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# Pathogenicity of *Lecanicillium muscarium* against *Ricania simulans*

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## Abstract

*Lecanicillium muscarium* (Petch) Zare et Gams is a widely occurring entomopathogenic fungus. The effect of *L. muscarium* against *Ricania simulans* (Walker) (Rhynchota Ricaniidae) was studied under laboratory and field conditions. In laboratory studies, six isolates of *L. muscarium* were assessed against nymphs of *R. simulans*, at a single dose (1 x $10^7$ conidia/ml) on tea leaflets. Mortality percentage caused by *L. muscarium* isolates after a seven-day period varied from 50.95 to 74.76% and median lethal time ($LT_{50}$) values ranged from 2.34 to 3.90 days. In a field experiment, *L. muscarium* strain Lm4 was assessed against nymphs and adults of *R. simulans*, at a single dose (1 x $10^7$ conidia/ml) on kiwifruit plants. The $LT_{50}$ values for nymphs and adults of *R. simulans* were 4.18 days and 6.49 days, respectively. The results of the field study indicated that *R. simulans* nymphs were more susceptible to the fungus than adults. *L. muscarium* strain Lm4 could be considered as an environmentally friendly alternative for biocontrol of *R. simulans*.

**Key words:** Biological control, *Lecanicillium muscarium*, entomopathogenic fungi, *Ricania simulans*.

## Introduction

The family Ricaniidae is a group of hemipteran insects (Rhynchota) containing over 40 genera and 400 species worldwide. Thus, it is one of the smaller families in the planthopper superfamily (Fulgoroidea). The family is mainly distributed in tropical Africa, Asia and in Australia, with a few species occurring in the Palearctic regions (Xu *et al*., 2006). Most have triangular wings either opaque or with delicate lacy brown patterning. A few species have glassy-clear wings. The Ricaniidae consists of common herbivores in both agricultural and natural systems, often causing severe damage to their host plants. Planthoppers have also been known worldwide as vectors of different plant pathogens such as viruses and bacteria (Nault and Ammar, 1989). *Ricania simulans* (Walker) is widespread in the Black Sea Region of Turkey with an extensive host range including fruits, vegetables and ornamentals. It is a serious pest of kiwifruit *Actinidia deliciosa* (Chevalier) Liang et Ferguson and tea (*Camellia sinensis* L.) plants in this region of Turkey.

The negative aspects of conventional pest control have led to the investigation of alternative methods such as biological control. In the recent years, biological pest control, including the use of entomopathogenic fungi, has been attracting much attention. Entomopathogenic fungi have shown their great potential to control insect pests, both in field and under greenhouse conditions (Inglis *et al.,* 2001). Entomopathogenic fungi species formerly within the genus *Verticillium*, section Prostrata were recently reclassified using morphological characteristics and Internal Transcribed Spacer region (ITS) sequences, as genus *Lecanicillium* (Zare and Gams, 2001). In this new classification, *Lecanicillium lecanii* (Zimmermann) Zare et Gams is the type species and considered as a species complex, which includes *L. lecanii*, *Lecanicillium muscarium* (Petch) Zare et Gams and *Lecanicillium longisporum* (Petch) Zare et Gams. Species of *Lecanicillium* have a wide host range and have been isolated from a variety of insect orders (Zare and Gams, 2001).

Strains of *L. muscarium* have been isolated from aphids, scales, whiteflies, thrips and other insects in various regions of the world and have also been proved to be pathogenic against different insects (Hall, 1981; Cuthbertson and Walters, 2005; North *et al.,* 2006; Askary and Yarmand, 2007; Goettel *et al.,* 2008; Anand and Tiwary, 2009; Anand *et al.,* 2009). *L. muscarium* has also been shown to be an important natural enemy of *Scolypopa australis* (Walker) (Rhynchota Ricaniidae) in kiwifruit orchards (Marshall *et al.,* 2003). It has been commercialized worldwide as the biopesticides Mycotal against whiteflies and thrips and Verticillin against whiteflies, aphids and mites (Faria and Wraight, 2007).

The objective of this study was to determine the pathogenicity of *L. muscarium* against *R. simulans* under laboratory conditions for the nymphal stage and under field conditions for nymphal and adult stages.

## Materials and methods

*L. muscarium* isolates Lm1, Lm2, Lm3, Lm4, Lm5 and Lm6 were originally obtained from infected *Issus* sp. (Rhynchota Issidae,) at Trabzon province (Black Sea Region of Turkey). Fungal strains, maintained in tubes containing Sabouraud dextrose agar and deposited in the fungal culture collection of the Department of Plant Protection, Faculty of Agriculture, Atatürk University,

Erzurum-Turkey, were cultured on potato dextrose agar and incubated at 25 °C for 10 days. Conidia were harvested with deionised water containing 0.03% Tween 80 and filtered through 4 layers of sterile cheesecloth to remove mycelium. Conidia were counted under a compound microscope using a haemocytometer to calibrate a suspension of 1 x $10^7$ conidia/ml of each strain.

The viability of conidia was evaluated using a method modified from Wekesa *et al*. (2005). The conidial suspension was adjusted to $10^6$ conidia/ml and 0.1 ml sprayed on to water agar plates. After 24 h at 25 °C, cover slips were applied and the number of germinated conidia counted. Conidia were examined under a microscope at 400X magnification. A conidium was considered germinated when the germ tube of any length was visible. A total of 200 conidia were evaluated and relative percent germination was calculated.

### Virulence of *L. muscarium* isolates to *R. simulans*

*L. muscarium* isolates Lm1, Lm2, Lm3, Lm4, Lm5 and Lm6 were screened for their virulence to fourth nymphal stages of *R. simulans* collected directly from tea (*C. sinensis*) plants in commercial orchards in the Rize province of Turkey.

Tea leaves were placed in a Petri dish (15 cm in diameter) and sprayed with conidial suspensions using a hand sprayer. Two millilitres of a single dose of 1 x $10^7$ conidia/ml were sprayed on both leaf surfaces, left to dry for twenty minutes in a laminar flow hood before placing the leaves on wet cotton wool in Petri dishes. Nymphs of *R. simulans* were introduced into each Petri dish and the dishes were loosely capped to prevent their escape. The control leaves were treated with sterile distilled water containing 0.03% Tween 80. All dishes were incubated at 23 ± 2 °C for 5 days with a 16L:8D photoperiod and inspected daily. Dead nymphs were surface sterilized and transferred to Petri dishes lined with moist tissue paper to allow the growth of the fungus on cadaver surface. The presence of *Lecanicillium* was verified by microscopical inspection of the fungi for the presence of diagnostic verticils of conidiogenous cells on the cadavers. The experimental design was a randomized complete block with three replicates, and each replicate consisted of 30 nymphs of *R. simulans*.

### Field efficacy of Lm4 strain of *L. muscarium* against *R. simulans*

For the field experiments, fourth nymphal stages and adults of *R. simulans* were collected directly from kiwifruit (*A. deliciosa*) plants in commercial orchards in the Rize province. The kiwifruit was used as the bioassay plant.

Humidity, temperature (min, max) and rainfall at orchards trails were monitored daily for the duration of the trials (June to August 2009) at a meteorological station located at the Atatürk Tea and Horticulture Research Institute in Rize, neighbouring the experimental site (figure 1).

The kiwifruit plants were selected and cheesecloth cages (30 x 75 cm) were attached to each twig. In each cage, 10 nymphs of *R. simulans* were introduced. In the second set of bioassays 10 adults of *R. simulans* were used in each cage. After introducing the nymphs and adults of *R. simulans*, ten milliliters of *L. muscarium*-LM4 suspension containing 1 x $10^7$ conidia/ml, were sprayed with a standard plastic hand sprayer onto leaf surfaces and twigs were then labelled. Control twigs were treated only with water. The total number of dead nymphs and adults was recorded every day until 7 days after treatment. *L. muscarium*-LM4 was consistently reisolated from dead nymphs and adults. The experimental design was a randomized complete block with four replicates, and each replicate consisted of 40 nymphs and adults of *R. simulans*.

### Data analyses

The median lethal time ($LT_{50}$) was calculated according to Berón and Diaz (2005). Only data on mortality caused by mycosis were used for statistical analysis. Data were evaluated by analysis of variance (ANOVA) with CoStat Version 6.2 software (CoHort Software, Monterey, CA), and differences are presented by the results of Duncan's multiple range test. Values of $P < 0.05$ were considered significant.

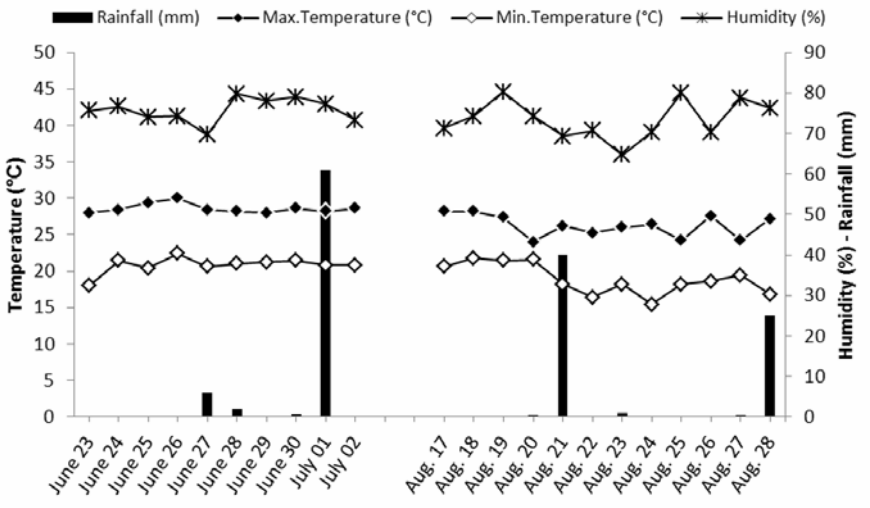


**Figure 1.** Summary of weather data in the Rize province during the 2009 field experiments.

## Results and discussion

Conidia viability was assessed before each experiment and almost 100% of the conidia of all isolates germinated. The median lethal time ($LT_{50}$) for the six isolates was varied from 3.90 to 4.80 days and the total percentage mortality varied from 80 to 100% at 5 days post-treatment at laboratory conditions (figure 2). Significant differences were found in the $LT_{50}$ values of *R. simulans* nymphs between the *L. muscarium* isolates (table 1). *R. simulans* nymphs treated with *L. muscarium* strain Lm4 had the fastest mortality rate, with 100% of the nymphs dead within 5 days (figure 2). Thus, *L. muscarium* strain Lm4 was considered to have the highest pathogenicity to the *R. simulans* nymphs among the isolates tested. Fungal infections were confirmed with light microscopy for all dead nymphs of *R. simulans*, and the fungus was re-isolated from the cadavers.

The high pathogenicity of *L. muscarium* strain Lm4 against nymphs of *R. simulans* in the laboratory indicated that the strain had potential as a microbial control agent. The high mortality observed might be because of the high concentration used in the bioassay, which could mask variation in susceptibility between developmental stadia. Several isolates of *L. muscarium* have been shown to be effective in controlling nymphs of *S. australis* on vine [*Pandorea pandorana* (Andrews) Steenis] in laboratory trials (Marshall *et al*., 2003).

Nymphs and adults of *R. simulans* were treated with the conidia of *L. muscarium* strain Lm4 during the field trials. Following inoculation with the Lm4 strain of *L. muscarium*, the $LT_{50}$ values for *R. simulans* nymphs and adults were 4.18 days and 6.49 days, respectively. Figure 3 shows the mortality of nymphs and adults after treatment. After 2, 4 and 6 days of treatment, 15.86%, 60.95% and 85.71% of *R. simulans* nymphs had died, respectively. Adult mortality was about 5.55% two days after treatment and it increased to 16.82, 36.84 and 56.31% after four, six and eight days after treatment, respectively. Nymphal stages appear to be more susceptible to infection than the adults.

The field experiments were conducted during the summer, when the average temperature ranged between 18.4 °C at night and 28.3 °C during the day, with a relative humidity average of 74% and rainfall was 11.6 mm (figure 1). In particular, the pathogenicity of *L. muscarium* is associated with high moisture levels (Jackson et al., 1985). However, Vidal *et al.* (2003) found that *Lecanicillium* isolates were virulent over a range of moisture levels (53-98.5%).

The significance of *L. muscarium* as one of the effective biological control agents against insects in the world have been reviewed (Hall, 1981; Marshall *et al*., 2003; Cuthbertson and Walters, 2005; North *et al*., 2006; Askary and Yarmand, 2007; Goettel *et al*., 2008; Anand and Tiwary, 2009; Anand *et al*., 2009). Entomopathogenic fungi have shown great potential for the management of various arthropod pests (Butt *et al*., 2001; Eken and Demirci, 1997; Hajek and St. Leger, 1994; Inglis *et al*., 2001). The use of biological control agents such as pathogens as part of an integrated pest management strategy could reduce the dependence on chemical control.

**Table 1.** Median lethal time ($LT_{50}$) of *R. simulans* nymphs treated with different isolates of *L. muscarium* at a concentration 1 x $10^7$ conidia/ml.

| Isolate | $LT_{50}$ (days) |
|---|---|
| Lm1 | 4.43 ab* |
| Lm2 | 4.55 ab |
| Lm3 | 4.58 ab |
| Lm4 | 3.90 b |
| Lm5 | 4.47 ab |
| Lm6 | 4.80 a |
| Control | n.o. |
| LSD | 0.65 |

*P < 0.05, within columns, means followed by different letters are significantly different.

n.o. - Mortality caused by mycosis was not observed in the bioassays.

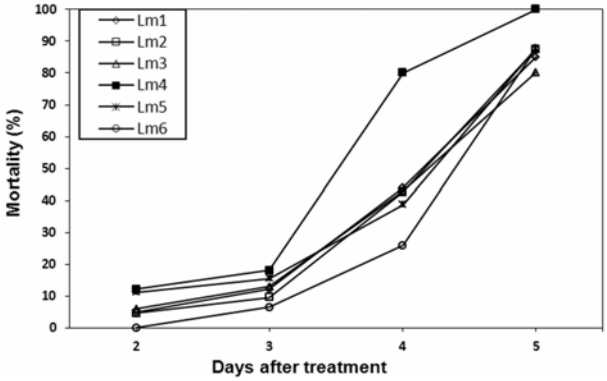


**Figure 2.** Mortality rates of *R. simulans* nymphs treated with different strains of *L. muscarium* under laboratory conditions.

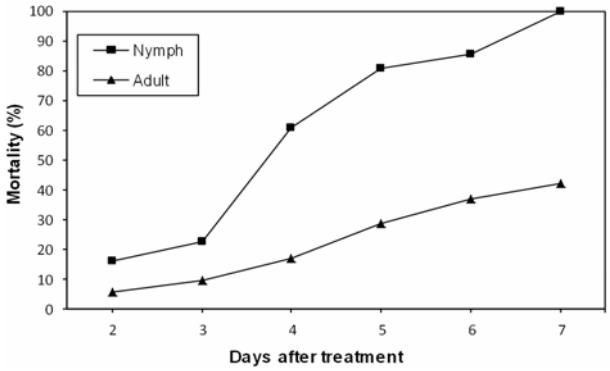


**Figure 3.** Mortality rates of *R. simulans* nymphs and adults treated with *L. muscarium* strain Lm4 under field conditions.

The susceptibility of different developmental stages of *R. simulans* to strain Lm-4 of the entomopathogenic fungus *L. muscarium* was shown in this study. This strain had nearly equal virulence against *R. simulans* under laboratory and field conditions. It means that a control of *R. simulans* with *L. muscarium* Lm-4 seems possible under field conditions. A method to increase

the effectiveness of *L. muscarium* Lm-4 in field is needed if this strain is to be used effectively in the microbial control of *R. simulans.* This is the first report to demonstrate the pathogenic effect of *L. muscarium* against *R. simulans*.

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