Nicotinamide and phytohormones as biostimulants in common bean

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ABSTRACT

The cultivation of beans (*Phaseolus vulgaris* L.) is of great importance to the national economy since they are grown in almost all regions of Brazil and the use of biostimulants based on phytohormones and plant vitamins can improve the yield of the crop. This work aimed to evaluate the effects of the application of the vitamin nicotinamide and phytohormones as biostimulants on bean plants. The experimental design was carried out in randomized blocks in a 2 x 5 factorial scheme with 4 replicates. The treatments consisted of the application of five doses of nicotinamide (0, 200, 400, 600 and 800 mg L^{-1}) in the presence or absence of phytohormones containing auxin, gibberellin and cytokinin. The growth characteristics, production components and productivity of the bean grains were evaluated. The use of phytohormones and nicotinamide alone or together favored the growth, production components and grain productivity of the bean plant. The range of 366 to 469 mL L^{-1} nicotinamide achieved the highest values for the growth variables, production components and grain productivity of the bean plant, with 414 mL L^{-1} being the most suitable to achieve higher grain productivity.

Keywords: *Phaseolus vulgaris*; auxin; cytokinin; gibberellin; vitamins.

INTRODUCTION

Beans are a prominent crop on the national scene, being among the most relevant in socioeconomic terms (Castro *et al.*, 2019). Its production, in the 2022/2023 harvest, reached 2,699.5 thousand tons (Conab, 2024), giving Brazil the title of the third largest producer of this legume in the world, behind India and Myanmar (FAO, 2022).

Beans are grown in almost all regions of the country, using more rudimentary techniques, under varied environmental conditions, and at different times of the year. (Tavares *et al.*, 2017), Therefore, the application of new technologies for the adaptation and good development of the common bean plant can become a differentiator in increasing grain productivity. As a result, the use of substances such as biostimulants has been highlighted in some research, due to the benefits provided to the plant and, consequently, gains in grain productivity (Perin *et al.*, 2016; Abreu *et al.*, 2020; Frasca *et al.*, 2020; Santos *et al.*, 2020).

Biostimulants are defined as substances consisting of vitamins, phytohormones, amino acids, ascorbic acid, micronutrients and seaweed, which, when applied in-furrow, via foliar or seed treatment, act as plant regulators, so that on contact with the plant they promote the uptake and utilization of nutrients, tolerance to biotic and abiotic stress and enable the plant to reach its maximum production potential (Nardi *et al.*, 2016; Vasconcelos & Chaves, 2019; Silva *et al.*, 2021).

Research shows that biostimulants containing phytohormones such as indolebutyric acid (auxin), kinetin (cytokinin) and gibberellic acid (gibberellin) can improve plant performance, as the composition and concentration of each

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compound contribute to plant growth, cell division and the plant's uptake of water and nutrients. The combined effect of these three hormones therefore gives it the function of mediating physiological processes (Bhupenchandra *et al.*, 2022; Sosnowski *et al.*, 2023).

Auxins are phytohormones that promote plant growth (Wang *et al.*, 2015) by supporting the regulation of cell division and cell elongation, which leads to the formation of new structures in plants (Cassel *et al.*, 2021). Gibberellic acid, which belongs to the group of gibberellins, has a function related to the growth of plant stems and its exogenous application ensures the elongation of internodes and increases the insertion of the first pod (Pedó *et al.*, 2018). Kinetin, the first molecule of the cytokinin group to be identified, acts in the regulation of cell growth, the formation of nodules in the roots of nitrogen-fixing plants, the development of apical meristems and the control of senescence (Mangena, 2022).

Another important compound that can promote plant growth is nicotinamide, a B-complex vitamin also known as niacin or vitamin B3. Its functions include promoting root development, alleviating biotic or abiotic stress, activating enzymes that break down carbon during photosynthesis, and functioning as a component of NAD+ and NADP+, electron transporters in metabolism. (Kirkland & Meyer-Ficca, 2018).

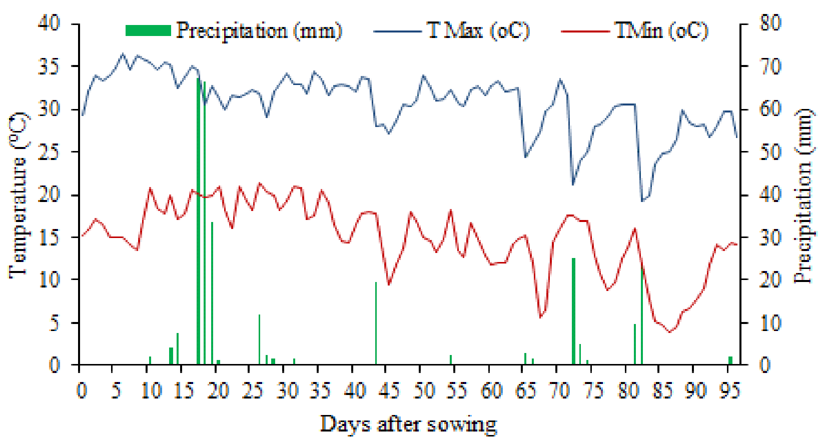
Exogenous foliar application of this vitamin can lead to an increase in productive and biometric traits, as seen in beans, leading to an increase in all productive components of the crop (Abreu *et al.*, 2020). Specifically for soybean crop, the application of nicotinamide has a marked effect on productivity, increasing it more significantly in places where there are effects of periods of water stress, due to poor rainfall distribution (Lima *et al.*, 2024).

This research considered the hypothesis that the isolated or combined use of nicotinamide and phytohormones can promote improvements in bean cultivation and increase grain productivity. The aim of the work was therefore to evaluate the effects of using the vitamin nicotinamide and phytohormones as biostimulants on common bean.

MATERIAL AND METHODS

The work was carried out in the experimental area of the Federal University of Mato Grosso do Sul, Chapadão do Sul/MS Campus, with a latitude of 18°48'459" South, a longitude of 52°36'003" West and an altitude of 820 m, in a typic Dystrophic Red Latosol of clayey texture (Santos *et al.*, 2018). According to Köppen, the climate is humid tropical (Aw), with a rainy season in summer and a dry season in winter and an average annual rainfall of 1,850 mm. The average annual temperature varies between 13 °C and 28 °C. The precipitation and air temperature data during the experiment are shown in figure 1.

During the crop cycle, 297.8 mm of precipitation fell, a value below that required for good development and reaching the maximum production potential of common bean, which according to Marco *et al.* (2012) should be between 300 and 600 mm. In addition, in 88% of the growing period, daily rainfall did not reach the required average value, which is 3 to 4 mm per day (Figure 1).



Source: INMET

Figure 1: Maximum temperature, minimum temperature and rainfall in the experimental area on the campus of the Federal University of Mato Grosso do Sul-MS, in the period from 02/03/2020 to 05/04/2020.

The experimental design was in randomized blocks, in a 2 x 5 factorial scheme, with four replicates, with five doses of nicotinamide in foliar application (0, 200, 400, 600 and 800 mg L^{-1}), with the presence or absence of the phytohormone Stimulate® (cytokinin 0.09 g L^{-1} + gibberellic acid 0.05 g L^{-1} + 4-indol-3-ylbutyric acid 0.05 g L^{-1}) at a dose of 1000 mL ha^{-1}. Once the plants had reached the V4 stage, the treatments were applied using an 8-liter constant-pressure backpack sprayer at a flow rate of 270 L ha^{-1}.

The plots consisted of five lines, each 5.0 m long and spaced 0.45 m apart. For the usable area, the three central lines were taken into account, excluding 0.5 m from each end, giving a total of 3.6 m^2. The cultivar used was BRS Estilo, which is characterized by upright growth, indeterminate growth type II, high production potential and production stability.

Before the experiment was carried out, soil samples were taken from the experimental area in the 0 – 0.20 m layer and a chemical analysis was carried out according to the method proposed by Van Raij *et al.* (2001). This analysis yielded the following values: pH ($CaCl_2$) = 5.3; P (Mel.), K, S, B, Cu, Fe, Mn and Zn = 10.8; 62; 4.2; 0.16; 1.4, 50, 15.2 and 5.7 mg dm^{-3}, respectively; Ca, Mg, H+Al and CTC = 2.20, 0.50, 5.4 and 8.3 cmolc dm^{-3}, respectively; V% = 34.6 and MO = 29 g dm^{-3}. The values for soil texture were: Clay = 52.5%, silt = 5% and sand = 42.5%. To correct the acidity of the soil, 3.3 t ha^{-1} of dolomitic limestone (PRNT = 90%) was applied to increase the base saturation to 70%.

Sowing took place on 3 february 2020 with a 4-row tractor seed drill, which applied 12 seeds per meter and 230 kg ha^{-1} MAP fertilizer (10% N and 46% P_2O_5). For seed treatment, the fungicide carboxanilide + dimethyldithiocarbamate was used at a dose of 93.75 + 93.75 g a.i. ha^{-1} 100 kg^{-1} seed and the insecticide thiophanate methyl + fluazinam at a dose of 63.0 + 15.75 g a.i. ha^{-1} 100 kg^{-1} seed. When the plants had reached the V4 growth stage, a top dressing was applied consisting of the application of 80 kg ha^{-1} N and 60 kg ha^{-1} K_2O, using the fertilizers urea and potassium chloride as a source.

Crop protection management consisted of weed and pest control applications. The first herbicide application was at 10 days after emergency (DAE) (with fluasifop-P-butyl (125 g a.i. ha^{-1}), followed by the application of the insecticides thiamethoxan (17.6 g a.i. ha^{-1}) + lambda-cialothrin (13.2 g a.i. ha^{-1}) and chlorfenapyr (240 g a.i. ha^{-1}) at 13 DAE. Then the herbicide bentazon (720 g a.i. ha^{-1}) was applied at 14 DAE and finally, at 18 DAE, the herbicide haloxifope-P-methyl (60 g a.i. ha^{-1}).

At 45 DAE, 5 plants were sampled from the crop area of each plot to determine plant height, stem dry mass and leaf dry mass. To determine the height, the plants were measured from the neck to the tip. To determine the dry mass of the stem and leaves, these parts of the collected plants were packed separately in paper bags and placed in the oven at 65 °C until they reached a constant mass.

When the plants had reached full flowering, the chlorophyll reactivity index was assessed using the Falker instrument ClorofiLOG. Five plants were assessed per plot, measuring the first leaf from top to bottom that was fully developed and exposed to the sun.

The harvest took place at 91 DAE and the variables evaluated were: height of the first pod attachment, measured from the base (neck) to the attachment of the first pod; number of pods per plant, counting all pods on the 5 plants; number of grains per pod, counting the grains of 10 pods; grain mass per plant, determined by weighing the grains of all pods, with humidity set at 13%; productivity, determined by weighing and sieving the grains of all plants in the three crop lines of the plot; mass of 100 grains, determined by weighing 100 grains from each plot, with humidity adjusted to 13%.

Before proceeding with the analysis of variance, the homogeneity of variance and normality of errors were verified. The data were subjected to analysis of variance with the means of the factors compared by the Skott-Knott test at a probability of 0.05 using the Sisvar software (Ferreira, 2019).

RESULTS AND DISCUSSION

The variables plant height, stem dry mass, number of pods per plant, grain mass per plant and grain yield showed a significant interaction between the factors of nicotinamide doses, in the presence or absence of phytohormones. Leaf dry mass, height of first pod set and number of grains per pod were separately affected by the two factors. The variable mass of one hundred grains was only affected by the nicotinamide doses, while total chlorophyll showed no significant response to any of the factors tested (Table 1).

Plant height was increased by the application of phytohormones up to a maximum dose of 469.0 mg L^{-1}

Table 1: Mean squares for total plant height (HT), stem dry mass (SM), leaf dry mass (LM), height of first pod insertion height (HP), number of pods per plant (PP), number of grains per pod (GP), the grain mass per plant (GM), the mass of one hundred grains (100W), the total chlorophyll (CL) and the productivity (GY) as a function of different doses of nicotinamide (N) in the presence or absence of phytohormone (P) for bean crops

		Mean Square				
		HT	**SM**	**LM**	**HP**	**PP**
Block	3	5.06	0.29	0.08	1.18	0.32
P	6	50.85**	42.87**	40.08**	4.36*	16.00**
N		174.33**	81.19**	43.00**	8.37**	14.04**
PxN		14.57*	5.50*	0.75^{ns}	0.02^{ns}	1.15*
Error	18	5.10	1.96	1.18	0.82	0.34
CV (%)		4.79	6.69	6.33	5.84	6.24
Average		47.18	20.92	17.16	15.54	9.38
		Mean Square				
		CL	GP	100W	GM	GY
Block	3	3.04	0.02	1.10	0.09	5931.00
P	6	4.37^{ns}	0.48**	0.67^{ns}	8.05**	152390.14**
N		5.97^{ns}	0.28**	3.38**	5.56**	125658.19**
PxN		0.71^{ns}	0.01^{ns}	0.09^{ns}	1.01**	3255.98**
Error	18	2.35	0.04	0.77	0.05	624.82
CV (%)		3.48	5.43	3.67	2.96	1.38
Average		44.06	3.35	23.83	7.46	1817.17

*, ** and ns – Significant at $p \leq 0.05$, 0.01 and, not significant by t-test, respectively

nicotinamide (Figure 2A). In the variable stem dry mass, there was an increase in the doses of nicotinamide in the presence of phytohormones up to a dose of 453.0 mg L^{-1} of the vitamin (Figure 2B). Both the stronger growth of the plant and the accumulation of stem phytomass may be due to the presence of the phytohormones gibberellic acid, indole-butyric acid and kinetin, which act on the stem and meristematic development of the plant to provide stronger growth and development (Park *et al.*, 2017).

Leaf dry mass showed a positive response to the presence of the phytohormone, with an increase of 12.4% compared to treatments without application (Figure 3A). The variable height of the first pod set was negatively affected by phytohormone spraying, remaining 4.2% below the values observed when no phytohormones were applied (Figure 3B), however, the average values of this variable are still within the appropriate range for mechanical harvesting, which according to Bisognin *et al.* (2019) is 15 cm. The number of grains per pod was also favored by the presence of phytohormones, resulting in an increase of 6.4% compared to the absence of these products (Figure 3C).

As reported by Yadav *et al.* (2021), the application of phytohormones promotes several changes in plant metabolism associated with the regulation, growth control, transport, storage and mobilization of nutrients. These factors together possibly led to an increase in leaf dry mass and the number of grains per pod of the bean plants studied. Regarding the negative effect on the height of the first pod set, a similar result was obtained by Ávila *et al.* (2010) when working with the same product in field bean cultivation.

Leaf dry mass was affected by the application of nicotinamide up to a dose of 426.2 mg L^{-1}, which produced the greatest increase compared to the other doses used (Figure 3A). The application of the vitamin nicotinamide can favor various processes in the plant, considering that its action as an NAD+ pathway promotes greater energy production and thus an increase in growth rates (Berglund *et al.*, 2017).

At the height of insertion of the first pod, the dose of 465.5 mg L^{-1} of the vitamin was the one that promoted the highest average values for this variable, reaching 16.4 cm (Figure 4B). According to El-Bassiouny *et al.* (2014), the application of nicotinamide favors the increase in indoleacetic acid and therefore growth rates, since this hormone acts in cell division and contributes to the metabolic activities of the plant.

The number of pods per plant reached a higher value in the presence of phytohormones up to a dose of 384.5 mg L^{-1} nicotinamide (Figure 4C), resulting in a 22.8% increase in this variable compared to the control. This result is

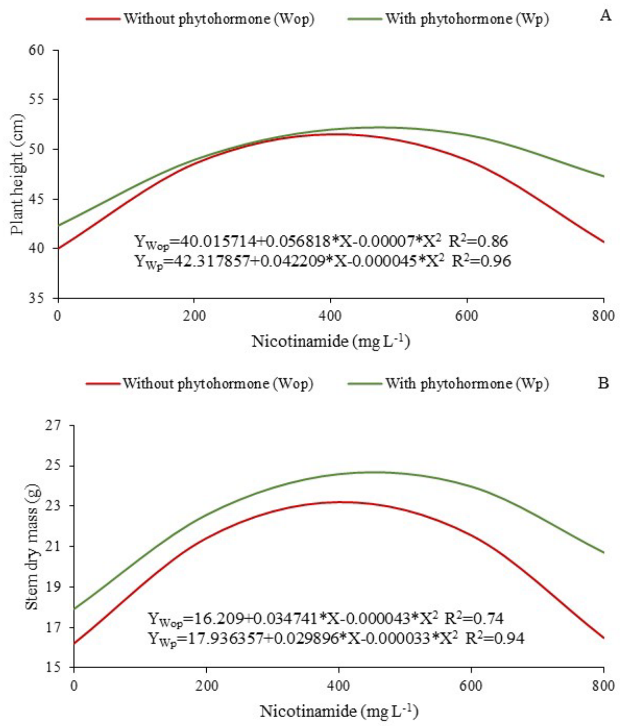


Figure 2: Total height of bean plants and dry mass of the stem as a function of different doses of nicotinamide in the presence or absence of phytohormone.

consistent with that of Albrecht *et al.* (2011), who observed positive responses on the number of pods per plant when soybean seeds were treated with phytohormones. The exogenous application of nicotinamide stimulates the plant's physiological system (Colla *et al.*, 2021) and promotes stronger vegetative and reproductive development due to a higher production of photoassimilates (Dong *et al.*, 2015), which are consumed in the production of new structures in the plant, such as pods.

The application of nicotinamide at a dose of 454.5 mg L^{-1} resulted in a greater number of grains per pod compared to the other treatments (Figure 5A). This is probably due to the regulatory role of B vitamins in carbon metabolism and their activity in protein synthesis as a functional coenzyme in the transport of photoassimilated compounds. These two factors enable plants to better utilize these compounds during grain filling, which increases the analyzed variable (Kaya *et al.*, 2015).

The variable mass of one hundred grains increased as a function of nicotinamide application up to the maximum dose of 366.4 mg L^{-1} and amounted to 24.4 g (Figure 5B). However, even in the treatment that reached the highest average value for this variable, the value was below the expected value, which would be 26.0 g depending on the characteristics of the cultivar (Melo *et al.*, 2011). This could be due to the water stress to which the plants were subjected during the experiment, a factor that prevented the cultivar from reaching its full potential for the average mass of one hundred grains.

Grain mass per plant was favored by the application of phytohormones up to the maximum dose of 382.4 mg L^{-1} nicotinamide (Figure 5C). The variable grain productivity in the presence of the phytohormone increased up to a dose of 414.6 mg L^{-1} (Figure 5D), at which 2009.1 kg ha^{-1} of beans were produced, corresponding to a productivity increase of 4.7 bags ha^{-1} or 16.1% compared to the productivity without application of nicotinamide.

This increase in productive variables could be related to the auxin contained in the phytohormone-based product so that its action on the roots favors their development and consequently increases their ability to absorb water and nutrients (Qin & Wang, 2018; Sosnowski *et al.*, 2023). As a result, these substances are rapidly allocated to the main outlets of the plant, such as the grains (Dourado Neto *et al.*, 2014). In addition, it is possible to link the increase in grain mass per plant to the effect of nicotinamide on the chloroplasts, which promotes a biochemical stimulus for the accumulation of nitrogenous compounds that provide more mass during physiological maturation (Bassuony *et al.*, 2008).

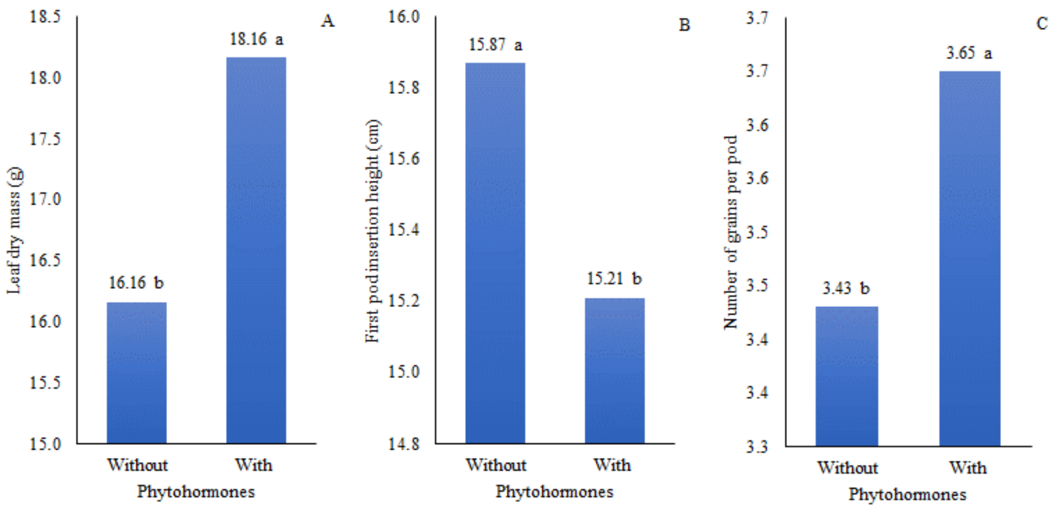


Figure 3: Dry mass of leaves, height of first pod set and number of grains per pod in the presence and absence of phytohormones.

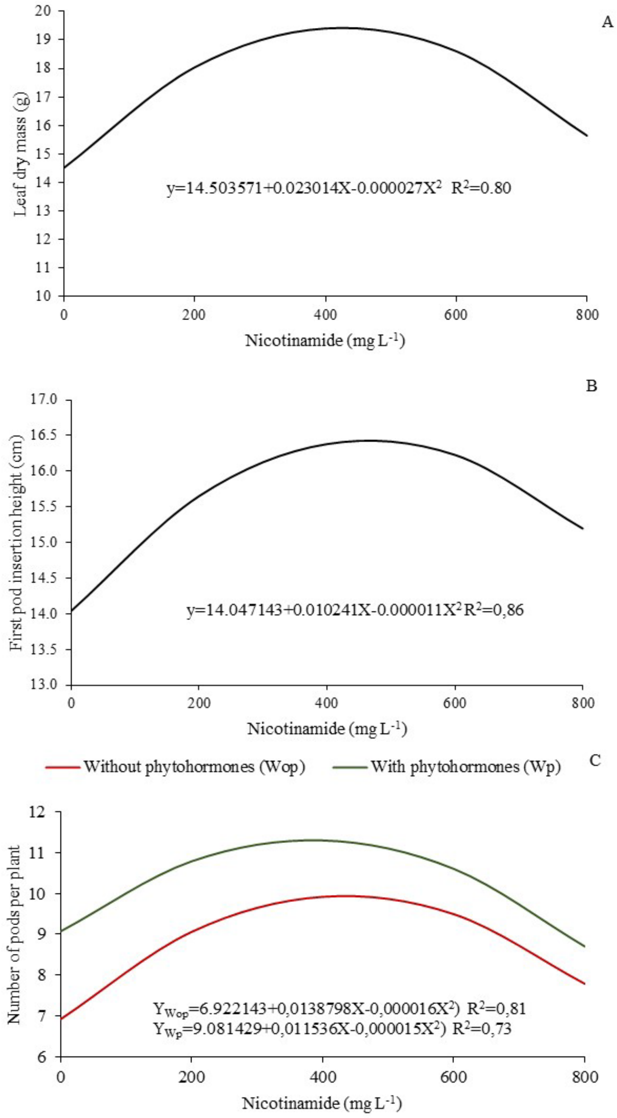


Figure 4: Dry mass of leaves (A), insertion height of the first pod (B) and number of pods per bean plant (C) as a function of different nicotinamide doses, in the presence or absence of phytohormones.

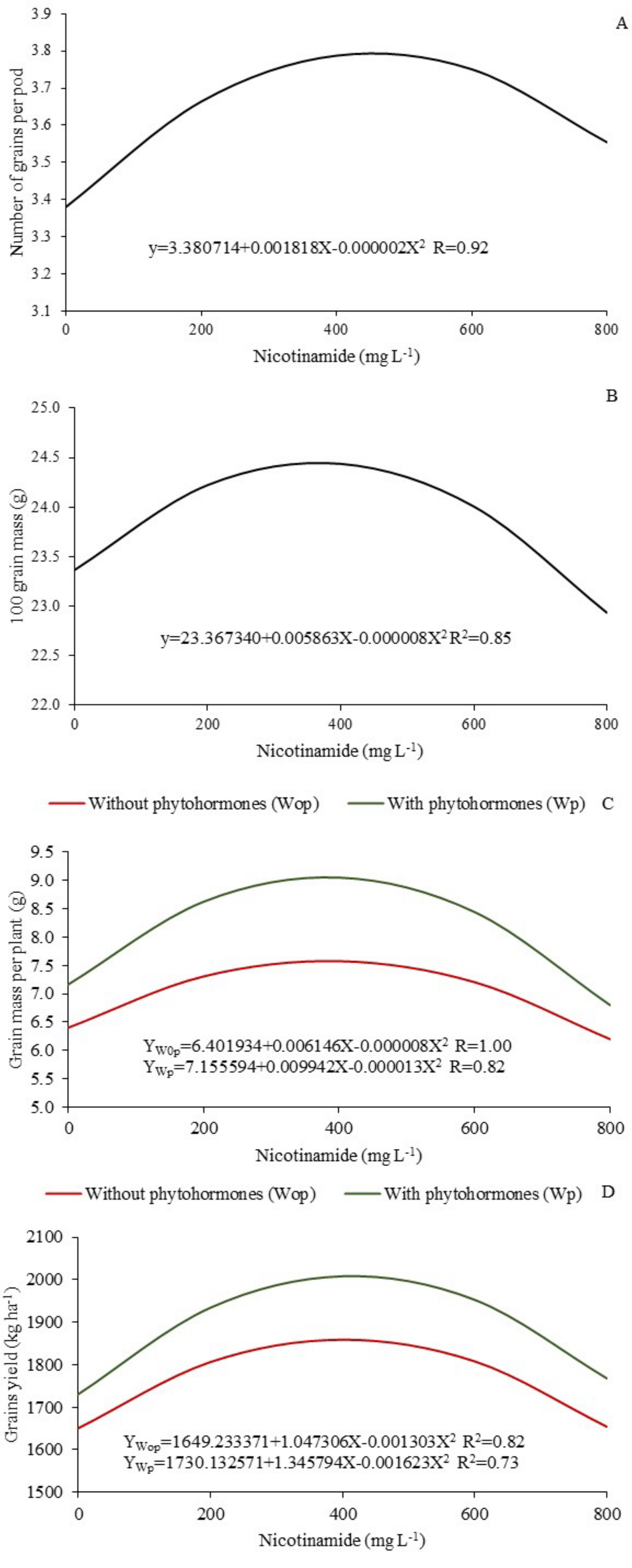


Figure 5: Number of grains per pod (A), mass of hundred grains (B), mass of grains per plant (C) and grain productivity (D) of beans as a function of different doses of nicotinamide in the presence or absence of phytohormones.

CONCLUSIONS

The use of phytohormones and nicotinamide alone or together favored the growth, production components and grain productivity of the bean plant.

In the range of 366 to 469 mg L^{-1} nicotinamide, the highest values were obtained for the growth variables, production components and grain productivity of the bean plant, with 414 mg L^{-1} being the most suitable to obtain higher grain productivity.

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