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Determination of the median lethal concentration (LC_{50}) of mycoinsecticides for the control of *Ceratitis capitata* (Diptera: Tephritidae)

Determinación de la concentración letal media (CL_{50}) de micoinsecticidas para el control de *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract: The aim of this study was to determine the median lethal concentration (LC_{50}) of the commercial products Boveril WP® (*Beauveria bassiana*) and Metarril WP® (*Metarhizium anisopliae*) on the larvae and pupae of the fruit fly, *Ceratitis capitata*. Insects used in this study came from a laboratory colony. The evaluated product concentrations were 10.00, 15.00, 20.00 and 25.00 g/L of water, which correspond, respectively, to $5.00x10^9$, $7.50x10^9$, $10.00x10^9$ and $12.50x10^9$ viable conidia/L of water for the two products, and in the control only water was applied. Third instar larvae and pupae of *C. capitata* were used in this study. Results showed an overall mortality of larvae with all conidial concentrations of *M. anisopliae*. The LC_{50} values for larvae were 2.99 and 2.97 g/L for Boveril® and Metarril®, respectively, while for pupae they were 3.12 and 4.74 g/L for Boveril® and Metarril®, respectively. The high pathogenicity demonstrated by lower conidial concentrations of the tested products may mean greater efficiency from both economic and environmental points of view.

Key words: Mycoinsecticides. Mediterranean fruit fly.

Resumen: El objetivo de este estudio fue determinar la concentración letal media (CL_{50}) de los productos comerciales Boveril WP® (*Beauveria bassiana*) y Metarril WP® (*Metarhizium anisopliae*) para el control de larvas y pupas de la mosca mediterránea de la fruta, *Ceratitis capitata*. Los insectos utilizados en el experimento provinieron de una cría de laboratorio. Las concentraciones de los productos evaluados fueron: 10,00; 15,00; 20,00 y 25,00 g/L de agua, lo que corresponde, respectivamente, a $5,00x10^9$; $7,50x10^9$; $10,00x10^9$; $12,50x10^9$ conidias viables/L de agua para los dos productos; en el control sólo se aplicó agua. Se usaron larvas de tercer estadio y pupas de *C. capitata*. Se registró la mortalidad general de las larvas de tercer instar en todas las concentraciones de conidios de *M. anisopliae*. Los valores de CL_{50} para las larvas fueron 2,99 y 2,97 g/L para Boveril® y Metarril®, respectivamente, mientras que para las pupas fueron 3,12 y 4,74 g/L para Boveril® y Metarril®, respectivamente. La alta patogenicidad demostrada en una baja concentración de conidios de los productos probados, indica una mejor eficiencia desde el punto de vista económico y ambiental.

Palabras clave: Micoinsecticidas. Mosca mediterránea de la fruta.

Introduction

The fruit cultivation has stood out as an excellent agricultural activity in the irrigated areas from northeastern Brazil. The fruit cultivation for export has shown promise, but the occurrence of fruit flies is one of the limiting factors for achieving success in this activity (Carvalho and Nascimento 2002), in addition, it has been verified a growing increase in the frequency of these insects, as a result of the natural dispersion processes or the unintentional transport of infested fruit from one region to another.

The mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), is considered cosmopolitan, infesting more than 250 host plant species widely distributed in South America (Gallo *et al.* 2002). The indiscriminate use of pesticides to control fruit flies causes a serious ecological imbalance and triggers the emergence of other pest populations by eliminating natural enemies, and lead to human and environmental infection. The biological control of fruit flies interacts with a set of integrated management strategies meeting the demands of consumer markets (Oliveira 2010). Biological control can serve as a tool available to the producer to control this pest, resulting in fruit with lower levels of pesticide residues and lesser impact on the environment (Oliveira *et al.* 2010a).

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuill and *Metarhizium anisopliae* (Metsch) Sorok. are well-known and being used worldwide as biocontrol agents of many agricultural pests, they have potential to control several insects of different orders (Bridge *et al.* 1990; Alves 1992; Pereira *et al.* 1993; Silva 2001). The use of entomopathogenic fungi for pest control can be alternative candidates to solve the problems of resistance (Omoto *et al.* 1994). The microbial control of fruit flies can be a process that can partially replace other control methods in integrated management programs for these insects, especially the use of agrochemicals, presenting economic and environmental advantages for tropical fruit (Oliveira *et al.* 2010b).

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Information about effects of different concentrations of commercial products produced by these entomophatogenic micro-organisms in the mortality of immature stages of Tephritidae are still very incipient, this way, this study aims at determining the mean lethal concentration (LC_{50}) of the fungal commercial products Boveril WP® (*Beauveria bassiana*) and Metarril WP® (*Metarhizium anisopliae*), on the larvae and pupae of *Ceratitis capitata* (Diptera: Tephritidae) as a first step towards effective mycoinsecticidal control of this destructive pest.

Materials and Methods

Insects. The research was carried out in the Entomology Laboratory of the Plant Department, Universidade Federal da Paraíba - UFPB/CCA, Areia-PB. The *C. capitata* fruit flies were obtained from the Entomology Laboratory rearing from CCA-UPFB, Areia-PB. The larvae were fed with artificial diet consisting of beer yeast (120 g), raw carrot (600 g) and Nipagin (5 g). Adult flies were kept in cages and fed daily with a solution of 25% honey in water, provided in cotton placed on top of the cage (50x50x60 cm) during the oviposition period. All stages were kept at 25 ± 2°C, 12 h of photophase and relative humidity of 80 ± 10%.

Bioassay. In this study, the commercial fungal products used were for *B. bassiana* and *M. anisopliae*, (Boveril WP® and Metarril WP® respectively) (ITAFORTE Bioproducts Company, Brazil). The concentrations used were 10.00, 15.00, 20.00 and 25.00 g/L of water, which is equivalent to a minimum of $5.00x10^9$; $7.50x10^9$; $10.00x10^9$ and $12.50x10^9$ viable conidia/L of water from each product, and de H_2O was used for control (0.0 g/L of water). The viability of isolated evidence of pathogenicity and virulence were evaluated according to the method described by França *et al.* (2006). In the viability test two Petri dishes containing PDA (potato dextrose agar) as culture media were used, incubated in BOD at 25 ± 2°C, 12 h of photophase and relative humidity of 80 ± 10% for 24 hours, later to perform readings in a light microscope by determining the percentage of germinated and not germinated. To test the pathogenicity and virulence of the isolates used in this study, there was a bioassay spraying a suspension of $5.00x10^9$ conidia/L, 25 larvae of *C. capitata* first instar for each species of fungus, these larvae were used because are highly susceptible to entomopathogenic stage and due to its ease of laboratory rearing (Oliveira 2010; Oliveira *et al.* 2010b).

Larvae were kept on artificial diet in Petri dishes, while pupae were subjected to substrate containing washed sand. Fungal suspensions of each concentration from either fungal product were topically applied with the deposition of 5 µl of the suspensions of the fungi on the whole larvae or pupae using micro-spray, later the insects were transferred to Petri dishes lined with filter paper, at 25 ± 2°C, 12 h of photophase and relative humidity of 80 ± 10%. To ensure fungal effect, mortality of treated larvae or pupae was verified eight days post-fungal application in each concentration of either fungi. The mortality was confirmed by sporulation. Mortality was then calculated and compared to a control (un-treated) group using the suitable statistical analysis.

Statistical analysis. The experimental design was completely randomized for five replicates (20 insects each) (larvae or pupae) for each concentration from each fungus. However mortality data (%), presented in the figure, were corrected by Abbott's formula Ma = (Mt-Mc)/(100-Mc)x100, where Ma = mortality corrected for control treatment; Mt = mortality observed in dealing with the mycoinsecticides and Mc = mortality observed in the control treatment (Abbott 1925). Data were analyzed by probit analysis (Finney 1971) (SAS Institute Inc. 2003), and significance was assessed by the degree of overlap of 95% CL, i.e.

Results and Discussion

The entomopathogenic fungi have affected the mortality of *C. capitata*. A high mortality of larvae and pupae at all concentrations of conidia of *M. anisopliae* and *B. bassiana* was recorded (Fig. 1). None of the concentrations of conidia of both products adopted in this study provided a lower mortality LC_{50} (Fig. 1, Table 1).

The values of LC_{50} for larvae were 2.99 g/L and 2.97 g/L products Boveril® and Metarril®, respectively, while the pupae were 3.12 g/L and 4.74 g/L products Boveril® and Metarril®, respectively (Table 1). The lower concentrations needed to obtain the LC_{50} for larvae perhaps are associated with an increased exposure route of infection, in case, coat and digestive tract of the insect fungal spores contained in the fungal solution.The results shown in this study are similar to those reported by Loureiro and Moino Jr. (2006) about the total mortality caused by *B. bassiana* in aphids, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) and *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphidae) and to these of Almeida *et al.* (2007) on *Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphidae). In fact, we reported in this study high levels of mortality in all concentrations applied both for specimens that received conidia of *B. bassiana* and *M. anisopliae* (Fig. 1). The value found by Loureiro and Moino Jr. (2006) of LC_{50} of Boveril® (*B. bassiana*) in aphids was 0.233 g/L.

Entomopathogenic fungi can be used in programs to control *C. capitata* through the application in the soil against their larvae and pupae, offering great advantages because it allows the multiplication of pathogens in agroecosystems. Mochi *et al.* (2006) found that the application of conidial suspension of *M. anisopliae* on the soil surface has provided a decrease in the survival of the pupal and adult phase of *C. capitata*. According to Onofre *et al.* (2002) this is a promising alternative

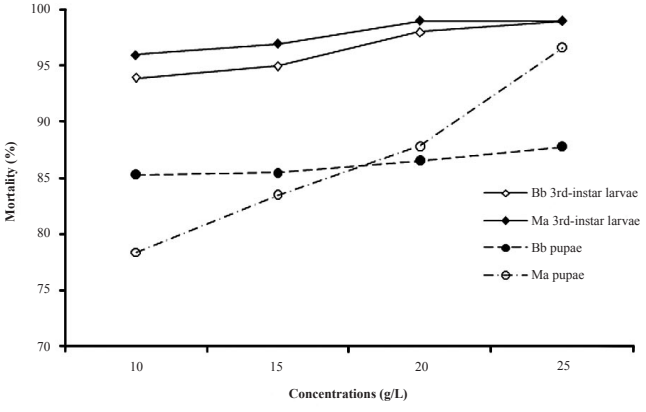

Figure 1. Average mortality of 3rd-instar larvae and pupae of *Ceratitis capitata* treated topically (n = 100) with *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma).

Table 1. Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to third instar larvae and pupae of *Ceratitis capitata* (Diptera: Tephritidae) via topical exposure at 25 ± 2°C, 12h of photophase and relative humidity of 80 ± 10%. The value of each χ^2 refers the probability of the angular coefficient > 0.LC - values and slopes (in g/L) were estimated by probit procedure (SAS Institute Inc., 2003).Values followed by the same letter within row are not significantly different if their 95% CL overlap.

Fungi	Stage	Slope ± SE	LC_{50} (95%FL)	χ^2 ; P
B. bassiana	3rd-instar larvae	1.11 ± 0.23	2.99 (2.33 – 3.48) a	63.45; 0.0001
M. anisopliae		1.03 ± 0.03	2.97 (2.44 – 3.37) a	80.05; 0.0001
B. bassiana	Pupae	1.76 ± 0.34	3.12 (2.80 – 3.43) b	179.10; 0.0001
M. anisopliae		1.63 ± 0.42	4.74 (3.08 – 5.25) c	98.66; 0.0001

for controlling fruit flies, as they are known to be pathogenic to Diptera like African fly tsé-tsé *Glossina morsitans morsitans* Westwood, 1851 (Diptera: Glossinidae) (Kaaya and Munyinyi 1995), *C. capitata* (Castillo *et al.* 2000; Ekesi *et al.* 2002; Dimbi *et al.* 2003; Alves *et al.* 2004; Mochi *et al.* 2006) and *Musca domestica* L. (Diptera: Muscidae) (Steinkraus *et al.* 1990).

The results of this study encourage the use of *B. bassiana* and *M. anisopliae* as a further measure of *C. capitata* control, because the larvae present in infested fruits lying on the ground represent a great advantage in the multiplication of entomopathogenic microorganisms and in preventing the spread of these insects in the field. Results obtained by Reys (2003) on the effect of the *M. anisopliae* on the larvae of Mexican fly, *Anastrepha ludens* (Loew, 1873) (Diptera: Tephritidae), revealed high levels of pathogenicity of the *M. anisopliae* to larvae of this insect pest. Quesada-Moraga *et al.* (2006) observed lethal suspensions of *B. bassiana* and *M. anisopliae* in *C. capitata*, causing mortality in adult *C. capitata*, ranging from 30 to 100%. Dimbi *et al.* (2003), reported an overall mortality of adult *C. capitata*, *C. rosa* variety fasciventris Karsch and *C. cosyra* (Walker, 1849) (Diptera: Tephritidae) submitted applications with low concentrations of conidia of *B. bassiana* and *M. anisopliae.*

The high pathogenicity levels demonstrated in lower conidial concentrations of the tested products, means a better efficiency from the point of economy and environment. The alternate application of spray products mycoinsecticides increases the possibility of success, because the entomopathogenic fungi are more susceptible to the action of adverse environmental factors such as high temperature, low humidity and high incidence of UV that can occur after each spraying, thus jeopardizing its survival in the environment (Ignoffo 1992; Grijalba *et al.* 2009). Moreover the efficiency spraying as it allows lower costs to the products and also through the use of low conidia concentrations of *B. bassiana* and *M. anisopliae* can enhance the selectivity of these organisms to the complex of natural enemies involved in agroecosystems.

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