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Efficacy of *Nomuraea rileyi* and Spinosad against olive pests under laboratory and field conditions in Egypt

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ABSTRACT

Isolates of the entomopathogenic fungus, *Nomuraea rileyi* and the microbial pesticide spinosad were tested against the olive insect pests: *Bactrocera oleae, Ceratitis capitata* and *Prays oleae* under laboratory and field conditions. Results obtained showed that the LC50 of *N. rileyi* for *P. Oleae, C. capitata* and *B. oleae* were 142, 145 1nd 155 spores/ml after treated with different concentrations the fungus under laboratory conditions. The corresponding figure for Spinosad were 165, 176 and 178 spores/ml. Under field conditions, results showed that during season 2011, The percentage of *P. Oleae, C. Capitata* and *B. oleae*, infestations were significantly decreased in plots treated with *N. rileyi* to 10± 3.1; 13± 3.0 and 10± 2.2 individuals as compared to 38±3.4, 31±2.4 and 33±3.2 individuals of the corresponding pests in the control, during season 2011 in El-Esraa (Nobaryia) after 90 days of post application. When *Spinosad* were applied in the field, the percentage of infestations were significantly decreased in all both two seasons. During the harvest season, the olive fruits weight were 2998 ±32.32 Kg/Feddan in the plots treated with *N. rileyi* and plots treated with *Spinosad the olive fruits collected weighted 2847* ±42.51 Kg/Feddan as compared to 2219± 24.82 Kg/Feddan in the control during season 2011. During season 2012 the treatments trees with *N. rileyi scored the highest weight 3090* Kg/ Feddanas compared to 2169± 80.53 Kg/Feddan among the control trees.

Key words: *Bactrocera oleae, Ceratitis capitata, Prays oleae* ; *Nomuraea rileyi, Spinosad.*

Introduction

Olive (*Olea europaea* L.) has become one of the important economical crops in Egypt. Its cultivated area has been expanded largely in the last decade, particularly in new reclaimed arid areas (Western side of the Nile). Its area reached 49000 Hectares in 2010 (productivity = 6327 Kg/ Hectare) (Mohamed, 2009). Olive tree is subjected to attack by many insect pests that affect yield quality and quantity. Among the most common pest species surveyed in Egypt are: *Bactrocera oleae* (Rossi), *Prays oleae* Bern. and *Ceratitis capitata* (Wied.) (Mohamed, 2009). *B. oleae* is the key pest damaging olive in the world (Rice, 2000) as well as in Egypt (Eid, 2003) it was a native to Mediterranean countries which has 98% of the world's cultivated olive trees (Montiel and Jones, 2002). *P. oleae* is one of the most important insect pests of olives in Egypt and other Mediterranean countries. The moth develops three generations per year (Montiel and Jones, 2002). In Egypt the first generation of moths appears in April the female lays its eggs on the flower buds, the newly hatched larvae feed on the buds and flowers (Montiel and Jones, 2002). Contact and oral bioassays with fungi, revealed that moderate to high mortality rates for the olive fruit fly occurred when the adults were exposed to conidia of *Mucor hiemalis, Penicillium aurantiogriseum, P. chrysogenum* and *B. bassiana* isolates (Mohamed, 2009). He also reported that,a strain of *M. hiemalis* isolated from *S. nonagrioides* larvae was the most toxic fungus that resulting in 85.2% mortality to the olive fruit fly adults. *B. brongniartii* and *B. bassiana* were the most pathogenic to the C. capitata adults causing 97.4 and 85.6% mortality, respectively (Mohamed, 2009). Metabolites collected from the *M. hiem alis* and *P. chrysogenum* isolates were toxic to adults of both species (Mohamed, 2009). The Mediterranean fruit fly *C. capitata* (Wiedermann) and the olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) are from the serious insect pests which attack the olive fruits and cause an economical destruction to the olive trees. These pests were controlled by chemical insecticides which pollute the environment and causes cancer diseases, where bioinsecticides could control these pests safely (Roberts and Humber, 1981; Tanda and Kaya, 1993; Hajek and St. Leger, 1994). Strains of *M. anisopliae* and *Paecilomyces fumosoroseus* were pathogenic to *C. capitata* adults under laboratory bioassay (Castillo *et al*., 2000). Konstantopoulou and Mazomenos (2005) reported that the usage of *B. bassiana* and *B. brongniartii* fungi were the most pathogenic to *C. capitata* causing 97.4 and 85.6% mortality. *M. anisopliae* cause a highly mortality rates to *C. capitata* and *B. oleae* adults and the rate of larval mortality was 85.2%. In Egypt, Mohamed (2009) reported that *Lecanicillim lecanii* and *M. anisopliae* fungi, and the interaction between *B. bassiana* and *M. anisopliae* fungi are suitable candidates to be used for control of *P. oleae.* Spinosad is a relatively new insecticide that is made up of two complex organic compounds, spinosyn A (right) and spinosyn D. These compounds are produced by certain microbes that were first discovered in soil found at an abandoned rum factory. Spinosad is a broad-spectrum, organic insecticide. The term "broad-spectrum" means that it is toxic to a wide variety of insects. It is, however,

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relatively non-toxic to mammals and beneficial insects. If used carefully only insects that actually eat something that has been treated, such as a leaf, are affected. This is different than a lot of other broad-spectrum insecticides that are toxic if the insect merely comes in contact with dry insecticide residues Qiao *et al*.(2012).

The present study aims to evaluate the pathogenicity of the isolates the entomopathogenic fungus, *Nomuraea rileyi* and Spinosad (bio-insecticide) against olive pests under laboratory and field conditions. It is necessary to find alternative safety insecticides to reduce the heavy doses of chemical insecticides which is used for olive pests control.

Materials and Methods

Laboratory tests:

Insects:

B. oleae and *C. capitata* adults used in the present work were obtained from laboratory colonies maintained in our laboratory at 25±2°C and 60–65% relative humidity (RH) and 12:12 (L:D) photoperiod. Adults were provided with water and a solid diet consisting of 40% sugar, 10% hydrolyzed yeast, 5% egg yolk. The olive Moth, *Prays oleae* (Bernard, 1788) (Lepidoptera: Yponomeutidae), was reared on olive leaves under the same laboratory conditions. Adults reared in cylinder glass cages (15cm diameter x 22cm height), covered with muslin, and fed on 10% sucrose solution.

The commercial Spinosad-baised, GF-120 (Conserve 0.24% CB) as a microbial insecticide (macrocyclic lactone insecticides, Saccharopolyspora spinosa Martz & Yao),were used against the olive pests according to Sameh *et al*., (2009)

Isolation of the fungi:

The fungus *Nomuraea rileyi* was isolated from the diseased insect pests (*C. Capitata, B. oleae & P. oleae*). Isolates were subcultured on nutrient PDA medium. Isolates were identified at National research Centre (NRC) Plant Pathology Department. The spores of *N. rileyi*, were collected from agar surface of the fungus culture in 15cm diameter Petri-dish. Spore suspension in water + 0.1% Tween-80 was prepared. The strength of original culture was 1x108 spore/ml. It was used as stock suspension and kept in a refrigerator at 4°C. From this stock, dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 L cellculture glass bottles according to Rombach *et al*., (1988) and modified by El-Husseini *et al*., (2004).

Bioassays against target pests:

All fungal isolates concentrations of *N. rileyi*, ranged from 1x102 to 1x108 spores/ml were prepared by 1-10 fold dilution from the main stock culture (1x108) and tested under controlled conditions (25±2ºC and 65±5% RH) against *C. Capitata, B. oleae & P. oleae* adults. Ten 3-day-old flies were collected in test tubes, immobilized on ice and carefully transferred to PDA dishes (9 cm diameter) containing the six fully developed fungal colonies. The flies were allowed to walk on the fungal colonies for 5–10 min depending on fly mobility until the flies collected spores on their body. The flies were then removed from the Petri dishes and placed in small cages (10 cm x 10 cm x 10 cm). The same number of flies treated similarly but with uninoculated PDA plates was used as controls. Solid diet and water were offered to flies and kept under rearing conditions. Dead flies were counted and removed from the cages daily for 21 days. Each treatment was replicated five times The percentages of mortality were calculated after seven days and corrected according to Abbott's formula (Abbott, 1925), while the LC50 value was calculated through Probit analysis according to Finney equation (Finney, 1971).

Field experiments:

Esraa village- El-Nobaryia region, during the two successive seasons 2011&2012 starting from the first of July till the end of August to evaluate the efficacy of the tested fungi against the target insect pests under field conditions. Three random patches of Olive trees were selected, each comprised 12 trees (12 trees for Spinosad-baised and 12 trees for *N. rileyi*, applications and 12 trees for control) to carry out the field experiment. *N. rileyi*, was applied, each as a single treatment at the rate of $1x10^8$ spores/ml. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten liters sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of the application. Each treatment was replicated four times. Four plots were treated with water as control. Random samples of leaves

and fruits olives plants were weekly collected from each treatment and transferred to laboratory for examination. The infestation of *C. capitata, B. oleae & P. oleae* were estimated in each case.

After harvest, yield of each treatment was weighted as Kg/Feddan. Yield loss was calculated according to the following equation:

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

Potential yield was that yield obtained after *N. rileyi*, treatment, which gave the best results among the tested pathogens, and was taken as a base for comparing with the other treatments

Results:

Under laboratory conditions:

Data in table (1) show that the LC_{50} of *N. rileyi* fungus for *P. oleae, C. Capitata* and *B. oleae* were, 142, 145 1nd 155 spores/ml , respectively. The corresponding figure for Spinosad was 165, 176 and 178 spores/ml (Table 2).

The percentage of *P. Oleae, C. Capitata* and *B. oleae*, infestations were significantly decreased in plots treated with *N. rileyi* to 10± 3.1; 13± 3.0 and 10± 2.2 individuals as compared to 38±3.4, 31±2.4 and 33±3.2 individuals of the corresponding pests in the control, during season 2011 in El-Esraa (Nobaryia) after 90 days of post application. When *Spinosad* were applied in the field, the percentage of infestations were significantly decreased in all both two seasons (Table 3). The obtained results are similar to other studies carried out by Castillo *et al.* (2000) and Espin *et al*. (1989) on their work on *C. capitata*. After harvest the olive fruits weight were 2998 ±32.32 Kg/Feddan in the plots treated with *N. rileyi* and plots treated with *Spinosad the olive fruits collected weighted 2847* ±42.51 Kg/Feddan as compared to 2219± 24.82 Kg/Feddan in the control during season 2011. During season 2012 the treatments trees with *N. rileyi scored the highest weight 3090* Kg/ Feddanas compared to 2169± 80.53 Kg/Feddan among the control trees. In all cesses, during the both seasons 2011 and 2012 the yield loss ranged between 29.80 – 25.98 % in the control and significantly decreased to 5.03 and 3.23% after *Spinosad* treatments during two successive seasons (Table 4). These results agree with Sabbour & Shadia Abd El-Aziz, (2002 and 2010) and Shadia Abdel Aziz & Nofel (1998), who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with Castillo *et al.* (2000) who reported that the virulence of *B. bassiana* against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. Espin *et al*. (1989) recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments. Konstantopoulou and Mazomenos (2005) reported that the fungi *B. bassiana* and *B. brongniartii* application considered the most pathogenic to *C. capitata* causing 97.4 and 85.6% mortality, while *M. anisopliae* cause a highly mortality rates to *C. capitata* and *B. oleae* adults and the rate of larval mortality was 85.2%. In Egypt, Mohamed (2009) reported that the fungi *Lecanicillim lecanii, M. anisopliae* and inter action between *B. bassiana* and *M. anisopliae* are suitable candidates to be used for control of *P. oleae*. Abdel-Rahman & Abdel-Mallek (2001), Abdel-Rahman (2001) and Abdel-Rahman *et al*. (2004), controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab (2005, 2007) and Sahab and Sabbour (2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

Table 1: Effect of *N. rileyi* on the target insect pests under laboratory conditions.

Target pests	LC_{50} (spores/ml)	Slope	Variance	95% Confidence limits
Prays oleae	142	0.01	0.02	156-111
Ceratitis capitata	154	0.01	0.03	176-113
Bactrocera oleae	155	0.02	0.01	181-133

Table 2: Effect of Spinosad, on the target insect pests under laboratory conditions.

Target pests	LC_{50} (spores/ml)	Slope	Variance	95% Confidence limits
Prays oleae	165	0.01	0.03	186-122
Ceratitis capitata	176	0.02	1.02	198-144
Bactrocera oleae	178	0.01	0.04	199-154

Table 3: Infested plants with target insect pests after treatment with the fungi *N. rileyi* and *M. anisopliae* under field conditions through out the two seasons.

Treatment	Days after treatment	El-Esraa (Nobaryia)					
		Season 2011			Season 2012		
		P. oleae	*C. capitata*	*B. oleae*	*P. oleae*	*C. capitata*	*B. oleae*
Control	20	2.1±2.1	3.2±14	2.1±3.5	5.4±2.3	6.2±2.4	5.1±1.4
	50	21±2.3	10±.2	21±2.2	27±3.4	22±3.4	25±2.5
	90	38±3.4	31±2.4	33±3.2	38±3.7	36±4.6	35±3.4
	120	49±1.2	51±4.0	51±1.2	51±3.3	55±6.7	57±3.4
N. rileyi	20	0±0.0	1.1±1.2	0±0.0	1.5±2.1	2.4±5.3	.12±3.9
	50	4±2.2	5±3.1	6±2.2	8±4.5	10±4.4	10±3.4
	90	10±3.1	13±3.0	10±2.2	14±3.4	18±3.4	20±3.7
	120	15±4.2	16±2.3	15±2.3	20±3.5	24±2.9	20±4.5
Spinosad	20	0±0.0	0±0.0	0±0.0	1±1.1	3.6±2.8	3.3±4.7
	50	2±1.1	3±2.1	4±2.3	5±3.4	13±2.8	11±5.4
	90	16±2.2	17±1.2	19±3.8	18±3.4	29±1.7	19±2.6
	120	25±2.3	20±2.2	19±3.5	27±3.5	36±1.9	29±4.5

Table 4: Weight of harvested olive fruits and percentage of yield loss after treatment with the fungi against target insect pests.

Treatment	El-Esraa (Nobaryia) during			
	Season 2011		Season 2012	
	Kg/Feddan	% Yield loss	Kg/Feddan	% Yield loss
Control	2219± 24.82	25.98	2169±80.53	29.80
N. rileyi	2998± 32.32	--	3090±65.31	-
Spinosad	2847± 42.51	5.03	2990±69.43	3.23
F-value	30.11		32.14	
LSD 5%	79		77	

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