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Assessment the effect of *Thymus vulgaris* (Lamiales: Lamiaceae) on biology of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae)

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Abstract
The insecticidal toxicity of *Thymus vulgaris* against *Pectinophora gossypiella* newly hatched larvae and its predator; the 2nd instar larvae of *Chrysoperla carnea* were evaluated under laboratory conditions. The LC_{50} values for *P. gossypiella* treated newly hatched larvae with *T. vulgaris* extract was 1.86 (g/100g diet) after ten days of treatment, While, the LC_{50} value was 626.97 (g/100g diet) for the 2nd instar larvae of *C. carnea* after seven days of treatment with *T. vulgaris* extract. The extract treatment elongated the larval and pupal period compared with control with a 92.31% adult emergence. Whereas, the total immature stages of *C. carnea* was 18.63 days after treatment compared with 16.1 days for the control with 75% adult emergence.

Keywords: toxicity, *Thymus vulgaris*, Biology, *Pectinophora gossypiella*, *Chrysoperla carnea*

Introduction
The pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the destructive cotton insects and a key pest and an economically important insect pest in many of major cotton growing countries. Determine the best time for application of any type of control program against *P. gossypiella*, that minimize cost of control and preserve cotton bolls from infestation and achieved greatest economic of yield production with a great protect of environmental system (El-Lebody *et al*. 2015) [8].
Green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), is a polyphagous predator has a high predatory capacity and adaptation to different ecosystems (Souza and Carvalho 2002; Costa *et al*. 2010) [18, 4] and prefer the eggs and the early instar larvae of *P. gossypiella* (Orphanides *et al*. 1971). According to the importance of *C. Carnea* as a biological control agent of *P. gossypiella* (Moustafa 2016 and Moustafa *et al*. 2019) [8], it is important to study the toxicity of different type of insecticides on both of them.
Botanical insecticides have received more attention because of their low human toxicity, their risk assessment against non-target organisms is necessary, rapid degradation, and reduced environmental impact revealed an important tool in pest management programs. The plant families, particularly; Meliaceae, Rutaceae, Labiateae, Asteraceae and Pedaliaceae are promising sources of the plant-based insecticides (Schmutterer 1990; Isman 2000) [17, 9].
Thymus vulgaris is a flowering plant in the mint family Lamiaceae, order Lamiales. It is growing up to 15-30 cm tall by 40 cm wide (Christopher Brickell 2008). The genus *Thymus* is an aromatic plant that includes about 215 species particularly in the Mediterranean area (Morales R. 2002) [14].
In view of the importance of botanical insecticides this study was aimed to evaluate the toxic effect of *Thymus vulgaris* against *P. gossypiella* and its predator *C. carnea*.

Material and Methods
Plant material
The leaves of the plant (*Thymus vulgaris*) were collected from Supermarket in Khulias, Jeddah, Kingdom of Saudi Arabia, in February 2019.

Preparation of extracts
The dry leaves of *T. vulgaris* (100 g) were grounded and then macerated in 200 ml of 70% aqueous ethanol solution. After leaving the solution 7days, it was filtered through Whatman No. 40 filter paper. The solvent was removed under reduced pressure using a rotary evaporator to obtain 16.12 g of extract.

Insect used
The newly hatched larvae of pink bollworm *Pectinophora gossypiella* (Saund.) used in this study were obtained from a standard laboratory colony reared in Bollworm Department, Plant Protection Research Institute; Agriculture Research Center (ARC), Giza, Egypt, on an artificial diet for several generations away from any insecticidal contamination, that described by Abd-El Hafez *et al*., (1982).
The larvae of *C. carnea* used in this experiment were reared in Trichogramma mass production unit, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt, and reared for one generation at bollworms department, Plant Protection Research Institute on *P. gossypiella* eggs for using in the experiment.

Toxicity assay
The toxicity of the different dilutions of the tested plant extract against the newly hatched larvae of *P. gossypiella* and *C. carnea* 2nd instar larvae were studied. For obtaining the LC_{50}, serial concentrations of 10, 5, 2.5, 1.25 and 0.625 (gm extract dilution/ 100 ml distilled water) were prepared then mix 1 ml from each concentration with diet.

Fifty newly hatched larvae of *P. gossypiella* were transferred individually to the surface of the treated diet, after 48 hours, the live larvae were transferred on untreated diet and kept in glass tubes (2 x 7.5 cm). And dip egg cards of *P. gossypiella* with each concentration and prepare thirty 2nd instar larvae *C. carnea* for exposure to the treated eggs. For the control test untreated diet was used. All tubes were capped with cotton stopper and incubated at 26±2 ºc and 70-85% RH and inspected daily until pupation.

Mortality was recorded at intervals after 2, 5, 7 and 10 days after larval treatments. Mortality percentages were corrected according to Abbott's formula (1925) in each case. Larval, pupal durations, weights and adult emergence were determined. The values of LC_{50} were calculated to investigate the effect of *T. vulgaris* extract on the developmental stages of *P. gossypiella* larval and pupal duration (immature stages).

Statistical analysis was calculated by ANOVA and Duncan's multiple range tests (ANOVA of arcsine square root transformed percentages), Duncan (1955).

Results and Disscusion

The toxicity of the plant extract of *T. vulgaris* on *P. gossypiella* newly hatched larvae and the 2nd instar larvae of its predator *C. carnea* was presented in Tables (1 and 2).

Table 1: Toxicity of *T. vulgaris* against newly hatched larvae of *P. gossypiella*

Treatment	Corrected mortality (%)			
	2 days	5 days	7 days	10 days
Control	0.00	0.00	0.00	0.00
10.00 [gm/ 100 ml D.W.]	63.5[a]	67.94[a]	68.86[a]	69.21[a]
5.00 [gm/ 100 ml D.W.]	51.28[b]	57.18[b]	59.14[b]	59.19[b]
2.50 [gm/ 100 ml D.W.]	38.94[c]	44.74[c]	49.94[c]	52.73[c]
1.25 [gm/ 100 ml D.W.]	27.63[d]	32.80[d]	40.75[d]	46.30[d]
0.625 [gm/ 100 ml D.W.]	18.23[e]	22.41[e]	32.04[e]	39.97[e]
LC_{50}	4.65	3.35	2.51	1.86
Slope	1.04±0.26	1.04±0.26	0.77±0.25	0.53±0.24
F	108.90***	545.6***	657.5***	495.5***
LSD	0.55	0.79	1.81	1.57

Values in the column are all significantly different from each other (ANOVA, Duncan's multiple range test, $P < 0.05$).

The LC_{50} values for *P. gossypiella* newly hatched larvae treated with *T. vulgaris* extract were 4.65, 3.35, 2.51, 1.86 (gm/100 ml D.W.) after two, five, seven and ten days of the treatment respectively, table (1). While, LC_{50} values was 626.97 after treatment with *T. vulgaris* extract after two, five and seven days after treatment of *C. carnea* 2nd instar larvae as shown in table (2).

Table 2: Toxicity of *T. vulgaris* against *C. carnea* 2nd instar larvae

Treatment	Corrected mortality (%)		
	2 days	5 days	7 days
Control	0.00	0.00	0.00
10.00 [ml/ 10 ml D.W.]	22.45	22.45	22.45
5.00 [ml/ 10 ml D.W.]	18.84	18.84	18.84
2.50 [ml/ 10 ml D.W.]	15.61	15.61	15.61
1.25 [ml/ 10 ml D.W.]	12.77	12.77	12.77
0.625 [ml/ 10 ml D.W.]	10.31	10.31	10.31
LC_{50}	626.97	626.97	626.97
Slope	0.43±0.51	0.43±0.51	0.43±0.51
F	48.67***	48.67***	48.67***
LSD	2.18	2.18	2.18

The results in Table (3) showed the latent effect on larval, pupal period and total immature stage, for the newly hatched larvae of *P. gossypiella* treated with the LC_{50} value of *T. vulgaris* extract compared with the control.

Table 3: Effect of LC_{50} concentration of *T. vulgris* extract on some biological parameters of *P. gossypiella*.

Treatments	Larvae stage	Pupal period		Total Immature stages	% Adult Emergence
	Larval duration (days) (Mean ±SE)	% Pupation	Pupal duration (days) (Mean ±SE)		
2.50 [ml/ 10 ml D.W.]	21.08[a]	43.33	8.27[a]	29.35[a]	92.31[a]
Control	16.5[b]	100	7.17[a]	23.67[b]	100[b]
F value	60.84**	80.55***	1.94[ns]	44.17**	192***
LSD	2.02	17.63	1.73	2.95	1.60

Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, $P < 0.05$)

Generally, the extract was significantly prolonged the duration of larval stage. These periods were estimated by 21.08 days/larvae compared with 16.5 days in control. Also, the used extract caused little or non-significant changes in pupal period, by 8.27 days/ pupa, compared to that of the control, 7.17 days. The percentage of the pupation was 43.33% in the treatment compared with 100% in the control. The total immature stage of *P. gossypiella* was 29.35 days,

compared with 23.67 days for the control. The adult emergence percentage was 92.31% after the treatment compared with 100% in the control.

Data in Table (4) showed the latent effect on larval and pupal period of the 2nd instar larvae of *C. carnea* after the treatment with the concentration of 10gm/ 100 ml D.W. of *T. vulgaris* extract compared with the control. The duration of larval stage for the 2nd and 3rd instar larvae was 10.00 days after treatment with *T. vulgaris* extract compared to 8.20 days for the control. Also, cocoon duration after extract treatment was 8.63days/cocoon, compared to 7.90 days in the control, with the percentage of cocoon formation (pupation) by 70.00%. The total immature stage of *P. gossypiella* was 18.63 days, compared with 16.10 days in control. On the other hand, the percentage of adult emergence was 75.00% compared with 80.00% in control.

Table 4: Effect of LC_{50} concentration of *T. vulgaris* extract on some biological parameters of *C. carnea*

Treatments	Larval duration (2nd and 3rd instars)	Pupal period			
		% Pupation (Cocoon formation)	Cocoon duration (days) (Mean ±SE)	Total Immature stages	% Adult Emergence
10.00 [ml/ 10 ml D.W.]	10.00[a]	70.00	8.63[a]	18.63[a]	75.00[a]
Control	8.20[a]	80.00	7.90[a]	16.10[b]	80.00[a]
F-value	6[ns]	1.5[ns]	3.62[ns]	35.82**	1.35[ns]
LSD	2.27	22.67	2.91	2.09	18.30

Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, $P < 0.05$)

In this respect, there are many authors that treated different insect pests with *Thymus vulgaris* plant extracts like; Klaudia *et al*. (2020) [10] determine the effect of *T. vulgaris* plant extract, the results showed that plant species had a significant influence on the choice of feeding site of *Acrobasis advenella* (Lepidoptera, Pyralidae) and lower number of eggs and larvae was observed for *T. vulgaris*. Also, Yazdani *et al* (2014) found that the *T. vulgaris* essential oils was toxic on the lesser mulberry pyralid *Glyphodes pyloalis* and affected the nutritional indices of the 4th instar larvae of *G. pyloalis* and therefore, the used essential oil concentrations may be considered as alternatives to the classic pest control agents. Khosravi and Sendi (2013) [12] found that *T. vulgaris* has possessed the greatest effect on *Xanthogaleruca luteola* beetle larval development. Also, Abdelaziz *et al.* (2012) [2] found that *T. vulgaris* leaves extract caused (95%) mortality as insecticidal effect on the larvae of *Tuta absoluta* under the laboratory conditions.

On the other hand the effect of the extract of the plant under studying on *p. gossypiella* predator *C. carnea* was investigated by some authors but in contrary with our results like, Irannejad and Samih (2012) evaluated side effects of *T. vulgaris* on the survival rate and the reproductive parameters of *C. carnea* in controlled condition. The life duration of the predator on eggs treated on *T. vulgaris* was 69 days showed that it had the lowest survival period. The minimum fecundity of the females was 231.10 eggs in *T. vulgaris*. And, Mohammad *et al.* (2012) [13] evaluated the side effects of the plant extracts of *Calotropis procera*, *Teucrium polium*, *Fumaria parviflora* and *Thymus vulgaris* on *C. carnea* and they found that *C. procera* and *T. polium* are the most suitable choices.

In conclusion, *Thymus vulgaris* extract highly toxic effect against *p. gossypiella* larvae, on contrast the extract showed little effect on the predator *C. carnea* at the different concentrations used. The LC_{50} concentration of the extract prolonged larval and pupal periods of *p. gossypiella* larvae compared with the control. *Thymus vulgaris* extract can be considered as new insecticide in the *p. gossypiella* control.

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