Efficacy of Some Microbial Control Agents Against Onion Insect Pests in Egypt

Sabbour, M. M. and M. H. Abbass
Department of Pests and Plant Protection, National Research Centre, Dokki, Giza, Egypt
E.mail: sabbourm9@yahoomail.com
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ABSTRACT

The entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* were tested against onion insect pests. Results showed that they significantly decreased the infestations with *Thrips tabaci*, *Delia alfrii* and *Eumerus amoenus*. Under the laboratory conditions, 26±2°C and 70-80% RH, the LC_{50} s recoded were 113, 87 and 71 spores/ml for the respective pests species by *M. anisopliae*. Using *B. bassiana*, the LC_{50}s were 110, 98 and 66 for the same insect species, respectively. Respective LC_{50} s of *Verticillium lecanii* were 161, 150 and 141 spores/ml. Under green house conditions, results showed that the LC_{50}s of *B. bassiana* were 130, 87 and 65 spores/ml on *T. tabaci*, *D. alfrii* and *E. amoenus* while respective LC_{50} s of *M. anisopliae* were 121, 81 and 70 spores/ml. Under field conditions, the infestation's percentages with *T. tabaci* were72 ± 2.5, 83± 3.4 and 97± 5.1% in the untreated plot and decreased to 31± 4.7, 28±2.1 and 21±2.2% after 20, 50 and 90 days in *B. bassiana* treated areas, respectively. Lesser effects on the target insect pests under the field conditions were achieved by *V. lecanii* and *Bacillus thuringiensis*. Generally, relative yield loss significantly decreased when onion plants were treated with any of the *B. bassiana*, *M. anisopliae*, *V. lecanii* and *B. thuringiensis*.

Key Words: *Bacillus thuringiensis; Beauveria bassiana; Metarhizium anisopliae; Verticillium lecanii, Thrips tabaci , Delia alfrii, Eumerus amoenus*, Efficacy.

INTRODUCTION

In Egypt, onion is the 2nd major export crop after cotton. This is mainly due to the increasing demand of the crop for local markets and export. Onion becomes less important for export as a cache crop due to the current infestations with different insect pests and diseases either in the field or in the store. The major insect pests attacking onion plants in the field are *Thrips tabaci* (Thripidae) onion maggots, *Delia* sp. (Anthomyidae) and *Eumerus amoenus* (Syrphidae) which damage the onion crop when the larvae tunneled into the base of the plant. These insect pests were almost destructive to the young plants. As a result of infestation, the plant leaves become yellow and dry, finally leads to the death of the plant. Castineiras *et al.*, (1996) stated that *Thrips* had affected the yield and quality of vegetables especially onion and strawberry. In the recent years, management of these pests has become difficult. This difficulty increased due to the introduction of economically destructive pest species. *Thrips* managements are difficult because of high levels of pesticide resistance resulting from years of exposure to different active ingredients.

Chemical insecticides are widely applied for control of *Thrips*. More than 20 insecticides, including formulations for foliar spraying and soil applied granules are currently registered for *Thrips* control (Plant Protection Manual, 2000). A desirable management strategy advocated biological control as a central component. The usage of microbial control agents could give good results against the insect pests without polluting the environment.

The ubiquitous fungi *Beauveria bassiana* (Balsamo), *Verticiliium lecanii* and *Metarhizium anisopliae* (Metschnikoff) are common disease agents associated with dead insects in nature, (Mc Coy *et al.*, 1988). These fungi have been scrutinized worldwide as microbial control agents of soil inhabiting insects in particular, McCoy (1995) and Quintela and McCoy (1998) reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* and *M. anisopliae* caused 90 - 100% larval mortality. Also the fungus infects the adult maggots and causes the fly to stick to the tip of the plants and die. Federici and Maddos (1996) suggested that the entomopathogenic fungi causing such disease outbreak in the insect pests. Lacy and Kaya (2000) mentioned that most of the insect species can be reduced by assortment of entomopathogenic fungi.

The present work aims to evaluate the effect of some entomopathogens against onion insect pests under several conditions.

MATERIALS AND METHODS

An experimental area of about 200 m^2 was divided into 20 equal plots (10 m^2 each). Onion plants (Giza-20 cultivar) were cultivated at the

National Research Centre farm at El-Noubaria (El-Emam Malek district) on 15th of December during the two successive seasons (2004 and 2005). The plants received regular agricultural practices but without using pesticides through the whole growing seasons.

Laboratory trials

Rearing of the target insect pests

T. tabaci adults and nymphs were reared on green onion leaves under the laboratory conditions; 26±2°C and 70-80% RH. The leaves were changed every other day. Onion maggots *Delia alfrii* and *Eumerus amoenus* were also reared on onion bulbs under the same laboratory conditions.

Effect of *Bacillus thuringiensis kurstaki*, Dipel 2X (23000 IU)

Pieces of onion leaves were sprayed with 6 concentrations (500, 250, 125, 63, 32 and 16 ug/ml) of Dipel 2X. Two drops of tween 80 were added to each concentration as a wetting agent. The leaves were left for drying under laboratory conditions. Thereafter, they were placed in Petri dishes (one/ dish) lined with moistened filter paper. Twenty adults of the *T. tabaci* were introduced to each dish to feed for 24 hours. The adults were then transferred to similar Petri-dishes and fed on untreated onion leaves till death. The experiments were replicated four times. Control was made by feeding the adults on untreated onion leaves. Percentages of mortality were calculated and corrected according to Abbott, 1925, while LC_{50}s were calculated through probit analysis (Finney, 1964). The experiments were carried under the laboratory conditions of 26 ± 2° C and 60-70% R.H.

Isolation of the fungus *V. lecanii*

The fungus *V. lecanii* was isolated from the dead and/or infected insect pests. The infected larvae were stored individually in tightly closed sterilized vials in a refrigerator at 4°C. Refrigerated individuals were examined 24-48h post storage, then dipped in 2% sodium hypochlorite for 3-5 min and washed with sterile distilled water. Isolates were subcultured on nutrient PDA Medium; isolates were identified at N.R.C. Microbiology Department. The spores of *V. lecanii* were collected from the surface of mycelium growth. The original culture of *V. lecanii* was $8X10^8$spores/ ml. 0.5 ml was suspended in 100ml of distilled water and 2 drops of emulsifying agents were added (Tween-80). This gave rise to a concentration of $8X10^7$ spores/ml. The pathogen suspension undergoes 1-2 fold dilutions 6 times.

Cultivation of the fungi *B. bassiana* and *M. anisopliae* and recovery of spores

B. bassiana (BR3) and *M. anisopliae* (RM3) were kindly provided by Prof. Dr Alain Vey, Mycology Unite; National De La Research Stentifique, Univ. Montpellier. The fungi were received in agar plates as sporulated cultures. Conidiospores from these plate provided inocula for all experiments. The spores of *B. bassiana* and *M. anisopliae* were collected from the surface of mycelium growth and spores suspensions were mixed with Tween-80 (2drops). The preparation was diluted in water and adjusted at a concentration of 16.5X108 conidia/ml. Conidiospores of both fungi were harvested from fungal cultures that were produced on potato dextrose agar plus 0.4% yeast extract (PDAY) and incubated for 10-15 days at 25± 1 C°. Conidial inoculum was taken from pure fungal cultures, with no more than two serial passages from a host insect. Conidial viability was determined by counting germ tubes produced on PDAY after 18h using light microscope at 400X. Conidial viability was 95-100%. The surface of the cultures was gently brushed in the presence of 20 ml of sterilized water, in order to free the spores, the suspension was then filtered through muslin. The concentration of the spores' suspension was adjusted using a haemocytometer to 16.5 $X10^8$ conidia/ml.

Effect of the fungi *B. bassiana, M. anisopliae* and *V. lecanii* on the target insects

B. bassiana; *M. anisopliae* and *V. lecanii* were used in 6th concentrations 16.5, 8.25, 4.125, 2.02, 1.06 and $0.5X10^8$ spore/ ml. Pieces of onion leaves were dipped in the last prepared preparations and left to dry under laboratory conditions then placed in Petri dishes (one/dish) for each concentration (4 replicates/ each). Third instar larvae of each of the target insects were transferred into each Petri dish. Control was made by feeding the larvae on untreated onion leaves. The percentages of mortality were calculated and corrected according to, Abbott (1925), while LC_{50}s were calculated through probit analysis Finny (1964). The experiments were carried under the laboratory conditions of 26±2° C and 60-70% R.H.

Semi field (green house) trials

Onions were planted in a green house (40 plants per plot). Natural infestation took place, the plants were sprayed by the biopreparations of *B. t., B. bassiana, M. anisopliae* and *V. lecanii* at the concentrations of 300 ug/ml of Dipel 2X and 8.25 x 10^8 conidia/ml for each of the fungi. Control samples were left without treatments. The onion plants were examined every 2 days by transferring

the onion leaves to the lab. Percentage of infestations was calculated until end of the experiment. In the control, the larvae were fed on untreated onion leaves. Each treatment was replicated 4 times. The percentages of mortality were calculated and corrected according to Abbott (1925), while LC_{50} s was calculated through probit analysis (Finney, 1964).

Field trials

Trials were carried out in onion cultivations naturally infested with the *E. amoenus, D. alfrii* and *T. tabaci* (300 m^2 apart from each other) at N.R.C. farm at El- Noubaryia during 2004 and 2005 seasons. Five onion plots, in each area were treated by the rate of 2 g/5ml/10m^2 of *B. thuringiensis*. The fungi; *B. bassiana, M. anisopliae* and *V. lecanii* were applied by the concentration $16.5x10^8$ spores/ ml. Each treatment was sprayed weekly and replicated 4 times. The percentages of infestations by *T. tabaci, D. alferii* and *E. amoenus* after 20, 50 and 90 days from treatments were estimated. Four plots were treated by water as check.

Yield assessment

Yield weight was estimated by kilograms for the treated and untreated plots (Kg/feddan). Yield loss was calculated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield - Actual yield}}{\text{Potential yield}}$$

Potential yield was the yield of *B. bassiana* treated onion (which gave the best results among the tested pathogens, taking as a base for comparing other products)

RESULTS AND DISCUSSION

Data in table (1) show that the LC_{50} of *B. t.* under the laboratory conditions against *T. tabaci,* reached 220 ug/ml and 292 ug/ml under the green house conditions. The LC_{50} s of the same pathogen on *D. alfrii* and *E. amoenus* were 190 and 165 ug/ml under the laboratory conditions and 207 and 191 ug/ml under the semi field conditions (Table 1). Federici and Maddos (1996) mentioned that *B.t.* is the most used entomopathogen in the United States with thousands of tons applied annually to control the pests of vegetables and field crops. Since 1987, number of projects (Project report, 2003) reveled that the entomopathogens (viruses, fungi, microspordia and nematodes) reduced numbers of *Thrips* on vegetables plants.

By treating the target insect pests under laboratory conditions by the isolated fungi *V. lecanii,* the LC_{50}s for *T. tabaci, D. alfrii* and *E. amoenus* were, 161, 150 and 141 spore/ ml, respectively. While under green house conditions, they were 142, 138 and 111 spores/ml, respectively (Table 1). The results show that after treating the *T. tabaci* by the fungi *B. bassiana* and *M. anisopliae* under laboratory conditions, the LC_{50} were 110 and 113 spores/ml, respectively (Table 2). After treatments with the same fungi under semi field conditions the LC_{50}s ranged between 70 and 130 spores/ml (Table 2).

In field experiments, the data showed that percentage of infestation with *T. tabaci* decreased significantly to 26% compared to 72% in the control (Table 3). Percentage of infestation with *D. alfrii* decreased to 23±3.2 after 20 days post treatment by *M. anisopliae* compared to 65±3.4 in the control (Table 3). When the *E. amoenus* was treated by *B. bassiana,* percentage of infestation was 27, 23 and 29% compared to 68, 85 and 96% in the control after 20, 50 and 90 days of treatments, respectively (Table 3).

Castineiras *et al.* (1996) and Frantz and Mellinger (1998) indicated that *B. bassiana* occurs in Florida soil and this strain infected *Thrips* under laboratory, semi field and field conditions. Also, *Thrips* was controlled by this pathogen in the fields.

Table (1): Effect of *Bacillus thuringiensis* and *Verticillium lecanii* on three target insect pests under laboratory and green house conditions

Agent & Target pests	Green house				Laboratory			
	LC_{50}	v	s	95% confidence limits	LC_{50}	v	s	95% confidence limits
B. thuringiensis								
T. tabaci	220	0.001	1.02	233-143	292	0.002	2.5	210-149
D. alfrii.	190	0.001	1.03	178-111	207	0.001	1.03	188-112
E. amoenus	165	0.001	1.03	155-87	191	0.001	1.10	1 78-130
V. lecanii								
T. tabaci	161	0.001	1.02	133-143	142	0.002	2.5	110-143
D. alfrii.	150	0.001	1.03	78-111	138	0.001	1.03	88-122
E. amoenus	141	0.001	1.03	55-87	111	0.001	1.10	78-132

Table (2): Effect of fungi on three target insect pests under laboratory and green house conditions.

Pathogens target insects	*B .bassiana*				*M. anisopliae*			
	LC_{50} (Spores/ml) x 10^8	Variance	Slope	95% confidence Limits x10^8	LC_{50} (Spores/ml) x 10^8	Variance	Slope	95% confidence Limits x10^8
Laboratory								
T. tabaci	110	0.001	1.12	127-88	113	0.001	1.50	132-100
D alfrii.	98	0.002	1.03	112-76	87	0.002	1.27	112-75
E. amoenus	66	0.004	1.33	85-55	71	0.002	1.22	81-55
Green house								
T. tabaci	130	0.001	1.02	146-98	121	0.001	0.001	132-90
D alfrii.	87	0.002	1.02	110-66	81	0.002	0.002	100-65
E amoenus	65	0.002	1.30	85-57	70	0.002	0.002	91-50

Table (3): Effect of different treatments on three target insect pests under field conditions

Post 1st application date	Treatments	% of infestations (mean) ±s.e		
		T. tabaci	*D. alfrii*	*E. amnenus*
20	Control	72±2.5	65±3.4	68±1.6
50		83±3.4	74±3.3	85±2.3
90		97±5.1	95±3.1	96±3.3
20	*B. thuringiensis*	52±2.6	55±2.4	55±4.5
50		53±3.3	54±2.3	54±2.7
90		90±5.2	59±2.1	59±3.2
20	*B. bassiana*	31± 4.7	24 ±4.5	27± 4.5
50		28± 2.1	27±2.6	23± 5.2
90		21± 2.	20±3.5	29±4.5
20	*M. anisopliae*	26±3.4	23± 3.2	22±3.3
50		21±3.2	20± 3.4	32±4.4
90		20±4.2	15± 2.5	21±2.6
20	*B. thuringiensis*	35±5.3	32±3.3	455.7
50		30±2.2	44±5.1	48±4.6
90		33±3.6	30±4.2	39±2.1
20	*V. lecanii*	44±2.3	40±2.1	31±3.3
50		55±1.4	51±6.2	39±4.3
90		67±2.7	60±5.3	46±3.6

Table (4): Assessments of damage caused among treatments

Treatments	Season 2004		Season 2005	
	Weight kg/feddan	% of yield loss kg/feddan	Weight kg/feddan	% of yield loss kg/feddan
Control	1117±55.74	73	1002 ±57.9	80
B. thuringiensis	2012±76.93	51	2981±79.28	40
B. bassiana	4156±80.51	0	5011±73.90	0
M. anisopliae	4023±69.84	3	4561±65.82	9
V. lecanii	3001±61.36	27	3120±89.53	37
F value=		33.9		
LSD%=		111.46		

Insects can also spread the fungus through mating (Long *et al.* 2000). High humidity and free water enhanced activity of the conidia and the subsequent infection of the insect. Fungal spores infected pests in cool to moderate temperatures and were readily killed by solar radiation (Goettel *et al.* 2000 and Wraight and Ramos, 2002). Frantz and Mellinger (1998) found that the fungi *B. bassiana* gave good results for controlling *Thrips*, especially at high infestations. They also found that the fungus *B. bassiana* could infect *Thrips* and decrease its number.

One formulation of *B. bassiana*, Mycotrol™, was reported to be sensitive to high temperatures with best results at temperatures between 70 and 80°F. Slow growth at warmer temperatures may make this a poor option for growers in southern states (Kuepper, 2003). Kovach and English-Loeb (1997) worked on the major pests of strawberries, indicated that the plant bugs populations and their damage could be reduced to about half by four applications of a product containing *B. bassiana.*

Data in table (4) show that the weight of the onion bulbs in *B. bassiana* treated cultivations reached 5011 and 4156 kg/feddan compared to 1002 and 1117 kg/feddan in the control plots in 2005 and 2004 crop seasons, respectively. The weight of onion bulbs in all treatments ranged between 4023 and 2012 kg/feddan, this led to significant decrease of the yield ranged between 3 -51% kg/feddan during season 2004 (Table 4). During season 2005, the yield of onion bulb weight was significantly increased after treatment with the different types of entomopathogens compared to 1002 kg/feddan in the untreated plots. The percent of yield reduction ranged between 9 - 40 % compared to 80 % in the control (Table 4). Similar results were obtained by Seweify (1998), Quintela and McCoy 1998, Mansour (1999), Long *et al.* (2000), Wraight and Ramos (2002) and Sabbour and Sahab (2005) and (2007) who reported that the crop yield increased after treatments with the fungi.

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