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OVICIDAL ACTIVITY AND LATENT ADVERSE EFFECT OF *Syzygium aromaticum* AND *Pinus brutia* OILS AGAINST *Pectinophora gossypiella* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was undertaken to investigate the impacts of the two essential oils; *Syzygium aromaticum* (Clove) and *Pinus brutia* (Turpentine) against 1-3 days old eggs of pink bollworm *Pectinophora gossypiella*; the toxicity and the adverse impact of these products on some biological and physical characters were studied under laboratory conditions at 25 ±1 °C and 65±5% R.H.

The obtained results revealed that the eggs of *P. gossypiella* pest were highly susceptible to Pinus than Syzyginum. The LC_{50} value were 9.38% ppm when PBW eggs treated with Turpentine oil, while it increased to 11.57% when 1-3 days old eggs of *P. gossypiella* dipping with Syzyginum. Some biological aspect studies show a prolongation in larval and pupal stages developments resulted from treated 1-3 day old eggs by *S. aromaticum* and *P. brutia*.It estimated by 9.0 and 20.0 days/ larvae, respectively for larvae and 9.3 and 10.2 days/ pupae. . The life cycle were significant affected, as it was elongated to 34.1 and 36.9**,** respectively, compared with 26.2 days in control.

In contrast, in adult stage, the results indicated that the percentage of adult emergence was highly decreased to 56.0 and 61.0% emerged from eggs treated with LC_{50} of Syzygium and Turpentine, respectively, compared with (94.00%) control, with high reduction in total eggs laid, percentage of hatchability. The average number of eggs deposited by females were 107 and 97 eggs laid at 11.1 and 7.6 days oviposition/ female, resulted from Syzygium and Turpentine, respectively compared with 226.0 eggs/ female in control deposited at 11.6 days.

The physical result indicated that latent adverse effect in biochemical larvae resulted from eggs treated with two essential oil Syzygium and Turpentine. It caused high decreased in total protein to 15.0 and 8.6 (total protein (mg/g.b.wt.), respectively compared with17.0 mg/g.b.wt. /larvae in control, with decreased the Phenoloxidase, to 14.8 and 13.5 (O.D. units/g.b.wt), respectively, compared with 26.0 Phenoloxidase (O.D. units/g.b.wt) in control. In contrast, increased in free-amino acid to 137.3 and 298.0 (µg D, L- alanine/g.b.wt) respectively, compared with 118.0 in control, also, the total lipid increased to 27.9 and 22.0 (mg/g.b.wt.), respectively, compared with16.9 (mg/g.b.wt.) in control..

Keywords: *P. gossypiella; Syzygium and Pinus brutia; biological and physical.*

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1. INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larvae is considered to be one of the most injurious cotton pest, because it is difficult to control with insecticides. On contrary, the eggs considerable easy to control with insecticides, because are laid on the sutures or under the bracteoles at the base of the boll, particularly on bolls up to 14 days old. Upon hatching destructive larvae can penetrate feeds on flowers making rosetted bloom, later it enters bolls within 20–30 min to 2 hr. [1]. It feeds on seeds therein, which results in malformation, rotting, premature or partial boll opening, and great loss as in both quality and quantity and reduction of cotton yield [2].

Wide used of several chemical pesticides sprays have been applied, it had created some problems in natural fields; such as pest resistance and toxicity against beneficial predator or parasite and honey bees, in additional high effect on environmental pollution and high input costs, health risks and the presence of pesticide residues in plants at the end resistant pest.

New, essential natural oils are being tried as potential candidates for pest and disease management. Because, it products play an increasingly prominent role as alternatives to synthetic for used to control some insect pests due to the increasing injurious on environmental pollution, negative effects on non-target organisms and health hazards, [3].

Several essential oils of botanical origin have been reported for their repellant, toxic and developmental inhibitory activities.

From theses essential natural oil products*;* Oil of clove, also known as clove oil, is an essential oil extracted from the clove plant, *Syzygium aromaticum* (Clove) belongs to the Myrtaceae family which considers as an important medicinal plant with wide range of biological activities such as anti-bacterial or anti-oxidant activities [4,5]. The essential oils obtained from some other aromatic plants showed toxic effects on many insects [6,7].

Pinus brutia, common name; Turpentine oil, Family**:** Pinacea: the essential oils obtained by steam distillation from plant naturally grown. Analysis of turpentines showed that alpha- and beta-pinene are the only major components in essential oil [8].

Under the laboratory condition, many researchers used mineral and natural oils products as an insecticide for some pests; against eggs and newly hatched larvae of the *P. gossypiella* or *Earias insulana* and *Spodoptera littoralis* in laboratory and field experiments [9,10] mentioned that jojoba oil proved efficiency against the 2^{nd} and 4^{th} instars larvae of *S. littoralis* after 24 hrs of treatment.

In additionally; many studies on plant essential oils (Eo) were screen for their effects on some metabolic, physiological and behavioral functions of some insects. They also have fumigant high toxicity and repellent activity against a variety of Lepidoptera insects **[**11].

The effect of oils on their relation sheep between some biological aspects and physiology of digestive were studied by many authors' [12, 13, 14, 10, 15] Also, the inhibition of carbohydrates hydrolyzing enzymes , free amino acid, total protein and lipids and subsequently their latent effects on the insect growth and biology high affected [16, 17].

The experiment amid to knowledge of information the toxicity and adverse effects of natural oil products on some biological aspects and physiology of digestive effect on *P. gossypiella*.

2. MATERIAL AND METHODS

2.1 Insect Used

The target insect: eggs (1-3 day old) of pink bollworm, *P. gossypiella* used in this experiments was obtained from the laboratory colony of Bollworm Department, Plant Protection Research Institute; Agriculture Research Center. This work was carried out under the controlled conditions; 25±1°C and relative humidity 65±5% R.H. It reared on semi-artificial diet for several generations described by Amer [18].

2.2 Oil used for the Investigated

Family: Myrtaceae
English name: Clove oil
Scientific name: *Syzygium aromaticum*

Clove oil is a mixture of different compounds, with the three main active ingredients: being eugenol, eugenyl acetate and caryophyllene.

Analysis of Syzygium aromaticum found the 23 constituents, with eugenol (76.8 %), followed by β-caryophyllene (17.4 %), α-humulene (2.1 %), and eugenyl acetate (1.2 %) as the main components. [19] reported eugenol (89.6 %), β-caryophyllene (8.6 %) and eugenol acetate (1.7 %) Jirovetz et al. [20].

- Family: Pinacea: 2
English name: Turpentine oil

Scientific name: Pinus brutia Ten.

Analysis of turpentines showed that alpha- and beta-pinene are the only major components in essential oil (turpentine) and it major was determined. Gas chromatography-mass spectroscopy analysis [6].

2.3 Procedure

2.3.1 Prepared syzygium and turpentine oils concentrations

To study the ovicidal activity of Syzygium and Turpentine oils against 1-3 days egg of *P. gossypiella*, Serial concentrations in water were prepared. Five concentrations (25, 12.5, 6.25, 3.125 and 1.562%) for **Syzygium and Turpentine oils** were freshly prepared for the stock solution of each compound (100 ml liter water).

2.3.1 Ovicidal effect of the tested Turpentine and Syzygium oils on the eggs

Dipping technique was used as a method of application in the present work. The eggs age of *P. gossypiella* used from 1- 3 days old , treatment of eggs was done by dipping a piece of paper containing eggs on the different concentrations of each essential oil. Three replicates from eggs were used, each replicate (100 eggs on paper) was dipped in each concentration of each oil. After that the papers were left until dried. In additional; other three replicates of 1-3 old day egg were dipped in water and left as control. Then, the treated and control eggs were kept in an incubator under constant conditions 25±1°C and 75±5% R.H. The eggs were observed daily until hatching, the percentages of hatchability were estimated after 4-7 days for each treatment. Data were corrected and LC_{50s} of Syzygium and Turpentine were calculated according to [21] by using proban software.

2.3.2 Some biological aspects for immature and mature stages resulted from eggs treated

For some biological aspects studies; newly hatched larvae resulted from treated eggs with LC_{50} of Syzygium or Turpentine oils were transferred individually to the untreated diet by camel hair brush. Three replicates use each replicate 40 tubes, each tube (2 cm X 7.5 cm) containing 4 gm of diet were used [9].

The same was done with the newly hatched larvae resulted from untreated eggs. All tubes were capped with cotton, and kept in an incubator in laboratory at 25 ±1 °C and 65-5% RH and inspected every two days until pupation. Pupae formed from each treatment were removed and placed in clean tubes till adults' emergence. Some biological aspects larval, pupal stages and life cycle duration, percentage of adult emergence, malformation adults and sex ratio were estimated.

The newly emerged moths resulted from larvae hatched from eggs treated by LC_{50s} of Syzygium or Turpentine oils were sexed and transferred to chimney glass cage (five pairs /cage). Each treatment was replicated three times. The adults were fed on 20% sucrose solution. All cages kept under the previous conditions in an incubator in laboratory at 25 ±1 °C and 65-5% RH. It were examined daily to record pre ovipostion, ovipostion and post ovipostion times and the total numbers of eggs laid per day and percentage of hatchability. In additional; estimated the females and males longevity for each treatment

2.4 Statistical Analysis

All biological data obtained in the present studies were subjected to data analysis by standard errors and using statistically analyzed with one – way analysis of variance (ANOVA; $P < 0.05$ %) [22] and Duncans multiple range test of means [23] were used.

2.5 Biochemical Assay

2.5.1 Preparation of samples for biochemical assay

Larvae samples (14 days old) of *P. gossypiella* resulted from eggs dipping in LC_{50} of Syzygium or Turpentine oils tested were collected to study the adverse impact of two tested natural oils on biochemical assay. At the same time, anther sample were collected from control. These larvae samples were homogenized in distilled water using a Teflon homogenizer. it were centrifuge at 5000 r. p. min. at $5C^0$ in refrigerated centrifuge, and kept in deep freezer at $-20C^0$ till use for biochemical assays. All samples of larvae were analyzed bio-chemically in Physiological Dept. of plant Protection Researches Institute.

2.5.2 Technique of biochemical analysis

The colorimetric determination of total soluble protein, lipid and carbohydrate in total homogenate larvae was carried out, as described by Bradford [24]. Free amino acid and Phenol- oxidase activity was determined according to modification of [25].

3. RESULT AND DISCUSSION

The numbers of *P. gossypiella* larvae completed the embryogenesis for hatchability (egg completed incubation to hatchability) were decreased with the increase the concentration from two oils.

The most efficacy of tested oils was recorded in case of Turpentine followed by Syzygium oil against *P. gossypiella* eggs. The number of hatchability were (17, 44, 63 and 84 eggs) in case of Turpentine and increased to (21, 46, 76 and 91 eggs) in case of Clove oil at (25, 12.5, 6.25 and 3.125 %) concentrations, respectively. The two natural products oils (Syzygium and Turpentine) showed ovicidal activity at the high concentrations applied (25 and 12.5).

Toxicity of two oils against the eggs of the pink bollworm from 1-3 days old.

The corresponding $LC_{25\ \&50}$ values were 4.398 and 9.38% when *P. gossypiella* eggs dipped in Turpentine, respectively, and it increased to 6.041 and 11.572 when eggs dipped in **Syzygium** oil, respectively. the corresponding LC_{95} values were 56.46 and 49.58%, when *P. gossypiella* eggs dipping in **Syzygium and Turpentine,** respectively.

Effect of two potential oils on different stages of pink bollworm resulted from eggs treated.

3.1 Iincubation Period

Data presented in Table 3 recorded that the increase in *the time required for* completion of *P. gossypiella* embryogenesis for hatchability, *it were 5.8 and 6.7 days/ egg when eggs dipping in LC_{50} values for* Syzygium and Turpentine, respectively, compared *with 3.6 days in control.*

3.2 Larval and Pupal Durations

According to the Data in Table 3 illustrated obvious increase in developmental larval times for two oils, as it recorded 19.0 days of resulted from treated eggs with LC_{50} of Syzygium, while it increased to 20.0 days when treated with Turpentine oil, compared with 14.6 days in the untreated. In addition, the average of pupal duration increased to 9.3 and 10.2 days for the previous compounds compared to 7.3 days in control.

The obtained data presented in Table 3 indicate an increase in the times required for total immature stages, it reach to 28.2 and 30.2 days when different stages resulted from treated eggs with LC_{50} of Syzygium and Turpentine oils, respectively, compared with 21.9 in the untreated check.

Table 1. Effect of two potential oils on number hatchability of the pink bollworm eggs treated under laboratory conditions

Tested eggs		Con.	Initial number of eggs	Time of hatchability				Total hatchability	%hatchability Reduction
				4 (days)	5 (days)	6 (days)	7 (days)		
		25	100	0	7	10	0	17	83
		12.5	100	0	15	20	9	44	56
		6.25	100	0	25	30	8	63	37
1-3 days	**Clove**	3.125	100	11	39	34	0	84	16
		25	100	0	0	71	4	21	79
		12.5	100	5	13	18	10	46	54
		6.25	100	14	40	22	0	76	24
		3.125	100	30	44	17	0	91	9
Control		0.0	100	61	33	0	0	94	6.00

Table 2. Toxicological evaluation of Syzygium and Turpentine against eggs of pink bollworm under laboratory conditions

Ages of eggs	Compounds used	Toxicity: 95% Confidence limits			
		LC_{25} (%)	LC_{50} (%)	LC_{95} (%)	Slope± SE
P. gossypiella	**Syzygium**	6.041	11.572	56.46	.2.389
1-3 days' old eggs	**Turpentine**	4.398	9.38	49.58	2.04

Table 3. immature stages deterrent effect of tested oils against pink bollworm under laboratory conditions

Natural oil used		Conc. (%)	Duration immature stags (days+ SE)			Total immature stage (days+ SE)	Life cycle (days+ SE)
			Eggs	Larvae	Pupae		
P.	**Syzygium**	11.572	**5.8±0.10** [b]	19.0±1.2b	9..3.±0.3a	28.3±0.8a	34.1±1.6
	Turpentine	9.38	**6.7±0.2a**	20.0±1.4a	10.2± 0.2a	30.2±1.3a	36.9±2.4
	Control	**0.0**	**3.6**± 0.3**c**	14.6± 0.7c	7.3± 0.5b	21.9± 1.4b	26.2± 1.5
LSD			**0.446**	3.419	1.120	2.683	2.636
F			.152.6	8.450	21.141	31.522	60.923
P			0.0001	0.0017	0.0019	0.0007	0.0001

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's Multiple range tests, $P < 0.05$)

Table 4. Effect of tested two oils against pink bollworm malformed and mortality of different stages under laboratory conditions

Compounds used		No. of initial used	Malformed of immature stags			% of emergence adults	Sex ration as female
			% Accumulated Larvae mortality	Malformed % Larvae	% Total malformed and dead pupae		
P. gossypiella	**Syzygium**	**140**	**31.4**	12.5	19	61	0.4
	Turpentine	**140**	**49.28**	18.36	14	56	0.3
	Control	**100**	**7**	2	1	94	0.54

3.3 Life Cycle

Data in Table 3 proved that the life cycle time (= estimated from eggs to adult emergence) resulted from eggs treated with LC_{50} of Syzygium and Turpentine oils. Significant affected were found between treated and untreated, as it was elongated to 34.1 and 36.9 days, respectively, compared with 26.2 days in control.

3.4 Effect of two Tested Oils on Mortality and Malformation of Pink Bollworm Resulted from Eggs Treated under Laboratory Conditions

Data in Table 4 clear that the two tested oils high increased the accumulated mortality and malformation of larval or pupal stage resulted from eggs treated with **Lc**$_{50}$ under laboratory conditions than that of untreated. The percentage of accumulated larvae mortality after hatched from treated eggs until pupation formed were 31.4 and 49.28 % for Syzygium and Turpentine oils, respectively, compared with 7.0% in untreated. Malformed recorded 12.5 and 18.36 % for Syzygium and Turpentine, respectively, compared with 2.0% in untreated. The most morphological deformed was pupal-adult intermediate resulted or the adult not completed the emergence from pupae lead to dead the adult before emergence.

Also, the percentage of adult emergence was highly significantly affected by two oils, it decreased to 61.0 and 56.0% for adults resulted from eggs treated with LC_{50} of Syzygium and Turpentine oils, respectively, compared with control (94.0%) Table, 4.

3.5 Sex Ratio

The sex ratio of *P. gossypiella* was 0.54 as female for untreated in Table 4. Sex ratio parameters were high differed according to treatment. It was 0.4 and 0.3 as female when resulted from eggs treated with Syzygium and Turpentine oils, respectively.

3.6 Ovipositional Period and Reproductive Potential

Statistical analysis of data summarized in Table 5 demonstrated that highly significant differences between pre- oviposition, oviposition period, reproductive and fertility for adults resulted from eggs treated with Syzygium and Turpentine, respectively comparative with control. The respective, average number of eggs deposited (fecundity) by females were 107 and 97 eggs laid at 11.1 and 7.6 days oviposition/ female resulted from Syzygium and Turpentine, respectively, compared with 226.0 eggs/ female in control deposited at 11.6 days. Hatchability percentages, also were high affected by the Syzygium and Turpentine oils treatments. The results shows those post - ovipostion periods were significantly influence from 0.7 to reached 3.2 days . respectively, compared with 2.1 days in control.

The longevity of adult female increased when resulted from Syzygium , longevities of females estimated by 18.4 days/ female, and the Longevities for males recorded 14.3 days. In contrary, longevity of adult female and male high decreased when resulted from egg treated with turpentine, it were 12.4 and 9.3 days for female and male**,** respectively, compared with16.0 and 13.6 days in control Table,5.

This data in Table 5 indicated that latent toxic effect in biochemical parameters larvae resulted from eggs treated with previous two essential oil Syzygium and Turpentine. It caused high decreased in the level of total protein to 15.0 and 8.6 (total protein (mg/g.b.wt. /larvae resulted), respectively compared with17.0 mg/g.b.wt. /larvae in control, with increased free-amino acid to 137.3 and 298.0 (µg D, L-alanine/g.b.wt) respectively, compared with 118.0 (µg D, L- alanine/g.b.wt) in control. In additional, the two essential oil Syzygium and Turpentine cussed high increased in total lipid, it was 27.9 and 22.0 mg/g.b.wt. /larvae resulted), respectively, compared with16.9 mg/g.b.wt. /larvae in control. While, two essential oil Syzygium and Turpentine decreased the Phenoloxidase, it estimated by 14.8 and 13.5 (O.D. units/g.b.wt) respectively compared with 26.0 Phenoloxidase (O.D. units/g.b.wt) in control. Additionally, the present study clearly that the total protein and Phenoloxidase appeared as the most affected with high level of reduction with turpentine. These percentages were - 49.4 total protein (mg/g.b.wt. /larvae resulted) and - 48.1, respectively (Table, 6).

4. DISCUSSION

The results of these experiments indicate that the two natural oils are high ovicidal activity toxic. The data recorded that eggs high susceptibility to Turpentine than Syzygium: the LC_{50} for Syzygium and Turpentine were 11.572 and 9.38, respectively. In additional; promising as a prolonged the incubation period of eggs. It significant difference in times require for completed the embryogenesis to hatchability (TH). At the same time, it cussed reduction in hatchability eggs. The results are in congruence with those of [8, 17, 26 and 27] they studied that the toxicity of essential oil Syzygium and others oils against, *Tribolium castaneum,* Aedes aegypti and other Lepidoptera insects also, [14] they recorded that the clove oil was high toxicity and repellency against *Rhyzopertha dominica, Sitophilus oryzae* and *T. castaneum*. [28]. [29,30] revealed that, the LC_{50} and LC_{90} values of pepper oil against 1st instars larvae of cotton leaf-worm recorded by 37.35 and 64.07 %. On the other hand, the newly hatched larvae of spiny bollworm after one day from treatment at concentration 70 % clear increased in percent larval mortality to 42 %. While the mortality decreased to 31 % at 60 % concentration. In case of spiny bollworm, pepper oil had the least effect of on the newly hatched larvae after one day of treatment. Also, Donald et al., (2008) recorded that, black pepper and red pepper are high toxicity and causing mortality of different insect species. [31]. [22] found that the combination of long pepper oil (*P. hispidinervum*) and clove (*Syzygium aromaticum*) oils in two concentrations with Xentari WG (Bta) a more toxic for *S. frugiperda*. Also, essential oil Clove (EOC) has recorded a larvicidal activity against field larva of *Ae. egypti* with LC_{50} of 92.56 and 62.3ppm in two different reports [8].

In additional; the present studies recorded that the two oils Syzygium and Turpentine cussed a significant prolonging of the time required for larvae development, which may be; due to, adverse effect as ante-feeding and repellent for larvae, it led to high effect on the metabolism and some biological parameter and Physical activity larvae; these appearance in latent effect on elongated development of pupae, life cycle for *P. gossypiella* among the Turpentine followed by Syzygium. *It* may be reflecting for their different chemical compositions in content the two essential oils. Thus a very careful study is needed to select the optimum combinations for further development. The results are in congruence with those of [13, 14, 20,32, 33] recorded that the ovicidal effect of essential oil Syzygium is probably the major factor in the suppression of eggs hatchability and the development of immature stages and adults resulted from treated eggs, also, 14. [10] recorded that the Syzygium oil were very toxicity and repellency against *Rhyzopertha dominica, Sitophilus oryzae* and *Tribolium castaneum*. Also, suggesting that the activity of clove oil was solely due to this major compound. Also, [23,24] they recorded that the Proclaim and mineral oil caused high effect on the metabolism of *P. gossypiella*, it lead to affect the growth, development for different stages of insect.

Table 5. Effect of two natural oils on fecundity and fertility of the pink bollworm adult resulted from eggs treated under laboratory conditions

Natural oil used		Ovipositional period (in days)			Longevity -(in days)		Total eggs laid	Fecundity % hatch
		Pre- oviposition	Oviposition	Post- oviposition	Female	Male		
P. gossypiella	**Syzygium**	**3.9±0.10b**	**11.1±0.6b**	3.2±**0.1**a	18.4±**1.2**a	14.3±**0.7**a	**107.0±9.6**	68
	Turpentine	**4.1±0.3a**	**7.6±0.2c**	0.7±**03**c	12.4±**0.1c0**	9.3±**0.3b**	**97.0±8.1**	77
	Control	**2.3±0.10c**	**11.6±0.5a**	2.1±**0.1**b	16.0±**1.2b**	13.6±**0.5**a	**226.0±11.5**	98
SLD		**0.103**	0.362	0.121	1.030	1.613	6.214	3.240
F		114	163	106	6.841	10.335	109.0	27.3
P		0.0001	0.0017	0.000	0.000	0.001	0.000	0.00

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's Multiple range tests, P < 0.05).

Table 6. Biochemical parameters in larvae resulted from eggs treated with Syzygium and Turpentine

Biochemical aspects	Total protein (mg/g. b. w. t		Free-amino acid (µg D,L-alanine/g. b. wt		Total lipid (mg/g .b. wt		Phenol oxidase (O.D. units/g. b. w	
	Treatment	**decrease (-)or increased(+)%**	**Treatment**	**Decrease (-) or increased(+)%**	**Treatment**	**increased(** by times	**Treatment**	**decrease(-)**
Syzygium	**15.0±1.10 b**	**-11.76**	**137.3±10.99b**	+	**27.9±0.21 a**	**1.65**	**14.8±0.9 b**	- 43.07
Turpentine	**8.60±4.1 c**	**-49.4**	**298.0±13.2 a**	+	**22.0±16.25 b**	**1.302**	**13.5±4.9 b**	- 48.1
Control	**17. 0±0.28 a**	**0.0**	**118.0±2.3 c**	**0.0**	**16.9±0.5 c**	**00**	**26.0±3.2 a**	0.0
LSD	**0.133**	**…**	**7.421**		**2.531**	**..**	**1.404**	..
P	**o.oo1**	**---**	**0.0003**		**0.001**	**…**	**0.21**	

Also, the results clear that the differences in relative performance of the two oils in the toxicity or adverse effect on mortality and malformed may be indicating likely differences in the nature of the chemicals responsible for the arrest we observed. In addition, the latent effect of two tested oils have an appeared on the adult performance and reproductive potential resulted from treatment of 1-3 day old eggs after dipping in LC_{50} value of Syzygium and Turpentine. Generally, results indicated that all treatments decrease adult emergence, reduction in longevity and fecundity and eggs hatchability.

All these results agree with [7,25,9, 23] they revealed that the effects of mineral and natural oil against eggs and newly hatched larvae of Pink bollworm and cotton leaf-worm, it cussed reduction in hatchability eggs with high mortality larvae after egg hatchability with increased in developmental stages. Also, [25] who found that the essential jojoba oil high toxicity and ant - feeding effect, it lead to development inhibitor. [8, 10,7,14, 27, 34,35] studied that the Larvicidal efficacies and chemical composition of essential oils of *Syzygium aromaticum* against mosquitoes. It high increased the time of developmental stages.

The present physical study for *P. gossypiella* clearly that; the protein, free amino acid and lipids are a very important in body insect for released the energy required to development the different stages.

These results are in congruence with those of [14, 15,25,28,11,24,29,34,35,36,37] they recorded that the inactivation of digestive enzymes with reduction in total protein or lipids results physical analyzed leading to poor nutrient utilization, development for different immature stages for insects , because all these are of vital importance to insects' growth, where they require for energy growth, longevity, and reproduction.

In addition, [6, 3,19,20,32,36,38,37,39] mentioned the combination of pepper oil, Piper *hispidinervum* and clove, *Syzygium aromaticum* essential oils considerable a more effective control of *S. frugiperda*. The both oil high effected on biological and reproductive parameters, in additional, changes in the levels of phenoloxidase and nitric oxide in the hemolymph of *S. frugiperda*.

5. CONCLUSION

The two essential oils of **Syzygium and Turpentine** can be involved in a promising alternative for the integrated management program in controlling the pest, to reduce the population of *P. gossypiella* in the fields. Because it caused high reduction in hatchability of eggs with latent effect on different immature stages, with the adverse effects on decrease for the amount of eggs laid by females' resulted and high reduction of hatchability. In additional, it play very important role in change physical body insect for released the energy required to development growth, longevity, reproduction and fertility in all stages. It lead to reduction in population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ingram WR. *Pectinophora. Gossypiella* (Lepidoptera: Gelechiidae) In Insect pests of cotton". Edited by G.A. Matiews and JP Tunstall, Wallngford CAB. 1994;107- 149.
2. Kandil Mervat AA. Studies on the predaceous and parasitic insects on the pink and spiny bollworm. Ph.D. Thesis, Faculty of Agriculture, Benha Branch, Zagazig University; 2001.
3. Sharma VK, Savalia CV, Selvam DT and Darekar SD. Seroprevalence of caprine and ovine brucellosis in mehsana and patan districts of Gujara INTAS., 2006; POLIVET. 2006; 7(ll):316-3'18
4. Thonggoom O, Punrattanasin N, Srisawang N, Promawan N, Thonggoom R. In vitro controlled release of clove essential oil in self-assembly of amphiphilic polyethylene glycol-block-polycapro-lactone. J Microencapsul. 2016;33(3):239–248. [PubMed] [Google Scholar]
5. Sebaaly C, Charcosset C, Stainmesse S, Fessi S, and Greige G H. Clove essential oil-in-cyclodextrin-in-liposomes in the aqueous and lyophilized states: From laboratory to large scale using a membrane contactor. Carbohydr Polym. 2016;138:75–85. [PubMed] [Google Scholar].
6. Tripathi AK, Prajapati V, Aggarwal KK, Khanuja SBS and Kumar S. Repellency and toxicity of oil from Artemisia annual to certain stored product beetles. J. Econ. Entomol. 2000; 93(1): 43-47.
7. Choi WL, Lee SG, Park HM and Ahn YJ. Toxicity of plant essential oil to *Tetranychus urticae* (Acari: Tetranchidae) and *Phytoseiulus persimilis* (Acari: phytoseiidae). J. Econ. Entomol. 2004;97:553-558.
8. Alejandro LP, Audno G and Masur H. Larvicidal effect of Eucalyptus Grandis

essential oil and turpentine and their major components on *Aedes egypti* larvae Journal of the American Mosquito Control Association. 2007; 23-3:299-303

9. Rofail, MM, Nada AM, El-Sisi G and Rashad AM. Time of spraying some natural oils as a limiting factor for controlling cotton bollworm, *Pectinophora gossypiella* (Saunders). Egypt. J. Agric. Res. 2000;78:4- 1499-1507.
10. Gaaboub A, El-Kady HA, El-Khayat EF, El-Shewy, AM. Biochemical and histological effect of some plant extracts, insecticide (methomyl) and bio insecticide (protecto) against cotton leafworm, *Spodoptera littoralis* (Boisd.). 1st International Conference On Biotechnology Applications In Agriculture. Benha University, Moshtohor and Hurghada, 2012 18-22, February Egypt; 2012.
11. Fayemiwo K A, Adeleke MA, Okoro OP, Awojide SH and Awoniyi IO. Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. Asian Pac J Trop Biomed. 2014; -1-30–34. [PMC free article] [PubMed] [Google Scholar]
12. El-Barky NM, Dah HF and El-Sayed, YA. Toxicological evaluation and biochemical impacts for radient as a new generation of spinosyn of Spodoptera littoralis (Boisd.), larvae. Egypt. Acad. J. Biolog. Sci. 2008;1(2): 85-97.
13. Zhang S, Xiao-zhen YE. Impacts of chemical insecticides on extracellular protease and chitinase activities of *Metarhizium anisopliae*. J. Fujian College Forest. 2010;4:289-292.
14. Zeng L, Lao CZ, Cen VG, Zeng and liang GW. Study on the insecticidal activity compounds of the essential oil from Syzygium aromaticum against stored grain insect pests.: 2010; Proceedings of the 10th International Working Conference on Stored Product Protection
15. Assar AA; Abo El-Mahasen MM, Dahi HF and Amin HS. Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Journal of Bioscience and Applied Research. 2016;2(8): 587-594.
16. Mohammad M, Izadi H. Cooling rate and starvation affect supercooling point and cold tolerance of the Khapra beetle, *Trogoderma granarium* everts fourth instar larvae (Coleoptera: Dermestidae). J. Therm. Biol. 2018;71: 24–31.
17. Kandil Mervat AA, EL-Shennawy RA, Ahmed DA. Arbohydrate eydrolyzing enzymes as a target for Pink Bollworm *Pectinophora gossypiella* (Saund.) Control agents. J. of Mansoura; 2020.
18. Amer AEA. Economic artificial diets for rearing spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae). J. Plant Prot. and Path., Mansoura Univ. 2015;6(3):527 –534.
19. Santin GR, Marivane L, Luiz CKJ. Gastroprotective activity of essential oil of the Syzygium aromaticum and its major component eugenol in different animal models. Naunyn-Schmied Arch Pharmacol. 2011;383: 149–158.
20. Jirovetz L, Buchbaue Gr, Stoilova I, Stoyanova S, Krastanov A, Erich S. Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem. 2006; 23;54 (17):6303-7.
21. Finney DJ. Probit analysis. A Statistical Treatment of the Sigmoid Response Curve. 7th Ed. 1971; Cambridge University Press, Cambridge.
22. Snedecor GW. Statistical methods 5th Ed, Iowa State Col.N.Y.; 1952.
23. Duncan DB. Multiple range and multiple F test. Biometrics. 1955;11: 1-42.
24. Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. Anal.Biochem.1976;72: 248-254.
25. Ishaaya, I. Observation on the phenoloxidase system in the armored scale *Aonidiella aurantii* and *Chrysomphalus aonidum.* Comp. Biochem. *Physiol.* 1971; 39 B:935-943.
26. Hosseini-Naveh H, Bandan AR, Azmayeshfard P, Hosseinkhan S, Kazzazi M. Digestive proteolytic and amylolytic aactivities in *Trogoderma granarium* everts (Dermestidae: Coleoptera). J. Stored Prod. Res. 2007;43(4): 515–522
27. Cruz GS, Wanderley TV, Oliveira V, Correia AA, Breda MO, Alves TGS, Cunha FM, Teixeira AAC, Dutra KA, Navarro DMAF. Bioactivity of *Piper hispidinervum* (Piperales: Piperaceae) *and Syzygium aromaticum* (Myrtales: Myrtaceae) oils, with or without formulated Bta on the Biology and Immunology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Econ. Entomol. 2014;107 - 1:144-153.
28. El-Mesallamy AMD, Raslan SA, Nagar ME, El-Medany WAZ. Toxicity and latent effect of pepper plant essential oil (*Capsicum annum*) on Some Cotton Pests. Middle East Journal of Applied Sciences. 2015;2077-4613-5;1184-1191.

29. Ahmed DA. Comparative studies, under laboratory conditions, of four selected insecticides on Pink Bollworm, *Pectinophora gossypiella* (Saund.). Egypt. Acad. J. Biolog. Sci. (F. Toxicology and Pest control). 2020; 12(2):47- 61.
30. Kandil MA, Fouad EA, Hefny DE and Abdel-Mobdy YE. Toxicity of fipronil and emamectin benzoate and their mixtures against cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) with relation to GABA content. Journal of Economic Entomology. 2020;113: 385–389.
31. Yacoub SS. Efficacy of some plant extracts in controlling *Sesamia cretica* Led. and *Ostrinia nubilalis* (Hubn.) in maize fields. Ph.D. Thesis, Fac. Agric., Benha Univ. 2006;289.
32. Mina M, and Khalequzza M. Toxicity of essential oils against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). bio- sci. 2007; 17: 57.
33. Mina M, and Khalequzza M. Ovicidal activity of essential oils against red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. bio- sci. 2009;17:57-62, 2
34. Żółtowska K, Lipiński Z, Łopieńska-Biernat E, Farjan M and Dmitryjuk M. The activity of carbohydrate-degrading enzymes in the development of brood and newly emerged workers and drones of the Carniolan Honeybee, *Apis mellifera* carnica. J. Insect Sci. 2012;12(1):Article 22. Available:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3471771/.
35. kandil, Mervat AA, Ahmed F and Moustafa Hemat Z. Toxicological and biochemical studies of lufenuron, chlorfluazuron and chromafenozide against *Pectinophora gossypiella* (*Saunders*). Egypt. Acad. J. Biolog. Sci. 2012;1: 37- 47.
36. Kandil Mervat AA, Salem MS and Adly AM. Biological and biochemical changes in pink bollworm, *Pectinophora gossypiella* after treatment with Hexaflumuron and Chlorfluazuron. Annals of Agric. Sci. Moshtohor. 2013;51-4- 427- 432.
37. Kanat M and Alma MH. Insecticidal effects of essential oils from various plants against larvae of pine processionary moth (Thaumetopoea pityocampa Schiff) (Lepidoptera: Thaumetopoeidae). Pest Management, Sci., 2004;60(2):173-177.
38. Costat Statistical Software. Microcomputer program analysis version. CoHort Software, Monterey, California. 1990;6:311.
39. Magda S and Shadia A. Roll of three essential oils and their Nano against *Ephestia cautella* (Lepidoptera-Pyralidae) under laboratory and store conditions /International Journal of Pharm Tech Research. 2016;9(10):194-200.