

Evaluation of Some Terpenes and Entomopathogenic Fungi on Three Sugar Beet Insect Pests

Sabbour, M. M. and A. Abdel-Rahman

Pests and Plant Protection Department, National Research Center, Dokki, Giza, Egypt.

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ABSTRACT

Phyllotreta cruciferae (Goeze), *Pegomyia hyoscami* (Witt) and *Cassida vittata* (Vill) were considered as serious pests infesting sugar beet leaves leading to their dryness. The insecticidal effects of the entomopathogenic fungi *Verticillium lecanii* (V.l), *Nomuraea rileyi* (N.r) and *Paecilomyces fumosoroseus* (P.f) and the terpenes; α -pinene; camphene and citronellol were tested under laboratory and field conditions. Laboratory results showed that the LC₅₀s of the tested fungi against the three pests ranged between 5.4×10^6 and 1.43×10^7 spores/ml. The tested terpenes at the 1% concentration level induced reasonable mortality (ranged between 42 and 60%) among third instar larvae of the target insects. Field applications showed that, among plots treated with *P. fumosoroseus*, the average of reduction percentages in infested plants ranged between 83.79 and 73.21% for the three tested insect pests during seasons 2005 and 2006. The best results were obtained in the field among the plots treated with *P.f* combined with α -pinene the percentage of reduction scored higher precedent ranged between (86.23 and 90.87) and (94.70 and 92.56%) at 2005 and 2006 seasons, respectively. The sucrose content recorded the highest amount in the plots treated with *P. fumosoroseus* and α -pinene (15.6 and 15.8%/beet) in the two successive seasons, respectively. The yield losses were significantly decreased after application of *P. fumosoroseus* during both seasons. The yield losses were ranged between (4.6 and 53%) and (7.7-53%) when combined fungi and terpenes were applied in 2005 and 2006 crop seasons, respectively.

Key Words: Terpenes; α -pinene; camphene; citronellol, entomopathogenic fungi *Verticillium lecanii*, *Nomuraea rileyi*, *Paecilomyces fumosoroseus*, *Phyllotreta cruciferae*, *Pegomyia hyoscami*, *Cassida vittata*, Sugar beet

INTRODUCTION

Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. The sugar beet has currently been infested with many insect pests which cause high losses in the crop yield and decrease its sugar content (Bassyouny, 1993 and Mesbah, 2000). Among these insects, *Cassida vittata* (Vill) and *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) and *Pegomyia hyoscami* (Witt) (Diptera: Anthomyidae). Chemical insecticides were used to control these insect pests, but they were always causing a lot of pollution to the environment. Thereafter microbial control agents were advocated to be used against such pests (Castillo *et al.*, 2000; Sabbour, 1992; 2003 and 2006). The entomopathogenic fungi have long been known to cause epizootics among certain insect pests under laboratory and field conditions (Barson *et al.*, 1994; Watson *et al.*, 1998; Reitheiner *et al.*, 1997). Feng *et al.*, (1990), reported that the fungi are very effective in controlling many insect pests. Also, the use of many plant derivatives gave promising results for controlling many insect pests (Ismail, 1994; Schmidt *et al.*, 1997, Ismail and Sabbour 2002).

entomopathogenic fungi and entomotoxic terpenes on *C. vittata*; *P. hyoscami* and *P. cruciferae* pests under laboratory conditions and to explore how far they can protect the crop in the field and increasing its yield.

MATERIALS AND METHODS

The sugar beet insects; *C. vittata*; *P. hyoscami* and *P. cruciferae* were reared under laboratory conditions (26 ± 2 C° and 60 ± 5 % R.H.) in cages 50 x 50 x 60 cm per each. The third larval stage was used in the experimental work.

Cultivation of the fungi

The fungus, *Verticillium lecanii* (V.l) was kindly obtained from Prof. Dr Alain Vey, Mycology Unite, National De La Recherche Scientifique, Univ. Montpellier. *Paecilomyces fumosoroseus* (P.f) (Apopka strain 97) and *Nomuraea rileyi* (N.r) (Farlow) Samson were obtained from Florida University and reproduced in Microbiology Dept., N. R. C. Cairo, Egypt. The fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 C°.

This study aims to evaluate the effect of these

Seven-days old cultures with well developed

spores were harvested by washing with 10 cc sterilized water then added 3ml, Tween-80 and completed to 100 ml water and used as stock suspension with known spore concentration then kept in a refrigerator at 4 C°, from which the fungi were sub-cultured to be used in laboratory evaluation tests (infectivity and bioassay tests) adjusted as conidiophores concentration of 1×10^8 /ml. Large amount of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1L cell culture glass bottles according to Rombach *et al.*, 1988.

Evaluation of the fungal effects on the target insect pests

The fungi, *P. fumosoroseus*, (*P. f.*), *V. lecanii* (*V.l*) and *N. rileyi* (*N.r*) at concentrations ranged from 1×10^2 to 1×10^8 spores/ml were tested against *C. vittata*; *P. hyoscamii* and *P. cruciferae* third instar larvae under laboratory conditions ($26 \pm 2^\circ$ C and 65 ± 5 %RH.). Fresh leaves of sugar beet were sprayed with the desired diluted suspension to the point of run off, left to dry, then put in 1 L plastic container (5 containers were used/concentration/ treatment). Twenty newly larvae of each species were placed in each container and covered with muslin. Untreated leaves were sprayed by water only and used as control. The leaves were changed every other day. The experiment was repeated 4 times. The percentages of mortality were calculated after seven days and corrected according to Abbott, (1925), while LC_{50} s were calculated through probit analysis of Finney, (1964).

Tested terpens

The pure terpens; α -pinene; camphene and citronellol are commercial samples obtained from Aldrich chemical Co. Ltd. Emulsions were prepared as follows: 1ml of each was mixed thoroughly with 5 drops of Tween-80, the appropriate amounts of water were added to obtain the desired concentrations (1; 0.5 and 0.25%).

Green sugar beet leaves were sprayed with each concentration of the tested terpenes to the point of run off then left to dry. Twenty newly hatched larvae of each species were placed on the treated leaves in each container, then covered with muslin and kept under laboratory conditions for three days. Thereafter, the numbers of alive and dead larvae were counted while survivors subsequently were allowed to feed on untreated green leaves in clean containers and were examined every other day till pupation. Control vials contained leaves treated with water only. Each experiment was replicated four times.

Field trials

The field trials were carried out in the growing sugar beet during the two successive growing seasons 2005 and 2006. Sugar beet was cultivated at Ebn-Malek Farm at El -Nobaryia region, Egypt, N. R. C. The sugar beet was planted in November 15th in an area of about one feddan. The area was divided into plots (each about 40 m²). Four plots were assigned for each treatment and for control as well, two rows of plants were left untreated between plots. Application of the fungi occurred at the rate of 1×10^8 spores/ml. sprayed at the sunset. Four applications were made at 4-week's intervals during crop growing season. Terpens were applied at the 5% concentration levels and repeated four times at 4- weeks intervals. The combination fungus which revealed the best results at the concentration of 1×10^8 with each plant extract at 0.2%. Control plots were left without any treatments. Examinations of 40 plants /plot /treatment were carried out just before the first application and seven days after last application to calculate the average reduction percentages in the target insect infestation percentages which was calculated in each treatment according to Henderson and Tilton (1955). The agricultural practices followed the recommendations of the Ministry of Agricultural.

Twenty tubers were taken from the first 5 rows in each treatment and in the control as well. Sugar (sucrose) content per beet was determined in the Biochemistry Department in the National Research center.

Yield Assessments

Yield weight was determined in kgs for treated and untreated plots. Yield loss was calculated according to the following equation:

$$\text{Yield loss} = \frac{\text{potential yield} - \text{actual yield}}{\text{Potential yield}} \times 100$$

Potential yield was based on the highest obtained yield in the different treatments for each season.

RESULTS AND DISCUSSIONS

The LC_{50} s of the three tested fungi (Table 1) were ranged between 5.4×10^6 and 1.32×10^7 spores/ml against the three insect pests. *P.f* scored the best results against the target insect pests. The LC_{50} s for *P. cruciferae*, *P. hyoscamii* and *C. vittata*, were 6.5; 5.4 and 8.7×10^6 spores/ml, respectively, while *N.r* caused the least effect against all tested insect pests (Table 1). Satisfactory results with the entomopathogenic fungi were reported by Sabbour (1992, 1995, 2003, 2005, 2007); Sharaf El-Din

Table (1): Evaluation of fungi on the sugar beet pests under laboratory conditions

Fungi tested	<i>P. cruciferae</i>				<i>P. hyoscami</i>				<i>C. vittata</i>			
	LC ₅₀	s	v	95% CL	LC ₅₀	s	v	95% CL	LC ₅₀	s	v	95% CL
<i>V.l</i>	9.7x 10 ⁶	0.01	1.2	12.2-7.7	8.8 x 10 ⁶	0.01	1.2	13.3-7.6	13.2 x 10 ⁷	0.02	1.1	16.7-9.8
<i>N.r</i>	1.31 x 10 ⁷	0.02	1.1	14.4-9.8	1.01x 10 ⁷	0.02	1.1	15.6-9.6	1.43 x 10 ⁷	0.03	1.1	17.7- 11.1
<i>p.f</i>	6.5 x 10 ⁶	0.01	1.2	8.9-4.3	5.4 x 10 ⁶	0.01	1.2	11.2-4.1	8.7 x 10 ⁶	0.01	1.2	12.3-6.6

(1999). Long *et al.*, 2000, Sabbour and Ismail 2001; El-Husseini *et al.* (2003 and 2004) found that *Beauveria bassiana*, *Metarhizium anisopliae*; and *Paecilomyces lilacinus* were effective against some sugar beet pests.

Regarding, terpenes α -pinene at all concentration levels scored the highest mortalities among *P. cruciferae*, *P. hyoscami* and *C. vittata*, 3rd instar larvae, followed by camphene while citronell induced less effects. The obtained results classified that α -pinene and camphene at the 1% concentration level achieved reasonable mortalities ranged between 44 and 60% among all tested insects. (Table 2). These results were in a harmony with Meisner *et al.*, (1982); Ismail (1994) Sharaby (1987) who reported that α -pinene and camphene showed insecticidal properties against *Spodoptera littoralis*. Ismail and Sabbour (2002) found that α -pinene and camphene showed positive correlations between concentrations and larval mortality.

Table (2): Percentage of larval mortality of three sugar beet insect pests fed on trepens treated green sugar beet leaves for seven days

Treatments	Conc. %	Mortality%		
		<i>P. cruciferae</i>	<i>P. hyoscami</i>	<i>C. vittata</i>
α -pinene	1.00	58	60	60
	0.5	44	54	58
	0.25	31	41	44
Camphene	1.00	44	59	57
	0.5	30	55	50
	0.25	29	38	33
Citronellol	1.00	42	50	52
	0.5	25	28	27
	0.25	22	22	23
Control	0.0	1.0	0.0	5.0

Infestation percentages of the three tested insects evaluated in all experiments plots in the sugar beet fields before 1st application (15/12/2005) when the infestations reached about 5.5%, 5.5 and 6.5% with *P. cruciferae*, *P. hyoscami*, and *C. vittata*, respectively. The percentage of reductions in *P. cruciferae* ranged between 83.79 and 52.23% one week after the fourth application (Table 3). On the other hand, the percentage of reductions ranged

between 78.57 and 66.51 % when the fungus *V.l* was applied against *P. cruciferae*, *P. hyoscami*, and *C. vittata* during both seasons (Tables 3 and 4). The best results were obtained in the plots treated with *P.f* combined with α -pinene, respectively, the percentage of reduction were 90.87 and 94.70% for *P. cruciferae* during seasons 2005 and 2006 (Tables 3and 4).

The sucrose contents ranged between 8.3 and 15.6% in the plots treated with different treatments during 2005 season. The highest sucrose content increased to 15.8% was revealed in plots treated with the fungus *P.f* and α -pinene in 2006 crop season (Table 4). Goodwine *et al.* (2007) reported that the sugar beet sucrose contents increased after bioagents treatments especially after fungi treatments. These results coincide with that claimed by Ismail and Sabbour (2002) who found that the combinations of terpenes and *B. bassiana* fungus increased the efficacy of the fungus against the cotton bollworms. Mansour (1999), Sabbour, (1992 and 1995, 2003 and 2006), Sabbour and Sahab (2005 and 2007) reported that many insect pests could be controlled by the bio-insecticides under field conditions.

Data in (Table 5) show that the weights of the sugar beet among *P.f*-treated cultivations were amounted to 4612 and 4731 kg/feddans as compared to 980 and 977 kg/feddans in the control plots during 2005-2006, crop seasons, respectively. Meanwhile, they were ranged between 2321 and 3400 kg/feddans in 2005 crop season and 2546 and 3583 kg/feddans in 2006 crop season in the other tested treatments. This lead to significant decrease of the yield loss ranged between 7.7 -53 % and 14-53% kg/feddans during seasons (2005 and 2006) as compared to 80 and 82% in the control plots, respectively (Table5). After addition of the terpenes to the fungus *P.f* the trepens showed an enhancement to the fungus and the weight of the sugar beet ranged between 4999 and 4766 kg/feddans during season 2005 and 5531 and 4999 kg/feddans during season 2006. In this respect, Sabbour and Sahab (2005 and 2007), reported that the different entomopathogenic fungi could reduce many insect pests under laboratory and field conditions and causing yield increase under field conditions. Long, *et al.* (2000) found that the yield

Table (3): Effect of different treatments against the sugar beet insects in the field during 2005

Treatments	% of sucrose content/beet	<i>P. cruciferae</i>			<i>P. hyoscami</i>			<i>C. vittata</i>		
		Infestation%		R%	Infestation%		R%	Infestation%		R%
		1	2		1	2		1	2	
Control	5.0	8.5	29.5	----	8.5	27.5	---	6.5	25.5	---
<i>V.l</i>	14.0	8.5	8.0	72.88	6.0	6.5	66.51	7.0	6.5	73.33
<i>P.f</i>	13.0	8.0	4.5	83.79	7.5	6.5	73.21	6.5	6.0	76.47
<i>N.r</i>	11.0	6.0	4.5	75.30	6.0	7.5	61.36	6.0	7.5	68.13
α -pinene	9.4	7.0	8.5	65.01	5.5	8.0	55.04	6.5	8.5	66.66
Camphene	9.0	8.0	10.0	63.98	6.0	8.0	58.78	5.5	8.5	60.60
Citronellol	8.3	7.0	9.5	60.89	5.5	8.4	52.23	5.5	8.5	60.60
<i>P.f</i> + α -pinene	15.6	6.0	1.9	90.87	5.0	2.1	87.63	5.0	2.7	86.23
<i>P.f</i> +Camphene	15.1	5.0	2.1	87.89	5.0	2.0	87.01	5.0	2.9	86.23
<i>P.f</i> + Citronellol	15.0	5.0	2.5	85.59	5.0	2.2	86.4	5.0	3.1	84.19

1: Before infestations (15/12/2005) 2: One week after 4th application (31/3/2006) R: Reduction in infestation

Table (4): Effect of different treatments against the sugar beet insects in the field during 2006

Treatments	% of sucrose content/beet	<i>P. cruciferae</i>			<i>P. hyoscami</i>			<i>C. vittata</i>		
		Infestation%		R%	Infestation%		R%	Infestation%		R%
		1	2		1	2		1	2	
Control	5.0	8.5	31.5	----	9.5	29.5	----	7.5	27.5	----
<i>V.l</i>	15.6	5.5	4.5	67.01	5.5	4.5	73.65	7.0	5.5	78.57
<i>P.f</i>	14.3	8.0	7.5	81.65	6.0	5.0	75.98	6.5	5.0	79.02
<i>N.r</i>	12.2	7.0	3.0	59.90	5.0	5.5	64.57	5.0	6.5	64.54
α -pinene	10.2	4.5	8.0	52.02	5.0	7.5	51.69	5.5	8.0	60.33
Camphene	9.6	5.0	8.5	54.14	5.5	8.5	50.23	5.5	8.5	57.85
Citronellol	9.6	5.0	8.5	54.14	5.0	8.5	45.25	6.5	8.5	64.33
<i>P.f</i> + α -pinene	15.8	5.1	1.0	94.70	6.5	1.5	92.56	6.5	1.5	93.53
<i>P.f</i> +Camphene	15.4	5.0	2.0	89.20	6.0	2.5	86.58	5.0	2.5	89.51
<i>P.f</i> + Citronellol	15.3	6.0	2.5	88.75	7.5	2.5	89.26	6.0	2.5	88.63

1: Before infestations (15/12/2005) 2: One week after 4th application (31/3/2007) R: Reduction in infestation

Table (5): Assessments of damage caused after treatment with different treatments

Treatment	Season 2005		Season 2006	
	Wt (kg/feddan)	yield loss %	Wt (kg/feddan)	yield loss %
<i>V.l</i>	3432 \pm 54.66	31	3619 \pm 63.43	34
<i>N.r</i>	3400 \pm 66.71	31	3583 \pm 80.12	35
<i>P.f</i>	4612 \pm 48.92	7.7	4731 \pm 71.23	14
α -pinene	2976 \pm 87.4 1	40	2998 \pm 66.43	45
Camphene	2765 \pm 76.31	44	2872 \pm 58.22	48
Citronellol	2321 \pm 65.43	53	2546 \pm 66.11	53
Control	980 \pm 51.55	80	977 \pm 64.67	82
<i>P.f</i> + α -pinene	4999 \pm 43.61	0	5531 \pm 49.55	0
<i>P.f</i> +Camphene	4768 \pm 54.87	4.6	5100 \pm 51.89	7.7
<i>P.f</i> + Citronellol	4766 \pm 72.45	4.6	4999 \pm 65.87	9.6
F value		34.6		32.9
Lsd5%=		122.7		126.5

was increased after treatments with *B. bassiana*. Mesbah *et al.* (2004) reported that some microbial control agents were mainly effective as biocides and reduced the infestations of the sugar beet insect pests and increased the yield in Kafer El-Sheikh. Seweify (1998) and Mansour (1999) found that the crop yield increased after treatments with fungi. Sabbour (2006) found that the yield losses of the potatoes were significantly decreased in the plots which treated with *B. bassiana* and *M. anisopliae*. Similar results were obtained by Sabbour, (2003 and 2007), Sabbour and Sahab (2005) and 2007); Sabbour and Ismail (2001) and Ismail and Sabbour (2002).

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