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PATHOGENICITY OF THREE ENTOMOPATHOGENIC FUNGI AGAINST *GEOTROGUS DESERTICOLA* UNDER LABORATORY CONDITIONS

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Abstract

Description of the subject: *Geotrogus deserticola* (Blanch, 1850) (Coleoptera: Scarabaeidae) commits major damage on the roots of the most varied plants and especially on cereals.
Objectives: The application of biological control methods in exploring soil micro flora is interesting.
Methods: After collecting the *Geotrogus deserticola* larvae sampled from the soil, entomopathogenic fungi were isolated, purified and characterized. Selection tests of entomopathogenic fungi isolates with the most effective insecticidal activity against white grubs were carried out, followed by pathogenicity tests on larvae of the first and second larval stages (L_1 and L_2).
Results: The pathogenicity tests show that the mortality rate is very high from the 48 hours of treatment (from 48 to 196 hours), and that for *Beauveria bassiana* (Bals.-Criv) (B7, B9), an isolate of *Metarhizium anisopliae* (Metsch.) (M) and an isolate of *Paecilomyces fumosoroseus* (Wise) (P3). In fact, the corrected mortality rate obtained after treatment of L_1 and L_2 larvae of *Geotrogus deserticola* by the high concentrations of the isolates tested CL_{90}, reached 100% after 196 h only.
Conclusion: With the high mortality rate and the diversity of isolates obtained, it is very interesting to continue the characterization of other isolates and their bioactive metabolites specifically toxins for the control of *Geotrogus deserticola* larvae.
Keys words: *Geotrogus deserticola*; entomopathogenic fungi; virulent; $LC_{50;}$ LT_{50}

PATHOGÉNICITÉ DE TROIS CHAMPIGNONS ENTOMOPATHOGÈNES CONTRE *GEOTROGUS DESERTICOLA* DANS LES CONDITIONS DE LABORATOIRE

Résumé

Description du sujet : *Geotrogus deserticola* (Blanch, 1850) (Coleoptera : Scarabaeidae) commet des dommages importants sur les racines des plantes les plus variées et en particulier sur les céréales.
Objectifs : Application des méthodes de lutte biologique à l'exploration de la microflore du sol.
Méthodes : Les champignons entomopathogenes ont été isolés, purifiés et caractérisés, suivis de tests de pathogénicité sur les larves des premier et deuxième stades larvaires (L_1 et L_2).
Résultats : Les tests de pathogénicité montrent que le taux de mortalité est très important dès les 48 heures de traitement (de 48 à 196 heures), et cela pour *Beauveria bassiana* (Bals. -Criv) (B7, B9), un isolat de *Metarhizium anisopliae* (Metsch.)(M) et un isolat de *Paecilomyces fumosoroseus* (Wise) (P3). En effet, le taux de mortalité corrigée obtenus après traitement des larves L_1 et L_2 de *Geotrogus deserticola* par les fortes concentrations des isolats testées CL_{90}, atteints 100% au bout de 196h uniquement.
Conclusion : les isolats obtenus sont très intéressants de poursuivre la caractérisation d'autres isolats et de leurs métabolites bioactifs spécifiquement les toxines pour le contrôle des larves de *Geotrogus deserticola.*
Mots clés : *Geotrogus deserticola* ; champignons entomopathogènes ; virulence ; CL_{50} ; LT_{50}

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INTRODUCTION

Geotrogus deserticola is a polyphagous and nefarious pest of specific significance as it adversely affects the economic status of the farmers [1]. These insects feed on roots of various crops in different agro-ecological areas [2]. The life cycle shows that the larvae have a two-year cycle with the emergence of adults in the spring. Mating takes place on the ground surface. Egg laying, which begins two to three weeks after mating, takes three to four days. After three weeks, the 1st larval stage appears. The first moult is observed in September of the first year. The longevity of 2nd larval stage can exceed one year. The second moult is observed in September of the second year. The life span of 3rd larval stage is six months. Pupation, which lasts a month, takes place in March. The emergence of new imagos takes place in spring (April, May). *G. deserticola* pests are difficult to control because of their hidden nature, sometimes combined with insufficient knowledge on their life cycles and below-ground ecology [3]. Difficulties in achieving consistent and effective control of *G. deserticola* with current insecticides [4]. As well as public concern about pesticide use, point to the importance of developing biological control strategies for these pests. Entomopathogenic fungi have shown varying degree of virulence under different environmental conditions and against different stages of insect life cycle [5]. Members of the genera *Beauveria, Metarhizium, Entomophthora, Verticillium* and *Paecilomyces* have received maximum attention and are widely used against *G. deserticola* [6]. The more extensive use of *M. Anisopliae* world-wide for biological control of scarabs may be due to its broader host range when compared to the limited host range of *Beauveria brongniartii*. However, host range is much more restricted when individual and specific fungal isolates/strains are considered [7]. The study purposes mention as follows: to use entomopathogenic fungi effectively in biological control programs, and test their capacity to kill target host and find isolates suitable for efficient artificial production. The aims of current study were evaluation of entomopathogenic fungi *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Metarhizium anisopliae* on 1st and 2nd larval stages of *G. deserticola* under laboratory conditions.

MATÉRIEL ET MÉTHODES

1. Sampling sites

Naturally infected insects' cadavers were collected from four cereals lands in three areas: in the province of Mascara, area of Oued Abtal (35° 26'47.36 "N-00° 37'16.63" E), province of Saïda area of Khrichefa (35° 5'29.67 "N - 000° 36'29.39" E) and province of Tiaret area of Rahouia, Temba (35° 29'33.30 "N - 001° 7'5.87" E). All the provinces located in the North West of Algeria. The soil type in these three provinces is salty claythecollection was realised from November 2015 to June 2018. The dead cadavers with external signs of mycosis were collected and transported promptly to the laboratory.

2. Collection of entomopathogenic fungi isolates

The fungi isolations were performed by using two different methods the first one was conducted by disinfect the cadavers with sodium hypochlorite at 2% for two minutes then rinced at list two times with sterilized distilled water and then dried by using sterilized filter paper. The *G. deserticola* corpse was then culture on Potatoes Dextrose Agar in Petri-dishes. The Petri-dishes (90×15 mm) were incubated at 25±2˚C for seven days. Where the second one the entomopathogenic fungi were isolated using a modified procedure of Thakur [8]. The fungi were isolated directly from dead cadavers by transferring external conidia from dead insects of *G. deserticola* into 1.5% water agar with 0.05 mg/l chloramphenicol and incubating at 25±2˚C.

3. Morphological identification of fungi isolates

Morphological identification of collected entomopathogenic fungi was conducted by using a slide culture technique **[9, 10]**. Microscopic examination of fungi is mainly based on cultural characteristics and morphology. The morph taxonomic characteristics of conidia- forming mycelia and conidia structure were identified based on the key of Luangsa [11-13].

4. Pathogenicity tests of fungal inoculation on the 1st and 2nd larval stage of G. deserticola

The ability of fungal isolates to cause infection and disease in insects and recovering the fungus from dead insect bodies were used as another parameter in completing the identification process. Fully sporulated 15 days old colonies on PDA Petri-dishes were selected for fungal inoculums preparation.

Conidia were scrapped from dishes in the sterilized condition in micro flow and transferred to a sterilized 100 ml flask. Ten ml of sterile distilled water containing 0.01 percent Tween® 80 was added to each flask as surfactant and agitated gently for 10 min by using vortex stirrer and this to allow maximum release of the spores. Then the suspension filtered by using sheet cloths. Concentration of conidia in each suspension was estimated using the Malassez cell. Three concentrations were prepared 10^4, 10^5 and 10^7conidia /ml from each isolate. Forty five instar larvae of each the of 1st and 2nd larval stage of *G. deserticola* were dipped in each suspension for 30 s. Control larvae treated with sterilized distilled water containing 0.01 percent Tween® 80. Batches of ten larvae placed in each 9 cm diameter plastic Petri dishes experiment was repeated twice with three replicates. After fungal application, treatments were maintained at 25±2°C, 70% RH, and a 12:12 h (L:D) photoperiod. The mortality of insects was recorded every 48hours. Dead larvae were removed daily and placed in new Petri dishes covered with wet Whattman paper for fungal emergence. Conidia from dead insects of each isolate were transferred separately to PDA medium in a sterile condition. Light microscopic studies and colony form proved that recovered fungus is the same as inoculated fungus [14].

5. Mortality rates

After applications of the suspensions of two isolates of *Beauveria bassiana* (B7 and B9), an isolate of *Metarhizium anisopliae* (M) and an isolate of *Paecilomyces fumosoroseus* (P3), on the larval stages of *G. deserticola*, the insects were being paralyzed to death. Brown spots are visible in the thorax corresponding to the presence of the mycelium [15, 16]. In the presence of moisture, the mycelium pierced the cuticle, especially at the inter-segmental membranes, and begins to sporulate. Soon after, the corpse becomes covered with mycelium. Mortality was assessed each 2 days. All insects, both dead and alive, were examined at the end of the experiment to assess the overall levels of infection. The dead insects were culture on media and moistened filter paper to confirm that mortality by fungi.using Abbott formula [17].

$$\text{Mortalité corrigée (\%)} = \frac{\text{Mortalité du lot traité (\%)} - \text{Mortalité du lot non traité (\%)}}{100 - \text{Mortalité du lot non traité (\%)}} \times 100$$

6. Statistical analysis of data

The statistical analyses were performed by using the XLSTAT Version 2014.5.03 software. The mortality was estimated using the dose effect analysis tool of XLSTAT. The Probit regression was used to calculate the lethal concentrations (LC_{50}, LC_{90}) and the lethal time (LT_{50}, LT_{90}), including their confidence limits, at the various concentrations. For all tests, the level of significance was assessed at the 5% and 1% thresholds. If necessary, the comparison of the averages is made on the basis of the Newmann-Keuls Student's test in order to distinguish homogeneous groups according to the mean values of the two variables tested.

RESULTS

All tested isolates, in the concentration of 1×10^7conidia/ml showed a larvicidal effect with significant differences in the rate of mortality among the genera of the isolates. The mean rate of corrected mortality of the two isolates of *Beauveria* (B7 and B9) was varied (95 to 100%) (p<0.0001), the isolate of *Metarhizium* (M) caused mortality at (91 and 100%) (p<0.0001) and the isolate of *Paecilomyces* (P3) provoqued mortality at (82 and 71%) (p<0.0001). Respectively (Table 1, 2) toward1st and 2nd larval stage, the isolate of *Beauveria* being more pathogenic. Pathogenicity demonstrates the capacity of the isolate of an entomopathogenic fungus to infect the insect causing disease, whereas virulence quantifies such effect based on the time (post-infection) of mortality or the degree of colonization of host tissues [18].

Table 1: Cumulative mortality of the 1st larval stage of *Geotrogus deserticola* by different isolates

Isolates	Concentrations		
	10^3conidia /ml*	10^5conidia /ml*	10^7conidia /ml*
P3	33.33±0.13 [a]	44.44±0.10 [a]	82.22±0.03 [b]
B7	48.88±0.10 [a]	88.88±0.07 [b]	100±0.00 [b]
B9	28.88±0.25 [a]	73.33±0.13 [b]	95.55±0.07 [c]
M	35.55±0.31 [a]	75.55±0.10 [b]	91.11±0.03 [b]

***** Values are averages ± standard errors. For each trait, the means followed by different letters are significant (Kruskal-Wallis test (KW)

Table 2: Cumulative mortality of the 2nd larval stage of *Geotrogus deserticola* by different isolates

Isolates	Concentrations		
	10^3conidia /ml*	10^5conidia / ml*	10^7conidia / ml*
P3	22,22±0.10[a]	53,33±0.11[b]	71,11±0,03 [c]
B7	46,66±0.13 [a]	82,22±0.03[b]	100.00±0.00 [b]
B9	22,22±0.10[a]	68,88±0.26[b]	100±0.00 [b]
M	46,66±0.13[a]	68,88±0.16 [b]	95,55±0.07[c]

* Values are averages ± standard errors. For each trait, the means followed by different letters are significant (Kruskal-Wallis test (KW)

All tested genera of entomopathogenic fungi exhibited a pathogenic effect to the 1st and2nd larval stage of *G. deserticola*, all the isolates showed mean pathogenicity higher. *B. bassiana*, *B. Brongniartii* and *M. anisopliae* have been reported to be highly effective against *G. deserticola* with concentration 1×10^8 spores/ml [19]. These cultures produced 100% mortality of *G. deserticola* after 20–24 days of treatment. Application of *M. anisopliae* in sugarcane with 4×10^9 conidia/ha registered 92% reduction in grub population of *Holotrichea serrata*. Both *Metarhizium spp.* and *Beauveria spp*. produce insecticidal toxins [20, 21]. Reported infection of *Paecilomyces fumosoroseus* from *M. melolontha*, *Isaria fumosorosea* have shown efficacy against coleopterans and some scolytines.

The fungi *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Metchnikoff), and *Isaria fumosorosea* (Wize) have been studied widely as biological control agents, including as mycoacaricides [22, 23]. The larval mortality differed significantly and increased with increasing of conidial concentration. The data of two isolates of *Beauveria* (B7 and B9) were the most virulent toward the first and second larval stage of *G. deserticola.* The LC_{50} value of the isolate B7 was 1.10×10^4 and 1.47×10^4conidia/ml for the 1st and 2nd larval stage respectively while the LC_{90} was 9.5×10^6 and 4.29×10^6 conidia/ml for the 1st and 2nd larval stage respectively (p=0.868). The LC_{50} value of the isolate B9 was 1.18×10^4 and 1.58×10^5 conidia/ml for the 1st and 2nd larval stage respectively (Table 3, 3Bis).

Table 3: Lethal concentration LC_{50} and $LC_{90}$of*G.deserticola* on first larval stagetreated with different isolates.

Isolates	LC_{50}	LC_{90}	Slope ±SE	X^2	df	R^2	F	Pr > F
P3	6.00×10^5 ($0.00\text{-}1.67\times10^6$)	$8.70\text{x}10^9$ ($0\text{-}5.19\times10^{10}$)	0.05±0.84	33.37	4	0.94	99.70	< 0.0001
B7	1.10×10^4 ($3.21\times10^3\text{-}1.89\times10^4$)	9.5×10^6 ($5.21\times10^5\text{-}1.27\times10^8$)	0.07±1.11	52.04	4	0.98	373.54	< 0.0001
B9	1.18×10^4 ($8.16\times10^2\text{-}2.28\times10^4$)	4.41×10^6 ($0\text{-}1.33\times10^7$)	0.06±0.84	48.01	4	0.98	352.54	< 0.0001
M	3.32×10^3 ($0.00\text{-}8.05\times10^3$)	9.23×10^6 ($0\text{-}3.39\times10^7$)	0.94±0.84	29.97	4	0.97	324.50	< 0.0001

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

Table 3Bis: Lethal concentration LC_{50} and LC_{90} of *G. deserticola* on second larval stage treated with different isolates

Isolates	LC_{50}	LC_{90}	Slope ±SE	X^2	df	R^2	F	Pr > F
P3	1.14×10^6 ($0.00\text{-}2.70\times10^6$)	1.50×10^{10} ($0\text{-}7.71\times10^{10}$)	0.6±0.12	33.50	4	0.98	100.0	< 0.0001
B7	1.58×10^5 ($0.00\text{-}4.03\times10^5$)	1.58×10^7 ($0\text{-}6.24\times10^7$)	0.16±0.15	77,22	4	0.978	295.0	< 0.0001
B9	1.47×10^4 ($1.02\times10^3\text{-}2.83\times10^4$)	4.29×10^6 ($0\text{-}1.32\times10^7$)	1.33±0.27	50.82	4	0.917	55.10	< 0,0001
M	2.17×10^4 ($0.00\text{-}6.78\times10^4$)	8.40×10^7 ($0\text{-}4.52\times10^8$)	0.71±0.21	37.98	4	0.934	92.88	< 0.0001

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

The LC_{90} for this isolate on the 1st and 2nd larval stage was 4.41×10^6 and 1.58×10^7 conidia/ml for the 1st and 2nd larval stage respectively (p=0.995). The isolate of *Metarhizium* (M) was having a pathogenic effect toward the 1st and 2nd larval stage where the LC_{50} value was 3.32×10^3 and 2.17×10^4conidia/ml for L_1 and L_2 respectively. The LC_{90} was 9.23×10^6 and 8.40×10^7 conidia/ml for the 1st and 2nd larval stage respectively (p=0.990). The estimated LC_{50} value for the isolate P3 for the 1st and 2nd larval stage was $6.00\times10^5$and 2.17×10^4 conidia/ml respectively while the LC_{90} was 8.70×10^9 and 8.11×10^7 conidia/ml for the 1st and 2nd larval stage respectively (p=0.994). Compared to our results, Harizia [24]. Found that the LC_{50} value was 4.73×10^4 and 2.13×10^5 conidia/ml and the LC_{90} was 1.11×10^5 and 8.24×10^8conidia/ml respectively for the 2nd and 3rd larval stage of *G. deserticola* treated with *P. fumosoroseus* P83. The virulence of the entomopathogenic fungal isolates tested in this study was evaluated through the analysis of mean lethal time (LT_{50}) of the pest insect after its exposure to the pathogen. In general, there was a high mortality of larvae in the first few days after inoculation almost after two days the mortality was reached 100%. Lethal time values (LT_{50}) varied among the tested isolates on the first instar of the white grubs the isolate of *Beauveria* (B9) showed lethal time values (LT_{50}=9.11 d) (p=0.876) than the isolate of *Beauveria* (B7) with lethal time values (LT_{50}=8.79 d) (p=0.870) these isolates were having shorter average of lethal time, the isolate of *Metarhizium*, showed lethal time values ($LT_{50}=1.36\times10^1$d)(p=0.486), the isolate of *Paecilomyces* P3, showed lethal time values ($LT_{50}=7.06\times10^1$d) (p=0.486). These isolate showed the lowest LT_{50} values, but did not differ significantly among them (Table 2). In the same case this isolate showed short lethal time value against the second instar the isolates of *Beauveria* (B7) showed a short lethal time values (LT_{50}=7.79 d) (p=0.998) the isolate (B9) was having lethal time values ($LT_{50}=4.12\times10^1$d) (p=0.999), the isolate (M) was having lethal time values ($LT_{50}=3.63\times10^1$d) (p=0.999), the lethal time of the isolate (P3) was ($LT_{50}=9.50\times10^1$d) (p=0.886). The results demonstrated significant variation in virulence among isolates of the tested genera (Table 4, 4Bis).

Table 4: Median lethal time (LT_{50} and LT_{90}) of the first larval stage of *G. deserticola* treated with different isolates and different concentration

Isolates	LC_{50}	LC_{90}	Slope ±SE	X^2	df	R^2	F	Pr > F
P3	7.06×10^1 (0-1.56×10^2)	2.19×10^3 (0-1.98×10^4)	0.05±0.84	33.37	3	0.033	5.54	0.020
B7	8.79 (0-8.75×10^1)	3.56×10^2 (0-2.7×10^3)	0.07±1.11	52.04	3	0.007	6.84	0.011
B9	9.11 (0-8.845×10^1)	5.2×10^2 (0-4.58×10^3)	0.06±0.84	48.01	3	0.008	6.86	0.011
M	1.36×10^1 (0-7.05×10^1)	2.95×10^2 (0-1.32×10^3)	0.94±0.84	29.97	3	0.013	7.73	0.007

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

Table 4Bis: Median lethal time (LT_{50} and LT_{90}) of the second larval stage of *G. deserticola* treated with different isolates and different concentration

Isolates	LC_{50}	LC_{90}	Slope ±SE	X^2	df	R^2	F	Pr > F
P3	9.50×10^1 (0-2.17×10^2)	5.62×10^3 (0-7.21×10^4)	0.6±0.12	33.50	3	0.030	5.31	0.022
B7	4.12×10^1 (0-1.57×10^2)	7.31×10^2 (0-6.33×10^3)	0.16±0.15	77.22	3	0.008	6.65	0.012
B9	7.79 (0-8.98×10^1)	6.93×10^2 (0-7.60×10^3)	1.33±0.27	50.82	3	0.034	4.36	0.037
M	3.63×10^1 (0-9.05×10^1)	3.04×10^2 (0-1.09×10^3)	0.71±0.21	37.98	3	0.043	6.77	0.011

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

This study suggested that although the genus was more pathogenic to the 1st and 2nd larval stage, since the lethal time of the isolates of *Beauveria* were for the two stages of *G. deserticola*. Entomopathogenic fungus have great promise for use as biological control agents against different insects however, their infectivity is quite different depending on fungus species and developmental stage of the insects [25]. Laboratory bioassays were performed to prove the potential of different isolates of pathogens on the first and second larval stage of *G. deserticola* while the bioassay was performed using the dipping method [26]. Our results showed that the mortality of *G. deserticola* larvae was depending to the concentration of conidia and the larval stage. The four isolates of the entomopathogenic fungi were more effective to control both larval stages. This finding was similar to that obtained by Kulkarni [27]. The pathogenicity however, varies with strains or isolates [28]. Therefore, the selection of effective strains of entomopathogenic is essential for the development of microbial insecticides against M. *melolontha* by spraying blastospores on adults in Europe. He observed a significant decrease in pest population during the second generation [29]. Reported that grubs of *H. serrata*, *H. consanguinea*, *H. froges* and *Autoserica nathani* were highly susceptible to *B. brongniartii*. Our study shows that the efficacy of the different isolates tested on *G. deserticola* larvae did not show the same insecticidal reaction [30].

The virulence of entomopathogenic may vary depending on the intrinsic factors of the isolates. The main characteristic that plays an important role in the virulence of entomopathogenic fungi is the production of enzymes necessary for the penetration n 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 [31]. When the fungus enters the haemolymph, the cellular defence system plays a key role through the reaction of the haemocyte cells and / or lipid cells of the insect. The secretion of antimicrobial substances and the detoxification of microbial toxins reduce the effectiveness of the pathogen [32].

DISCUSSION

M. anisopliae, B. bassiana and *P. fumosoroseus* are pathogenic microorganisms for many insects. Their effectiveness has led researchers to isolate their toxins and produce them in several commercial forms [15]. They have been used in the control of many crop pests such as *Adoryphorus couloni* (Coleoptera: Scarabaeidae), in Australia, the locust (Acrididae) in Africa [20]. *Cleonus punctiventris* (Coleoptera: Scarabaeidae) and *Anisopliae austriaca* (Coleoptera: Curculionidae) in America [33]. The results obtained in our study show that the mortality of *G. deserticola* larvae depends on the concentration of conidia they receive. The four isolates of entomopathogenic fungi were effective in both larval stages (L_1 and L_2). Our results are consistent with that of Kulkarni [27]. The larval stage L_1 showed sensitivity towards the concentrations 10^5 and 10^7 spores / ml of *M. anisopliae* where the mortality rates are higher. This mortality rate is significantly lower for the L2 larval stage; this is probably due to the size and sensitivity of the L2 larvae, compared to that of the L1. These results are similar to those obtained by Clarkson & Charnley [33]. Who found that the infection of locust larvae by entomopathogenic isolates is a function of their weight similarly, Gundannavar [34], observed that the application of different concentrations (10^3, 10^5 and 10^7conidia / ml) of *B. bassiana* to *H. armigera* larvae was more sensitive than adults. The newly formed cuticle has less rigidity and the thin layers of wax and cement are particularly susceptible to fungal attack [35]. Before the pathogen penetrates the cuticle, the preformed cuticular structure plays a key role in the preventive defense mechanism against the infectious agent. The waxy layer of the epicuticle contains antifungal substances that significantly reduce the germination of conidia. The saturated fatty acid chains of the larval cuticle of insects inhibit the germination of *B. bassiana* conidia, whereas the long chain fatty acids (C: 14) stimulate conidial germination and / or tube growth. germination [36]. The presence of free fatty acids in the cuticular structure would also have a regulatory role in the specificity of certain fungi. The cellular defense mechanism of the host can influence the infection cycle and the specificity of the entomopathogenic fungus. In the cuticle, structural defense systems suppress spore germination by inhibiting the stimulants necessary to trigger germination.

In addition, these systems inhibit microbial enzymes via protease inhibitors and melt fungal structures through the activation of propenoloxidase [37]. When the fungus enters the haemolymph, the cellular defense system plays a key role through the reaction of the haemocyte cells and / or lipid cells of the insect. The secretion of antimicrobial substances and the detoxification of microbial toxins reduce the effectiveness of the pathogen [38]. Our study shows that the efficacy of the different isolates tested on *G. deserticola* larvae did not show the same insecticidal reaction. The virulence of entomopathogens may vary depending on the intrinsic factors of the isolates [30]. It is also dependent on relative humidity, and temperature [40]. *B. bassaina*, *M. anisopliae* and *P. fumosoroseusse* develop and sporulate well at 20-25°C. As with many species of fungi, it requires a high percentage of relative humidity (more than 92% for 14 to 20 hours) to germinate, grow and sporulate [21].

CONCLUSION

In the north-western Algerian, the white grubs *Geotrogus deserticola* is the most formidable pest of the cereal crop. The viability of biological control agents for this pest is expected to reduce most of the environmental problems associated with the use of chemical insecticides. That is what our work is all about. Three entomopathogenic fungi, have been isolated from the mummified insects of *Geotrogus deserticola* in three area; Mascara, Tiaret and Saïda. These strains were the subject of a study of the entomopathogenic power of entomopathogenic fungi isolated from the larvae of *Geotrogus deserticola* revealed very encouraging results. Indeed, preliminary tests carried out on several isolates, have allowed us to select the best performers. Very significant mortality rates were recorded in a time not exceeding 48h. The results of this study will provide the key knowledge needed for the future application of native white grubs strains in Algeria. In addition, future applications will involve the exact identification of isolated entomopathogenic fungi with the determination of their mode of action in order to be able to manufacture an industrial product for large-scale applications.

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