# Evaluation of Different Isolates of Entomopathogenic Fungi against *Metopolophium dirhodum* (Walker) (Homoptera: Aphididea) from Constantine, Algeria

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**Abstract:** The aim of the present study was to investigate the effect of seven entomopathogenic fungi *(Aspergillus californicus, Beauveria bassiana, Fusarium oxysporium, Metharizium flavoride, Cladosporium cladosporioides, Trichoderma viride and Verticillium alfalfae*) against aphid insects: *Metopolophium dirhodum.* The selected entomopathogenic fungi were isolated from the agricultural soil of the National Institute of Plant Protection of Constantine, Algeria, and were tested against aphid insects that were gathered from the same area. The aphids were exposed to each fungal spore suspension ($10^7$ conidia/ml) for 10 seconds. The viability/mortality of the insects was evaluated on the 1st, 3rd, 5th, and 7th day after inoculation. After 7 days of inoculation, all the fungi species, except *F. oxysporum,* presented a significant effect ($P < 0.05$) against the studied aphid. The mortality rate was estimated between 21 and 96%. *B. bassaian, C. cladosporioides* and *V. alfalfae* presented the most potent effect on *M. dirhudum* with a percentage above 50% (95.83, 63.98 and 51.83%, respectively). *A. californicus* and *M. flavoride* showed the same effect: 41.97%. *T. viride* and *F. oxysporium* had the lowest effect with 31.44% and 20.83%, respectively. The inter/intra specificity of the fungi was mostly reported, besides other factors, as the modulator of their effectiveness.

**Keywords:** *Entomopathogenic fungi, M. dirhodum, Mortality rate.*

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## INTRODUCTION

The aphids are the most harmful insects affecting wheat plant *Diuraphisnoxia* (Mordvilko), *Sitobionavenea (F.), Schizaphis graminium* (Rondani), *Rhopalosiphumpadi* (L.), *Metoplophium dirhodum* (Walker), and *Siphamydis* (Passerini) are considered as the most important insects that depreciate substantially cereal cultures (Rassipour et al., 1996; Blackman and Esatop, 2000). The rose grain aphid (*M. dirhodum*) is the most important species that could destroy grain worldwide (Dixon,1987), in fact, they choose hazardly a host during the cold season and then emigrate towards drought cereals in summer (Carter et al., 1980). For instance, *M. dirhodum* insect is able to transmit a *Luteovirus*, and it provides a wide resistance against exterminators (Carter et al., 1980). Such situation compels us to focus on methods that can stop these insect pests and consequently disease deployment. The biocontrol using entomopathogenic fungi, has been recently considered the most effective method. Moreover, these fungi are important through their aptitude to fight against exterminators by invading insect crusts. These opportunities make them as the first candidate against this plant culture epidemic (Lacey and Goettel, 1995; Barta and Gagan, 2006).

The most common fungal species belong to the order of Entomophtorales. Entomopathogenic fungi such as *B. bassiana* (Balsamo) and *Peacilomyces ssp*. showed a promising level of activity against aphids (Milner, 1997). The purpose of the present study is to determine the most effective fungi between the studied seven fungal species against *M. dirhodum*, which is the most widely spread aphids in the culture region.

### Materials and Methods

#### *Insect rearing*

*Metopolophium dirhodum* (Walker) were collected from a wheat called Cirta HD.122 (*Triticum eastivum* L.) of the National Institute of Plant Protection' suburb (INPV) of Constantine, Algeria.

#### *Fungal isolates*

The virulence of seven fungal isolates (Aspergillus californicus, Beauveria bassiana, Fusarium oxysporium, Metharizium flavoride, Cladosporium cladosporioides, Trichoderma viride and Verticillium alfalfae) was tested with a pathogenicity test. The fungi used in this study were obtained from a private collection from agricultural soil of the INPV and then they were isolated.

One gram (1g) of the soil was diluted in 9ml of distilled water. Then 100µL of dilutions which are $10^{-3}$, $10^{-4}$, $10^{-5}$ was taken and planted in Potatoes Dextrose Agar (PDA: 200 g potatoes, 20 g D. Glucose, and 20 g Agar) that was supplemented by Chloramphenicol (10mg/l). The dishes were incubated for two weeks at 28°C. The macroscopic and microscopic of any resultant fungal growth were compared in terms of taking into account the standard description of mycelium and spore.

***Bioassay***

The aphids were attacked for 10 seconds in each fungal suspension prepared at the concentration of $1.10^7$ conidia/ml of sterile distilled water and supplemented with a drop of Tween 80 (0.05%). Dishes were kept at room temperature at 25°C and humidity Aw=60%. The viability/mortality of the insects were evaluated on the 1st, 3rd, 5th and 7th day after inoculation day. All the treatments were repeated six times.

***Statistical analysis***

The calculation of insect mortalities was corrected using Abbot's (1925) calculation method "Formula percent mortality rates". The data were subjected to one-way ANOVA and significant differences were detected. Furthermore, multiple comparisons were made through Tukey's HSD using SPSS ® for data analysis (version 17.00 Software. 2006 SPSS.Inc.Chicago.il, USA).

**Results and Discussion**

At 7 days after application, the effect of the isolated fungi against the aphid insect *M. dirhodum* varied widely and ranged between 21% and 96%. The results revealed that *B. bassiana* had the most effective toxicity with 95.83%, followed by *C. cladosporioides* with 63.98%, then *V. alfalfae* with 51.83%. *A. californicus* and *M. flavoride* presented approximately the same effect with 41.97%. Compared to the other fungal species, the effect of *T. viride* and *F. oxysporium* against *M. dirhodum* were the lowest with 20.83% and 31.44%, respectively (Fig. 1).

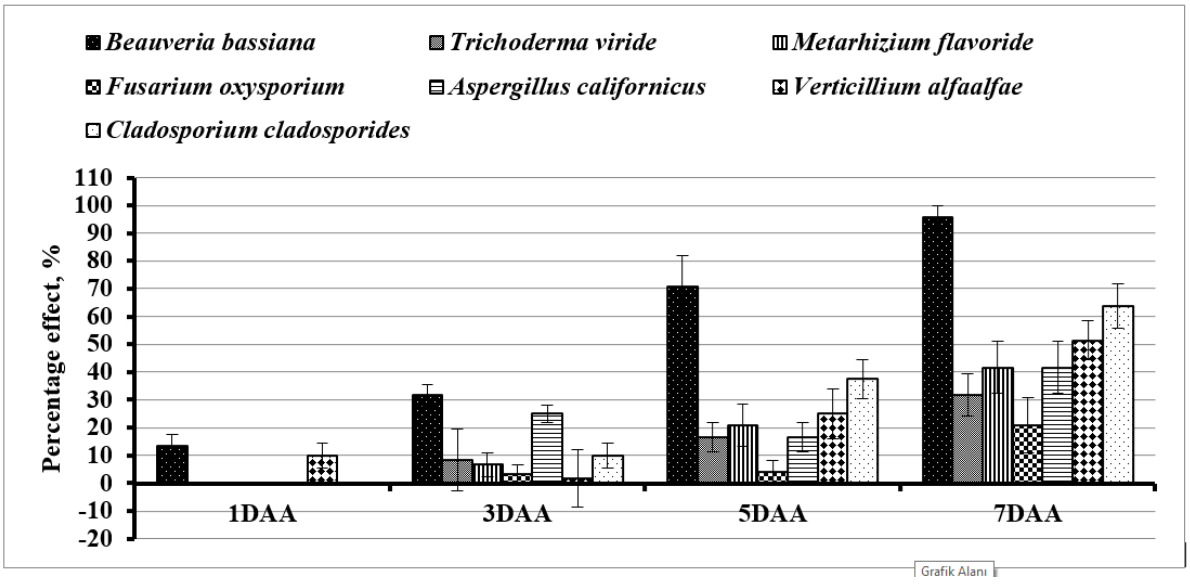


**Figure 1.** Effect of fungi on *M. dirhudum* (%)

The death rate presented in our study by the density of the aphids after application revealed in the same way, that *B. bassiana* presented the most significant fatal effect at all times of treatment ($P < 0.05$) compared to the control and to the other fungal species. Its effect began on the first day of application

with a death rate of 13.33%. In contrast, *T. viride* had no pronounced effect compared to the control at all times of incubation, it did not affect the aphids at the third day after application with only 6.6% of death rate. In addition to *B. bassiana, A. californium* presented significant toxicity against *M. dirhodum* (3.50; $P < 0.05$) on the third day of application, thereby *C. caladosporioides* had exercised significant fatal effect beside *B. bassiana* with 2.33 ($P < 0.05$) on the fifth day after application. However, after the seventh day, all fungal species, except *F. oxysporium* presented significant fatal effect against the aphid compared to the control (Table 1).

**Table 1**. Effect of *M. dirhodum* (Walker) population density before and after the fungal suspension application

| | **1DAA** | **3DAA** | **5DAA** | **7DAA** |
|---|---|---|---|---|
| ***Aspergillus californicus*** | 0.00 $^{b}$ | 25.00 ± 3.16 $^{ab}$ | 16.67 ± 5.27 $^{bc}$ | 41.67 ± 9.38 $^{bc}$ |
| ***Beauveria bassiana*** | 13.33 ± 4.22 $^{a}$ | 31.67 ± 3.80 $^{a}$ | 70.83 ± 10.92 $^{a}$ | 95.83 ± 4.17 $^{a}$ |
| ***Fusarium oxysporium*** | 0.00 $^{b}$ | **0.83** ± 5.83 $^{b}$ | 4.17 ± 4.17 $^{c}$ | 20.83 ± 10.03 $^{c}$ |
| ***Metharizium flavoride*** | 0.00 $^{b}$ | 4.33±0.21 $^{ab}$ | (2.83±0.17$^{ab}$ | 2.00±0.26$^{bc}$ |
| ***Cladosporium cladosporioides*** | 0.00 $^{b}$ | 10.00 ± 4.47 $^{ab}$ | 37.50 ± 7.05 $^{b}$ | 63.89 ± 7.59 $^{ab}$ |
| ***Trichoderma viride*** | 0.00 $^{b}$ | 6.67 ± 4.22 $^{ab}$ | 20.83 ± 7.68 $^{bc}$ | 41.67 ± 9.38$^{bc}$ |
| ***Verticillium alfalfae*** | 10.00 ± 4.47 $^{ab}$ | 1.67 ±10.38 $^{ab}$ | 37.50 ± 7.05 $^{bc}$ | 63.89 ± 7.95$^{bc}$ |
| **P.value (P ≤ 0.05)** | 0.000 | 0.000 | 0.000 | 0.000 |
| ***S.E.M*** | 0.062 | 0.23 | 0.21 | 0.26 |

**1DAA**: 1$^{st}$ day after application; **3DAA**: 3$^{rd}$ day after application; **5DAA**: 5$^{th}$ day after application; **7DAA**: 7$^{th}$ day after application; **P**: Probability; a. b, c, means with different superscripts letters within the same line are significantly different according to Tukey's HSD multiple range test ($P < 0.05$). **S.E.M**: Standard Error of Mean.

The pathogenic effect of fungi against insect's pest populations has been observed for a long time (Butt et al., 1994; Hesketh et al., 2008; Freed et al., 2012). Several fungi species were developed as biological control agents for aphids (Shah and Pell, 2003; De Faria and Wraight, 2007). The rose grain aphids *M. dirhodum* (Walker) (Hemiptera: Aphididea), is one of the most serious species found in almost all grain–producing regions of the world. *M. dirhodum* is a vector of *Luteovirus* and barley yellow dwarf **(**Carter et al., 1980).

The development of aphid colonies is highly threatened by the presence of entomopathogenic fungal species considered as their most important cause of death as reported by Remaudière et al. (1981). Hence, more than 750 fungal species were reported as a potent agents against insects propagation, their ability to modulate the insects population was well discussed in literature (McCoy et al., 1988; Gillespie and Moorhouse, 1989). The most studied fungal species in the biocontrol of aphids are ascomycetes belonging to the order of *Hypocreales* (*Beauveria, Metarhizium, Nomura, Verticillium et Peacilomyces*). *Beauveria bassianan* and *Metarhizium anisopliae* were considered as the most effective ones regarding

the recorded high mortality rate (epizooties) **(**Burges, 1981; Carruthers and Soper, 1987; McCoy et al., 1988).

In our study, *B. bassaian*, *C. cladosporioides* and *V. alfalfae* presented the most potent effect on *M*. *dirhudum* with a percentage above 50% (95.83, 63.98 and 51.83%, respectively).

In contrast, *Aspergillus californicus* and *Metarhizium flavoride* showed an equal effect estimated by 41.97%. Finaly, *Trichoderma viride* and *Fusarium oxysporium* showed the lowest effect with 31.44% and 20.83%, respectively.

*B. bassiana* have killed 90% of the aphids after 3 days of application and these results agreed with those of Mburu et al. (2009) who reported that *B. bassiana* developed a considerable activity against *M. dirhudum*. It has been shown, in the same context, that this species presented a large spectrum and strong virulence, affecting the host with a simple contact action. Its safety for the vertebrate make it used, in the same case, in classical biocontrol by introduction technique (Meyling and Eilenberg, 2007).

Kim et al. (2013) found that among 47 cultures of *B. bassiana*, Bb08 showed the highest mortality (78%) against green peach aphid three days after treatments.

The significance of *Cladosporium spp*. as one of the effective biological control agents against whiteflies, aphids and scale insects in the world have been reviewed (Roberts and Humber, 1981; Hulden, 1986; Pan et al., 1989; Humber, 1991; Thumar and Kapadia, 1994; Han *et al.*, 1997; Abdel-Baky et al., 1998). *C. cladosporioides* exercised significant fatal effect beside *B. bassiana* with 63.93% ($P < 0.05$) after the seventh day of application.

Saranya et al. (2008) showed that the conidial suspension of *C. oxysporum* generated corrected mortality of 77.5% in *Aphis craccivora* Koch individuals, when the suspension was used at a concentration of $10^8$ conidia/ml incorporated in the Teepol solution. However, Bensaci et al. (2015) revealed that maximum mortality of *C. oxysporum* against *A. fabea* (67.96%) was obtained with conidial suspensions at a concentration of $10^8$ conidia/ml.

In addition, Abdel-Baky and Abdel-Salam (2003) showed in a laboratory test, that maximum mortalities of *A.gossypii* (37.5%) and *A. craccivora* (38%) were recorded on the third day after application with conidial suspensions of *Cladosporium* spp. at a concentration of $10^6$ conidia/ml.

In the present study, the results describing the effect *V. alfalfae* on aphids were similar to those reported by Chavan et al. (2008) who showed significantly higher efficacy controlling aphids with 68.23% to 89.54% mortality of *V. locanii* .

The effect of *M. flavoride*, registered at 7 days after application (41.67%) was inconsistent with the average effect with 60-86% reported by Murerwa et al. (2014) in their study on the effect of *M.*

*anispliae* on the aphids *M. dirudum* and *R. padii*. Similar results were reported by Won et al. (2015) on green peach aphids (*Mysuspersicae*).

In the same level, *A. californicus* has registered an effect (below 60%) consistent with that reported by Won et al., (2015). *Aspergillus* species displayed a wide diversity of lifestyles including in clinical, industrial and agricultural environments; some of them may be opportunistic pathogens of a wide range of organisms including agricultural pests (Gibbons and Rokas, 2012). It has been reported through toxicity test that *Aspergillus* species could be useful in aphid control as pest control agents. However, these saprophytic fungi are not targeting only insects, but can also affect immune depressed humans, mammals and birds (Tell, 2005). Non-aflatoxin-producing and non-toxigenic *A. flavus* strains are currently studied in biological control to reduce pre-harvest contamination of crops with aflatoxin (Ehrlich, 2014).

As a part of selection process, five different concentrations of *Aspergillus clavatus* (Desmazie`res), *Aspergillus flavus* (Link) and *Metarhizium anisopliae* ((Metschnikoff) Sorokin) spora were tested against the pea aphid, *Acyrthosiphon pisum* (Harris). *Aspergillus* isolates induced higher mortalities than *M. anisopliae,* which is well known as entomopathogen in the literature (Seye et al., 2014).

Our results showed that *T. viride* presented mortality rate estimated at 31.94%. These results were completely different in comparison to those reported by Ganassi et al. (2001) who registered a massive effect (100%) after 3 days of application of *Trichoderma* against three varieties of *Schizaphis graminum* isolated from a rice plant. The same results were reported by Omar et al. (2012) on *Cockrociches periplaneta* (American insect) and *Pestalotia psidii*, respectively. These reports indicated that the effectiveness of the fungi depended on their metabolites capacity to stimulate the aphids infection.

About *Fusarium*, the rate of mortatlity registered in our study was inconsistent with that obtained by Guesmi-Jouani et al. (2010) who reported a fatal rate exceeding 90%. The effectiveness of *Fusarium* species was high but particularly reported on *Aphids gossypii Glover* species (Ganassi et al., 2001). The selection of entomopathogenic fungi for biological control of insects remains difficult. It depends on the insect‘s susceptibility and vulnerability towards fungus and consecutively the inter- and intra specificity of each fungus against the insects (Glare et al., 2012).

**Conclusion**

Under the present experimental conditions, the results revealed that *B. bassiana*, had infection ability unlike *M. falvoride*, that showed a rate less than 50% even if it was the most important species used in the biocontrol against insects. *C. cladosporides* and *V. alfalfae* encouraging results were obtained, regarding *T. viride* and *F. oxysporium* that had rates with 31.94% and 20.89 %, respectively. Even though, these two species are very important in biocontrol against insects. In conclusion, the

present study revealed that a specialization could be found to every kind of fungi against a specific insect (interspecific and intraspecific).

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