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 LC_{50} value of the fungus was obtained as 5.9×10^5 and 3.2×10^6 conidia/ml for *S. maydis* and *M. dirhodum*, respectively. The LT_{50} 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 LC_{50} and LT_{50} indicated that *L. longisporum* was more virulent to *S. maydis* than to *M. dirhodum*. The LT_{50} and R_0 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), *Rhopalosiphum 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

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L. muscarium, respectively) and are recommended for applications against greenhouse aphids, and against whiteflies and thrips, respectively (Faria and Wraight 2007). Many isolates of *Lecanicillium* demonstrate high pathogenicity to several species of aphids such as *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas), and *Myzus persicae* (Sülzer) (Askary *et al*. 1998; Alavo *et al*. 2001; Kim *et al*. 2007).

Several studies have been carried out on fungal pathogens against cereal aphids. Ganassi *et al.* (2010) investigated the effects of a strain of the fungus *Lecanicillium lecanii* (Zimm.) on the aphid *S. graminum*. They found that the *L. lecanii* formulation affected the survival of the aphids and interacted differently with the studied morphs; the lethal time values being lower for alate compared to apterous morphs and nymphs. Virulence of *V. lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Bals.-Criv.) Vuill. were evaluated on six species of cereal aphids. The results showed pathogenicity of both fungal species on all aphid species with *B. bassiana* having more efficacy than *V. lecanii* (Feng *et al*. 1990). In Iran, the efficacy of some entomopathogenic fungi including *Metarhizium anisopliae* (Metchnikoff) Sorokin (Mohammadipour *et al*. 2010a), *B. bassiana* (Mohammadipour *et al*. 2010b), as well as *L. muscarium* and *L. aphanocladii* (Mohammadipour *et al*. 2010c) was demonstrated on the Russian wheat aphid, *D. noxia* in laboratory conditions.

The entomopathogenic fungus *L. longisporum* (Hypocreales: Ascomycota) is a capable alternative control agent against important pests (Zare and Gams 2001). There is no data about the pathogenicity of the entomopathogenic fungi on the aphid *S. maydis*. Furthermore, management of *S. maydis* through biological control is difficult because the common aphid parasitoids attacking the other wheat aphids do not prefer this aphid as a host (Rakhshani *et al*. 2008). It was recently demonstrated that detached leaf bioassays may provide different results as compared to whole plant spraying. An isolate of *Paecilomyces farinosus* (Holmsk.) was efficacious against powdery mildew colonies in detached leaf cultures but not on whole plants (Szentivanyi *et al*. 2006). In the present study, we aimed to evaluate the potential of *L. longisporum* for controlling *S. maydis* and *M. dirhodum* on whole plants. We support practical control management strategies and the prevention of further aphid outbreaks.

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 sanborni* 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 ($F_{(4,59)} = 2.87$; $p < 0.001$), 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 morality 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

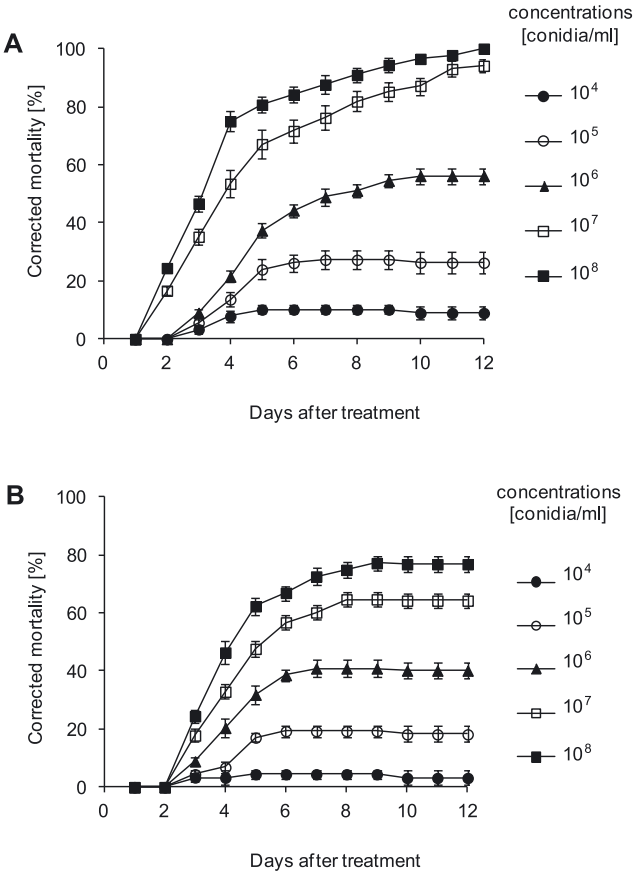

Fig. 1. Cumulative mortality of *S. maydis* (A) and *M. dirhodum* (B) adults following treatment by different concentrations of the fungus *L. longisporum*

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*

Concentration [conidia/ml]		Mean (±SE)*	
		S. maydis	*M. dirhodum*
10^4		10.15 (±1.42) a	4.44 (±1.40) a
10^5		27.30 (±3.07) b	19.28 (±2.00) b
10^6		54.52 (±2.23) c	40.87 (±2.85) c
10^7		85.16 (±3.39) d	64.68 (±2.32) d
10^8		94.36 (±2.68) d	77.14 (±2.49) e
Results of ANOVA	$F_{(4, 29)}$	185.715	178.338
	p	< 0.001	< 0.001

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

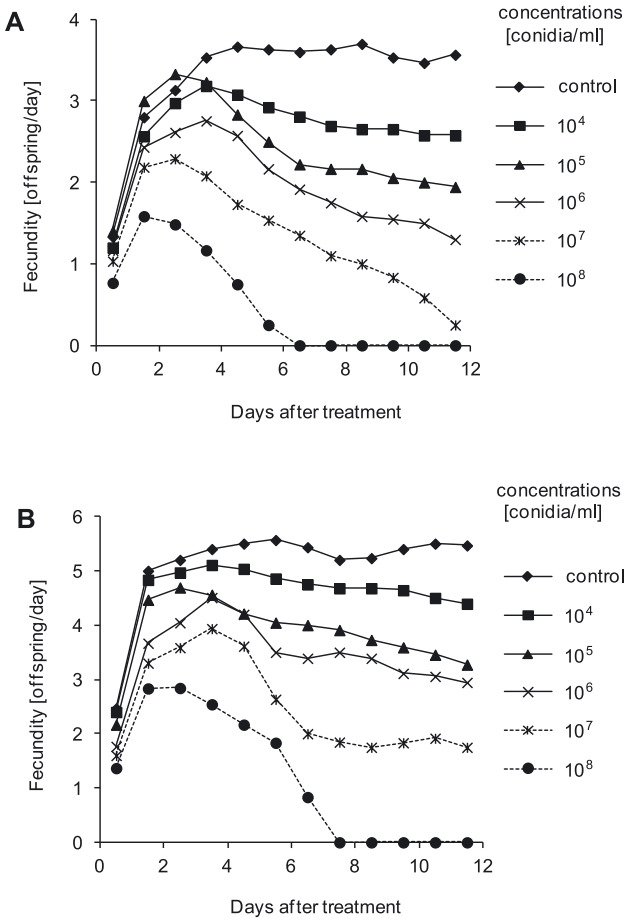

Fig. 2. Fecundity of *S. maydis* (A) and *M. dirhodum* (B) (n = 15) following treatment by different concentrations of the fungus *L. longisporum*

Table 2. One-way analysis of variance (ANOVA) on R_0 (mean ±SE, n = 6) of *S. maydis* and *M. dirhodum* adults over 12 days, following treatment by different concentrations of the fungus *L. longisporum*

Concentration [conidia/ml]		Mean (±SE)*	
		S. maydis	*M. dirhodum*
The control		19.05 (±0.49) a	28.92 (±0.52) a
10^4		14.33 (±0.14) b	25.65 (±0.71) b
10^5		11.15 (±0.19) c	18.10 (±0.28) c
10^6		7.27 (±0.14) d	13.07 (±0.51) d
10^7		3.70 (±0.20) e	8.05 (±0.46) e
10^8		1.44 (±0.09) f	4.87 (±0.19) f
Results of ANOVA	$F_{(5, 35)}$	693.339	394.175
	p	< 0.001	< 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* pathogencity after nine days, against adults of *S. maydis* and *M. dirhodum*

Aphid species	n[a]	Probit mortality-concentration		*t* ratio	Hetero-geneity	*g* factor (0.95)	Concentration [conidia/ml × 10^4]* (95% CL)	
		slope (±SE)	intercept (±SE)				LC_{50}	LC_{90}
S. maydis	540	0.74 (±0.65)	–1.32 (±0.15)	11.47	0.34	0.029	59.27 a (35.40–95.45)	3050.12 a (1583.66–7161.99)
M. dirhodum	540	0.58 (±0.56)	–1.45 (±0.15)	10.32	0.69	0.036	315.89 b (178.51–569.79)	49788 b (18368–200830)

[a] 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. LT_{50} and LT_{90} of *L. longisporum* pathogencity against adults of *S. maydis* and *M. dirhodum*

Aphid species	Concentration [conidia/ml]	Probit mortality-time		*t* ratio	Hetero-geneity	*g* factor (0.95)	Time [day] (95% CL)	
		slope (±SE)	intercept (±SE)				LT_{50}	LT_{90}
S. maydis	10^7	3.08 (±0.20)	−1.81 (±0.16)	14.99	0.28	0.017	3.86 (3.53–4.18)	10.07 (9.17–11.30)
	10^8	3.54 (± 0.23)	−1.68 (±0.16)	15.05	0.78	0.016	2.99 (2.71–3.25)	6.84 (6.37–7.54)
M. dirhodum	10^7	2.75 (±0.33)	−2.07 (±0.26)	8.11	0.84	0.058	5.65 (5.18–6.16)	16.48 (13.23–23.41)
	10^8	2.94 (±0.34)	−1.89 (±0.25)	8.63	1.09	0.097	4.41 (3.74–4.95)	12.03 (9.75–17.54)

ratio test of parallelism ($\chi^2 = 4.00$; df = 1; p = 0.045). The heterogeneity factors of all the bioassays were less than 1. A heterogeneity factor less than 1 indicated that there was no sign of systematic deviations in the chi-square (χ^2) values. For both aphid species, the regression tests (*t* ratio) were greater than 1.96 and the potency estimation tests (*g* factor) were less than 0.5 at all probability levels (Table 3). The intercepts of probit mortality regressions were significantly different between *S. maydis* and *M. dirhodum*, as revealed by rejection of the likelihood ratio test of equality ($\chi^2 = 26.48$; df = 2; $p < 0.001$). The above differences in slopes and/or intercepts of the probit mortality regressions among experimental treatments were reflected in the LC_{90} or LC_{50} estimates. At 9 DAT, the LC_{50} values were obtained as 5.9×10^5 and 3.2×10^6 conidia/ml against *S. maydis* and *M. dirhodum*, respectively (Table 3). Thus *L. longisporum* was significantly less virulent to *M. dirhodum*.

The results of LT_{50} estimates at 10^8 conidia/ml, also indicated that the aphid *S. maydis*, (LT_{50} = 2.9 days) was more vulnerable to the pathogenecity of *L. longisporum* than *M. dirhodum* (LT_{50} = 4.4 days) (Table 4).

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 LC_{50} 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 LC_{50} of 3.2×10^6 conidia/ml. Using the topical application method, the LC_{50} 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 LT_{50} 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 (10^8 conidia/ml) of this fungus, the LT_{50} 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 susceptibile 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 LC_{50} and LC_{90} indices (Robertson and Preisler 1992), indicated the LC_{50} and LC_{90} 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

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 LC_{50} 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 10^8 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 LT_{50} 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 LT_{50} 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 LT_{50} 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

Abbott W.S. 1925. A method of computing the effectiveness of an insecticide. Econ. Entomol. 18 (2): 265–267.

Alavo T.B.C., Sermann H., Bochow H. 2001. Biocontrol of aphids using *Verticillium lecanii* in greenhouse: factor reducing the effectiveness of the entomopathogenic fungus. Arch. Phytopathol. Plant Prot. 34 (6): 407–424.

Ashouri A., Arzanian N., Askary H., Rasoulian G.R. 2004. Pathogenicity of the fungus, *Verticillium lecanii*, to the green peach aphid, *Myzus persicae* (Hom.: Aphididae). Commun. Agric. Appl. Biol. Sci. 69 (3): 205–209.

Askary H., Carrier Y., Belonger R.R., Brodeur J. 1998. Pathogenicity of the fungus, *Verticillium lecanii* to aphids and powdery mildew. Biocontrol Sci. Technol. 8 (1): 23–32.

Asman K. 2007. Aphid infestation in field grown lettuce and biological control with entomopathogenic fungi (Deuteromycotina: Hyphomycetes). Biol. Control. Hortic. 25 (2): 153–173.

Blackman R.L., Eastop V.F. 2000. Aphids on the World's Crops: An Identification and Information Guide. John Wiley & Sons, Chichester, England, 476 pp.

Blackman R.L., Eastop V.F. 2006. Aphids on the World's Herbaceous Plants and Shrubs. John Wiley & Sons, Chichester, England, 1439 pp.

Corrales C.E., Castro A.M., Ricci M., Dixon A.F.G. 2007. *Sipha maydis*: Distribution and host range of a new aphid pest of wintercereals in Argentina. Econ. Entomol. 100 (6): 1781–1788.

Cortez-Madrigal H., Alatorre-Rosas R., Mora-Aguilera G., Bravo-Mogica H., OrtIz-Garcia C.F., Aceves-Navarro L.A. 2003. Characterization of multisporic and monosporic isolates of *Lecanicillium* (= *Verticillium*) *lecanii* for the management of *Toxoptera aurantii* in cocoa. Biocontrol 48 (3): 321–334.

Diaz B.M., Oggerin M., Lastra C.C.L., Rubio V., Fereres A. 2009. Characterization and virulence of *Lecanicillium lecanii* against different aphid species. Biocontrol 54 (6): 825–835.

Dixon A.F.G. 1987. Cereal aphids as an applied problem. Agric. Zool. Rev. 2 (1): 1–57.

Faria M., Wraight S. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control. 43 (3): 237–256.

Feng M.G., Johnson J.B., Kish L.P. 1990. Virulence of *Verticillium lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal-infesting aphids (Homoptera: Aphididae). Environ. Entomol. 19 (3): 815–820.

Fournier V., Brodeur J. 1999. Biological control of lettuce aphids with the entomopathogenic fungus *Verticillium lecanii* in greenhouses. Bulletin OILB/SROP 22 (1): 77–80.

Fournier V., Brodeur J. 2000. Dose-responses susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the entomopathogenic fungus *Verticillium lecanii*, azadirachtin and insecticidal soup. Environ. Entomol. 29 (3): 568–578.

Ganassi S., Grazioso P., Moretti A., Sabatini M.A. 2010. Effects of the fungus *Lecanicillium lecanii* on survival and reproduction of the aphid *Schizaphis graminum*. Biocontrol 55 (2): 299–312.

Harper A.M., Huang H.C. 1986. Evaluation of the entomophagous fungus *Verticillium lecanii* (Moniliales: Moniliaceae) as a control agent for insects. Environ. Entomol. 15 (2): 281–284.

Helen Y., Pell J.K., Alderson P.G., Clark S.J., Pye B.J. 2003. Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species. Pest. Manag. Sci. 59 (2): 156–165.

Jackson C.W., Heale J.B., Hall R.A. 1985. Traits associated with virulence to the aphid *Macrosiphoniella sanborni* in eighteen isolates of *Verticillium lecanii*. Ann. Appl. Biol. 106 (1): 39–48.

Kim J.J., Goettel M.S., Gillespie D.R. 2007. Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Biol. Control. 40 (3): 327–332.

Kim J.J., Goettel M.S., Gillespie D.R. 2008. Evaluation of *Lecanicillium longisporum*, Vertalec for simultaneous suppression of cotton aphid, *Aphis gossypii*, and cucumber powdery mildew, *Sphaerotheca fuliginea*, on potted cucumbers. Biol. Control. 45 (3): 404–409.

LeOra software 1987. POLO-PC: A User's Guide to Probit for Logit Analysis. LeOra software, Berkeley, CA, USA.

Mohammadipour A., Ghazavi M., Bagdadi A. 2010a. Efficacy of Iranian isolate of *Metarhizium anisopliae* (Metschnikof.) Sorokin against Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) on the laboratory condition. p. 98. In: Proc. 19th Iranian Plant Protection Congress, Tehran, Iran, 31 July – 3 August 2010, 630 pp.

Mohammadipour A., Ghazavi M., Bagdadi A., Nikpour N. 2010b. Efficacy of Iranian isolate of *Beauveria bassiana* against Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) on the laboratory condition. p. 96. In: Proc. 19th Iranian Plant Protection Congress, Tehran, Iran, 31 July–3 August 2010, 630 pp.

Mohammadipour A., Ghazavi M., Bagdadi A., Zare R. 2010c. Efficacy of entomopathogenic fungi *Lecanicillium muscarium* and *L. aphanocladii* against Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) on the laboratory condition. p. 97. In: Proc. 19th Iranian Plant Protection Congress, Tehran, Iran, 31 July – 3 August 2010, 630 pp.

Nazemi A.H. 2012. Evaluation of Pathogenecity of *Lecanicillium longisporum* on the Aphid *Cinara pini*. MSc Thesis, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, 104 pp.

Rakhshani E., Tomanovic Z., Stary P., Talebi A.A., Kavallieratos N.G., Zamani A.A., Stamenkovi S. 2008. Distribution and diversity of wheat aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) in Iran. Eur. J. Entomol. 105 (5): 863–870.

Rassipour A., Radjabi G., Esmaili M. 1996. Part II – Country Reports. Sunn Pests and Their Control in the Near East. http://www.fao.org/docrep/v9976e/v9976e05.htm [Accessed: March 17, 2013].

Robertson J.L., Preisler H.K. 1992. Pesticide Bioassays with Arthropods. CRC Press, Florida, 127 pp.

Sabzalian M.R., Hatami B., Mirlohi A. 2004. Mealybug, *Phenococcus solani*, and barley aphid, *Sipha maydis*, response to endophyte-infected tall and meadow fescues. Entomol. Exp. Appl. 113 (205): 205–209.

SPSS 2007. SPSS Statistical Software CD-ROM Version 16.0 for Windows ChicagoIL, SPSS Inc., USA.

Stearns S.C. 1992. The Evolution of Life Histories. Oxford University Press, New York, 248 pp.

Steinkraus D.C. 2006. Factors affecting transmission of fungal pathogens of aphids. J. Invertebr. Pathol. 92 (3): 125–131.

Szentivanyi O., Varga K., Wyand R., Slatter H., Kiss L. 2006. *Paecilomyces farinosus* destroys powdery mildew colonies in detached leaf cultures but not on whole plants. Eur. J. Plant. Pathol. 115 (3): 351–356.

Tabashnick B.E., Cushing N.L. 1987. Quantitative genetic analysis of insecticide resistance: variation in fenvalerate tolerance in a diamondback moth (Lepidoptera: Plutellidae) population. Econ. Entomol. 82 (1): 5–10.

Vu V.H., Hong S.I., Kim K. 2007. Selection of entomopathogenic fungi for aphid control. J. Biosci. Bioeng. 104 (6): 498–505.

Wang Z.G., Kundsen G.R. 1993. Effect of *Beauveria bassiana* (Fungi: Hyphomycetes) on fecundity of the Russian wheat aphid (Homoptera: Aphididae). Environ. Entomol. 22 (4): 874–878.

Yokomi R., Gottwald T. 1988. Virulence of *Verticillium lecanii* isolates in aphids determined by detached-leaf bioassay. J. Invertebr. Pathol. 51 (3): 250–258.

Zare R., Gams W. 2001. A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium*. Nova Hedwigia 73 (1–2): 1–50.