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Entomopathogenic potential of *Purpureocillium lilacinum* against the model insect *Galleria mellonella* (Lepidoptera: Pyralidae)

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Abstract

Pathogenicity of the entomopathogenic fungus *Purpureocillium lilacinum* against *Galleria mellonella* larvae was studied using two application methods, immersion and injection. *G. mellonella* last instar larvae were immersed and injected with fungal suspensions of different conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}) and mortality was recorded daily. *P. lilacinum* showed pathogenicity at the different concentrations by both methods. *P. lilacinum* was highly infectious for *G. mellonella* causing 100% larval mortality within 7 days post-immersion with 1×10^8 concentrations. The median lethal time (LT_{50}) was 1.83 days. Using the injection method, 1×10^8, 1×10^7, 1×10^6 and 1×10^5 concentrations caused 100% larval mortality within 1, 2, 3 and 4 days after injection respectively. The median lethal time (LT_{50}) was 1, 1.47, 1.96, aand 2.05 days, respectively. For both methods (immersion and injection) the lethal concentration of *P. lilacinum* that caused 50% larval mortality (LC_{50}) was 3.1×10^4 and 4.7×10^3 conidia mL^{-1}, respectively. Both methods were effective on larvae, but the injection method was more effective than immersion method at 4.7×10^3 conidia mL^{-1} causing 50% larval mortality. For immersion and injection methods, the median lethal times (LT_{50}) at the concentrations of 1.0×10^8 conidia mL^{-1} were 1.83 and 1 days, respectively. The pathogenicity results obtained from this isolate using the model insect *G. mellonella* showed that *P. lilacinum* can be used as a potential biopesticidal agent against lepidopteran pests.

Key words: biopesticidal activity, entomopathogen fungus, Lepidopteran pest, pathogenicity.
Abbreviations: LT_{50}, 50% lethal time; LC_{50}, 50% larval mortality.

Introduction

It is known that insects, one of the most important elements of biological diversity, have various biological and non-biological factors affecting their growth and development in the environment. One of the important factors that insects are exposed to during the development process is entomopathogenic fungi. Entomopathogenic fungi are also known to be more effective in the control of various insect pests, compared to other microorganisms (Deacon 1983). Previous studies reported that several species of entomopathogenic fungi such as *Beauveria bassiana, Isaria fumosorosea, Lecanicillium lecanii, Metarhizium anisopliae, Paecilomyces lilacinus* and *Pandora neoaphidis*, have shown good potential for biological control of insects and root-knot nematode (Hajek, Leger 1994; Klingen et al. 2002; Kiewnick, Sikora 2006; Ibrahim et al. 2016; Majeed et al. 2017). These abovementioned studies suggested that entomopathogenic fungus could be used in integrated pest management programmes as eco-friendly biopesticides. For this reason, in this study, we selected *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones, and Samson (KUKENS WDCM101) [the old name is *Paecilomyces lilacinus* (Thom)], an entomopathogenic fungus and a common soil fungus (Demirel et al. 2005). *P. lilacinum* has been used as a biocontrol agent against eggs of plant root nematodes (Khan et al. 2003). Several studies about the entomopathogenic effects of *Paecilomyces* species showed that P. *lilacinus, Paecilomyces farinosus* and *Paecilomyces fumosoroseus* have pathogenic potential on some insect pests (Meitkiewski et al. 1997; Pedro et al. 2001; James 2003; Michalaki et al. 2007). Also, it has been shown that different strains of *P. lilacinus* have entomopathogenic effects against some insects (Esser and El-Gholl 1993; Bustillo et al. 1999; Beron and Diaz 2005; Ghazavi et al. 2005). Therefore, the first aim of this study was to test the entomopathogenic effects of P. *lilacinum*, isolated from agricultural lands of Eskişehir province, on the model insect *Galleria mellonella* (Lepidoptera: Pyralidae) last instar larvae.

To investigate the pathogenicity of *P. lilacinum* on the insects, we selected the greater wax moth, *G. mellonella* larvae because of two reasons. First, this species causes significant economic losses in the beekeeping industry by feeding on honeycomb by larval stages and laying eggs in the adult stage on the honeycombs of *Apis mellifera*

(Hymenoptera: Apidae) during storage (Charriere, Imdorf 1997). Several fumigant insecticides such as sulphur dioxide, acetic acid, formic acid, para-dichloro benzene, methyl bromide and phosphine, have been used to control wax moth on beeswax combs during storage. Use of these chemicals is harmful to bee populations (Kwadha et al. 2017). Therefore, we think that eco-friendly pesticides, such as entomopathogenic fungus, could be used as an alternative bioinsecticide. Second, *G. mellonella* is an excellent model insect used in pathogenic and toxicological investigations of various xenobiotics, and also quite easy to grow in the laboratory (Altınçiçek et al. 2007; Altuntaş et al. 2016; Maguire et al. 2016). Furthermore, the second aim of this study was to determine the pathogenicity of *P. lilacinum* against *G. mellonella* larvae using immersion and injection methods. Thus, we also determined which application method was more effective.

Materials and methods

Fungus culture

The entomopathogenic fungus *Paecilomyces lilacinum* was isolated from the agricultural lands of Eskişehir province, Turkey and was identified according to Pitt (1978) by Rasime Demirel (Demirel et al. 2005). This strain is also maintained in the KUKENS culture collection (WDCM101). The isolated strain of *P. lilacinum* was grown on malt extract agar at 28 °C in the dark. Conidial suspensions for assays were prepared by scraping conidia from 14-day-old well sporulated cultures into an aqueous solution of 1% Tween 80. Different conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}) were determined using a Neubauer hemocytometer.

Insect rearing

A *Galleria mellonella* colony was maintained by feeding the insects on an artificial diet including 340 g bran, 20 g pollen, 75 mL filtered honey, 150 mL glycerol, 100 g ground old dark honeycomb and 75 mL distilled water, modified by Bronskill (1961). All stock and experimental laboratory cultures were maintained at Anadolu University, Eskişehir, Turkey at 25 ± 2 °C, 60 ± 5% relative humidity, and constant darkness.

Bioassay of P. ilacinum

Pathogenicity of the entomopathogenic fungus Purpureocillium against the *G. mellonella* larvae was studied based on immersion and injection methods. Infection of *G. mellonella* last instar larvae was carried out by immersion at selected conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}) for 5 s. Also, the last instar larvae were immersed in an only aqueous solution of 1% Tween 80 as a control (n = 100). Using the injection method, last instar larvae of *G. mellonella* were treated by injection with 10 µL of each conidial suspension of different conidial concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}) and also 10 µL of the aqueous solution of 1% Tween 80 was used as a control (n = 100). Infected larvae were incubated at 25 °C in the dark for 15 days. Using both methods, mortality rates were observed daily for 15 days and recorded. All assays were replicated five times with 20 larvae completely randomized from different populations.

Statistical analysis

The median lethal time (LT50) was calculated according to Beron and Diaz (2005) and 100% mortality was determined in both methods. All observed mortality percentages were corrected using Abbott's formula (1925). According to the larval mortality data, the median lethal concentrations of *P. lilacinum* with associated 95% confidence levels ($P < 0.05$) for both methods were determined using Probit Analysis in Statistical Package for the Social Sciences (SPSS) software (version 18.0 for Windows, SPSS Science, Chicago, IL).

Results and discussion

In this study, it was determined that the entomopathogenic activity of *P. lilacinum* against *G. mellonella* larvae differed according to the application method (Table 1 and 2). *P. lilacinum* caused high mortality for *G. mellonella* resulting in approximately 100% mortality at 7 days post-immersion

Table 1. Percentage of mortality and median lethal time (LT_{50}) values of *P. lilacinum* suspensions against last instar larvae of *G. mellonella*. *Mortality percentages (%) were corrected using Abbott's formula (1925). **The Median lethal Time (LT_{50}) was calculated by Σ ($days_n$ × $infected\ larvae_n$) / total of infected larvae (Beron, Diaz 2005)

Application method	Fungal concentration (conidia mL^{-1})	Mortality (%)*	LT_{50} (days)**
Immersion	1×10^6	60	2.66
	1×10^7	74	2.61
	1×10^8	100	1.83
Injection	1×10^4	52	2.88
	1×10^5	100	2.05
	1×10^6	100	1.96
	1×10^7	100	1.47
	1×10^8	100	1.00

Table 2. Mortality percentage of *G. mellonella* larvae exposed to different condial suspansions of *P. lilacinum* in both application methods

Fungal concentration (conidia mL^{-1})	Log concentration	Mortality (%)		Corrected mortality (%)	
		Immersion	Injection	Immersion	Injection
Control	0	1	1	1	1.0
1×10^3	3	40	27	40	27.0
1×10^4	4	45	52	45	52.0
1×10^5	5	48	100	48	97.5
1×10^6	6	60	100	60	97.5
1×10^7	7	74	100	74	97.5
1×10^8	8	100	100	98	97.5

with 1×10^8 conidial concentration. However, fungal concentrations at 1×10^8, 1×10^7, 1×10^6 and 1×10^5 conidia mL^{-1} caused approximately 100% mortality after injection at 1, 2, 3 and 4 days respectively (Table 1). At the same time, we observed 100 % larval mortality at 1×10^8 conidia mL^{-1} in immersion and injection methods and the median lethal time (LT_{50}) was determined at 1.83 and 1 days, respectively. The LT_{50} values of fungal concentrations at 1×10^7, 1×10^6 and 1×10^5 conidia mL^{-1} were 1.47, 1.96 and 2.05 days, respectively using the injection method for *G. mellonella* larvae (Table 1). For the immersion method, the LT_{50} values of fungal concentrations at 1×10^7 and 1×10^6 conidia mL^{-1} fungal suspensions were 2.61 and 2.66 days, respectively (Table 1). Similar to the results of our study, Ibrahim et al. (2016) reported that *P. lilacinum* at the high concentration of 10^9 conidia mL^{-1} applied with immersion method caused 87.5% mortality of *G. mellonella* larvae and also LT_{50} was 2.2 days. However, in a previous study using the agar surface technique, *P. lilacinus* had very low infection with 30% larval mortality on *G. mellonella* in 10 days and a LT_{50} value 16.16 days (Baydar et al. 2016). the reason for the differences between this previous study and our study could be due to application method and fungus strain. Based on these results, we conclude that our strain of *P. lilacinum* had the shortest LT_{50} values against last instar larva of *G. mellonella* using the applied two methods.

The percentage of larval mortality using the tested methods at different conidial concentrations of *P. lilacinum* is summarized in Table 2. Our findings showed that fungal concentration of 1×10^8 conidia mL^{-1} caused 100% larval mortality using immersion method. On using the injection method, 100% larval mortality occured at 1×10^5 conidia mL^{-1}. Probit analysis showed that the LC_{50} value of the conidial concentration of *P. lilacinum* was 3.1×10^4 (confidence limit: $2.3 \times 10^4 - 5.4 \times 10^5$) condia mL^{-1} ($\chi^2 = 27.041$; $df = 4$; $P = 0.000$) for the immersion method. For the incetion method, LC_{50} value of the conidial concentration of *P. lilacinum* was 4.7×10^3 (confidence limit: $1.4 \times 10^3 - 1.3 \times 10^4$) conidia mL^{-1} ($\chi^2 = 16.89$; $df = 4$; $P = 0.002$). The present findings indicate that the effective virulence of *P. lilacinum* against *G. mellonella* larvae differed between the application methods. In other words, the injection of fungal suspension was more effective than the immersion bioassay for model insect *G. mellonella*.

A previous study reported that bioassay isolate PDRL812 of *P. lilacinus* showed higher mortality rates on mustard aphid (*Lipaphis erysimi*) with a LC_{50} value at 5.1×10^3 spores mL^{-1} and 100% mortality of insect population at 10^7 spores mL^{-1} (Ujjan, Shahzad 2012). In agreement with the above-mentioned study, we observed almost similar LC_{50} values using the injection method in *G. mellonella*. Our findings are also in line with the findings of Ibrahim et al. (2016) who reported that different fungal isolates including *P. lilacinus* caused varying mortality rates of 2nd instar larvae of *G. mellonella* at different times with immersion methods. On the other hand, it was noted that *P. fumosoroseus* was more virulent than *P. lilacinus* on white fly nymphs *Trialeurodes vaporariorum* (Gokce, Er 2005). Mwamburi et al. (2010) also found that *P. lilacinus* was non-pathogenic to adult house flies *Musca domestica*. Pathogenicity differences of *P. lilacinum* may be associated with the application method and the different conidia concentrations attached to the cuticle of the studied insect groups (Clarkson, Charnley 1996; Rambadan et al. 2011). Since we eliminated the cuticle barrier with the injection method, *P. lilacinum* was more pathogenic against *G. mellonella* at lower concentrations and short exposure times. Furthermore, the present results obtained with the model insect *G. mellonella* indicate that *P. lilacinum* may be used for biological control of the lepidopteran pests instead of dipterous pests.

In conclusion, the obtained results showed that this isolate of *P. lilacinum* had high pathogenicity on *G. mellonella* larvae considering the low LT_{50} and LC_{50} values. These fungal formulations would be beneficial for field studies in future. Therefore, we suggest that further research is done to determine the efficacy and viability of this isolate for longer periods in field or storage environments as a contact biopesticide against target lepidopterous pests. Considering human and environmental health, biopesticides obtained from entomopathogenic fungi could be used as alternative biocontrol agents in integrated pest management programmes. Thus, research on the use of entomopathogenic fungi for the control of insect pests should be continued.

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