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Pathogenicity of the Entomopathogenic Fungi *Beauveria bassiana* (Balsamo) and *Verticillium lecanii* (Zimmerman) Against Aphid *Macrosiphum rosae*, Linnaeus (Hemiptera: Aphididae) under Laboratory Conditions

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Abstract

The effects of two entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii* were studied on aphid adults of *Macrosiphum rosae* under laboratory condition (25 ± 1 ºC, 80 ± 5% RH and a photoperiod of 12 L: 12 D h). Bioassay was conducted by putting 1 µl from each concentration on insect body by micro-applicator as topical application method. This experiment was conducted with six replications on CRD design. Laboratory bioassay studies were carried with six different concentrations (10^4, 10^5, 10^6, 10^7, 10^8 spore/ml) of *B. bassiana* and *L. lecanii* on adult aphids of *M. rosae*. The LC_{50} values *L. lecanii* and *B. bassiana* were obtained 1.38 $\times10^4$ and 2.66 $\times10^5$ spores/ml, respectively. The LT_{50} values ranged from 1.80 to 3.05 days with *L. lecanii* concentrations and from 2.30 to 3.16 with *B. bassiana* concentrations on adult aphids. Results showed that LC_{50} and LT_{50} values *L. lecanii* were lower than *B. bassiana.* Therefore, *L. lecanii* has higher virulence compared with *B. bassiana* on adult aphids of *M. rosae*.

Keywords: *Macrosiphum rosae, Beauveria bassiana, Verticillium lecanii*, LC_{50}, LT_{50}.

1. Introduction

The rose aphid, *Macrosiphum rosae* (L.), is an important pest on rose plants. This pest caused the deformation of the leaf blades, the shortening of shoots, petioles and deformation of the flowers (Salem and Abdel-Raheem, 2015). In addition, this aphid secretes honey-dew and was caused saprophytic fungi developing on plants (Cichocka, 1980; Jaskiewicz, 2006). Aphids may persist throughout the year as colonies on roses (especially in mild winters) and sometimes over winter as eggs on roses (Blackman and Eastop, 2006).

M. rosae has been controlled predominantly by using chemical insecticides, such as primicarb, imidacloprid, parathion, malation, but this method has caused problems for the environment (Talebi Jahromi, 2011). In addition, some studies indicate that chemical insecticides caused resistance in pest populations (Foster *et al.*, 1998).

The biological control with entomopathogenic fungi is gaining importance in pest management programs. In addition, among the different biological agents, entomopathogenic fungi have several advantages compared with the conventional insecticides. Entomopathogenic fungi are, for example, inexpensive, easy for application, high efficiency, non-hazardous for human and ecosystem (Lacey *et al.*, 2001). Therefore, these advantages have led to the commercialization of a large number of new fungus-based biopesticide products (Faria and Wraight, 2007)

Lecanicillium lecanii (Zimmerman) and *Beauveria bassiana* (Balsamo) have been recognized as entomopathogenic fungi with high potential in biological control of aphids (Askary *et al.*, 1998; Derakhshan *et al.*, 2007; Abd EI-Salam and El-Hawary, 2011). The purpose of this research is to evaluate the bio-efficacy of *Verticillium lecanii* and *B. bassiana* on adult aphids of *M. rosae* under laboratory condition

2. Materials and Methods

2.1. Insect Rearing

Rose aphids were collected from the rose plants of the University of Mohaghegh Ardabili, Ardabil, Iran. Thereafter, aphids were transferred to rose plants of Floribunda cultivar. All the treated Petri dishes were

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maintained at 25 ± 1 °C, 80 ± 5 % RH and a photoperiod of 12: 12 h (L: D) in an incubator.

2.2. Entomopathogenic Fungi

Commercial formulation enthomopathogenic fungi of *B. bassiana* and *V. lecanii* were obtained from Sadrabiotech Company of Iran.

2.3. Bioassay

Five different concentrations ($1×10^8$, $1×10^7$, $1×10^6$, $1×10^5$, $1×10^4$ spores/ml) were prepared for *B. bassiana* and *V. lecanii.* Conidial suspensions were vortexes for 5 min to produce a homogeneous suspension. Each concentration was replicated six times and each replication consisted of 10 adult aphids. Pathogenic fungi bioassay was conducted by putting 1µl from each concentration on insect body by microapplicator as topical application method. One day old adult aphids were transferred using a camel hairbrush on broad rose leaves into Petri dishes (11 cm diameter) that were filled with thick layer of 0.1% agar. Totally, 60 aphids were used for each treatment. For controls, adult aphids were treated with distilled water. The mortality data were recorded over a period of five days and rose fresh leaves were used each day for feeding aphids.

2.4. Statistical Analysis

The LC_{50} and LT_{50} values were calculated using the Probit procedures with SPSS for Windows® release 19.0. The percent corrected cumulative mortality of each fungus was subjected to ANOVA test and the means were compared by the Tukey test, using SPSS 19.0 software program (SPSS, 2009). For the correction mortality data with that in control used the Abbott's formula (Abbott, 1925).

3. Results and Discussion

The data presented in Table 1 shows the LC_{50} values of *V. lecanii* and *B. bassiana* at 3th day post treatment on *M. rosae*. According to this table, the LC_{50} value of *V. lecanii* is lower than *B. bassiana* on aphids (Figures 1 and 2). Therefore, *V. lecanii* is more pathogenic compared with *B. bassiana* on adult aphids *M. rosae*. Abd El-salam and El-Hawary (2011) reported that LC_{50} *L. lecanii* is lower than *B. bassiana* on *Aphis craccivora* (Koch). In addition, Sarnyaya *et al.* (2010) indicated that *V. lecanii* was more pathogenic than *B. bassiana* against adults *Brevicoryne brassica* (Linnaeus). Therefore, the similarity between the present and the above-mentioned studies indicates that *L. lecanii* is more pathogenic compared with *B. bassiana* on aphids. Abdel-Raheem *et al*. (2015) studied the efficacy of *B. bassiana* and *V. lecanii* against three life phases of *Tuta absoluta*. Results indicated that LC_{50} *B. bassiana* is lower compared to *V. lecanii* on larvae (Neonate- 2nd and 3rd instare). Therefore, *B. bassiana* is more effective than *V. lecanii* on *T. absoluata*, which disagrees with our findings. Therefore, this difference might be due to the difference in the virulence of fungal isolates and the host species.

LC_{50} values of *V. lecanii* and *B. bassiana* were obtained: 1.38×10^4 and 2.66×10^5 spore.ml^{-1}, respectively. Nazemi *et al.* (2014) found that LC_{50} of *Lecanicillium longisporum* on the aphid *Cinara pini* (Linnaeus) was 1.2×10^6 spore.ml^{-1}. Vu *et al.* (2007) reported that the LC_{50} value of *L. lecanii* against *Myzus persicae* (Sulzer) was 1.65×10^6 spore.ml^{-1} after six days of the treatment. In addition, Akmal *et al*. (2013) indicated that LC_{50} *B. bassian* on *B. brassica* and *Schizaphis graminum* (Rondani) were 6.28×10^5 and 6.76×10^6 spore.ml^{-1}, respectively. Comparing the above-mentioned studies with the present study indicated that LC_{50} *L. lecanii* and *B. bassiana* on *M. rosae* are lower

Lethal time values for 50% mortality (LT_{50}) ranged from 1.80 to 3.05 days with *L. lecanii* concentrations and from 2.30 to 3.16 days with *B. bassiana* concentrations on adult aphids *M. rosae* (Table 2). Results indicated that increasing concentration caused reducing the LT_{50} and the lowest LT_{50} were obtained at the highest concentration (10^8spore/ml). The LT_{50} values of *L. lecanii* in all concentration were significantly lower compared with *B. bassiana* on adult aphids *M. rosae*. Sarnyaya *et al.* (2010) reported that the LT_{50} values of *L. lecanii* and *B. bassiana* on *A. carcivora* were 3.90 to 7.25 and 3.63 to 6.88 days, respectively. Therefore, their findings disagree with the results of the present research.

Also, the present study indicated that mortality adult aphids increased with increasing the conidial concentrations and the time placed after treatment. A 100% mortality was obtained in concentrations 10^7 and 10^8 spore/ml with *L. lecanii* on the 4th day (Table 4). But *B. bassiana* caused 100% mortality by these concentrations on the 5th day post treatment (Table 3). Loureiro and Moino (2006) found that *B. bassiana* caused 100% mortality on *M. persicae* at 10^6 spore's ml^{-1} on the 7th day of treatment. Abd El-salam and El-Hawary (2011) recorded 100 % mortality of adults *A. carcivora* with *L. lecanii* applied at concentration 1.0 ml conidial suspension (1×10^6 spore/ml) after three days. So, Sarnya *et al.* (2010) reported that *L. lecanii* caused 100% mortality at 10^7 and 10^8 spore.ml^{-1}, but *B. bassiana* caused mortality 96% at 10^8 spore.ml^{-1} after 7 days. Results of the studies above are different from our findings; therefore, this difference might be due to the difference in the virulence of fungal isolates and the host species.

This leads to the conclusion that LC_{50} and LT_{50} of *V. lecanii* against *M. rosae* are lower compared with *B. bassiana*. Therefore, the present study suggests that the entomopathogenic, *V. lecanii* is more suitable for control *M.rosae* compared to *B. bassiana.*

Table 1. LC_{50} values of *V. lecanii* and *B. bassiana* against adult aphides, *M. rosae*.

Fungi	N	LC_{50} (spore/ml)	95% Confidence lower – upper	Slope ±SE	X^2
Verticillium lecanii	360	$1.38×10^4$	$8.82×10^2$-$6×10^4$	0.29±0.05	4.30
Beauveria bassiana	360	$2.66×10^5$	8.59- $6.79×10^6$	0.11±0.06	0.62

Table 2. LT_{50} values of *V. lecanii* and *B. bassiana* against adult aphides, *M. rosae.*

Fungi	Concentration(spore/ml)				
	10^8	10^7	10^6	10^5	10^4
Verticillium lecanii	1.80	2.12	2.52	2.83	3.05
Beauveria bassiana	2.30	2.60	2.84	3.07	3.16

Table 3. Mortality percentage of *M. rosae* treated with *B. bassiana*

Concentration (Spore/ml) \ Day	1	2	3	4	5
Control	0	0	0	0	0
10^4	0	16.66	45	71.66	100
10^5	0	20	48.33	71.66	100
10^6	0	26.66	50	78.33	100
10^7	15	31.66	55	86.66	100
10^8	18.33	38.33	65	93.33	100

Table 4. Mortality percentage of *M. rosae* treated with *V. lecanii.*

Concentration (Spore/ml) \ Day	1	2	3	4	5
Control	0	0	0	0	0
10^4	0	18.33	51.66	75	100
10^5	0	23.33	60	80	100
10^6	0	36.66	65	90	100
10^7	15	45	75	100	100
10^8	20	53	93.33	100	100

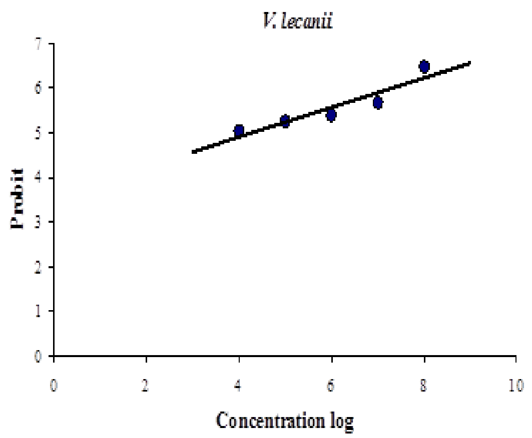

Figure 1. Probit graph for *V. lecanii* on 3 day

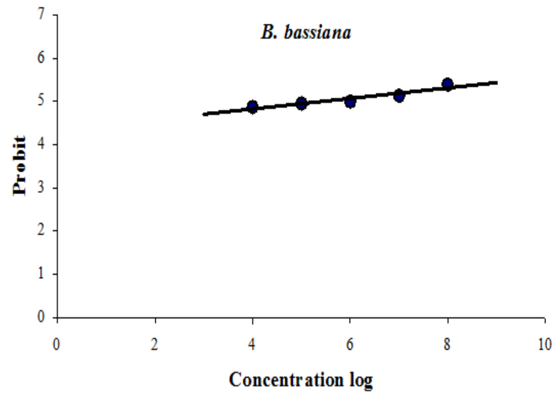

Figure 2. Probit graph for *B. bassiana* on 3 day

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