Efficacy of *Paecilomyces fumosoroseus* Isolate P83 (Ascomycota: Hypocreales) against *Dociostaurus maroccanus* (Orthoptera: Acrididae)

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ABSTRACT

An isolate of the fungal biocontrol agent *Paecilomyces fumosoroseus* (Wise) Brown and Smith was bioassayed to determine its efficacy against *Dociostaurus maroccanus* (Thunberg, 1815). Trials were conducted under optimal laboratory conditions. Males locust were dipped individually in fungal suspension at five different concentrations of *P. fumosoroseus* isolate P83 ranging from $1x10^4$ to $1x10^8$ conidia/ml. Experiments were carried out in triplicates. The treated locusts became infected and mortality increased significantly with increasing concentrations. The 5 days post- inoculation mortality was estimated to 49.5 ± 3.92 %, 51.83 ± 5.41 %, 69.41 ± 2.90 %, 94.66 ± 2.72 % and 100 ± 0.00 % at $1x10^4$, $1x10^5$, $1x 10^6$,$1x 10^7$and $1x10^8$ conidia/ml, respectively. The LC_{50} was $3.93 x 10^4$ conidia/ml while the LC_{90} and LC_{99} were $7.24 x 10^6$ and $4.31 x 10^8$, respectively. The estimated lethal concentration and the median lethal time values decreased when the conidial concentration increased.

Results of the present study revealed that the insecticidal effect of *P. fumosoroseus* isolate P83 to *D. maroccanus* males could be a promising area of investigation for biological control.

KEYWORDS: Conidial suspension, Moroccan locust, isolate P83, virulence, LT_{50}, LC_{50}

INTRODUCTION

The Moroccan locust, *Dociostaurus maroccanus* (Thunberg, 1815), is considered as one of the most dangerous agricultural pests. In its gregarious phase the locust has periodically caused significant yield losses in several Mediterranean and Asian countries [1, 2]. The current method for locust control is based on applying synthetic insecticides, a situation that is unlikely to change during outbreak years [3, 4]. Nevertheless, concerns about pesticide tolerance, environmental contamination and human safety have enabled researchers to look for alternative methods of control [5]. Such methods include the use of entomopathogenic fungi considered as host specific and one of possible candidates for eco-friendly pest control instead of chemical control agents [6,7,8,9]. More than 700 species of fungi belonging to 90 genera were isolated from various insect species [7,10]. At least, 12 species or subspecies of fungi have been used as active ingredients for mycoinsecticides including *Paecilomyces fumosoroseus* [11]. Of the 31 species of *Paecilomyces spp.*, 14 species are known pathogens of arthropods. [17,18] , *P. fumosoroseus* has been isolated from most regions of the world and has been reported to infect several insects, over 40 species, belonging to many different orders, but is not reported from locust [12,13,14,15]. This fungus can target all stages in the life cycle of insects [16] and is one of the most common fungal pathogens found causing rapid infection and death of Whitefly [12,19,20,21] , and has become a commercial product to control especially whitesflies, thrips, spider mites and aphids in greenhouses and interiorscapes [22,23]. Most studies of *P. fumosoroseus* focus on whiteflies. Therefore, in this study we conducted tests at various conidial concentrations of *P. fumosoroseus* isolate P83 to assess the insecticidal potential , evaluate the virulence (median lethal concentration, i.e. LC_{50}), estimate the LC_{90} and determine the lethal time (TL_{50}, LT_{90}) of the fungal isolate against adult males of *D. maroccanus* under laboratory condition for possible use as a biological control agent.

MATERIALS AND METHODS

Dociostaurus maroccanus

D. maroccanus, obtained from the laboratory culture at the University of Mascara (Algeria), were reared under a 12 h light : 12 h dark photoperiod in a controlled temperature room at 26°C, 60-70 % relative humidity. Each cage made of wooden frames and metallic mesh (30 cm x 30 cm x 30 cm) was equipped with a 75 W light bulb that were mounted in the middle of the upper wall of the cages. Locusts were provided with wheat bran, distilled water, and fresh lettuce. Moroccan locust males, aged between 10-14 days were used in the experiment.

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Preparation of conidial spore suspensions

The fungal isolate *P. fumosoroseus* P83 obtained from soil of agricultural fields was cultured at 25 ± 1 °C on Potatos Dextractos Agar (PDA) in constant light for 15 days. This fungus requires light for optimal production of aerial conidia [24].

Conidia were harvested by adding 100 ml of sterile distilled water supplemented with 0.03% Tween-80. The conidial suspension was vortexed for 10 min and then filtered through layers of sterilized cheese cloth. Conidial concentrations were measured using a hemacytometer. From the stock conidia suspensions the following concentrations were prepared: 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml. The control consisted of sterile water containing 0.03% Tween80. Viability of the isolate P83 conidia was tested by spraying 0.1 ml of conidia suspension containing 10^6 conidia ml^{-1} onto PDA. An examination of these cultures after 24 h in an incubator at 25°C showed that 86.33 ± 1.45 % of the conidia was viable.

Inoculation of locust

Each *D. maroccanus* male was dipped in 20 ml of fungal suspension at the different concentrations [25]. Adults were left in the suspension for 20 s and then left to air dry for 1 min. After inoculation, locusts were transferred into 30 cm x30 cm x 30 cm wooden-framed cages with 10 individuals per cage at a controlled temperature of 26° C, and a relative humidity between 60 and 70%. The experiment was replicated on three different occasions, each time with 10 adult for each concentration. Controls were treated with sterile water containing 0.03% Tween-80.

Recording mortalities

Mortality was assessed each day for 5 days. All insects, both dead and alive, were examined at the end of the experiment to assess the overall levels of infection. Only the dead insects were used to assess the production of pathogen spores. Locusts were removed from cages, placed singly in Petri dishes containing 2 sheets of Whatman filter paper saturated with distilled water to provide humidity, and then incubated at 25°C for 2 days to encourage fungal growth [26]. Locusts were considered to be mycosed if mycelia protruded through the cuticle after death and subsequently formed distinctive condition.

Analysis of data

Data were analyzed by 1-way ANOVA- test. The mortality rate of the locusts exposed to test was corrected using Abbott's formula. Statistical significance of differences in mortality was examined using the Chi-square test (χ^2). The level of significance was $P < 0.05$. The effect of increasing concentrations of the fungal isolate on the mortality of locust was estimated using the dose effect analysis tool of XLSTAT. The Probit regression was use to calculate the lethal concentrations (LC_{50}, LC_{90}, LC_{99}) and the lethal time (LT_{50}, LT_{90}), including their confidence limits, at the various concentrations.

RESULTS

Fungal isolate was pathogenic to adults *D. maroccanus* and increasing fungal concentrations resulted in mortality rise (Table 1). All of the locust adults treated with the pathogens became infected. Fungal growth was observed on cadavers treated with *P. fumosoroseus* P83 within 5 days post inoculation. Sites of infection occurred on the membranes between head capsule and thorax, on intersegmental membranes and areas under the elytra (Fig. 1). Furthermore, we showed that mortality increased with increasing concentrations. The 5 days post- inoculation mortality was found to be 49.5 ± 3.92 %, 51.83 ± 5.41 %, 69.41 ± 2.90 %, 94.66 ± 2.72 % and 100 ± 0.00 % at 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml, respectively. The LC_{50} value was 3.93×10^4 conidia/ml while the LC_{90} and LC_{99} values were 7.24×10^6 and 4.31×10^8, respectively (Table 2). We gave evidence that when fungal concentrations increased, the LT_{50} and the LT_{90} of the *D. maroccanus* population decreased, indicating a faster killing speed by *P. fumosoroseus* P-83. At the highest concentration (1×10^8 conidia/ml), LT_{50} and LT_{90} were 2.87 days and 4.27 days, respectively. On the other hand, at the lowest (1.0×10^4 conidia/ml) they were 24.47 days and 36.39 days, respectively. According to the Chi square statistic test, for each fungal concentration, the values of lethal time were significantly different ($P < 0.05$) (Table 3).

Table 1. Mortality percentage (means $\pm$ SE) of *D. maroccanus* treated with *P. fumosoroseus* P83 at various concentrations 5 days post-inoculation.

Concentration conidia/ml	Mortality (%)	χ^2	df	P
1.0×10^8	100 ± 0.00	1.03	2	0.60
1.0×10^7	94.66 ± 2.72	0.73	2	0.69
$1. \times 10^6$	69.41 ± 2.90	1.06	2	0.59
1.0×10^5	51.83 ± 5.41	1.73	2	0.28
1.0×10^4	49.5 ± 3.92	4.04	2	0.13

For each treatment, the mortality rate was no significantly different (P < 0.05).

Fig 1. Cadaver of *D. maroccanus* infected by *P. fumosoroseus* P83, 5 days after treatment.

Table 2. Probit analysis and lethal concentration (LC) (conidia/ml) values of dose-mortality response assays 5 days post-inoculation of *D. maroccanus*

	LC[a]	95% CL[b]
LC$_{50}$	3.93 x 10^4	0,65 x 10^4 to 1,17 x 10^5
LC$_{90}$	7.24 x 10^6	2, 15 x 10^6 to 6,47 x 10^7
LC$_{99}$	4.31 x 10^8	5,18 x 10^7 to 3.55 x 10^{10}
χ^2 (df)	38.13 (1)	
P (χ^2)[c]	< 0,0001	
Slope ± SE	0.60 ±0.11	

[a] Probit analysis was used to calculate the lethal concentration.
[b] The criteria for significance was the failure of the 95% confidence limits (CL).
[c] LC values and there upper or lower confidence limits (conidia/ml) are significantly different at P < 0.05.

Table 3. Median lethal time (LT$_{50}$, LT$_{90}$) of *D. maroccanus* treated with *P. fumosoroseus* P83 at various concentrations.

concentration conidia/ml	LT$_{50}$ (days) and 95% CL	LT$_{90}$ (days) and 95% CL[a]	χ^2 [b]	P [c]
1.0 x10^8	2,87 (2,51 - 3,18)	4,27 (3,90 - 4,82)	208,09	< 0,0001
1.0 x10^7	5,52 (5,07 - 5,93)	8,01 (7,50 - 8,71)	195,78	< 0,0001
1.0 x10^6	16,27 (15,26 - 17,26)	25,47 (24,28 - 27,08)	219,41	< 0,0001
1.0 x10^5	18,10 (18,85 - 19,60)	26,79 (25,01 - 29,24)	115,51	< 0,0001
1.0 x10^4	24,47 (22,7 - 27,07)	36,39 (32,45 - 43,21)	323,25	< 0,0001

Probit analys was used to calculate the LT$_{50}$ and LT$_{90}$.
[a] The criteria for significance was the failure of the 95% confidence limits.
[b] Chi-square test statistic (χ^2) indicates a satisfactory goodness-of-fit.
[c] LT values followed by different upper or lower case letters within a column are significantly different (P < 0.05).

In this study, *D. maroccanus* adults were susceptible to isolate *P. fumosoroseus* P83 and appeared to be highly virulent and kill faster at the highest concentration. Mortality increased with increasing concentrations, all adults died after exposure to 1x10^8 conidia/ml within 5 days post-inoculation.

DISCUSSION

The present study reveals the efficiency of P. *fumosoroseus* isolate against *D. marocanus* at various spore concentrations. The highest mortality was 100 % at the end of 5 days with $1x10^8$ conidia/ml. Our results are consistent with those of several other previously published papers. Under optimal conditions, the death of an insect usually takes between 3 and 5 days from the time of application [7]. Similar effects have been shown for insect pests by research under laboratory conditions. Pathogenicity of *P. fumosoroseus* against *Culex quinquefasciatus* and *Tetranychus urticae* showed respectively 97.73% and 79.16% mortality, 8 days after treatment with $1x10^8$ conidia/ml[27,36]. It has been found that the mortality rate of diamondback moth *Plutella xylostella* and *Eutetranychus orientalis* induced by this pathogen was 76.2% 6 days after inoculation and 72 % on the 9th day post-treatment [15, 37]. In small-scale field trials, multiple applications of *P. fumosoroseus* at 4-7 days intervals provided more than 90% mortality of late-instar whiteflies [28]. It was also observed that an isolate *P. fumosoroseus* FG340 caused 100% mortality of *Spodoptera exigua* larvae 6 days after treatment at a low concentration 1×10^4 conidia/ml [9].

Presumably, susceptibility of most insects to entomopathogens is related to spore dosage. The speed of kill is influenced by the number of infection propagules in contact with cuticle [34]. In the current study, the lethal concentration (LC_{50}) and median lethal time for the *D. maroccanus* adult males were 3.93 x 10^4 conidia/ml and 2, 87 days at the highest concentration (1×10^8 conidia/ml), respectively. Panyasiri *et al.* (2007) [29] found that *P. fumosoroseus* isolate FWA3 was highly virulent against thrips and whiteflies species. The LC_{50} were 9.51 x 10^2 and 9.41 x 10^4 for *Ceratothripoides claratris* and *Bemisia tabaci*, respectively. According to [37], the LT50 value was 4.31 days against adult females of *T. urticae* traeted *with a* commercial preparation of *P. fumosoroseus* (Priority) at a concentration of 2.0×10^9 conidia/ml. Regarding our results, the isolate P83 seems to be more effective. Virulence variations depend, among others, on the diversity of pathogenic isolates and type of tested insects. Fungal isolates can highly differ in their efficacy to specific targets, such intraspecific variability for pathogen efficacy has been previously reported by other authors [30, 31, 12]. The virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity. Fungi can infect insects via several pathways including the gut (ingestion), through the spiracles, and through direct penetration of the integument. The pathogenicity is primarily mediated by entry through the external integument. Mycelial penetration through the integument favored by conidia which have evolved both physical and enzymatic mechanisms. The hydrophobic nature of the epicuticle is generally considered a good substratum for adhesion of fungal spores [38]. Conidia attach to the cuticle, germinate and penetrate the cuticle, this can occur between 24 to 48 h under ideal conditions [28]. Once in the hemocoel, the mycelium grows and spreads throughout the host, forming hyphae and producing blastospores [32]. Host death often occurs due to a combination of fungal toxins, physical obstruction of blood circulation, nutrient depletion and organ invasion. The main characteristic playing an important role in the virulence of entomopathogenic fungi is the production of enzymes necessary for the penetration of the arthropod cuticle. The extracellular proteases enzymes are considered the most important to penetrate the cuticle allowing the toxic compounds to invade the host's haemolymph. Moisture is a key factor for high and rapid killing of insects by entomopathogenic fungi, and further development on cadavers [32,33,35]. The current work reveals the insecticidal effect of *P. fumosoroseus* isolate P83 on the *D. maroccanus* males, due to the high mortality of locust, this pathogen could be a promising area of investigation for biological control. However, efficacy and the effects of environmental factors, temperature and humidity, should also be tested in field experiments for its effective usage as an alternative to chemicals.

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