Pathogenicity of Three Commercial Products of Entomopathogenic Fungi, *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii* against Adults of Olive Fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) in the Laboratory

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Abstract

Mahmoud M.F. (2009): **Pathogenicity of three commercial products of entomopathogenic fungi,** *Beauveria bassiana*, *Metarhizum anisopilae* **and** *Lecanicillium lecanii* **against adults of olive fly,** *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) **in the laboratory.** Plant Protect. Sci., **45**: 98–102.

The pathogenicity of entomopathogenic fungi, *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii*, was evaluated against adults of the olive fly *Bactrocera oleae* (Gmelin) under laboratory conditions by two ways, contact bioassays and oral bioassays. The results showed that oral bioassays caused higher mortality after four treatments than the used contact bioassays. Moreover, the virulence of *L. lecanii* was higher than the virulence of *B. bassiana* and *M. anisopilae* in both ways of experiment. Lethal time (LT_{50}) was shorter in oral bioassays than in contact bioassays in all treatments. It was 14.67, 8.30 and 5.43 days for *B. bassiana*, *M. anisopilae* and *L. lecanii* with oral treatment while it was 16.6, 26.07 and 12.59 days for *B. bassiana*, *M. anisopilae* and *L. lecanii*, respectively, with contact treatment. The slope values were 2.41, 2.55 and 2.37 for contact bioassays and 1.64, 1.69 and 1.61 for oral bioassays of *B. bassiana*, *M. anisopilae* and *L. lecanii*, respectively. The mortality response to the interaction between *B. bassiana* and *M. anisopilae* was synergistic while the interaction between *B. bassiana* + *L. lecanii* and *M. anisopilae* + *L. lecanii* showed an antagonistic response.

Keywords: *Bactrocera oleae*; *Beauveria bassiana*; *Metarhizum anisopilae*; *Lecanicillium lecanii*; pathogenicity

The olive fruit fly *Bactrocera oleae* (Gmelin) is a major pest of olives in the Mediterranean region where over 98% of the world's olives are produced. In Egypt it is probably the most important agricultural pest (El-Basha 2002).

The predominant method to control the olive fly has been the use of traditional insecticides. However, the continued use of insecticides has caused enormous problems; environmental pollution, development of insecticide resistance and contamination of products (Roessler 1989; Cabras *et al*. 1997).

As an alternative to chemical control or as a part of Integrated Pest Management (IPM) programs, there is a resurgence of interest in the use of microbial insecticides for the biological control of insect pests. Fungal agents belong to the most promising group of biological control agents against insect pests. Particularly, the Deuteromycete fungi are known to cause epizootics in fly populations under laboratory and field conditions (Barson *et al*. 1994; Watson *et al*. 1996: Reithinger *et al*. 1997). *Metarhizum anisopilae* (Metchnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin

and *Paecilomyces fumosoroseus* (Wize) Brown & Smith have been recognised as some of the most important entomopathogens of dipteran insects (Steinkraus *et al*. 1990; Kuramoto & Shimaku 1992; Samson *et al*. 1994; Watson *et al*. 1995).

The objective of this study was to evaluate the pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizum anisopilae* (Metchnikoff) Sorokin, *Lecanicillium lecanii* (Zimmermann) Zare & W. Gams when used alone as contact and oral bioassays and to determine the mortality response to the interaction among fungi against the olive fruit fly *Bactrocera oleae* (Gmelin) under laboratory conditions.

MATERIALS AND METHODS

Insect. The culture of olive fly collected from infested olive fruits, Ismailia Governorate, was used for the study. This culture of *Bactrocera oleae* was maintained in the laboratory of the Plant Protection Department, Faculty of Agriculture, Suez Canal University, under controlled conditions 25 ± 2°C, 65–75% relative humidity and light: dark photoperiod of 12 h. Adult diet consisted of 1 part of protein hydrolysate and 3 parts of sugar by weight (Mahmoud 1997).

Bioassays. (1) Contact bioassay: Twenty-five newly emerged adults were placed in experimental cages (10 cm × 20 cm ×15 cm). Adult diet and water were supplied and kept under rearing conditions. The doses of entomopathogenic fungi were prepared as 1×10^8 conidia/ml and put each one in a small sprayer and then adults were sprayed carefully for 30 seconds. The same amount of adults was used for the control, which was sprayed only with sterile distilled water. Dead adults were counted four times (after 5, 10, 15 and 20 days from the start of the experiment). Each variant of the experiment was replicated five times.

(2) Oral bioassay: Suspension (1 ml) of *B. bassiana* (1×10^8 conidia), *M. anisopilae* (1×10^8 conidia) and *L. lecanii* (1×10^8 conidia) was mixed with 1 ml of the diet for adults. Flies consumed the treated diet within 2–3 days. Diet without fungal conidia was supplied to adults used as controls. Twenty-five adults were placed in cages (10 cm × 20 cm × 15 cm) under rearing conditions. Each treatment was replicated five times and mortality was recorded four times (Konstantopoulou & Mazomenos 2005).

(3) Interaction among entomopathogenic fungi: The effect of interaction among *B. bassiana*, *M. anisopilae* and *L. lecanii* was tested. The concentrate composition of the fungi was added to the diet for adults and diet without fungal conidia was supplied to adults used as controls. Each treatment was replicated five times and mortality was recorded after 20 days as total mortality. The analysis for synergistic and antagonistic interactions was based on a comparison of mortality rates of entomopathogenic fungi when used alone and mixed in the diet for adults.

Statistical analysis. Data were statistically analyzed by ANOVA (SAS Institute 1999). When the F-test was significant, means were separated using Duncan's multiple range test (DMRT) at the 0.05 level of significance. LT_{50} and slope were calculated by probit analysis (Finney 1971).

RESULTS AND DISCUSSION

The virulence of *B. bassiana*, *M. anisopilae* and *L. lecanii* against adults of *B. oleae* was estimated after four treatments with spraying the adults (Table 2). Significant differences were found in the virulence of all entomopathogenic fungi tested ($F = 5.48$; $P \leq 0.0087$ for 5 days, $F = 16.84$; $P \leq 0.0000$ for 10 days, $F = 43.08$; $P \leq 0.0000$ for 15 days and $F = 44.9$; $P \leq 0.0000$ for 20 days). The results obtained in this study are similar to other studies carried out on different insects; for example, Castillo *et al*. (2000) found out the virulence of *B. bassiana* against *Ceratitis capitata* from 8 to 30%. Espin *et al*. (1989) observed 69–78% mortality in *Ceratitis capitata* adults with *M. anisopilae*.

Table 1. Compounds tested against the olive fruit fly, *Bactrocera oleae*

Product	Entomopathogenic fungus	Concentration	Manufacturer
1 – Bio-Power	*Beauveria bassiana*	1×10^8 conidia/ml	
2 – Bio-Magic	*Metarhizum anisopilae*	1×10^8 conidia/ml	T. Stanes & Company Ltd., India
3 – Bio-Catch	*Lecanicillium lecanii*	1×10^8 conidia/ml	

Table 2. The virulence and time mortality response of *Bactrocera oleae* adults sprayed with *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii*

Entomopathogenic fungi	Concentration (conidia/ml)	Virulence (%)				LT_{50}	Slope
		5*	10*	15*	20*		
Beauveria bassiana	1×10^8	10.4^{ab}	27.2^{ab}	47.2^{b}	60.8^{b}	16.06	2.41
Metarhizum anisopilae	1×10^8	2.4^{bc}	18.4^{b}	25.6^{c}	39.2^{c}	26.07	2.55
Lecanicillium lecanii	1×10^8	15.2^{a}	34.4^{a}	70.4^{a}	77.6^{a}	12.59	2.37
Control	–	0.0^{c}	2.4^{c}	6.4^{d}	9.6^{d}	–	–

*days after treatment; means followed by the same letters in the same column are not significantly different (LSD at $P \leq 0.05$)

Probit analysis of the time mortality response (LT_{50}) was 12.59 days shorter for *L. lecanii* than that of *B. bassiana* 16.06 days and *M. anisopilae* 26.07 days. Riba *et al.* (1985) recorded LT_{50} values between 1.2 and 5.4 days for mosquitoes inoculated with *M. anisopilae*.

Similar results were obtained when *B. oleae* adults were fed a diet containing conidia of the above entomopathogenic fungi. Data showed significant differences in virulence among the fungi *B. bassiana*, *M. anisopilae* and *L. lecanii* after four treatments. The high virulence of entomopathogenic fungi observed in adults fed a diet containing conidia of *L. lecanii* followed those treated with *M. anisopilae* and *B. bassiana*. Mortality was significantly different from the control ($F = 23.79$; $P \leq 0.0000$ for 5 days, $F = 67.24$; $P \leq 0.0000$ for 10 days, $F = 245.5$; $P \leq 0.0000$ for 15 days and $F = 293.7$; $P \leq 0.0000$ for 20 days). *L. lecanii* was the most pathogenic fungal species to *B. oleae* adults, the virulence was 100% after 20 days of exposure. These results are opposite to Castillo *et al.* (2000), who reported that *L. lecanii* and *P. fumosoroseus* caused low mortality (>10%) of *C. capitata*. The *M. anisopilae* and *B. bassiana* also showed high virulence of *B. oleae* 92 and 80%, respectively. Konstantopoulou and Mazomenos (2005) found out moderate pathogenicity of *B. bassiana* when it was tested as oral bioassay against *B. oleae* adults. Probit analysis of the time mortality response revealed that *L. lecanii* killed adults of *B. oleae* more rapidly than *B. bassiana* and *M. anisopilae* ($LT_{50} = 5.43$). Rosa *et al.* (2002) obtained LT_{50} values of 2.8, 3.9, 4.6, 5.7 and 5.9 days for *Anastrepha ludens* adults after exposure to different isolates of *B. bassiana*. Riba *et al.* (1985) obtained LT_{50} values of 2.2, 1.9 and 1.6 days for *Aedes aegypti*, *Anopheles stephensi* and *Culex pipens* adult mosquitoes, respectively, after exposure to *M. anisopilae*.

The slope values of entomopathogenic fungi toward adults are presented in Tables 2 and 3. The steepest slope of 2.55, 2.41 and 2.37 was observed in contact bioassays while the flattest one was recorded in oral bioassays at 1.69 and 1.61.

The effect of combined application of *B. bassiana*, *M. anisopilae* and *L. lecanii* on the mortality

Table 3. The virulence and time mortality response of *Bactrocera oleae* adults fed a diet containing *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii*

Entomopathogenic fungi	Concentration (conidia/ml)	Virulence (%)				LT_{50}	Slope
		5*	10*	15*	20*		
Beauveria bassiana	1×10^8	4.8^{ab}	14.4^{ab}	48.0^{b}	80.0^{b}	14.67	1.64
Metarhizum anisopilae	1×10^8	13.6^{bc}	70.4^{b}	88.0^{c}	92.0^{c}	8.30	1.69
Lecanicillium lecanii	1×10^8	44.0^{a}	88.0^{a}	98.4^{a}	100^{a}	5.43	1.61
Control	–	0.0^{c}	0.8^{c}	1.4^{d}	7.2^{d}	–	–

*days after treatment; means followed by the same letters in the same column are not significantly different (LSD at $P \leq 0.05$

Table 4. Effect of the interaction among *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii* on the mortality response of *Bactrocera oleae*

Entomopathogenic fungi	Concentration (conidia/ml)	Mortality* (%)	Response
Beauveria bassiana + *Metarhizum anisopilae*	1×10^8	100[a]	synergistic
Beauveria bassiana + *Lecanicillium lecanii*	1×10^8	72.0[b]	antagonistic
Metarhizum anisopilae + *Lecanicillium lecanii*	1×10^8	62.4[c]	antagonistic
Control	–	6.4[d]	–

*mortality after 20 days

response of *B. oleae* is presented in Table 4. Data indicated that the combination of *B. bassiana* + *M. anisopilae* gave a synergistic response while the combination of (*B. bassiana* + *L. lecanii*) and (*M. anisopilae* + *L. lecanii*) gave an antagonistic response. Theoretically, the combined application of different species of insect pathogens can increase the efficacy of pest control. Entomopathogens have different types of relationship with the investigated insects *in vivo* including independent development, antagonism, synergism and others. There is little information about the results of mixed entomopathogenic fungi of insects. Gouli *et al*. (2008) found out that the principal possibility of using the tank mixtures of different species of entomopathogenic fungi for the control of western flower thrips, *Frankliniella occidentalis*, gave a good result for mass production. Batta (2008) also observed the synergistic interaction between the effect of the fungi *Beauveria bassiana*, *Metarhizum anisopilae* and the diatomaceous earth dusts and found the negligible effect on the viability of created conidia.

This study demonstrated that *L. lecanii*, *M. anisopilae* and the interaction between *B. bassiana* and *M. anisopilae* are suitable candidates to be used for the control of *B. oleae* adults. The next step of work will be directed toward the development of an effective field trial.

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