DOI: 10.1590/1808-1657v78p4712011

SCIENTIFIC COMMUNICATION

COMPATIBILITY OF NEUTRAL DETERGENT WITH THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* (METSCH.) SOROK.

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ABSTRACT

Entomopathogenic fungi have been widely used in pests control. The main diluent for its application in the field is water, and due to its hydrophobic character, its dispersion in the environment is complicated. Several products may be mixed to the sprays, allowing the dissolution and the dispersion of the fungus in the spray. Yet, some of these products may influence in the viability, sporulation or even in its genetic composition, changing its virulence. This paper aimed to evaluate the effect of a neutral detergent about the viability of the entomopathogenic fungus *Metarhizium anisopliae*. Three different concentrations of a neutral detergent (Ipê®) were used: 0.01%, 0.02%, 0.03% and an adhesive spreader (Tween 80) was used in the control. The percentage of conidia germination, the number of colony forming units (CFU), vegetative growth and the number of produced conidia were evaluated. There was no effect of the different concentrations of the neutral detergent on the germination. All detergent concentrations affected negatively in the vegetative growth of the fungus. Only the detergent at 0.01% of concentration did not affect the CFU number and the conidia production of the fungus. So, this is the only concentration classified as compatible for *M. anisopliae*, which may be recommended to promote the conidia dispersion of this fungus species in water.

KEY WORDS: Entomopathogen, biological control, microbial control.

RESUMO

COMPATIBILIDADE DE DETERGENTE NEUTRO COM O FUNGO ENTOMOPATOGÊNICO *METARHIZIUM ANISOPLIAE* (METSCH.) SOROK. Fungos entomopatogênicos têm sido amplamente utilizados no controle de pragas. O principal diluente para sua aplicação no campo é a água e, devido seu caráter hidrofóbico, a sua dispersão no meio é dificultada. Vários produtos podem ser misturados às caldas, permitindo a solubilização e dispersão do fungo na calda. Porém, alguns destes produtos podem influenciar na viabilidade, esporulação ou até mesmo na composição genética, alterando a sua virulência. Este trabalho objetivou avaliar o efeito do detergente neutro sobre a viabilidade do fungo entomopatogênico *Metarhizium anisopliae.* Foram utilizados como tratamentos três concentrações diferentes de detergente neutro Ipê®: 0,01%; 0,02%; 0,03% e o Tween 80 como testemunha. Avaliou-se a porcentagem de germinação dos conídios, o número de unidades formadoras de colônia, o crescimento vegetativo e o número de conídios produzidos. Não houve efeito das diferentes concentrações do detergente neutro sobre a germinação. Todas as concentrações do detergente interferiram de forma negativa no crescimento vegetativo do fungo. Apenas o detergente a 0,01% não interferiu no número de UFC e na produção de conídios do fungo, sendo a única concentração classificada como compatível com o fungo *M. anisopliae*, podendo ser recomendada para promover a dispersão dos conídios dessa espécie de fungo em água.

PALAVRAS-CHAVE: Entomopatógeno, controle biológico, controle microbiano.

Entomopathogenic fungi may be a viable alternative to control pests, due to their production and application facilities and their mass production. The application is generally made through pulverization with water, being its main diluent (Alves et al., 2008). It is known conidia present a hydrophobic character which difficults their dispersion in water (Nitschke; Pastore, 2002).

The surfactants may be used as adjuvants to the spray of the pulverization, because they are amphipathic molecules formed with a hydrophobic portion and a hydrophilic one. Due to these factors,

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the surfactants tend to dispose into the interfaces among liquid phases with different degrees of polarity (oil/water and water/oil). These products deserve notability in the industries, because they are used to produce several products, such as cleansing products. This is possible due to its broad use involving: detergency, emulsification, lubrication, foaming and wet properties, solubility and dispersion of phases (Nitschke; Pastore, 2002).

The detergent belongs to the surfactants group and may be used as an alternative aiming at a better dispersion of the conidia from entomopathogenic fungi in water, due to its similarity with other adjuvants, whose function is to diminish the superficial tension of water and to favor conidia homogenization (Kotz; Treichel, 2002). However, the usage of these products associated with entomopathogenic fungi may interfere in the development of these microorganisms, affecting their viability, sporulation or even its genetic composition, changing its virulence (Batista Filho et al., 1998).

Up to this moment, in specialized literature, there is no work with neutral detergent mixed in the spray with entomopathogenic fungi. So, this work aimed to verify the compatibility of neutral detergent with the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883.

It was used *M. anisopliae* fungus (IBCB 425). For evaluating the compatibility tests, a neutral detergent was utilized (Ipê®) which presents the following composition: anionic surfactant, adjusting agent, glycerine, sequestrant, deriveds from isothiazolinone, dye, thickener and water. Three different concentrations of detergent (0.01%, 0.02%, 0.03%) were used and for the control treatment, it was used an adhesive spreader (Tween 80) at 0.01%. The effect of the neutral detergent upon the fungus was studied *in vitro* and so germination, vegetative growth, conidial production and the colony forming unit (CFU) with the presence or absence of the product were all evaluated.

For evaluated the viability, it was used a suspension of 1.0×10^8 conidia/mL, which was kept resting for 2 hours in different concentrations of detergent. After this period, 0.2 mL of the suspension was aplied to the surface of Petri dishes containing the culture medium (PDA). After the inoculation, the dishes were incubated for 18 hours in an acclimatized room at 25 ± 1º C, RH of 70 ± 10% and a 12h photoperiod (Alves; Moraes, 1998). After the incubation period, the number of conidia was counted, sharing the dishes in four quadrants with 100 conidia in each one, between those germinated and non-germinated ones, establishing later, the germination percentage.

For quantifying the CFU, it was used a suspension containing 1.0×10^6 con/mL kept in resting for 2 hours in different concentrations of detergent. After this period, 0.2 mL of the suspension was applied to the surface of Petri dishes containing PDA. After the dishes were packed in an acclimatized room at 25° ± 1° C, with RH of 70 ± 10% and 12h photoperiod for 3 days. The counting of CFU was made based on the technique of counting dishes (Alves; Moraes, 1998).

For evaluating the vegetative growth and the conidia production of the fungus, Tween 80 and neutral detergent were added to the mixture medium. After medium solidification, the inoculation was effected at three points on the dish. After, the dishes were packed in an acclimatized room at 25 ± 1º C, RH of 70 ± 10% and 12h photoperiod for 10 days to promote the vegetative and reproductive growth of the fungus. For evaluating the vegetative growth of the fungus, the colonies were measured, previously marked in the outer part of the dish in orthogonal directions, to determine the mean diamenter (cm). The counting of the conidia was achieved, using the technique of direct counting in a microscope with a Neubauer chamber (Alves; Moraes, 1998).

The statistical design was fully randomized with 4 treatments with 3 replicates for each treatment, and each one formed by 3 Petri dishes. The data of conidia viability were transformed to arcsen $(x/100)^{1/2}$, the data of CFU were transformed to $(x + 1)^{1/2}$ and the data of vegetative and reproductive growth were transformed to $(x + 0.5)^{1/2}$. Then, all were submitted to the analysis of the group means of Scott Knott test at 5% of probability, with the assistance of Sisvar 5.0 statistical program (Ferreira, 2005).

The obtained data were also submitted to the calculation of the compatibility (BI = Biological Index) proposed by Rossi-Zalaf et al. (2008), which allows the classification of the products in selectivity/compatibility classes, according to the observed effect, in relation to the evaluated parameters.

There was no significant difference on the percentage of germinated conidia in relation to the treatments (Table 1), the values above 95% for this parameter were observed, presenting a high viability. Evaluating the oil as an emulsifier for the formulations of *Beauveria bassiana* (Balsamo) Vuillemin, 1912, Luz et al. (2004) verified levels of germination higher than 98%. Silva *et al.* (2006), while analysing the germination percentage of *M. anisopliae* due to different products from mineral and vegetal oils, noted that from 15 tested products only one (Oppa) was lower in the control, inhibiting the fungi germination; and this loss had been little significance, since the germination rate continued higher than 93%. In the experiments with adjuvants in the development *in vitro* of the entomopathogenic fungi *M. anisopliae* and *B. bassiana*, Costa et al. (2003), verified that from the 4 tested products, AgRho DEP 775 at 0.07% and 0.09% did not differ in the control after 20h and 48h of these fungi inoculation, with a germination higher than 99%.

Table 1 – Conidia germination (%) (± EP), number of CFUs (± EP), mean diameter of colonies (± EP) and mean prodcution of conidia (± EP) of *Metarhizium anisopliae* isolated IBCB 425, submitted to different concentrations of neutral detergent.

Treatment	Germination (%)[1]	CFU	Diameter (cm)	Conidia (x 10^8 con/mL)
Control	96.75 ± 0.63	648.25 ± 11.77a	4.02 ± 0.03 a	0.92 ± 0.04 a
0.01%	96.00 ± 0.41	638.00 ± 16.49 a	2.53 ± 0.02 b	0.89 ± 0.07 a
0.02%	96.25 ± 1.03	523.25 ± 22.07 b	2.27 ± 0.06 c	0.39 ± 0.04 b
0.03%	97.00 ± 0.30	516.75 ± 26.14 b	1.40 ± 0.02 d	0.33 ± 0.03 c
VC (%)	2.82	3.53	1.70	10.28

[1]There was no significant difference between treatments by F test ($P < 0.05$).

When CFU numbers were evaluated, it was verified that there was no significant difference between the detergent in the lower concentration and the control. In the other concentrations there was a significant reduction in the development of the fungus (Table 1). Silva *et al.* (2006) noted that from the 14 tested products in the development of the fungus *M. anisopliae*, 6 presented a significant reduction in the produced CFU numbers in relation to the control. According to these authors, these confirmations may be a signal of inhibition on the vegetative growth, as suggested by Silva; Neves (2005), since germination was not affected in these mixtures.

There was a significant interference on the vegetative growth due to the different concentrations of neutral detergent used, verifying a reduction in the mean diameter of the colonies. In relation to the conidia production, only the 0.01% treatment was similar to the control (Table 1). Despite the 0.01% treatment had inhibited the vegetative growth, there was no significant reduction in the conidia production when compared to the control. These results agree with the observation reported by Zimmermann (1975), who states that the inhibition of the vegetative growth is not necessarily an indication of a sporulation reduction or a conidial viability.

In relation to the BI determination, only the 0.01% treatment was compatible for the entomopathogenic fungus *M. anisopliae*. The treatment at 0.02% was classified as moderately toxic and at 0.03% as toxic (Table 2).

Table 2 – BI values for the classification of the neutral detergent effect on *M. anisopliae* fungus.

Treatment	T Value	Classification
0.01%	82	Compatible
0.02%	54	Moderately toxic
0.03%	41	Toxic

In vitro studies have the advantage to maximum expose the microorganisms to the action of the used chemical product, which does not occur under field conditions due to several factors. So, since the product in laboratory is considered innocuous, it is expected it may be efficient in the field. On the other hand, the toxicity of the product in vitro does not always suggest a high toxicity in the field, but rather indicates only the possibility of the occurrence of damage of this nature (Moino Junior; Alves, 1998).

The adverse effects caused by the detergent only upon some evaluated parameters from *M. anisopliae* fungus may have occurred due to a specific component, present on the formulation of the used detergent, the derivatives of isothiazolines. These compounds present antimicrobic action (Lemus; Hernández, 2007), which are widely used in industry as preservatives. According to Capelletti (2006), the isothiazolines are considered non-oxidizing biocides which interfere on the metabolism and/or provoke a desintegration of the cell wall, effects that depend on the dosages and on the period of microorganism exposition to these compounds. As the period of exposition from the fungus, at different concentrations of the neutral detergent, was the same in each one of the tests, it may be credited the negative observed effects for the detergent at 0.02 and 0.03%; the highest quantity of derivatives of isothiazolines found in these treatments.

In this work, the obtained results suggest that the neutral detergent on its lowest concentration may be used together with the spray of the enthomopathogen, due to their characteristics similar to the adjuvants, decreasing the superficial tension of water and assiting on the homogenization of conidia and also to the low interference on the fungus development when compared and analysed all the evaluated parameters.

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Received on 9/4/10
Accepted on 26/4/11