Revista Brasileira de Agroecologia
Rev. Bras. de Agroecologia. 9(1): 195-204 (2014)
ISSN: 1980-9735

In vitro assay of alternative phytosanitary products and plant extracts on *Metarhizium anisopliae* (Metsch.) Sorok. (Clavicipitaceae)

Avaliação in vitro de produtos fitossanitários alternativos e extratos vegetais sobre *Metarhizium anisopliae* (Metsch.) Sorok. (Clavicipitaceae)

FORMENTINI, Marina Andressa[1]; ALVES, Luis Francisco Angeli[2]; PINTO, Fabiana Gisele da Silva[3]; MAMPRIM, Ana Paula[4]

1Manejo Agrícola Ltda, marina@manejoagricola.com.br; 2 Laboratório de Biotecnologia, Centro de Ciências Biológicas, Universidade Estadual do Oeste do Paraná, Cascavel/PR - Brasil, luis.alves@unioeste.br; 3Laboratório de Biotecnologia, Centro de Ciências Biológicas, Universidade Estadual do Oeste do Paraná, Cascavel/PR - Brasil, fabiana.pinto@unioeste.br; 4Laboratório de Biotecnologia, Centro de Ciências Biológicas, Universidade Estadual do Oeste do Paraná, Cascavel/PR - Brasil, anamamprim@hotmail.com

RESUMO: O objetivo deste estudo foi avaliar o efeito in vitro dos produtos comerciais Dalneem®, Ecolife® e Stubble-Aid® na concentração rotulada (CR), na metade (½CR) e no dobro dessa concentração (2CR) e dos extratos aquosos de raiz de *Curcuma longa* (Zingiberaceae) e de folhas de *Cymbopogon citratus* (Poaceae), *Corymbia citriodora* (Myrtaceae) e *Rosmarinus officinalis* (Lamiaceae), na concentração de 10% (p/v) sobre *Metarhizium anisopliae*. Os tratamentos foram pulverizados sobre o fungo previamente inoculado no meio de cultura BDA (batata, dextrose e ágar), em placas de Petri. Foram avaliadas as unidades formadoras de colônias (UFC), crescimento vegetativo, produção de conídios, germinação de conídios e atividade inseticida sobre lagartas de *Diatraea saccharalis* (Lepidoptera: Crambidae). Apesar dos produtos comerciais terem afetado significativamente alguns parâmetros biológicos do fungo, somente Dalneem® 2CR foi classificado como incompatível com o fungo. Os extratos vegetais ocasionaram efeito significativo somente sobre o crescimento vegetativo do fungo (variando de 4 a 7%), sendo todos classificados como compatíveis com o isolado de *M. anisopliae*.

PALAVRAS-CHAVE: controle associado, conservação, controle alternativo

ABSTRACT: This study aimed to evaluate in vitro effect of commercial products Dalneem®, Ecolife® and Stubble-Aid® in recommended concentration of the product (RC), half that concentration (½RC) and twice that concentration (2RC) and the aqueous rhizomes extracts of *Curcuma longa* (Zingiberaceae), aqueous leaves extracts of *Cymbopogon citratus* (Poaceae), *Corymbia citriodora* (Myrtaceae) and *Rosmarinus officinalis* (Lamiaceae) in 10% concentration (w/v) on *Metarhizium anisopliae*. The treatments were sprayed onto the fungus previously grown in Petri dishes with PDA (potato, dextrose and agar) culture medium. There were analyzed the colony forming units (CFU), vegetative growth, conidial production, conidial germination and insecticidal activity against *Diatraea saccharalis* larvae (Lepidoptera: Crambidae). Although commercial products significantly affected some biological parameters of the fungus, only Dalneem® 2RC was classified as incompatible with the fungus. The plant extracts caused significant effect only to vegetative growth (ranging from 4 to 7%), and were all classified as being compatible with the strain of *M. anisopliae*.

KEY WORDS: associated control, conservation, alternative control

Correspondências para: luis.alves@unioeste.br
Aceito para publicação em 29/11/2013

Introduction

In modern agriculture, many synthetic phytosanitary products are used in order to increase productivity. However, its improper use causes environmental problems, besides causing problems to the health of producers and consumers. Due to the increasing demand for food free of residues, many producers have opted for the use of alternative practices in the management of their crops.

Currently, there are several alternative phytosanitary products based on micro-organisms, plant extracts or citrus biomass that have antimicrobial, insecticidal and/or inducing plant resistance, protecting them from attack by pathogens and insects (CAVALCANTI et al., 2006). These products are safer than chemical products, since they are selective to natural enemies, keeping them in the environment (ALVES et al., 1998a). Studies have demonstrated the action of extracts of *Rosmarinus officinalis* L. (Lamiaceae) (TAGAMI et al., 2009), *Corymbia citriodora* Hook (Myrtaceae) (KUBERAN et al., 2012), *Curcuma longa* L. (Zingiberaceae) (SHUKLA AND DWIVEDI, 2012) and *Cymbopogon citratus* Stapf (Poaceae) (SILVA et al., 2012) in the management of phytopathogenic fungi in different crops.

Entomopathogens are known to be important pest population density reduction factor in Integrated Pest Management (IPM) programs, whether they occur naturally or when they are applied or introduced to insect control, as sugarcane or pasture froghoppers control with the fungus *Metarhizium anisopliae* (Clavicipitaceae) (ALVES et al., 2008).

The conservation of such entomopathogens in the environment is an important action to avoid outbreaks of pest populations. However, the use of incompatible products may inhibit the development of these pathogens, affecting IPM, and studies about the interaction of alternative phytosanitary products and entomopathogenic fungi are scarce. Also, interactions between them can be both positive and negative, and so, there is the necessity of carry out further studies with other products and methodologies, and also assessing other parameters to verifying the compatibility among them and arrange the best practices to more sustainable agriculture (ALVES et al., 1998a; MERTZ et al., 2010; RIBEIRO et al., 2012; MAMPRIM et al., 2013).

This study aimed to evaluate in vitro effect of some alternative commercial products and plant extracts on biological parameters of *M. anisopliae*.

Material and Methods

The fungus *M. anisopliae* (strain Unioeste 22) was from Entomopathogenic Fungi Collection of the Agricultural Biotechnology Laboratory from Western Parana State University, Unioeste, in Cascavel, PR, Brazil. This strain was cultured as Alves et al. (1998b), in a complete medium for conidial production (agar 20 g, yeast extract 5 g, salt mixture 4.6 g, glucose 10 g, water distilled 1000 mL^{-1}) previously autoclaved and poured onto sterilized Petri dishes. Plating was performed according to the full dish method, the conidia were transferred from Eppendorf vial to the dish containing medium by platinum loop and then streaked with a Drigalsky loop. Dishes were incubated at 26±1°C and photophase of 12 h. After 7 days, conidia were scraped and transferred to flat-bottom vials, containing 10mL sterilized water with Tween 80® (0.01%). The conidial concentrations in the suspensions were determined by quantify under optical microscope, with a Neubauer chamber.

The alternative phytosanitary products evaluated are used in the organic or agroecological production for various purposes, and were tested at three concentrations: recommended concentration of the product (RC), half that concentration (½RC) and twice that concentration (2RC), based on information of their respective label. Moreover, crude aqueous extracts of rhizomes of *Cu. longa* and leaves of *Cy. citratus*, *Co. citriodora* and *R.*

officinalis were also evaluated, tested at a concentration of 10% (Table 1).

We planned to test the vegetal extracts as they are prepared by farmers. Vegetal parts were collected in rural area from Palotina city (24° 17' 02" S 53° 50' 24"W), western region of Paraná and taken to the laboratory where they were weighed and ground in a blender to prepare the crude extracts at 10% (w/v^{-1}), in the proportion of 50 g leaves/rhizomes to 500 mL of sterile distilled water, whose concentration is considered a threshold in the economic standpoint. Then, the plant extracts were filtered in gauze, sterilized by vacuum filtration through a 0,45 µL nitrocellulose membrane filter and stored in sealed vials and protected from light at - 10 °C for a maximum period of seven days.

Table 1: Alternative phytosanitary products used in treatments, with their composition/scientific name, concentration recommended by label and biological activity.

Nature	Product	Composition/Scientific name	Average recommendation ha^{-1}	Activity
Comercial products	Dalneem®	Emulsifiable oil from neem seeds (*Azadirachta indica*)	1 L 100 L^{-1}	Insecticide
	Ecolife®	Bioflavonoids + citrus phytoalexins (16.6 g L^{-1}) Ascorbic acid (16.5 g L^{-1}) Lactic acid (9.5 g L^{-1}) Citric acid (13.0 g L^{-1}) Vegetal glycerin (66.0 g L^{-1})	0,75 L 100L^{-1}	Inductor resistence
	Stubble-Aid®	Copper sulfate (28.0 g L^{-1}) Ferrous sulfate (51.6 g L^{-1}) Manganese sulfate (12.0 g L^{-1}) Zinc sulfate (42.0 g L^{-1})	1 L 100L^{-1}	Biofertilizer
Plant extracts	Rosemary leaves	*Rosmarinus officinalis*	10%	Insect repellent
	Eucalyptus leaves	*Corymbia citriodora*	10%	Insecticide
	Lemon Grass leaves	*Cymbopogon citratus*	10%	Insect repellent
	Turmeric rhizomes	*Curcuma longa*	10%	Fungicide

The method used to evaluate the effect of the products on the biological parameters of *M. anisopliae* is that best represents the field conditions, as described by Silva and Neves (2005), with modifications.

In the conidial germination test, 100 µL of conidial suspension (1 × 10^6 conidia mL-1) were spread on the surface of PDA (potato, dextrose, agar) culture medium in a Petri dish (6 cm diameter), followed by spray of 250 µL of the alternative phytosanitary products using Sagima SW775 airbrush spray apparatus at a constant pressure of 0.84 kgf cm^{-2} and a distance of 20 cm. The dishes were incubated at 26 ± 1 °C and photophase of 12 h during 16 h to quantify the number of conidia germinated and non-germinated under a light microscope.

For the vegetative growth and conidial production, the fungus was inoculated with a platinum wire loop at three points on the surface of PDA in Petri dishes which were incubated under the same conditions for 48h. After that, products were sprayed as described before, and dishes were incubated again for five days. Colony diameter were assessed of two colonies per dish and these were cut close to the edge, and transferred individually to glass tubes with 10 mL of distilled water + Tween 80® (0.01%) and agitated until conidia were totally released from the medium surface for conidia quantification in a Neubauer chamber. We performed an average of the two colonies evaluated.

Data obtained were submitted to ANOVA (F test) and means were compared by Tukey test ($p<0.05$), using the program Sisvar (FERREIRA, 2011). Products toxicity was classified according Rossi-Zalaf et al. (2008), as follows: BI = 47[VG]+43[SPR]+10[GER]/100. In this model, values for vegetative growth (VG), sporulation (SPR) and germination (GER) are given in relation to the control (100%). Where BI = Biological Index is: Toxic (BI = 0 to 41); Moderately Toxic (BI = 42 to 66) and Compatible (BI> 66).

Colony forming units (CFU) were determined by plating aliquots of 100 µL of conidial suspension (1 × 10^3 conidia mL^{-1}) on PDA media surface followed by spraying of the products and then the dishes were incubated as described before, for three days. After that, the colonies formed were counted.

For all treatments, five dishes were prepared, and each dish was considered a replication. For the control, dishes containing the fungus were sprayed with only sterilized distilled water + Tween® 80 (0.01%).

Insecticidal activity was evaluated plating conidia on PDA culture medium surface in Petri dishes and then the different products were sprayed. To control, plates were sprayed with sterile distilled water. The dishes were incubated for seven days at 26±1 °C and photophase of 12 h. After this period, conidia were scraped and a suspension of 1 × 10^8 conidia mL^{-1} was prepared, whose concentration was determined previously, in order to cause approximately 80% of mortality of *Diatraea saccharalis*. This insect was chosen for its susceptibility to fungus (ZAPPELINI et al., 2010) and due to the disponibility in our laboratory.

Based on study by Oliveira (2009), 3rd instar caterpillars were immersed and manually shaken for 1 minute in conidial suspensions (1 × 10^8 conidia mL^{-1}) from culture media + products or from control plates. Insects were transferred to sterilized Petri dishes lined with filter paper and feed with artificial diet. For each treatment was prepared five plates (replicates) with 10 caterpillars each one. Insects were incubated at 26 ±1 °C and photophase of 12 h. Food was replaced daily and mortality was evaluated for 10 days. Dead insects were removed and placed in a moist chamber for confirmation of the pathogen mortality (ALVES et al., 1998b). These data were also submitted to ANOVA (F test) and means were compared by Tukey test ($p<0.05$),

using the program Sisvar (FERREIRA, 2011).

Results and Discussion

Effect of commercial products on biological parameters of *M. anisopliae*

It was observed distinct effects of alternative phytosanitary products on *M. anisopliae*, depending on the parameter analyzed and the concentration used (Table 2).

The conidia viability was not significantly affected by any one of the products evaluated. This fact is important because germination is one of the most relevant factor in the infection process of the fungus since it initiates the disease in the host insect. Furthermore, the conidia represents the form of the fungus resistance and its integrity unchanged guarantees the conservation of the natural enemy at environment (ALVES, 1998; NEVES et al., 2001).

Regarding the CFU, Dalneem® (½RC) presented the best result, with significant increase

Table 2: Mean percentage of conidia viability , colony forming units (CFU), colony diameter , conidia production, "BI" values and compatibility of *Metarhizium anisopliae* (strain 22-Unioeste), in contact with different alternative commercial products (26±1 °C and photophase

	Viability		Colonies		Diameter		Conidia mL^{-1}		
Treatment*	(%)	PV**	CFU	PV	cm^2	PV	× 10^6	PV	BI***
Control	7.6^a	0	122^b	0	2.5^a	0	54^a	0	-
Dalneem® ½RC	64.0^a	-19.7	174^a	42.6	2.45^a	-2	40^b	-20.4	122.98/C
Dalneem® RC	68.0^a	-14.7	115^b	-5.7	2.2^b	-12	28^c	-48.1	88.23/C
Dalneem® 2RC	70.2^a	-11.8	107^b	-12.3	1.5^c	-40	4^d	-92.6	42.22/MT
C.V. (%)	7.24		3.02		1.56		7.03		
Control	104^a	0	122^a	0	2.5^a	0	54^a	0	-
Ecolife® ½RC	76.6^a	-11.4	104^b	-14.8	2.3^b	-8	36^b	-33.3	123.82/C
Ecolife® RC	74.5^a	-13.8	113^{ab}	-7.4	2.2^b	-12	30^b	-44.4	93.46/C
Ecolife® 2RC	74.8^a	-13.5	104^b	-14.8	2.3^b	-8	42^b	-24.1	120.18/C
C.V. (%)	8.84		3.13		0.80		14.84		
Control	73.9^a	0	360^a	0	2.65^a	0	51^a	0	-
Stubble-Aid® ½RC	83.5^a	13.1	195^c	- 45.8	1.9^c	- 28.30	17^b	- 66.7	72.07/C
Stubble-Aid® RC	78.3^a	5.9	279^b	- 22.5	2.3^b	- 13.21	25^b	- 50.1	70.12/C
Stubble-Aid® 2RC	68.3^a	-7.6	267^b	- 25.8	2.3^b	- 13.21	24^b	- 52.1	88.96/C
C.V. (%)	2.67		1.38		0.93		6.47		

Means followed by same letter in the columns, to each product, do not differ by Tukey test ($p<0.05$)
Data of viability transformed into $\sqrt{x}$ and data of CFU, diameter and conidia production transformed into arcsen $\sqrt{x}/100$
*Products concentrations: ½RC= Half of field recommendation; RC= Field recommendation; 2RC= Twice of field recommendation
**PV = Percentual variation; PV = [(Treatment mean/Control mean × 100) − 100]
***BI between 0 a 41 = toxic; between 42 e 66 = Moderately toxic; > 66 = compatible

of 42% in relation to the control treatment, whose fact has been common in studies that follow this methodology in vitro evaluation of products effects on entomopathogens (NEVES et al., 2001; TAMAI et al., 2002; MAMPRIM et al., 2013). Suggest two hypotheses for these results: the fungus can metabolize substances of the product to use as a nutrient source or the fungus, in a toxic medium, could be making a reproductive effort, increasing its development, according with Moino Jr. and Alves (1998). However, there was a significant reduction for all other products. Ecolife® (½RC and 2RC) and Stubble-Aid® (½RC), for example, caused reduction in the colony formation around 15 and 45%, respectively.

The vegetative growth was not only affected by Dalneem® ½RC. The others treatments, however, presented significant reductions relative to control, especially Dalneem® 2RC and Stubble-Aid® ½RC that reduced the size of the colonies in 40 and 28%, respectively.

Conidia production was significantly lower in the presence of the products, except from the Ecolife® that although it caused reduction of up to 44%, this was not significant as compared to the control.

Barguil et al. (2005) observed a complete inhibition of this parameter on *Phoma constarricensis* Echandi, using Ecolife® 1% into the culture medium. Also, Mertz et al. (2010) observed reduction in vegetative growth of *Beauveria bassiana* Bals. Vuill. (Cordycipitaceae) by Ecolife® mixed in PDA at the highest concentrations tested.

Dalneem® and Stubble-Aid® in all concentration reduced significantly conidia production, ranging from 92 to 52% in 2RC, respectively. This corroborate Hirose et al. (2001) who observed reduction of 54% in conidia production and 37% in diameter of colonies of *M. anisopliae* in contact with neem seed oil, and the viability of conidia was reduced only 17%. Marques et al. (2004) reported similar findings, since they found that 2.5% of the product NIM-I-GO, based on nem oil and other plants, inhibited 84.4% of conidiogenesis of *M. anisopliae* and germination was not affected. Recently, Pinto et al. (2012) also observed reduction of vegetative growth and conidial germination of *B. bassiana* by neem oil.

The inhibition caused by Dalneem® can probably be attributed to components of oil and not to the azadirachtin, its mainly active ingredient. In this way, Shashi and Gupta (1998) did not observed reduction on mycelial growth of *B. brongniartii* in culture media with high azadirachtin concentrations. Also, it was observed effects of aqueous extracts from neem seeds and neem oils on *B. bassiana*, and it was observed that neem oil caused more negative effect on growth vegetative and conidia production than plant extract (RIBEIRO et al. 2012).

Liquid biofertilizers, such as Stubble-Aid®, present recognized fungistatic activity due to the presence of metabolites (toxins, esters, acids and phenols) which are also part of these products (GALLO et al., 2002), explaining their negative action on *M. anisopliae*. However, there are no related studies of this product on this fungi specie.

Based on the toxicity formula proposed by Rossi-Zalaf et al. (2008), only Dalneem® 2RC was incompatible to the fungus (Table 2) due to high reduction in the conidia production in this product concentration. Castiglioni et al. (2003) observed that Nimkol-L® (based on extracts of neem leaves) was compatible with *M. anisopliae* in concentrations of 0.2; 0.5 and 1% applied on PDA, after the pathogen being inoculated, but they used another formula proposed previously by Alves et al. (1998a) that does not take into account the influence of the fungal germination. Accordingly, Neves et al. (2001) discuss the relevance of conidia germination in the study of compatibility since the inhibition of this initial stage of the fungus affects its entire development. However, Pinto et al. (2012) also classified the neem oil product studied by them

as moderately toxic using the same formula proposed in this study.

Insecticidal activity was not reduced significantly by fungus developed in contact with alternative phytosanitary products (confirmed mortality ranged between 74 and 97%). Despite of being an important parameter to be considered in this type of study, it was not found similar studies with this technique used in this research to discuss the data here obtained.

Effect of plant extracts on biological parameters of *M. anisopliae*

Plant extracts presented no significant reductions on fungus, except for vegetative growth, but these reductions were not reached 8% (Table 3). Furthermore, it is noteworthy that there are few studies about the effect of plant extracts on entomopathogenic fungi.

With respect to *CY. citratus*, others studies also observed detrimental effect on entomopathogenic fungi. Mertz et al. (2010) observed higher reduction of viability, vegetative growth and conidia production of *B. bassiana*, respectively 85; 26 and 23%, when the crude extract at 10% was mixed to the culture medium. Mamprim et al. (2013) also reported significant inhibition at the same biological parameters of *M. anisopliae* analyzed in the present study to *Cy. citratus* aqueous extracts at 10%, especially the viability, with reduction of 42% relative to the control, using the same technique of contact between fungus and product in this study, however, the methodology to obtain the extract was different.

Table 3: Mean percentage of conidia of viability, colony forming units (CFU), colony diameter, conidia production, "BI" values and compatibility of *Metarhizium anisopliae* (strain 22-Unioeste), in contact with different aqueous plant extracts at concentration 10% (26±1°C and photophase of 12h).

Treatment	Viability		Colonies		Diameter		Conidia/mL		BI**
	(%)	PV*	CFU	PV	cm²	PV	× 10⁶	PV	
Control	75.4ª	0	155.5ª	0	2.6ª	0	41.5ª	0	-
R. officinalis	68.6ª	-9	151.0ª	-2.9	2.4ᵇ	-7.7	34.0ᵇ	- 18.1	87.70/C
C.V. (%)	5.75		3.03		1.82		7.38		
Control	100ª	0	155.5ª	0	2.6ª	0	44.5ª	0	-
Co. citriodora	98.9ª	-1.1	148.2ª	-4.7	2.4ᵇ	-7.7	42.0ª	- 5.6	93.86/C
C.V. (%)	5.04		4.24		1.56		3.97		
Control	75.4ª	0	361.5ª	0	2.6ª	0	41.5ª	0	-
Cy. citratus	72.7ª	-3.6	350.3ª	- 3.1	2.4ᵇ	- 7.7	43.3ª	4.3	97.83/C
C.V. (%)	5.22		0.9		1.22		4.19		
Control	100ª	0	155.5ª	0	2.6ª	0	44.5ª	0	-
Cu. Longa	98.8ª	-1.2	155.0ª	-0,3	2.5ª	-3.9	39.5ª	- 11.2	93.23/C
C.V. (%)	1.65		3.33		1.59		4.79		

Means followed by same letter in the columns, to each product, do not differ by Tukey test ($p<0.05$)
Data of viability transformed into $\sqrt{x}$ and data of CFU, diameter and conidia production transformed into arcsen$\sqrt{x}/100$
*PV = Percentual variation; PV = [(Treatment mean/Control mean × 100) − 100]
**BI between 0 a 41 = toxic; between 42 e 66 = Moderately toxic; > 66 = compatible

Mertz et al. (2010) also observed strong inhibition in *B. bassiana* development by contact to *Cu. longa* at 10%, with reduction of 73% of viability, 25% of vegetative growth and 79% of conidia production. Besides, Mamprim et al. (2013) related significant reductions of 15 and 30% of viability and CFU, respectively, of *M. anisopliae* by *Co. citriodora* and *Cu. longa* aqueous extracts, when compared to only 1 and 5% reductions at the same parameters studied here.

The results observed in others studies with the same plant extracts here evaluated were more significant, however, it is important to take into account the variation in the technique adopted for contact of products with fungus, either by incorporation into the culture medium (MERTZ et al., 2010), or by superficial application on culture medium (as used in this study). Moreover, another important point to explain the differences between studies is the different methods to obtain the extracts, once Mamprim et al. (2013) used dried and ground plant material to prepare the extract in cold distilled water, followed by filtration with paper filter.

Thus, these results might be explained by the different methodologies used, either by greater exposure of entomopathogen to products in the culture medium, or by presence of higher concentrations of active ingredients in the extracts obtained from dried material (BARA et al., 2006). However, in this study we chose to techniques closer to the reality of farmer condition, both for the preparation of plant extracts as of application that simulates the field conditions.

Table 4: Mean percetage mortality (± SE) of *Diatraea saccharalis* caterpillars treated with *Metarhizium anisopliae* (strain 22-Unioeste) cultured on PDA, followed by spraying of alternative phytosanitary products (26 ± 1ºC and photophase of 12h).

Nature	Treatment*	Mean mortality confirmed (%)
Comercial products	Water control	0.0 ± 0.00^{b}
	Fungus control	74.1 ± 13.35^{a}
	Dalneem® ½RC	85.4 ± 5.29^{a}
	Dalneem® RC	96.3 ± 3.70^{a}
	Dalneem® 2RC	97.6 ± 2.38^{a}
	Ecolife® ½RC	84.6 ± 11.75^{a}
	Ecolife® RC	85.0 ± 7.64^{a}
	Ecolife® 2RC	91.1 ± 4.84^{a}
	Stubble-Aid® ½RC	92.3 ± 4.44^{a}
	Stubble-Aid® RC	83.3 ± 8.33^{a}
	Stubble-Aid® 2RC	87.2 ± 6.78^{a}
	C.V.(%)	15.79
Plant extracts	Water control	0.0 ± 0.00^{b}
	Fungus control	84.3 ± 4.56^{a}
	Rosmarinus officinalis	63.6 ± 7.56^{ab}
	Corymbia citriodora	93.1 ± 4.52^{a}
	Cymbopogon citratus	82.7 ± 4.14^{a}
	Curcuma longa	61.2± 14.64^{ab}
	C.V.(%)	26.66

Means followed by the same letter in the column, to each nature, do not differ by Tukey test ($p<0.05$). *Products concentrations: ½RC= Half of field recommendation; RC= Field recommendation; 2RC= Twice of field recommendation.

With regard to toxicity, all plant extracts were compatible to the fungus (Table 3), similar to observed by Mamprim et al. (2013).

Insecticidal activity of the fungus was not significantly affected by contact with plant extracts in the culture media, although *Cu. longa* and *R. officinalis* have formed an intermediate group (Table 4).

In a similar study evaluating insecticidal activity of *B. bassiana* produced in culture media containing extracts of *Cu. longa* and *Cy. citratus* to *Galleria mellonella* L. (Lepidoptera: Pyralidae) it was also observed the fungus activity was not affected by contact with the plant extracts (MERTZ et al. 2010). However, as well as to the commercial products, there are no others studies that evaluate this parameter.

Thus, when an IPM strategy is devised, it is important to take into account the compatibility of products sprayed on the crop, avoiding the use of toxic ones with the natural enemy, in this case *M. anisopliae*. Present investigations showed that both alternative commercial products (except Dalneem® 2RC) as the plant extracts can be used in areas where *M. anisopliae* occurs, once compatibility is proved in vitro, where contact between products and fungi is higher than in the field. So, it is expected the same will be occur in the field.

Conclusion

Alternative commercial products caused significant negative effects on colony forming units, vegetative growth and conidial production of *M. anisopliae*. In other wise, plant extracts significantly affected only vegetative growth of the fungus. All alternative phytosanitary products, except Dalneem® 2RC (Moderately toxic), were compatible with the entomopathogenic fungus *M. anisopliae*.

Acknowledgements

To financial support of CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico), agreement nº 303209/2007-0.

References

ALVES, S.B. Fungos entomopatogênicos. In: ALVES, S.B. (Ed.). **Controle microbiano de insetos**. Piracicaba: FEALQ, 1998. Cap.11, p.289-381.

ALVES, S.B. et al. Produtos fitossanitários e entomopatógenos. In: ALVES, S.B. (Ed.). **Controle microbiano de insetos**. Piracicaba: FEALQ, 1998a. Cap.8, p.217-238.

ALVES, S.B. et al. Técnicas de laboratório. In: ALVES, S.B. (Ed.). **Controle microbiano de insetos**. Piracicaba: FEALQ, 1998b.Cap.20, p.637-711.

ALVES, S.B. et al. Fungos entomopatogênicos usados no controle de pragas na América Latina. In: ALVES, S.B.; LOPES, R.B. (Ed.). **Controle microbiano de pragas na América Latina**.Piracicaba: FEALQ, 2008. p.69-104.

BARA, M.T.F. et al. Determinação do teor de princípios ativos em matérias-primas vegetais. **Revista Brasileira de Farmacognosia**, Curitiba, v.16, n.2, p.211-215, 2006.

BARGUIL, B.M. et al. Effect of extracts from citric biomass, rusted coffee leaves and coffee berry husks on *Phoma costarricencis* of coffee plants. **Fitopatologia Brasileira**, Brasília, v.30, n.5, p.535-537, 2005.

CASTIGLIONI, E. et al. Compatibilidad de *Beauveria bassiana* y *Metarhizium anisopliae* com Nimkol-L® para el combate de *Heterotermes tenuis*. **Manejo Integrado de Plagas y Agroecologia**, Costa Rica, v.69, p.38-44, 2003.

CAVALCANTI, F.R. et al. Acibenzolar-s-metil e Ecolife® na indução de respostas de defesa do tomateiro contra a mancha bacteriana (*Xanthomonas vesicatoria*). **Fitopatologia Brasileira**, Brasília, v.31, n.4, p.372-380, 2006.

FERREIRA, D.F. SISVAR: a computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v.35, p.1039-1042, 2011.

GALLO, D. et al. **Entomologia Agrícola**. Piracicaba: FEALQ, 2002.

HIROSE, E. et al. Effect of biofertilizers and neem oil on the enthomopatogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok.Tecapar. **Brazilian Archives of Biology and Technology**, Curitiba, v.44, n.4, p.419-423, 2001.

KUBERAN, T. et al. In vitro evaluation certain plant extracts against *Glomerella cingulata* causing brown blight disease of tea. **World Journal of Agricultural Sciences**, Índia, v.8, n.5, p.464-467, 2012.

MAMPRIM, A.P. et al. Efeito de defensivos agrícolas naturais e extratos vegetais sobre parâmetros biológicos de *Metarhizium anisopliae* (Metsch.) Sorok. **Semina: Ciências Agrárias**, Londrina, v.34, p. 1451-1466, 2013.

MARQUES, R.P. et al. Crescimento, esporulação e viabilidade de fungos entomopatogênicos em meios contendo diferentes concentrações de óleo de Nim (*Azadirachta indica*). **Ciência Rural**, Santa Maria, v.34, n.6, p.1675-1680, 2004.

MERTZ, M.R. et al. Efeito de produtos fitossanitários naturais sobre *Beauveria basiana* (Bals.) Vuill. in vitro. **Biossay**, Piracicaba, v.5, n.3, p.1-10, 2010.

MOINO JR., A.; ALVES, S.B. Efeito de imidacloprid e fipronil sobre *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. e no comportamento de limpeza de *Heterotermes tenuipes* (Hagen). **Anais da Sociedade Entomológica do Brasil**, Londrina, v.27, n.4, p.611-619, 1998.

NEVES, P.M.O.J. et al. Compatibility of entomophatogenic fungi with neonicotinoid insecticides. **Neotropical Entomology**, Londrina, v.30, n.2, p.263-268, 2001.

OLIVEIRA, D.G.P. Proposta de um protocolo para avaliação da viabilidade de conídios de fungos entomopatogênicos e determinação da proteção ao calor conferida a *Beauveria bassiana* e *Metarhizium anisopliae* pela formulação em óleo emulsionável. Piracicaba, 2009. 89p. Dissertação (Mestrado em Ciências - Entomologia) - Escola Superior de Agricultura "Luiz de Queiroz".

PINTO, A.P.F. et al. Patogenicidade de *Beauveria bassiana* ao psilídeo *Diaphorina citri* e compatibilidade do fungo com produtos fitossanitários. **Pesquisa Agropecuária Brasileira**, Brasília, v.47, n.12, p.1673-1680, 2012.

RIBEIRO, L.P. et al. Compatibility of *Beauveria bassiana* comercial isolate with botanical insecticides utilized in organic crops in Southern Brazil. **Biological Agriculture & Horticulture**, Coventry, v.28, p. 223-240, 2012.

ROSSI-ZALAF, L.S. et al. Interação de microrganismos com outros agentes de controle de pragas e doenças. In: ALVES, S.B.; LOPES, R.B. (Ed). **Controle microbiano de pragas na América Latina: avanços e desafios**. Piracicaba: FEALQ, 2008. p.279-302.

SHASHI, S.; GUPTA, R.B.J. Compatibility of *Beauveria brongniartti* with pesticides and organic manures. **Pesticide Research Journal**, Ghaziabad, v.10, p.251-253, 1998.

SILVA, R.Z.; NEVES, P.M.O.J. Techniques and parameters used in compatibility tests between *Beauveria bassiana* (Bals) Vuill and in vitro phytosanitary products. **Pest Management Science**, Inglaterra, v.61, n.7, p.667-674, 2005.

SILVA, J.L. et al. Atividade antifúngica de extratos vegetais sobre o crescimento in vitro de fitopatógenos. **Revista Verde**, Mossoró, v.7, n.1, p.80-86, 2012.

SHUKLA, A.; DWIVEDI, S.K. Bioefficacy of plant extracts against fusarium species causing wilt in pulses. **IOSR Journal of Engineering**, Índia, v.2, n.1, p.136-144, 2012.

TAGAMI, O.K. et al. Fungitoxidade de *Bidens pilosa*, *Thymus vulgaris*, *Lippia alba* e *Rosmarinus officinalis* no desenvolvimento in vitro de fungos fitopatogênicos. **Semina: Ciências Agrárias**, Londrina, v.30, n.2, p.285-294, 2009.

TAMAI, M.A. et al. Toxicidade de produtos fitossanitários para *Beauveria bassiana* (Bals.) Vuill. **Arquivos Instituto Biológico**, São Paulo, v.69, n.3, p.89-96, 2002.

ZAPPELINI, L.O. et al. Seleção de isolados do fungo entomopatogênico *Metarhizium anisopliae* (Metsch.) Sorok. visando o controle da broca da cana-de-açúcar *Diatraea saccharalis* (Fabr. 1794). **Arquivos do Instituto Biológico**, São Paulo, v.77, n.1, p.75-82, 2010.