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Pathogenicity of *Metarhizium anisopliae* (Metsch) on *Ceratitis capitata* L. (Diptera: Tephritidae)

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ABSTRACT

Several chemical substances are used to control insects, diseases and weeds; however many of these are toxic to mankid and the animals besides reducing the potential of pest control by predators, parasitoids and pathogens. Because biopesticides are thought to be more rapidly degradable than synthetic chemicals while having a lower ecotoxicological effects, the interest in these products for pest control has grown substantially. The insecticidal activity of an isolate local strain of an entomopathogenic fungus, Metarhizium anisopliae (Metsch) Sorok, was tested at four doses, against fourth larval stage and adults (male & female) of Ceratitis capitata (Wied) under laboratory conditions. The percentage of accumulated mortalities of the fourth larvae at the seventh day after inoculation were 26.13% for the lower dose, 6.5 $x10^5$spore/ml and increased to 89.05 for the highest dose represented by 52 x 10^5 spore/ml. Obtained results showed that the susceptibility of males to the fungus was higher the females with mortality of 88.21% and 76.05 respectively with the of 52 x 10^5 spore/ml. These toxicity assays allowed the determination of the different lethal doses (CL_{50}, CL_{90}) for both stages. The assay of the female adult of C. capitata exposed to DL_{50} = 25.4 $x10^5$; DL_{90} = 68.6 $x10^5$ spore/ml) of M. anisopliae showed a significant decrease ($p<0.05$) of the egg number laid after treatment. The cuticular proteins amounts have been were determined on the fourth instar larvae of C. capitata at 3 and 6 days after treatment with the two lethal doses (DL_{50}=24.8 $x10^5$; DL_{90}= 49.6 $x10^5$ spore/ml). Results showed a significant decrease in protein amounts after treatment as compared to control series. The bioassays reveal that Metarhizium anisopliae presents better potential for the biological control of Ceratitis capitata.

Key words: Biocontrol, Proteins, Cuticle, *Metarhizium anisopliae, Ceratitis capitata.*

INTRODUCTION

The Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Wied.) (Diptera: Tephritidae), is one of the most serious fruit pests worldwide. The medfly is a polyphagous species its infestation causes huge economic losses [1]. Is control depends upon chemical spray with organophosphate pesticides then environmental impacts and resistance development justified the search for alternative products, like the growth regulators of the insects (IGRs) and the biopesticides**.** The progressive undertaking conscience, of environmental dangers to the abusive use of conventional insecticides, contributes to the interest given to the biological control. The use of biocontrol agents including entomopathogenic fungi as alternative to synthetic chemical control is being explored for management of wide range of fruit fly pests [2]; [3]; [4]; [5] and disease vectors [6]; [7]. *Mearhizium anisopliae***,** with asexual reproduction, belongs to the class of Hyphomycetes**,** was the subject of many research related to the mode of infestation [8] and the toxicity mechanisms of the insect [9] where it was demonstrated that the spore penetration varies according to the degree of contamination and the thickness of the cuticule of the host [10]. The biopesticides, which are the crude soluble extract (CSPE)from the fermentation product of the entomopathigenic fungi, *Metarhizium* and *Beauveria* species, have appeared in the literature as promising insecticides for the control of the pest fruit flies [11]; [5]; [12]; [13]. Several of these biopesticides intended for the control of *Ceratitis capitata* [14]; [15]; [16].were extracted starting from *M. anisopliae* [17]; [13]; [18]. These products are characterized by the presence of toxins called destruxines, secreted by *M. anisopliae* [19].

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.) is a major pest of fruit cultures in Algeria [20], and the damage degree varies according to the area, the climatic, biological and farming factors, which are directly related on the life of the insect [21]. The present study was conducted to evaluate the effects of a local isolated strain on entomopathogenic fungus *Metarhizium anisopliae* (Metch.) on various developmental stages of *Ceratitis capitata.*

MATERIALS AND METHODS

Fungal isolate

Metarhizium anisopliae strain, used in this study, originally isolated from a larval mummies of colding moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) and four doses were tested (6.5 $x10^5$; 13 $x10^5$; 26 $x10^5$ and 52 $x10^5$ Spore/ml) during these bioassays. The isolation of this fungus was made on Potato Dextrose Agar (PDA) medium, and then the purification was carried out by the transfer of single spore colonies in to Sabouraud Dextrose Agar (SDA) and culture incubated at 25°C and 75% humidity, for a week, in complete darkness. Conidia obtained from the first subculture were used for mass production of inoculums.

Bioassays

The fourth-instar larvae and adults, male and female, of *Ceratitis Capitata* used for the assays were obtained from a stock colony of the laboratory. The inoculation of the larvae with *Metarhizium anisopliae*, was carried out on filter paper imbibed with 5ml of each dose, by deposing the larvae in the centre and allowed to move for 3mn. Flies, newly emerged were exposed to the fungus, by spraying 20 ml of conidia solution of every dose, on insect and the cloth covering the cage (25 x 25 x 25cm) containing 25 individuals. The toxicity assays were carried out on 25 individuals with 3 repetitions for all series. After inoculation the larvae and adults were kept under the same conditions of rearing (25°C; 80% RH) in laboratory.

Cuticular proteins bioassays

The cuticular proteins bioassay was carried out according to the method of [22] using the blue shining of coomassy as reagent and albumin like standard protein. The treatment of the fourth larvae of *C. capitata* was carried out with the 2 lethal doses: DL_{50} = 24.8 $x10^5$; DL_{90} = 49.6 $x10^5$

spore/ml and cuticular protein rates were measured at 3 and 6 days with 5 different larval pools per series.

Statistical analysis

The mortality percentage observed for each concentration was corrected [23] and subjected to probit analysis [24] LC_{50}, LC_{90}, 90% confidence limits and the slope were calculated [25].Data from insecticidal tests were subjected to analysis of variance [26] after angular transformation of observed mortality percentages. The data of control and treated series relating to the cuticular protein rates and the numbers of eggs were submitted the test *t* of Student.

RESULTS

Dose-response relationship was determined for *Metarhizium anisopliae* applied to the fourth-instar larvae and adult flies of *Ceratitis. capitata* (Weid). The product exhibited an insecticidal activity against the treated stages. The accumulated mortality recorded, during 7 days; for the inoculated larvae with the different doses, exhibited a dose response relation-ship **(Figure 1)**. The mortality varies between 26.13%, for the tested dose of 6.5 x 10^5spore/ml, and 89.05% for the highest dose = 52 x 10^5 spores/ml **(figure 1)**. With probit analysis for the fourth stage larvae, the LC_{50} was calculated as 24.8 x10^5 (n= 75; 95%; CL = 22.6 x10^5-28.30 x 10^5 spore/ml; Slope= 2.61) and the LC_{90} was 49.6 x10^5 spore/ml) (95% CL = 46.6 x10^5-52.3 x 10^5 spore/ml).

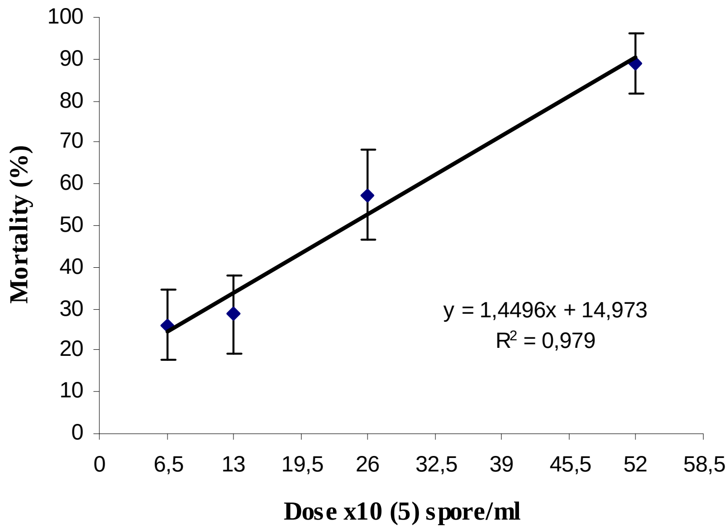

Figure 1. Dose-response relationship of inoculated larvae, of the fourth stage development of *Ceratitis capitata*, with the *Metarhizium anisolpiae*, during 15 days.(R^2 = coefficient of determination).

M. anisolpiae was efficacious in controlling adults of *C. capitata* and the mortality was dose-related **(Fig. 2)**. The fungus applied on the adults of *C. capitata* showed a toxic effect for both sexes. For the dose = 6.5 10^5 spore/ml the mortality was 12.44% for the females and 14.85% ($p<0.05$) for the males. This mortality increased significantly ($p<0.005$) when the flies inoculated with a dose = 52 x 10^5 spore/ml to 76.05% and 89.05% for the females and males, respectively (Fig. 2). These results were submitted to probit analysis where LC_{50} was calculated as 25.4 x10^5 spore/ml (n = 75; 95%; CL = 24.8 x10^5- 26.3 x 10^5 spore/ml; Slope = 2.31) and the LC_{90} was 68.6 x10^5 spore/ml) (95% CL = 66.8 x10^5-71.3 x 10^5 spore/ml) for the females. The lethal doses were also calculated for the males, LC_{50} was 20.6 x10^5 spore/ml (n = 75; 95%; CL = 16.8 x10^5- 22.3 x 10^5 spore/ml; Slope = 4.21) and the LC_{90} was 60.6 x10^5 spore/ml) (95% CL = 56.8 x10^5-63.3 x 10^5 spore/ml; Slope= 4.21). The results revealed that there were a significant decrease ($p<0.05$) in the survival of males. It was noticed that males were more sensible to the fungus than females ($p<0.05$) (**Figure 2**).

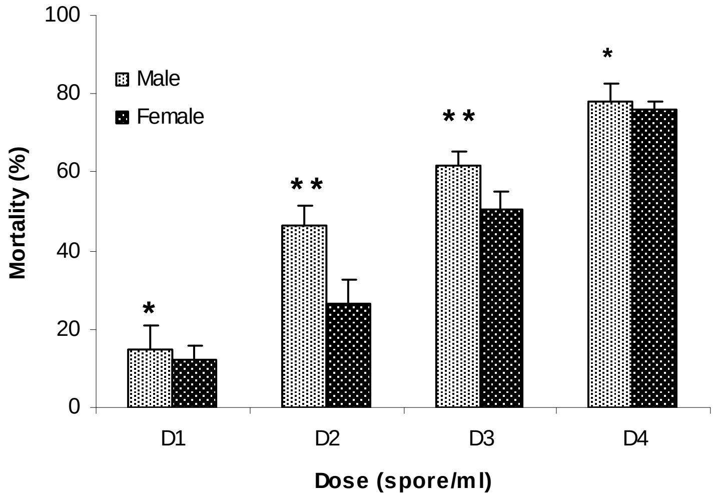

Figure 2: Comparison of dose-response relationship of inoculated flies (Male and female), of *Ceratitis capitata*, with different doses of *Metarhizium anisolpiae*, during 15 days (n=15)

Moreover, the results indicated a significant decrease ($p<0.05$) in the average eggs number laid per female throughout 15 days, when DL_{50}=25.4 x10^5; DL_{90}= 68.6 x10^5 spore/ml of *M. anisopliae* were inoculated to the female newly emerged (**Figure 3**).

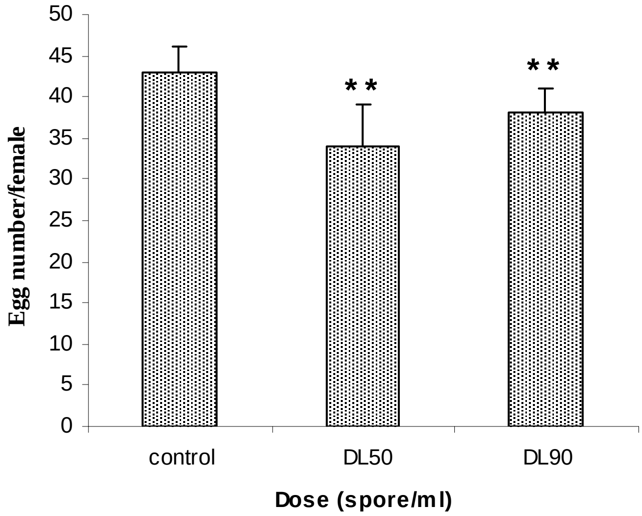

Figure 3: Mean number of eggs laid by *Ceratitis capitata* following inoculation of adults with two doses (DL_{50}= 25.4 x 10^5; DL90= 68.6 x 10^5 spore/ml) of *Metarhizium anisolpiae*, during 15 days.

M. anisopliae was tested at lethal doses (DL_{50}=24.8 x10^5 and DL_{90}= 49.6 x10^5 spore/ml) on the changes cuticular protein amounts, for the fourth instar larvae of *C. capitata*, during 2 periods (3 & 6 days) of the developmental stage. The results showed that the protein amounts decreased significantly during the development for the control and treated series (**Table 1**). Whereas the fungus-inoculated larvae was found to undergo a significant ($P<0.05$) suppressed cuticular protein levels, as compared to control, during the periods of bioassay 3, and 6days.

Table 1: Effect of *Metarhizium anisopliae*, on the amount of Cuticular proteins (mg/ml; m ± s; n = 15), of the fourth instar larvaes of *Ceratitis capitata* inoculated with two doses (DL_{50} = 10.8 x 105 & DL_{90} = 48.9x 10^5 spore/ml).

Time after inoculation	Control	CL_{50}	CL_{90}
3 Days	57.37 ± 1.33	54.07 ± 2.13 **P** = 0.395	52.85±1.51 **P** = 0.165
6 Days	30.49 ± 3.09	30.68 ± 2.65 **P** = 0.090	26.49 ± 2.13 **P** = 0,315

DISCUSSION

The entomogenous, hyphomycete fungus *Metarhizium anisopliae* (Metsch) Sorokin, is a pathogenic micro-organism for many insects. Its effectiveness led the researchers to isolate and produce its toxins [10]. *M. anisopliae* has been studied extensively for the control of wide range pests [27]; [28] and showed much promise for the control of subteraranean pests [29]; [30]. It was used in the control against many pests of the cultures like *Adoryphorus couloni* (Coleoptera) in Australia [31], the locust (Acrididae) in Africa [32], *Cleonus punctiventris* (Coleoptera), and *Anisoplia austriaca* (Coleoptera) in America [9]. Recently, a new insecticide compound, which is the crude soluble extract from the fermentation product of the entomopathogenic fungus *Metarhirium anisopliae*, has appeared as promising insecticide for the control of the Mediterranean fruit fly, *Ceratitis capitata* [13]. The effect of the autochthonous *M. anisopliae* against the fourth larvae and the adults of *C. capitata* showed a high toxicity with a dose-response manner with a mortality started at the day 3 to 6 days confirmed the results of [2] and [33]. Our results clearly show that *M. anisopliae* is highly efficacious on both developmental stages of *C. capitata*, accord with [27]; [30]; [14]; [15]. The toxicity is due to the selective action of enzymes secreted by the fungus that acts on the chemical components of the cuticule of the insect [17]; [34]; [2]. Even though the ingestion of this compound produces serious injuries in the midgut epithelium, it has a very low antifeedant effect on the flies [13]. The author [35] reported that *M. anisopliae* did not have any effect on non target organisms found in cowpea cropping system. The toxic effect of this fungus was recorded on various stages of Lymantria dispar [36], *Cydia pomonella* [33] and *Liriomyza huidobrensis* [37]. The insects are differently sensitive to these bio-insecticides and it was shown that some species of Lepidoptera are more sensitive [38]. The injection of a DL_{50} amount of destruxine has a weak effect in larvae of the silkworm [39], contrary to the larvae of *Galleria mellonella* [40].

Effect of *M. anisopliae* against the adult flies of *C. capitata*, caused a cumulated mortality for both sexes, whereas the male mortality is higher than of the female. This result suggests that the male adults were more susceptible than females and this confirm the results reported by [14]. However, it was documented that the treated larvae of *Cydia pomonella*, [41]; [33], and *Blattella germanica* [42] with the same fungus the female larvae were more sensitive than the males one. Moreover, the results indicated significant decrease in the average number of eggs laid per female through out 15 days after inoculation with *M. anisopliae* of new emerged females. Similar results were reported by [14] in *C. capitata* and in *Liriomyza huidobrensis* (Diptera: Agromyzidae) [37] and this is in agreement with a previous works [14]. *M. anisopliae* was more effective in reducing fecundity, fertility and oviposition delay [43].

The quantification of the cuticular proteins amounts of treated larvae with DL_{50} and DL_{90} of *M. anisopliae* showed significant decreases on the cuticular protein rates. This effect would be caused by the enzymes secreted by the spores of *M. anisopliae*, mainly the chitin enzyme, diacethylase, which acts on the degradation of cuticular chitin and facilitates the penetration of the hyphae through the cuticule during the first 24 hours of treatment [44]. Theses decreases could be also justified by the use a certain quantity of cuticular proteins like energy source [45]. In conclusion, the results of the present study suggest that *M. anisopliae* could be considered as a strong micro-organism for developing biological control of C. *capitata*.

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