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EVALUATION OF BIOTHERAPICS FOR THE CONTROL OF PLANT DISEASES

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ABSTRACT: Beans and tomatoes are crops of great importance in Brazil, and particularly in the state of Paraná. Bean angular leaf spot and tomato septoria are important diseases in these crops. Agroecological-based crops are gaining relevance in different parts of the world, and have been encouraged in the state of Paraná. Homeopathic science has been used as a tool for this type of agriculture. Biotherapics are produced according to homeopathic pharmacotechnique, but they do not follow the same healing principles as homeopathy. In this work, the control of bean angular leaf spot and tomato septoriosis was evaluated through two biotherapics, called Pse02 (*Pseudocercospora griseola* 02) and Sep01 (*Septoria lycopersici* 01), produced respectively with the bean pathogen and with diseased tomato leaves. Two experiments were conducted with each biotherapic in a completely randomized design in greenhouse. 0DH, 12DH, 19DH, 20DH, 21DH, 24DH, 30DH dynamizations of biotherapic Pse02 and 0CH, 9CH, 12CH, 15CH, 17CH, 19CH dynamizations of biotherapic Sep01 were sprayed in plant shoots and irrigated in the soil on four different dates. In only one experiment of each biotherapic, there was significant effect of dynamization in relation to control. Biotherapics Pse02 in 19DH dynamization, and Sep01 in 15CH dynamization promoted significant reduction in the size of the angular leaf spot lesion in bean as well as in the severity and number of septoria lesions in tomato in relation to control, respectively.

KEYWORDS: angular leaf spot, *Pseudocercospora griseola*, beans, septoriosis, *Septoria lycopersici*, tomato, homeopathy.

AVALIAÇÃO DE BIOTERÁPICOS PARA O CONTROLE DE DOENÇAS DE PLANTAS

RESUMO: O feijão e o tomate são culturas de grande relevância no Brasil, e particularmente no estado do Paraná. A mancha angular do feijão e a septoriose do tomate são doenças importantes nestas culturas. Os cultivos de base agroecológica estão ganhando relevância em diferentes partes do mundo, e vêm sendo estimulados no estado do Paraná. A ciência homeopática tem sido divulgada como uma ferramenta para este tipo de agricultura. Os bioterápicos são produzidos segundo a farmacotécnica homeopática, mas não seguem os mesmos princípios de cura da homeopatia. Neste trabalho avaliou-se o controle da mancha angular do feijão e da septoriose do tomate através de dois bioterápicos, denominados Pse02 (*Pseudocercospora griseola* 02) e Sep01 (*Septoria lycopersici* 01), produzidos respectivamente com o patógeno do feijão e com folhas doentes de tomate. Foram conduzidos dois experimentos com cada bioterápico em delineamento inteiramente casualizado em casa de vegetação. As dinamizações 0DH, 12DH, 19DH, 20DH, 21DH, 24DH, 30DH do bioterápico Pse02 e as dinamizações 0CH, 9CH, 12CH, 15CH, 17CH, 19CH do bioterápico Sep01 foram pulverizadas na parte aérea e irrigadas no solo em quatro datas diferentes. Em apenas um experimento de cada bioterápico houve efeito significativo de uma dinamização em relação ao controle. Os bioterápicos Pse02 na dinamização 19DH, e Sep01 na dinamização 15CH promoveram redução significativa no tamanho da lesão de mancha angular bem como na severidade e número de lesões de septoriose do tomate em relação ao controle, respectivamente.

PALAVRAS CHAVE: mancha angular, *Pseudocercospora griseola*, feijão, septoriose, *Septoria lycopersici*, tomate, homeopatia.

INTRODUCTION

The state of Paraná is one of the largest bean producers (*Phaseolus vulgaris*) in the country. In the three 2017/18 harvests, it produced 613,000 tons of beans, in a cultivated area of around 408,000 hectares (Salvador, 2018a). Angular leaf spot, caused by the fungus *Pseudocercospora griseola*, is distributed in all bean producing regions in Paraná, and is considered one of the main crop diseases (Bianchini et al., 2005).

Tomato is another important crop in the state of Paraná, in addition to being the second most consumed vegetable in Brazil. The northern region of the state is the largest producer, with 47% of production, followed by the southern region with 35% of production (Salvador, 2018b). Septoria or septoria leaf spot, caused by *Septoria lycopersici*, is an important tomato disease in the rainy season throughout Brazil, as it causes the progressive destruction of leaves, reducing the photosynthetic area and exposing fruits to sunburn (Reis et al., 2006).

Agroecology is a priority topic in research and extension activities of IDR-Paraná (IDR-Paraná, 2020). Normative Instruction No. 7 of MAPA, of 1999, introduced homeopathy as an input for use in organic/agroecological agriculture aiming at the control of diseases and pests. The homeopathic science was developed by the German physician Samuel Hahnemann, and is based on four pillars: the principle of healing by similarity, experimentation of medicines on healthy individuals, use of dynamized medicines and prescription of simple substances (single medicine). Homeopathy was created based on the rigorous observation of the effect of drugs on the human body, using as a scientific methodology the cure through the similitude of symptoms and the experimentation of medicinal substances in healthy individuals (Teixeira, 2011).

On the other hand, biotherapics, isotherapics, auto-isotherapics and nosodes are used for isopathic or isotherapeutic treatment. The term isotherapeutic, derived from “equal therapy”, means treatment by the substance or agent that caused the disease or imbalance. Thus, the term isotherapeutic is used to designate the treatment that uses dynamized organic or inorganic substances, substances that are the cause or are associated with a certain imbalance. Biotherapics are produced using homeopathic pharmacy techniques, and are a practical way for agroecological farmers to intervene in plant health (Carneiro, 2011a).

Treatment with isotherapics has been used to reduce the toxic effect of substances on plants (Jaeger et al., 2011) and to control diseases and pests. In a review of the use of homeopathy in agriculture, Carneiro et al. (2011b) observed that thirty percent of articles analyzed used at least one isotherapic treatment produced from pathogens, pests, among other products. Effects of biotherapics have already been detected in reducing leaf area consumption by *Cerotoma tingomarianus* (Fazolin et al., 1997), in reducing damage by *Epilachna varivestis* (Hernández et al., 2017), and in increasing germination of normal bean seedlings (Pinheiro et al., 2019). On the other hand, Trebbi et al. (2016) evaluated the effect of biotherapy made with cauliflower leaves infected with *Alternaria brassicicola* on disease control and did not observe significant effect on disease reduction using this form of treatment.

Considering the importance of organic agriculture in the state of Paraná, the aim of this work was to verify whether two biotherapics, produced from a pathogen or from diseased plants, are efficient, respectively, in the control of bean angular leaf spot and tomato septoria.

MATERIAL AND METHODS

The experiments were carried out in greenhouse at the experimental station of the `Instituto de Desenvolvimento Rural do Paraná` - IAPAR-EMATER, Londrina-PR.

Production of biotherapics

Biotherapics were produced at the Laboratory of Mycology of the Plant Protection Area of IDR-Paraná. *Pseudocercospora griseola* biotherapic, coded as Pse02, used as active input a *Pseudocercospora griseola* isolate grown in V-8 culture medium (Sartorato and Rava, 1994). The preparation followed the grinding protocol on the decimal scale by the Hahnemannian method, according to the Manual of Technical Norms for Homeopathic Pharmacy (ABFH, 2007). The starting point for the preparation of treatments applied to plants was the 9DH dynamization stored in 70% hydroalcoholic solution (w/w).

The biotherapic coded as Sep01 was produced by crushing the shoot of a tomato plant with symptoms caused by *Septoria lycopersici* to extract the juice from

leaves. The preparation of the biotherapic followed the grinding protocol on the centesimal scale by the Hahnemannian method, according to the Manual of Technical Norms for Homeopathic Pharmacy (ABFH, 2007). The starting point for the preparation of treatments was the 6CH dynamization, stored in 70% (w/w) hydroalcoholic solution.

Production of pathogen inoculum

The *Pseudocercospora griseola* inoculum used in the experiment was produced using V-8 culture medium (Sartorato and Rava, 1994), with the addition of 500 ppm of streptomycin sulfate. Petri dishes with the fungus were incubated in B.O.D. at temperature of 24 ±1ºC, under conditions of absence of light, for 15 days. After this period, spores were suspended in sterilized distilled water with 0.01% Tween 80. The spore concentration for plant inoculation was adjusted to 1.5 x 104 conidia mL-1 with the aid of a Neubauer chamber.

The *Septoria lycopersici* inoculum was also produced in V-8 medium, and incubated under the same conditions as the previous one. The spore suspension for plant inoculation had spore concentration adjusted to 3 x 104 conidia.mL-1 with the aid of a Neubauer chamber.

Experiments with beans

The first test was carried out with the `Tangará` bean cultivar, which was sown in pots with capacity of 2 kg of soil, filled with ravine soil and sand in a 3:1 ratio. Treatments evaluated consisted of 0DH, 12DH, 19DH, 20DH, 21DH, 24DH, 30DH dynamizations of biotherapic Pse02 applied at concentration of 0.1%, in addition to control with distilled water. The hydroalcoholic solution without succussion was considered as the 0DH treatment, used as a diluent in the preparation of medicines. All stages of preparation of dynamizations in liquid form were carried out in the dynamizing equipment, model Denise 10-50, with 100 cycles.

Applications of biotherapic dynamizations were carried out 14, 15, 16 and 19 days after sowing (d.a.s.), when the central leaflet of the first trifoliate leaf had 3 cm in width. The spraying of treatments was carried out with the aid of a manual sprayer, on the lower and upper sides of leaves, up to the point of beginning of runoff. Then, 30 mL of the treatment were applied to the soil around plants. After the last application of treatments, plants were inoculated with *Pseudocercospora griseola* spore suspension and kept for 48 hours in a fog chamber, at temperature of 24ºC and relative humidity of approximately 90%, with regime of 24 hours in the dark and 12 hours of light plus 12 hours of dark. Subsequently, plants remained in greenhouse in a completely randomized experimental design, with four pots and two plants per pot. Symptom assessments occurred 17 days after inoculation, by counting the number of angular leaf spot lesions and estimating the size of lesions using a scale (Bassanezi, 1995).

The second experiment was carried out with the `Carioca` bean cultivar under the same conditions as in the previous experiment, with the difference that treatments were applied at 21, 24, 25 and 26 d.a.s. The test was carried out with five replicates of two plants per pot, and the evaluation of symptoms occurred 15 days after inoculation, using the same criteria already described.

Experiments with tomato

Treatments evaluated consisted of 0CH, 9CH, 12CH, 15CH, 17CH, 19CH dynamizations of biotherapic Sep01 applied at concentration of 0.1%, in addition to control with distilled water. The hydroalcoholic solution without succussion, used as diluent in the preparation of medicines, was considered as the 0CH treatment. Treatments were applied by spraying leaves and then 30 mL were applied to the soil around the plant. In both tests, `Cereja` tomato cultivar was used, which was sown in pot with capacity of 2 kg of soil, filled with ravine soil and sand in a 3:1 ratio.

The applications of biotherapic dynamizations occurred when plants had between 3 and 6 leaves, at 28, 30, 35 and 37 days after sowing (d.a.s.) in the first test and at 26, 28, 33 and 35 d.a.s. in the second test. After the last application of treatments, plants were inoculated with pathogen spore suspension and remained for 48 hours in fog chamber at temperature of 24° ±1°C and minimum relative humidity of 90%. Then, plants were taken to the greenhouse in a completely randomized design with six replicates with one plant per pot. The disease was evaluated on two leaves of each plant, sixteen days after inoculation, by counting the number of lesions on the leaf and estimating the severity using a diagrammatic scale adapted from Boff (1991).

Statistical analysis of data from all tests was performed using the SISVAR statistical software (Ferreira, 2011), and means were compared using the 5% LSD test.

RESULTS AND DISCUSSION

In the first experiment with biotherapic Pse02 conducted with the `Tangará` bean cultivar, the F test of the analysis of variance was not significant at 5% of probability, and therefore, no treatment differed from control, both for variable lesion size and number of angular spot lesions (Table 1).

Table 1. Effect of biotherapic Pse02 from *Pseudocercospora griseola* on `Tangara` bean angular leaf spot.

Treatment	Lesion size (cm^2)	Number of lesions
Control	1.42 n.s.	3.97 n.s.
0DH	1.52	3.40
12DH	1.80	4.46
19DH	1.55	4.37
20DH	1.61	4.96
21DH	1.27	4.30
24DH	1.74	6.40
30DH	1.68	3.14
Mean	1.57	4.37
c.v.	47%	58%

n.s.: not significant

In the second experiment with biotherapic Pse02, conducted with `Carioca` cultivar, the F test was significant (<0.05) for lesion size, but not for number of lesions (Table 2). In the comparison of means test, the 19DH dynamization significantly reduced the size of lesions in relation to control.

Table 2. Effect of biotherapic Pse02 from *Pseudocercospora griseola* on `Carioca` bean angular leaf spot.

Treatment	Lesion size (cm^2)	Number of lesions
Control	2.73 a*	14.35 n.s.
0DH	2.87 a	16.87
12DH	2.96 a	17.33
19DH	2.03 b	15.47
20DH	2.43 ab	16.36
21DH	2.40 ab	13.37
24DH	2.73 a	11.75
30DH	2.90 a	13.50
Mean	2.63	14.8
c.v.	24%	35%

* Means followed by different letters indicate significant difference by the 5% LSD test. n.s. = not significant

The `Carioca` cultivar is more susceptible to angular leaf spot than `Tangará` cultivar, and this was demonstrated in experiments because the control treatment presented for variables lesion size and number of lesions, respectively, 1.4 and 3.9 for `Tangará` cultivar, and 2.7 and 14.3 for `Carioca` cultivar.

Regarding biotherapic Sep01 produced with diseased tomato leaves, in the first experiment (Table 3), the F test of the analysis of variance was significant, and the 15CH treatment differed significantly from control both for severity and number of lesions. Thus, 15CH dynamization of Sep01 reduced by about 50% the severity and number of lesions in the first experiment. In the second experiment with the same biotherapic (Table 4), no significant difference between treatments was observed.

Table 3. Effect of biotherapic Sep01 on tomato septoria.

Treatment	Lesion size (cm^2)	Number of lesions
Control	3.1 a*	8.8 a*
0CH	2.6 ab	7.1 a
9CH	2.8 ab	8.1 a
12CH	2.5 ab	6.8 ab
15CH	1.6 b	3.7 b
17CH	3.6 a	8.9 a
19CH	2.9 a	8.4 a
Mean	2.7	7.4
c.v.	25%	26%

* Means followed by different letters indicate significant difference by the 5% LSD test.

Table 4. Effect of biotherapic Sep01 on tomato septoria.

Treatment	Severity (%)	Number of lesions
Control	2.5 n.s.	8.0 n.s.
0CH	2.6	8.5
9CH	2.4	6.4
12CH	3.3	9.4
15CH	2.1	7.1
17CH	2.8	8.5
19CH	2.5	7.7
Mean	2.6	7.9
c.v.	47%	51%

n.s.: not significant

Rossi et al. (2007) evaluated biotherapic produced from *Xanthomonas campestris* pv. *vesicatoria* in the control of bacterial spot on tomato and observed that the form of application had an effect on the result, and application via spraying in plant shoot was not efficient, but when applied via irrigation, the 24CH dynamization reduced the disease severity. In the present work, to avoid the interference of the form of application, spraying and irrigation were both used. Rissato et al. (2016) found that the biotherapic produced from *Sclerotinia sclerotiorum* in 24CH dynamization reduced by 100% the production of fungus sclerotia in culture medium.

On the other hand, Diniz et al. (2006) did not observe the effect of the biotherapic from tomato leaves with late blight on the disease control. Shah-Rossi et al. (2009) evaluated, among other treatments, nosode made from healthy *Arabdopsis thaliana* leaves and leaves infected with *Pseudomonas syringae* in reducing disease symptoms, but did not observe efficient disease control by the nosode. Lorenzetti et al. (2016) evaluated the effect of *Macrophomina phaseolina* biotherapic produced from the pathogen mycelium, and observed that it had no effect on the pathogen in culture medium or on the disease in plants in greenhouse.

Different products can be used to prepare biotherapics, which makes it difficult to compare results. Modolon et al. (2012) worked with biotherapic produced from healthy *Solanum lycopersicum* and *Solanum aculeatissimum* plants. The dynamizations evaluated significantly reduced septoria under greenhouse conditions, but not in the field.

Carneiro et al. (2010) observed variable results in experiments carried out to verify the effect of *Alternaria solani* biotherapic produced with the pathogen on tomato black spot. The dynamizations evaluated did not affect the germination of spores or the development of the fungus in the culture medium, but reduction of up to 72% in the disease severity in plants in greenhouse was observed. However, there was no significant effect of dynamizations evaluated in two of the four experiments reported. In addition to the starting point for biotherapic preparation, the preparation method, the dynamization scale and the evaluated dynamization itself, other factors may interfere with the control efficiency.

Some authors have studied the influence of factors linked to plant physiology on the result of treatments with substances dynamized by the homeopathic technique. Baumgartner et al. (2008) in a study carried out with peas, considered that the physiological state of seeds used in tests had an influence on the results obtained. In a study with *Lemna gibba*, the authors observed that the plant would need to be at a certain stage of development to respond to dynamized gibberellic acid (Majewsky et al., 2014). The species responded to gibberellic acid dynamizations in only one of the three experiments in the article, probably due to small changes in the plant developmental stage. Thus, the reproducibility of research experiments with substances prepared by the homeopathic technique requires careful attention aiming at uniformity among plants used and analysis of their physiological state.

Since the mechanism of action of substances dynamized by the homeopathic method is still unknown, all variables related to the organisms under study must be carefully controlled. Jaeger et al. (2015) analyzed the reproducibility of results in research with homeopathy and pointed out different experimental parameters that should be considered in research, from the way of preparing the homeopathic treatment to the physiological state of the treated organism. Furthermore, it is important to repeat the experiments, something ignored in many published articles.

The results reported by some authors suggest that the use of biotherapics can be a potential approach for the use of sustainable agriculture, even if they are not strictly considered “homeopathic medicines”, in view of not satisfying the four pillars of homeopathy, as they have not been submitted to pathogenetic experimentation, nor are they applied according to the similarity of signs/symptoms between those described for the medication and those observed in the diseased individual (Carneiro and Teixeira, 2018). However, in the present work, only the 19DH dynamizations of biotherapic Pse02 and 15CH of biotherapic Sep01 promoted significant reduction in relation to control in some analyzed variable. As this effect occurred in only one experiment of each biotherapic, evaluating other dynamizations of these biotherapics is considered important.

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