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Research Article

Virulence of Some Entomopathogenic Fungi against the Oliver Black Scale Insect, *Saissetia oleae* (Oliver) on Olive Trees in Egypt

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ABSTRACT

The olive black scale, *Saissetia oleae* attacks the olive crop in Egypt. The present studies aims to study the virulence of some Entomopathogenic fungi against the olive black scale insect, *Saissetia oleae* (Olivier) infesting olive trees. The virulence of some Entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Virticillium lecanii* were tested under laboratory at concentrations 2x102, 2x103 and 2x10 4 spores /ml and experiments were conducted under laboratory conditions at 25±2 o C and 65±5 %RH.. The nymphs and The adults of *Saissetia oleae* were treated with the each concentrations includes three replicates Each replicate contained five insects and field conditions the tested fungi against *Saissetia oleae* , during the two successive seasons 2014&2015 starting from the first of April 2014 to evaluate the virulence of the tested fungi against the Saissetia oleae under field conditions. All tested Entomopathogenic fungi (*B. bassiana, M. anisopliae* and *V. lecanii*) were able to decrease the infestation with the olive black scale insect on olive trees through the three post treatment counts. The Entomopathogenic fungus, *M. anisopliae* was the highest virulence against *S. oleae* than *B. bassiana* and *V. lecanii*. Using of Entomopathogenic fungi due to reduction in number the insects after being treated with *B. bassiana*, *M. anisopliae* and *V. lecanii* as compared the control. *M. anisopliae* caused the highest reduction in black scale Insect, *Saissetia oleae* than *B. bassiana* and *V. lecanii*.

Keyword: Virulence; entomopathogenic fungi; olive black scale insect; *Saissetia oleae.*

INTRODUCTION

The edible olive has been cultivated for at least 5,000 - 6,000 years, with the most ancient evidence of olive cultivation having been found in Syria, Palestine, and Crete. The olive tree is native to the Mediterranean region and Western Asia, and spread to nearby countries from there. The olive tree, *Olea europaea*, has been cultivated for olive oil, fine wood, olive leaf, and the olive fruit. 90% of all harvested olives are turned in to oil, while about 10% are used as table olives. Olive is one of the most economically horticultural crops in Egypt. The cultivated area of olive trees in Egypt has been rapidly expanded year after year. In 2010 it was 163273 feddans and the quantity of production reached about 390932 tons, [1].

Olive trees are infested with different scale insects among them the olive black scale insect, *Saissetia oleae* (Olivier) [2]. It causes weakness of trees and yield loss. As a result of intensive use of bio- and microbial pesticides, [3,4] Many

studies were carried out to test the efficiency of Entomopathogenic fungi as bio insecticides for controlling scale insects; i.e., [5-16].

The aim of this investigation is to carry out the present experiment to study the virulence of some Entomopathogenic fungi against the olive black scale insect, *Saissetia oleae* (Olivier) infesting olive trees.

MATERIALS AND METHODS

Study site

This study was carried out at El-Nubaria, El-Behira, Governorate, Giza, Egypt.

Fungi cultures

The tested Entomopathogenic fungi *Metarhizium anisopliae* (Metchinkoff) Soroken isolated from larvae and adults of *Scrobipalpa ocellatella* and *Beauveria bassiana* (Balsamo) Vuillemin isolated from *Cassida vittata*, [17] and *Verticillium lecanii* were grown on peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract, 15g Agar and 500 ml. Chloramphenicol and completed to one litre by distilled water). The media was autoclaved at 120°C for 20 minutes, and poured in Petri-dishes (10 cm diameter x 1.5 cm) then inoculated with the three entomopathogenic fungi and kept at 25 ±2°C and 85 ±5 R.H. The fungal isolates were re-cultured every 14-30 days and kept at 4°C.

To obtain a huge numbers of conidia, *B. bassiana*, and *M. anisopliae* and *V. lecanii* isolates were propagated on wetted rice. Two Kilos gram wetted rice was washed in boiled water for 10 min. and put in thermal bags. These bags were autoclaved at 120ºC for 20 minute then infected by isolates and incubated at 26 ± 1 ºC for 15 days. The Conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added.

Preparing of the concentrations

Conidia of fungal isolates harvested by rising with sterilizing water 0.5% Tween 80 from 14 days old culture rice media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. Conidia were counted in the suspension by using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm 2). The suspension was put in plastic bottles (2 litre). To restore the virulence of the isolates it was passed through their natural host, wax moth larvae *Galleria mellonella*. Three concentrations were prepared, (C1) 2x102, (C2) 2x103 and (C3) 2x10 4 Spores /ml.

Bioassay procedure

Effect of *B. bassiana, M. anisopliae and V. lecanii* isolates on nymphs of *Saissetia oleae*

The nymphs of *Saissetia oleae* were fed on branches of olives treated with the following concentrations (2x102, 2x103 and 2x104 spores / ml.) each concentrations includes three replicates. Each replicate contained five nymphs at 25±2 o C and 65±5 %RH.

Effect of *B. bassiana, M. anisopliae and V. lecanii* isolates on the adults of *Saissetia oleae*

The adults of *Saissetia oleae* were put in Petri dishes (12 cm diameter) placed a wetted filter paper and sprayed with the previous concentrations at 25±2 o C and 65±5 %RH.

Field experiments

Experiments were carried out to study the effectiveness of the tested fungi against the target insect pests, during the two successive seasons 2014&2015 starting from the first of April 2014 to evaluate the virulence of the tested fungi against the *Saissetia oleae* under field conditions. Four random patches of Olive trees were selected; each comprised 10 trees (10 trees for *B. bassiana*, 10 trees for *M. anisopliae*, 10 trees for *V. lecanii* applications and 10 trees for control) to carry out the field experiment.

B. bassiana, M. anisopliae and V. lecanii were applied, each as a single treatment at the rate of 2x104 spores / ml. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed early in the morning. Percentage of mortality /sample was calculated after 15, 30, 45 and 60 days of the application. Each treatment was replicated three times. Four plots were treated with water as control. Random samples of branches of olives plants were 2 weeks collected from each treatment and transferred to laboratory for examination.

The reduction percentages in the population density of *S. oleae* in relation to the pre-treatment count were calculated according to [18] as follows:

$$\text{Reduction percentages} = 1 - \frac{Ta\ Cb}{Tb\ Ca} \times 100$$

Where: Tb and Ta are pre- and after-treatment counts, respectively.

Cb and Ca are untreated checks before and after treatment.

Data of the percentages reduction were subjected to simple analysis of variance.

RESULTS

Effect of *B. bassiana, M. anisopliae* and *V. lecanii* isolates on *Saissetia oleae* nymphs.

Mortality in the nymphs were occurred in the third day of treatment (Table 1) and gradually increased until reached to 100% in the eighth day. The highest percent of mortality was observed in the ninth day (the third concentration in *M. anisopliae* isolate treatment).

Table 1: The % Mortality of *Saissetia oleae* nymphs treated with *B. bassiana, M. anisopliae* and *V. lecanii* isolates at 25±2 o C and 65±5 %RH.

Days after treatment	Percent of mortalities								
	Con.	*B. bassiana*			*M. anisopliae*			*V. lecanii*		
		*C1	C2	C3	C1	C2	C3	C1	C2	C3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	3.0	4.0	14.7	26.5	34.4	38.7	3.0	4.5	11.3
4th	0.0	4.7	5.6	21.6	32.4	39.9	43.9	4.2	5.1	19.6
5th	0.0	7.7	7.9	30.0	37.1	42.3	47.7	7.0	7.2	29.0
6th	0.0	8.4	15.6	56.2	55.5	67.3	78.8	8.0	15.0	55.0
7th	0.0	15.6	27.9	75.8	79.9	83.2	96.9	15.0	25.0	75.0
8th	5.0	29.1	36.4	88.4	92.7	98.3	100	30.0	35.0	87.0
9th	10.0	50.3	77.6	100	100	100	100	49.0	75.0	97.0

**(C1) 2x102, (C2) 2x103 and (C3) 2x10 4 Spores /ml.*

The percent of mortality was 100 in all concentrations of *M. anisopliae* isolate and the third concentration only in *B. bassiana* isolate but reached to 97.0 % in *V. lecanii* in the ninth day from concentration. This mean that the nymphs of *Saissetia oleae* was affected by *M. anisopliae* isolate than *B. bassiana* and *V. lecanii* isolates. Also the virulence of the M. anisopliae is the most compared with the virulence of the *B. bassiana* and *V. lecanii.* Also the virulence of the *B. bassiana* was more than *V. lecanii.*

Effect of *B. bassiana, M. anisopliae and V. lecanii* isolates on *Saissetia oleae* adults

As mentioned in Table (2) mortalities in adults were occurred in the fourth day in all concentrations of *B. bassiana, M. anisopliae and V. lecanii* treatment. Mortality occurred in all concentrations in the fourth day from treatment and gradually increased until reached to 100% in the eighth day in the third concentration of *B. bassiana* and *M. anisopliae* isolate only and reached to 95 % of V. lecanii.

Table 2: percent Mortality of *Saissetia oleae* adults treated with *B. bassiana, M. anisopliae and V. lecanii* isolates at 25±2 o C and 65±5 %RH.

Days after treatment	Con.	Percent of mortalities								
		B. bassiana			*M. anisopliae*			*V. lecanii*		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4th	0.0	2.0	2.3	9.5	21.2	28.8	34.3	2.0	2.0	9.0
5th	0.0	9.0	15.9	34.4	22.7	32.3	36.6	7.0	13.9	30.0
6th	0.0	15.7	48.9	50.3	28.5	54.4	59.4	13.0	45.0	49.0
7th	0.0	27.9	49.9	52.3	35.5	84.0	96.4	27.0	49.0	51.0
8th	6.0	48.8	68.8	100	69.9	89.4	100	49.0	67.0	95.0

**(C1) 2x102, (C2) 2x103 and (C3) 2x10 4 Spores /ml.*

Filed Experiments

In season 2014 the reduction percentages in the different stages of *Saissetia oleae* (Olivier) are presented in Table (3) these data showed the superior virulence of the three entomopathogenic Fungi.

Percent of sampled substrate infested with black scale Insect, *Saissetia oleae* (Olivier) before and after treatment with *B. bassiana* reduction from 92.0% in the first week from treatment to 9.2 % after seven weeks from treatment.

Also when we treated the trees with *M. anisopliae* reduction in population of the black scale Insect, *Saissetia oleae* was 2.1% from 93.0% after seven weeks from treatment.

Also when we treated the trees with *V. lecanii* reduction in population of the black scale Insect, *Saissetia oleae* was 9.5% from 90.0% after seven weeks from treatment.

M. anisopliae caused the highest reduction in black scale Insect, *Saissetia oleae* than *B. bassiana* and *V. lecanii.*

Table3. Percent of sampled substrate infested with black scale Insect, *Saissetia oleae* (Olivier) before and after treatment with *B. bassiana, M. anisopliae and V. lecanii* isolates 2014.

Treatment Date	Control	*B. bassiana*	*M. anisopliae*	*V. lecanii*
April 10	90.0	92.0	93.0	90.0
April 24	93.0	40.3	35.3	40.2
May 8	94.0	33.5	20.7	34.5
May 22	90.0	9.2	2.1	9.5

In season 2015 the reduction percentages in the different stages of *Saissetia oleae* (Olivier) are presented in Table (4) these data showed the superior efficiency of the three entomopathogenic Fungi.

Percent of sampled substrate infested with black scale Insect, *Saissetia oleae* (Olivier) before and after treatment with *B. bassiana* reduction from 84.0% in the first week from treatment to 8.3 % after seven weeks from treatment.

Also when we treated the trees with *M. anisopliae* reduction in population of the black scale Insect, *Saissetia oleae* was 1.0 % from 86.0% after seven weeks from treatment.

Also when we treated the trees with *V. lecanii* reduction in population of the black scale Insect, *Saissetia oleae* was 8.5% from 85.0% after seven weeks from treatment.

M. anisopliae caused the highest reduction in black scale Insect, *Saissetia oleae* than *B. bassiana* and *V. lecanii.*

Table4. Percent of sampled substrate infested with black scale Insect, *Saissetia oleae* (Olivier) before and after treatment with *B. bassiana, M. anisopliae* and *V. lecanii* isolates 2015.

Treatment Date	Control	*B. bassiana*	*M. anisopliae*	*V. lecanii*
April16	85.0	84.0	86.0	85.0
April 30	90.0	37.7	30.3	38.2
May 14	91.0	30.2	19.0	32.5
May 28	87.0	8.3	1.0	8.5

DISCUSSION

We find in our study that the entomopathogenic fungus *M. anisopliae* was the most virulence than *B. bassiana* and *V. lecanii*. The yield weight of potatoes increased in plots treated with *B. brongniartii* and *N. rileyi* the infestations with the potato tuber moth *P. operculella* were significantly decreased [19-21]. The grain weevil *S. granaries* decreased significantly after entomopathogenic fungus *B. bassiana* treatments. The isolates of the fungi, *Beauveria bassiana* and *Metarhizium anisopliae* were highly toxic against the three treated insect pests: the fruit fly, *Ceratitis capitata*, the olive fruit fly, *Bactrocera oleae* and the olive Moth, *Prays oleae*, either under laboratory or field conditions [22, 23]. The yield loss was markedly reduced compared to the check (control) trees.

CONCLUSION

We find in our study that the three entomopathogenic fungi due to reduction in number the insects after being treated with *B. bassiana, M. anisopliae and V. lecanii* as compared the control. Many studies found that the fungi *B. bassiana* and *M. anisopliae* reduced insect infestations of the potato tuber moth, stored product insects and tomato pests under laboratory and field conditions. *M. anisopliae* was the most virulence than *B. bassiana* and *V. lecanii* against black scale Insect, *Saissetia oleae*.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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