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Poultry litter composting and its effects on the occurrence of clubroot

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ABSTRACT

This study aimed to evaluate the effects of the application of poultry litter with different composting times on the occurrence of clubroot and the development of cauliflower plants. Plots inoculated with *Plasmodiophora brassicae* (*Pb*) and fertilized with non-composted poultry litter (PL-0 + *Pb*) and with poultry litter composted for 15 (PL-15 + *Pb*) and 30 days (PL-30 + *Pb*), were tested. As control treatments, we used inoculated plots without poultry litter (Control + *Pb*), non-inoculated plots (NI) and treated with composted poultry litter for 30 days (PL-30 - NI), and without poultry litter (Control - NI). The degree of stabilization and maturation of the poultry litter affected the severity of the clubroot, the volume and mass of healthy roots and galls, and shoot growth. The treatments PL-15 + *Pb* and PL-30 + *Pb* were equivalent to Control + *Pb* in terms of disease intensity. In the presence of the pathogen (*Pb*), the application of PL-15 resulted in the lowest volume and mass of healthy roots and shoot mass. The PL-0 treatment was equivalent to the non-inoculated treatments. Further studies are needed to elucidate the processes and interactions related to the effect of maturation of poultry litter composts on root infection and the occurrence of clubroot.

Keywords: *Brassica oleracea* var. *botrytis, Plasmodiophora brassicae,* Compost, Organic matter, Suppressiveness.

Compostagem de cama de aviário e seus efeitos na ocorrência da hérnia das crucíferas

RESUMO

Este trabalho objetivou avaliar os efeitos da aplicação de cama de aviário com diferentes tempos de compostagem sobre a ocorrência da hérnia das crucíferas e o desenvolvimento de plantas de couve-flor. Utilizaram-se como tratamentos parcelas inoculadas com *Plasmodiophora brassicae* (*Pb*) e adubadas com cama de aviário fresca (CA-0 + *Pb*) e com cama de aviário compostada por 15 (CA-15 + *Pb*) e por 30 dias (CA-30 + *Pb*). Como testemunhas utilizaram-se parcelas inoculadas e sem cama de aviário (Testemunha + *Pb*), parcelas não inoculadas (NI) e tratadas com cama de aviário compostada por 30 dias (CA-30 - NI), e sem cama de aviário (Testemunha - NI). O grau de estabilização e maturação da cama de aviário interferiu na severidade da hérnia das crucíferas, volume e massa de raízes sadias e das galhas e no crescimento da parte aérea. Os tratamentos CA-15 + *Pb* e CA-30 + *Pb* foram equivalentes a Testemunha + *Pb* quanto à intensidade da doença. Na presença do patógeno (*Pb*), a aplicação de CA-15 resultou em menor volume e massa de raízes sadias e menor massa de parte aérea. O tratamento CA-0 foi equivalente aos tratamentos não inoculados. Novos estudos são necessários para se elucidar os processos e interações relacionados ao efeito da maturidade de compostos de cama de aviário na infecção das raízes e ocorrência da hérnia das crucíferas.

Palavras-chave: *Brassica oleracea* var. *botrytis, Plasmodiophora brassicae,* Composto, Matéria orgânica, Supressividade.

The Brassicaceae (=Cruciferae) family comprises several species of vegetables important to Brazilian and world agriculture. Among them, there is *Brassica oleracea* L., widely cultivated worldwide and has different botanical varieties such as cauliflower (*Brassica oleracea* L. var. *botrytis*). In Brazil, the cultivation of these vegetables is commonly carried out in an intensified way in regions of higher altitude, such as mountain regions, due to the climatic requirements of these species (Filgueira, 2008; Bhering et al., 2017; Santos et al., 2017; Santos et al., 2022a).

The production of cauliflower and other brassicas presents challenges regarding phytosanitary management. The disease known as clubroot, caused by the soil-dwelling protozoan *Plasmodiophora brassicae* Woronin, is one of the main limitations to the production of *Brassica* spp. in Brazil and the world. Significant losses and unfeasible areas for cultivating brassicas have been frequent in the main producing regions. It is known that the disease is favored in acidic or partially acidic soil conditions (pH<6.2), humid, and with medium temperatures, 20 to 25 ºC (Gossen et al., 2014; Bhering et al., 2017; Santos et al., 2022a). However, information and knowledge about the effect of organic matter on disease development are scarce.

The addition of organic composts and fertilizers to the soil is mentioned in the literature as a factor related to the occurrence of clubroot; however, with variable results (Tilston et al., 2002; Niwa et al., 2007; Bhering et al., 2017; Condé et al., 2017; Santos et al., 2018; Santos et al., 2021; Santos et al., 2022b). Many factors are involved, such as the source and amount of organic matter, level of compost, chemical composition, initial soil characteristics, and time of application, among others.

Poultry litter is one of the organic fertilizers applied in vegetable crops in Brazil and the most used in the mountainous region of the state of Rio de Janeiro (Santos et al., 2022a, b). It is waste generated in large quantities in poultry production, rich in nutrients such as N, P, and K, and widely used in agriculture as an organic fertilizer (Bolan et al., 2010; Rogeri et al., 2016), mainly in vegetable production areas (Bhering et al., 2020; Santos et al., 2022a).

Recent field surveys were carried out by Bhering et al. (2017) and Bhering et al. (2020) in cauliflower production areas in Nova Friburgo, RJ, the largest cauliflower producing region in Brazil (IBGE, 2017). These studies recorded greater intensity of clubroot in soils with low pH and higher organic matter content (Bhering et al., 2017; Bhering et al., 2020). The authors associated this relationship with using high doses of fresh poultry litter, without going through any treatment or composting process, and with no soil liming. It is known that under these conditions, the mineralization of organic matter can contribute to the soil acidification process (Haynes and Mokolobate, 2001; Bhering et al., 2020).

Among the best-known techniques for adequately using the organic waste of animal origin is composting, which is the biological process of decomposing waste (Pergola et al., 2018). The degree of stability and maturity of organic composts are considered requirements for compost to be safely used in soil (Stoffella and Kahn, 2001). Little is known, however, about the effects of the application of poultry litter, in different degrees of compost, in the occurrence of clubroot. This study aimed to evaluate the effects of the application of poultry litter, with different composting times, on the occurrence of clubroot and the development of cauliflower plants.

The experiment was carried out in a greenhouse from July to September 2017 in Seropédica, RJ, Brazil. Six treatments were evaluated, four with inoculation with *P. brassicae* (*Pb*) being: 1) fresh poultry litter (PL-0 + *Pb*), that is, with 0 days of composting; 2) poultry litter composted for 15 days (PL-15 + *Pb*) and 3) composted for 30 days (PL-30 + *Pb*); 4) control without poultry litter (Control + *Pb*); and two non-inoculated treatments (NI): 5) poultry litter composted for 30 days (PL-30 - NI) and 6) without poultry litter (Control – NI). The partial characterization of the materials used was carried out by Souza et al. (2019) (Table 1). The N_{total} was determined in a LECO Trupec© CHN. $N_{mineral}$ was quantified according to Tedesco et al. (1995), while the $N_{organic}$ was obtained by the difference between both (Table 1).

Cauliflower plants, cultivar Verona (Seminis), were grown in 8 L pots containing sandy textured soil: $pH_{(water)}$=4.90; Ca = 2.00 $cmol_c$ dm^{-3}; Mg = 1.00 $cmol_c$ dm^{-3}; CEC = 19.6 $cmol_c$ dm^{-3}; K = 236.0 mg dm^{-3}; P = 56.0 mg dm^{-3} and V = 25%. Before transplanting, the respective composts were added and homogenized to the soil at a dose equal to 0.957 g pot^{-1}, on a dry basis (Bhering et al., 2017).

Table 1. Chemical attributes of fresh (PL-0 days) and poultry litter composted for 15 (PL-15 days) and 30 days (PL-30 days).

Composts	C	$N_{mineral}$	$N_{organic}$	P	K	Ca	Mg	pH	EC
	%			g kg^{-1}				(H_2O)	dS m^{-1}
PL-0 day	31.85	6.21	19.69	1.62	13.73	13.71	2.88	8.35	3.39
PL-15 days	29.20	3.85	17.63	10.70	19.66	20.32	3.38	8.69	2.92
PL-30 days	25.60	3.27	18.51	10.33	20.39	18.69	5.32	8.82	4.20

In addition, to each pot was added: 1.2 g of N, 2.8 g of P_2O_5, and 2.1 g of K_2O. Urea, simple superphosphate, and potassium chloride were used as N, P, and K sources, respectively. Two topdressing fertilizations with N and K were carried out 30 and 60 days after transplanting (DAT) using the same doses and sources. Control treatments received only mineral fertilization.

Inoculation was performed one day after transplanting with the addition of 50 mL of *P. brassicae* resistance spore suspension (2.3 x 10^6 spores mL^{-1} of water) per pot (Santos et al., 2018). The same amount of water was added to the control treatments. A randomized block design with six treatments and seven replications was adopted. At 90 DAT, the following variables were determined: number of leaves per plant; stem length (cm); and leaf and stem dry mass (g) after drying in an oven at 60 ^oC until constant weight. The shoot dry mass (g) was obtained by the sum of the respective parts.

The roots were carefully washed and evaluated for severity of clubroot using a scale of notes containing levels of 0%, 8%, 20%, 42%, 68%, 87%, and 95% of root galls (Santos et al., 2017). Subsequently, the volume and fresh mass of healthy fractions and galls were obtained (Santos et al., 2018). The data obtained were submitted to Analysis of Variance (ANOVA), and the means were compared by the Tukey test ($p \leq 0.05$) using the SISVAR software (Ferreira, 2011).

The temperatures recorded between 22 and 35 °C affected flowering, and the inflorescences were not evaluated. However, the vegetative development was normal and allowed the evaluation of roots and shoots and the intensity of the disease. There was a significant effect of treatments ($p \leq 0.05$) on all variables analyzed (Table 2).

In general, the plants in the treatments without the presence of the pathogen - poultry litter composted for 30 days (PL-30 - NI), and the control, as well as the inoculated and fertilized treatment with fresh poultry litter (PL-0 day + *Pb*), showed greater shoot development when compared to the other treatments. The plants treated with PL-15 days + *Pb* and Control + *Pb* showed lower shoot development, while those treated with PL-30 days + *Pb* showed intermediate development (Table 2).

In these last three treatments (PL-15 + *Pb*; Control + *Pb*; and PL-30 + *Pb*), greater disease intensity was registered, expressed by greater severity and greater volume and mass of galls. Regarding the volume and mass of healthy roots, the plants of treatments PL-30 + *Pb* showed intermediate values, while those of PL-15 + *Pb* and Control + *Pb* showed significantly lower values. The plants in the PL-0 + *Pb* treatment presented healthy root mass and volume and mass and volume of roots with galls equivalent to the values observed in the non-inoculated treatments (PL-30-NI and Control - NI) (Table 2).

Table 2. Effect of fresh poultry litter (PL-0 day) and composted for 15 (PL-15 days) and 30 days (PL-30 days) and inoculation with *Plasmodiophora brassicae* (*Pb*) or not (NI) on the dry mass of leaves, stem, and shoot, number of leaves, stem length, severity of clubroot (%), volume (mL) and fresh mass of healthy roots and roots with galls (g) in cauliflower plants.

Treatments	Dry Mass (g)			Nº Leaf	Stem (cm)
	Leaf	Stem	Shoot		
Control – NI	39.24 ab	8.93 a	48.18 a	18.28 a	16.40 abc
PL-30 days – NI	42.53 a	11.14 a	53.94 a	19,14 a	18.24 a
Control + *Pb*	10.07 cd	3.47 bc	13.55 bc	10.42 b	12.54 cd
PL-0 days + *Pb*	38.61 ab	9.14 a	47.75 a	18.28 a	16.72 ab
PL-15 days + *Pb*	3.10 d	0.76 c	3.90 c	9.32 b	9.92 d
PL-30 days + *Pb*	23.10 bc	4.83 b	27.93 b	14.42 ab	13.38 bcd
CV(%)	25.11	21.94	23.71	13.65	16.5

Treatments	Severity[1] (%)	Root Volume (mL)		Root Fresh Mass (g)	
		Healthy[1]	Galls[1]	Healthy[1]	Galls[1]
Control – NI	0,00 c	43.21 a	0.00 b	47.15 a	0.00 b
PL-30 days – NI	0,00 c	33.42 ab	0.00 b	33.95 ab	0.00 b
Control + *Pb*	87.42 a	3.42 c	37.30 a	1.91 cd	31.74 a
PL-0 days + *Pb*	29.42 b	31.50 ab	3.32 b	29.47 ab	3.85 b
PL-15 days + *Pb*	95.00 a	0.01 c	22.88 a	0.01 d	24.19 a
PL-30 days + *Pb*	84.00 a	16.25 bc	39.91 a	20.38 bc	42.98 a
CV(%)	22.59	29.18	28.64	26.82	27.65

*For each treatment, means followed by the same letter in the column do not differ from each other by Tukey's test at the 5% probability level. [1]= Data transformed by $\sqrt{x + 1}$.

The clubroot negatively affected the development of plants. However, among the treatments with inoculation, the application of fresh poultry litter (PL-0 + *Pb*) contributed to the reduction of disease severity and increased the volume of the healthy fraction of the roots and, consequently, for the better development of the plants. It is possible to infer that this effect may be related to the greater supply of nutrients to the plants, mainly $N_{mineral}$, from the fresh poultry litter (Table 1) (Bolan et al., 2010; Souza et al., 2019). The higher concentration of $N_{mineral}$ in fresh poultry litter (PL- 0 day) results in the ready availability of this nutrient for the plants, which favors their initial development compared to other composts based on poultry litter. It should also be noted that the concentration of N in fresh organic waste is mainly in the ammoniacal form (Bernal et al., 2009). Microbial degradation of organic waste, such as poultry litter, generates toxic and volatile compounds, such as ammonia, which may explain part of the reduced activity of soil pathogens (Meghvansi and Varma, 2015).

Bhering et al. (2017) and Bhering et al. (2020) consider that the continuous application of fresh poultry litter, associated with a high input of fertilizers and the lack of soil liming, can contribute to greater acidification of the soil over time and, thus, favoring the development of clubroot. That is, the chemical changes in the soil resulting from the high input of poultry litter and fertilizers over time, associated with other variables such as the high natural acidity of the soils and their effects on the pathogen and the rhizosphere, can contribute to an environment more conducive to the pathogen.

Differently, however, from the effects mentioned by Bhering et al. (2017 and 2020) that result from the massive application of fresh poultry litter and chemical fertilizers in the long term, in the present study, there is the effect of a single application and in the short term. In the condition of this trial, with inoculated plots (*Pb*) and with a single application, fresh poultry litter (PL-0+*Pb*) resulted in beneficial effects for the plant and reduced disease damage compared to treatments with poultry litter only partially composted (PL-15 + *Pb* and PL-30 + *Pb*), which were equivalent to the control treatment.

The treatment PL-15 + *Pb* was harmful to the development of the roots, expressed by the lower mass and volume of healthy roots and shoot, followed by the treatment PL-30 + *Pb*, which presented intermediate values. Composts of low maturity and stability (Bernal et al., 2009; Souza et al., 2019), such as the one in the PL-15 treatment of this work, can result in at least partial aerobiosis, the release of toxic compounds, and immobilization of N, that harm plant development (Bernal et al., 2009). The PL-15, compared to PL-0, is characterized by higher hydrophobicity, lower polarity index, and high ammonia release (Souza et al., 2019). These characteristics, associated with low maturity and stability, may also have contributed to the reduction of water availability and phytotoxicity to plants.

In general, the effects of organic composts based on poultry litter on the plant, the pathogen, and the development of the disease must involve multiple factors usually associated with the suppression of diseases, such as changes in pH, content and bioavailability of nutrients, such as Ca, N, and micronutrients (Dixon and Tilston, 2010; Bhering et al., 2017; Santos et al., 2018; Santos et al., 2022b). In addition to these factors, the release of substances that are beneficial and/or harmful to the development of plants and the pathogen by microbial activity during the decomposition and mineralization of organic matter (Dixon and Tilston, 2010; Bonanomi et al., 2018) may also be involved in the promotion and/or suppression of disease and plant development.

The results of this study show that the degree of stabilization and maturity of the poultry litter can affect the development of clubroot and cauliflower plants. In treatments with inoculation, the composts based on poultry litter (PL) with 15 and 30 days of composting are equivalent to the inoculated control and without application of PL regarding the occurrence of the disease.

In the presence of *P. brassicae*, less disease severity and better healthy root and shoot development are obtained with poultry litter without composting, equivalent to treatments without inoculation. New studies should be carried out to elucidate the interactions involved in the process of mineralization of poultry litter and its effect on the development of the disease, considering, as a priority, materials with a longer period of composting as the long-term effects of the application of poultry litter.

Authors' Contribution

Jéssica de Oliveira Lima: investigation, writing of the original draft and formal analysis. Carlos Antônio dos Santos: investigation, writing, review, editing and formal analysis. Camila da Costa Barros de Souza: investigation, writing, review and editing. Cíntia Luiza Batalha Xavier: investigation. Nelson Moura Brasil do Amaral Sobrinho: conceptualization, writing, review and editing. Margarida Goréte Ferreira do Carmo: conceptualization, writing, review, editing and supervision.

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