**Pathogenicity of the fungus *Lecanicillium longisporum* against *Sipha maydis* and *Metopolophium dirhodum* in laboratory conditions**

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**Abstract:** This study aimed to evaluate the susceptibility of two cereal aphids, *Sipha maydis* (Passerini) and *Metopolophium dirhodum* (Walker), to the entomopathogenic fungus, *Lecanicillium longisporum* (Zimm.) Zare and Gams strain LRC 190, under controlled conditions. The conidial suspension of the fungus was administered using a sprayer on the whole plant over apterous adult aphids. The results indicated that both aphid species were susceptible to *L. longisporum* and that aphid populations were significantly reduced, compared to the control. Nine days after treatment, the LC50 value of the fungus was obtained as $5.9 \times 10^5$ and $3.2 \times 10^6$ conidia/ml for *S. maydis* and *M. dirhodum*, respectively. The LT50 value of the fungus at a concentration of $10^8$ conidia/ml was obtained as 2.9 and 4.4 days for *S. maydis* and *M. dirhodum*, respectively. The results demonstrated that there was a varying susceptibility to the fungus between aphid species. The estimated LC50 and LT50 indicated that *L. longisporum* was more virulent to *S. maydis* than to *M. dirhodum*. The LT50 and LT50 decreased as the conidial concentration increased. This is the first study to demonstrate the susceptibility of *S. maydis* to the entomopathogenic fungi. The present study suggests that *L. longisporum* has high virulence against the aphids *S. maydis* and *M. dirhodum*. Further research with an emphasis on greenhouse and field tests are required, however, before making any decision about using the fungus in a control program.

**Key words:** biological control, Entomopathogenic fungi, *Lecanicillium longisporum*, *Metopolophium dirhodum*, *Sipha maydis*

**Introduction**

Aphids are one of the major constraints to wheat production worldwide. *Diuraphis noxia* (Mordvilko), *Sitobion avenae* (F.), *Schizaphis graminum* (Rondani), *Rhopalosipham padi* (L.), *Metopolophium dirhodum* (Walker), and *Sipha maydis* (Passerini) are the most important cereal aphids (Rassipour et al. 1996; Blackman and Eastop 2006). *Sipha maydis* (Hemiptera: Chaitophoridae) feeds on numerous species. It feeds on over 30 genera of Gramineae (Blackman and Eastop 2006), in which wheat and barley are the most preferred hosts (Corrales et al. 2007). This aphid can transmit *Cucumber mosaic virus* (CMV) and *Barley yellow dwarf virus* (BYDV) (Blackman and Eastop 2000). The aphid is widespread in Eastern Europe, the Middle East, Central Asia, North and South Africa, and South America (Blackman and Eastop 2006; Corrales et al. 2007). In Iran, *S. maydis* presents in wheat fields at the seedling and maturing stages of host plants and can severely damage wheat (Rassipour et al. 1996; Sabzalian et al. 2004). The rose grain aphid, *M. dirhodum* (Walker) (Hemiptera: Aphididae), is one of the most serious species found in almost all grain-producing regions of the world (Dixon 1987). *Metopolophium dirhodum* feeds on leaves and is considered as a vector of BYDV (Blackman and Eastop 2006). The primary hosts are wild and cultivated *Rosa* spp. and the secondary hosts are numerous species of cereals and grasses.

In the last few decades, biological control, including the use of entomopathogenic fungi, is an emerging strategy used for controlling aphids. This strategy is especially used in high-value crops to substitute or complement traditional control. Traditional control refers to control based mainly on the use of traditional chemical insecticides. The entomopathogenic fungi play an important role in aphid biological control because aphids have morphological, biological and ecological characteristics making them susceptible to fungal pathogens. The pathogenic fungi are able to cause epizootics, drastically reducing aphid populations (Steinkraus 2006).

Recently, the entomopathogenic fungi belonging to the species *Verticillium lecanii* (Zimm.) Viegas have been attributed to a new genus, *Lecanicillium*, and in addition have been split into different species (Zare and Gams 2001). Highly virulent and epizootically efficient strains of *Lecanicillium* spp. have been mass-produced, and considered as biocontrol agents (BCAs) against some insect pests. Vertalec® and Mycotal® (Koppert Biological Systems, The Netherlands) are commercial formulations of developed *Lecanicillium* spp. strains (*L. longisporum* and...
Materials and Methods

Aphid culture

The apterous aphids S. maydis and M. dirhodum were collected from barley and oat plants, respectively. The aphids were reared on wheat (Triticum aestivum L.) cv. Pishtaz in a greenhouse at 25 : 20°C (day : night) and a 16 : 8 h (day : night) photoperiod. The species were identified by Olivera Petrovic-Obradovic, Belgrade University, Serbia. The aphid colonies were cultured on wheat for several generations before the commencement of the experiment. Test aphids were obtained by allowing viviparous aphids to produce nymphs for 24 h on wheat plants. Neonate nymphs were then reared as synchronous cohorts.

Preparation of fungus inoculum

The fungus L. longisporum strain LRC 190 was provided by Dr. Reza Talaei-Hassanloei, the University of Tehran, Iran. This fungus was isolated from Macrosiphoniella sanbornii in England by Mark S. Goettel. The fungus was incubated on Potato Dextrose Agar (PDA) medium, in 9 cm diameter glass Petri dishes for 12–14 days at 28°C in darkness. Conidia were harvested from the media by flooding with sterile 0.2% (v/v) Tween 20 and stirring with a glass bar. The conidia suspension was filtered through sterile cheesecloth to remove mycelium and was enumerated with an improved Neubauer haemacytometer. The conidia suspension was then diluted to give a series of concentrations between 10^4 to 10^8 conidia/ml. The viability of conidia (> 95% germination after a 24-h incubation on PDA medium) was confirmed before the onset of bioassay.

Pathogenicity bioassay

The pathogenicity of L. longisporum was determined on newly emerged apterous adults using five concentrations from 10^4 to 10^8 conidia/ml in three replicates. Three one-week-old seedlings of wheat cv. Pishtaz grown in a pot were used as the experimental unit. Fifteen apterous adults of barley aphid S. maydis and rose-grain aphid M. dirhodum were transferred separately to the wheat seedlings of each pot and allowed to settle on leaves for 3–4 h. A camel hair brush was used to transfer the aphids to the seedlings. The seedlings of each pot were treated with 10 ml conidia suspension using a small handheld sprayer. The control was sprayed with sterile distilled water and 0.2% Tween 20. The plants were air-dried for 30 min to remove excess suspension. The pots were then covered individually with a transparent sleeve sheet to prevent aphid escape. Humidity was maintained by placing a water dish on the bottom of each pot. The experiment was conducted in a greenhouse at 25 : 20°C (day : night), 16 : 8 h (day : night) photoperiod and 70–75% (day : night) relative humidity (RH).

Aphid mortality was recorded daily for 12 days. Newly born nymphs were counted and removed daily from the plants. Aphid cadavers were disinfected using 2% sodium hypochlorite, and rinsed with sterile distilled water. The cadavers were then incubated in a humidity chamber (100% RH) into a Petri dish on damp filter paper to ensure that death was due to fungal treatment. Only aphids which exhibited fungal sporulation were considered to have died from the fungus treatment. The whole experiment was conducted twice.

Statistical analysis

The bioassays were arranged as a randomized complete design. Aphid mortality was corrected for the control using Abbott’s formula (Abbott 1925). The control mortality never exceeded 5%. The Kolmogorov-Smirnov test was used to ensure that data satisfied the assumptions of the analysis of variance (ANOVA). At 9 days after treatment (DAT), the mortality rate of the aphid species was subjected to two-way ANOVA. For mean
comparisons, the mortality rate of each aphid species was subjected to one-way ANOVA followed by Tukey HSD test. The analyses were conducted using SPSS 16.0 software (SPSS 2007). The LC$_{50}$ values at 9 DAT and LT$_{50}$ at two concentrations ($10^7$ and $10^8$ conidia/ml) were obtained by probit analysis using the POLO-PC program (LeOra software 1987). The LC$_{50}$ or LT$_{50}$ values were considered to be significantly different when their associated 95% Confidence Intervals (CIs) were not overlapped (Tabashnick and Cushing 1987). For a group of 15 aphids, the net reproductive rate ($R_0$) over the 12 days following the treatment, was estimated as:

$$R_0 = \sum l_x m_x,$$

where $l_x$ – the probability of surviving from day $x$ to day $x + 1$, and $m_x$ – the average number of offspring produced by an individual on day $x$ (Stearns 1992).

**Results**

The results expressed as corrected percentage mortality are depicted in figure 1. In all the tests, the control mortalities were below 5%. Mortality of *S. maydis* at high concentrations ($10^7$ and $10^8$ conidia/ml) started two DAT. However, mortality of *M. dirhodum* at all the concentrations started three DAT (Fig. 1). At 9 DAT, the lowest concentration ($10^4$ conidia/ml) caused 10.15 and 4.44% mortality on *S. maydis* and *M. dirhodum*, respectively. At this time, the highest concentration ($10^8$ conidia/ml) caused 94.36% mortality on *S. maydis* and 77.14% on *M. dirhodum*. In contrast to *S. maydis*, mortality of *M. dirhodum* did not exceed thereafter (Fig. 1). The results demonstrated that adult mortality increased significantly as the conidial concentration increased (Table 1). There were significant differences in the susceptibility of two aphid species on day nine. The results indicated that the main effects of aphid species ($F_{(1,59)} = 64.24; p < 0.001$), fungal concentration ($F_{(4,59)} = 333.59; p < 0.001$), and the interaction effect of aphid × fungal concentration ($F_{(4,299)} = 2.87; p < 0.001$), were significant on aphid mortality. Most infected aphids deposited nymphs before being killed by the pathogen, although the daily fecundity was often much lower in treated groups than in the control (Fig. 2). The results indicated that $R_0$ decreased significantly as conidial concentration increased (Table 2).

A more appropriate comparison was obtained using probit analysis. *Lecanicillium longisporum* showed pathogenic activity against aphids with varying levels of virulence between aphid species. The dose-mortality responses of two aphid species to *L. longisporum* were compared in terms of differences in slope and/or intercept of probit regressions, and the LC$_{50}$ values (Table 3). The slopes of probit mortality regressions for the fungus on *S. maydis* (0.74) was significantly greater than that on *M. dirhodum* (0.58), as revealed by rejection of the likelihood significance.

**Fig. 1.** Cumulative mortality of *S. maydis* (A) and *M. dirhodum* (B) adults following treatment by different concentrations of the fungus *L. longisporum*.
Table 1. One-way analysis of variance (ANOVA) of percent mortality (mean ±SE, n = 6) of *S. maydis* and *M. dirhodum* adults nine days after treatment by different concentrations of the fungus *L. longisporum*

<table>
<thead>
<tr>
<th>Concentration [conidia/ml]</th>
<th>Mean (±SE)*</th>
<th><em>S. maydis</em></th>
<th><em>M. dirhodum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁴</td>
<td>10.15 (±1.42) a</td>
<td>4.44 (±1.40) a</td>
<td></td>
</tr>
<tr>
<td>10⁵</td>
<td>27.30 (±3.07) b</td>
<td>19.28 (±2.00) b</td>
<td></td>
</tr>
<tr>
<td>10⁶</td>
<td>54.32 (±2.23) c</td>
<td>40.87 (±2.85) c</td>
<td></td>
</tr>
<tr>
<td>10⁷</td>
<td>85.16 (±3.39) d</td>
<td>64.68 (±2.32) d</td>
<td></td>
</tr>
<tr>
<td>10⁸</td>
<td>94.36 (±2.68) e</td>
<td>77.14 (±2.49) e</td>
<td></td>
</tr>
</tbody>
</table>

Results of ANOVA

\[ F(4, 29) = 185.715 \]

\[ p < 0.001 \]

*values in columns with similar letters are not significantly different (Tukey HSD test at 5% level)*

![Graph A](image1)

Fig. 2. Fecundity of *S. maydis* (A) and *M. dirhodum* (B) (n = 15) following treatment by different concentrations of the fungus *L. longisporum*

Table 2. One-way analysis of variance (ANOVA) on *R₀* (mean ±SE, n = 6) of *S. maydis* and *M. dirhodum* adults over 12 days, following treatment by different concentrations of the fungus *L. longisporum*

<table>
<thead>
<tr>
<th>Concentration [conidia/ml]</th>
<th>Mean (±SE)*</th>
<th><em>S. maydis</em></th>
<th><em>M. dirhodum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>The control</td>
<td>19.05 (±0.49) a</td>
<td>28.92 (±0.52) a</td>
<td></td>
</tr>
<tr>
<td>10⁴</td>
<td>14.33 (±0.14) b</td>
<td>25.65 (±0.71) b</td>
<td></td>
</tr>
<tr>
<td>10⁵</td>
<td>11.15 (±0.19) c</td>
<td>18.10 (±0.28) c</td>
<td></td>
</tr>
<tr>
<td>10⁶</td>
<td>7.27 (±0.14) d</td>
<td>13.07 (±0.51) d</td>
<td></td>
</tr>
<tr>
<td>10⁷</td>
<td>3.70 (±0.20) e</td>
<td>8.05 (±0.46) e</td>
<td></td>
</tr>
<tr>
<td>10⁸</td>
<td>1.44 (±0.09) f</td>
<td>4.87 (±0.19) f</td>
<td></td>
</tr>
</tbody>
</table>

Results of ANOVA

\[ F(5, 35) = 693.339 \]

\[ p < 0.001 \]

*values in columns with similar letters are not significantly different (Tukey HSD test at 5% level)*
Table 3. Probit analyses of L. longisporum pathogenicity after nine days, against adults of S. maydis and M. dirhodum

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>n*</th>
<th>Probit mortality-concentration</th>
<th>t ratio</th>
<th>Heterogeneity</th>
<th>g factor (0.95)</th>
<th>Concentration [conidia/ml × 10^9]*</th>
<th>LC50</th>
<th>LC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. maydis</td>
<td>540</td>
<td>0.74 (±0.65)</td>
<td>-1.32 (±0.15)</td>
<td>11.47</td>
<td>0.34</td>
<td>0.029</td>
<td>59.27 a (35.40–95.45)</td>
<td>3050.12 a (1583.66–7161.99)</td>
</tr>
<tr>
<td>M. dirhodum</td>
<td>540</td>
<td>0.58 (±0.56)</td>
<td>-1.45 (±0.15)</td>
<td>10.32</td>
<td>0.69</td>
<td>0.036</td>
<td>315.89 b (178.51–569.79)</td>
<td>49788 b (18368–200830)</td>
</tr>
</tbody>
</table>

*total number of test aphids including control
*values in columns with similar letters are not significantly different using the method of overlapping limits; CL – Confidential Limits

Table 4. LT50 and LT90 of L. longisporum pathogenicity against adults of S. maydis and M. dirhodum

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Concentration [conidia/ml]</th>
<th>Probit mortality-time</th>
<th>t ratio</th>
<th>Heterogeneity</th>
<th>g factor (0.95)</th>
<th>Time [day] (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. maydis</td>
<td>10^7</td>
<td>3.08 (±0.20)</td>
<td>-1.81 (±0.16)</td>
<td>14.99</td>
<td>0.28</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>10^8</td>
<td>3.54 (±0.23)</td>
<td>-1.68 (±0.16)</td>
<td>15.05</td>
<td>0.78</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>10^9</td>
<td>2.75 (±0.33)</td>
<td>-2.07 (±0.26)</td>
<td>8.11</td>
<td>0.84</td>
<td>0.058</td>
</tr>
<tr>
<td>M. dirhodum</td>
<td>10^7</td>
<td>2.94 (±0.34)</td>
<td>-1.89 (±0.25)</td>
<td>8.63</td>
<td>1.09</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Discussion

The present study demonstrated the pathogenicity of L. longisporum LRC 190 against S. maydis and M. dirhodum in whole plant assays. Many studies have shown that various aphid species were susceptible to Lecanicillium spp. (Kim et al. 2007; Vu et al. 2007; Diaz et al. 2009; Ganassi et al. 2010). This is the first data about the susceptibility of S. maydis to an entomopathogenic fungus. At 9 DAT, the LC50 value of L. longisporum LRC 190 against S. maydis was 5.9 × 10^5 conidia/ml. In contrast to S. maydis, the fungus had less virulence against M. dirhodum with the LC50 of 3.2 × 10^6 conidia/ml. Using the topical application method, the LC50 value of 1.2 × 10^6 conidia/ml was obtained for L. longisporum LRC 190 on the aphid Cinara pini 9 DAT (Nazemi 2012). In the present study, the LT50 value of the fungus at a concentration of 10^8 conidia/ml was obtained as 2.9, and 4.4 days, for S. maydis and M. dirhodum, respectively. At the same concentration (10^6 conidia/ml) of this fungus, the LT50 of 4.2 days was obtained against C. pini adults (Nazemi 2012). These results indicated that L. longisporum LRC 190 is less virulent against C. pini than S. maydis, and that the susceptibility of M. dirhodum and C. pini to L. longisporum LRC 190 appears to be similar. That S. maydis was more susceptible to L. longisporum than M. dirhodum and C. pini may be due to the small size of the S. maydis aphid compared to M. dirhodum and C. pini. The small soft (weakly sclerotized) bodies of aphids appear to present relatively few barriers to penetration by fungal pathogens. These pathogens specialize in infecting aphids. The small size of aphids may be related to the relatively short time required for some fungal pathogens to kill the host (Steinkraus 2006).

A comparison between the susceptibility of S. maydis and M. dirhodum to L. longisporum on the basis of LC50 and LC90 indices (Robertson and Preiser 1992), indicated the LC50 and LC90 of the fungus against the M. dirhodum were 5.3 and 16.3 times those against S. maydis. Our studies demonstrated that the pathogenicity of L. longisporum differed according to aphid species as previously demonstrated by Jackson et al. (1985) and Yokomi and Gottwald (1988). Also, differences in virulence on some aphid species were found among different isolates of L. lecanii (Vu et al. 2007), which could probably be explained as due to differences in their physiological characteristics (Jackson 1988).
et al. 1985; Cortez-Madrigal et al. 2003; Steinkraus 2006). It was also recorded by Helen et al. (2003) that A. fabae showed more susceptibility than M. persicae to the same fungus under the same temperatures.

By immersing aphids in conidial suspensions Feng et al. (1990) showed that the LC50 of V. lecanii (DNVL8701) was 7.0 × 10^5 conidia/ml on M. dirhodum under controlled conditions, suggesting that V. lecanii (DNVL8701) is more virulent than L. longisporum LRC 190 against M. dirhodum. In addition to variances among fungi, the difference might be due to the bioassay methods used. One of the most important parameter affecting virulence of the entomopathogenic fungi is germination speed (Steinkraus 2006). A positive relationship has been found between the speed of germination and the virulence of some L. lecanii isolates (Yokomi and Gottwald 1988; Diaz et al. 2009).

In another work, an isolate of the fungus V. lecanii from Alberta, was pathogenic under controlled conditions, to the aphid M. dirhodum, and there was a 32–85% decrease in aphid population (Harper and Huang 1986). In the present study, up to 77.14% mortality in M. dirhodum was induced by the concentration of 10^6 conidia/ml from L. longisporum LRC 190.

In the present study, the net reproduction rate of both aphids decreased significantly as the conidial concentration increased; a similar result was reported by Ashouri et al. (2004), Fournier and Brodeur (1999) and Vu et al. (2007). Daily fecundity of aphid females infected with L. longisporum initially increased but later decreased, probably in response to fungal invasion of host tissues (Askary et al. 1998). The daily fecundity of aphids decreased as the conidial concentration increased (Askary et al. 1998; Fournier and Brodeur 2000). This result differs from that of Wang and Kundsen (1993) who reported that reproduction by individual aphids did not decrease due to pathogen treatment but total reproduction significantly decreased due to high mortality.

In the present study, we used the pure culture of the fungus, while in several studies on pathogenicity of L. longisporum, commercial formulations have shown high mortality rates in aphids. A formulation of L. longisporum (Vertalec) resulted in 100% mortality on Aphis gossypii at 11 DAT, with the LT50 value of 6.9 days (Kim et al. 2008). In the present study the highest concentration of L. longisporum LRC 190 caused 100% mortality on adult S. maydis at 12 DAT, and 77.22% mortality on M. dirhodum at 9 DAT, with the LT50 values of 2.9 and 4.4 days, respectively. Furthermore, Asman (2007) found that three species of Lecanicillium sp. were more pathogenic to the lettuce aphid than Vertalec. Also, Diaz et al. (2009) found that the L. lecanii isolate ICAL6 was more virulent than the commercial product Vertalec for M. persicae. However, L. longisporum (Vertalec) showed high virulence against Aulacorthum solani (Kaltenbach), M. euphorbiae, and M. persicae with the LT50 values of 1.8, 2.0, and 2.4 days, respectively (Kim et al. 2007).

In conclusion, the pathogenecity of L. longisporum was demonstrated on the cereal aphids, S. maydis and M. dirhodum reared on potted whole wheat plants. Further research in field conditions using more isolates, especially native ones, will be required to consider the fungus’ potential as an agent in biocontrol programs.

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References


