (٧٥,٥١ &٥١،٧٩٪) و (٨٧,٧٢ & ٦٦،٦٢ ٪) على الترتيب ، مقارنة بـ (٩٠,١٨٪) في الكنترول.