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# Organic Wastes and Nodulation of Soybean Plants

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## ABSTRACT

The need to reduce the use of mineral fertilizers and environmental pollution, together with the vigorous development of soybeans is of great economic, environmental, and social interest. Thus, studies of practices that lead to increased productivity and reduced production costs, such as the use of organic residues, for example, chicken manure and filter cake, in their fertilization are necessary. Thus, the objective was to evaluate the influence of the use of chicken manure and filter cake as organic fertilizer under the nodulation of soybean culture. For this, the following treatments were evaluated: T1 - Chicken manure; T2 - Chicken manure + mineral fertilization; T3 - Filter cake; T4 - Filter cake + mineral fertilizer; T5 - Mineral fertilization; T6 - Witness. The evaluations were in stages R1, R3, and R5, and the parameters evaluated: Fresh mass of the aerial part; Dry mass of the aerial part; Fresh root mass; Dry root mass; Root length; Plant height; Number of nodules; Viability of nodules. The application of chicken manure and filter cake had a beneficial influence on both root development and soybean nodulation, be it the number of nodules or their viability. The application of chicken manure and filter cake as fertilizer in the cultivation of soybean culture proved to be viable, as it brought benefits to both plants and the environment, as it is a correct option for the disposal of organic waste.

## INTRODUCTION

Soybean is the most cultivated legume in the world, in Brazil over two decades (1996/1997 to 2015/2016 harvests) the crop showed notable increases both in cultivated area and in total productivity and production (BALBINOT JUNIOR *et al.*, 2017). Reaching the mark of 36.9 million hectares in the 2019/2020 harvest and production of approximately 120.8 million tons (CONAB, 2020).

The increase in crop productivity is due to the improvement of the techniques used in its cultivation, and mainly to the efficient supply of nutrients. Among the nutrients, nitrogen is the most required by the crop due to the protein characteristic of its grains (BELLALOUI *et al.*, 2015). Under Brazilian production conditions, it can be supplied entirely by the biological nitrogen fixation process (HUNGRIA *et al*., 2007; MOURTZINIS *et al*., 2018), carried out by bacteria of the genus Bradyrhizobium (KASCHUK *et al.*, 2010). However, culture needs other elements such as phosphorus and potassium, which are indispensable for the ideal development of culture.

The search for greater productivity, the intensive use of fertilizers leads to higher production costs, and in some producing regions in Brazil, the cost of fertilization is the most expensive (SEIXAS *et al.*, 2020). Brazil imported 29.5 million tons of fertilizers in 2019 (ANDA, 2020), showing a great dependence on Brazilian agriculture for foreign inputs. To reduce this dependence, and the need to use sustainable practices for food production (KHAN *et al.*, 2020), an alternative for soil fertilization would be the addition of organic residues. And in southern Brazil, two options for fertilizing the soybean crop are filter cake and chicken manure, due to the volume generated and its chemical characteristics.

The filter cake is characterized by a residue from the mixture of milled bagasse and sludge from decantation and the sugar clarification process (FRAVET *et al.*, 2010). We have that for each ton of ground sugar cane, 30 to 40 kg of the filter cake is produced (SANTOS *et al.*, 2010). As it is an organic compound rich in calcium, nitrogen, and potassium (depending on the variety of cane, maturation time, soil type, clarification process, among others) it has been viewed as a fertilizer, that is, a source of nutrients for the plants (FRAVET *et al*., 2010; GONÇALVES *et al.*, 2018; RAMARI *et al.*, 2018).

Brazil is the world's largest exporter of chicken meat and the third-largest producer of this bird, according to the Brazilian Animal Protein Association (ABPA, 2020). Poultry production produces a considerable volume of chicken manure, a residue that consists of material distributed on the floor of the aviary to serve as a bed, receiving excreta, feed scraps, and feathers. This residue can be a valuable input due to the high concentration of nutrients (SZOGI *et al*., 2010), high availability in some regions, and low cost (SAINJU *et al.*, 2010; TAGOE *et al*., 2010; GONÇALVES *et al.*, 2018).

The use of these residues as a source of nutrients for soybean crops is an alternative, but the effect of this use on the population of soil microorganisms, including nitrogen-fixing bacteria, can be positive or negative. Thus, studies on the impact of this practice on the soybean nodulation process proved to be essential, so the objective was to evaluate the influence of the use of chicken manure and filter cake as organic fertilizer under the nodulation of soybean culture.

## MATERIAL AND METHODS

The work was carried out in the Phytopathology laboratory of Unicesumar, Maringá-PR-BR. For the implementation of the experiment, the soil was collected at a depth of 0-10 cm in an area with more than 10 years of planting with soybean, corn, and wheat crops.

The experiment was conducted in pots using the BS 2606 IPRO variety. The experimental design used was completely randomized with 6 treatments: T1- Chicken manure (5 ton.ha-1); T2– Chicken manure (5 ton.ha-1) + mineral fertilizer (139 kg.ha-1 of the formulated 04-30-10); T3– Filter cake (25 ton.ha-1); T4– Filter cake (25 ton.ha-1) + mineral fertilizer (139 kg.ha-1 of the formulated 04-30-10); T5 - Mineral fertilization (257 kg.ha-1 of the formulated 04-30-10) and T6 - Control (without fertilization). Four replicates per treatment were used, consisting of 3 vigorous plants per pot.

The inoculation was performed with liquid inoculant containing $6 \times 10^9$colony forming units (CFU) / mL of bacteria of the species Bradyrhizobiumjaponicum strains SEMIA 5079 and SEMIA 5080, in the dose of 150 mL for each 50 kg of soybean seeds as a technical recommendation. After treatment, planting was carried out in 2.0 L pots, and initially, each pot received five seeds, however, after emergence the three most vigorous plants were selected.

The evaluations took place in three phenological stages: R1, characterized by containing a flower completely open in any node of the main stem; R3, having as reference the presence of pods of 0.5 to 2.0 cm in one of the last four nodes in the main stem and; R5, the reproductive stage that presents the beginning of grain filling (<10% of granulation) in one of the last four nodes and also in the main stem.

In each phenological stage, 4 plants from each treatment were used, and the plants were removed with the aid of running water, thus preventing the root structures from being damaged or compromised. Then the plants were transferred to the laboratory where the following parameters were evaluated:

Fresh aerial mass (FAM): the plants were sectioned at the height of the neck, separating the aerial part from the roots, then obtained the fresh mass in grams with the aid of a precision scale;

Dry aerial mass (DAM): after obtaining the FAM, the plants were taken to a greenhouse at 65ºC, where they remained for 48 hours until reaching the constant weight, then the dry mass was obtained in grams, with the aid of a precision scale;

Fresh root mass (FRM): the roots of each plant were washed, removing any residue adhering to them, then they were kept at room temperature in the shade, to eliminate excess water, then obtained the fresh mass in grams, with the aid of a precision scale;

Dry root mass (DRM): after obtaining the FRMm the roots were taken to an oven at 65ºC, where they remained for 48 hours until reaching the constant weight, then the dry mass was obtained in grams, with the aid of a precision scale;

Plant height (PH): the aerial part of the plant was taken to the bench, then with the aid of a ruler, it was measured in centimeters from the beginning of the stem formation to the end of the furthest leaf;

Root length (RL): the fresh roots of the plant were taken to the laboratory bench surface and then measured in centimeters using a ruler;

Number of nodules (NNod): the nodules existing in the roots were removed and then counted;

Nodule viability (VNod): the nodules removed from each plant were cut in half and their color checked: red (viable nodules); white/yellowish (non-viable nodules).

The variables were subjected to analysis of variance and the means compared with the Tukey test at 5% probability, using the Sisvar software (FERREIRA, 2007).

**RESULTS**

The influence of the use of residues on the growth of soybean seedlings was verified through the height of the aerial part and length of the roots, in stage R1 the largest plants were obtained in the treatment in which filter cake was applied together with mineral fertilization (T4), differing significantly from the others. In R3, the T4 plants continued to show a greater significant height and, at this stage, the lowest plant height was observed in plants that did not receive any type of fertilization (T6). At the end of the crop cycle, R5, there was no significant difference in plant size among all treatments that received some type of fertilization, mineral or organic (T1, T2, T3, T4, and T5), with only the control plants presented reduced size, differing statistically from the others (Table 01).

As for the mass of the aerial part, in R1, there were plants with greater fresh vegetable mass expressed in T2, T3, and T4, followed by T1 and T5 and with less development in the control (T6). While for a dry mass of the aerial part there was no significant difference between treatments. In R3, the best statistical result for both fresh mass of the aerial part and dry mass was observed no treatment with chicken manure (T1), intermediate values for T2, T3, T4, and T5, and the worst accumulation of mass in the witness (T6). For R5, both for fresh shoots and dry matter there are no differences between treatments (Table 01).

**Table No. 01: Growth of aerial part of soybean plants submitted to different treatments with organic wastes.**

| Treatments[1] | FAM (g)[2] | | | DAM (g)[3] | | | PH (cm)[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1[5] | R3 | R5 | R1 | R3 | R5 | R1 | R3 | R5 |
| T1 | 13,06ab | 25,07a | 23,33a | 02,83a | 25,07a | 20,02a | 24,66b | 51,83ab | 53,66a |
| T2 | 14,36a[6] | 17,16ab | 12,93a | 03,03a | 17,16ab | 12,05a | 28,50ab | 47,33bc | 54,00a |
| T3 | 14,13a | 16,36ab | 14,49a | 02,75a | 16,36ab | 14,23a | 27,33ab | 51,33ab | 54,00a |
| T4 | 17,98a | 16,53ab | 21,12a | 03,36a | 16,86ab | 19,25a | 31,50a | 54,33a | 60,00a |
| T5 | 13,79ab | 16,36ab | 19,02a | 02,38a | 19,47ab | 17,21a | 28,16ab | 51,33ab | 57,66a |
| T6 | 08,38b | 07,62b | 20,01a | 02,15a | 07,62b | 16,08a | 27,33ab | 42,00c | 45,00b |

[1]T1- Chicken manure (5 ton.ha-1); T2– Chicken manure (5 ton.ha-1) + mineral fertilizer (139 kg.ha-1 of the formulated 04-30-10); T3– Filter cake (25 ton.ha-1); T4– Filter cake (25 ton.ha-1) + mineral fertilizer (139 kg.ha-1 of the formulated 04-30-10); T5 - Mineral fertilization (257 kg.ha-1 of the formulated 04-30-10) and T6 - Control (without fertilization).

[2]FAM (g): Fresh aerial part mass in grams.

[3]DAM (g): Dry aerial part mass in grams.

[4]PH (cm): Height of plants in centimeters.

[5]Phenological stages that the plant was in when the evaluation was carried out (R1, R3, and R5).

[6]Medias followed by the same letter, in the columns, do not differ from each other, by the Tukey test (5%).

As for root growth, at R1 there was no significant difference between treatments. In R3, the longest was observed in treatments that received only organic residues (T1 and T3), and root development was observed in plants that did not receive any fertilization (T6). In R5, the roots of plants treated with chicken litter (T1) proved to be more developed, and again the worst performance was observed as expected in non-fertilized plants (T6) (Table 2).

For the parameters fresh and dry root mass, as well as for height, in stage R1 there was no significant difference for fresh mass, whereas for dry mass there was a difference between treatments, with emphasis on T6. In the R3 stage, the control plants did not show the same performance as in the previous stage, presenting the lowest values of fresh and dry mass between treatments. At this stage, plants fertilized only with chicken manure stood out in terms of fresh and dry mass (Table 01). In R5, there were no significant differences between treatments for both fresh and dry root mass evaluation. Although the control plants had a shorter average root length, differing from the other treatments, both fresh and dry masses at the end of the experimental period were like the other treatments (Table No.2).

**Table No. 02**: **Root development of soybean plants submitted to different treatments with organic wastes.**

| Treatments[1] | FRM (g)[2] | | | DRM (g)[3] | | | RL (cm)[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1[5] | R3 | R5 | R1 | R3 | R5 | R1 | R3 | R5 |
| T1 | 2,55a[6] | 2,10a | 1,25a | 0,50b | 1,88a | 1,03a | 25,66a | 28,66a | 29,66a |
| T2 | 2,71a | 0,99ab | 1,23a | 0,51b | 0,62ab | 0,68a | 25,00a | 26,00ab | 24,33ab |
| T3 | 2,77a | 1,12ab | 1,24a | 0,75ab | 0,79abc | 1,01a | 27,33a | 29,00a | 26,66ab |
| T4 | 3,52a | 1,49ab | 1,09a | 0,54b | 1,23abc | 1,12a | 27,33a | 25,00ab | 26,33ab |
| T5 | 2,59a | 1,80a | 0,95a | 0,78ab | 1,66ab | 0,86a | 27,00a | 23,66ab | 21,33b |
| T6 | 2,07a | 0,57b | 1,43a | 1,08a | 0,39c | 0,79a | 11,83a | 16,66b | 12,66c |

[1]T1- Chicken manure (5 ton.ha-1); T2– Chicken manure (5 $ton.ha^{-1}$) + mineral fertilizer (139 $kg.ha^{-1}$ of the formulated 04-30-10); T3– Filter cake (25 $ton.ha^{-1}$); T4– Filter cake (25 $ton.ha^{-1}$) + mineral fertilizer (139 $kg.ha^{-1}$ of the formulated 04-30-10); T5 - Mineral fertilization (257 $kg.ha^{-1}$ of the formulated 04-30-10) and T6 - Control (without fertilization).

[2]FAM (g): Fresh root mass in grams.

[3]DAM (g): Dry root mass in grams.

[4]RL (cm): Root length in centimeters.

[5]Phenological stages that the plant was in when the evaluation was carried out (R1, R3, and R5).

[6]Medias followed by the same letter, in the columns, do not differ from each other, by the Tukey test (5%).

In addition to the growth of aerial and root parts, an important factor in soybean culture is the development of nodules, where biological nitrogen fixation occurs by mutualistic bacteria. In the evaluation of this parameter, it was found that in the R1 stage the number of nodules in the roots the treatment that treated filter cake associated with mineral fertilization (T4) stood out, however, their viability among all treatments was statistically similar. At stage R3, there was no difference in the number of nodules between treatments, however, the viability in T4 stood out from the rest. And no last evaluated stage (R5), plants fertilized only as poultry litter showed a greater number of nodules in their roots, followed by plants of T2, T3, T4, and T6, and the treatment with filter cake associated with mineral fertilization (T5) showed plants with a smaller number of nodules. As for viability, there were no differences between treatments (Table No. 3).

**Table No. 03: Quantity and viability of root nodules in soybean plants submitted to different treatments with organic wastes.**

| Treatments[1] | NNod[2] | | | VNod[3] | | | %VNod[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | R1[5] | R3 | R5 | R1 | R3 | R5 | R1 | R3 | R5 |
| T1 | 2,55a[6] | 2,10a | 1,25a | 0,50b | 1,88a | 1,03a | 25,66a | 28,66a | 29,66a |
| T2 | 2,71a | 0,99ab | 1,23a | 0,51b | 0,62ab | 0,68a | 25,00a | 26,00ab | 24,33ab |
| T3 | 2,77a | 1,12ab | 1,24a | 0,75ab | 0,79abc | 1,01a | 27,33a | 29,00a | 26,66ab |
| T4 | 3,52a | 1,49ab | 1,09a | 0,54b | 1,23abc | 1,12a | 27,33a | 25,00ab | 26,33ab |
| T5 | 2,59a | 1,80a | 0,95a | 0,78ab | 1,66ab | 0,86a | 27,00a | 23,66ab | 21,33b |
| T6 | 2,07a | 0,57b | 1,43a | 1,08a | 0,39c | 0,79a | 11,83a | 16,66b | 12,66c |

[1]T1- Chicken manure (5 ton.ha-1); T2– Chicken manure (5 $ton.ha^{-1}$) + mineral fertilizer (139 $kg.ha^{-1}$ of the formulated 04-30-10); T3– Filter cake (25 $ton.ha^{-1}$); T4– Filter cake (25 $ton.ha^{-1}$) + mineral fertilizer (139 $kg.ha^{-1}$ of the formulated 04-30-10); T5 - Mineral fertilization (257 $kg.ha^{-1}$ of the formulated 04-30-10) and T6 - Control (without fertilization).

[2]NNod: Number of nodules on the root of the soybean plant.

[3]VNod: - Vibiality of the nodules of the root of the soybean plant.

[4]%VNod:% of Viable Nodules in each treatment.

[5]Phenological stages that the plant was in when the evaluation was carried out (R1, R3, and R5).

[6]Medias followed by the same letter, in the columns, do not differ from each other, by the Tukey test (5%).

Plants fertilized with chicken litter associated with mineral fertilizer presented 100% viable nodules in stage R1, and plants fertilized with chicken manure only showed 67.8% viable nodules. The use of filter cake residue alone resulted in plants with 55.9% nodule viability and when associated with mineral fertilizer, 77%. In R3 the control treatments, filter cake associated with mineral fertilization, and chicken manure stood out from the others to the percentage of viable nodules to the total nodules presented by the plants. In the R5 stage, the viability of nodules in all treatments suffered great reductions, not exceeding 24%.

**DISCUSSION**

Padovan *et al*. (2002) affirm that early cultivars can present high production and accumulation of biomass and nutrients when combined with organic management, which indicates good possibilities for using filter cake and chicken manure. In this research, the use of organic residues favored the accumulation of fresh mass in stages R1 and R3 of soybean plants and when elected or in association with mineral fertilizers they provided even greater growth of plants in height.

The greater stature of the plants may be related to the greater root development. Therefore, it was observed that the plants that received organic fertilization had larger roots during the different phenological stages evaluated. And according to Farias et al. (2007), the increase in the root system influences the increase in the performance of the plant in all its metabolic activity, due to the greater absorption capacity. Deak et al. (2019) found that the co-inoculated treatments induced the soy root system to improve in length, volume, surface area, and root diameter, showing superiority to the uninoculated control in the temperature range of 20 to 30ºC.

There was no negative influence on the use of filter cake or chicken manure on the number and viability of the root nodules of soybean plants. This fact is important because nitrogen is an essential nutrient for culture, mainly in the flowering and grain filling phases (SEIXAS et al., 2020; HUNGARY et al., 2007. Nodules are specialized structures formed as a result of the symbiotic association of bacteria and plants where biological nitrogen fixation occurs and availability to the plant (HUNGARY et al, 2001).

Plants that have 10 to 30 nodules between the physiological stages R1 and R2 have sufficient conditions to achieve high productivity (CÂMARA; HEIFFIG, 2000). According to Moreira and Siqueira (2006), the chemical signaling process between soybean plants and diazotrophic bacteria is hampered by the availability of high doses of nitrogen in the soil, resulting in a smaller number of nodules. Thus, even with the use of the organic residues evaluated, the plants in all treatments showed more than 10 nodules in R1 and R2, with emphasis on the treatments with filter cake.

Any factor that interferes negatively in the establishment of the association or the viability of the nodules can cause significant losses for the soybean culture. Since, with less biological fixation, the amount of nitrogen made available to plants will be reduced. Because, the element required in greater quantity by soy is nitrogen (MALAVOLTA, 2006), and for each ton of grains produced by this crop, 80 kg $ha^1$ of nitrogen are needed (HUNGARY et al., 2007).

According to Khan et al. (2020), with the population increase coupled with the need for preservative practices, the excessive use of fertilizers cannot be ignored as long as practices aimed at clean agriculture are encouraged, with ownership of the supply being transferred to inoculants. crop nitrogen. Therefore, any factor that promotes an increase in nodulation and, consequently, biological nitrogen fixation, or even the replacement of mineral fertilizers without affecting this process, will be of great value for the sustainability of this production chain.

Silva et al. (2008) state that for organic fertilization to have significant effects on productivity, it is necessary to apply it for several years, as its effect is maximized in the long run, promoting improvements in soil fertility, in addition to providing adequate physical conditions for the development of the soil. soybean culture. These results corroborate those of Liu et al. (2009), who also indicates that the development of effective practices, especially with the manipulation of the amount and type of organic waste can improve the sustainability of ecosystems in the long run.

Gonçalves et al. (2019) found in their work that the use of filter cake and chicken manure as a source of nutrients for soybean cultivation improved soil properties and did not negatively impact the microbial population. And they also observed that the residues favored the germination and productivity of the soybean crop, without interfering in the nodulation process.

It is noteworthy that in stage R5, where grain filling is still occurring, the nodules evaluated in the plants in this study showed less quantity and viability than in R3 and R1 in all treatments since at the end of the reproductive stage of the plant, there is no longer an effective symbiosis between bacteria and plant because the need for nitrogen is already reduced (MOREIRA; SIQUEIRA, 2006).

## CONCLUSION

The organic residues filter cake and chicken litter provided greater growth in height for the soybean plants, however, there were no significant differences at the end of the experimental period regarding the fresh and dry mass of the aerial part to the control.

Filter cake and chicken litter residues provided greater root growth for soybean plants, resulting in benefits in the absorption of water and nutrients.

The evaluated residues had a beneficial influence on the modulation of soybean plants in terms of the number of nodules and did not interfere negatively in viability.

The application of chicken litter and filter cake as fertilizer for the cultivation of soybean culture proved to be viable, as it brings benefits to both plants and the environment, as it is an appropriate destination for these residues.

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