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Thiamine, cobalt and molybdenum applied as seed treatment influence the development of soybean crops[1]

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ABSTRACT - Cobalt (Co) and molybdenum (Mo) are essential elements with a fundamental role in biological nitrogen fixation in legumes. Vitamins such as thiamine, despite being required in small quantities, influence plant growth. This study aimed to assess the efficiency of different doses of thiamine applied as seed treatment, combined or not with Co and Mo, in enhancing the development of soybean crops. The experiment was conducted in a greenhouse according to a randomized block design with a 6 × 2 factorial arrangement, comprising six thiamine doses (0, 10, 25, 50, 100, and 200 mg kg^{-1}) in the presence or absence of Co and Mo at the recommended rate of 100 mL ha^{-1}. At 40 days after sowing, plants were analyzed for root length, root dry weight, shoot length, shoot dry weight, nodule number, and nodule dry weight. Principal component analysis showed that combined application of Co and Mo with thiamine was negatively associated with all variables and that the thiamine doses most positively associated with the analyzed variables were 50 and 200 mg kg^{-1}. In treatments containing thiamine alone, there was a linear direct relationship between thiamine dose and nodule dry weight. Root and shoot lengths and dry weights were highest in plants treated with thiamine only at a dose of 122 mg kg^{-1}. Application of Co and Mo combined with thiamine via seed treatment does not promote the development of soybean crops. Thiamine application is a promising treatment to increase shoot length, root dry weight, and nodule dry weight in soybean.

Key words: Micronutrient. Nodulation. Seed coating. Vitamin B1.

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INTRODUCTION

Legumes are renowned for their ability to fix atmospheric nitrogen (N) through biological nitrogen fixation (BNF), owing to their symbiosis with bacteria of the genus *Bradyrhizobium*. This ability indirectly accounts for the great economic viability of soybean crops, given that N-fixing bacteria are low-cost and atmospheric N is a virtually inexhaustible resource. By contrast, N fertilization is a costly procedure. Thus, in legume cultivation, BNF minimizes production costs associated with N application. Interestingly, mineral elements such as cobalt (Co) and molybdenum (Mo) are essential for BNF in legumes and were shown to reduce the need for N fertilizers (Santos Neto *et al*., 2018).

Mo participates in the BNF process as a catalyst of nitrogenase, an enzyme responsible for the conversion of atmospheric N into ammonia, in addition to acting as an electron donor to nitrate reductase, an enzyme complex responsible for the assimilation of nitrate by plants (Taiz; Zeiger, 2004). Co, on the other hand, is necessary for the synthesis of cobalamin (vitamin B12). Cobalamin participates in the formation of leghemoglobin, which has great affinity for oxygen and regulates oxygen concentration in root nodules, preventing nitrogenase inactivation. Furthermore, it has been demonstrated that N-fixing microorganisms require Co, regardless of whether they are growing within nodules or not (Minz *et al.*, 2018).

Although needed in low quantities, vitamins are essential for plant metabolism (Vannucchi; Cunha, 2009). At adequate concentrations, these organic compounds modulate the production of growth factors, exerting a profound influence on key physiological processes, such as enzyme synthesis (Hathout, 1995).

Thiamine (vitamin B1), a vitamin belonging to the B vitamin complex, serves as a cofactor for several plant enzymes. This vitamin plays an essential role in carbohydrate metabolism and is capable of enhancing the absorption of nutritional elements, which helps in maintaining and increasing energy reserves, in addition to activating plant defense mechanisms against biotic and abiotic stresses (Abdallah *et al.*, 2016; Kaya *et al.*, 2015). These effects can generate gains in the vegetative and reproductive development of plants (Colla *et al*., 2021; Soltani; Saffari; Moud, 2014; Vendruscolo; Oliveira; Seleguini, 2017), in addition to enhancing photosynthetic capacity under stress conditions (Ramos *et al*., 2021; Vendruscolo *et al*., 2022), and increasing the levels of photosynthetic pigments (Vendruscolo et al., 2021).

Thiamine application by seed immersion before sowing improved the development and yield of wheat crops (El-Bassiouny *et al.*, 2014). Thiamine applied via immersion or foliar treatment increased macronutrient levels in different genotypes of corn (Kaya *et al.*, 2015). Moreover, thiamine application increased seed vigor, stimulated seedling emergence, and improved yield parameters in common bean (Vendruscolo *et al.*, 2018a).

Considering the above, this study examined the hypothesis that seed treatment with thiamine combined with Co and Mo enhances the development of root nodules for BNF. The aim was to assess the effect of seed application of different doses of thiamine associated or not with Co + Mo on nodulation, root development, and shoot development in soybean crops.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse using seeds of soybean 97R50 IPRO. The experimental design was completely randomized according to a 6 × 2 factorial arrangement, with four replications. Treatments comprised six thiamine doses (0, 10, 25, 50, 100, and 200 mg kg^{-1}) in the presence or absence of Co + Mo at the recommended rate of 100 mL ha^{-1} commercial product.

Thiamine hydrochloride (99.3% a.i.) was used to prepare a 10,000 ppm solution. This stock solution was then diluted to the desired concentrations. Application was performed shortly before sowing. Seeds were placed in transparent plastic bags, and a pipette was used to add the solution (2 mL of solution for every 200 seeds). Bags were shaken for about 2 min to distribute the product over the seeds. The Co + Mo treatment was applied after the vitamin. Subsequently, seeds were inoculated with *Bradyrhizobium japonicum* at the recommended dose of 2 mL kg^{-1} seed.

The soil collected for the experiment (0–20 cm layer) was evaluated for chemical properties and texture (Table 1). Subsequently, the soil was limed to meet the requirements of soybean crops, following the recommendations of Sousa and Lobato (2004), without N application. The collected soil was added to 5 dm^3 pots (20 cm height, 20 cm width, and 17 cm bottom diameter), and sowing was carried out.

Sowing was performed by adding eight seeds per pot in an equidistant manner over the total area of the pot at a depth of 3 cm. After 20 days, plants were thinned to five plants per pot to minimize competition. Irrigation was carried out daily by using a manual watering can. Analyses of shoot length, root length, shoot dry weight, root dry weight, nodule number, and nodule dry weight were performed at 40 days after sowing, when plants were at the R1 stage. This period corresponds to the peak activity of N-fixing bacteria. Seedlings were removed from the pots. Subsequently, the roots were washed and separated from the shoots. Shoot and root lengths were measured in centimeters.

Table 1 - Chemical properties and texture of the 0–20 cm layer of the soil used in the experiment

pH $CaCl_2$	OM g.dm^{-3}	P1	K+	Ca^{2+}	Mg^{2+}	H+Al	SB*	CTCT*	BS*
-------- mg.dm^{-3} --------		-------------------------- cmol$_c$.dm^{-3} ----------------------------					----------- % -----------		
4.8	21.9	12.7	0.11	1.9	0.3	5.2	2.31	7.51	30.75
-- Classification* -------------------------------------									
Medium	Medium	High	Adequate	Adequate	Adequate	-	-	High	Medium
S	B	Cu	Fe	Mn	Zn	Clay	Silt		Sand
------------------------------------ mg.dm^{-3} ------------------------------------						---------------------- (g.dm^{-3}) ----------------------			
11.1	0.12	0.5	27	8.3	4.1	455	75		470
-- Classification* --									
High	Low	Medium	High	High	High	-	-	-	-

OM, organic matter; SB, sum of bases; CTCT, total cation-exchange capacity; BS, base saturation P, K, Fe, Zn, Mn, and Cu were extracted with Mehlich^{-1}. SB = Ca2+ + Mg2+ + K+. CECT = K+ + Ca2+ + Mg2+ + H + Al. BS = (K+ + Ca2+ + Mg2+)/CECT * Sousa and Lobato (2004)

Root, shoot, and nodule dry weights were measured by drying samples in kraft bags in a forced air circulation oven at 65 °C for about 72 h. After this period, nodules were separated from the roots and counted. Nodules, roots, and shoots were weighed separately. Results are the mean of five plants per treatment.

The data were subjected to analysis of variance. Differences between plants treated or not with Co + Mo were assessed using Tukey's test ($p < 0.05$). Thiamine doses were compared using polynomial regression ($p < 0.05$). Principal component analysis (PCA) was performed using R *software*. For PCA, variables were normalized to mean 0 and variance 1.

RESULTS AND DISCUSSION

The interaction effects of thiamine dose and Co + Mo treatment were significant for nodule dry weight, root length, shoot length, and root dry weight (Table 2). Nodule number and shoot dry weight were significantly influenced by the main effects of Co + Mo. The parameters achieved their highest values in the absence of Co + Mo (Table 2). The reduction in the shoot dry weight of seedlings may be associated with the harmful effect of increased doses of mineral elements in seed treatment. Excess Co can generate oxidative stress, impairing N metabolism, photosynthesis, and the antioxidant system, which also results in a decrease in dry matter accumulation (Ali *et al.*, 2010).

Negative effects of Co + Mo application in soybean seeds were also reported by Silveira *et al.* (2021). The authors observed that the salinity of the formulated products impaired water absorption during germination, drastically affecting bacterial survival, nodulation, and N_2 fixation. Therefore, it is important to know the quality and source of the chosen Co + Mo product, especially when treating seeds.

Shoot length was higher in seedlings that were seed-treated with 50 or 200 mg kg^{-1} thiamine in the absence of Co + Mo (Figure 1A). Similar results were observed for nodule dry weight (Figure 1C) in the 200 mg kg^{-1} thiamine treatment and root dry weight in the 50 mg kg^{-1} thiamine treatment (Figure 1D). The absence of thiamine and presence of Co + Mo positively influenced root length (Figure 1B). The combined presence of Co + Mo and thiamine was detrimental to these parameters.

The response of soybean to Co and Mo fertilization in Brazil has been variable. Such fertilization may be carried out as seed treatment or foliar treatment at V3–V5. Campo and Hungria (2002) stated that products based on Co and Mo are recommended as seed treatment in soybean crops, as they are easier to apply, more efficient, and more economical. However, direct contact of inoculant bacteria with salts containing Co and Mo during seed treatment is one of the limiting factors for BNF. The authors demonstrated that treatment of soybean seeds with 2.5 or 5.0 g ha^{-1} Co + Mo did not cause a significant effect on number of nodules per plant. These factors may explain the results obtained in the current research, with thiamine promoting nodulation in the absence of Co + Mo.

The lower nodule number in plants seed-treated with Co + Mo (Table 2) may also be explained by the toxic effect of Mo on the microbial inoculant applied to seeds. The population of bacteria responsible for nodulation is reduced, resulting in fewer nodules. The same effect was observed by Albino and Campo (2001), who found that Mo, applied in the form of sodium molybdate via seed treatment, promoted a 40% decrease in nodule number.

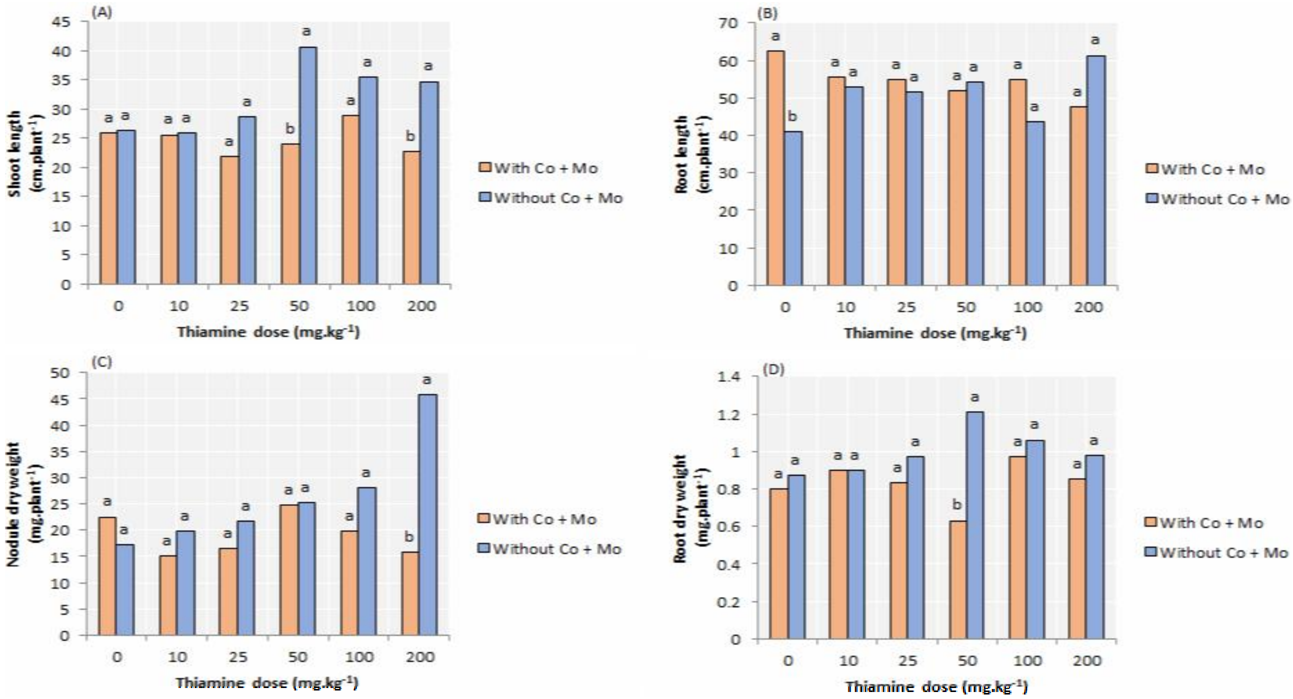
Calendula officinalis L. development was stimulated by thiamine application. Doses of up to 100 ppm, applied via foliar spraying, increased the development of aerial and reproductive organs and leaf pigment content. The treatment also promoted significant increases in biometric parameters and sugar content (Soltani; Saffari; Moud, 2014). These factors may explain why thiamine enhanced shoot and root growth in the current study. Thiamine alone may sufficiently meet the plant's nutritional needs, not requiring Co + Mo application.

Table 2 - Mean number of nodules (NN), nodule dry weight (NDW), root length (RL), shoot length (SL), root dry weight (RW), and shoot dry weight (SW) of soybean plants seed-treated with different thiamine doses associated or not with cobalt and molybdenum (Co + Mo)

Treatment Co + Mo	NN	NDW (mg plant^{-1})	RL (cm plant^{-1})	SL (cm plant^{-1})	RW (mg plant^{-1})	SW (mg plant^{-1})
Presence	28.21 b	19.05	54.50	24.81	0.83	1.19 b
Absence	35.40 a	26.41	50.74	31.99	1.00	1.40 a
Thiamine dose (mg kg^{-1})						
0	28.72	20.15	51.75	26.14	0.83	1.23
10	33.07	17.35	54.23	25.75	0.90	1.20
25	32.30	19.13	53.11	25.31	0.90	1.21
50	32.87	25.05	52.91	32.31	0.92	1.35
100	31.50	23.92	49.28	32.17	1.02	1.46
200	32.35	30.79	54.45	28.71	0.92	1.30
Co + Mo (C)	18.81 *	5.97 *	1.62^{ns}	21.66*	11.94*	10.12*
Thiamine (T)	0.62^{ns}	1.71^{ns}	0.27^{ns}	2.87*	0.97^{ns}	1.50^{ns}
C × T	1.75^{ns}	0.03*	2.73*	2.89*	3.15*	1.95^{ns}

ns, not significant; *, significant at $p < 0.05$ by Tukey's test; CV, coefficient of variation. Means in a column followed by different letters differ significantly by Tukey's test ($p < 0.05$)

Figure 1 - (A) Shoot length, (B) root length, (C) nodule dry weight, and (D) root dry weight of soybean seedlings seed-treated with different thiamine doses in the absence or presence of cobalt and molybdenum (Co + Mo). Within each thiamine dose, means followed by different letters differ from each other at $p < 0.05$ by Tukey's test



For treatments without Co + Mo, a positive linear equation provided a good fit to nodule dry weight data, demonstrating that nodule dry weight increases with increasing thiamine dose (Figure 2A). Therefore, thiamine facilitates symbiotic relationships between seedlings and N-fixing bacteria. These microorganisms benefit from their host by using the available carbohydrates, vitamins, and minerals. The effects of vitamin application are mainly protective. Vitamins activate the secondary metabolism of plants (Vendruscolo *et al.*, 2019). In particular, thiamine acts in carbon metabolism, protein synthesis, and protection against biotic and abiotic stresses, also serving as a coenzyme in different metabolic pathways (Kaya *et al.*, 2015).

The relationship of seed length and root dry weight with thiamine dose was explained by quadratic equations (Figures 2B and 2C). Maximum values of seed length and root dry weight were found to be achieved with thiamine doses of 122.6 and 122.0 mg kg^{-1}, respectively. These doses possibly afforded an increase in metabolic activities, promoting plant growth. This process occurs through improvements in cell division and expansion (El-Bassiouny *et al.*, 2014). Thiamine may act as a stimulant of photosynthetic pigments, promoting an increase in biometric characteristics of plants (Soltani; Saffari; Moud, 2014). A similar result was observed by Vendruscolo *et al.* (2018b) which thiamine doses of up to 100 mg plant^{-1} applied to the substrate positively influenced shoot growth in *Guazuma ulmifolia* seedlings.

PCA revealed two principal components, which together explained 83.94% of the total variance in the dataset (Figure 3). The PCA biplot showed that Co + Mo negatively influenced all plant variables. By contrast, thiamine doses of 50, 100, and 200 mg kg^{-1} were positively associated with all variables.

Thiamine at 50 and 200 mg kg^{-1} was plotted on the same quadrants of plant variables, showing a positive correlation with principal component 1 (Figure 3). All correlation values equal to or greater than 0.60 were considered relevant (Rencher; Christensen, 2012). Therefore, according to this criterion, all variables presented discriminatory power with at least one component (Table 3).

This study showed that seed treatment with thiamine and Co + Mo does not promote soybean development. Thiamine application in the absence of Co + Mo increased nodule dry weight, root dry weight, and shoot length. However, despite the positive effect of vitamin treatment, varying results may be obtained, depending on the crop and application method. Future studies are needed to identify the optimal application method and doses for soybean.

Figure 2 - (A) Nodule dry weight, (B) shoot length, and (C) root dry weight of soybean plants seed-treated with different thiamine doses in the absence of cobalt and molybdenum

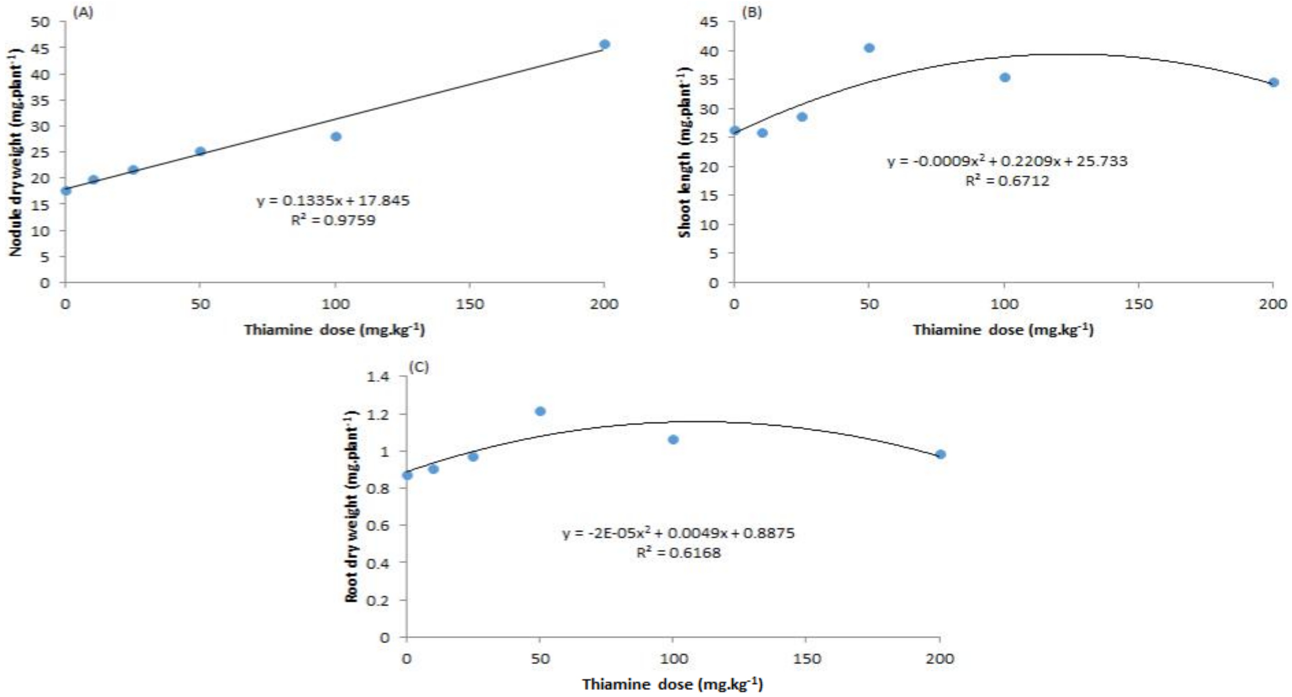


Figure 3 - Principal component analysis explaining the effects of cobalt + molybdenum (presence, P, and absence, A) and thiamine dose (0, 10, 25, 50, 100, and 200) on nodule number (NN), nodule dry weight (NDW), root length (RL), shoot length (SL), root dry weight (RW), and shoot dry weight (SW) of soybean plants

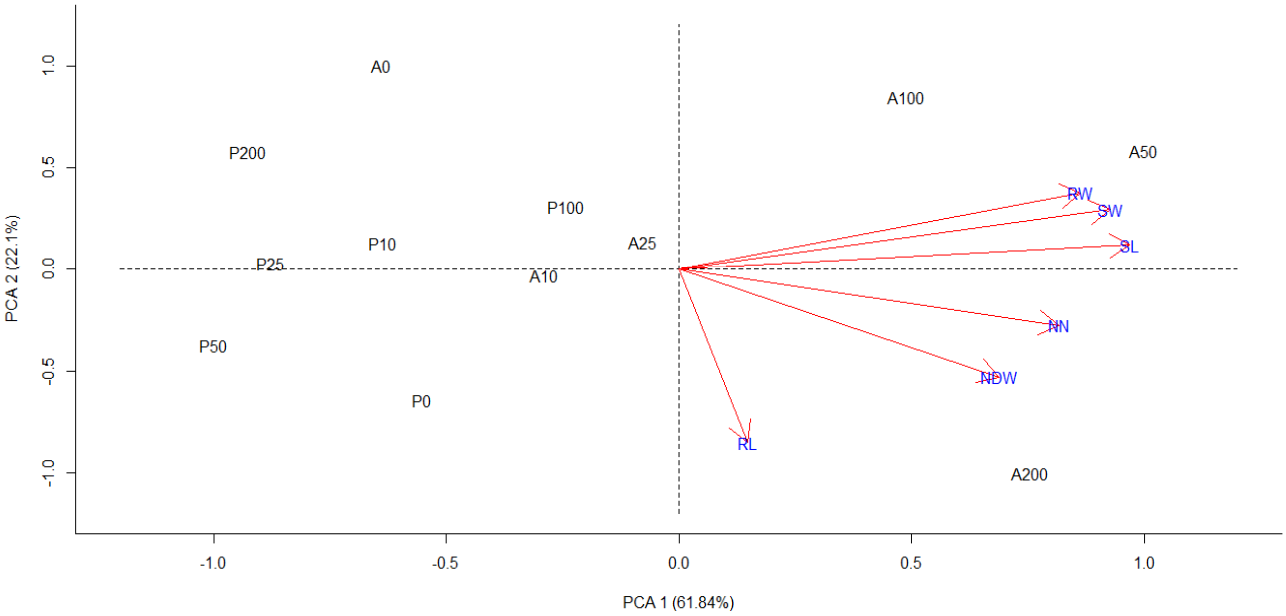


Table 3 - Factor loadings, eigenvalues, and variance of principal components explaining the effects of different thiamine doses in the absence or presence of cobalt and molybdenum in soybean plants

Variable	Principal component 1	Principal component 2
Nodule number	0.82	−0.27
Nodule dry weight	0.69	−0.53
Root length	0.15	−0.85
Shoot length	0.97	0.12
Root dry weight	0.86	0.38
Shoot dry weight	0.93	0.29
Eigenvalue	3.71	1.32
Variance (%)	61.84*	22.10
Total variance (%)	83.94	

* Percentage variation retained by the principal component in the original dataset. Values in bold were considered significant (|>0.60|)

CONCLUSIONS

1. Combined application of Co + Mo and thiamine as seed treatment does not enhance soybean development;
2. Thiamine application improves shoot length, root dry weight, and nodule dry weight in soybean crops.

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