HOMEOPATHY IN ORGANIC CHIVE CULTIVATION: DIFFERENT CONCENTRATIONS OF NOSODE OF COLLETOTRICHUM SPP

HOMEOPATIA EM CULTIVO ORGÂNICO DE CEBOLINHA: DIFERENTES CONCENTRAÇÕES DE NOSÓDIO DE COLLETOTRICHUM SPP

HOMEOPATÍA EN EL CULTIVO ORGÁNICO DEL CEBOLLINO: DIFERENTES CONCENTRACIONES DE NOSODE DE COLLETOTRICHUM SPP

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ABSTRACT

Anthracnose is a fungal disease that affects several crops, including chives, and can cause losses of up to 100% of production in some regions. And in organic agriculture there is a growing search for alternative controls that enable the consumption of safe food. In this context, the objective of this work focused on verifying the influence of different nosode dilutions on the control of anthracnose, caused by *Colletotrichum spp.*, in organically grown chives. The delimitation adopted was randomized blocks. The experiment was conducted in a greenhouse on a property that has used an organic system since 2008. The chive plants of the "every year" cultivar received nosode sprays at strengths of 6CH, 12CH, 18CH, 24CH, 30CH and purified water as a control, twice a week, for 10 weeks. The active input of the nosodes were leaves with symptoms of the disease. All medicines applied were prepared on the property of the experimental area. The results indicated inefficiency of nosodes of *Colletotrichum spp.* in the control of anthracnose and yield in chives in organic production.

Keywords: *Allium fistulosum.* Organic Agriculture. Isotherapy. Anthracnose.

RESUMO

A antracnose é uma doença fúngica que acomete diversas culturas, incluindo a cebolinha, podendo causar perdas de até 100% da produção em algumas regiões. Na agricultura orgânica, é crescente a busca de controles alternativos que possibilitem o consumo de alimentos seguros. Nesse contexto, o objetivo deste trabalho foi avaliar a influência de diferentes diluições de nosódio no controle da antracnose, causada por *Colletotrichum* spp., em cebolinha de cultivo orgânico. O delineamento adotado foi o de blocos casualizados. O experimento foi conduzido em casa de vegetação, em uma propriedade que utiliza sistema orgânico desde 2008. As plantas de cebolinha da cultivar "todo ano" receberam pulverizações de nosódios nas potências 6CH, 12CH, 18CH, 24CH e 30CH e água purificada

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como testemunha, na frequência de 2 vezes por semana, durante 10 semanas. O insumo ativo dos nosódios foram folhas com sintomas da doença. Todos os medicamentos aplicados foram preparados na propriedade da área experimental. Os resultados indicaram ineficiência de nosódios de *Colletotrichum* spp. no controle da antracnose e na produtividade na cultura cebolinha em cultivo orgânico de produção.

Palavras-chave: *Allium fistulosum.* Agricultura Orgânica. Isoterapia. Antracnose.

RESUMEN

La antracnosis es una enfermedad fúngica que afecta a diversos cultivos, incluido el cebollino, y puede causar pérdidas de producción de hasta el 100% en algunas regiones. En la agricultura ecológica, existe una creciente búsqueda de controles alternativos que permitan el consumo seguro de alimentos. En este contexto, el objetivo de este estudio fue evaluar la influencia de diferentes diluciones de nosodes en el control de la antracnosis, causada por *Colletotrichum spp.*, en cebollino cultivado ecológicamente. Se adoptó un diseño de bloques completos al azar. El experimento se llevó a cabo en un invernadero en una finca que ha utilizado un sistema ecológico desde 2008. Se pulverizaron plantas de cebollino del cultivar "Todo Año" con nosodes a las potencias 6CH, 12CH, 18CH, 24CH y 30CH, con agua purificada como control, dos veces por semana durante 10 semanas. El ingrediente activo de los nosodes fueron las hojas que presentaban síntomas de la enfermedad. Todos los medicamentos aplicados se prepararon en la finca del área experimental. Los resultados indicaron la ineficacia de los nosodes de *Colletotrichum spp.* para controlar la antracnosis y mejorar la productividad del cebollino en producción orgánica.

Palabras clave: *Allium fistulosum*. Agricultura Orgánica. Isoterapia. Antracnosis.

1 INTRODUCTION

Anthracnose is a fungal disease that attacks several species of plants, including chives, impairing the quality and quantity of production. For this, it is necessary to use control methods that do not affect the environment and human health (SANTANA, 2015).

Chives are a spiced vegetable consumed in all Brazilian regions, produced by family farming, which contributes to the culinary identity and the generation of work and income. In addition, it has medicinal properties used in folk medicine for the treatment of respiratory and digestive diseases (CLEMENTE et al., 2001; DIAS et al., 2019; ZÁRATE, 2010).

The losses caused by anthracnose in the production of chives are capable of reaching 58% in spring and 66% in summer in Rio Branco, Acre (PINHEIRO, 2023), and may reach 100% of production in some regions (WORDELL FILHO; STADNIK, 2008).

The Amazon Region is classified as an area of humid tropical climate, offering ideal conditions for the pathogen to establish itself and present itself in a severe way in several crops (WEIR; DAMM, 2012; ROSSI, 2022). There is no registration at the Ministry of Agriculture, Livestock and Supply (MAPA) of fungicides for the chemical control of the fungus *Colletotrichum* spp. in the chive crop (AGROFIT, 2023), as it is a short-cycle crop and exploited on a small scale (SANTANA, 2015). However, the excessive use of agrochemicals can induce resistance on the part of pests and diseases, and can also harm the health of consumers, causing problems such as allergies, poisoning and even cancer (BORSOI; HATAGIMA, 2014; KOIFMAN, 2003).

The importance of organic cultivation of chives and studies with alternatives that can strengthen sustainable production systems in olericulture, respecting natural resources and protecting human health, is highlighted. In this context, homeopathy is an alternative that has been recurrently studied in agriculture, and, in addition to being accepted by the principles of organic production, it is a low-cost and easily accessible technology, since the farmer has the autonomy to prepare homeopathic medicines on his property.

Homeopathy became officially recommended in 1999 through Normative Instruction No. 007, on organic production in Brazil, by MAPA (BRASIL, 1999). Currently, Ordinance No. 52, of May 15, 2021, includes homeopathy as a treatment method in organic production (BRASIL, 2021).

In agroecology, nosodes or isotherapies are medicines produced from diseased plants or animals, to treat the disease itself, based on the principle of equality and elaborated according to Homeopathic Pharmacotechnics (CARNEIRO et al., 2011; BRAZIL, 2011).

Maute and Maute (2018) recommend using homeopathic medicines that combat specific causes and balance the vitality of the plant, stressing the need to test various dynamizations.

The objective of this study was to evaluate the influence of different nosodium dilutions on the control of anthracnose in chives grown in an organic production system.

2 MATERIAL AND METHODS

The experiment was carried out at the Seridó Ecological Site, in Rio Branco - AC, located on the José Ruy Lino branch, km 1.7, on the left bank of the Porto Acre road, km 5, at latitude 9º 53' 16" S and longitude of 67º 49' 11" W, with an altitude of 150 m. The experimental area has been cultivated with organic agriculture since 2008.

The driving period was from July 4, 2022 to October 5, 2022. The average temperature recorded in the region during the experiment was 27.2 °C, relative humidity of 67.8% (INMET, 2023). According to Köppen (1918), the climate of the region is hot and humid, of the Am type.

The design adopted was randomized blocks evaluating 20 plants (clumps) per treatment, with five treatments and four replications, at the spacing of 0.20 x 0.10 m between plants and at the density of 2 bulbs/hole.

The installation of the experiment was carried out in beds on the soil of an open-sided greenhouse. The beds were erected with a hoe at a height of 0.20 m incorporating organic compost (15 t $^{ha-1}$ – dry basis) as fertilization. White and black polyethylene *mulching* plastic was used as a ground cover, to prevent invasive plants and keep soil moisture uniform. Irrigation was performed by daily micro-sprinkling of 6 mm.

The species of chives used was *Allium fistulosum* cultivar "todo ano", previously cultivated on site, planted asexually after the seedlings were prepared from the removal of excess roots and harvesting of commercial leaves.

The morphological identification of *Colletotrichum gloeosporioides and C. capsici.* It was based on the size and shape of 50 conidia and 50 appressoria of each isolate, randomly chosen from colonies with 15 days of growth. For the morphological and morphometric characterization of the conidia, a suspension was prepared at a concentration of 105 conidia mL-1 for each isolate, and semi-permanent microscopy slides were prepared for observation under a Zeiss optical microscope, under a 40-fold objective. All laboratory analyses were performed at the Phytopathology Laboratory of the Federal University of Acre.

Following the instructions of the Brazilian Homeopathic Pharmacopoeia (BRASIL, 2011), the elaboration of the mother tincture is always the first step in the elaboration of the

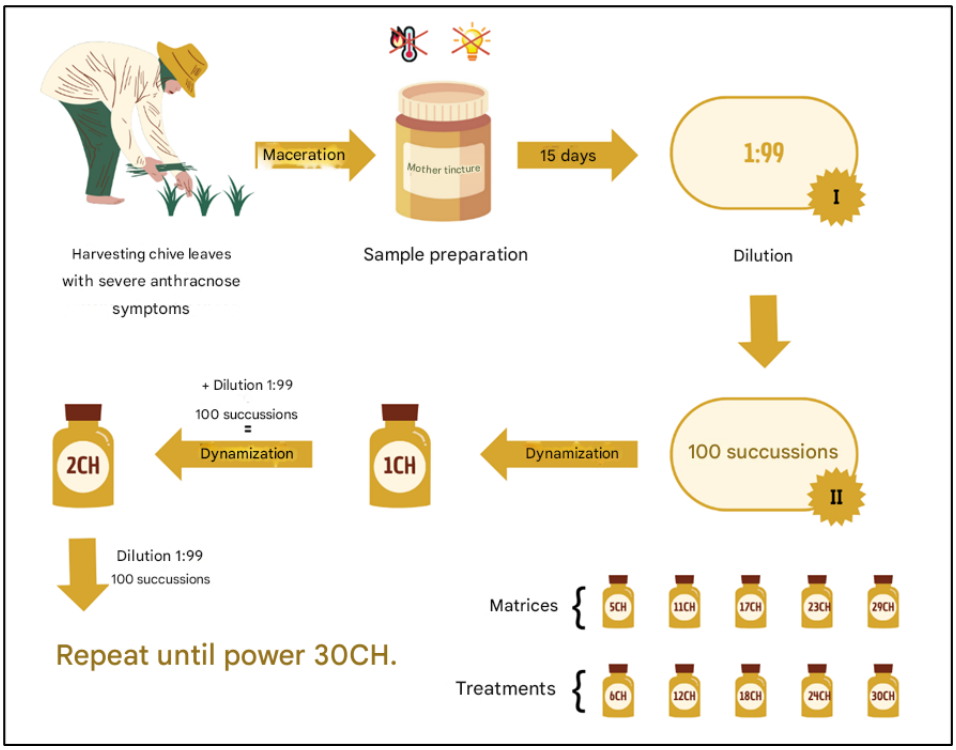
biotherapeutics. Then, chives with severe symptoms of *Colletotrichum* spp. were collected from the same area where samples were collected for the morphological identification of the fungi. Through the maceration process, the active ingredient was immersed for 15 days in its extracting liquid (70% ethanol), in a glass bottle wrapped with aluminum foil, stored in a thermal box at room temperature, protected from heat and light. During the maceration process, the container was shaken a few times. After 15 days, the extract was filtered and rested for 48 hours in a glass container. And then, the process of dynamization began.

The preparation of the homeopathic remedies was made from the filtered extract of the mother tincture, diluted in a 1:99 ratio, in 30% ethanol. Therefore, the scale adopted was the centesimal (1 part of active input to 99 parts of inert input). After dilution, the manual succussion process (vigorous and rhythmic vertical agitation of the forearm against the semi-rigid bulkhead) was initiated, thus obtaining the first Hanemanian centesimal order (1CH). This dynamization process was repeated up to the power of 30CH, always obeying the filling of 2/3 of the volume of each bottle.

The preparations used as treatments in the experiment were 6CH, 12CH, 18CH, 24CH, 30CH and purified water as a control. Therefore, the matrices stored in dark glass bottles deposited in a cooler at room temperature, from which the drugs for field use were made, were: 5CH, 11CH, 17CH, 23CH and 29CH, respectively. All homeopathic preparations used as treatment were dynamized on the day of their spraying, as recommended by the literature (BOFF, 2008) and illustrated in Figure 1.

Figure 1

Scheme of the elaboration of homeopathic preparations



Source: Authored by the author.

Twenty-two days after planting, the first application of the drugs was carried out, which was repeated until the end of the experiment, at a frequency of 2 times a week (MAUTE; MAUTE, 2018). On this day, the number of diseased leaves per plant was also quantified, which ranged from 1.70 to 2.10, so there was no difference in the initial severity between the treatments (Table 1 and Table 2). The initial infection occurred only by natural dissemination from adjacent plantations. The planting area has a history of anthracnose.

The evaluations began forty-three days after planting, and commercial and diseased leaves were counted. These were harvested manually near the base. The variables analyzed were the Fresh Commercial Leaf Mass (MFFC), weighing only the commercial leaves, the Number of Commercial Leaves (NFC) and the Number of Diseased Leaves (NFD). Fifty-two days after planting, fungi grown in the laboratory were inoculated to observe some increase in the severity of the disease. The inoculated species were *Colletotrichum gloesosporioides* and *C. capsici, at a concentration of 105* conidia per mL.

The MFFC was calculated by the sum of the fresh mass of commercial leaves in each harvest. Yield was estimated by the MFFC product multiplied by the planting density (50 plants/m2), expressed in g/m2. (the quotient of the fresh mass of commercial leaves by the number of leaves. Severity was considered the percentage of diseased leaves in relation to the number of total leaves.

The variables were submitted to the normality tests of the Shapiro-Wilk error (1965), homogeneity of variances of Bartlett (1937), followed by Analysis of Variance (ANOVA), with the aid of the SISVAR software version 5.6 (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

The centesimal concentrations of Hahnemann (CH) of nosodium at the powers CH6, CH12, CH 18, CH24 and CH30 did not affect the severity, the fresh mass of the commercial leaf, the yield and the technical indicators in comparison to the control, water (Table 1).

Table 1

Summary of the analysis of variance table, with the mean square and significance for the variables analyzed

Source of variation	GL	MFFC	PROD	Initial severity	Ultimate severity
Block	3	338.29ns	845926.2ns	26.5ns	5,069ns
Treatment	5	164.03ns	409779.3ns	10.1ns	5,179ns
Residue	15	178,47	446035,7	15,8	9,738
C.V.(%)		19,09	19,09	5,69	6,40

GL= degrees of freedom; MFFC= fresh commercial leaf pasta; PROD= productivity.
ns = not significant by the F test at 5% probability of error.
Source: Authors.

The fresh mass of commercial leaves per plant ranged from 61.6 to 78.6 g/plant, with no difference between concentrations. The average yield of commercial leaves was 3,498.2 g/m², with an average loss of 48.8% (Table 2). Even with the loss, the productivity achieved was higher than that researched by Simões et al. (2016), who reported commercial productivity of 2,667.1g/m². According to Souza et al. (2015), this productivity is economically viable, and may exceed R$23.00/m² in unupdated values.

Table 2

Fresh mass and yield of commercial fresh leaves and anthracnose severity in chives treated with own nosodium in different Hahnemann centesimals in an organic production system

Treatment	Fresh commercial leaf mass (g/plant)	Commercial sheet yield (g/m²)	Initial severity (%)	Final Severity (%)
Water	67,5	3.375,0	66,7	49,3
CH6	76,4	3.460,0	70,3	48,1
CH12	76,4	3.820,0	70,8	48,6
CH18	66,5	3.325,0	70,9	49,1
CH24	78,6	3.930,0	70,6	50,5
CH30	61,6	3.079,2	69,7	47,2
Average	71,2	3.498,2	69,8	48,8

Source: Authors.

The initial severity was high (69.8% of diseased leaves), this phenomenon occurred as a result of the lack of cleaning of diseased leaves, ensuring intentional initial contamination. The reduction to 48.8% occurred due to the reduction of the source of inoculum, with the cleaning of diseased leaves weekly and the reduction of leaf density by commercial harvesting (host reduction), an important culture method to help control phytopathological diseases.

In Brazil, there are still no published studies that specifically quantify the efficacy of nosodes (isotherapeutics) in vegetables, however Carneiro et al. (2011b) reference 20 scientific articles with isotherapy in vegetables, which in 20% of these the results did not differ between the treatments and the control. It is necessary to carry out literature reviews that analyze scientific works on isotherapy, evaluating not only the results, but also the rigor of the scientific methodology and the detailed description for later repetition.

In view of the results, three factors should be considered: climatic conditions favorable to the disease and unfavorable to plants; possible inefficiency of nosodium as a control of anthracnose in chives; and multifactorial conditions to which field experiments are subjected.

Despite the good adaptation to the temperatures of the city of Rio Branco, the climatic conditions are far from meeting the ideal recommended meteorological indicators. Analyzing that the average temperature of the region during the experiment was 27.2 ºC (INMET, 2023) and the range recommended by Filgueira (2008) as ideal for chives is the range of 8 ºC to 22 ºC, a possible stress for the plant should be considered. Pillar (1995) explains the adaptive value to high temperatures, stating that there are energy expenditures with increased perspiration and tissue production for the roots. This implies the breakdown of homeostasis treated in homeopathy and hinders the induction of resistance to the disease, intended in this work. On the other hand, high temperatures are one of the abiotic factors that favor the pathogen, along with the high air humidity, characteristic of the Amazon.

Other studies using nosode have shown similar results of inefficiency in the control of diseases in vegetables, as reported by Ferreira et al. (2009), who applied nosodium of *Fusarium subglutinans* (12CH) and homeopathy of *Ocimum gratissimun* (alfava-cravo 12CH) to control fusarium in pineapple. However, there was no significant reduction in the incidence of the disease and the homeopathic treatment with alfava-rangpur resulted in symptoms of apex curvature in the leaves. Rauber et al. (2007), who used 60CH late light nosode, also did not obtain significant results in controlling the disease in organic potato plants.

On the other hand, Rossi et al. (2004) reported a reduction in the severity of *Xanthomonas campestres* disease in tomato plants, applying nosodium to the 4CH and CH6 powers. While Erdmann (2008) evidenced the reduction of the incidence of rust in *Hypericum*

inodorum plants (stonewort) by applying the nosodium of the same disease at 30DH potency. Almeida et al. (2003) used *Spodoptera* sp. nosodium preparations in corn. (insect-pest) 30CH and homeopathic preparations in high dilutions of *Dorus* sp. (natural enemy of the caterpillar) 4CH and *Euchlaena* (botanical species similar to corn) 6CH, showing significant results in the control of the corn caterpillar. Although there are several studies reporting the positive effect of alternative treatments with nosode for the control of pests and diseases in plants, in some cases this may not occur, as is the case of the present work.

Analyzing that there are internal changes in the plant's metabolism that are not visible (BONATO, 2004) and that field experiments are subject to multifactorial conditions, it may not be possible to verify the subtle effects of the dynamizations of the isotherapeutics, as well as the potency and adequate dosage, requiring preliminary studies under partially controlled conditions (BOFF, 2005).

4 FINAL CONSIDERATIONS

New trials should be conducted to evaluate the effect of other nosode potencies and different application frequencies for the control of anthracnose in chives. As well as the use of agroecological techniques and organic system technologies associated with the use of other homeopathic medicines already evaluated as capable of inducing resistance and increasing productivity in crops, cited in the references of this work.

Another interesting alternative for future experiments is to evaluate the application of homeopathic preparations using raw milk as an active ingredient, since authors (ZATARIM, 2002; CAMPANHOLA; BETTIOL, 2003; CASALI, 2004b) report efficiency in the control of plant diseases. According to Campanhola and Bettiol (2003), the effect of dynamizations with raw milk is due to the fact that it contains several salts, amino acids and germicidal properties that can induce resistance to plant diseases. In addition to stimulating natural biological control and positively altering the physical, chemical and biological characteristics of the leaf surface, increasing the vitality of the plant.

The data from this work may be used for further studies in order to implement other dynamizations or doses at other frequencies.

Homeopathic research in agriculture should continue due to the importance of defining a well-structured Booklet of Homeopathy in Vegetables, in order to strengthen the organic production system and offer autonomy to the rural producer, with alternative medicines of low cost and easy access, thus contributing to the economy, protection of the environment, human health and food sovereignty.

5 CONCLUSION

The homeopathic preparations of *Colletotrichum* spp. nosodes dynamized on Hahnemann centesimal scales (CH) at the strengths CH6, CH12, CH 18, CH24 and CH30 were not efficient in the control of anthracnose in chives grown in an organic production system.

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