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APPLICATION OF DIFFERENT DOSES OF *Bacillus subtilis* IN NEMATOIDS CONTROL IN BEAN CULTURE

APLICAÇÃO DE DIFERENTES DOSES DE Bacillus subtilisNO CONTROLE DE NEMATOIDES NA CULTURA DO FEIJOEIRO

Isabella de Oliveira Miranda[1], Ana Clara Fardin[1], Jefferson Martins[1], Edilson Ramos Gomes[2]

[1] Eng. Agrônoma, Faculdades Integradas de Bauru, Vila Ipiranja Bauru - SP, Brasil.
[2] Prof. Doutor em Agronomia, Faculdades Integradas de Bauru, Vila Ipiranja Bauru - SP, Brasil.

ABSTRACT: Nematodes are phytopathogenic agents that directly interfere with the absorption of water and nutrients by plants, causing losses of up to 60% in production. Its control is hard, as they have a high range of hosts and resistance structures, thus, bio stimulants are a viable alternative, to promote growth and control these organisms. Therefore, this test aimed to evaluate different doses of Bacillus subtilis in response to nematode control, as well as bean development and production.The experiment was conducted in a protected environment in Bauru, state of São Paulo,and the design was completely randomized (DIC), being: T1: the witness, with autoclaved soil and without inoculum and 0.0 mL L-1 of Bacillus subtilis, T2: 0, 0 mL L-1 of Bacillus subtilis, T3: 5.0 mL L-1of Bacillus subtilis, T4: 10.0 mL L-1 of Bacillus subtilis and T5: 15.0 mL L-1 of Bacillus subtilis, T6: 20.0 mL L-1 of Bacillus subtilis, with eight replications. The biological parameters of the culture were evaluated at 45 and 70 days after sowing. Treatments T3 and T4 were able to control and/or reduce nematodes of the genera Meloidogyne spp., Pratylenchus spp. and Helicotylenchusspp, as well as an increase in the evaluated biological parameters.

Keywords: *bio stimulant, biological control, production.*

RESUMO: Os nematoides são agentes fitopatogênicos que interferem diretamente na absorção de água e nutrientes das plantas, causando perdas de até 60% na produção. Seu controle é dificultoso, pois possuem uma alta gama de hospedeiros e estruturas de resistência, assim, os bioestimulantes são uma alternativa viável, uma vez que, além de promoverem o crescimento, podem controlar estes organismos. Com base no exposto, esse ensaio teve a finalidade de avaliar diferentes doses de Bacillus subtilis em resposta ao controle de nematoides, bem como o desenvolvimento e produção do feijoeiro. O experimento foi conduzido em ambiente protegido em Bauru/SP, o delineamento foi inteiramente casualizado (DIC), sendo: T1: a testemunha, com solo autoclavado e sem inoculo e 0,0 mL L-1 de Bacillus subtilis, T2: 0,0 mL L-1 de Bacillus subtilis, T3: 5,0 mL L-1 de Bacillus subtilis, T4: 10,0 mL L-1 de Bacillus subtilise T5: 15,0 mL L-1 de Bacillus subtilis, T6: 20,0 mL L-1 de Bacillussubtilis, com oito repetições. Foram avaliados os parâmetros biológicos da cultura aos 45 e 70 dias após semeadura. Os tratamentos T3 e T4 conseguiram controlar e/ou diminuir os nematoides dos gêneros Meloidogyne spp., Pratylenchus spp. e Helicotylenchusspp, assim como aumento dos parâmetros biológicos avaliados.

Palavras-chave: *bioestimulante, controle biológico, produção*.

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* Autor correspondente: edilsonevj@hotmail.com
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INTRODUCTION

Agricultural production has numerous adversities, however, it can be mentioned the appearance of pests and diseases, among them, the nematode, which are phytopathogens that survive in the soil, causing harm both directly, such as galls or root lesions, interfering with water absorption and nutrients, and indirectly, helping the entry of other phytoparasites (OLIVEIRA et al., 2017).

The genera that most affect beans are Meloidogyne (ALVES, 2020) and Pratylenchus (SIMÃO et al., 2010), respectively, popularly known as the root-knot nematode and root-lesion nematode. Its control is hard, mainly due to its high range of hosts and resistance structures, which can remain viable in the soil for a long period (MOENS et al., 2009).

Studies on the genus Helicotylenchusare limited; however, it is known that the hot and humid weather favors the reproduction of this species (BRIDA, 2012). This pathogen species, after dead, begin to exhibit a coiled body, adopting a spiral shape, more or less closed, called in English as "spiral nematodes" (GARBIN; COSTA, 2015). Chemical control is still more common, even though, an ecological alternative is the biological way, which does not leave residues in the soil or in the environment, and it is safer for humans, avoiding intoxication. Furthermore, it is more efficient in the long term than other types of control, avoiding the creation of resistance in phytopathogens (COSTA, 2015). The use of growth-promoting bacteria in biological control is gradually increasing, becoming an option for many producers.

Bacillus subtilis is used to limit the growth of harmful organisms. Fernandes et al. (2013), affirm the effectiveness in reducing the amount of M. javanica eggs with the use of Bacillus subtilis.

According to Ferreira (2015), in addition to controlling phytopathogens and increasing productivity (NGUGIA et al., 2005; YAO et al., 2006), these bacteria also cause the phenomenon of induced systemic resistance, preventing or limiting the prevalence of phytopathogenic microorganisms (RYU et al., 2004; ONGENA et al., 2007).This work aimed to evaluate different doses of Bacillus subtilis in response to nematode control, as well as the development and production of common bean.

MATERIAL AND METHODS

The experiment was carried out in a protected environment (agricultural greenhouse), in partnership with the phytopathology laboratory of the Agronomy course, at Integrated Colleges of Bauru (FIB), in Bauru city, São Paulo State, with coordinates 22º20'41.1"Sand 49º06' 24.7"W at 530 altitude meters.

Before the experiment implementation, itwas collected soil from nine different points with a depth of 0 to 40 cm, in order to homogenize the sample. Subsequently, the sample was sent to the laboratory for analysis its physicochemical properties, as well as the presence of phytonematodes (Tables 1 and 2).

Table 1. Result of the chemical-physical analysis of the soil used in the experiment.

pH	**M.O.**	**P**	**Al^{3+}**	**H**	**$H+Al^{3+}$**	**K**	**Ca**	**Mg**	**V**	**SB**	**CTC**
$Ca\ Cl_2$	g dm^{-3}	mg dm^{-3}	----------	----------	mmolcdm^{-3}	----------	----------	----------	%	mmolcdm^{-3}	
4.4	7.3	170.40	2.64	18.88	21.52	5.03	32.60	18.60	72	56.23	77.75

B	**Cu**	**Mn**	**Zn**	**Fe**
----------	----------	mgdm^{-3}	----------	----------
0.29	0.70	1.20	0.30	86

Coarsesand	**thinsand**	**total sand**	**Clay**	**silt**	**Texture**
		g kg^{-1}			
668	199	867	100	33	Sandy

Table 2. Result of nematode analysis

City	Culture	Analysistype	Report	Species	Soil quantity	Root quantity
Bauru-SP	beantree	soilnematode	6666/2020	*Meloidogyne*spp	50	20
Bauru-SP	beantree	soilnematode	6666/2020	*Pratylenchus*spp	70	40
Bauru-SP	beantree	soilnematode	6666/2020	*Helycotilenchus*spp	30	10

All soil was manually sieved. After that, it was placed in plastic bags of approximately 26 kg to be autoclaved. In this process, it was exposed to temperatures of 121 ºC, for two hours with a pressure of one atm, in order to sterilize, avoiding undesirable microorganisms. 48 plastic pots with 14.3 L capacity were used and filled with a mix of 13 liters of soil and 2 liters of Carolina Soil substrate. According to the methodology Raij et al. (1997), 4.5g of limestone, aiming at raising the Percentage to 80 and fertilization in such a way as to meet the needs of the crop, a pre-planting correction was not necessary.

Four bean seeds, of the BRS Embrapa´s growth methodwere sown per pot, on 2020, October 10. The first inoculation occurred at five DAS and the second one at 20 DAS with nematodes of the genera Meloidogyne spp., Pratylenchus spp. and Helycotilenchusspp.s using a solution of 200 mL of contaminated soil per 800 mL of water. 100 mL of this solution was applied per pot, the same proportion used for roots that contained the inoculum, except in the treatment (control).

After 20 days, thinning was done, leaving only two plants per pot. The second inoculation was intended to add nematodes also when the crop and its root system were more developed to confirm the effectiveness of the inoculum. This inoculation was carried out at 23 DAS, using the same proportions aforementioned.

Irrigation management was based on the water retention curve, aimingto maintain soil water content at field capacity (CC), according to the methodology of Gomes et al., (2015). In this sense, tensiometers with a 12 cm depth were installed, in order to monitor soil tension and perform the application of irrigation depths to maintain the soil in CC of all treatments.

Fertilizations were carried out weekly, starting with 1.5g of urea + 0.5g of potassium chloride and after three weeks, 1.0g of urea + 0.8g of potassium chloride was added per pot (Table 3).

Figure 1.Percentage of water uptake of rice seeds in substrate moistened with water with electrical conductivities of 1.0 dSm in (D)

Table 3. Common bean fertilization.

DAS	Fertilization	
	Urea (g pot^{-1})	KCl (g pot^{-1})
15	1.50	0.50
22	1.50	0.50
29	1.50	0.50
36	1.00	0.80
43	1.00	0.80
50	1.00	0.80
57	1.00	0.80
64	1.00	0.80

The design was completely randomized (DIC), being: T1: the control, with autoclaved soil and without inoculum and 0.0 mL L-1 of Bacillus subtilis and inoculation, T2: 0.0 mL L-1 of Bacillus subtilis and inoculation, T3: 5.0 mL L-1of Bacillus subtilis and inoculation, T4: 10.0 mL L-1 of Bacillus subtilis and inoculation and T5: 15.0 mL L-1 of Bacillus subtilis and inoculation, T6: 20, 0 mL L-1 of Bacillus subtilis and inoculation, with eight repetitions. The application of Bacillus subtilis was divided into two applications at 25 DAS and 50 DAS. The evaluations were performed at 45 DAS and 70 DAS. At 45 DAS, the evaluated parameters were plant height (cm), considering the soil to the apical part and with the aid of a ruler. To measure the stem diameter (cm) a caliper was used, as well as the total leaf count (unit) for the number of leaves. The fresh and dry biomass of the aerial part (stem plus leaves) and root (g) was determined with a precision balance. To determine the dry mass of the plant, it was placed in a drying oven for 72 hours at 70 ºC.

In addition to the fresh and dry biomass of the plant, at 70 DAS, plant height (cm), stem diameter (cm), number of lateral branches (unit), number of leaves (unit), number of nematodes present in the soil and number of pods per plant (unit).

The data obtained in the experiment were submitted to analysis of variance and comparison of means to the Tukey test at 5% significance in the SISVAR program (FERREIRA, 2008).

RESULTS AND DISCUSSION

The values shown in table 4 refer to the first evaluation. It was observed that there were no statistical differences between all treatments.

However, treatment 6 (20.0 mL L-1 of Bacillus subtilis + inoculum) showed the lowest results when compared to the other ones. Even though there was no difference between treatments, the plants that received the 5.0 mL L-1 dose of Bacillus subtilis + inoculum showed better height (AP) and leaf number (NF) performance, indicating the effect of Bacillus subtilis action in the soil that resulted in better conditions for them (Table 4).

Table 4. Result of plant height (AP), stem diameter (DC), fresh shoot biomass (MSPA), dry hoot biomass (MSPA), number of leaves (NF), fresh root biomass (MFR) , root dry biomass (MSR) and root volume (RV) at 45 days in response to the following treatments: T1: the control, with autoclaved soil and without inoculum and 0.0 mL L^{-1} of *Bacillus subtilis*, T2: 0.0 mL L^{-1} of *Bacillus subtilis*, T3: 5.0 mL L^{-1} of *Bacillus subtilis*, T4: 10.0 mL L^{-1} of *Bacillus subtilis* and T5: 15.0 mL L^{-1} of *Bacillus subtilis*, T6: 20 .0 mL L^{-1}*of Bacillus subtilis.*

TREATMENTS	AP (cm)	DC (cm)	MFPA (g)	MSPA (g)	NF (Unit.)	MFR (g)	MSR (g)	VR (mL)
1	9.28 a	0.35 a	23.39 a	4.53 a	13.33 a	36.12 a	2.85 a	10.0 a
2	9.08 a	1.52 a	12.09 a	3.01 a	14.66 a	12.07 a	1.33 a	8.33 a
3	10.25 a	0.53 a	10.58 a	2.03 a	17.00 a	18.06 a	2.08 a	10.0 a
4	9.28 a	0.44 a	17.36 a	3.14 a	15.00 a	14.49 a	2.96 a	6.66 a
5	9.66 a	0.40 a	13.40 a	2.85 a	14.66 a	23.22 a	1.16 a	8.33 a
6	8.66 a	0.36 a	5.97 a	1.61 a	10.33 a	11.08 a	0.77 a	6.66 a
CV (%)	**16.04**	**43.72**	**69.87**	**52.43**	**30.8**	**62.41**	**47.43**	**28.98**

* Means followed by the same letter in the column do not change from each other according to Tukey's test at 5% probability.

With the application of Bacillus in the soil at an intermediate dose (10.0 mL L-1 of Bacillus subtilis + inoculum), the highest mean root dry mass (MSR) was observed, in addition, the second highest means for fresh and dry mass of shoots and number of leaves (NF).

Cardozo and Araújo (2011) pointed out that Bacillus spp. provided an increase in plant height and dry biomass of the aerial part because Bacillus reduced the reproduction of root-knot nematodes, considering one of the possibilities for increasing these parameters in common bean also (Table 4).

For the 70 DAS (Table 5) the evaluated parameters showed a statistical difference between treatments T3, T4 and T5, for the parameters dry biomass of the aerial part, number of pods and root dry biomass. These results show the beneficial effects of Bacillus on the soil/plant relationship, where these microorganisms promote improvements in nodulation and N2 fixation, and their ability to solubilize nutrients.

Such confirmations can be noticed for treatment 5 (15.0 mL L-1 of Bacillus subtilis + inoculum) which demonstrated greater plant height, stem diameter, number of branches, fresh mass of roots, root volume, and number of green beans. Pavezi (2017) also proved that the use of bio stimulants in beans increased the number of pods and root length.

Table 5. Result of plant height (AP), stem diameter (DC), fresh shoot biomass (MFPA), dry shoot biomass (MSPA), number of leaves (NF), number of branches (NR) , number of pods (NV), root fresh biomass (MFR), root dry biomass (MSR) and root volume (RV) at 70 days in response to the following treatments: T1: the control, with autoclaved soil and without inoculum and 0, 0 mL L-1 of Bacillus subtilis, T2: 0.0 mL L-1 of Bacillus subtilis, T3: 5.0 mL L-1 of Bacillus subtilis, T4: 10.0 mL L-1 of Bacillus subtilis and T5: 15.0 mL L-1 of Bacillus subtilis, T6: 20.0 mL L-1 of Bacillus subtilis.

TREATMENT	AP (cm)	DC (cm)	MFPA (g)	MSPA (g)	NR (Unit)	NV(unit)	MFR (g)	MSR (g)	VR (mL)
1	11,12 a	0,46 a	33,56 a	7,54 b	10,75 a	1,75 ab	17,20 a	2,87cb	8,75 a
2	11,11 a	0,56 a	50,59 a	19,05 a	13,50 a	2,50 ab	16,02 a	5,03 a	8,50 a
3	11,62 a	0,54 a	49,23 a	14,70 ab	12,25 a	2,00 ab	15,14 a	3,26 abc	10,0 a
4	10,68 a	0,55 a	42,95 a	12,08 ab	16,25 a	2,00 ab	15,14 a	3,77 ab	6,25 a
5	12,18 a	0,59 a	48,01 a	14,02 ab	16,50 a	5,25 a	17,48 a	2,77 cb	13,75 a
6	9,93 a	0,52 a	27,33 a	6,30 b	10,25 a	0,25 b	12,71 a	1,61 c	7,50 a
CV (%)	**16,97**	**15,78**	**34,65**	**35,18**	**37,32**	**32,55**	**41,94**	**27,62**	**37,59**

* Means followed by the same letter in the column do not change from each other according to Tukey's test at 5% probability

In Figure 1, it is showed the amount of nematodes in the soil according to the different treatments. It is understood that the number of nematodes of the genus *Meloidogyne* spp., decreased significantly compared to the first analysis (Table 2) in treatments 3, 4, 5 and 6. It was also noticed that even with a lower number of nematodes present in the rhizosphere root of the plant there was still the presence of galls in the root system. The genus *Pratylenchus*spp. was the one that showed the greatest reduction due to the action of treatments with bio stimulant. Although, for the *Helicotylenchus* spp. control, the bio stimulants were not very effective on this genus in all treatments.

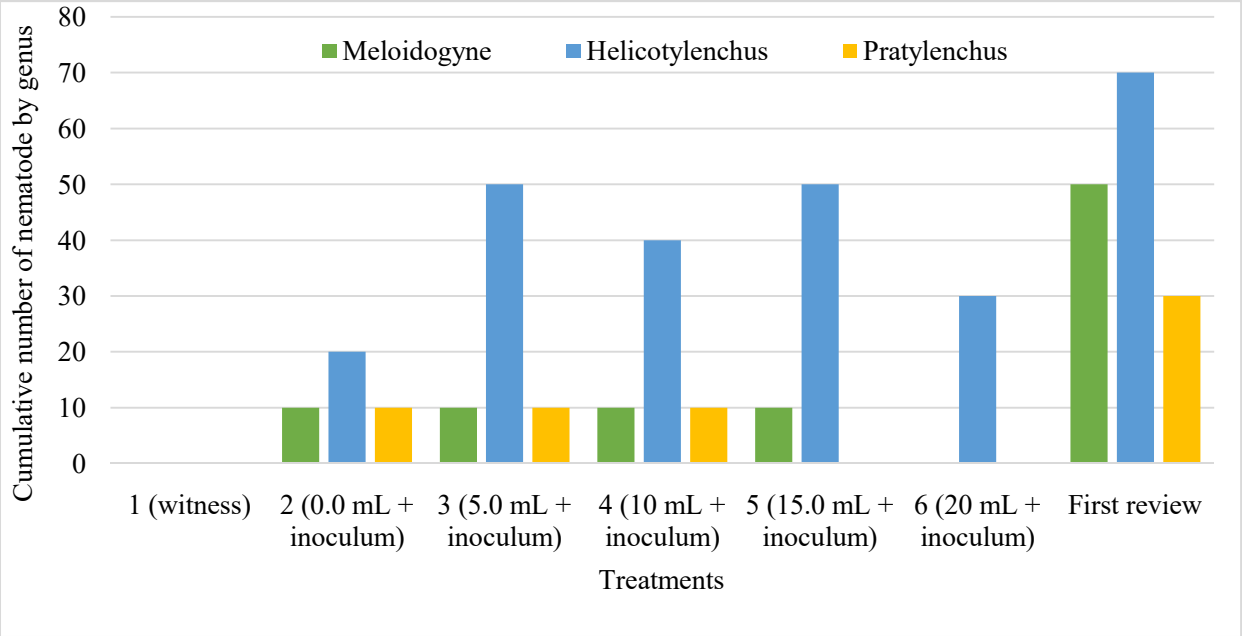

Figure 1. Graph with the initial and final amount of nematodes of the genus *Meloidogyne* spp., *Pratylenchus* spp. and *Helicotylenchus*spp.

However, treatment 2 (0.0 mL L^{-1} + inoculum) stands out, where the amounts of nematodes should be higher, since there was no application of *Bacillus subtilis*, although, it was not possible to know the exact number which was inoculated per pot, but it´s supposed that one of the possibilities is that in this treatment, the amount was smaller than the others.

We must highlight that, even in the same treatment, the amount of *Helicotylenchus* was lower than in the first evaluation. This fact is also correlated with the application of the bio stimulant, since, in all treatments that the application was carried out, the amount of the nematode was greater.

Fernandes et al. (2014) has found that the microbiolization of bean seeds with *Bacillus spp*., reduced the number of eggs of *M. incognita*, but, the use of bacteria + fungus, reduced 80% of eggs of *M. javanica* compared to its separately use, one of the hypotheses for the amounts of microorganismsreduction present in the soil.

CONCLUSIONS

The application of*Bacillus subtilis* can reduce the amount of nematodes, as well as increase the growth of the bean plant.The doses of 5.0 mL L^{-1} and 10.0 mL L^{-1} of *Bacillus subtilis* were the ones that showed expressive results, both in the reduction of pathogens and in the biological parameters analyzed.

It is extremely important to provide more researches about the influence of

Bacillus subtilis in this culture, both for the promotion of growth and for the control of pathogens, since the nematode *Helicotylenchus spp*., with the presence of the bacteria reproduced more, thus, it is necessary to evaluate if it helps in the reproduction and permanence of this phytoparasite.

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