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ORIGINAL ARTICLES

Laboratory and Field Evaluations of Two Biocides and an Insecticide Against *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) and *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

Laboratory and field experiments was conducted to evaluate the toxicity of two biocides i.e. onion oil and byrovect (mixture of *Bacillus thuingiensis* var *kurestaki* and *Spodotera littorales* NPV) against *Pectinophora gossypiella* (PBW) and *Earias insulana* (SBW). The LC_{50} & confidence limits (95%) for values of onion oil and byrovect after 2 hours, 2 days and 7 days of treatment were determined. Moreover, the biological effects of LC_{50} of the onion oil against pre-adult and adult stages of PBW were studied. Also, the two biocides and Chlorofet EC (48%) (As a chemical insecticide) were evaluated in Qalyoubia Governorate cotton field, individually (each one alone in sequence of 4 applications) as well as the three compounds in 2 different sequences program against cotton bollworm complexes, SBWand/or PBW. Present results cleared that the sequencing spray of onion oil (1st spray), chlorofet EC (48%) (2nd spray) and byrovect (3rd spray) was the most effective program against the PBW cotton boll infestation and larval content. In this respect chlorofet EC (48%) individually revealed high potential efficacy against SBW and boll complexes.

Key words: *Pectinophora gossypiella*, (PBW), *Earias insulana* (SBW), biocides, chemical insecticide, toxicity & biological effects.

Introduction

The pink bollworm (PBW) *Pectinophora gossypiella* and spiny bollworm *Earias insulana* (SBW) are known as very destructive pests of cotton, *Gossypium barbadense* L. in Egypt. One unit infestation degree by either the PBW and/or SBW cause a reduction of total yield weight equal to 10% and 6–9% (on an average), respectively. Also, that is depending on the larval content averages and infestation percentages/100 green bolls for each of the two involved insect-species (El-Saadany *et al.*,1975). Durán *et al*., (2000) mentioned that each SBW larva can damage up to 3 squares and 1 boll in 1-2 plants.

The chemical control with conventional insecticides did not overcome on the PBW problem. Also, the use of chemicals against SBW is proven to be insufficient due to the overlapping of the bollworm's different development stages and its location (Henneberry, 1986 and Durán *et al*., 2000).On other hand, many natural enemies as well as many of biocides such as bactericides, viricides ……etc. and /or their mixture(s), moreover botanically derived insecticides as Neem, Pyrethrum, Rotenone, Nicotine, Sabadilla, Ryania and a number of different available botanicals were gained favor in recent years and used to protect agricultural crops from the ravages of insects, mites and nematodes in different parts of the world. They are comparatively safe to natural enemies and higher organisms (Gurusubramanian **&** Krishna, 1996;Solsoloy *et al*., 2001,Karima *et al*., 2001, Karima & Abd El-Razek, 2005, Abdel- Hafez *et al*., 2007, Ahmad, 2007,Massoud *et al*., 2009 and Dimetry, 2012).

Plant extracts have more attention in controlling many pests, that are non-toxic to man and animals, possess distinct toxicity and also lead to antifeeding activity and inhibition growth of some pests (Sharaby & Ammar, 1997,Badr *et al*., 2000,Amer, 2004,D'Andrea *et al*., 2001 and Anwar *et al*., 2007).The present work was carried out under laboratory and cotton field cultivated with the cotton variety "Giza-86". The laboratory studies directed to evaluate the toxicity of two biocides i.e. extract of onion seed oil and byrovect (mixture of *BT kurestaki* and *S. littorales* NPV) against PBW. In addition, the biological effects of the LC_{50} of the onion oil against PBW were studied. In cotton field, these biocides and chlorofect (as a chemical insecticide) were evaluated individually as well as in sequence(s) against the PBW, the SBW and/or their complexes in Qalyoubia Governorate, in 2010 cotton season.

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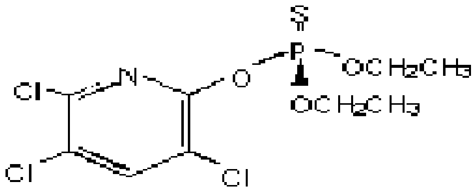
Materials And Methods

Toxicological and biological studies were carried out to evaluate the effect of onion oil and byrovect (*Bacillus thuingiensis* var *kurstaki* & *Spodoptera littoralis* NPV) against pink bollworm (PBW). Also, the former biocidies as well as chlorofet 48%EC (chloropyrifos) were evaluated against the cotton bollworm PBW, SBW and their complexes in cotton field.



Chlorofet 48%EC (*O, O*-diethyl-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate)

1. Laboratory studies:

Preparation of onion seed oil:

The onion (*Allium cepa* Linn) seed oil was obtained by cold mechanical pressing from Oils Press Unit in National Research Center in Cairo, Egypt.

Rearing of P. gossypiella:

Newly hatched larvae of *P. gossypiella* were obtained from a colony maintained in the laboratory for several generations at 27 ± 1°C and 75 ± 5% relative humidity (RH). Larvae were reared on a modified artificial diet as described previously by Abd El-Hafez *et al.*, (1982).

Toxicological studies:

All bioassay trials for onion oil and byrovect were tested on the newly hatched larvae of the PBW. Tested materials were homogenate mixed with 100 g of artificial diet to obtain the tested concentrations, i.e.,0.05, 0.1, 0.2, 0.4 and 0.6 ml/100 g diet for onion oil and 0.125, 0.025, 0.05, 0.1 and 0.2 ml/100 g diet for byrovect (without antimicrobial agent). After preparation of tested diets, each one was individually folded into 3 Petri dishes (9 cm in diameter). Ten newly hatched larvae of PBW were placed on the surface of the diet using a soft brush. Another group of 3 Petri dishes was prepared containing the same diet but mixed with equal volume of distilled water (used as control) and an equal number of the maintained larvae were placed on their surface. Larvae were allowed to feed on the tested diets for two hours. Afterwards any alive larvae were transferred individually to glass vials (2 X 7 cm) containing normal diet. Vials were plugged with absorbent cotton and incubated at 27 ± 1°C and 75 ± 5% RH. Larval mortalities were recorded 2 hours, 2 days, and 7 days after treatment. Percentages of mortalities were corrected according to Abbott's formula **(Abbot, 1925)** as follows:

$$\%\text{Corrected mortality} = \frac{\text{T - C}}{\text{100 - C}} \times 100$$

Where; T: %mortality in treatment
C: %mortality in check

The variability in response to the tested materials was calculated based on LC_{50} and the slope values according to Metcalf & Luckman, (1994) and Jyoti & Brewer,(1999). Concentration-mortality regression lines were illustrated according to Finney, (1971). The toxicity index was calculated using the equation of Sun, (1950) as follows:

$$\textbf{Toxicity index} = \frac{\textbf{LC}_{50}\textbf{ of A}}{\textbf{LC}_{50}\textbf{ of B}} \times 100$$

Where; A: the most effective material
B: the other tested material

Biological effects of onion oil on PBW:

This assay was applied to onion oil only. Newly hatched larvae of PBW were fed on diets containing LC_{50}

(inspected after 2 days) placed in Petri dishes (9 cm in diameter). Three replicates of (400 larvae/replicate) were used. After two days, the survivors in each assay were transferred to glass vials (2 X 7 cm) containing control diet and held at 27 ± 1°C and 75 ± 5% RH. Larvae of control bioassay were fed on control diet containing water instead of tested material. After adult moths' eclosion, ten replicates each contained three cages (5-pairs/cage) of emerged moths that appeared morphologically not impaired were placed in glass chimney for mating .The upper and lower surfaces of each were covered with muslin cloth held in position by rubber bands. Moths were fed on sucrose solution 10% by providing each cage with soaked piece of cotton wool .The cages were examined daily until death of moths. Biological observations were made to determine larval and pupal periods, pupation%, pupal mortality%, moth emergence, sex ratio, survival rate and adult longevity.

Field experiments:

Field experiments were carried out in an area of 1/2 Fadden cultivated with the cotton variety "Giza-86" during season 2010 in Qalyoubia Governorate to evaluate the efficacy of onion oil(L/Fadden), byrovect (*Bacillus thuingiensis* var *kurstaki* & *S. littoralis* NPV) (300 g/Fadden) and chlorofet 48%EC (chloropyrifos) (1L/Fadden) against the PBW, SBW and/or their complexes.

2.1. The experimental area was divided into 3 treatments (onion seed oil, byrovect and chlorofet 48% EC). Each treatment splited to 3 replicates and each replicate about ($42m^2$). In addition, three replicates were used as control. The recommended agronomic practices were followed in all plots including the untreated check. The treatments was applied on cotton plants in complete randomize block design, at 6 days intervals (19 th Aug., 25th Aug., 31st Aug. and 7th Sep., 2010) using solo motor sprayer at 400 L/Fadden. Each treatment was sprayed individually 4 times.

2.2. Sequence(s) of the former treatments were tested by the same technique in another experiment as the following 2 sequences:

Sequence (1) = oil (1st spray), chlorofet (2nd spray) and byrovect (3rd spray).

Sequence (2) = chlorofet (1st spray), oil (2nd spray) and byrovect (3rd spray).

Green cotton bolls samples (25 bolls /replicate) were collected randomly before spray and at 7 days post each spray from all treated and untreated area. The samples were examined and dissected in the laboratory, to estimate the total number of infested bolls with PBW, SPW and/or their complex as well as the total larval number of each pest alone. Efficacies of tested treatments against the cotton boll infestation and/or the larval content of either PBW or SBW were calculated according to equation of (Henderson and Tilton, 1955).

Statistical analysis:

Toxicological data were statistically calculated through a Proban program, software computer program (Jedrychowski, 1991). The variability in response to the tested materials was determined based on LC_{50} and slope in addition to illustrating concentration-mortality regression lines. Analysis of variance (ANOVA) was conducted on all data using Costat program software computer. Means were compared by Duncan's multiple range test (Duncan, 1955).

Results And Discussion

Laboratory studies:

Toxicological studies of tested materials:

Table (1) summarizes the concentration-mortality responses of feeding PBW newly hatched larvae for two hours on various concentrations of tested materials (onion oil and byrovect). Results indicate that the mortality rates increased with the increase of the used concentration and the period after treatment. The corrected mortality percentages after 2 hours of onion oil treatment ranged from 13.71% using the lowest concentration (0.05 ml/100g diet) to 74.00% using the highest concentration (0.6 ml/100g diet). As for byrovect, the corrected mortality percentages ranged from 13.53 to 55.26% at the lowest and the highest concentrations, respectively. The illustrated concentration-mortality regression lines (Figure1) confirmed the same results as a positive relationship between the applied concentration and the mortality percentage. After 2days of treatment with onion oil mortality % ranged from 57.30 to 92.59%.While, the mortality percentages after 7 days of treatment were increased and ranged between & 58.34 to 96.11%. Nearly, the same was obtained with byrovect the mortality percentage. After 2days of treatment ranged between 29.57 to 70.89% , and after 7 days 33.46 to 75.59% in the cases of byrovect treatment, respectively.

Table 1: Efficacy of onion oil and byrovect tested against newly hatched larvae of *P. gossypiella* at different periods.

	Conce. (ml/100g diet)	Corrected mortality (%) After		
		2 hours	2 days	7 days
Onion oil	0.05	13.71	57.30	58.34
	0.10	27.12	70.17	73.11
	0.20	45.04	81.08	85.28
	0.40	64.05	89.19	93.28
	0.60	74.00	92.59	96.11
Correlation (r) ±S.E.	0.64 ± 0.21 **			
Byrovect	0.0125	13.53	29.57	33.46
	0.0250	21.38	38.90	41.93
	0.0500	31.39	49.49	52.62
	0.1000	43.01	60.47	64.37
	0.2000	55.26	70.89	75.59
Correlation (r) ±S.E.	0.48 ± 0.24 ns			

***= significance, ns= none significance*

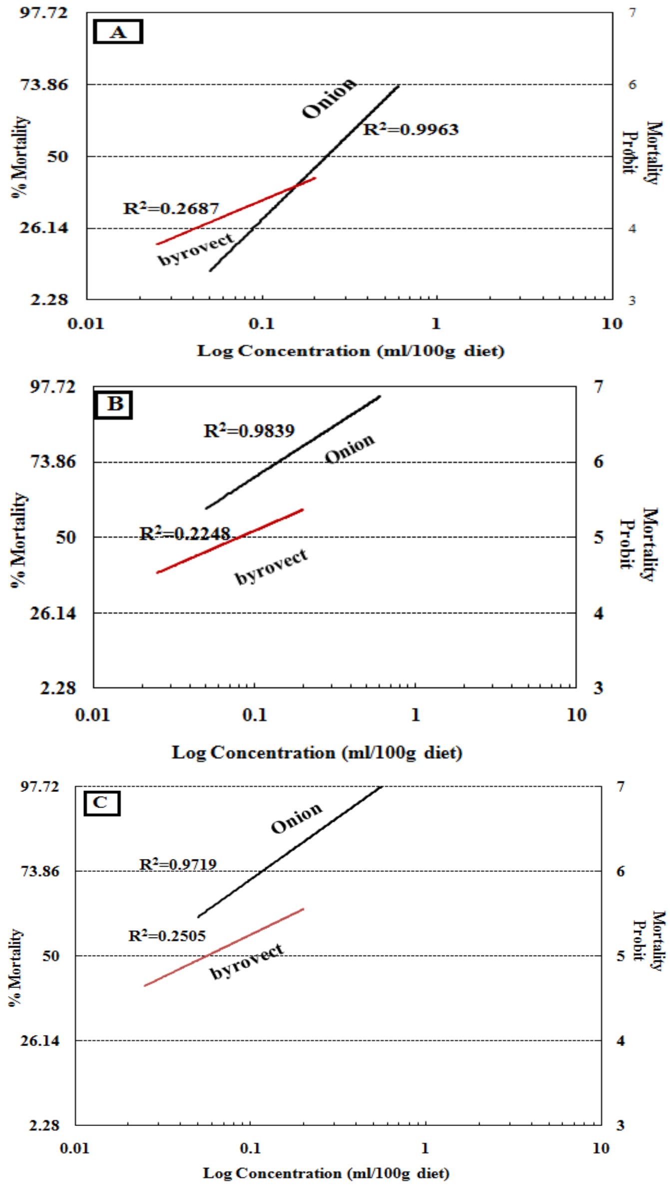


Fig. 1: Concentration log probit toxicity lines of onion seed oil and byrovect against newly hatched larvae of *P. gossypiella* after 2 hours (A), 2 days (B) and 7 days (C) of treatment.

According to the reports of many authors the onion oil was observed effective in eliminating some species in addition to its insecticidal activity on other (Metspalu *et al*., 2013) & Popoola (2013). Regarding the cumulative mortality as depicted in Figure (2), it could be noted that the highest percentage of mortality (≈ 40%) was occurred within the next two days following treatment with onion oil at low concentrations. While mortality% distributed between the first two intervals at low concentration of byrovect. On the other hand, higher concentrations of tested materials caused an acute effect. For example, most larvae died (74.00 & 55.26%) within the first two hours following treatment with the highest concentration of tested materials, respectively. Thus, 22.11 & 20.33% of larvae died until the seventh day of treatment with the onion oilt and byrovect, respectively. So, due to the high mortality in younger larval stages we may predict that crop damage will remain at a lower level.

Generally, this study demonstrated that tested materials were effective in causing mortality of the PBW. The mortality was concentration dependent. Such results coincide with Ignoffo (1962), Graves & Watson (1970), Rizk *et al.* (1981), Abd El-Hameed (1995) and Amer (2004). Morality among larvae was increased by increasing either the concentration or the feeding period. Great percentage of mortality almost following the first two hours from treatment and continued among larvae even after they being transferred to an untreated diet. These results are in agreement with those obtained by Taher *et al*., (1994) and Zidan *et al*., (1998). El-Nemaky (2000) found that the pink and spiny bollworms larvae had moderate susceptibility to the action of M-peed and MVP II *B. thuringiensis* products in comparable with the efficiency of the recommended insecticides i.e. Curacron, Atabron and Cutabron.

Table 2: LC_{50} (ml/100 g diet) and slope values of onion oil and byrovect tested against the newly hatched larvae of *P. gossypiella* at different periods.

Treatment	*2 hours after treatment*			*2 days after treatment*			*7 days after treatment*		
	LC_{50} (95%FL)	Slope ± SE	Toxicity index at LC_{50}	LC_{50} (95% FL)	Slope ± SE	Toxicity index at LC_{50}	LC_{50} (95%FL)	Slope ± SE	Toxicity index at LC_{50}
Onion oil	0.24 (0.16-0.39)	1.61 ± 0.27	62.5	0.04 (0.0004-0.09)	1.21 ± 0.30	100	0.06 (0.04-0.07)	1.59 ± 0.18	100
Byrovect	0.15 (0.08-1.14)	1.03 ± 0.24	100	0.07 (0.03-0.30)	1.00 ± 0.24	57.14	0.08 (0.04-0.22)	1.25 ± 0.27	75

LC: lethal concentration,FL: fiducial limits:

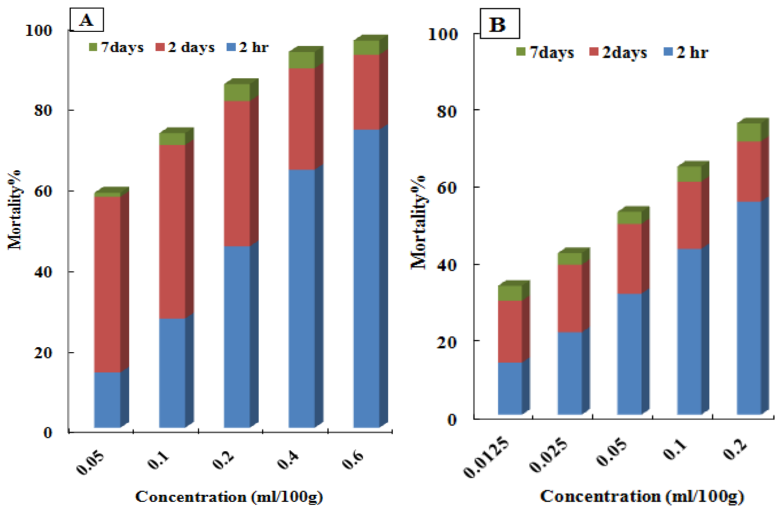


Fig. 2: Cumulative mortality of *P. gossypiella* larvae after 2 hours, 2 days, and, 7 days of treatment with onion seed oil (A) and byrovect (B)

Based on the LC_{50} values (Table 2 & Figure 1) after 2 hours of treatment, byrovect was the most toxic material. In this case, the LC_{50} reached 0.15 ml/100 g diet with toxicity index 100 and slope value of 1.03± 0.24 against the newly hatched larvae of the PBW. On contrary, onion oil was the least effective as LC_{50} values = 0.24 ml/100 g diet, toxicity index was 62.5 and slope value of 1.61± 0.27 after the aforementioned period. While, after 2 & 7 days of treatment, The LC_{50} values for onion oil were 0.04 & 0.06 ml/100 g diet. The toxicity index was 100 and slope value of 1.21±0.3 & 1.59± 0.18, respectively). The LC_{50} values were 0.04 & 0.06 for onion oil and 0.07 & 0.08 for byrovect after 2 and 7 days after treatment, respectively. According to Metcalf & Luckman (1994) and Jyoti & Brewer (1999) the LC_{50} value alone does not reveal an accurate picture of the total pathogenic effect. They reported that insects respond to increasing doses of pathogenic organisms by increased infection and mortality, just as they respond to increased doses of insecticides. Thus, the mortality effect of an insect pathogen on its host can be expressed as an LC_{50} and LC_{90} values and can also be characterized by the slope of the log-probit curve.

The slope of the concentration-mortality curve is a measure of variability in response to treatment within the insect population tested. As the value of the slope increases, mortality associated with changes in concentration increases. Conversely, as the value of slope decreases, less change in mortality is seen per unit change in concentration of the mortality agent. In the present study, the highest slope value was estimated for onion oil after 2 and 7 days suggested that this product was the most toxic testes material.

2. Biological studies of onion oil:

The newly hatched larvae of PBW were fed on artificial diet treated with LC_{50} concentration of onion oil after two days. Two hours post-treatment, the surviving larvae were transferred to continue its development on untreated artificial diet until full-grown. Observations were done on pre-adult and adult stages.

Pre-adult stage:

Data given in Table (3) showed that a considerable variation in the larval and pupal duration was achieved as result of onion oil application. In the control insects, male and female larvae required an average of 17.33± 2.55 & 17.5 ± 1.88days to pupate and an additional of 8.21± 1.29 & 8.0± 1.24 days (pupal period) to reach the adult stage. Accordingly, the total male and female period required to reach the adult stage was 25.54 & 25.65 days.

Table 3: Effect of LC_{50} of onion oil on pre- adult stage of *P. gossypiella.*

Treatment	Larval period (days)*± SD		Pupal period (days)*± SD		Pupation% ± SD		Inhibition of pupation %		Pupal Mortality% ± SD	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Control	17.33^{b}± 2.55	17.65 ^{b} ± 1.88	8.21 ^{a} ± 1.29	8.00 ^{a} ± 1.24	100.0 ^{a} ± 0.00	86.68 ^{a} ± 4.98	0.0	0.0	7.41 ^{b} ± 6.93	25.19 ^{b} ± 22.34
Onion oil	19.54 ^{a} ± 2.40	19.34 ^{a} ± 2.05	7.85 ^{b} ± 1.68	7.73 ^{b} ± 1.24	50.0 ^{b} ± 2.74	50.23 ^{b} ± 3.54	50.0	36.45	54.15 ^{a} ± 27.48	37.89 ^{a} ± 34.62
LSD (5%)	0.22	0.17	0.14	0.11	0.24	0.24			2.36	3.17

**Means followed by the same letter at the same column are not significantly different.*

Onion oil caused significant retardation in larval development as it prolonged to 19.54 ± 2.40& 19.34 ± 2.05days for male and female, respectively. Conversely, shortened pupal periods (7.85 & 7.73 days for male and female, respectively) were achieved as a result of newly hatched larvae treatment with LC_{50} of onion oil. Accordingly, the survived larvae after treatment required an average of 27.39 and 27.07 days for male and female, respectively to reach the adult stage.

Accordingly, onion oil prolonged the periods required to reach the adult stage. These results are in agreement with Khalil *et al.,* (2006) & Metspalu *et al*., (2013) who found that the larval development of cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae) was significantly slower and mortality was higher on *A. cepa* ,this could be due to the repellent effect of the onion oil .Pupation% was highly affected when larvae were fed on a diet containing LC_{50} of onion oil, since the average percent was 100 & 86.68% in the male & female control, respectively. This average decreased significantly to 50 & 50.23% in the treated male & female, respectively. The highest percentage in inhibition of pupation percentage (50%) occurred with male larvae compared with female larvae (36.45%). Concerning the pupal mortality%, there was a clear variance between two treatments. The average percentages were 7.41 & 18.89% in the male and female control treatment, respectively. Otherwise, this average increased significantly to 54.15 & 37.89% for the male and female onion oil, respectively. Khalil *et al.,* (2006) reported that the mortality of *M. brassicae* larvae and pupae was also significantly affected by food plant. The pupal mortality on *A. cepa* reached 19%.

Adult-stage: Moth emergence decreased significantly to 65.77% & 70.55% for male & female, respectively, when the larvae were fed on a diet containing LC_{50} of onion oil. While, 85.67% & 83.60% for male & female were recorded in control, respectively (Table 4). It was remarkable that the onion oil treatment affected sex ratio of PBW. These values differed insignificantly with control. The recorded ratio was (61.02% male & 37.32% female) and (51.08% male & 48.92% female) in treatment and control, respectively. Khalil *et al.,* (2006) found that the *M. brassicae* fed on *A. cepa* had a male-biased sex ratio.

Concerning the survival rate, the highest rates from egg to adult emergence were recorded as 85.67 & 83.60 for male and female control treatment, respectively. These values for onion oil treatment being considerably lower (22.39 male & 26.27 female). In comparison with control insects, the adult longevity was significantly affected when newly hatched larvae were fed on diet containing LC_{50} concentration of the tested extract. Life-span of PBW male and moths resulted from onion oil treatment were 40.13 & 23.98 days, respectively. These values were significantly higher than those achieved with control insects (16.97 & 16.26 days for male and female, respectively) (Table 4).

Table 4: Effect of LC_{50} of onion oil on adult stage of *P. gossypiella* .

Treatment	% Moth emergence* ± SE		% Sex ratio%* ± SE		Survival rate** ± SE		Adult longevity (days)* ± SE	
	♂	♀	♂	♀	♂	♀	♂	♀
Control	85.67^{a} ± 3.82	83.60^{a} ± 2.46	51.08^{a} ± 0.74	48.92^{a} ± 0.74	85.67^{a} ± 3.82	83.60^{a} ± 2.46	16.97^{b} ± 0.19	16.26^{b} ± 0.14
Onion oil	65.77^{b} ± 6.18	70.55^{b} ± 3.64	61.02 ^{a} ± 2.35	37.32^{a} ± 2.24	22.39^{b} ± 2.69	26.27^{b} ± 2.81	40.13^{a} ± 1.61	23.98^{a} ± 0.22
LSD (5%)	15.51	9.27	12.87	15.66	10.35	9.96	14.19	2.62

** Means followed by the same letter at the same column are not significantly different.*
*** Survival rate = (Normal moth/total larvae)*100*

All the obtained results of the present experiment are in agreement with those of Amer (2004) who reported that Biorepel 10% E (a.i. garlic juice) induced gradual increase in larval mortality, elongation in larval & pupal duration period and high decrease in adult emergence with reduction in adult longevity of PBW.

Generally, our study indicates that the extract of *A. cepa* is endowed with higher larvicidal efficacy and subsequently has latent effect on pupal and adult stages. The biocidal properties of onion are attributed to sulphur volatiles produced during degradation of *Allium* tissues. The primary emitted compounds are thiosulphinates and zwiebelanes mainly converted in *Allium* product (extract) to disulphides (Arora *et al.*, 2012). Further characterization of bioactive molecules of *A. cepa* extract will provide greater clarity about insecticidal nature of these bioactive compounds. This could become alternative to the conventional insecticides used for the regulation of *P. gossypiella*.

II- Field experiments:

The efficiency of onion seed oil extract, byrovect (*Bacillus thuingiensis* var *kurstaki*) and chlorofet 48% EC (chloropyrifos) individually and/or their sequence(s) were tested against the PBW, SBW and/or their complexes. Sequence (1): oil (1st spray), chlorofet (2nd spray) and byrovect (3rd spray) while sequence (2): chlorofet (1st spray), oil (2nd spray) and byrovect (3rd spray). % infestation of cotton bolls with each pest & the pest complexes and the efficiency of tested treatments against the larval content of each pest were presented in Tables (5, 6 & 7).

1- Efficiency of tested treatments against the cotton boll infestation:

As shown in Table (5), the efficiency of onion seed oil against the infestation of cotton bolls with the bollworm complexes ranged between 61.3% & 77.8%. The corresponding efficiencies for byrovect and chlorofet range(s) were 47.8% & 72.8% and 65.0% & 86.4%, respectively. In addition, the efficiency of sequence (1) ranged between 61.8% & 79.3%. The corresponding efficiency range for sequence (2) was 48.5% & 65.1%.

In case of cotton boll infestation with SBW and PBW the efficiencies range(s) of onion oil extract, byrovect and chlorofet were (66.7 to 84.6% & 23.8 to 81.0 %), (52.6% to 80.9% & 23.8% to 71.4%), and (75.3% to 89.8% & - 42.8 to 71.4), respectively. In the other hand, the efficiency of sequence (1) on SBW and PBW ranged between (61.7% to 73.9% & 61.8 to 79.3,while, the efficacy range for sequence (2) was (44.0 to 60. 0 &50.0% to 70.0%)), respectively (Table 5).

Table 5: The efficiency % of tested treatments individually and/or in sequence(s) against the cotton boll infestation with complexes of *E. insulana* and/or *P. gossypiella* and the infestation with each pest during cotton season 2010.

Treatment	Efficiency % after spray(s) against the cotton boll infestation											
	Bollworm complexes				*E. insulana*				*P. gossypiella*			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Onion oil	61.3	77.8	67.7	65.1	70.2	84.6	66.7	-	33.3	23.8	81.0	23.8
Byrovect (*Bt* +NPV)	47.8	72.8	70.9	54.3	52.6	69.2	80.9	-	66.7	71.4	52.4	23.8
Chlorofet (48% EC)	71.7	65.0	86.4	67.8	77.4	75.3	89.8	-	66.7	- 42.8	71.4	-28.6
Sequence 1	70.7	79.3	61.8	-	61.7	80.4	73.9	-	93.3	80.0	41.7	0.0
Sequence 2	49.9	65.1	48.5	-	44.0	55.0	60.0	-	70.0	50.0	60.0	0.0

Sequence (1): oil (1st spray), chlorofet (2nd spray) and byrovect (3rd spray).
Sequence (2): chlorofet (1st spray), oil (2nd spray) and byrovect (3rd spray).

However, as shown in Table (7), the mean program efficiencies of the onion oil extract against the infestation of cotton bolls with the bollworm complexes, SBW and/or PBW against each pest infestation alone were 68.9%, 73.8% & 46.5%, respectively. The corresponding efficiencies for byrovect and chlorofet were 63.6%, 67.6% & 63.5% and 74.4%, 80.8% & 31.8%, respectively. In addition, the mean efficiencies of the tested sequence (1) and sequence (2) were 70.6%, 72.5% & 71.7% and 54.6%, 53.0% & 40.0%, respectively. In this regard, Karima *et al.*, (2001) revealed an opposite relation between the percentages of small cotton bollworm larvae of either PBW and/or SBW before the beginning of spray and efficacy of bio-insecticides, chemical insecticides and/or their mixture. Amer (2004) observed that Chinmix 5% EC (Beta-cypermethrin) was the most effecient compound against cotton bollworms followed by Spintor and Biorepel 10%E (*a.i.* Garlic juice). The annual percent of infestation reduction recorded 39.90, 63.72 and 34.71 & 94.31, 69.21 and 37.72, respectively during the two seasons of experiments. Also, Massoud *et al*., (2009) found that, Dursban gave the highest infestation reduction (66.9% & 74.00%) followed by Achook (55.42% & 70.41%) and Agerin (44.38% & 37.21%) in first and second season, respectively. Moreover, they added that the sequence Achook, Pindelta, Dursban and Larvin and the second sequence Pindelta, Achook, Dursban and Larvin gave general reduction means of 75.30 and 76.15%, respectively.

2- Efficiency of tested treatments against the larval content of PBW and/or SBW inside the cotton boll:

As shown in Tables (6 & 7), the efficiency of onion oil extract against the larval contents of both SBW and PBW ranged from (54.3% to 80.0% with mean of 67.3%) and (11.8% to 76.2% with mean of 36.5%), respectively. The corresponding efficiencies range(s) for byrovect and chlorofet were 42.8% to 77.1% (with mean of 64.1%) and 7.6% to 64.3% (with mean of 55.8%) and 7.55% to 95.9% (with mean of 81.3%) and (-91.2%) to 64.3% (with mean of 22.1%), respectively. In addition, the efficiency ranges of tested sequence (1) were 61.7% to 80.4% (with mean of 70.9%) and 35.7% to 94.3% (with mean of 70.0%) against the larval contents of SBW and PBW, respectively. The corresponding efficiencies range for sequence (2) were 44.0% to 60.0% (with mean of 51.3%) and -12.5% to 60.0% (with mean of 29.2%), respectively.

In this respect, Ghobrial & Dittrich (1980) reported that, chlorpyrifos was intermediate efficient insecticide against *E. insulana* in the late season. Also, Gurusubramanian and Krishna (1996) found that hatchability of exposed freshly laid eggs (<24 h old) of *Earias vittella* Fabricius to volatiles from bulbs of *Allium sativum* was significantly reduced, and only 30% of those of *E. vittella* that hatched from treated eggs managed to complete their metamorphosis. Solsoloy *et al.,* (2001) evaluated the potentiality of betel as a botanical pesticide (the crude volatile oil, its fractions and formulated product) against pests of cotton. Their findings showed that the oil and the formulated product at 5% rate effectively inhibited oviposition of *Pectinophora gossypiella* and showed promise ovicidal effect against cotton bollworm, *Helicoverpa armigera* and pink bollworm.

Table 6: The tested treatments individually and/or in sequence(s) against *E. insulana* and/or *P. gossypiella* during cotton season 2010

Treatment	Efficiency after spray(s) against the larval content of							
	E. insulana				*P. gossypiella*			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Onion oil	67.6	80.0	54.3	0.0	16.7	16.7	76.2	11.8
Byrovect (*Bt* +NPV)	42.8	72.4	77.1	0.0	64.3	64.3	38.8	7.6
chlorofet(48% EC)	7.55	72.4	95.9	0.0	58.3	- 56.2	64.3	- 91.2
Sequence 1	61.7	80.4	70.6	0.0	94.3	80.0	35.7	0.0
Sequence 2	44.0	50.0	60.0	0.0	60.0	40.0	- 12.5	0.0

Sequence (1): oil (1st spray), chlorofet (2nd spray) and byrovect (3rd spray).
Sequence (2): chlorofet (1st spray), oil (2nd spray) and byrovect (3rd spray).

Table 7: Mean program efficiency% of tested treatments individually and/or in sequence(s) against the cotton boll infestation with the bollworm complexes of *E. insulana* and/or *P. gossypiella* and the larval content of each pest during cotton season 2010

Treatment	Mean program efficiency% against				
	Cotton boll infestation			Larval content	
	Bollworm complexes	*E. insulana*	*P. gossypilla*	*E. insulana*	*P. gossypiella*
Onion oil	68.9	73.8	46.5	67.3	36.5
Byrovect(*Bt* + NPV)	63.6	67.6	63.5	64.1	55.8
Chlorofet (48% EC)	74.4	80.8	31.8	81.3	22.1
Sequence 1	70.6	72.5	71.7	70.9	70.0
Sequence 2	54.6	53.0	40.0	51.3	29.2

Sequence (1): oil (1st spray), chlorofet (2nd spray) and byrovect (3rd spray).
Sequence (2): chlorofet (1st spray), oil (2nd spray) and byrovect (3rd spray).

Field evaluation of the formulated betel product (60 EC) indicated a reduction in bollworm population at a lower rate than the check insecticides, deltamethrin and profenofos.

In addition, Karima & Abd El-Razek (2005) found that, the field application with two formulations of B.T., PoGv and their binary mixture are effective on its own against PBW larval population and PBW infestation of cotton bolls. But, they were not reliably effective when the percentages of PBW small larval population (%) increase and full-grown larval population (%) decrease at zero time of spraying of cotton plants.

Present results cleared that the sequencing spray of onion oil (1st spray), chlorofet EC (48%) (2nd spray) and byrovect (3rd spray) was the most effective program against the PBW cotton boll infestation and larval content. In this respect chlorofet EC (48%) individually revealed high potential efficacy against SBW and bollworm complexes.

Conclusions:

In light of the aforementioned results, it could be concluded that, the tested materials were effective in causing mortality of the PBW. The mortality was concentration dependent. Onion oil is endowed with higher larvicidal efficacy and subsequently has latent effect on pupal and adult stages. In field experiments results cleared that the sequencing spray of onion oil (1st spray), chlorofet EC (48%) (2nd spray) and byrovect (3rd spray) was the most effective program against the PBW cotton boll infestation and larval content. In this respect chlorofet EC (48%) individually revealed high potential efficacy against SBW and boll complexes.

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