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In vitro assay on homeopathic solutions on *Metarhizium anisopliae* (Metsch) Sorok (*Ascomycota: Clavicipitaceae*)

Efeito in vitro de soluções homeopáticas sobre Metarhizium anisopliae *(Metsch) Sorok* (Ascomycota: Clavicipitaceae)

Silvana Damin[1]*, Luis Francisco Angeli Alves[1], Andreia Kusumota Bonini[1], Talita Moretto Alexandre[1]

RESUMO: O presente trabalho teve por objetivo avaliar o efeito *in vitro* das soluções homeopáticas *Arsenicum album* 24CH; *Calcarea carbonica* 30CH; *Kali iodatum* 100CH; *Phosphorus* 3CH; *Silicea* 30CH; *Staphysagria* 6, 30 e 100CH; *Spodoptera frugiperda* 30CH; *Sulphur* 100 e 200CH e *Thuya occidentalis* 200CH sobre parâmetros biológicos do fungo *Metarhizium anisopliae*. As soluções medicamentosas foram diluídas em água destilada (esterilizada) (0,1%) e pulverizadas sobre o fungo previamente inoculado no meio de cultura BDA. Os parâmetros biológicos avaliados foram: germinação de conídios, unidades formadoras de colônias, crescimento vegetativo, produção de conídios e atividade inseticida contra lagartas de *Diatraea saccharalis* (Lepidoptera: Crambidae). As soluções não afetaram os parâmetros avaliados. Todos os tratamentos foram considerados compatíveis ao *M. anisopliae*.

PALAVRAS-CHAVE: fungos entomopatogênicos; soluções dinamizadas; conservação de espécies; compatibilidade.

ABSTRACT: This study aimed to evaluate the *in vitro* effect of homeopathic solutions *Arsenicum Album* 24CH; *Calcarea carbonica* 30CH; *Kali iodatum* 100CH; *Phosphorus* 3CH; *Silicea* 30CH; *Staphysagria* 6, 30 and 100CH; *Spodoptera frugiperda* 30CH; *Sulphur* 100 and 200CH and *Thuya occidentalis* 200CH on biological parameters of the fungus *Metarhizium anisopliae*. The solutions were diluted in sterile distilled water (0.1%) and were sprayed on the previously inoculated fungus on PDA culture medium. Germination, colony forming units, vegetative growth, conidial production and insecticidal activity of the fungus against larvae of *Diatraea saccharalis* (Lepidoptera: Crambidae) were evaluated. Homeopathic solutions did not affect negatively the parameters evaluated. Thus, all treatments were considered compatible to the fungus *M. anisopliae.*

KEYWORDS: entomopathogenic fungus; dynamized solution; species conservation; compatibility.

[1]Laboratório de Biotecnologia Agrícola; Universidade Estadual do Oeste do Paraná (UNIOESTE) – Cascavel (PR), Brazil.
*Corresponding author: sil_damin@hotmail.com
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INTRODUCTION

The entomopathogenic fungi have great potential in pest control, due to its capacity to suppress insect populations in all stages of host development (Leite et al., 2003). They are the major microbial agents controlling insect pests. Around 80% of diseases in insects have entomopathogenic fungi as etiologic agent (Alves, 1998).

The fungus *Metarhizium anisopliae* (Metsch.) Sorok. stands out as a natural pathogen of several agricultural pests, being recommended in Brazil to control sugarcane froghopper (*Mahanarva fimbriolata* and *M. posticata*), pasture spittlebugs (*Notozulia entreriana, Deois flavopicta, D. incompleta, D. schach, Aeneolamia selecta*), sugarcane borer (*Diatraea saccharalis*) and locusts (*Rhammatocerus schistocercoides*) (Alves et al., 2010; Brasil, 2013).

Products based on *M. anisopliae* represented about 30% of the market mycoinsecticides in the Latin America in 2007/2008 (Faria; Wraight, 2007; Alves; Lopes, 2008). However, when considered in addition to the products available, the products under registration process, these represented 55% of the market in 2009 (Michereff Filho et al., 2009).

This growth mainly comes from organic or agroecological farmers who have opted for alternative practices such as the use of natural pesticides (NP) in pest control. Out of which, the products based on microorganisms, especially bacteria and fungi, protective mixtures (Bordeaux mixture, Viçosa mixture, and lime sulfur), plant extracts, essential oils and homeopathic products are included.

Among the products taken as alternative, those homeopathic are the latest in agricultural use. In Brazil, the first reports were described by Brunini; Arenales (1993) who used *Staphysagria* in vegetables and ornamental plants, resulting in increased plant resistance to aphids, as well as improvement in the general conditions of plants. In recent years, several studies demonstrate the potential of homeopathic products for control and insect repellence (Almeida et al., 2003), phytopathogens control (*Phakopsora euvitis* Ono and *Alternaria solani* Ellis & Martin) (Bonato et al., 2006; Toledo, 2009, Modolon, 2010), induction resistance in plant, increase both secondary metabolites production and plant productivity (Castro et al., 2002; Duarte, 2007).

Commonly used in farms based on agroecological techniques, the NP are considered safe and healthy for agricultural production, and selective to natural enemies (Alves et al., 1998a). For Alves; Lopes (2008), preservation of microorganisms in the soil is of great importance, since they are components of a sustainable system of pest control, and, in case of positive associations of biological agents with synthetic pesticides (SP) or NP and other control techniques, higher productivity and healthier foods can be obtained.

By the practical, economical and sustainable peculiarities, NPs have received special attention in studies to evaluate their efficiency, their mode of action and action on biological control agents. Accordingly, it was found that some commercial products such as Ecolife® and Stubble-Aid® and others neem-oil-based affected conidia production and the vegetative growth of *M. anisopliae* (Marques et al., 2004; Formentini et al., 2013). Mamprim et al. (2013) highlighted the negative effect of lime sulfur broth on all biological parameters of *M. anisopliae*, classified as moderately toxic. However, the same authors have shown that aqueous and alcoholic plant extracts of some insecticide plants have proved compatible to the fungal development.

The entomopathogenic microorganisms can be considered in the agroecosystem under two aspects, whether natural or a component of microbial product (biopesticide), and, thus, the studies to understand the interaction between them and NP are justified (Rossi-Zalaf et al., 2008).

Regarding the homeopathy action on microorganisms, the few existing studies seek to control the phytopathogens, with inhibition of spore germination of *Fusarium roseum* (Khanna; Chandra, 1976), reduced mycelial growth and sporulation of *Alternaria solani* submitted to homeopathic treatments (Toledo, 2009; Modolon, 2010).

The evidence of these early studies on the action of homeopathic products on phytopathogenic fungi alert for possible interactions with entomopathogenic fungi also demonstrating the need for evaluating homeopathic remedies for such biological agents.

Thus, the present study aimed at evaluating the effect of homeopathic medicines recommended for various agricultural purposes on the fungus *M. anisopliae.*

MATERIAL AND METHODS

Fungal isolate and homeopathic medicines

The fungus *Metarhizium anisopliae* (strain UNIOESTE 22) from the Collection of Entomopathogenic Fungi UNIOESTE was cultured on potato–dextrose–agar (PDA) plates, and conidia were produced by subculturing the fungus on a sporulation medium (SM) containing 0.36 g KH_2PO_4, 1.05 g $Na_2HPO_4 \cdot 7H_2O$, 0.6 g $MgSO_4 \cdot 7H_2O$, 1 g KCl, 10 g glucose, 1.58 g $NaNO_3$, 5 g yeast extract, and 20 g agar in 1,000 mL water, incubated at 26°C and 12h photoperiod for growth and spore production for 8 days. The conidia were collected by scraping the medium surface, transferred to sterile glass tubes, stored at -10°C for a period not exceeding 10 days until performing the experiments.

The homeopathic products *Arsenicum album* 24CH; *Calcarea carbonica* 30CH; *Kali iodatum* 100CH; *Phosphorus* 3CH; *Silicea* 30CH; *Staphysagria* 6, 30 and 100CH; *Spodoptera*

frugiperda 30CH; *Sulphur* 100 and 200CH; and *Thuya occidentalis* 200CH were evaluated, whose selection was based on the results of scientific studies on the effects of these drugs on various plant species, insects and microorganisms (Table 1). The preparation of drugs was performed in homeopathic pharmaceutical laboratory based in the Farmacopeia Homeopática Brasileira (Brasil, 1997).

Thus, 0.2 mL of each of homeopathic solution were added separately in 19.8 mL (1/100 dilution) in 70% ethanol with 100 vigorous shaking or sucussion in mechanical arm dynamizer (Model 50-Autic Denise) to produce the fourth Hahnemannian centesimal dilution (4CH). In successive dilutions, they were obtained in the following dilutions of each drug, which were stored for later use.

Each treatment was prepared by adding 20 µL of homeopathic solution to 19.98 ml of sterile distilled water in a sterile glass tube (solution at 0.1% concentration homeopathic/water), these treatments being then sprayed on the fungus according to each biological parameter.

General procedures for in vitro evaluation

Sterile glass tubes containing the fungus produced as described before were used, in which were added 10 mL of sterile distilled water + Tween® 80 (0.01%). The suspension was stirred, quantified in Neubauer chamber and standardized in the concentration of 1×10^9 conidia mL^{-1} with subsequent dilutions, appropriate to the evaluated parameters, namely: vegetative growth, conidial production and insecticidal activity, according to Silva; Neves (2005) and Oliveira (2009).

Conidia germination – The PDA medium (200 g potato, 20 g dextrose, 0.05 g streptomycin, 15 g agar and 1,000 mL sterile distilled water) was poured into the Rodac-type plate and after PDA medium solidification, 150 uL fungal suspension (1×10^6 conidia/mL) were inoculated on its surface, spread on with manual and circular movement of the plate. Then, 150 µL of each of homeopathic solutions sprayed with a micro-sprayer coupled to an air compressor (constant pressure of 0.84 kgf/cm^2 output). The control plates received the spraying of 150 µL sterile distilled water + Tween® 80 (0.01%). For each treatment, five plates were prepared, each one corresponding to a repetition. All plates were incubated for 18h at 26 ± 1ºC and 12h photoperiod.

After incubation, the conidia were counted under optical microscope (400 × magnification) by dividing each dish into four quadrants and counting at least 100 conidia per quadrant, thus quantifying germinated and non-germinated conidia.

Colony forming units (CFU) – 100 µL of a fungal suspension (1×10^3 conidia/mL) were inoculated and distributed on the surface of PDA culture medium in Petri dish with a Drigalski loop. Then, 100 µL of treatments were sprayed on the inoculated fungus. In the control, plates containing fungus received 100 µL sterile distilled water + Tween® 80 (0.01%). The plates were incubated for three days at 26 ± 1ºC and 12h photoperiod with subsequent quantification of colonies formed. Five plates were prepared for all treatments, each of them considered as a repetition.

Vegetative growth – The fungus was inoculated on the culture medium surface in a Petri dish with a platinum loop at three points. The plates were incubated at 26 ± 1ºC and 12h photoperiod for 2 days. After incubation, 250 µL treatment/plate were sprayed. The control plates were treated with sterile distilled water + Tween® 80 (0.01%) onto culture media surface. Plates were again incubated for five days under the same conditions described above. The vegetative growth was determined

Table 1. Homeopathic medicines used with respective dilutions, mode of action and indications from the literature.

Treatments	Dilution	Mode of action/ Indication	References
Arsenicum album	24CH	Production of secondary metabolites, control of phytopathogens, soil detoxification and plants	Casali et al., 2009; Bonato et al., 2012;
Calcarea carbonica	30CH	Vegetative growth in plants, soil with calcium deficiency	Bonato et al., 2012
Kali iodatum	100CH	Control of phytopathogens; plant development	Toledo, 2009; Casali et al., 2009; Bonato et al., 2012
Phosphorus	3CH	Production of secondary metabolites (essential oil), control of phytopathogens	Duarte, 2007; Casali et al., 2009; Andrade, 2000
Silicea	30CH	Control of phytopathogens and pests; plant development	Bonato et al., 2006; Casali et al., 2009; Bonato et al., 2012
Staphysagria	6; 30 e 100CH	Control of insects, mites and phytopathogenic diseases	Bonato et al., 2012; Rupp et al., 2012;
Spodoptera frugiperda	30CH	Control of insects	Almeida et al., 2003; Bonato et al., 2012
Sulphur	100 e 200CH	Control of phytopathogens and pests	Sinha; Singh (1983); Bonato et al., 2007; Casali et al., 2009
Thuya occidentalis	200CH	Control of phytopathogens, insects and mites	Casali et al., 2009; Bonato et al., 2012

based on two perpendicular measurements of the colonies with a caliper rule in order to obtain the average diameter.

Conidia production – After verification of vegetative growth, the colonies obtained previously were cut and transferred individually to sterile glass tubes, to which were added 10 mL of sterile distilled water + Tween® 80 (0.01%). After stirring for about 2 minutes, the conidia quantification was performed in Neubauer chamber and optical microscope. For each treatment, 10 colonies were evaluated, 2 of each repetition.

Effect of different hydroalcoholic solutions on *M. anisopliae*

Whereas homeopathic products are made of alcoholic solutions, an experiment was conducted in order to verify the effect of this solvent on the biological parameters of the fungus.

General procedures were adopted for *in vitro* evaluation, as previously described, having the spraying of hydroalcoholic solutions as treatments in the concentrations ranging from 0.5 to 2.5% of alcohol/distilled sterile water. The percentage values were chosen based on the recommendation for preparing the "broth" applied in the field, it should not be applied alcoholic solutions above 1%, given the possibility of toxicity to plants and biological agents (Bonato et al., 2012). Sterile distilled water was applied in the control. For each treatment and also the control, five replicates were prepared.

Effect of different homeopathic products on *M. anisopliae*

The same experimental procedures described in the general procedures for *in vitro* evaluation were used, with homeopathic products at 0.1% as treatment (Table 1). A non-diluted 0.1% hydroalcoholic solution and a control were used, in which was applied sterile distilled water + Tween® 80 (0.01%).

Besides the parameters previously mentioned, the effect of homeopathic products on the insecticidal activity of *M. anisopliae* was evaluated as follow.

The fungus was inoculated onto SM in Petri dishes and about one hour later they were sprayed with 250 µL each of the products, using a micro-sprayer coupled to air compressor (0.84 kg/cm^2 output constant pressure). In the control plates, 250 µL of sterile distilled water were applied on the fungus. The plates were incubated for 8 days at 26 ± 1ºC and 12h photophase. The conidia were collected by scraping the medium surface and transferred to sterile glass tubes, then preparing suspensions at 1 × 10^9 conidia/mL, which was previously determined as one to obtain approximately 80% mortality of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Crambidae).

Third-instar larvae of *D. saccharalis* from the laboratory rearing and fed on artificial diet were used in the bioassays (Parra, 1999).

The insects were placed in Petri dishes and received 2 mL of treatments using a Potter tower (1.05 kgf/cm^2). After 1 minute, the larvae were transferred to Petri dishes containing artificial diet and were incubated for 10 days at 26 ± 1°C and 12h photophase. Daily, the food replacement and mortality assessment were performed, with dead insects being removed, immersed in 70% alcohol for 15 seconds and in sterile distilled water for equal time, and transferred to a moist chamber for confirming the fungus mortality, noting signs and symptoms of fungal infection as Alves et al. (1998b). For each treatment and also for the control, they were used 60 larvae divided into 4 replicates.

Data analysis

Experimental design was completely randomized set-up and the data tested for normality (Shapiro-Wilk) and analysis of variance (F-test). The means were compared by the Scott-Knott test, both at 5% by using the statistical program Sisvar® (Ferreira, 2011).

The compatibility between treatments and fungus was based on the Biological Index formula (BI) proposed by Rossi-Zalaf et al. (2008) calculated as:

$$IB = \frac{47\left[CV\right] + 43\left[ESP\right] + 10\left[GER\right]}{100}$$, where:

IB = Biological Index; CV = percentage of colony's vegetative growth after 7 days, compared to the control; ESP = percentage of sporulation of colonies after 7 days compared to control; GER = percentage of germinated conidia, since that the CV, ESP and GER values should be previously corrected for the respective controls. The IB values (p = 0.05) for products classification were: Toxic – 0-41; Moderately Toxic – 42-66; and Compatible – > 66.

RESULTS AND DISCUSSION

Effect of different hydroalcoholic solutions of *M. anisopliae*

The different hydroalcoholic solutions, in general, did not affect the biological parameters of *M. anisopliae*. The viability of conidia, number of CFU and the vegetative growth were not affected by alcoholic solutions in culture medium. However, the hydroalcoholic solutions at 2.0 and 2.5% stimulated the conidia production (Table 2).

In a study on the phytopathogenic fungus *Alternaria solani,* diluted hydroalcoholic solutions (at 0.0015% alcohol) may have action on biological parameters of the fungus, differing from the control distilled water (Toledo, 2009).

The findings of Toledo (2009) reaffirm the potential energy of homeopathic medicines, coming from the dilution

process (with sucussion), as the alcohol concentration used by the author in the tests was very low. Perhaps the results contrary to this study are due to the different species of fungi used in both studies.

It is noteworthy that even the hydroalcoholic solutions with double of the alcohol present in homeopathic solutions showed no negative effect, discarding any possibility of the alcohol present in homeopathic solution to interfere with the biological parameters of *M. anisopliae*.

Action of homeopathic solutions on *M. anisopliae*

The viability of conidia in all treatments was above 93%, not differing significantly from the control (94%), corroborating the observations of Toledo (2009), who also found no effect of homeopathic products *Sulphur, Silicea terra, Staphysagria, Phosphorus* and *Kali iodatum* on the germination of the fungus *A. solani*.

In the germination phase, both the SP and NP are more likely to intervene in the development of entomopathogenic fungi because, when sprayed, the products come into direct contact with the conidia which have not yet penetrated the insect integument at this time and may compromise the control efficiency by entomopathogen (Alves et al., 1998a; Silva et al., 2005).

There was also no effect on the CFU formation, and even the solutions *Arsenicum album* 24CH, *Calcarea carbonica* 30CH, *Phosphorus* 3CH, *Staphysagria* 6CH, *Spodoptera frugiperda* 30CH, *Sulphur* 100CH and *Thuya occidentalis* 200CH stimulated this parameter when compared with the control (Table 3).

Table 2. Viability of conidia, colony forming units, vegetative growth and production of *Metarhizium anisopliae* (UNIOESTE 22), subject to different alcoholic degrees (26 ± 1°C and 12h photoperiod).

Treatments	Viability (%)	CFU	Vegetative growth (cm^2)	Conidia production (× 10^6/mL)
Control	97.8 ± 1.29 a	35.5 ± 4.15 a	2.0 ± 0.23 a	17.2 ± 1.64 b
0.5% hydroalcoholic solution	98.9 ± 0.80 a	29.8 ± 2.42 a	2.8 ± 0.30 a	13.9 ± 2.13 b
1.0% hydroalcoholic solution	98.1 ± 0.41 a	29.8 ± 3.53 a	2.8 ± 0.22 a	13.0 ± 2.53 b
1.5% hydroalcoholic solution	97.9 ± 0.42 a	29.5 ± 2.30 a	3.2 ± 0.05 a	16.7 ± 2.13 b
2.0% hydroalcoholic solution	98.7 ± 0.74 a	28.6 ± 3.96 a	3.6 ± 0.36 a	19.7 ± 2.56 a
2.5% hydroalcoholic solution	98.4 ± 0.96 a	26.8 ± 3.99 a	3.5 ± 0.05 a	22.3 ± 3.02 a
C.V. (%)	1.08	16.32	6.57	18.43
Factor F	0.70	1.71	1.46	2.96

Means (± SE) followed by the same letter in the column for each product tested does not differ by the Scott-Knott test at 5% significance.
CFU: colony forming units.

Table 3. Viability of conidia, colony forming units, vegetative growth, conidia production, "T" values and compatibility of *Metarhizium anisopliae* (UNIOESTE 22) subjected to different homeopathic medicines (26 ± 1°C and 12-h photophase).

Homeopathic drugs	Viability (%)	CFU	Vegetative growth (cm^2)	Conidia production (× 10^6/mL)	T/C value
Control	94.7 ± 0.77 a	39.0 ± 2.61 b	3.1 ± 0.07 a	31.7 ± 2.13 b	-
Hydroalcoholic sol. (0.1%)	94.8 ± 0.94 a	39.6 ± 3.77 b	3.1 ± 0.13 a	37.0 ± 4.32 a	-
A. album 24CH	94.0 ± 1.53 a	52.6 ± 2.78 a	3.0 ± 0.20 a	41.7 ± 5.76 a	111.11/ C
C. carbonica 30CH	95.4 ± 0.27 a	50.8 ± 2.95 a	2.4 ± 0.13 a	45.2 ± 6.84 a	107.72/ C
Kali iodatum 100CH	93.4 ± 1.10 a	44.2 ± 3.35 b	3.1 ± 0.06 a	27.2 ± 1.22 b	93.37/ C
Phosphorus 3CH	94.3 ± 1.26 a	54.0 ± 2.34 a	3.0 ± 0.11 a	37.5 ± 4.48 a	106.60/ C
Silicea 30CH	94.2 ± 0.74 a	41.4 ± 3.77 b	2.4 ± 0.11 a	25.3 ± 2.51 b	80.53/ C
Staphysagria 6CH	94.9 ± 1.62 a	55.4 ± 7.06 a	2.3 ± 0.24 a	29.1 ± 2.22 b	84.62/ C
Staphysagria 30CH	93.8 ± 1.26 a	42.2 ± 5.02 b	3.0 ± 0.12 a	28.7 ± 6.17 b	93.66/ C
Staphysagria 100CH	93.2 ± 1.10 a	43.6 ± 7.64 b	3.1 ± 0.10 a	36.5 ± 6.36 a	105.97/ C
S. frugiperda 30CH	93.4 ± 1.04 a	61.0 ± 3.80 a	3.0 ± 0.36 a	26.3 ± 2.39 b	90.57/ C
Sulphur 100CH	93.8 ± 1.27 a	51.6 ± 2.90 a	3.0 ± 0.30 a	38.1 ± 4.21 a	106.59/ C
Sulphur 200CH	93.8 ± 1.59 a	35.6 ± 4.99 b	3.1 ± 0.10 a	35.2 ± 1.82 a	104.58/ C
T. occidentalis 200CH	94.7 ± 0.87 a	50.8 ± 4.25 a	3.0 ± 0.16 a	25.6 ± 3.94 b	90.16 / C
C.V. (%)	2.13	16.04	5.20	22.33	-
Factor F	0.82	4.80	1.04	3.71	-

Means (± SE) followed by the same letter in the column for each product tested does not differ by the Scott-Knott test at 5% significance. T values, according to Rossi-Zalaf et al. (2008) between 0 and 41 = toxic (T); between 42 and 66 = Moderately toxic (MT); greater than 66 = compatible (C).
CFU: colony forming units.

This increase in CFU is a trend already observed by other authors in analyses of NP (commercial products, aqueous and alcoholic plant extracts) and biological parameters of *M. anisopliae*, which can likely be attributed to the degradation and utilization of substances present in the composition of products tested and may have been used as nutrients by the fungus (Formentini et al., 2013; Mamprim et al., 2013).

In contrast, in the dilutions whose homeopathic solutions were used in the tests (with the exception of medicine *Phosphorus* 03CH), the probability of having material from the original substance in solution is very remote, since from the twelfth dilution (1/100) it exceeds the number of Avogadro (6.02×10^{23}), leaving only the information of the original substance (Davenas et al., 1988). Thus, according to the homeopathy principles, the homeopathic drug action is the result of the stimulus of the information in this diluted medication. Thus, in the absence of matter, the possibility of the presence of active ingredients in homeopathic solutions may have caused the increased CFU in the present work is discarded, still not known to the mechanism of action of homeopathy on the organism at the cell level (Bonato et al. 2012).

Furthermore, the informational presence of *S. frugiperda* in homeopathic preparation may have been based on the homeopathy principles, a factor to stimulate the growth of fungus that is pathogenic to this insect (Carneiro et al., 2008).

It is noteworthy that in the few studies verifying the action of homeopathic solutions on the pathogenic fungi, the effect on the number of CFU is not evaluated (Toledo, 2009; Modolon, 2010), making it difficult to compare with this class of products.

The lack of effect on vegetative growth was confirmed in the diameter of colonies, which did not differ statistically among products and between them and the control.

However, the drugs *Sulphur, Silicea terra, Staphysagria, Phosphorus* and *Kali iodatum,* when incorporated into the PDA culture medium, reduced the vegetative growth of *A. solani* (Toledo, 2009). Similarly, *Arsenicum album* at *Staphysagria* at the dynamization 6, 12, 25, 30, 50, 60, 80 and 100CH promoted reduction in vegetative growth of *A. solani* when applied to the fungus inoculated on the PDA culture medium (Modolon, 2010). In such cases, variations may be caused by concentrations and especially by the difference between fungal species involved.

Conidia production was stimulated by drugs *Arsenicum album* 24CH, *Calcarea carbonica* 30CH, *Phosphorus* 3CH, *Staphysagria* 100CH and *Sulphur* 100 and 200CH, and promoted significant increase when compared to control water (Table 3).

Under field conditions, the rust infestation (*Phakopsora euvitis* Ono) was reduced with homeopathic solutions of *Silicea* 30CH over 90%, which proves the fact that homeopathic solutions have no broad spectrum action, i.e., in different fungi can cause different results (Bonato et al., 2006).

Regarding toxicity, according to the biological index proposed by Rossi-Zalaf et al. (2008), all homeopathic treatments were compatible to the fungus *M. anisopliae* with toxicity values above 80 (values greater than T = 66 compatible).

The absence of similar studies makes it difficult to discuss the results of this parameter, but, based on the absence of negative interaction of homeopathic solutions tested in this work with all biological parameters of *M. anisopliae,* it may be considered that the combined use in field is safe, given the conditions in the laboratory being of maximum exposure among drugs and fungus and field (Alves et al., 1998a).

Action of homeopathic treatments on the insecticidal activity of *M. anisopliae* on *D. saccharalis*

The *D. saccharalis* mortality by the fungus was not affected by the presence of homeopathic drugs in the culture medium by comparing the treatment containing the fungus produced in culture medium treated with water only (control fungi). Mortality confirmed was between 78 and 98% (Table 4).

The lack of effect of homeopathic treatments on the insecticidal activity of *M. anisopliae* reflects, besides the absence of effects on the above parameters and also because these drugs at such dilutions may not have specific action on the fungus *M. anisopliae* able to inhibit some step of its metabolism

Table 4. Mortality confirmed (± SE) of *Diatraea saccharalis* larvae undergoing homeopathic products and the fungus *Metarhizium anisopliae* (UNIOESTE 22) multiplied in amid ME + homeopathic medicines (26 ± 1°C and 12h photoperiod).

Treatment	Confirmed mortality (%)
Control	00.0 ± 0.00 b
Hydroalcoholic solution (0.1%)	00.0 ± 0.00 b
M. anisopliae	91.6 ± 7.28 a
M. anisopliae + *Arsenicum album* 24CH	96.6 ± 3.84 a
M. anisopliae + *Calcarea carbonica* 30CH	96.6 ± 2.22 a
M. anisopliae + *Kali iodatum* 100CH	94.7 ± 3.92 a
M. anisopliae + *Phosphorus* 03CH	91.6 ± 4.84 a
M. anisopliae + *Silicea* 30CH	86.6 ± 3.14 a
M. anisopliae + *Staphysagria* 6CH	78.3 ± 8.53 a
M. anisopliae + *Staphysagria* 30CH	95.0 ± 3.68 a
M. anisopliae + *Staphysagria* 100CH	90.0 ± 6.66 a
M. anisopliae + *Spodoptera frugiperda* 30CH	96.5 ± 2.30 a
M. anisopliae + *Sulphur* 100CH	88.3 ± 6.57 a
M. anisopliae + *Sulphur* 200CH	90.0 ± 2.22 a
M. anisopliae + *Thuya occidentalis* 200CH	98.3 ± 1.92 a
C.V. (%)	13.30
Factor F	69,18

Means (± SE) followed by the same letter in the column for each nature does not differ by the Scott-Knott test at 5% significance.

to influence its action on *D. saccharalis*, such as synthesis of enzymes or toxins, important on the insecticidal activity of this fungal species (Alves et al., 1998a; Freimoiser et al., 2005).

In the literature, studies on interaction, either SP or NP and entomopathogenic fungi have not been focused on the effects on the insecticidal activity of the fungus, which makes difficult the full understanding of the interactions that these agents can promote to the insect, since currently in several crops products with different purposes are being applied in combination, as a way to reduce costs and manpower.

It is also important to note that the non-interference of homeopathic treatments on the pathogenicity of *M. anisopliae* is very positive for the conservation of the natural enemy in the environment, thus becoming inoculum for probable epizooties (Alves; Lecuona, 1998).

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