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Research Article

The Strategy of Anti-Soft Scale Insect *Pulvinaria tenuivalvata* (Newstead) Infesting Sugar-Cane.

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ABSTRACT

The red-striped soft scale insect *Pulvinaria tenuivalvata* attacking sugar-cane at Attfieh region, Giza governorate is considered an economic important insect pest on sugar-cane, recently. Effective laboratory evaluation of Petroleum ether fraction of *Cressa cretica* and entomopathogenic fungi, *Beauveria bassiana* on the biological aspects of *P. tenuivalvata* was investigated. Different treatments had a great effect on nymphal mortality, the pre-oviposition period and oviposition period. Statistical analysis showed significant differences between tested compounds and control on the soft scale insect population. The tested compounds are increased sugar-cane yield significantly, at Attfieh region.

Keyword: *Pulvinaria tenuivalvata*; biology; *Beauveria bassiana;* Attfieh region

INTRODUCTION

Sugar-cane (*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. Sugar-cane the main source of sugar production; produces 70% 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 the mid 1990s [2-5] it spread within few years to most of the sugar-cane growing areas. Damage is caused by severe wilting due to sap depletion, and by sooty mould 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 more safe methods are needed.

Among the various avenues explored, bio-insecticides of plant origin may offer a better solution, [6-9] and used many isolates from entomopathogenic fungi against many insects to control it and studies the virulence of these entomopathogenic fungi on it [10-15].

The present work is, therefore, undertaken to elucidate the toxic and biological effects of petroleum ether fraction of *Cressa cretica* L., Fam.: Convolvulaceae and entomopathogenic fungi *Beauveria bassiana*on against the red-striped soft scale insect *Pulvinaria tenuivalvata* and the role of these materials in decreasing the population density of this pest and consequently their effect on sugar-cane yield. 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, *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. The 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.

Effect of different treatments on the biological aspects of *P. tenuivalvata*.

Treatments tested:

1. The petroleum ether fraction of *Cressa cretica* previously evaluated in laboratory tests was used in this experiment namely Petroleum ether 5%.
2. Entomopathogenic fungi, *Beauveria bassiana* with concentration 10^8 spores⁄ml were tested.
3. KZ oil 95 %: Mineral oil specifically formulated for pest control, the miscible type formulated by Kafr El-Zayat Company for chemicals and pesticides.

Preparation of Petroleum ether fraction:

Two hundred and fifty grams of the fine powder of *Cressa cretica* (Fam.: Convolvulaceae) were extracted by different solvents (petroleum ether (40-60 °C), methanol and butanol) in soxhlet apparatus according to methods [16]. The fraction of the *Cressa cretica* whole plant was isolated by column chromatography on silica gel. After exhaustion, each solvent was evaporated under reduced pressure to 30° C. After removal of the solvent, the dry residue of each fraction was used in bioassay tests.

Entomopathogenic Fungi

Beauveria bassiana (Balsamo) Vuillemin isolated from *Cassida vittata* [17] was grown on Peptone media (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media was autoclaved at 120 °c for 20 minutes and poured in Petri- dishes (10 cm diameter x 1.5 cm). Then incubated the fungi and kept at 24 ±2˚C and 65± 5 % RH. The fungal isolates were re-cultured every 14 – 30 days and kept at 4 ˚c.

Preparing of concentrations

Spores of fungal isolates harvested by rising with sterilized water 0.5 % Tween 80 from 14-day old culture Peptone media. The suspensions were filtered through cheesecloth to reduce mycelium clumping. The spores were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm^2). The concentration was used 10^8 spore /ml.

To test the effect of different compounds on the biological aspects of *P. tenuivalvata*, forty newly hatched 1st instar nymphs were individually transferred to virgin sugar-cane previously planted in pots. Each infested pot was directly sprayed with 5ml of an aqueous emulsion of each tested treatment. 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. Recently emerged adults obtained from the stock culture were sprayed with different treatments. Records of pre-oviposition, oviposition, post-oviposition periods as well as fecundity and longevity were determined.

Field experiments

Effect of different treatments on the population of P. tenuivalvata:

Experimental design:

One Feddan of sugar-cane, *Saccharum officinarum* L. naturally infested with the soft scale insect, *P. tenuivalvata*, was chosen in Attfieh region, at Giza Governorate, and it was divided into plots (6x7m^2 each). Tested treatments, (Kz oil, the most effective fraction of *Cressa cretica* and *Beauveria bassiana*) were distributed in a randomized complete block design and each treatment was replicated in 5 plots. Full coverage of the sugar-cane plants was secured by the use of a knapsack sprayer fitted with one nozzle. Five replicates (5 plots) of the untreated control treatment were sprayed with water only. The number of scales (nymphs + adult females) was used as a criterion to evaluate the effectiveness of the tested treatments.

A sample of 50 leaves/treatment (10 leaves / replicate) was randomly chosen before compound applications and also after 3, 7and 14 days from spraying as post treatment counts. Samples were kept in muslin bags until they were thoroughly examined in the laboratory. Counts of scales both on upper and lower surfaces of sugar-cane leaves were made under a stereomicroscope. For the purpose of statistical analysis, the percentages of reduction in pest population were calculated according to the equation of [18] as follows:

$$\text{Reduction (\%)} = \left(1 - \frac{T_a \times C_b}{T_b \times C_a}\right) \times 100, \text{ where:}$$

T_a = No. of individuals in treated plots after treatment.
T_b = No. of individuals in treated plots before treatment.
C_a = No. of individuals in control plots after treatment.
C_b = No. of individuals in control plots before treatment.

Effect of different treatments on sugar-cane yield

To determine the effect of different applications on the crop yield, after harvest, weights of sugar-cane stalks per plot ($6x7m^2$) were recorded. Five plots were taken for each treatment as well as untreated control, and then data were calculated.

Data were statistically analyzed by ANOVA using the Instat V2.03 computer programme test, and mean values were separated by the least significant difference (LSD) procedure [19] at a probability of 5%.

RESULTS AND DISCUSSION

Effect of different treatments on the *P. tenuivalvata* biological aspects:

Nymphal stage

Toxicity Effect:

Nymphal mortality as shown in Table (1) was affected by different treatments. As indicated from obtained adults, which had maximum survival when nymphs were treated with *C. cretica* (Petroleum ether) (15% adult obtained), while the percent survival decreased to 12.5 & 10% when nymphs were treated with *B. bassiana* or KZ oil. From the foregoing results, it can be concluded that different treatments had a great effect on percent mortality. KZ oil was the effective one where the least percentage of adults obtained followed by entomopathogenic fungi and plant fraction. Oils were usually recommended as insecticides against soft scale insects as early as 1763 but probably very little was used until the nineteenth century [20].

Table 1: Efficacy of tested materials against *P. tenuivalvata* nymphal stage

Treatment	Rate (ml/L)	% Nymphal mortality						Adult obtained
		1st		2nd		3rd		
		No.	% Mortality	No.	% Mortality	No.	% Mortality	No.
K.Z oil 95%	15	29	72.5	32	80	36	90	4
Cressa cretica (Petroleum ether)	50	27	67.5	30	75	34	85	6
Beauveria bassiana	10^8 spores/ml	28	70	32	80	35	87.5	5
Control	0.0	2	5.0	0	0.0	0	0.0	38

Effect on development

Data show that the 2nd nymphal instar duration decreased from 21.6±0.9 days at untreated control to 12.0±1.0, 11.0±0.7 and 10.9±1.3 days at *C. cretica* Petroleum ether 5%, *B. bassiana* and KZ oil 95%, respectively, table 2. Differences between control and treatments were significant.

Total nymphal duration decreased from 32.1±0.9days in untreated control to 16.1±1.3days when nymphs were treated with the fraction, it reached to 17.0±1.2 days when treated with oil and 17.8±0.9days with the *B. bassiana*, table 2. Differences between untreated control and treatment tests were significant. These results agree with that found by [21] which suggest that *Diplotaxis hara* extract possesses harmful effects throughout the entire developmental stages of *S. littoralis*.

Table 2: Biological aspects of *P. tenuivalvata* after treated

Treatment	Rate (ml/L)	Average nymphal stage duration in days			Average total nymphal duration in days
		1st Instar	2nd Instar	3rd Instar	
K.Z oil 95%	15	3.0±0.3 (1-4)	10.9±1.3 (2-19)	3.1±0.4 (1-7)	17.0±1.2 (4-29)
Cressa cretica (Petroleum ether)	50	2.9.0±0.3 (2-4)	12.0±1.0 (1-20)	4.1±0.5 (1-6)	16.1±1.3 (5-29)
Beauveria bassiana	10^8 spores/ml	2.9±0.2 (2-6)	11.0±0.7 (2-19)	3.9±0.4 (0-8)	17.8±0.9 (7-28)
Control	0.0	4.3±0.5 (2-6)	21.6±0.9 (12-29)	6.2±0.5 (3-9)	32.1±0.9 (20-38)
LSD 0.05	-	1.8	3.5	2.3	4.7

Adult stage

Effect on oviposition:

Results in table (3) show that the pre-oviposition period and oviposition period were affected by different treatments. The latter period decreased from 11.2±0.9days in control to 6.3±0.5 days, 5.7±0.5and 3.6±0.7days when adults were treated with Petroleum ether fraction, *B. bassiana* and KZ oil, respectively. Differences between the oviposition period in control and different treatments were significant. All treatments had no effect on the post-oviposition period of *P. tenuivalvata*.

Table 3: Efficacy of tested materials against P. tenuivalvata adult stage

Treatment	Rate (ml/L)	Average duration in days			Longevity in days	Total no. of eggs laid per female (Fecundity)
		Pre-oviposition	Oviposition	Post-oviposition		
K.Z oil 95%	15	3.0±0.4 (1-7)	3.6±0.7 (1-12)	2.1±0.3 (0-13)	8.7±0.9 (4-25)	33.4±2.2 (25-50)
Cressa cretica (Petroleum ether)	50	3.1±0.5 (1-8)	6.3±0.5 (0-13)	3.9±0.5 (0-10)	13.3±1.1 (1-22)	30.9±2.7 (15-35)
Beauveria bassiana	10^8 spores/ml	2.9±0.23 (1-7)	5.7±0.5 (0-14)	3.4±0.6 (0-7)	12.0 ±0.8 (1-21)	34.9±4.7 (5-55)
Control	0.0	2.3±0.3 (1-3)	11.2±0.9 (5-25)	2.5±0.4 (1-5)	16.0±0.9 (8-28)	200.0±25.7 (55-630)
LSD 0.05	-	1.7	2.2	0.9	2.9	33.7

Effect on longevity:

Data in table (3) show that the longevity of *P. tenuivalvata* was affected by different treatments. The longevity of scales decreased from 16.0±0.9days in the control to 13.3±1.1days, 12.0 ±0.8days and 8.7±0.9days with Petroleum ether fraction, *Beauveria bassiana*, and KZ oil, respectively. Differences between the longevity of scales in control and KZ oil were highly significant, being significant between the longevity of scales in control and Petroleum ether fraction and *B. bassiana*. These results agree with those found by [22] they stated that the mode of action of mineral oils against immature and mature stages was due to their developmental effects.

Effect on fecundity:

The fecundity of the red–striped soft scale *P. tenuivalvata* was affected by different treatments. Results in table (3) show that females laid the lowest average of eggs 30.9±2.7eggs, 33.4±2.2eggs and 34.9±4.7eggs / scale when treated with Petroleum ether fraction, KZ oil and *B. bassiana*, respectively in comparison to the control of 200.0±25.7eggs/scale. Differences between the fecundity of scales in control and all treatments were significant.

Field experiments:

Effect of different treatments on a population of *P. tenuivalvata* in Attfieh, Giza Governorate:

The efficiency of different tested treatments against adult stage of *P. tenuivalvata* on sugar-cane plants is presented in Table (4). The mean numbers of adults found on leaves before treatments, ranged from 58.6 to 68.2scales / 50 leaves, indicating a relatively uniform distribution of insect infestation. Three days after spraying, the treatments suppressed the levels of infestation to different degrees as compared to untreated control. *Beauveria bassiana* (10^8 spores/ml), Petroleum ether fraction of *Cressa cretica* with a rate of (50ml/L.) and significantly lowered the percentage of infestation to 39.9% and 44.6 %, respectively, although they didn't reach KZ oil (15ml/L.) activity (54.0%). Two weeks after treatments, *B. bassiana* (10^8 spores/ml), Petroleum ether fraction (50ml/L.) and KZ oil (15ml/L.) had almost similar activity, 65.8,69.1 and 79.4% reduction in the infestation, respectively. Statistical analysis showed significant differences between tested compounds and control on the soft scale insect population. The low susceptibility of the adult stage to the tested compounds may be attributed to the presence of protective scales which prevent the penetration of sprayings. These results are in harmony with those found by [23, 24] they reported that the mineral oils are the most effective against Diaspididae. Also, results of the present investigation are in general agreement with those obtained by [25-27] they stated that oils proved to be successful against scales, mealy bugs and some other sucking insects on different crops and fruit trees without phytotoxic effect of any kind.

Table 4: Efficacy of tested materials against *P. tenuivalvata* adult stage on sugar-cane plant in Attfieh, at Giza Governorate

Treatment	Rate (ml/L)	Mean number of adult /50 leaves and % reduction in infestation after spraying								
		Before treatment	After 3 days		After 7 days		After 14days		Average	
		Mean no.	Mean no.	% R.*	Mean no.	% R.*	Mean no.	% R.*	Mean no.	% R.*
K.Z oil 95%	15	66.1	30.8	54.0	20.2	71.4	14.6	79.4	21.9	68.5
Cressa cretica (Petroleum ether)	50	60.7	34.1	44.6	24.1	62.9	20.1	69.1	26.1	59.1
Beauveria bassiana	10^8 spores/ml	58.6	35.7	39.9	23.5	62.5	21.5	65.8	26.9	56.5
Control	0.0	68.2	69.1	0.0	72.9	0.0	73.2	0.0	71.7	0.0
LSD	-	2.7	1.8	-	1.7	-	1.9	-	2.6	-

% R = Percentages of reduction in the adult infestation*

Effect of different treatments on sugar-cane yield:

Results in Table (4) indicate that the different treatments had positive effects on sugar-cane yield. Increased percentages over the control ranged between 1.23 to 1.27 when used *Beauveria bassiana*, *Cressa cretica* Petroleum ether fraction 5% and KZ oil 95%, respectively. The average calculated yield in the control was 21710 Kg / feddan, as compared to 26750, 27180 and 27670 Kg / feddan after using the *Beauveria bassiana*, *Cressa cretica* Petroleum ether fraction 5% and KZ oil 95%, respectively, (Table 5). The difference between means of yield when using treatments were significant as compared to control. These results agree with that found by [28].

Table 5: Efficacy of different treatments against *P. tenuivalvata* on sugar-cane yield

Treatments	Concentration %	Mean yield Kg /plot ($42\ m^2$)	Calculated yield Kg / feddan	Increase in yield
KZ oil 95%	1.5%	276.7	2767	1.27
Cressa cretica	5.0%	271.8	2718	1.25
Beauveria bassiana	10^8 spores/ml	267.5	2675	1.23
Untreated control	0.0%	217.1	2171	1.00
LSD	-	22.4	-	-

CONCLUSION

According to the obtained results, it could be stated that the tested compounds can play an important role as natural insecticides in controlling the red-striped soft scale insect, *P. tenuivalvata*. These compounds may be used as components in (IPM) programmes for controlling this insect pest and to avoid pollution of environment and hazards to man or animals.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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