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Fungitoxicity activity of homeopathic medicines on *Alternaria solani*

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The black spot disease caused by the fungus *Alternaria solani* (Ellis and Martin) L. R. Jones and Gront is one of the most important diseases of tomato (*Lycopersicon esculentum* Mill.). The control is usually performed with fungicides, resulting in products contaminated with pesticide residues. In recent years, the use of homeopathic medicines has been highlighted in research for disease control. The aim of this study was to evaluate the *in vitro* fungitoxicity against *A. solani* by the homeopathic medicines *Propolis, Isotherapic of A. solani* and *Isotherapic of ash,* at 6, 12, 30 and 60CH (hahnemanian centesimal) dynamizations, and *Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricum and Kali iodatum* at 6, 12, 30 and 100CH dynamizations. Distilled water and 30% hydroalcoholic solution were used as controls at 12, 30, 60 and 100CH dynamizations. Mycelial growth, sporulation and conidial germination of *A. solani* were evaluated. The results indicated that for mycelial growth only in *Sulphur* and *Staphysagria* 100CH showed suppressive effect compared to both controls. For sporulation, *Propolis* 6, 30 and 60CH and *Ferrum sulphuricum* 6 and 30CH caused inhibition and differed from both controls. *Isotherapic of A. solani* 6CH, *Isotherapic of ash* 6CH and *Ferrum sulphuricum* 30CH reduced spores germination of the pathogen. It was also found that distilled water at 60 and 100CH inhibited mycelium growth. These results indicate the potential of some homeopathic medicines for trials aiming to control the black spot disease in tomato crops.

Key words: Homeopathy, alternative control, black spot, *Solanum lycopersicum*.

INTRODUCTION

The black spot caused by *Alternaria solani* (Ellis and Martin) L. R. Jones and Grout is one of the most important and common diseases of tomato (*Solanum lycopersicum* Mill.) crops, with high destructive potential, focusing on leaves, but also in stems, petioles and fruits, causing significant economic losses (Jones et al., 2014).

The fungus which causes this disease survives in crop debris and infecting other vegetables such as potatoes

and eggplant.

The small number of cultivars with genetic resistance to this disease, associated with the high cost of seeds, results in the control with chemical products to those traditionally grown tomato varieties that are susceptible to the pathogen (Kurozawa and Pavan, 2005). According to the Program for Analysis of Pesticide Residues in Food, 18% of tomato samples analyzed were unsatisfactory due to the use of unauthorized pesticides and presence of pesticide residues above the acceptable limits in the produce (Anvisa, 2008).

Thus, it is necessary to develop new strategies for management of tomato diseases with the use of natural pesticides (Hamerschmidt et al., 2012) such as extracts and essential oils from medicinal plants (Monteiro et al., 2013), fungal extracts (Stangarlin et al., 2011) and homeopathic medicines (Bonato et al., 2007a; Toledo et al., 2015). Homeopathy, science developed by Hahnemann for over 200 years, is an option with great potential in diseases control (Modolon et al., 2012). It is a low-cost alternative, easy to use by farmers, and it is also environmentally friendly (Bonato, 2007).

Few studies demonstrate the direct effect of homeopathic medicines on the pathogen. Khanna and Chandra (1992) observed inhibition of spore germination of *Alternaria alternata* (Fr.) Keissler, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., *Fusarium roseum* (Link) Snyd. et Hansen x gramineae and *Gloeosporium psidii* Delacr. by various drugs. Sinha and Singh (1983) found that *Sulphur* (200CH) inhibited in 100% the growth of *Aspergillus parasiticus*, while *Silicea terra* and *Dulcamara* caused 50% inhibition. Saxena et al. (1987) conducted a study on *Thuya ocidentalis, Nitric acidum* and *Sulphur* at 200CH observed inhibition of 22 fungi genera. This study was aimed at analyzing *in vitro* the antifungal effect of some homeopathic medicines on mycelial growth, sporulation and spore germination of *Alternaria solani* in order to develop alternative methods for controlling black spot disease in tomato.

MATERIALS AND METHODS

The study comprised two assays: a) test to determine the *in vitro* fungitoxicity activity of homeopathic medicines on the mycelial growth and sporulation of *Alternaria solani*; and b) test to verify the action of drugs on the spore germination of this fungus.

Obtaining *A. solani* isolates

The isolate 1707 from EMBRAPA-Hortaliças (CNBH) was used. This was recovered by subculture to Petri dishes containing about 20 mL PDA (potato dextrose agar), followed by new subculture to V8-agar medium. The isolate was incubated at 25°C and 12 h photoperiod (Balbi-Peña et al., 2006).

Choice of treatments

Choice of drugs was carried out according to the potential described in the literature review relevant to disease control and by analogy to the human medical field. Isopathy was also used, which is described by Bonato (2007b) as the use of the causal agent for medicine preparations. Treatments were separated into three study groups: one with the homeopathic preparations *Propolis, Isotherapic of A. solani* (IAS) and *Isotherapic of ash* of tomato leaves (ICF) with black spot lesions; another with *Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricum* and *Kali iodatum*; and a third with distilled water (DW) and hydroalcoholic solution (HS) with dynamizations. This separation was done in order not mix drugs that have gone through the process of classical homeopathy trial (Pustiglione, 2004).

The choice for propolis, though no papers in which this substance has been homeopatized, was due to characteristics cited in literature, as the mother-tincture is recommended for fungi control. According to Longhini et al. (2007), propolis is a resin collected by bees *Apis mellifera* L. and has antifungal action (Marini et al., 2012).

Considering this is a typical work into area of organic production, none of commercial pesticides were used as a pattern for comparing the efficiency of homeopathic drugs.

Preparation of homeopathic medicines and treatments

The homeopathic medicines *Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricum* and *Kali iodatum* were acquired in homeopathic pharmacy at 6CH and handled to 12, 30 and 100CH (CH: hahnemanian centesimal) according to the Brazilian Homeopathic Pharmacopoeia (2011), diluting 1:100 (1 part drug to 99 parts 30% alcohol p.a.) and succussing 100 times.

For the *Isotherapic of ash* (ICF) of tomato leaves infected with *A. solani*, leaves were dried at 60°C to constant weight and then incinerated to obtain ashes. It was used a piece of raw material and four parts of 70% ethanol P.A. at sterilized amber glass being left 15 days in the dark with daily stirrings (Bonato, 2007b). After the time required, material was filtered resulting in the mother-tincture. Subsequently, 1:100 was diluted (1 part mother-tincture to 99 parts 70% ethanol P.A.) and succussed 100 times, obtaining 1CH dynamization to 6, 12, 30 and 60CH dynamizations.

The drug *Propolis* was prepared with 20 g mass of propolis in 100 mL 70% ethanol P.A., left for 20 days for maceration, then filtered to obtain the different dynamizations (6, 12, 30 and 60CH).

For the *Isotherapic* of structures of *A. solani* fungus (IAS), one part of hyphae and spores was added to four parts 70% ethanol P.A., in sterilized amber glass and kept for 15 days in the dark with daily agitation. Afterwards, the material was filtered and succused. Distilled water (DW) and 30% hydroalcoholic solution (HS) were also prepared at 6, 12, 30, 60 and 100CH as Homeopathic Pharmacopoeia standards (2011) obeying the 1:100 proportion and using distilled water and 30% hydroalcoholic solution as solvents, respectively.

As controls were used distilled water and 30% hydroalcoholic solution (ethanol) by being solvents in homeopathic medicine preparations. All preparations from 6CH were performed with 30% ethanol P.A. and kept in dark amber glass.

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In vitro bioassays for antifungal activity determination

Test for mycelial growth inhibition

Treatments at appropriate dynamizations were incorporated into the V8 culture medium at 45°C and 0.005% concentration (Bonato, 2007b; Cupertino, 2004), and then poured into Petri dishes. One 7 mm diameter disk containing *A. solani* mycelium was sub-cultured to the center of Petri dishes and then sealed with plastic film and incubated at 25°C in the dark. Controls were also diluted to 0.005% obtaining the 0.0015% alcohol graduation in the application (Bonato and Silva, 2003).

The fungistatic activity effectiveness was evaluated according to the methodology described by Stangarlin et al. (1999) through daily measurements of colonies diameter (average of two diametrically opposed measures), starting 24 h after the experiment to date in which fungal colonies reached 75% culture medium surface at 144 h.

Test for spore production

Sporulation of each of these colonies, used in the assay for mycelial growth inhibition, was assessed at the end of the test for mycelial growth inhibition. For this, a suspension was prepared by adding 10 mL distilled water on the Petri dish, scraping the colony and filtration in cheese cloth, being determined the number of spores per mL with a Neubauer chamber in optical microscopy (Balbi-Peña et al., 2006).

Test for spore germination

To Petri dishes containing V8-agar medium and *A. solani* were added 10 mL sterile water and then scraping the colony with sterile stainless steel spatula with 7 days of age. After one hour of drying in flow chamber, plates were sealed with plastic wrap and then placed under a 12 h dark and 12 h light photoperiod according to methodology adapted by Pulz (2007), temperature ranging from 22 to 28°C until sporulation, which occurred 11 days after scraping the colony.

A 40 uL aliquot of spore suspension with 2 x 10^4 conidia/mL *A. solani* and another with 40 mL of each treatment corrected to maintain the same concentration as in the mycelial growth test (0.005%), were placed together on a microscope slide coated with a thin layer of water-agar (1%). These plates were incubated in a moist chamber in the dark at 25°C and germination percentage determined at the time of maximum spore germination (± 16 h), established in the curve of pathogen' spores germination (Balbi-Peña et al., 2006).

Data analysis

The experiment was arranged in randomized design with two factors: the group of homeopathic preparations factorial 5 x 5 (*Propolis,* IAS, ICF, DW and HS at 0, 6, 12, 30 and 60CH), the group of medicines factorial 8 x 5 (*Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricum, Kali iodatum*, DW e HS at 0, 6, 12, 30, 100CH) and the group of distilled water and hydroalcoholic solution factorial 2 x 6 at 0, 6, 12, 30, 60, 100CH, with four repetitions, with each Petri dish and slide considered a plot. Data were analyzed by group: homeopathic preparations, medicines and distilled water and 30% hydroalcoholic solution controls. Hydroalcoholic solution was considered the 0CH for each treatment. Data were subjected to analysis of variance (ANOVA), and means discriminated by the Scott-Knott test at 5% probability using the SISVAR program (Ferreira, 2011), version 5.1 (Build72).

RESULTS

Mycelial growth inhibition

Figure 1 presents the results obtained in the mycelial growth inhibition of *A. solani* in the presence of homeopathic preparations *Propolis, IAS* and *ICF*, compared with DW and HS (30%). Data indicate that *Propolis* (A) at 12 and 60CH did not differ from control DW. At 6 and 30CH they did not differ significantly from the HS control, but were 5.8 and 8.2% lower than DW. With *IAS* (B), at 6CH, it was equal to distilled water and the other dynamizations (12, 30 and 60CH) did not differ from the HS, but minimized mycelial growth at 4.78% (12CH) and 3.62% (30 and 60CH) compared to control DW.

With ICF (C), 12 and 30CH dynamizations differ from controls DW and HS but with intermediate values between them. Dynamizations 6 and 60CH were statistically equal to HS but reduced mycelial growth by 6.26 and 4.78% compared to control DW.

Results indicated that despite homeopathic preparations studied showing statistically lower values than the control DW, none had average colony diameter lower than the HS control and thus, there is a joint toxic action on the fungus, i.e. medicine and ethanol acting together with regard to mycelial growth inhibition of *A. solani*.

Figure 2 shows data regarding the mycelial growth inhibition of *A. solani* under the influence of medicines *Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricum* and *Kali iodatum* compared with controls and DW and HS. Data show that *Sulphur* (A) *Staphysagria* (C) at 100CH showed the lowest values and inhibited mycelia growth in 16.97% and 12.9% respectively, compared with controls. In addition, 6, 12 and 30CH were lower and statistically different from control DW but equal to HS. The medicines, *Silicea terra* (B), *Phosphorus* (D), *Ferrum sulphuricum* (E) and *Kali iodatum* (F) had similar behavior, showing values lower than the DW control at all dynamizations but statistically equal to HS.

Figure 3 shows the results of the average colony diameter of *A. solani* in the presence of DW and HS activated at 6, 12, 30, 60 and 100CH, compared with DW and 30% HS not activated.

Distilled water at 60 and 100CH inhibited 12.4 and 11.0% fungal mycelial growth different from the controls, while 6CH (8.7%) and 30CH (6.9%) were lower than non-activated DW but statistically identical to HS (Figure 3A). Due to these differences, the control group was considered a subgroup, i.e. treatment as well, since there was dynamization effect. Data suggest that water, when activated, behaves like a homeopathic medicine conferring specific properties not yet elucidated by science. With the HS, 60 and 100CH dynamizations were equal to the HS control to mycelial growth inhibition and

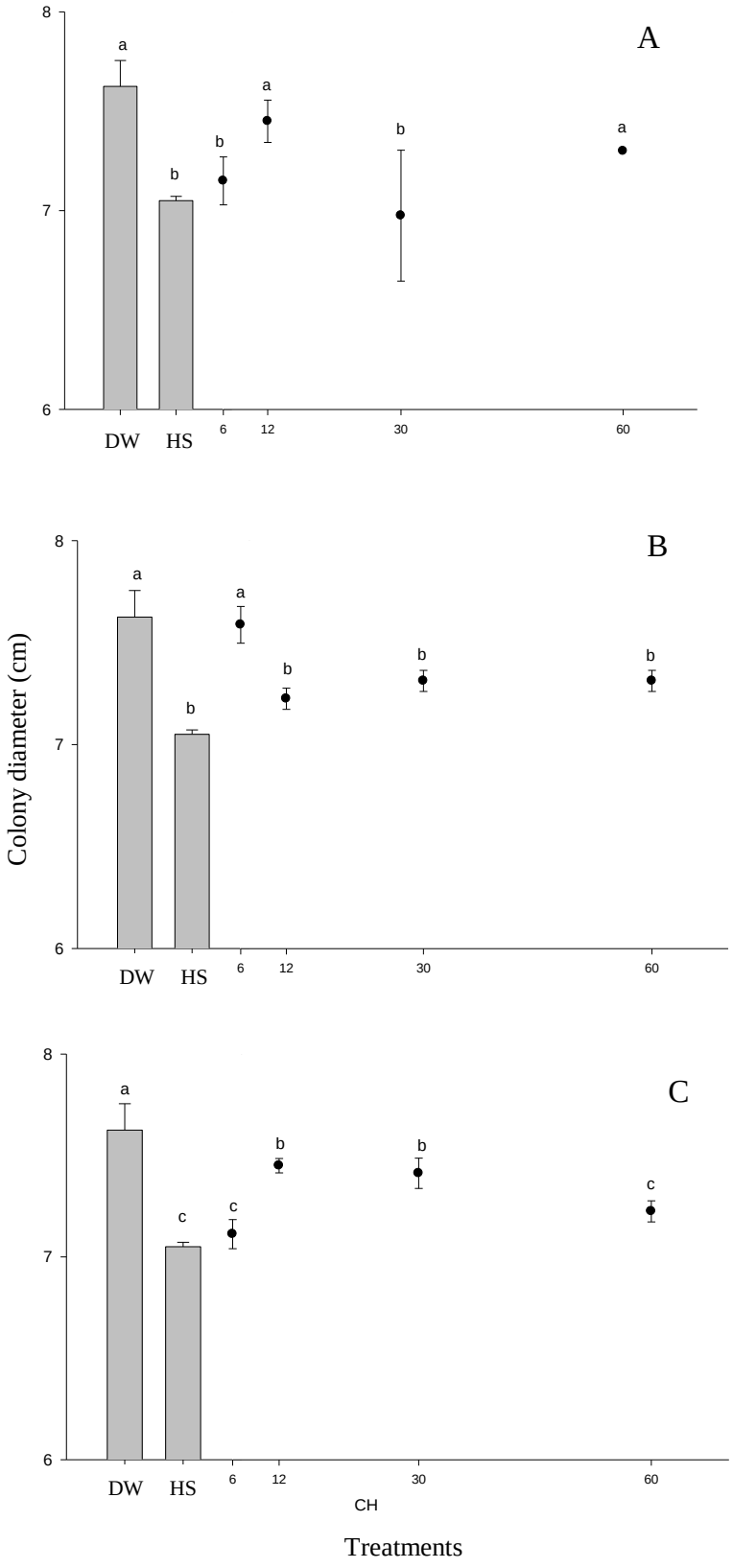

Figure 1. Effect of homeopathic medicines *Propolis* (A), IAS (B) and ICF (C) at 6, 12, 30 and 60CH on mycelial growth of *A. solani* compared with DW and HS. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 2.8%.

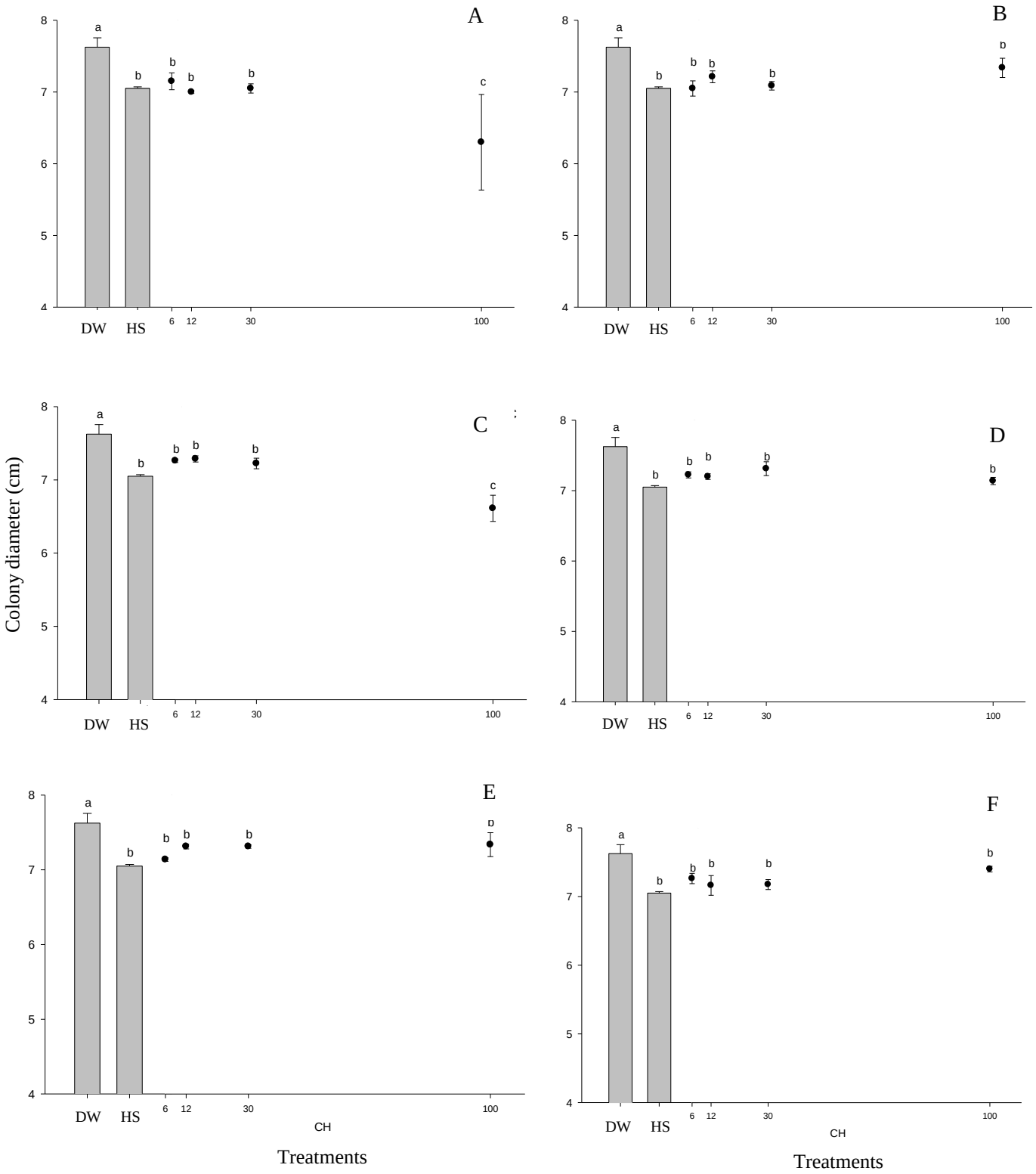

Figure 2. Effect of homeopathic medicines *Sulphur* (A), *Silicea terra* (B), *Staphysagria* (C), *Phosphorus* (D), *Ferrum Sulphuricum* (E) and *Kali iodatum* (F) at 6, 12, 30 and 100CH on the mycelial growth of *A. solani* compared with 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 3.76%.

different from DW. At 6, 12 and 30CH were higher than the SD but lower than the DW. Hydroalcoholic solution

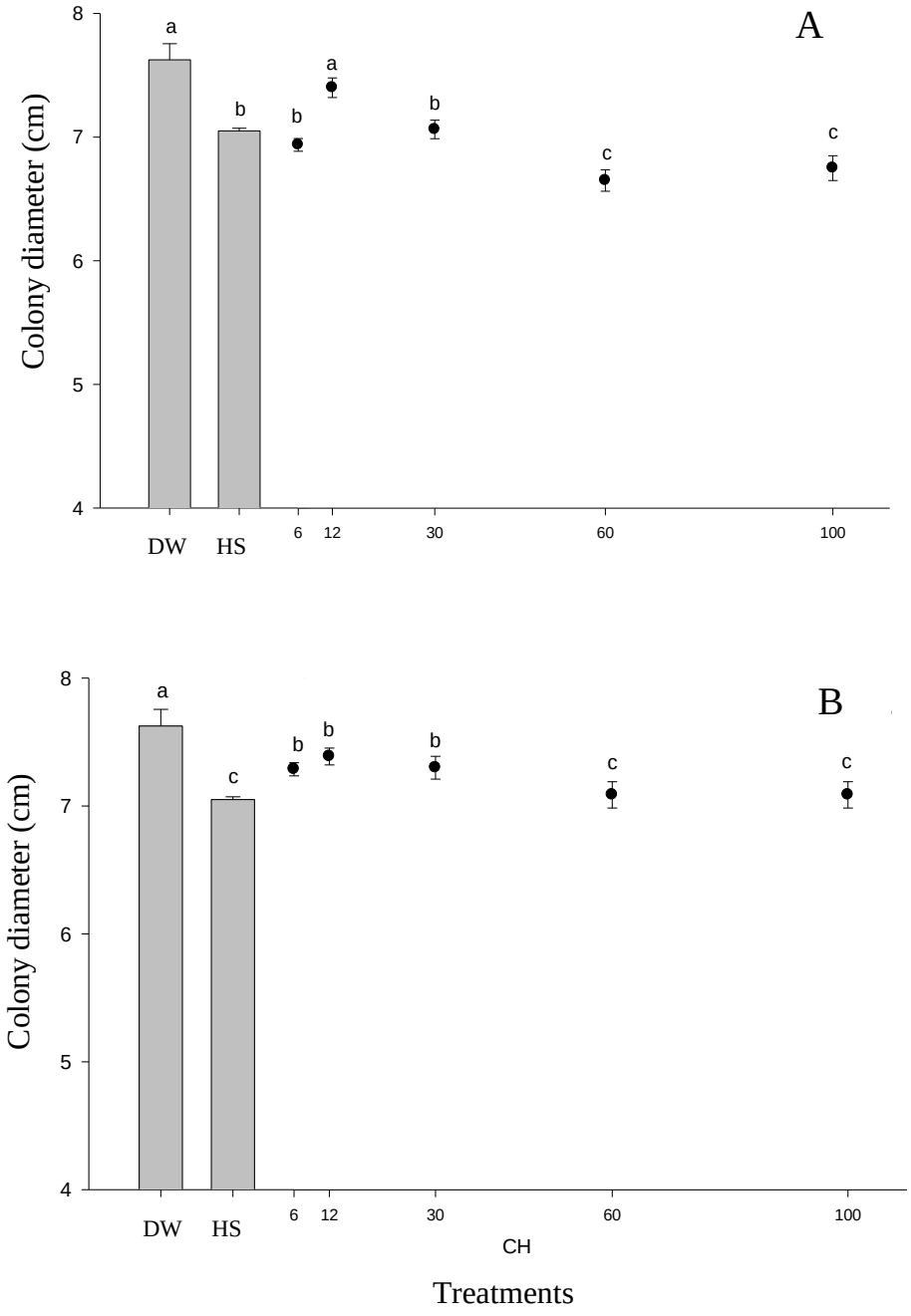

Figure 3. Effect DW (A) and HS (B) at 6, 12, 30, 60 and 100CH on the mycelial growth of *A. solani* compared with 30% HS and non-activated DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 2.56%.

inhibited 7.58% mycelial growth showing that alcohol had antifungal effect.

Spore formation inhibition

Figure 4 shows the results for sporulation inhibition of *A. solani* by homeopathic medicines *Propolis* (A), IAS (B) e ICF (C). *Propolis* at 6, 30 and 60CH had suppressive effect on sporulation (65.5, 58.5 and 52.1% respectively), whereas 12CH behaved equal to the 30% HS control but 20.0% lower that DW.

With IAS (B), 12, 30 and 60CH dynamizations did not differ statistically from hydroalcoholic solution, but inhibited sporulation in 32.90 and 52.52% compared to distilled water and with no effect at 6CH.

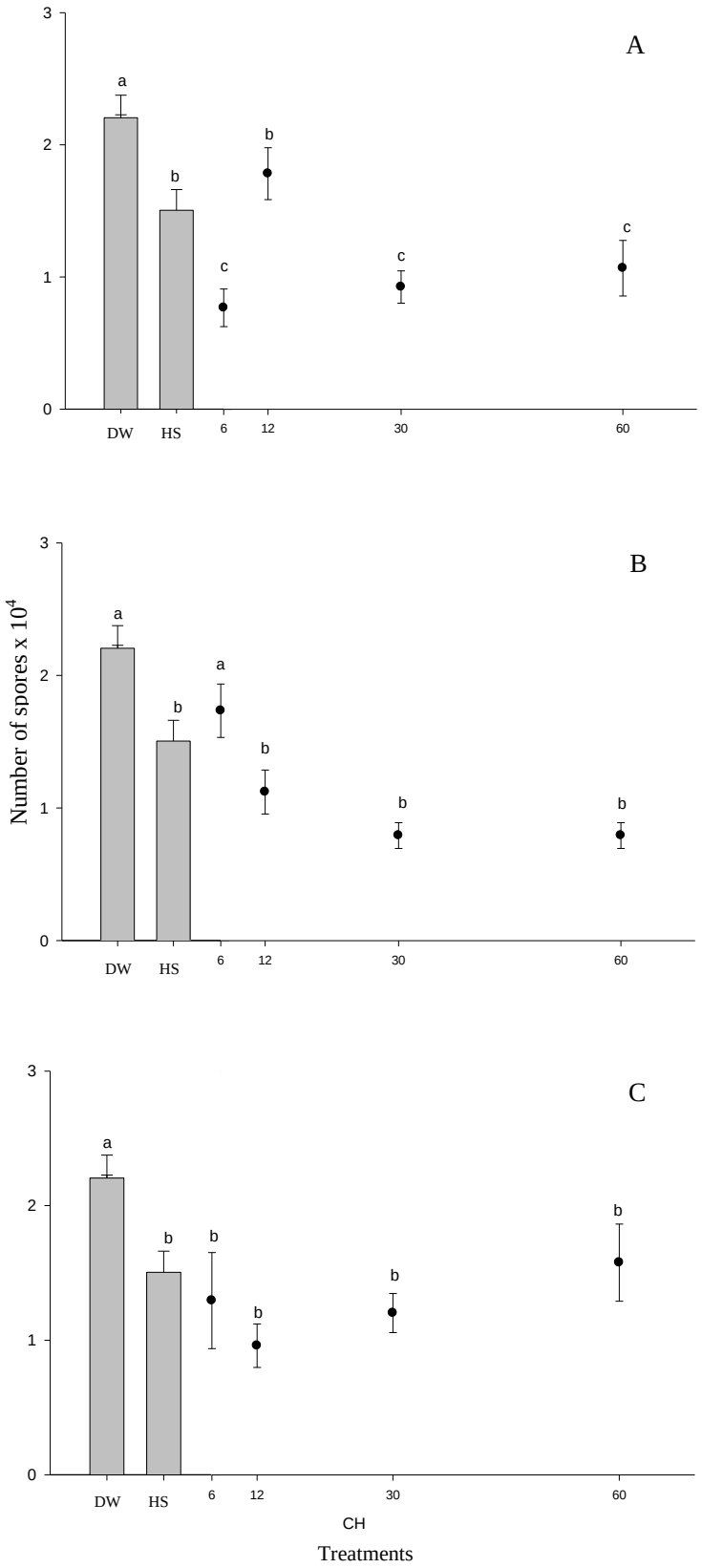

Figure 4. Effect of homeopathic medicines *Propolis* (A), IAS (B) and ICF (C) at 6, 12, 30 and 60CH on the sporulation of *A. solani,* compared with 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 8.47%. Transformed data (X +1.0) ^ 0.5.

All ICF (C) dynamizations were not different from HS control but different from DW. At 6, 12, 30 and 60CH sporulation was inhibited by 30.19, 56.9 and 46 and 29.2% respectively.

Results showed that isotherapics had no effect on sporulation compared with the control HS, but were effective when considering the control DW. This was also observed in the mycelial growth inhibition, indicating that there may be a sum of factors in the mixture of ethanol, water and medicine. *Propolis* at 6, 30 and 60CH were effective in inhibiting sporulation, since it had suppressive effect compared with both controls and presented as homeopathy with potential to control diseases in plants through the effect on the pathogen reproduction.

Figure 5 shows the results of the sporulation inhibition of *A. solani* by homeopathic medicines *Sulphur*, *Silicea terra*, *Staphysagria*, *Phosphorus*, *Ferrum sulphuricum* and *Kali iodatum* at 6, 12, 30 and 100CH.

Sporulation of *A. solani* was inhibited by *Ferrum sulphuricum* (E) in 45.0% and 30.2% at 6CH and 30CH, respectively compared with controls. At 12 and 100CH were equal to SH but lower in 36.36 and 21.86% than DW, respectively. Comparing 6CH with DW there is 63.1 and 53.1% inhibition at 30CH. These data confirm the results obtained with isotherapics in mycelial growth suppression, conferring a better result with the presence of ethanol plus medicine.

Other medicines, *Sulphur* (A), *Silicea terra* (B), *Staphysagria* (C), *Phosporus* (D) and *Kali iodatum* (F) were statistically different from distilled water but equal to 30% HS. *Staphysagria* at 6CH was the medicine that showed greatest suppression of sporulation (63.1%) compared to the DW. *Kali iodatum* (F) at 6CH increased sporulation compared with the control HS but was statistically equal to the DW (Figure 5).

Figure 6 shows the results of sporulation inhibition of *A. solani* by the effects of DW and HS at 6, 12, 30, 60 and 100CH compared with non-activated solutions.

The number of spores found in the presence of DW at 6 and 12CH were 56.6 and 36.1% lower than non-activated DW but equal to 30% HS. The dynamizations 30 and 60CH were different and higher than the HS and 100CH was 26.2% higher than the distilled water (Figure 6A). Results suggest that water, when activated, behaves as a homeopathic medicine, a phenomenon that also occurred in the mycelium growth, sometimes with a suppressive either promoter effect (100CH).

The number of spores with 30% HS at 12, 30, 60 and 100CH was 30.1, 51.6 and 30.2% lower than the DW and equal to it at 6CH. Data show significant effect of alcohol on sporulation of *A. solani* (Figure 6B).

Spore germination inhibition

Figure 7 shows the effect of homeopathic medicines *Propolis* (A), IAS (B) and ICF (C) at 6, 12, 30 and 60CH compared with controls DW and 30% HS on the spore germination of *A. solani.* Propolis did not differ from controls at tested dynamizations. For IAS (B) and ICF (C) there was 8.1 and 6.7% reduction on spore germination of *A. solani* at 6CH but other dynamizations were statistically identical to controls. The homeopathic medicines *Sulphur*, *Silicea terra*, *Staphysagria*, *Phosphorus* and *Kali iodatum* at all dynamizations studied had no effect on inhibiting spore germination of *A. solani* despite several dynamizations presenting lower averages than controls, especially *Sulphur* at 12 and 30CH (Figure 8).

Ferrum sulphuricum at 12 and 30CH suppressed the germination of *A. solani* at 4.6 and 3.10% compared with the control DW and 5.5 and 4.0% compared with HS. At 6 and 100CH were statistically equal to controls DW and 30% HS.

For spore germination of *A. solani*, there was no statistical difference for DW and HS compared among each other and with the controls (Figure 9).

DISCUSSION

Sinha and Singh (1983) studied the phytotoxic effect of several homeopathic medicines on *Aspergillus parasiticus* responsible for contamination in stored products and the toxin aflatoxin production. This study found that *Sulphur* at 200CH inhibited 100% fungal growth and *Silicea terra* and *Dulcamara* reduced the fungal growth by 50% and toxin production by more than 90%. *Phosphorus* had little effect on fungal growth inhibition (less than 10%) but decreased by almost 30% aflatoxin production.

In this study *Sulphur* at 100CH, *Staphysagria* at 100CH, DW at 60CH and 100CH reduced mycelial growth of *A. solani*, indicating thereby the potential in reducing injuries and development of black spot disease. This could be possible, since hemibiotrophic fungal diseases such as those caused by *A. solani*, have development based on the increased size of lesions, production of enzymes and toxins that cause death of host cells (Leite and Stangarlin, 2008).

In nature, pathogen's propagating structures are produced and disseminated to achieve a new site of infection, where they will infect, colonize and reproduce again. If the environment is favorable and there is host tissue available, multiple infectious cycles will be produced successively (Amorim, 1995). In this study several homeopathic medicines had no effect on reproduction of *A. solani* by inhibiting the inoculums production, which thus might act directly on its infectious cycles and reduce the rate of disease progression in a tomato tree- pathogen interaction.

The process of spore germination is one of the basic steps on the infectious process and directly linked to the pathogen-host relationships. The cycle of this relationship

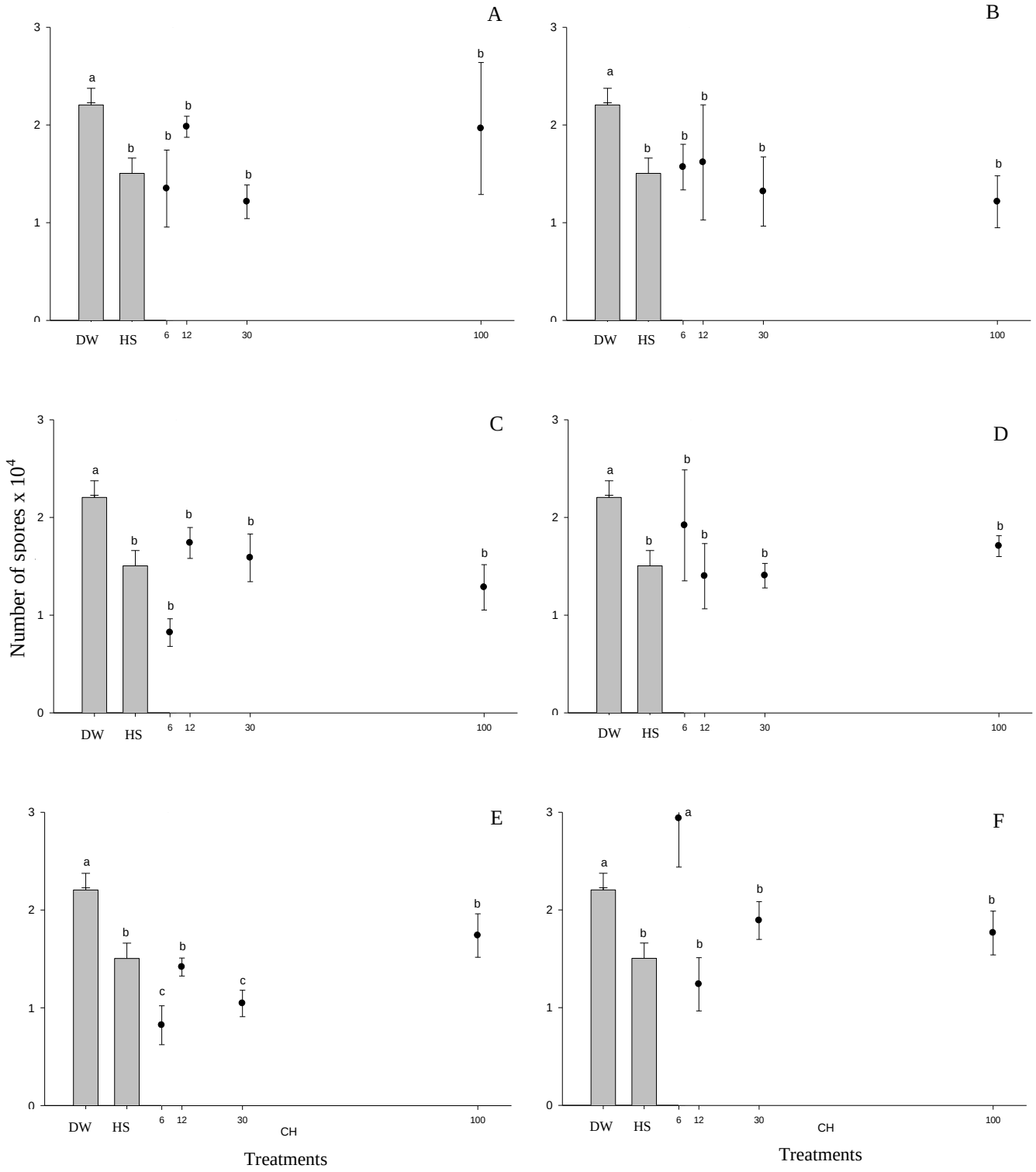

Figure 5. Effect of homeopathic medicines *Sulphur* (A), *Silicea terra* (B), *Staphysagria* (C), *Phosphorus* (D), *Ferrum sulphuricum* (E) and *Kali iodatum* (F) at 6, 12, 30 and 100CH on the sporulation of *A. solani* compared with 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV% = 10.36%. Transformed data (X +1.0) ^ 0.5.

according to Amorim (1995) consists of five basic sub-processes: survival, dissemination, infection, colonization and reproduction. The infection begins with phenomena linked to pre-penetration, adhesion and germination of

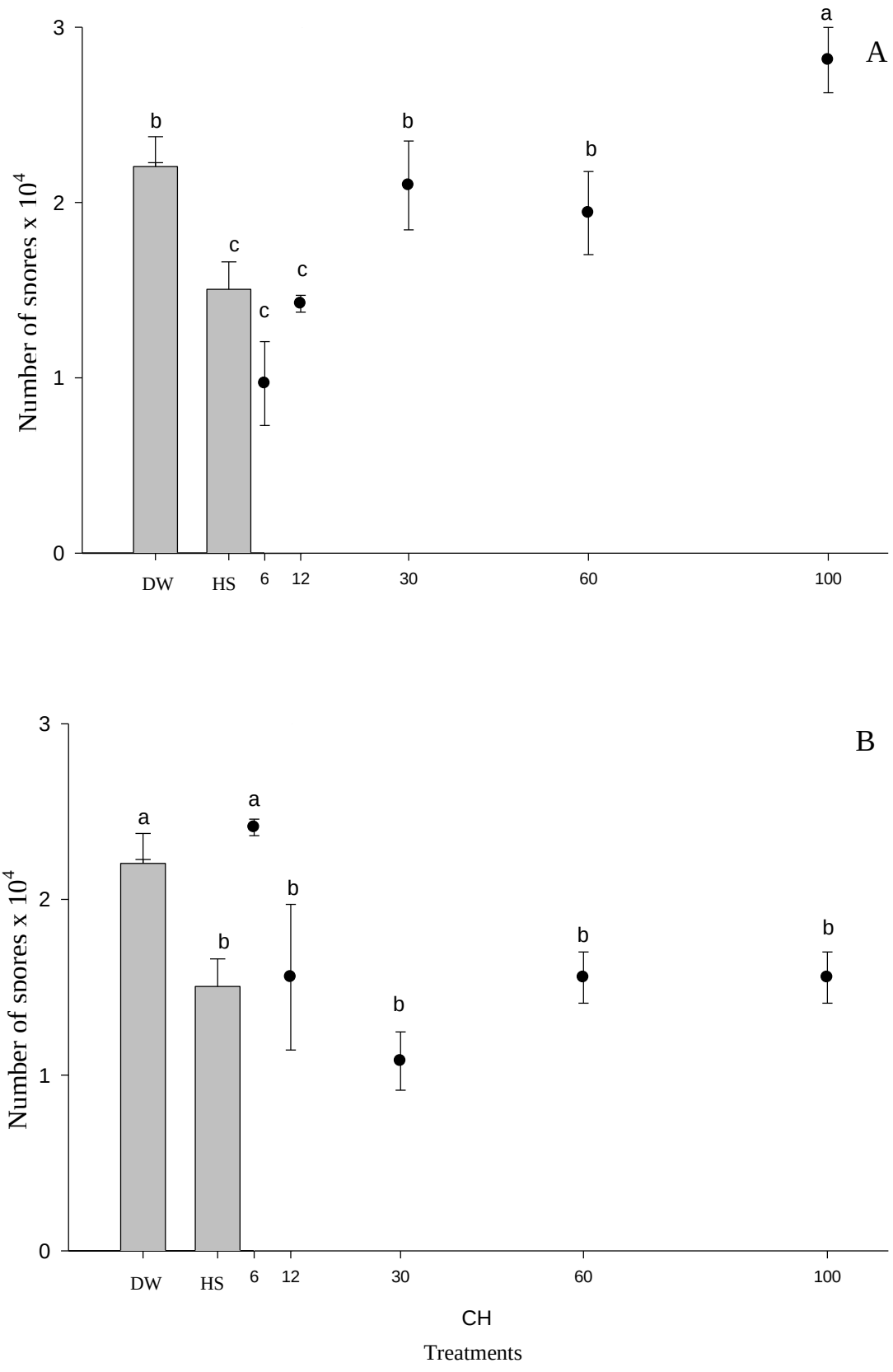

Figure 6. Effect of DW (A) and HS (B) at 6, 12, 30, 60 and 100CH on the sporulation of *A. solani* compared with non-activated 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 7.78%. Transformed data (X +1.0) ^ 0.5.

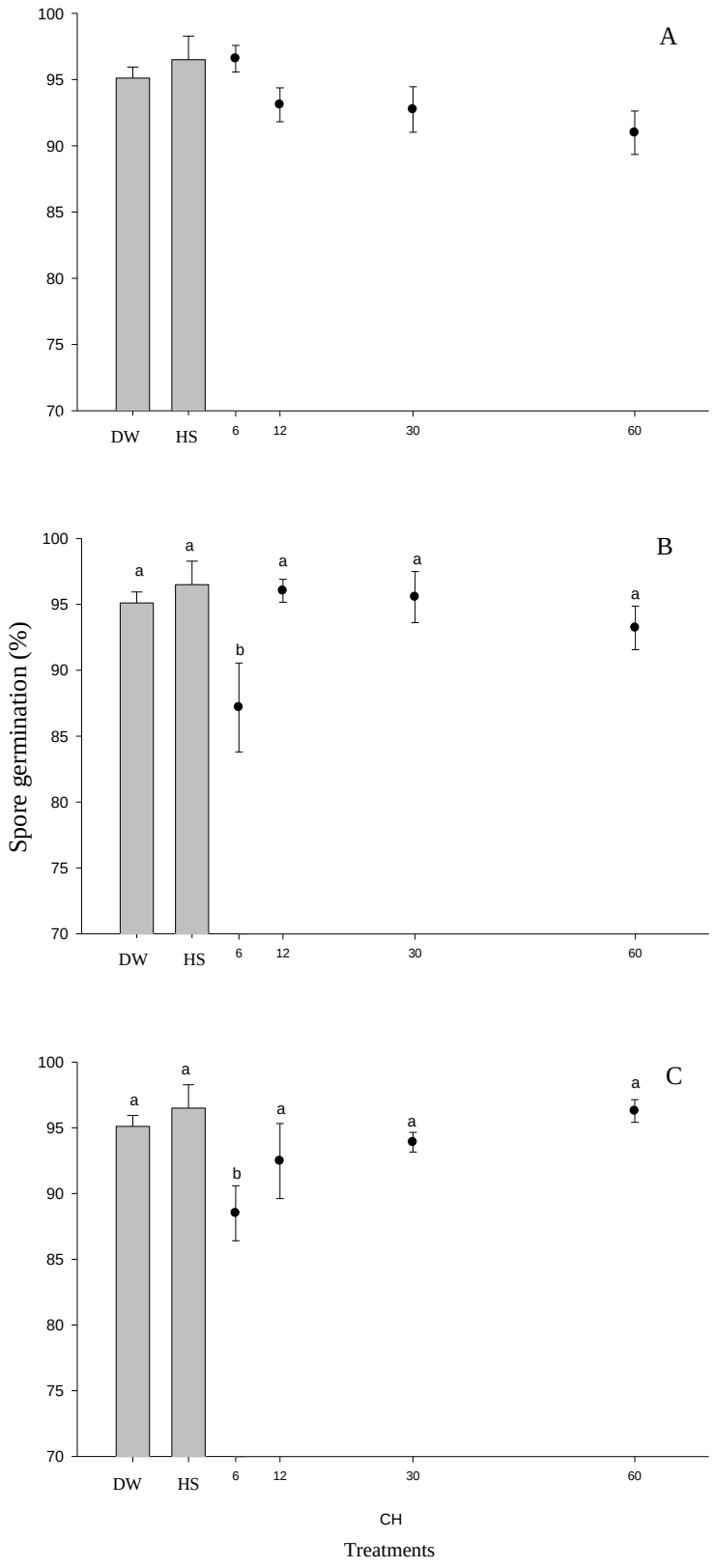

Figure 7. Effect of homeopathic medicines *Propolis* (A), IAS (B) and ICF (C) at 6, 12, 30 and 60CH on spore germination of *A. solani* compared with 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 3.93%.

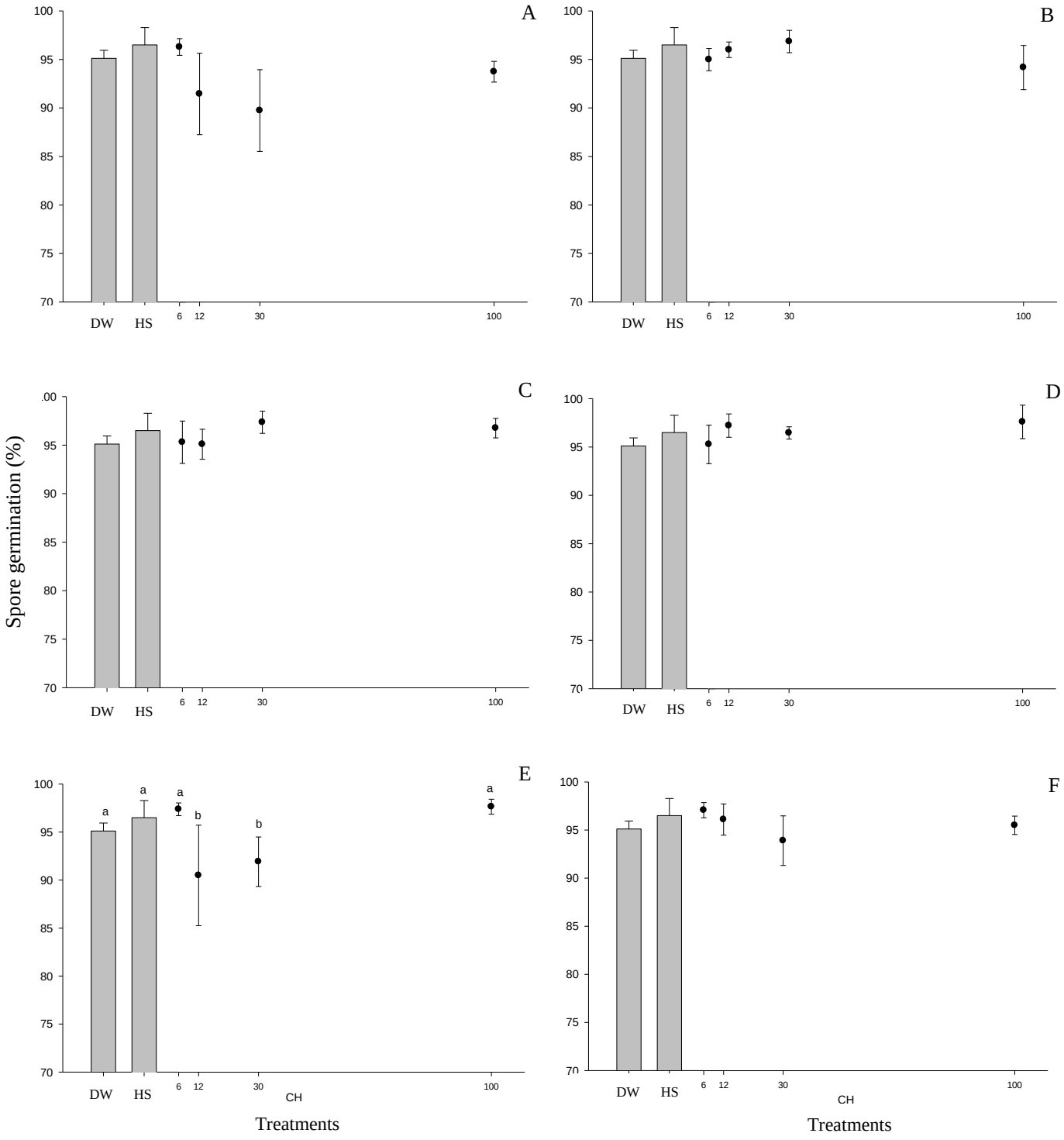

Figure 8. Effect of homeopathic medicines *Sulphur* (A), *Silicea terra* (B), *Staphysagria* (C), *Phosphorus* (D), *Ferrum sulphuricum* (E) and *Kali iodatum* (F) at 6, 12, 30 and 100CH on spore germination of *A. solani* compared with 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 4.37%.

spores and presents itself as a critical process, since it functions as a landmark for pathogenesis. Data from this trial showed that *Ferrum sulphuricum* may be a potential medicine for the control of black spot disease, working in reducing germination of spores and thus reducing the risk of pathogen penetration in the plant.

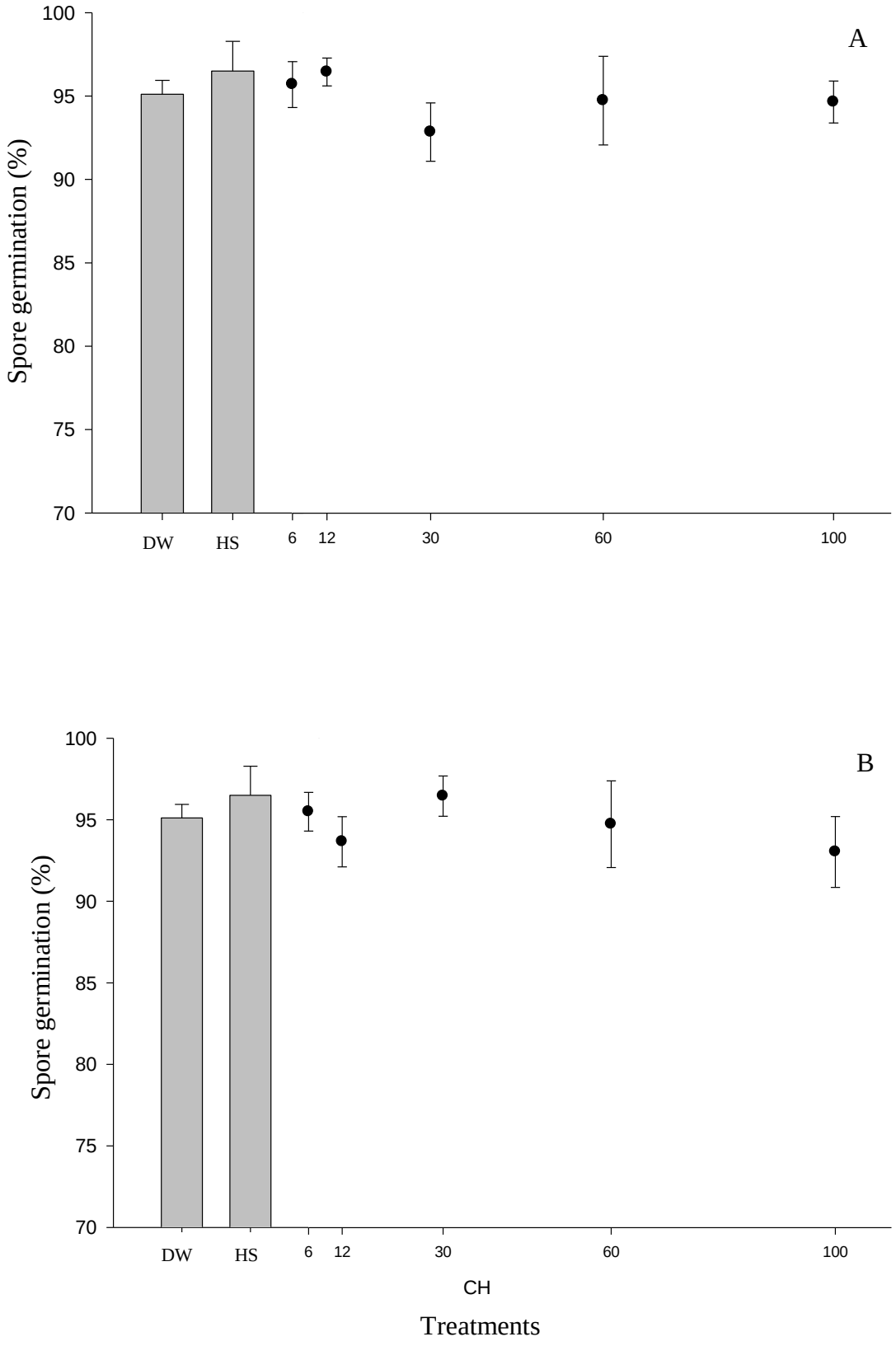

Figure 9. Effect of DW (A) and HS (B) at 6, 12, 30, 60 and 100CH on the spore germination of *A. solani* compared with non-activated 30% HS and DW. Bars represent + SD. Same letters do not differ by the Scott-Knott test ($p < 0.05$). CV = 3.45%.

Khanna and Chandra (1992) observed respiration suppression of the fungi *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium roseum* and*, Gloeosporium psidii* with various homeopathic medicines. Authors found correlation between the inhibitions of spores' germination with their respiration rate. In this study there was greater effect of homeopathic medicines analyzed on the sporulation of *A. solani*. This fact is important regarding the application method of these products in the field, since when there is the effect on the pathogen; results were more significant in the reproduction and thus, could interfere more effectively with the disease progress having little curative effect. In this sense, it is important to combine practical alternatives to control plant diseases aiming to add the effects of these products to have a more effective effect.

Although the coefficient of variation of the trials was low, there is a high standard deviation in the results, which demonstrates that data did not behave homogeneously. This fact leads us to speculate that other factors may interfere when using and researching with ultra high diluted solutions, since the control of environmental conditions is high in laboratory bioassays. Alcohol has effect on the homeopathic preparations, since it was significantly different from distilled water for mycelial growth and sporulation, demonstrating the importance of being used in the preparation of homeopathic medicines. There were also major differences when comparing medicines with the control DW than with HS, indicating a sum of effects when mixing ethanol and medicine.

Several homeopathic medicines had negative effect, or equal to control alcohol. Bonato (2007) mentioned that some dynamizations enhanced the values of variables measured while others show a suppressive effect.

Results of the tests were arranged in figures per variable and study group, that is, homeopathic preparations, homeopathic medicines and controls for each variable. Due to the cyclical or sinusoidal behaviors in dynamizations, despite the attempt, it was not possible to fit equations for these variables studied. Kolisko and Kolisko (1978) were the first to study the plant response to gradual and successive dynamizations of several ultra-high diluted solutions. These authors found that by treating plants with increasing dynamizations of ultra-diluted and succussed preparations could provide standard curves, similar to electromagnetic waves. Responses in form of waves had several peaks of maximum and minimum. Thus, responses could be higher or lower than the control even having no effect. Such cyclical behaviors may reflect the internal dynamics of the substance of which is activating and its similarity to the plant organism studied.

According to Bonato (2007), the plant could be a model of dynamic biorhythms of the substance to be used and they cited that this behavior has been observed in almost all previous studies. The author emphasizes that works on the area prove the results obtained by Kolisko and Kolisko (1978), which depending on the homeopathic medicine and studied plant, responses in the form of wave can be horizontal, upward or downward, but always in the wave form. These responses are still a mystery to researchers.

Silva (2006) investigated the effects of ultra-dilutions of *Apis mellifica* on the liquid photosynthesis in *Sphagneticola trilobata* (L.) Pruski and fit linear, quadratic and cubic equations but worked with 1CH to 12CH dynamizations, unlike the subject of this work which prioritized dynamizations more widely spaced (6, 12, 30, 60 and 100CH) and thus it was considered inappropriate to extrapolate data in case equations were adjusted. Homeopathic medicines have fungitoxic action against *A. solani*, which was dependent on the nature of the medicine and dynamization used, as *Propolis* for mycelial growth, *isotherapics* from pathogen and tomato leaf for spore germination, *Ferrum sulphuricum* for sporulation and spore germination and *Sulphur and Staphysagria* for mycelial growth.

Although none of commercial pesticides were used as a pattern for comparing the effectiveness of homeopathic drugs in this work, in the literature is possible to get some data showing the amount of *A. solani* inhibition by chemicals. Balbi-Peña et al. (2006) verified that curcumin at 400 mg L^{-1} inhibited about 25% the mycelial growth of *A. solani*. Franzener et al. (2007) found 20% of mycelial growth inhibition of *Alternaria brassicae* using azoxystrobin 80 mg L^{-1}. These values of inhibition are similar that one found in this work when we use high diluted solutions of the homeopathic drugs *Sulphur* and *Staphysagria* 100CH, with almost 20% of inhibition. Thus, our results showed the potential of homeopathic drugs for controlling *A. solani* in organic tomato production.

Conflict of Interests

The authors have not declared any conflict of interests.

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