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Impact of extracts treatment on biological aspects of soft scale insect *Pulvinaria tenuivalvata* (Newstead)

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Abstract
The red-striped soft scale insect *Pulvinaria tenuivalvata* attacking sugarcane at Attfieh region, Giza governorate is considered an economic important insect pest on sugarcane in Egypt. Laboratory evaluation of different extracts of *Cressa cretica* on the biological aspects of *P. tenuivalvata* was studied. The obtained results revealed a wide range difference in the response of the two stages (Adult and Nymphal) to the tested compounds. It was obvious that, the nymphal stage was most susceptible tested treatments while the adult stage was the most tolerant one. Petroleum ether was the most effective compound on nymphal and adult stages of *P. tenuivalvata* while water extract was the least toxic compound. Based on the obtained results, treatments could be used in IPM program against *P. tenuivalvata*.

Keywords: *Pulvinaria tenuivalvata*, biological aspects, plant extract, control

Introduction
Sugarcane (*Saccharum officinarum* L.), family: Graminace is considered as one of the most important field crops grown not only in Egypt, but also all over the world. Sugarcane the main source of sugar production; produces 80% of the total local sugar production (1.9 million tons) [1]. However, its production has been seriously threatened by the attack by many insect pests which lead to losses in the quality and quantity of the crop. The most effective pest is the red-striped soft scale insect, *Pulvinaria tenuivalvata* (Newstead). It appeared for the first time as a new pest attacking sugar-cane plantations at Attfieh region, Giza Governorate in mid 1990s [2- 4], it spread within few years to most of sugarcane growing areas. Damage is caused by severe wilting due to sap depletion, and by sooty mold growth on honeydew excretions coating leaf surfaces; both impair photosynthesis and cause leaf dryness causing leaves fall together with a pronounced reduction in quality and quantity of the yield.

Since sugar and /or sugar-cane juice are used for human consumption, it is better to avoid using conventional synthetic chemical insecticides for the control of this serious pest, and safer methods are needed.

Among the various avenues explored, bio-insecticides of plant origin may offer a better solution of pest control, [5- 10].

The present work is, therefore, undertaken to elucidate the toxic and biological effects of plant extract (*Cressa cretica* L., Fam.: Convolvulaceae) on *P. tenuivalvata*. Such studies may play a reliable role in exploring integrated pest management programme(s) in sugar-cane fields based on certain ecological parameters.

Materials and Methods
- **Stock Culture**

A colony of the red- striped soft scale, *P. tenuivalvata* (Newstead) was established in an insectary under constant conditions of 27±2 ° C and 70±5% r.h. at Pests and Plant Protection Department, National Research Centre. This colony was started by collecting samples of highly infested sugar-cane leaves from Attfieh region, Giza governorate. The stock culture was built on healthy virginal sugar-cane plants planted in pots 30 cm in diameter and 20 cm high. Artificial infestation was achieved by transferring a suitable number of first nymphal instars (crawlers) from the field-infested samples to each pot. The potted plants were irrigated when necessary. To avoid crowding, newly hatched nymphs (i.e., crawlers) were transferred to new virgin sugarcane previously planted in pots.

Laboratory evaluation of different extracts of *Cressa cretica* on the biological aspects of *P. tenuivalvata*.

The above-ground herb of *Cressa cretica* (Fam.: Convolvulaceae) was obtained from plants was collected from east desert. The above-ground herb was dried under shade to constant weight and crushed to fine powder. Two hundred and fifty grams of the fine powder were extracted by different solvents (petroleum ether (60-80ºC), ethanol and water) with increasing polarity in Soxhlet apparatus except water according methods **[11].** After exhaustion, each solvent was evaporated under reduced pressure to 30 ° C. After removal of the solvent, the dry residue of each extract was used in bioassay tests. Three concentrations (5%, 2.5% and 1.25%) of the tested extracts were prepared by dissolving the residue in water using few drops of tween-80 as emulsifier. To study the effect of tested plant extracts on different biological aspects of the red-striped soft scale insect, forty newly hatched 1st instar nymphs were individually transferred to virgin sugarcane previously planted in pots. Each infested pot was directly sprayed with 5ml of an aqueous emulsion of each concentration of tested extract. Each replicate was kept under small plastic cage as mentioned before. Equal numbers of insects were similarly

sprayed with water and the emulsifier served as control. The insects were observed daily until the adults emerged. Records of nymphal mortality, and nymphal duration were kept. Recent emerged adults obtained from the stock culture were sprayed with different concentrations of plant extract. Forty replicates were maintained for every tested concentration. Plastic cages were examined daily to record deposited eggs. Records of pre-oviposition, oviposition, post-oviposition periods as well as fecundity and longevity were determined.

To study the effect of extracts on the egg stage, two hundred newly laid eggs obtained from the stock culture were sprayed with the materials of treatments. These were divided into five groups, each consisting of forty eggs a representing one replicate. Incubation period and percent hatchability were determined for each treatment.

Data were statistically analyzed by ANOVA using the Instate V2.03 computer program test and mean values were separated by the least significant differences (LSD) procedure [12], at probability = 1%.

Results and Discussion

Laboratory evaluation of different extracts of *Cressa cretica* on the biological aspects of *P. tenuivalvata*:

1. Nymphal Stage

- **Nymphal Mortality**

The highest percentages of mortality of nymphal instar as indicated in Table (1) were with petroleum ether at extract and the lowest with water and ethanol. The percentages of nymphs that the adult stage obtained differed with the different treatments. Petroleum ether extract proved to be more efficient than the other tested extracts for e.g., this percentage reached the maximum when nymphs treated with 5% concentrations of petroleum ether, 82.5 % then decreased to 70% at Ethanol 5% and reached a minimum of 20.0 with Water 1.25%, (Table, 1). In general, the percentages of mortality of the nymphal instars at any concentration increased as development progressed because the survivals occurred during the younger nymphal instars. So that it could recommend use to this extract to control this pest.

These data indicate that the *P. tenuivalvata* nymphs were more susceptible to the extracts than the adult stage. The least susceptibility of the adult stage to the tested compounds may attribute to the presence of protective scales which prevent the penetration of extracts into bodies of nymphs. Also, the smooth touch and shape of their scales increases the loss of the spraying solution received by the insect. Related results have given by [13] who proved that the adult stage of scale insects was least susceptible to the tested insecticides, then any other insect stage. On the other hand, ethanol, 1.25% and water, 1.25% decreased the mortality to the minimum values.

Many authors stated that the use of natural products for the control of many insect pests; petroleum ether extract was effective against larvae of many insects' pest [14- 19]. The plant extract (Guosheng), produced in china, was more effective than chemical insecticides for controlling scales and mites [20]. They reported that 500ml /L. and 750ml /L. of solutions of Guosheng killed 87.9% - 97.9% of *ceroplastesrubens* nymphs. Bionsecticides based on plant essential oils are just beginning to emerge as useful plant protectants, [17]. Plant material can be quickly steam-distilled to create EOs, which are rich in phenolics and a variety of volatile, low-molecular-weight terpenes.

- **Nymphal Development**

To evaluate the effect of different extracts (petroleum ether, ethanol and water) of *Cressa cretica* on the development of nymphal stage of the red-striped soft scale insect *P. tenuivalvata*; newly hatched nymphal instars (crawlers) produced from stock culture in the laboratory were treated with different concentrations (5, 2.5 and 1.25%) of tested extracts and allowed to develop on sugar-cane plants as mentioned before.

The results obtained in table (2) show that the nymphal stage durations of this pest (the 1st, 2nd and 3rd nymphal instars) were affected when nymphs were treated with tested extracts, this effect was clear on the 2ndnymphal instar duration, decreased from 22.6±0.8 days in untreated control to 11.2±1.3 days when nymphs were treated with petroleum ether extract at a concentration of 5%. The differences between nymphs (2ndnymphal instar) treated with petroleum ether extracts, at all concentrations used and untreated control was significant. The average total nymphal stage duration decreased from (32.6±0.7 days) in untreated control to (18.1±1.4 days and 19.3±1.5 days) when they were treated with petroleum ether extract 5%, and 2.5% concentrations, respectively.

On the other hand, the total nymphal stage duration in days increased to 38.2±0.6 days and 35.3±0.8 days when nymphs were treated with water and ethanol extract 1.25%, respectively. Again, the differences between total nymphal stage duration when nymphs were treated with petroleum ether extracts and untreated control statistically was highly significant, table (2).

Thus, the nymphal duration was greatly affected by the extracts to which nymphs were exposed, especially petroleum ether extract.

In general, the tested extracts of *Cressa cretica* plant could be arranged according to their bio-detrimental effects on the nymphal stages of *P. tenuivalvata* in the following descending order: petroleum ether > ethanol > water.

From the foregoing results, it could conclude that petroleum ether extracts 5% has the highest effect on the duration of nymphal stage development and the percent mortality of *P. tenuivalvata*.

2. Adult Stage

- **Effect on oviposition period**

The pre-oviposition period of scales was prolonged in all treatments; elongated at petroleum ether treatment 1.25% being on an average 4.7±0.6 days, while it was shortened with water 5% being on an average 2.6±0.2 days. But in untreated control the pre-oviposition period was on an average 2.2±0.2 days, (table, 3).

Table 1: Effect of different extracts of *Cressa cretica* on nymphal mortality and adult emergence of *P. tenuivalvata*

Plant extract	% conc.	% Nymphal mortality						Adult obtained
		1st nymph		2nd nymph		3rd nymph		
		No*	% Mortality	No.	% Mortality	No.	% Mortality	No.
Petroleum ether	5	28	70.0	31	77.5	33	82.5	7
	2.5	24	60.0	26	65.0	28	70.0	12
	1.25	16	40.0	20	50.0	22	55.0	18
Ethanol	5	24	60.0	26	65.0	28	70.0	11
	2.5	19	47.5	21	52.5	23	57.5	17
	1.25	12	30.0	14	35.0	16	40.0	14
Water	5	18	45.0	20	50.0	22	55.0	18
	2.5	14	35.0	16	40.0	18	45.0	12
	1.25	8	20.0	2	5.0	0	0.0	30
Control	0	2	5.0	0	0.0	0	0.0	38

Table 2: Effect of different extracts of *Cressa cretica* on Nymphal development

Plant extract	Conc.%	Average nymphal stage duration in days			Average total nymphal duration in days
		1st Instar	2nd Instar	3rd Instar	
Petroleum ether	5%	3.1±0.4 (1-4)	11.2±1.3 (1-20)	3.8±0.3 (0-8)	18.1±1.4 (5-28)
	2.5%	3.0±0.4 (1-4)	12.2±1.2 (1-23)	4.1±0.8 (0-9)	19.3±1.5 (4-31)
	1.25%	4.0±0.8 (1-7)	16.0±1.1 (1-26)	5.2±0.6 (0-9)	25.2±1.5 (4-38)
Ethanol	5%	3.0±0.3 (2-4)	14.0±1.1 (2-22)	6.1±0.7 (0-8)	23.1±1.2 (6-30)
	2.5%	5.0±0.4 (3-6)	16.0±1.1 (2-23)	10.1±0.9 (1-14)	31.1±1.3 (7-38)
	1.25%	6.0±0.6 (2-7)	18.1±0.4 (16-27)	11.2±0.6 (4-15)	35.3±0.8 (22-44)
Water	5%	5.9±0.2 (5-6)	12.0±0.6 (2-18)	5.9±0.5 (0-9)	23.8±0.7 (8-26)
	2.5%	6.0±0.2 (5-6)	22.0±1.3 (2-30)	6.6±0.6 (0-15)	34.6±1.6 (7-46)
	1.25%	6.0±0.6 (1-7)	25.0±0.8 (15-30)	7.2±0.6 (0-13)	38.2±0.6 (25-42)
Control	0.0	4.0±0.5 (2-6)	22.6±0.8 (12-28)	6.0±0.3 (3-9)	32.6±0.7 (21-37)
LSD 0.05	-	1.9	4.5	2.4	5.6

As for the effect of different extracts on the oviposition period, the results show in table, 3 indicated that with petroleum ether 5% the shortest oviposition period occurred (4.6±0.6 days) being more than two time longer with untreated control (10.7±0.8 days). Differences between the oviposition period in control and different treatments were highly significant, while at ethanol concentration of 2.5% and 1.25% it was significant. Also, differences between different extracts were significant.

There was a negative correlation between concentrations and oviposition period. Thus, an increase in concentration decreased the oviposition period.

Statistical analysis revealed an insignificant difference between the effect of extracts on the post- oviposition period and untreated control.

- **Effect on adult longevity**

When the total longevity of *P. tenuivalvata* was showed in (table, 3), it was found that the average longevity of adults was shortened to 10.8 ±0.9 days and 10.1±0.8days for adults treated with petroleum ether 5% and water 5%, respectively. On the other hand, it was prolonged to 13.8±1.0 days and 15.3±0.9 days in adults treated with ethanol 1.25% and untreated control, respectively.

Differences between the mean longevities of *P. tenuivalvata* treated with petroleum ether 5% were highly significant with untreated control, but insignificant difference between control and other treatments.

- **Effect on adult fecundity**

The total number of eggs laid by each scale seems to be affected by the tested extracts. Results in table, 3 show that maximum number of eggs were laid when adults were treated with ethanol 1.25% (50.6±2.6eggs / scale) but minimum fecundity occurred at petroleum ether 5% (40.4±2.1eggs /scale), and in control scale laid 190.1±30.7 eggs.

The difference between the number of eggs laid at different treatments and control were highly significant, however it was insignificant among different treatments.

Thus, it could be concluded that the *Cressa cretica* different extracts affected the number of eggs laid by scales.

From the forgoing experiments on the effect of different *Cressa cretica* treatments on oviposition, longevity and fecundity, it can be concluded that treatments with *Cressa* ***cretica*** extracts affect the biology of this pest. Increased concentration decreased oviposition, longevity and fecundity. The most effective extract is petroleum ether at a concentration of 5% as the insects treated with this extract gave the shortest oviposition, longevity and minimum number of eggs laid per scale.

Again, the tested extracts could be arranged according to their effect on the adult stage of *P. tenuivalvata* in the following descending order: petroleum ether > ethanol > water.

The same conclusion about the effect of extracts on adult longevity was given by **[15],** who stated that Azadirachtin had post-effect on adult stage by reducing the longevity and fecundity female's as well as its effect as oviposition deterrent. *s. littoralis* moth lived a shorter time from 8 days in untreated control to 2 days when treated with *Diplo taxis spp* extracts. also, [11], found that Nicandra and curcuma extracts in petroleum ether 5% concentration are more efficient on adult stage and larval duration of *Bemisia tabaci*. Similar results were reported by **[21]** who found that seven vegetable oils resulted in 9.16 to 100% oviposition deterrence to potato tuber moth, *Phthorimaea operculella* (Zell).

Table 3: Effect of different extracts of *Cressa cretica* on adult stage

Plant extract	% Conc.	Average duration in days			Longevity in days	Total no. of eggs laid per female (fecundity)
		Pre-oviposition	Oviposition	Post- oviposition		
Petroleum ether	5%	4.0±0.4 (1-9)	4.6±0.6 (1-13)	2.2±0.3 (0-12)	10.8 ±0.9 (4-23)	45.4±2.1 (20-60)
	2.5%	4.1±0.5 (1-10)	6.0±0.7 (2-14)	2.4±0.6 (0-8)	12.5±1.2 (4-24)	47.6±1.2 (42-50)
	1.25%	4.7±0.6 (1-12)	7.4±0.6 (1-15)	2.5±0.4 (0-8)	14.6±1.1 (1-27)	50.0±2.4 (31-65)
Ethanol	5%	4.1±0.4 (1-7)	6.1±0.6 (0-14)	2.9±0.4 (0-9)	13.1±1.1 (1-23)	40.9±2.6 (10-45)
	2.5%	3.6±0.2 (1-9)	7.4±0.6 (0-14)	3.0±0.3 (0-7)	14.0±0.9 (1-25)	42.3±2.4 (0-50)
	1.25%	3.1±0.3 (1-9)	7.6±0.5 (0-13)	3.1±0.4 (0-8)	13.8±1.0 (1-26)	50.3±2.6 (0-62)
water	5%	2.6±0.2 (1-6)	5.1±0.5 (0-13)	2.4±0.5 (0-6)	10.1±0.8 (1-19)	50.9±3.7 (0-65)
	2.5%	2.8±0.3 (1-8)	5.4±0.5 (0-11)	2.5±0.3 (0-7)	10.7±0.8 (1-19)	50.9±4.3 (0-75)
	1.25%	3.4±0.4 (1-9)	6.5±0.6 (0-15)	3.2±0.4 (0-8)	13.1±1.2 (1-27)	50.4±4.7 (0-68)
Control	0.0	2.2±0.2 (1-4)	10.7±0.8 (5-27)	2.4±0.3 (1-4)	15.3±0.9 (7-27)	190.1±30.7 (50-633)
LSD 0.05		1.6	2.6	0.6	2.9	48.9

According to [22] adult *Heliothis armigera* oviposited very few numbers of eggs on strips of castor leaves treated with Thymol. Ismail [5], investigated the biological activity of certain solanaceous alkaloids against *P. operculella* (Zell) and found that these alkaloids reduced the number of eggs laid by the female. Kinbuluma [23], the mortality and oviposition by female *Sitophilus zeamais*, repellent effect of the compounds and their effects on maize seed germination, were evaluated relative to the synthetic chemical, pirimiphos-methyl and ethanol, as positive and negative controls.

3. Egg stage

- **Effect on the incubation period:**

The eggs incubation period of eggs decreased as the concentration increased, reaching a minimum of 2.4±0.3 days when eggs were treated with petroleum ether 5%, and reached a maximum period of 5.0±1.0 days at water1.25%. The difference between incubation period caused by different treatments and control was highly significant; also, the difference between treatment with petroleum ether 5% and 2.5% was significant, while difference between treated with petroleum ether 5% and ethanol 5% was insignificant, (table, 4).

- **Effect on the percent of hatchability:**

The percent of eggs hatching deferred with the different treatments with *Cressa cretica* extracts as shown in (table, 4). Percent of hatched egg is concentration dependent, i.e., the higher extract concentrations gave lower percentage of hatchability. Petroleum ether extract had a stronger effect on egg hatch than other extracts. When eggs were treated with petroleum ether5% the majority of eggs failed to hatch and the percentage dropped to 33%. But the maximum hatchability occurred when the eggs were treated with water extract 1.25% being 82.5%. These findings agree with those obtained by [5, 24] on *P. operculella* (Zell). Ebadollahi, [10, 25], Consequently, the essential oils of the Lamiaceae plant family and their main components, especially monoterpenoid ones with several bioeffects and multiple modes of action against diverse groups of damaging insects and mites, are safe, available, and efficient alternatives to The harmful synthetic pesticides.

Table 4: Effect of different extracts of *Cressa cretica* on the egg stage of *P. tenuivalvata.*

Plant extract	% Concentration.	Average of incubation period in days	% Hatchability
Petroleum ether	5%	2.4±0.3 (1-3)	33.0
	2.5%	4.0± 0.5 (1-5)	42.5
	1.25%	4.6± 1.03 (1-7)	50.6
Ethanol	5%	3.0± 0.5 (1-4)	57.5
	2.5%	4.3± 0.7 (1-5)	60.0
	1.25%	4.3± 0.7 (1-6)	82.2
Water	5%	4.4±0.1 (1-6)	50.5
	2.5%	4.3±0.7 (1-6)	52.7
	1.25%	5.0±1.0 (1-6)	82.5
Control	0.0	9.5±1.2 (5-14)	100
LSD		1.59	-

*No of egg treated 200 egg / concentration.

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