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A N I N T E R N A T I O N A L J O U R N A L

Effect of Inoculation Associated to Leaf Sprayed Co+Mo on the Yield and Grain Nutrients in Common Bean (*Phaseolus vulgaris* L)

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ABSTRACT

The objective of this work was to evaluate the efficiency of nitrogen fixing inoculum associated with Co + Mo leaf spray on the common bean grain yield and grain nutrients, cv. FT Nobre. Three dosages of the inoculant (0, 200 and 400 g/50 kg seeds), combined with four Co + Mo leaf spray levels (T0=0,0; T1=4.9,49; T2=7.3,73; and T3=9.7,97 g ha^{-1} of Co and Mo, respectively) were tested. The grain yield with the use of the inoculant (400 g / 5O kg seed^{-1}) associated with the higher level of Co+Mo (T2 and T3) was very similar to the mineral nitrogen condition fertilizer recommended for the bean (70 kg ha^{-1} of N). With the increased inoculant dosage, an increase of the protein content and of P and Mg in the grain was also observed. The results indicated that the mineral nitrogen source could be replaced by inoculation of the seeds with Rhizobium tropici *combined with Co + Mo leaf spray.*

Key words: Seed quality, phosphorus, magnesium, nitrogen

INTRODUCTION

There are many factors that limit the common bean production witch can decrease the efficiency of the *Rhizobium*-leguminosae association. Some factors are related to the plant physiology, which make the plant slow the ability of nodulating easily with native isolates of *Rhizobium* that are naturally present in the soil rizosphere. However, they are many times inefficient in fixing the nitrogen, thus imposing the limitations to biological nitrogen fixation (Hungria et al., 1994 and Denardin, 1991). In Latin America, the beans breeding programs have considered as priority for the release of early maturity cultivars, with high grain yield and highly responsive to the mineral nitrogen fertilization (Tsai et al., 1993). These characteristics confer negative effect to the biological nitrogen fixation, which, associated with the highly demand for nitrogen for the grain production, turn the crop highly dependent on the mineral nitrogen fertilizer (Velasquez et al., 1988). Although common beans show a high demand for the nitrogen, mineral nitrogen fertilizers usage has decreased on small farms, mainly because of its high price. Consequently, bean grain yield has been in low ranges, close to 900 kg ha^{-1} (D'Agostini et al., 2001). Under this condition, seeds inoculation with *Rhizobium* could be a more reasonable and economic advantageous way of releasing the nitrogen to the plants without jeopardizing the grain yield (Mendes et al., 1994).

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Seeds inoculation with *Rhizobium* must assure a number of *Rhizobium* cells in the root system that enable an abundant and effective nodulation (Katiyar and Pant, 1993). However, the nodulation success depends on the factors that are related to the efficiency of the isolate and ability to compete in the soil, inoculant quality and control of the environmental factors that may negatively affect *Rhizobium* survival (Hungria et al., 1994). Under favorable climate and soil fertility conditions, common bean inoculated with efficient *Rhizobium* isolates can yield from 1500 kg ha^{-1} (Franco and Balieiro, 1999) to 2500 kg ha^{-1} (Mendes et al., 1994). These values are higher than the Brazilian common bean grain yield average, which is around 745 kg ha^{-1} (IBGE, 2004).

The symbiotic common bean is more sensitive to climatic adversities and nutritional deficiency than other plants that have nitrogen available from the mineral sources. This is a consequence of the necessity that the symbiotic plant has to keep not only its physiological processes, but also the specific requirements of the symbiotic system (Munn and Franco, 1982). Thus, the proper micronutrients supply, such as molybdenum (Mo) and cobalt (Co), can contribute to increase the biological nitrogen fixation efficiency (Dechen et al., 1991). There are studies that show that bean inoculation with *Rhizobium* can increase the grain yield, especially when combined with Co + Mo leaf spray (Oliveira, 1996; Vieira et al., 1994).

In a study in which Mo was supplied at 40 g ha^{-1} under the acid soil conditions and without liming, there was a bean grain yield increase, even higher than the yield resulted from the treatment with soil pH correction (Oliveira, 1996). In other places in Brazil, Mo sprayed in the bean plants was responsible for the grain yield increase of 125 kg ha^{-1} (Vieira et al. 1994).

Cobalt use is justified because it is related to cobaltine enzyme, which catalyses the biochemical reactions involved in oxygen transport to the bacterioid inside the nodules (Campo et al., 1999). Due to its important role in nitrogen fixation, Co supply is considered highly important in the bean production (Oliveira et al., 1996). In preliminary studies with the application of Mo and Co in common beans, seeds production, straw production, and plant height increased to 100 and 130%, 61 and 49%, and 18 and 9%, respectively. Together, these micronutrients showed to be more effective than phosphate (P_2O_5) applied in the dosage of 1,000 kg ha^{-1} (Junqueira Netto et al., 1977).

Rhizobium inoculant combined with Co + Mo was tested in a greenhouse study, with a native soil type Latossolo vermelho distroférrico from the Brazilian Cerrado. In this study, with different levels of Mo (0, 7, 14 and 21 g ha^{-1} of Mo) and Co (0, 0.3; 0.6 and 0.9 g ha^{-1} of Co), the results showed that Mo added to the inoculant resulted in an increase in the number of the pods per plant, grain number per pod, and grain yield. The highest grain yield level was obtained from the treatment in which Mo and Co were added to the dosage of 14 and 0.6 g ha^{-1}, respectively (Corrêa et al., 1990).

Despite the variety of results already known, there are no available data regarding the association of the inoculant use on the bean seeds and application of Co + Mo through leaf spray, in the field conditions in State of Santa Catarina - Brazil. Thus, the objective of this work was to evaluate the effect of three dosages of inoculant, combined with four levels of Co + Mo sprayed on the bean leaves of the common bean cultivar FT Nobre on grain yield, as well as grain nutrient accumulation, such as the protein, phosphorus, magnesium and zinc.

MATERIAL AND METHODS

The soil from the experimental plot was classified as Nitossolo Vermelho distroférrico (Embrapa, 1999), with the following characteristics: 4% sand, 28% silt and 68% clay; 3.5% organic matter; pHH_2O 5.0; pHSMP 5.3; 2.8 mg P dm^{-3}; 45 mg K dm^{-3}; 2.1 mg Zn dm^{-3}; 1.2 $cmol_c$ dm^{-3} Al; 3.9 $cmol_c$ dm^{-3} Ca; 2.7 $cmol_c$ dm^{-3} Mg (Tedesco et al., (1995). Twelve treatments of combinations were tested between three dosages of the inoculant (0, 200 and 400 g of inoculant / 50 kg of seeds) and four levels of Co+Mo leaf spray (T0: 0 and 0; T1: 4.9 and 49; T2: 6.3 and 63; and T3: 9.7 and 97 g ha^{-1} of the Co and Mo, respectively). The mineral nitrogen fertilizer (70 kg ha^{-1} of N ha^{-1}) was used as reference for the grain yield in the year the experiment was conducted.

The experiment design were completely randomized blocks with four replicates. Each plot had six rows with six meters length, and 0.5 m between rows. For harvesting, four internal rows of each plot five meters center were used. The soil was prepared in the conventional way, with ploughing followed by two narrow wings. Soil pH correction was done and fertilizers (for phosphorus and potassium) according to the recommendations for the bean production by the Comissão de Fertilidade do Solo RS/SC (1995). Liming was applied three months before the sowing at the rate of 50% of the dosage in order to obtain pHH_2O 6.0 (Zago, 2001). The fertilizer was applied at the rate of 75 kg ha^{-1} of P_2O_5 (triple super phosphate) and 70 kg ha^{-1} of K_2O (Potassium chloride). Mineral nitrogen fertilizer (70 kg ha^{-1} of N) was applied only two times: at sowing (20 kg ha^{-1} of N), and dressing at 30 days after the emergence of the plant (50 kg ha^{-1} of N). The fertilizers were placed in the plating row, before sowing, 10 cm deep, and then they were incorporated in the soil in order to avoid the salinity effects in the seeds.

The common bean seeds (*Phaseolus vulgaris* L.) of FT Nobre cultivar, were inoculated with the commercial inoculant peat based with *Rhizobium tropici* isolates SEMIA 4077 and SEMIA 4080, with the minimum concentration of 1.0 x 10^8 viable cells per gram. The inoculants were applied at the rates of 200 and 400 g / 50 kg of the seeds. The inoculation was performed 2 h before the sowing, with the addition of 400 mL sugar solution (20%) to 50 kg seeds (Brandão Junior and Hungria, 2000). The sowing was performed in early December, adding 15 seeds per meter, achieving 250.000 plants ha^{-1}. The leaf spray with the micronutrients Co+Mo was performed when the plants were in the V4 and R5 stages, using a protective frame to avoid the contamination among plots.

During this study, the development of the weeds were kept under the control and the pests were controlled at the developmental stages V2/V3 and V4, with metamidofós (300 mL ha^{-1} of active ingredient). Benomyl (50 g ha^{-1} of active ingredient) was sprayed at stages R5 and R7 in order to protect the plants from *Colletotrichum lindemuthianum* infection. The harvest was done when all the plants from each plot were dried and grain yield and 1000 grain weight were estimated, taking into consideration the grain humidity of 13 % (Brasil, 1992).

Bean Grain evaluations

The grain total protein, phosphorus, magnesium and zinc was analyzed using the sulfuric acid digestion methodology (Malavolta et al., 1997). The results were statistically analyzed and regression curves were calculated, considering 12 treatments from the combinations between three inoculant dosages and four levels of Co+Mo leaf spray.

RESULTS AND DISCUSSION

The use of increasing doses of the inoculant showed a significant effect (P<0,05) in the grain yield and accumulation of the protein, phosphorus, magnesium and zinc in the grains. The Co+Mo leaf spray fertilizer and their interaction with inoculant presented significant effect (P<0.05) for the grain yield and accumulation of zinc in the grains, but the weight of a thousand seeds did not respond to the application of inoculant and or Co+Mo isolated (Table 1 and Table 2).

Table 1 - Analysis of variance of 1000 seed weight (M1000s), grain yield, protein accumulative in bean grains as a function of *Rhizobium* inoculation and Co + Mo leaf spray, either isolated or applied combined. DF = degree of freedom; MQ = medium square; CV= coefficient of variation.

Treatment	DF	MQ		
		M1000s	Grain yield	Protein
Bloco	3	20.67 ns	3013.13 ns	7.41 ns
Inoculant	2	26.20 ns	179503.20***	43.54 ***
Co+Mo leaf spray	3	14.29 ns	18702.31***	1.13 ns
Inoculant*Co+Mo leaf spray	6	26.70 ns	9835.83**	3.54 ns
Error	33	23.40	2527.20 ns	2.84
CV %		2.66	2.99	4.08

* - Statistically significant by test F, 5 % probability. ** - Statistically significant by test F, 1 % probability. *** - Statistically significant by test F, 0.1 % probability. ns - Statistically not significant.

Table 2 - Analysis of variance of Phosphorus (P), Magnesium (Mg), and Zinc (Zn) accumulative in bean grains as a function of Rhizobium inoculation and Co + Mo leaf spray, either isolated or applied combined. DF = degree of freedom; MQ = medium square; CV= coefficient of variation.

Treatment	DF	MQ		
		P	Mg	Zn
Bloco	3	0.0015 ns	0.0013 ns	0.00000011 ns
Inoculant	2	0.0076 ***	0.0122 **	0.00005015 ***
Co+Mo leaf spray	3	0.0040 ns	0.0049 ns	0.00000621**
Inoculant*Co+Mo leaf spray	6	0.018 ns	0.0049 ns	0.00001526***
Error	33	0.0016	0.0022	0.00000072
CV %		6.19	9.87	10.04

* - Statistically significant by test F, 5 % probability. ** - Statistically significant by test F, 1 % probability. *** - Statistically significant by test F, 0.1 % probability. ns - Statistically not significant.

Increasing inoculant dosage caused a linear positive effect on the grain yield at all the levels tested with the Co+Mo leaf spray and more significantly at the higher dosages of Co+Mo leaf spray (dosage: 9.7 with 97 and 7.3 with 73 g ha^{-1} of Co and Mo, respectively) (Fig. 1A). On the other hand, when the levels of Co+Mo leaf spray were applied, their beneficial effect on the grain yield was more significant upon the application of 400 g of the inoculant (Fig. 1B). The grain yield from the treatment with inoculant and Co+Mo (400 g inoculant and Co + Mo - T2 and T3) was 1,891 kg ha^{-1}, similar to the one from the mineral nitrogen fertilizer recommended for the beans (70 kg ha^{-1} of N ha^{-1}) which was 1,981 kg ha^{-1}. The use of the inoculant associated with Co+Mo could be an interesting approach to increase the grain yield.

When the inoculant was applied alone (T0, Fig. 1A), it caused increase in the grain yield, but when Co+Mo was applied alone, wasn't the same (without inoculante, Fig. 1B). This effect could be explained due to the better infection of the roots by the symbiotic bacteria (Ferrarezi, 2002). According to Hungria et al. (2001) and Vargas et al. (1979), a higher dosage of inoculant reflected in higher probability of nodulation by the required bacteria. However, the effect of Co+Mo was to contribute in better nitrogen fixation, increasing the nitrogen metabolism (Dechen et al., 1991).

Similar results were obtained by increasing dosages of the inoculant, on which the grain production was observed as a result of Co and Mo application. In this study, when Co was added to increasing dosage of inoculant, the grain yield increased 100% and when Mo was added, it increased 130%. The dry matter increased 61% when Co was added, whereas it increased 49% when Mo was added. Together, these micronutrients were as efficient as super phosphate equivalent to 1,000 kg ha^{-1} (Junqueira Netto et *al.*, 1977).

In an experiment conducted in the greenhouse, with the soil of Cerrado classified as Latossolo Vermelho Eutrófico, increasing levels of Mo (0, 7, 14 e 21 g ha^{-1} of Mo) and Co (0, 0,3; 0,6 e 0,9 g ha^{-1} of Co), applied to the seeds and also combined with the inoculant were able to increase the pods/plant, seeds/pod and grain yield. The highest grain yield was achieved when Co+Mo were added at the rates of 0.6 g ha^{-1} and 14 g ha^{-1}, respectively. In this study the satisfactory effect of Co was observed (Corrêa et al., 1990).

The absence of response to the increasing levels of the leaf spray of Co+Mo on the grain associated with the seeds inoculation using the lower inoculant dosages (Fig. 1A) could be due to the low efficiency of the soil native bacteria in nitrogen fixation (Denardin, 1991). In this kind of situation, the leaf spray with the micronutrients was not viable because it dit not contribute to increase the grain yield. Similar results were also obtained in other studies (Vieira, 1995; Zago, 2001).

Like grain yield, a positive response was also observed with the we increased inoculant dosage in protein content, P and Mg in the bean grain both with linear positive response (Fig. 2). The P increased from 0.62 mg grain^{-1} (no inoculant) to 0.66 mg grain^{-1} (400 g of inoculant / 50 kg of the seeds); whereas Mg increased from 0.45 mg grain^{-1} (no inoculant) to 0.50 mg grain^{-1} (400 g of the inoculant / 50 kg of the seeds). The protein content in the grain increased from approximately 40 mg grain^{-1} (in the treatments: no inoculant and 200 g of the inoculant / 50 kg of the seeds) to 43 mg grain^{-1} (400 g of the inoculant / 50 kg of the seeds).

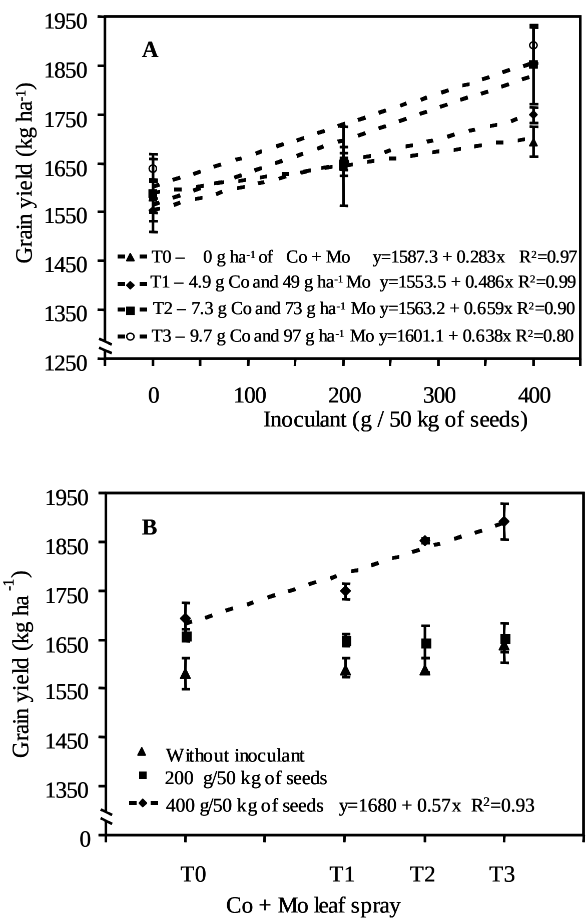

Figure 1 - Grain yield as a function of increasing dosages of Co + Mo leaf spray, and inoculation with *Rhizobium*. To T0 = 0 g ha^{-1} of Co+Mo; T1 = 4.9 g ha^{-1} of Co + 49 g ha^{-1} of Mo; T2 = 7.3 g ha^{-1} of Co + 73 g ha^{-1} of Mo; T3 = 9.7 g ha^{-1} of Co + 97 g ha^{-1} of Mo. Error bars (n=4).

The observed increases in P, Mg and protein accumulation in the grain due to the usage of higher dosage of the inoculant could be due to better biological nitrogen fixation that resulted in better nodulation. This could be explained by the high efficiency of the translocation of the biological nitrogen in the grains (Hungria et al., 1985). The dosage of 400 g of the inoculant / 50 kg of the seeds led to high yield (Fig. 1A) and P, Mg and protein accumulation in the grain (Fig. 2). This showed that the inoculant use could be a practice that substitute the mineral nitrogen fertilization. The use of increased doses of the inoculant associated only with Co+Mo should be considered carefully since Zn accumulation in the grains was reduced. This decrease in the Zn accumulation in the grains was from 10 µg $grain^{-1}$ to 4.7 µg $grain^{-1}$ (Fig. 3A).

The effect of the crescent levels of Co+Mo leaf spray on the inoculant was positive to Zn accumulation in the grains only with 200 g inoculant per 50 kg of the seeds (Fig. 3B). However, Zn accumulation in the grains decreased when the crescent levels Co+Mo leaf spray were used associated with 400 g the inoculant per 50 kg of the seeds (Fig. 3B). The decrease of the Zn accumulation in the grains could not be explained by the available biological nitrogen, since the nitrogen increase did not necessarily cause an increase in the amount of Zn in the grains, but caused significant increases in the grain yield (Farinelli et al., 2006).

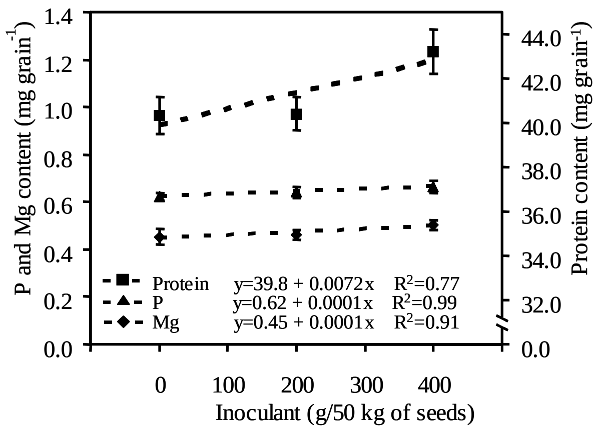

Figure 2 - Accumulation of phosphorus, magnesium and protein in bean grains as a function of increasing *Rhizobium* inoculant dosages. Error bars of n=4.

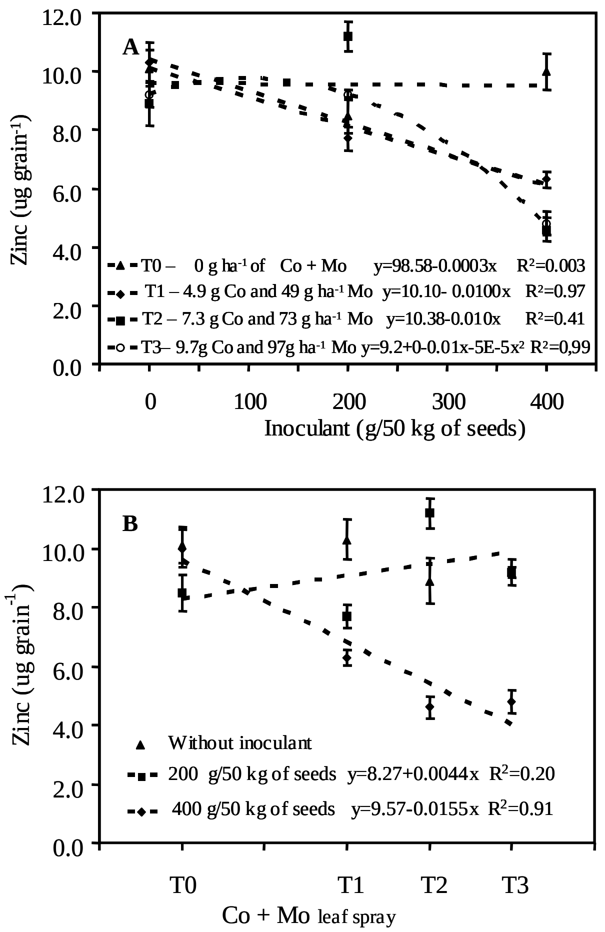

Figure 3 - Zinc accumulation in bean grain as a function of increasing dosages of Co + Mo leaf spray and inoculation with *Rhizobium*. To T0 = 0 g ha^{-1} of Co+Mo; T1 = 4.9 g ha^{-1} of Co + 49 g ha^{-1} of Mo; T2 = 7.3 g ha^{-1} of Co + 73 g ha^{-1} of Mo; T3 = 9.7 g ha^{-1} of Co + 97 g ha^{-1} of Mo. Error bars (n=4).

CONCLUSION

The use of the inoculant associated with the higher level of Co+Mo was favorable in the increased grain yield and the use of the inoculant alone increased the protein content, P and Mg in the grain. In this case, based on results, it could be recommend that the use of *Rhizobium* inoculation associated with Co+Mo leaf spray could be a substitute for the mineral nitrogen fertilizers recommended for the bean.

RESUMO

O objetivo do trabalho foi avaliar o uso do inoculante associado ao uso de adubação foliar de Co+Mo sobre a produtividade de grãos, acúmulo de nutrientes nos grãos de feijão comum, cv FT Nobre. Testaram-se 3 doses de inoculante (0, 200 e 400 g/ 50 kg de sementes) combinadas com 4 níveis de adubação foliar de Co+Mo, (T0=0 e 0; T1=4,9 e 49; T2=7,3 e 73; e T3=9,7 e 97 g ha^{-1} de Co e Mo, respectivamente).

A produtividade dos grãos com o uso de inoculante (400 g / 50 kg de sementes) associado a alto nível de Co+Mo (T2 e T3) foi semelhante ao fornecimento de nitrogênio mineral (70 kg ha^{-1}). O aumento das doses de inoculante também favoreceu o aumento dos teores de proteína, P e Mg nos grãos. Esses resultados indicam que o nitrogênio na forma de fertilizante mineral pode ser substituído pela prática da inoculação das sementes de feijão associado à adubação foliar de Co+Mo.

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