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Research Article

Pathogenicity of *Lecanicillium longisporum* (Ascomycota: Hypocreomycetidae) on the aphid *Cinara pini* (Hemiptera: Lachnidae) in laboratory conditions

Amir Hossein Nazemi[1], Gholamhossein Moravvej*[1], Javad Karimi[1] and Reza Talaei-Hassanlouei[2]

1. Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Plant Protection, Faculty of Agricultural Sciences and Engineering, University of Tehran, Karaj, Iran.

Abstract: The aphid species, *Cinara pini* (Linnaeus, 1758) reported in our previous work as a new aphid on pinus trees for Iran, was described using the classic method and through analysis of COI gene sequence. In the next step, we addressed the efficiency of the entomopathogenic fungus, *Lecanicillium longisporum* (Zimm.) Zare and Gams strain LRC 190, on the aphid. The fungus was administered to the second instar nymphs and adults using topical application procedure. The results indicated that the entomopathogen caused 90% mortality in adults over seven days at a concentration of 10^8 spores/ml, while the same control level was achieved for nymphs by 8×10^7 spores/ml. The LC_{50} values were obtained as 1.2×10^6 and 6.9×10^5 spores/ml for adults and nymphs, respectively. The present study suggests that the entomopathogenic fungus, *L. longisporum* could be considered as a potential candidate in biocontrol programs of *C. pini.* This is the first report on the pathogenicity of *L. longisporum* on *C. pini*.

Keywords: Biocontrol, Insect pathology, DNA Barcode, Pathogenecity

Introduction

Molecular methods have provided additional valuable characters for the resolution of taxonomic problems and the discovery of new species within the Aphididae (Foottit, 1997). DNA barcoding has been proposed as a standardized approach to species delimitation in numerous groups of living organisms (Hajibabaei *et al.*, 2007) including insects (Floyd *et al.*, 2009). The aphid family Lachnidae (c. 320 species), sister group to the economically devastating family Aphididae (c. 3300 species), encompasses a diverse array of associations with host plants and attendant hymenopterans and of life histories, including potentially long term parthenogenesis. Most-parsimonious phylogenetic trees were inferred from partial (905-coding-bp) sequences of elongation factor 1α (EF-1α) and complete (675-bp) sequences of cytochrome oxidase 2 (COII) The *Cinara* curtis genus was studied by Normark (2000), who used DNA sequences of nuclear elongation factor 1α and COII to reconstruct evolutionary relationship among species of Lachnidae. Most authors have included the genera *Lachnus*, *Moculoachnus*, *Pterochloroides*, *Tuberolachnus*, and *Nippolachnus* within a tribe Lachnini (Blackman and Eastop, 1994; Czylok, 1990; Ilharco and Harten, 1987; Lampel and Burgener, 1987). There is no evidence to support monophyly of this group of aphids. Universal primers have been developed for conserved mtDNA sequences flanking regions useful for phylogenetic and population studies (Folmer *et*

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* **Corresponding author**, e-mail: Moravej@um.ac.ir
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al., 1994; Lunt *et al.,* 1996; Simon *et al.,* 1994). Taxonomic and phylogenetic studies have used mitochondrial or nuclear genes as cytochrome oxidase I and II (CO-I and CO-II), nuclear elongation factor 1α, and leucine tRNA (Normark, 2000). Favert and Voegtlin (2004) provided an important molecular phylogenetic analysis of *Cinara* species, associated with pinyon pines in the southwestern United States, using CO-I and nuclear elongation factor 1α DNA sequences. In recent years, DNA barcoding has attracted attention of the researchers for studying different groups of insects. DNA barcoding is able to identify the sample in all stages of life by using short DNA sequences (e. g. eggs, larvae, nymphs or pupae) and it is an approach for identifying many invertebrate taxa which their morphological identification is problematic due to lack of availability of taxonomic keys for immature life stages. The standard sequence used for this purpose is a fragment of 5′ end of mitochondrial COI gene that is amplified by the "universal primers"(Folmer *et al.,* 1994). Several studies showed that it is a reliable tool for the molecular identification of Lepidoptera, Hymenoptera, Coleoptera and Diptera species (Hajibabaei *et al.,* 2006).

As a new aphid species in the region, attempt was made to focus on the entomopathogenic fungus, *Lecanicillium longisporum* as a biocontrol agent for aphid population management.Until recently, the form-genus *Verticillium* contained a wide variety of species with diverse host ranges, including arthropods, nematodes, plants and fungi. However, the genus has been redefined using rDNA sequencing, placing all insect pathogens into the new genus *Lecanicillium* (Zare and Gams, 2001). *Lecanicillium* spp. use both mechanical forces and hydrolytic enzymes to directly penetrate the insect integument and the cell wall of the fungal plant pathogen (Goettel *et al.,* 2008). *Lecanicillium* spp. usually use a general pathway of pathogenesis for entomopathogenic mitosporic fungi including adherence to the host cuticle by conidia; germination; penetration of the cuticle; production of blastospores within the haemocoel; ramification of the mycelia and invasion of tissues, which finally causes the death of the host. The last step is the production of conidia on the surface of the cadaver (Askary *et al.,* 1999). In addition to the nutrient utilisation and tissue digestion during infection of insect hosts, many entomopathogenic fungi produce toxic substances which may play a crucial role in the death process (Gindin *et al.,* 1994). Many isolates of *Lecanicillium* have shown high pathogenicity to several species of aphids such as *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) (Alavo *et al.,* 2002; Askary *et al.,* 1998; Kim *et al.,* 2001). In addition, recent studies have demonstrated that the genus *Lecanicillium*, including the isolate of *L. longisporum* (as the active ingredient of Vertalec®), have suppressive activity against powdery mildews (Askary *et al.,* 1998; Kim *et al.,* 2007; Kiss, 2003; Miller *et al.,* 2004; Romero *et al.,* 2003). This suggests that these fungi may play a dual role, controlling both aphids and powdery mildews (Askary *et al.,* 1998; Kim *et al.,* 2007; Kim *et al.,* 2008).

The rising costs of traditional chemical pesticides, increasing resistance of insects to these products and their undesirable effects on the environment (Corey *et al.,* 1993; Mukanganyama *et al.,* 2003; Scott *et al.,* 1998) have led to renew efforts to search for novel approaches to insect pest management (El-Salam and El-Hawary, 2011). In the last decades, biological control, including the use of entomopathogenic fungi, has emerged as a strategy to control aphids, especially in high-value crops. These have been viewed as a substitute or complement to traditional chemical measures. Aphids seem to be susceptible hosts for entomopathogenic fungi because of their morphological, biological and ecological characteristics (Steinkraus, 2006).

Due to a global trend toward application of COI gene as DNA barcode, we attempted to provide information about this gene sequence of *C. pini*, as a new aphid species for Iranian fauna collected in a previous work (Nazemi *et*

al., 2013). The main purpose of the present research is to study the pathogenicity of *L. longisporum* against this new urban pest.

Materials and Methods

Aphid collection

The aphid *Cinara pini* (Linnaeus) was collected from *Pinus mugo* Turra in Mashhad region in the northeast of Iran during spring 2009. The classic method using the available keys (Blackman and Eastop, 2006) diagnosed it as the genus *Cinara*. Final confirmation of the species was made by Olivera Petrovic-Obradovic from Belgrade University, Serbia.

Molecular study procedure

DNA extraction

Genomic DNA from the aphid was extracted using a Bioneer kit (Bioneer Co. Daejeon, South Korea). Individual aphids were kept at -20 °C for at least 12 h, crushed using a micropestle in 180 µl lysis buffer and 20 µl proteinase K. The homogenate was incubated at 56 °C for 4 h, followed by 10 min at 95 °C. The supernatant was stored at -20 °C until use.

PCR of the COI gene

The PCR reaction was performed in 25 µl reaction volume using an Eppendorf thermocycler. Each reaction mixture contained 2 µl DNA template, 3 µl (10X) buffer, 1 µl $MgCl_2$, 0.3 µl *Taq* assay buffer, 0.5 µl $dNTP_s$ (each in 10 mM concentration), 1 µl forward and reverse primers (10 picomoles/µl) and 0.25 µl *Taq* polymerase (5U). The primers used to amplify the COI region were LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (reverse) (Folmer *et al.,* 1994). The temperature profile consisted of an initial denaturation step at 94 °C for 1 min followed by 30 cycles (denaturation at 94 °C for 60 sec, annealing at 52 °C for 90 sec, and extension at 72 °C for 90 sec), with a final extension at 72 °C for 8 min. The PCR product was electrophoresed on 1% agarose gel at 90 V. The gel was stained by ethidium bromide and its image was saved. The PCR product was purified and sent to Macrogen Inc. for sequencing.

Sequencing and analysis

Chromatograms were checked using the BioEdit software (Hall, 1999). The resulting sense and antisense sequences were edited and used in alignment. Representative sequences for all known *Cinara* groups were retrieved from GenBank and included in the alignment file. Sequences were aligned using the default settings of Clustal W (Thompson *et al.,* 1994). Unweighted parsimony analysis of the alignments was conducted with PAUP*4.0b2 (Swofford, 1999). For the analyses, gaps were treated as missing characters, and the reliability of trees was tested with a bootstrap test (Felsenstein, 1985). Parsimony bootstrap analysis included 100 re-samplings using the branch and bound algorithm. The most appropriate model of sequence evolution was determined using hierarchical likelihood ratio tests (hLRTs) in the jModelTest 0.1.1 program (Posada, 2008). The number of trees held at each step during stepwise addition was 1, and the branch-swapping algorithm was tree-bisection-reconnection (TBR). The analysis was implemented in PAUP* with bootstrap replicates.

Biological assay

Aphid rearing

The original stock of *Cinara pini* aphid was collected from *P. mugo* in the campus of Ferdowsi University of Mashhad. A clone of aphids was established on 3–5-year-old *P. mugo* seedlings for several generations in a thermostatic chamber at 24 ± 2 °C on a 16/8 h day/night photoperiod. To provide the cohort aphids' age, parthenogenetic apterous females were maintained on branches enclosed by net-cloth bags (15 × 25 cm) to prevent the escape of aphids. Branches which were 15–20 cm in length were selected from the lateral parts of pine trees. Ten individual females were introduced into each bag using a paintbrush. The females were removed after 24 h, and newborn aphids were reared as a synchronous cohort.

Preparation of entomopathogenic fungus

The fungal isolate *L. longisporum* LRC 190 was obtained from the University of Tehran, cultured on potato dextrose agar (PDA) medium in 9 cm diameter glass petri dishes and incubated at 27 °C. After 10-12 days, the fungal mass was harvested by a glass rod in 10 to 15 ml sterile distilled water containing 0.2% Tween-20. The propagule was passed through sterile cheesecloth.

The conidial concentration of the stock suspension was estimated using an improved Neubauer bright line haemocytometer (Reichart) under a Leica Galen III Microscope (400 × magnification). Serial dilutions were made to give a range of concentrations depending on bioassay requirements. Suspensions were held overnight on ice at 4 °C and then routinely checked for conidial germination prior to use in bioassays as described by (Yeo *et al.*, 2003).

Virulence bioassay

The 8 cm long pieces of *Pinus* branches were sterilized with 96% ethanol and placed on filter paper into 9 cm glass petri dishes. The ends of the branches were kept wet using moist cotton to prevent desiccation. A net-covered opening in the Petri dishes lid facilitated ventilation (Yokomi and Gottwald, 1988). The Petri dishes were maintained in a controlled chamber at 25 ± 2 °C, 70% RH and 16:8 h light: dark period.

A group of 30 aphids were treated individually by 2 µl suspension of each of six fungal concentrations (10^4, 10^5, 10^6, 10^7, 8×10^7 and 10^8 spores/ml) using topical application method (Butt and Goettel, 2000). Control aphids were treated by 2 µl distilled water containing 0.2% Tween-20. Each concentration was repeated three times. Bioassays were performed on apterous adults and the second instar nymphs. The aphids were transferred on *Pinus* branches in above-mentioned Petri dishes. The mortality was daily recorded for 10 days. Aphids with no movement in antennae or legs were considered as dead. The aphid cadavers were removed from the dishes, sterilized with 96% ethanol and inspected under a stereomicroscope (× 40) for fungal infection.

Statistical analysis

Data on mortality of aphids at 5 and 9 days post-inoculation were subjected to analysis of variance with conidial concentration and aphid stage regarded as fixed factors. Mean comparisons were performed using Tukey HSD among fungal concentrations and unpaired t tests between aphid stages. The statistical analyses were done with SPSS 16. Furthermore, percentage mortality data up to the 9th day were analysed with the probit model (Finney, 1971) using a maximum likelihood program (POLO-PC, Leora Software, Berkeley, California).

The results include estimates of the LC_{50} (and other LCs if required) and the 95% confidence limits, the slope and intercept of the probit mortality regression and the relevant statistical tests (such as t-ratio, g factor and heterogeneity). For comparison of the probit mortality lines of treatments, the program also provides the likelihood ratio tests of equality and parallelism (Russel *et al.*, 1977). Comparisons were made based on the procedure described by Robertson and Preisler (1992). The estimates of parameters needed for computing the confidence limits of the resistance ratio were provided by individual probit analysis in the POLO-PC output. The lethal time values for 50% mortality (LT_{50}) with selected concentrations were calculated for adults and the second instar nymphs using the Probit analysis (Finney, 1971).

Results and discussion

Characterization of the aphid indicated that it belongs to the subfamily Cinarinae and family Lachnidae, identified as *Cinara pini* (Linnaeus, 1758) (Fig. 1) by Olivera Petrovic-Obradovic, University of Belgrade, Serbia. The mean adult length was 2.69 ± 0.06 mm. The base of the final antennal segment had fewer than four hairs. The final rostrum segment was longer than the basal diameter of the cornicles or

slightly longer than the second segment of hind tarsus legs.

Figure 1 The ventral view of *Cinara pini* (Linnaeus, 1758) adult aphid.

The study area for the *C. pini* COI gene was amplified and the 629 bp fragment was obtained. Sequences obtained in this way were compared with several other species in the genus *Cinara*, as well as phylogenetic tree species, using neighbour joining and employing MEGA4 software (Fig. 2).

Symptoms of fungal infection

Small, white and velvet-like colonies appeared on agar plates in 3–4 days. Microscopic observation revealed these to be an interweaving mycelia mat. On day 4, from the individual hypha of mycelium branches which grew and stretched out in all directions, spores formed on the tips of the sporophores (phialides) as clusters.

The *L. longisporum* caused increased activity of *C. pini* during the initial stages of infection, when the fungus was penetrating the host cuticle before colonizing the haemolymph. A pre-death behaviour was observed in *C. pini* in terms of slow, non-directional movement and shaking (Fig. 3). Similar symptoms have been described previously in the terminal stages of aphids exposed to some other entomopathogenic fungi (Jensen *et al.,* 2001; Mccoy *et al.,* 1988).

Fungus virulence on *C. pini*

At 5 days post-inoculation, the results of two-way ANOVA on aphid mortality showed that the main effects of fungal concentration ($F_{(5, 36)} = 243.74$, $P < 0.001$), aphid stage ($F_{(1, 36)} = 12.09$, $P = 0.02$) and interaction effect ($F_{(5, 36)} = 2.82$, $P = 0.039$) were significant. Likewise, at 9 days post-inoculation, the ANOVA detected significant effects of concentration ($F_{(5, 36)} = 401.66$, $P < 0.001$) and aphid stage ($F_{(1, 36)} = 15.09$, $P = 0.01$), but not the interaction effect ($F_{(5, 36)} = 1.64$, $P = 0.187$).

The virulence of the entomopathogenic fungus *L. longisporum* against *C. pini* adult and second nymphal stages are presented in Figures 4 and 5. The mortality of adults and nymphs increased with fungus concentration. In all concentrations, the first mortality occurred on the third day of application for both developmental stages. After nine days, the lowest concentration (10^4 spores/ml) elicited 8.05 and 8.53% mortality in adults and nymphs, respectively. At this time, the concentration of 10^8 spore/ml caused about 90% mortality in adults, while a similar level of mortality in nymphs was obtained by 8×10^7 spores/ml (Table 1). Harper and Huang (1986) showed that *V. lecanii* reduced the population of *Aphis pisum* by 60–90%, *Myzus dirhodum* by 32–85%, *M. persicae* by 50–100%, and *Trialeurodes maculata* by 37–75% (Harper and Huang, 1986).

El-salam and El-Hawary (2011) indicated that *L. lecanii* was more pathogenic than *Beauveria bassiana* against adults or nymphs of *Aphis craccivora*. In their work, *L. lecanii* caused 100% mortality on adults and nymphs with 5.0 ml conidial suspension (5×10^6 spores/ml) and 1.0 ml conidial suspension (1×10^6 spore/ml) after three days, respectively (El-Salam and El-Hawary, 2011). Hellen *et al*., (2003) recorded that *A. fabae* was more susceptible than *M. persicae* to the *Verticillium lecanii* isolate HRI 1.72 at 20 and 25 °C (Helen *et al.*, 2003).

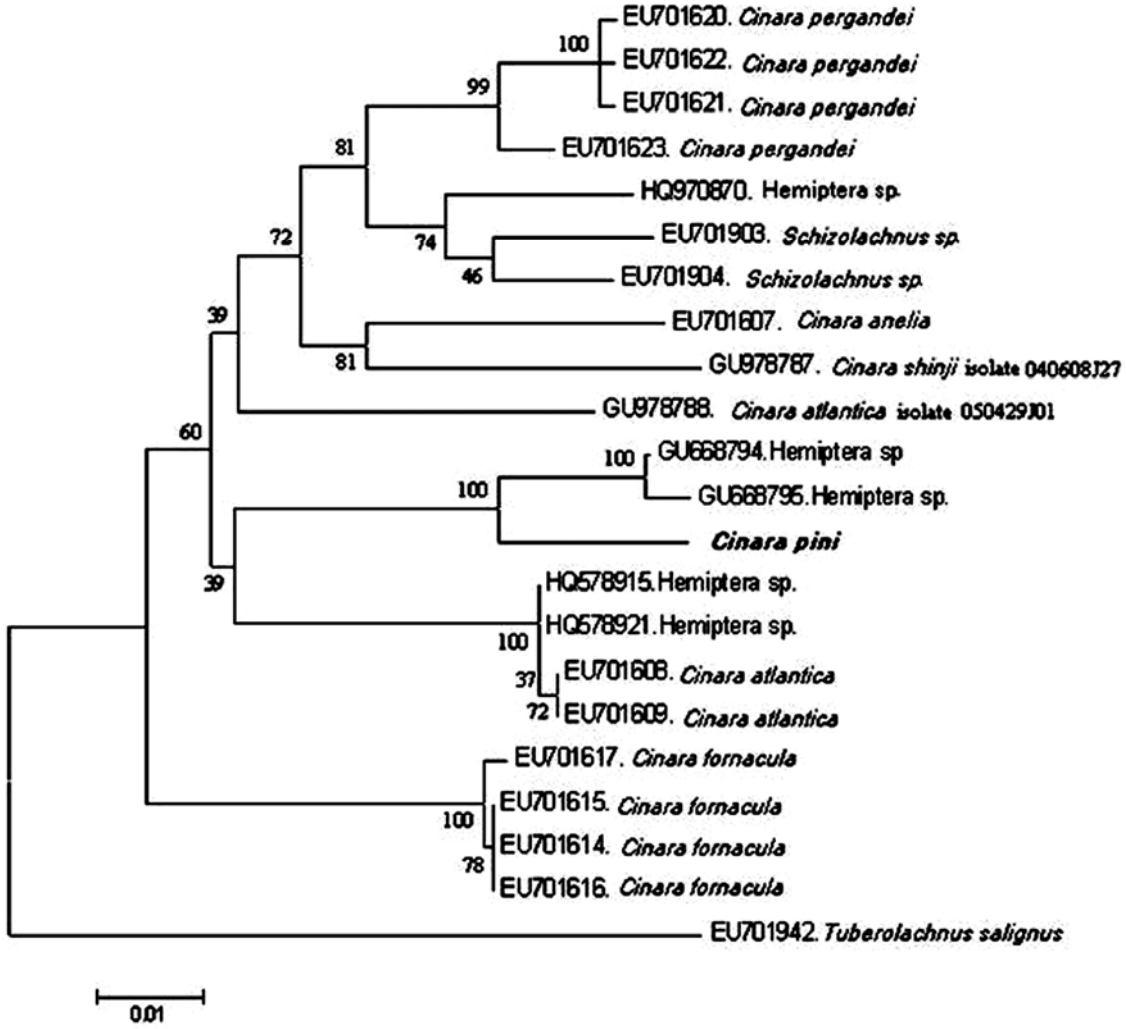

Figure 2 Phylogenic relationship of *Cinara* species and closely related aphids based on COI sequences and Neighbourhood Joining method.

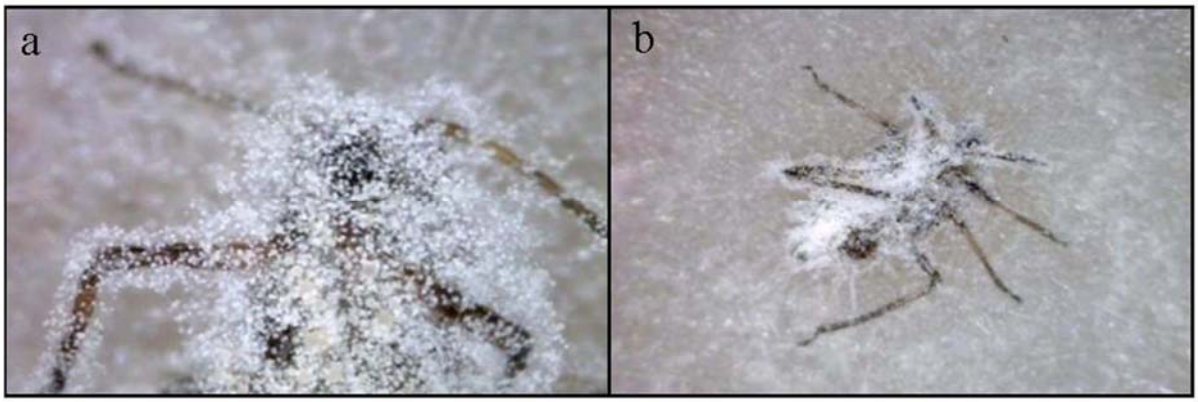

Figure 3 Symptoms of infection by *Lecanicillium longisporum* on adult aphid, *Cinara pini*.

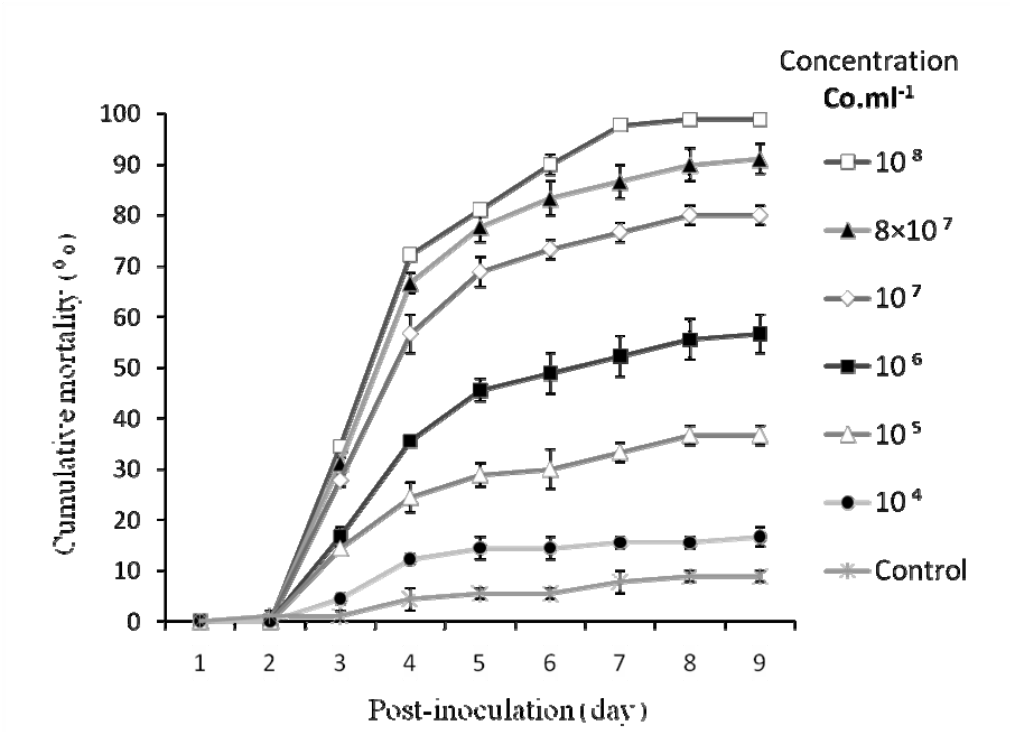

Figure 4 Cumulative mortality of the second instar nymphs of *Cinara pini* treated with the entomopathogenic fungus *Lecanicillium longisporum* during nine days from onset of application (n = 3, each replicate contained 30 aphids).

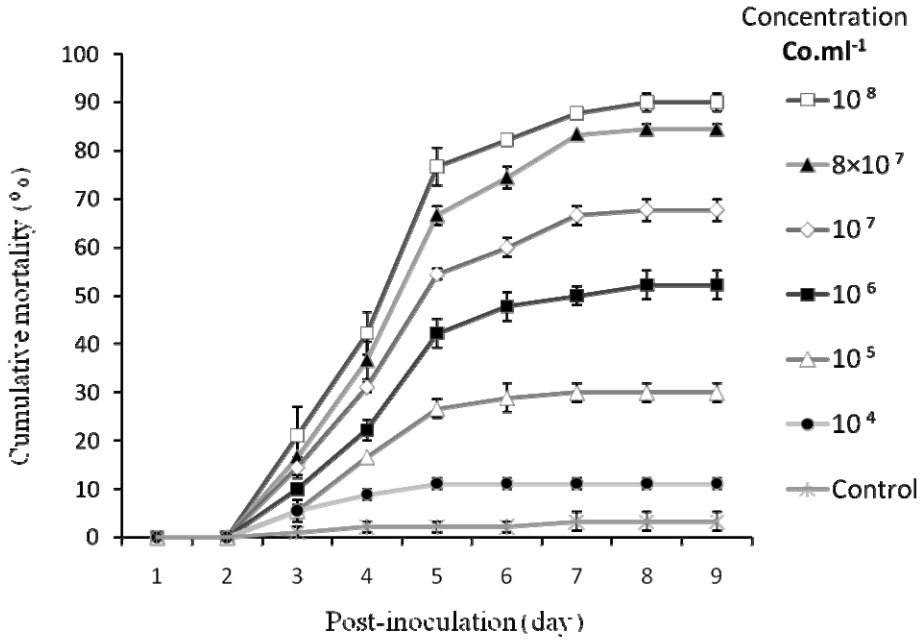

Figure 5 Cumulative mortality of the apterous adults of *Cinara pini* treated with the entomopathogenic fungus *Lecanicillium longisporum* during nine days from onset of application (n = 3, each replicate contained 30 aphids).

Table 1 Statistical analyses of corrected percent mortality (n = 3, Mean ± standard error) ¥ on second instar nymphs and adults of *Cinara pini* at five and nine days post-inoculation by different concentrations of the fungus *Lecanicillium longisporum*.

Post-inoculation (day)	Concentration ($co.ml^{-1}$)	%mortality ± SE		t-test (*df* = 4)
		2^{nd} instar nymph	Adult	
Fifth day	10^4	9.42 ± 2.35 a	9.09 ± 1.14 a	0.122 ^{NS}
	10^5	24.71 ± 2.35 b	25.00 ± 1.97 b	0.093 ^{NS}
	10^6	42.35 ± 2.35 c	40.91 ± 3.01 c	0.377 ^{NS}
	10^7	67.06 ± 3.11 d	53.41 ± 1.14 d	4.121 *
	8×10^7	76.47 ± 3.11 de	65.91 ± 1.97 e	2.870 *
	10^8	80.00 ± 1.18 e	76.14 ± 3.94 e	0.940 ^{NS}
	$F_{(5, 18)}$	136.07	109.57	
	P	< 0.001	< 0.001	
Ninth day	10^4	8.54 ± 2.11 a	8.05 ± 1.15 a	0.203 ^{NS}
	10^5	30.49 ± 2.11 b	27.59 ± 1.99 b	0.999 ^{NS}
	10^6	52.44 ± 4.24 c	50.57 ± 3.04 c	0.358 ^{NS}
	10^7	78.05 ± 2.11 d	66.67 ± 2.30 d	3.644 *
	8×10^7	90.24 ± 3.23 de	83.91 ± 1.15 e	1.850 ^{NS}
	10^8	98.78 ± 1.22 e	89.66 ± 1.99 e	3.910 *
	$F_{(5, 18)}$	175.83	246.02	
	P	< 0.001	< 0.001	

¥: At each post-inoculation day, means with the same letters in a column are not significantly different (Tukey HSD test at 5% level)
T-test shows statistical differences between developmental stages in each concentration. NS: not significant, *: significant at 5% level.

The probit analysis of nine days mortality data was conducted using POLO-PC. The slopes of the nymph and adult probit morality regressions for the fungus were not significantly different, as revealed by accepting the likelihood ratio test of parallelism ($X^2 = 2.91$, df = 1, $P = 0.088$). However, the intercepts of probit morality regressions between two developmental stages differed significantly, as revealed by rejecting the likelihood ratio test of equality ($X^2 = 8.11$, df = 2, $P < 0.05$) (Table 2). A susceptibility comparison between adults and the second instar of *Cinara pini* on the basis of LC_{50} and LC_{90} indices showed that the LC_{50} of the fungus against the nymphal stage was not significantly different from the amount in adults. However, the LC_{90} of the fungus was four times greater in adults than nymphs (Table 2). The 50% lethal time (LT_{50}) decreased significantly as the fungal concentration increased, ranging between 3.58 and 5.62 days. Furthermore, the LT_{50} values at adult stage were higher than those at nymphal stage (Table 3).

Vu *et al.,* (2007) found that the LC_{50} of *V. lecanii* (VL10) against *M. persicae* was 1.65×10^6 conidia/ml after six days of treatment. Leite *et al.,* (2005) found that the LC_{50} of the *V. lecanii* strain CG 904 indicated 100% mortality at concentrations of 10^8 conidia.ml^{-1}, LC_{50}, 2×10^5 conidia.ml^{-1}; and LT_{50}, 4.4 days (Leite *et al.,* 2005).

Table 2 Probit analyses of *Lecanicillium longisporum* pathogencity at nine days post-inoculation against second instar nymphs and adults of the aphid *Cinara pini.*

Aphid stage	n	Probit mortality-concentration		tratio	Heterogeneity	Factor g (0.95)	Concentration (Co.ml^{-1} × 10^4) (95% fiducial limits)[1]	
		Slope (± SE)	Intercept (± SE)				LC_{50}	LC_{90}
Adults	630	0.59 (± 0.50)	-3.04 (± 0.28)	11.77	0.56	0.027	126.53 70.25-215.27	17718 8386-46866
Second instar	630	0.72 (± 0.67)	-3.52 (± 0.38)	10.77	1.29	0.085	69.01 20.19-168.79	3961 1547-15539

n: The number of insects tested.
1: Lower-upper.

Table 3 LT_{50} and LT_{90} values for *Lecanicillium longisporum* on adults and second instar nymphs of the aphid *Cinara pini*.

Aphid stage	Concentration (Co.ml^{-1})	Probit mortality-time		t ratio	Heterogeneity	Factor g (0.95)	Time (day) (95% fiducial limits)	
		Slope (± SE)	Intercept (± SE)				LT_{50}	LT_{90}
Adult	10^7	3.76 (±0.28)	-2.82 (± 0.21)	13.14	2.52	0.08	5.62 4.97-6.37	12.30 9.98-17.54
	8×10^7	4.90 (± 0.32)	-3.28 (± 0.23)	15.22	2.31	0.05	4.67 4.20-5.13	8.54 7.51-10.29
	10^8	5.32 (± 0.33)	-3.36 (± 0.24)	15.83	2.70	0.06	4.28 3.81-4.72	7.54 6.60-8.86
Second instar	10^7	3.87 (± 0.26)	-2.49 (±0.19)	14.66	4.08	0.10	4.40 3.68-5.10	9.42 7.68-13.45
	8×10^7	4.77 (± 0.30)	-2.80 (± 0.21)	15.78	3.72	0.08	3.87 3.29-4.40	7.18 6.18-9.03
	10^8	6.67 (± 0.43)	-3.69 (± 0.27)	15.33	1.68	0.04	3.58 3.28-3.85	5.57 5.12-6.21

In conclusion, the present study suggested that the entomopathogenic fungus *L. longisporum* LRC 190 is a good candidate for a possible biocontrol programme of *C. pini* aphid. Additional studies are needed to confirm whether the laboratory results reflect the performance of this strain in the field. It is also suggested to evaluate the possible production of toxic metabolites by the fungus and to assess the risk of these metabolites with regard to natural enemies and human health.

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بررسی بیماریزایی قارچ *Lecanicillium longisporum* (Ascomycota: Hypocreomycetidae) روی شته *Cinara pini* (Hemiptera: Lachnidae) در شرایط آزمایشگاهی

امیرحسین ناظمی[۱]، غلامحسین مروج[*۱]، جواد کریمی[۱] و رضا طلایی حسنلویی[۲]

۱- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، صندوق پستی ۱۱۶۳–۱۷۷۵، مشهد، ایران.
۲- گروه گیاهپزشکی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: Moravej@um.ac.ir
دریافت: ۲۸ آبان ۱۳۹۱؛ پذیرش: ۱۱ آذر ۱۳۹۲

چکیده: در مطالعه حاضر، شته‌ی *Cinara pini* (Linnaeus, 1758) که اخیراً به‌عنوان یک گونه جدید برای ایران از روی درختان کاج گزارش شده بود، با استفاده از روش‌های کلاسیک و توالی ژن CO1 بررسی و توصیف گردید. سپس کارایی قارچ بیمارگر *Lecanicillium longisporum* (Zimm.) Zare and Gams استرین LRC 190 روی این شته مورد ارزیابی قرار گرفت. سوسپانسیون قارچ به‌روش تماس موضعی (قطره‌گذاری) روی حشرات کامل و پوره‌های سن دوم به‌کار برده شد. نتایج این بررسی نشان داد که تیمار قارچ در غلظت $۱۰^{۸}$ کنیدی بر میلی‌لیتر، هفت روز پس از کاربرد روی حشرات کامل ۹۰ درصد مرگ‌ومیر ایجاد کرد درحالی‌که میزان تلفات مشابه در همین مدت زمان، روی پوره‌ها با غلظت ۸×۱۰^{۷} کنیدی بر میلی‌لیتر به‌دست آمد. شاخص‌های LC_{50} قارچ علیه حشرات کامل و پوره‌های سن دوم به‌ترتیب $۱/۲ \times ۱۰^{۶}$ و $۶/۹ \times ۱۰^{۵}$ کنیدی بر میلی‌لیتر به‌دست آمد. براساس نتایج این بررسی، استفاده از قارچ بیمارگر *L. longisporum* به‌عنوان عامل بیوکنترل در برنامه‌های کنترل بیولوژیکی شته *C. pini* توصیه می‌گردد. این مطالعه، اولین گزارش روی ارزیابی بیماریزایی قارچ *L. longisporum* علیه شته *C. pini* می‌باشد.

واژگان کلیدی: بیوکنترل، بیماری‌شناسی حشرات، DNA بارکد، بیماریزایی