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Inoculation and co-inoculation of the winter bean and chemical treatment of the seeds[1]

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ABSTRACT - Given the large differences in the results of inoculation and co-inoculation in the bean crop, an experiment in cultivating winter beans was carried out in 2019 under greenhouse conditions with the aim of evaluating the performance of the beans and a possible increase in plant growth and grain production, using seeds inoculated or co-inoculated with *Rhizobium tropici* and *Azospirillum brasilense*, with and without fungicide and/or insecticide, in sandy and clayey soils. A total of 112 pots were used, and 14 treatments were applied in a completely randomised experimental design, with four replications. The Scott-Knott test was used to analyse the results, and showed that whether using untreated seeds or carrying out complete chemical treatment with fungicide and insecticide plus co-inoculation with *R. tropici* and *A. brasilense*, winter bean production was reduced in clayey soil, while the joint use of fungicide and insecticide, fungicide and *R. tropici*, or fungicide, insecticide and *R. tropici*, favoured shoot development. Inoculating with *A. brasilense*, without *R. tropici* and with insecticide, or insecticide and fungicide, favoured greater root growth in clayey soil. In sandy soil, grain production was higher using seeds treated solely with insecticide, solely with *R. tropici,* or with a combination of both. Production was lower in the joint treatment with fungicide, insecticide and the co-inoculation of bacteria, or when using insecticide together with *A. brasilense*.

Key words: *Phaseolus vulgaris. Azospirillum brasilense. Rhizobium tropici.* Fungicide. Insecticide.

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INTRODUCTION

The world's third largest bean producer (Food and Agriculture Organisation of the United Nations Statistics - FAOSTAT (2021)), Brazil has three growing seasons: the first from August to December, the second from January to April, and the third from May to July. According to the National Supply Company – CONAB (2020), total production for 2019/2020 was 3.22 million tons, while for 2020/2021, production was 1.1, 1.2 and 0.8 million tons, respectively, for the first, second and third harvests, with an average productivity of approximately 1.2, 0.85 and 1.33 t ha^{-1}.

The crop stands out for its high nutritional, social and economic importance to the country, with grain rich in crude protein, phosphorus, calcium, magnesium, iron, zinc and copper (Klasener *et al*., 2020), while its greatest requirement is nitrogen. (Vazquez; Sá, 2015).

For the cultivation of Brazilian soya beans, the nitrogen supply was resolved using biological nitrogen fixation (BNF), with the inoculation of elite strains of *Bradyrhizobium*, which has generated savings of USD 9 billion per year in fertiliser, and made the crop competitive on international markets, as well as helping to reduce the release of millions of tons of CO_2 from fertiliser production into the atmosphere (Hungria; Mendes; Mercante, 2013).

In this respect, research into inoculation has sought to replace, through the use of BNF, a part of the demand met by fertilisation (Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA (2016)). Furthermore, with the aim of improving the performance of the rhizobia and the efficiency of FBN, among other benefits, co-inoculation techniques are being studied; these consist in combining bacteria of genus *Rhizobium* (symbiotic) with those of genus *Azospirillum* (associative), which can have a synergistic effect, enhancing nodulation and affording greater growth, and surpassing the results of using them individually (Bárbaro *et al*., 2008; Bulegon *et al*., 2016; Mortinho *et al*., 2022).

However, although widely used in Brazil as a seed treatment (ST), inoculation and co-inoculation today show new possibilities with the search for forms of application that would guarantee greater survival of the bacteria and more effective BNF, which can be affected by direct contact of the fixing bacteria with fungicides, by high exposure to external factors, or even by competition between the bacteria, which, instead of their synergistic effect would maintain or reduce performance and productivity (Cardoso *et al*., 2021; Figueiredo *et al*., 2016; Kaneko, F. H. *et al*., 2010; Silva *et al*., 2020).

At the same time, it has been shown that whether initial nodulation fails or is successful, carrying out additional inoculations and co-inoculations by spraying at the different growth stages promotes further nodulation, greater N fixation and higher grain productivity in the soya bean (Hungria; Nogueira; Araujo, 2015; Moretti *et al*., 2018).

Brazil, as a world reference in the use of nitrogen-fixing bacteria and diazotrophic and/or growth-promoting bacteria, provides a lot of pioneering information concerning the potential and real benefits of these technologies in the different soils of each region.

In this respect, an experiment was conducted with the aim of evaluating the performance of winter beans in terms of plant growth and grain production using uninoculated seeds, and those inoculated or co-inoculated with *R. tropici* and *A. brasilense*, with no chemicals or treated with fungicide and/or insecticide, in clayey and sandy soils.

MATERIAL AND METHODS

The experiment was conducted during the winter of 2019, in pots, under greenhouse conditions, in the district of Ilha Solteira, in the northwest of the state of São Paulo, Brazil.

To fill the pots, soil samples were collected from the surface layer at a depth of 0 to 0.20 metres of a typical clayey dystrophic Red Latosol and a sandy dystrophic Yellow Red Latosol, classified according to the Brazilian System of Soil Classification (Santos *et al*., 2018), both under a pasture of brachiaria grass.

The composite samples were then air-dried, crushed and sieved through 2-mm mesh to obtain air-dried fine earth (ADFE) for chemical analysis (Raij *et al*., 2001).

The chemical attributes of the clayey soil in the 0 to 0.20 m layer were Phosphorus (P resin) 3 mg dm^{-3}, organic matter 20 g dm^{-3}, pH ($CaCl_2$) 4.2, potassium (K) 0.8, calcium (Ca) 3, magnesium (Mg) 1, potential acidity (H + Al) 42, aluminium (Al) 12, sum of bases (SB) 4.8, $mmol_c$ dm^{-3}, sulphur ($S\text{-}SO_4$) 5 mg dm^{-3}, cation exchange capacity (CEC) 46.8 $mmol_c$ dm^{-3}, base saturation (V) 10%, aluminium saturation (m%) 71%, boron (B) 0.20, copper (Cu) 1.3, iron (Fe) 23, manganese (Mn) 7.8 and zinc (Zn) 0.2 mg dm^{-3}.

The chemical attributes of the sandy soil in the 0 to 0.20 m depth layer were Phosphorus (P resin) 1 mg dm^{-3}, organic matter 18 g dm^{-3}, pH ($CaCl_2$) 5.0, potassium (K) 0.6, calcium (Ca) 11, magnesium (Mg) 8, potential acidity (H+Al) 18, aluminium (Al) 0, sum of bases (SB) 19.6 $mmol_c$ dm^{-3}, sulphur ($S\text{-}SO_4$) 4 mg dm^{-3}, cation exchange capacity (CEC) 37.6 $mmol_c$ dm^{-3}, base saturation (V) 52%, aluminium saturation (m%) 0%, boron (B) 0.18, copper (Cu) 0.5, iron (Fe) 18, manganese (Mn) 3.1 and zinc (Zn) 0.3 mg dm^{-3}.

Seven kilograms of soil were placed in each pot, which had two perforations for drainage at the bottom. Based on the results of the soil analysis, the limestone requirement was calculated using the formula:

$$NC = CTC \frac{(V2 - V1)}{10 \times PRNT} \quad (1)$$

where, LR = limestone requirement in t ha^{-1}, CEC = cation exchange capacity in mmol$_c$ dm^{-3}, V2 = desired base saturation for the crop, V1 = base saturation found with the soil analysis, and RTNV = relative total limestone neutralising value.

As such, 11.6 and 2.8 g of dolomitic limestone with an RTNV of 86% were applied per pot to correct the acidity of the clayey and sandy soils, respectively, equal to 3.3 and 0.8 t ha^{-1}, and incorporated into the soil. An incubation period of 30 days was used, during which the pots were constantly moistened to allow the limestone to react with the soil.

Fertilisation was then carried out before sowing, using 200 mg kg^{-1} N, 200 mg kg^{-1} P, 120 mg kg^{-1} K and 5 mg kg^{-1} Zn, as per Andreotti *et al*. (2000). As the source of these nutrients, 2.2 g ammonium sulphate, 8.4 g formula 04-30-10, 1.5 g potassium chloride and 0.16 g zinc sulphate were used in each pot. A top dressing was applied 36 days after sowing, using 2.2 g ammonium sulphate per pot.

On 17 June 2019, six seeds of the IAC-Sintonia bean cultivar were sown in each of the pots. The seeds were treated shortly before sowing, applying the following 14 treatments: 1. Control; 2. Fungicide (F); 3. Insecticide (I); 4. F + I; 5. *Rhizobium tropici* (R); 6. F + R; 7. I + R; 8. F + I + R; 9. *Azospirillum brasilense* (A); 10. F + A; 11. I + A; 12. F + I + A; 13. R + A; 14. F + I + R +A.

The treatments were prepared in 14 plastic cups, each containing 48.5 g of seeds, giving an average of 200 seeds per cup, to which were added in sequence, first the fungicide, followed by the insecticide, and finally *R. tropici* and *A. brasilense* when present, leaving an interval between the application of each commercial product, so that the next product would only be applied once the previous one had dried.

The fungicide treatments included 0.05 g (100 g 100 kg^{-1} of seeds) of the systemic fungicide Thiophanate-methyl 700 g kg^{-1}, precursor of the Benzimidazole chemical group, as a wettable powder (WP), classified as category 5 and class II, indicated for treating the seeds of bean crops to control dry root rot (*Fusarium solani f.sp phaseoli*) and anthracnose (*Colletotrichum lindemuthianum*).

The insecticide treatments included 0.485 mL (1 L 100 kg^{-1} of seeds) of the insecticide Imidacloprid 150 g L^{-1} + Thiodicarb 450 g L^{-1}, systemic, of the neonicotinoid chemical group (Imidacloprid), and contact and ingestion, of the oxime methylcarbamate chemical group (Thiodicarb), as a concentrated suspension used specifically for treating seeds, classified as category 3 and class II, and indicated for the control of such pests as whitefly (*Bemisia tabaci*), the Curcurbit beetle (*Diabrotica speciosa*) and the green leafhopper (*Empoasca kraemeri*) in bean crops.

When inoculating with *R.* tropici, a dose of 400 mL ha^{-1} of inoculant was used, considering 50,000 g of seeds per hectare, equal to 0.388 mL per 48.5 g of seeds. The same calculation was carried out to define the dose of *A. brasilense* (Ab-V5 and Ab-V6, guaranteeing 2 x 108 CFU mL^{-1}), using 0.388 mL in each cup.

The experimental design was completely randomised by soil texture class. Considering the 14 treatments and four replications, there were a total of 56 experimental units in the clayey soil and 56 in the sandy soil.

Irrigation throughout the experiment was based on readings from the PCR soil moisture meter, with the required amounts of water applied manually.

Thinning was carried out at stage V1 of the bean plant (seedling emergence), keeping only two plants per pot. Plant height was measured at V3 (first open compound leaf). At stage R5 (flowering), when more than 50% of the plants were in flower, the chlorophyll index in the third trefoil of each plant per pot was measured using a digital chlorophyll meter (Falker). Root length, root volume (using the water displacement method in a test tube), and the fresh and dry weight of the shoots and roots were all measured. The number of pods, number of grains, and grain production per plant were also determined.

The results were entered into the Sisvar statistical software (Ferreira, 2011) and submitted to analysis of variance using the F-test at 5% probability. The mean values were compared using the Scott-Knott method (1974).

RESULTS AND DISCUSSION

Clayey soil

The leaf macronutrient content (Table 1) showed no significant variations as a function of the seed treatments or inoculations. According to Ambrosano *et al*. (1996), with the exception of calcium, the levels of leaf nutrients in the bean plants were adequate. As each of the treatments received the same levels of correctives, fertilisers and irrigation throughout the experiment, there were no nutrient limitations on the plants.

Mortinho *et al*. (2022) report that co-inoculation of plant growth-promoting bacteria in winter bean cultivation allows the recommended dose of NPK mineral fertiliser to be reduced by 50% due to the greater use efficiency when using *R. tropici* + *P. fluorescens* and *R. tropici* + *A. brasilense* + *P. fluorescens*.

In this study, as also seen by Kaneko *et al.* (2010), although N values in the leaves are within the range (30 to 50 g kg^{-1}) recommended by Ambrosano *et al.* (1996), seed inoculation with *R. tropici* had no effect on either this parameter or grain productivity. While Gitti *et al.* (2012) found that when N was not applied as top dressing, seed inoculation with *A. brasilense* afforded greater levels of leaf N, but had no effect on plant development, the production components or grain productivity in bean plants evaluated in clayey soil.

With the morphological attributes and production components (Table 2), there were significant differences only for shoot fresh matter production and plant height. Based on the values for shoot fresh matter, the plants in treatments using only fungicide and insecticide, fungicide and *R. tropici*, and those with fungicide, insecticide and *R. tropici*, proved to be more vigorous. This is possibly related to the fact that these treatments kept the plants green for longer than the other treatments, whereas the use of *A. brasilense* did not stand out in fresh matter production per plant, proving to be inferior, especially when used together with fungicide + insecticide or with *R. tropici*, in addition to when used together with fungicide + insecticide + *R. tropici*.

Similarly, plant height was lower in two of the treatments, one of them using *A. brasilense* together with fungicide + insecticide (Treatment 12) and the other using *A. brasilense* together with *R. tropici* (Treatment 13). Meanwhile, in the treatment that included all four components, plant height was favoured in a similar way to the other treatments. The lower plant height in V3, found in Treatments 12 and 13, had no negative effects on grain production per plant.

There were no significant differences in the leaf chlorophyll index, which means that despite none of the treatments standing out in this respect, the high values show that the plants were fully developed. According to Xiong *et al*. (2015), in some crops, especially legumes, the LCI does not accurately indicate N sufficiency in the plants. In any case, the LCI, as well as the N content of the leaves (Table 1), even with no differences between the treatments, were high.

The number of pods did not differ significantly between treatments, however, each treatment resulted in a production of more than 10 pods per plant. The number of grains was similar between treatments, a fact that may have been masked by the availability of water and nutrients at the time of grain filling (Table 2).

Although there were differences in shoot fresh matter production, there were no significant differences for dry matter, showing that the higher values for fresh matter were probably due to the plants being at different stages of senescence, each with a different water content.

Table 1 - Mean macronutrient content of the leaves of winter beans in clayey soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	nitrogen	phosphorous	potassium	calcium	magnesium	sulphur
	(g kg^{-1})					
C (1)	48.8	4.1	26.2	7.0	4.5	4.2
F (2)	45.2	4.2	26.6	5.7	4.0	3.4
I (3)	45.3	3.7	23.4	5.5	3.9	3.5
F + I (4)	45.0	4.4	23.9	5.5	3.6	3.4
R (5)	50.1	3.9	28.1	5.9	4.1	3.1
F + R (6)	48.4	4.6	30.2	5.4	3.8	3.4
I + R (7)	48.5	4.8	28.5	5.1	3.5	3.5
F + I + R (8)	46.0	3.9	24.9	5.2	4.0	3.5
A (9)	41.2	3.9	26.8	5.1	3.6	3.5
F + A (10)	50.3	3.7	25.3	4.4	3.3	3.6
I + A (11)	45.6	4.1	25.0	3.9	3.1	3.6
F + I + A (12)	45.6	3.6	24.9	4.7	3.3	3.7
R + A (13)	45.7	3.7	26.8	5.0	3.6	3.1
F + I + R + A (14)	47.6	3.7	29.7	4.7	3.9	3.9

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Source: The author

Table 2 - Mean values for plant height (ph), leaf chlorophyll index (lci), shoot fresh matter production (sfm), shoot dry matter production (sdm), number of pods per plant (npp), number of grains per plant (ngp), and grain weight per plant at 13% moisture (gw), in bean plants in clayey soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	ph (cm)	lci	sfm (g)	sdm (g)	npp	ngp	gw (g)
C (1)	13.7 a	50.2	26.6 c	17.2	13.4	41.4	11.2 b
F (2)	14.1 a	51.6	29.8 c	19.1	12.0	40.3	13.2 a
I (3)	13.0 a	50.1	27.0 c	19.3	11.0	36.8	12.8 a
F + I (4)	12.5 a	52.1	37.5 a	20.1	11.9	36.8	12.7 a
R (5)	12.4 a	49.9	36.2 b	21.8	11.6	41.5	14.0 a
F + R (6)	12.7 a	49.1	40.3 a	20.5	13.0	42.8	12.8 a
I + R (7)	12.1 a	50.5	32.4 b	20.7	10.5	42.6	14.3 a
F + I + R (8)	12.3 a	48.9	38.3 a	23.6	13.1	42.6	13.3 a
A (9)	13.3 a	48.9	35.3 b	19.8	12.3	35.9	13.5 a
F + A (10)	13.5 a	50.5	36.1 b	21.3	13.1	39.8	12.7 a
I + A (11)	12.6 a	51.3	35.2 b	21.2	12.5	37.3	12.2 a
F + I + A (12)	10.1 b	49.4	29.4 c	19.9	10.8	38.3	12.3 a
R + A (13)	10.2 b	51.3	26.5 c	18.9	11.9	40.5	12.2 a
F + I + R + A (14)	12.0 a	51.4	25.9 c	18.0	10.4	29.5	9.3 b
CV%	11.12	4.86	7.32	12.08	15.98	15.01	11.35

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Mean values followed by different letters in a column differ by Scott-Knott test (P < 0.05). Source: The author

For Straliotto, Teixeira and Mercante (2003), inoculating seeds with bacteria from the *Rhizobium* group is one alternative for replacing nitrogen fertilisation in bean plants, even if only partially. However, according to Peres *et al*. (2018), bean cultivation under field conditions, with the application of 40 kg ha^{-1} N as top dressing and no inoculation, afforded satisfactory physiological seed quality, with no need to apply higher doses of nitrogen and inoculating with *R. tropici*, inoculating with *A. brasilense*, or co-inoculating with *R. tropici* and *A. brasilense*.

In relation to grain production, for Florentino *et al*. (2018), inoculation with *R. tropici* proved to be a viable practice when mineral nitrogen was not used or was used only as top dressing, as it promoted good plant development and productivity, albeit not comparable to complete fertilisation applied in the furrow and as top dressing.

Figueiredo *et al*. (2016) describe how native rhizobia can promote nodulation, plant growth, nitrogen accumulation in the shoots, and yields equivalent to treatments inoculated with *R. tropici*. Similar to Kraeski *et al*. (2021), who found that whether inoculated with *R. tropici* or not, there was no effect on the increased grain productivity of irrigated beans, an increase seen with the use of higher doses of N applied as top dressing.

These and various other research findings suggest that the main reason for the highly variable results when inoculating bean plants is the widespread and diverse existence of native bacteria in the soil, which compete with the inoculated bacteria and interact differentially with the various cultivars, resulting in a differing response to nodulation and the FBN (Cassini; Franco, 2006).

In this research, there was a significant difference in grain yield per plant (13% moisture) in Treatments 1 and 14, which gave poorer results than the other treatments, showing that not using any chemical treatment or seed inoculation, or treating the seeds with fungicide, insecticide and co-inoculation by *R. tropici* and *A. brasilense* together, can be detrimental to winter bean production in clayey soils (Table 2). Such a result was seen in Treatment 1, possibly due to the lack of chemical protection of the seeds, which, when not combined with any of the bacteria, led to a reduction in grain yield; whereas in Treatment 14, possibly due to the competitiveness of the organisms in the face of the intense use of chemicals, this had a harmful effect on grain production.

In the context of this experiment, it is assumed that it is better to use the fungicide together with the insecticide and only one choice of bacteria when sowing. It then becomes necessary to study a better time or method of applying the other bacteria. Based on the present case and on research focused on soybean cultivation, such as that of Hungria, Nogueira and Araujo (2015) and Moretti *et al.* (2018), it is interesting to consider the use of an inoculant based on *R. tropici* applied via the seeds, with subsequent additional spraying of inoculants based on both *R. tropici* and *A. brasilense*.

The findings of Hungria, Nogueira and Araújo (2013) showed an increase in the root system of the bean plant due to the use of *A. brasilense*. In the present study, no differences were seen in the length or volume of the roots in the different treatments (Table 3). However, Treatments 11 and 12 afforded higher values for fresh and dry root matter, showing that more reserves were accumulated with these treatments.

Under these conditions, it can be seen that treatments with *A. brasilense*, without *R. tropici* but with insecticide or insecticide + fungicide, can cause a denser accumulation of organic matter in the roots of winter beans in clayey soil. Furthermore, there is the possibility of an increase in cytokinin concentration due to the inoculation of *A. brasilense* causing hormonal changes that favour an increase in root matter to the detriment of the shoots.

Sandy Soil

Similar to the results for the macronutrient content of clayey soil (Table 1), there was no effect from the seed treatments or inoculations in sandy soil (Table 4). According to Ambrosano *et al*. (1996), the levels of leaf nutrients in the bean plants were adequate, with the exception of calcium, which was slightly less. It is worth noting that each of the treatments received the same adequate levels of corrective agents and fertilisers, and the soil in the pots was moistened throughout the experiment, so there were no nutrient limitations on the plants.

The plants showed greater height in treatments using fungicide together with some of the bacteria, including where the insecticide was also applied, and where fungicide, insecticide and co-inoculation were used together (Table 5), demonstrating that the fungicide, by generating an environment of less competitiveness in the rhizosphere, favours initial growth in plant height by contributing to the performance of the inoculated bacteria.

No differences were recorded in the leaf chlorophyll index (Table 5), demonstrating physiological uniformity in the fully developed plants regarding the use of N in forming photosynthetic pigments.

However, there were large differences in the number of pods per plant between treatments (Table 5). There was also a compensatory effect, with greater grain production due to the smaller number of pods,

Table 3 - Mean values for main root length (mrl), root volume per plant (rvp), root fresh matter production (rfm) and root dry matter production (rdm), in bean plants in clayey soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	mrl (cm)	rvp (cm³)	rfm (g)	rdm (g)
C (1)	36.5	16.8	5.8 b	5.1 b
F (2)	43.3	15.0	4.3 b	3.4 b
I (3)	50.0	15.0	6.4 b	5.3 b
F + I (4)	40.3	15.0	4.9 b	4.5 b
R (5)	39.8	15.0	4.7 b	4.1 b
F + R (6)	44.3	13.6	5.1 b	4.5 b
I + R (7)	49.0	15.0	5.6 b	4.8 b
F + I + R (8)	39.8	16.9	6.2 b	5.6 b
A (9)	45.3	17.5	6.4 b	5.7 b
F + A (10)	40.5	17.5	6.9 b	5.9 b
I + A (11)	46.8	16.3	9.1 a	8.3 a
F + I + A (12)	44.3	20.0	11.1 a	10.4 a
R + A (13)	40.0	17.5	7.7 b	6.2 b
F + I + R + A (14)	40.5	17.5	6.3 b	5.5 b
CV%	16.62	14.35	24.72	28.98

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Mean values followed by different letters in a column differ by Scott-Knott test ($P < 0.05$). Source: The author resulting in a smaller difference in the number of grains per plant between treatments. Even with this balance, there was a larger number of pods and grains in the treatment with insecticide + *R. tropici*, in addition to the lower yield in the treatment with fungicide + insecticide + co-inoculation (Treatment 14).

Table 4 - Mean macronutrient content of the leaves of winter beans in sandy soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	nitrogen	phosphorous	potassium	calcium	magnesium	sulphur
	(g kg^{-1})					
C (1)	39.7	3.9	25.3	5.1	3.6	5.7
F (2)	41.5	4.2	24.8	4.7	3.2	4.5
I (3)	45.0	4.4	26.1	5.9	3.1	4.8
F + I (4)	45.5	4.4	24.3	7.0	3.4	5.5
R (5)	43.5	3.8	22.3	6.5	3.4	5.7
F + R (6)	42.6	3.8	22.0	5.0	3.0	4.5
I + R (7)	45.6	4.6	25.7	7.0	3.9	4.9
F + I + R (8)	45.5	4.6	27.7	5.3	3.5	5.5
A (9)	33.4	4.2	26.8	7.2	4.5	6.1
F + A (10)	45.0	3.9	25.4	7.1	3.9	4.9
I + A (11)	47.2	4.3	27.8	5.6	3.6	5.9
F + I + A (12)	48.9	5.0	25.2	6.6	3.6	5.7
R + A (13)	43.5	4.2	24.5	6.3	3.7	5.9
F + I + R + A (14)	41.9	4.1	24.4	5.6	3.6	6.6

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Source: The author

Table 5 - Mean values for plant height (ph), leaf chlorophyll index (lci), shoot fresh matter production (sfm), shoot dry matter production (sdm), number of pods per plant (npp), number of grains per plant (ngp), and grain weight per plant at 13% moisture (gw), in bean plants in sandy soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	ph (cm)	lci	sfm (g)	sdm (g)	npp	ngp	gw (g)
C (1)	11.0 a	52.5	36.7 c	21.2 a	11.1 d	27.1 b	6.6 c
F (2)	9.8 b	50.6	45.3 b	25.4 a	14.5 b	34.6 a	9.3 b
I (3)	9.5 b	53.9	39.4 c	23.5 a	10.9 d	35.0 a	12.2 a
F + I (4)	10.1 b	54.8	37.3 c	23.2 a	13.0 c	35.8 a	10.7 b
R (5)	9.7 b	49.8	38.0 c	23.0 a	12.5 c	38.9 a	12.5 a
F + R (6)	11.3 a	52.1	49.4 a	22.9 a	14.3 b	37.8 a	10.7 b
I + R (7)	9.2 b	54.8	43.8 b	22.2 a	17.9 a	36.1 a	12.9 a
F + I + R (8)	10.7 a	51.2	36.0 c	20.0 a	12.4 c	29.6 b	7.7 c
A (9)	10.0 b	52.6	43.8 b	24.1 a	13.0 c	38.3 a	11.0 b
F + A (10)	10.5 a	54.0	44.0 b	18.1 b	14.8 b	34.8 a	9.9 b
I + A (11)	8.4 b	53.6	37.7 c	16.5 b	13.6 b	30.0 b	4.5 d
F + I + A (12)	10.6 a	49.7	38.2 c	15.6 b	10.6 d	28.8 b	6.7 c
R + A (13)	10.1 b	52.3	34.2 c	16.1 b	12.6 c	29.8 b	7.7 c
F + I + R + A (14)	12.2 a	51.7	28.6 d	15.9 b	9.9 d	18.9 c	4.5 d
CV%	10.44	4.59	6.17	10.20	12.18	17.46	12.99

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Mean values followed by different letters in a column differ by Scott-Knott test ($P < 0.05$). Source: The author

Based on the production of shoot fresh matter, it can be inferred that the plants in Treatment 6 (fungicide together with *R. tropici*) were more vigorous. Again, it can be seen that the use of *A. brasilense* (Table 5) did not stand out for shoot fresh matter production, which was also significantly lower in Treatment 14. Due to the shortening of the plant cycle with the advancement of leaf senescence, the loss of water probably resulted in lower values for fresh matter production in this treatment.

The use of *A. brasilense* together with fungicide, insecticide or *R. tropici* resulted in poor shoot dry matter production (Treatments 10, 11, 12, 13 and 14), but when used exclusively (with no seed treatment or co-inoculation – Treatment 9) afforded better results than the other treatments (Table 5).

Grain production (13% moisture) was higher in three of the treatments: Treatment 3 - where only insecticide was used, Treatment 5 - where only *R. tropici* was used, and Treatment 7 - where insecticide and R tropici were used together. In the other treatments, the values were lower, especially Treatment 11, where insecticide together with *A. brasilense* was used, and in Treatment 14, where a complete treatment of fungicide, insecticide and bacterial co-inoculation was carried out (Table 5), unlike the benefits of co-inoculation seen by Bulegon *et al.* (2016) under other conditions. Cardoso *et al*. (2021) found no differences in productivity in treatments using inoculant based on *R. tropici* via seed application, and related this to possible interference from the high levels of nutrients in the soil. In turn, Teixeira *et al*. (2022), using seed inoculation and reapplying the liquid inoculant at stage V4 (257 mL ha^{-1}) achieved higher productivity in 'BRS Cometa' beans with no mineral-N supplement.

It can be seen that, just as in clayey soil (Table 2), lower plant height during the initial phase (V3) does not lead to poorer production, since Treatments 3, 5 and 7 did not stand out for plant height (Table 5).

Unlike the results for clayey soil (Table 2), those for sandy soil show that when the aim of planting is to ensure safety, both in terms of preventing fungal disease, and in places under pressure from early attack by pests of the bean crop, one option would be seed application of the chemicals only, adding the bacteria later via another route, as in Moretti *et al*. (2018) in the soya bean, since Treatments 8 and 12, despite including seed application of only one of the bacteria, showed very low grain yields.

No significant differences were seen in root length (Table 6), however, root volume showed greater development in plants from seeds treated with *A. brasilense*, demonstrating its hormonal effect; except when used alone (Treatment 9), where it resulted in greater shoot dry matter production (Table 5), or together with fungicide + insecticide + *R. tropici* (Treatment 14), where its effect on dry matter and grain production in sandy soils was detrimental. There was a similar situation from the use of *R. tropici* which, together with the fungicide or insecticide, presented high values (Table 6).

Table 6 - Mean values for main root length (mrl), root volume per plant (rvp), root fresh matter production (rfm), and root dry matter production (rdm), in bean plants in sandy soil as a function of the seed treatment. Ilha Solteira, 2019

treatment	mrl (cm)	rvp (cm^3)	rfm (g)	rdm (g)
C (1)	40.8	24.3 b	11.5 a	10.9 b
F (2)	45.5	31.3 a	14.2 a	13.7 a
I (3)	39.4	24.5 b	14.6 a	14.0 a
F + I (4)	39.5	19.9 b	7.9 b	7.3 b
R (5)	43.3	35.0 a	14.4 a	13.6 a
F + R (6)	52.5	26.3 a	12.7 a	11.6 a
I + R (7)	47.0	26.8 a	12.5 a	11.9 a
F + I + R (8)	41.8	18.8 b	11.7 a	10.1 b
A (9)	47.5	21.3 b	8.2 b	7.8 b
F + A (10)	58.8	31.0 a	12.7 a	12.3 a
I + A (11)	44.5	27.5 a	14.0 a	13.2 a
F + I + A (12)	41.5	29.0 a	9.5 b	8.9 b
R + A (13)	48.3	30.0 a	10.5 b	9.9 b
F + I + R + A (14)	45.3	23.8 b	9.9 b	9.3 b
CV%	18.85	13.60	16.80	18.24

Note: C = Control; F = Fungicide; I = Insecticide; R = *Rhizobium tropici*; A = *Azospirillum brasilense*. Mean values followed by different letters in a column differ by Scott-Knott test (P < 0.05). Source: The author

The production of fresh and dry root matter was lower in treatments 4, 9, 12, 13 and 14 (Table 6), which included, respectively, fungicide + insecticide, *A. brasilense* alone, *A. brasilense* together with fungicide + insecticide, *A. brasilense* together with *R. tropici*, and the complete treatment of fungicide + insecticide and co-inoculation. This shows that when using *A. brasilense,* a greater accumulation of fresh and dry matter was only possible when the bacteria were combined either with the fungicide or the insecticide when treating the seeds. Except for the above treatments using *A. brasilense* together with *R. tropici*, the other treatments with *R. tropici* were superior in terms of fresh and dry root matter accumulation in sandy soil.

In order to increase the acceptance of inoculation and co-inoculation of bean plants as a tool by farmers, a larger database is needed to clarify in which situations such applications are the most effective.

Among the possible factors that still cause results to vary are the failure to adopt good inoculation practices (Nogueira *et al*., 2018), the harmful effects of the active ingredients of fungicide products used in treating the seeds in direct contact with the inoculant (Silva *et al*., 2020), or even competition between bacteria in the rhizosphere, whether due to the high population of native host bacteria in some soils or the joint application of the two species from the inoculants.

The present research shows that grain production (Tables 2 and 5) in both clayey and sandy soils was adversely affected by joint use of the fungicide and insecticide, both chemical, together with the co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* via the seeds, due to generating a harmful rhizospheric environment whose effects prevented the bacteria from realising their full potential for plant development.

CONCLUSIONS

1. The joint use of fungicide and insecticide, and the co-inoculation of *Rhizobium tropici* and *Azospirillum brasilense* via the seeds, reduced grain production in winter beans in clayey and sandy soils, an effect also seen when using the insecticide together with *A. brasilense* in sandy soil;
2. The use of fungicide + insecticide, fungicide + *R. tropici*, or fungicide + insecticide + *R. tropici* favoured shoot development, while the use of *A. brasilense*, without *R. tropici* and with insecticide or insecticide + fungicide, favoured greater root growth in clayey soil;
3. Grain production in the winter bean in sandy soil was greater when treating the seeds only with insecticide, only with *R. tropici*, or with a combination of both.

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