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Planting fertilization and *Metarhizium anisopliae* inoculation in the initial growth of sugarcane

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ABSTRACT

The study aimed to evaluate the interaction between fertilizer doses (N:P:K) at planting the inoculation of the *Metahizium anisopliae* fungus in sugarcane billets during the initial development of the plant. The experiment was conducted in pots, under full sun, in a 2 x 5 factorial scheme, specified as follows: with and without inoculation of the fungus in the billets and five doses of NPK fertilizer (0%, 50%, 75%; 100%, and 125% of recommended fertilization). Plant height was measured at 53, 77, and 91 days after planting (DAP), and the root and shoot dry matter (DM) was quantified at 91 DAP. There was no significant influence of the interaction between fungus and NPK fertilizer doses (P>0.05) on all variables analyzed. However, a significant difference was observed for inoculation (P<0.05) and doses of NPK fertilizer <0.05). Inoculation of *M. anisopliae* resulted in greater plant height and root and shoot DM than non-inoculated plants, regardless of fertilizer doses. In this sense, new studies must be conducted to find a way to reduce the use of NPK fertilizer in sugarcane by *M. anisopliae* inoculation.

Keywords: Entomopathogenic fungi, Fertilizers, Plant growth-promoting microorganisms.

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Adubação de plantio e inoculação de *Metarhizium anisopliae* no crescimento inicial da cana-de-açúcar

RESUMO

O objetivo do estudo foi avaliar a interação de doses da adubação de plantio (N:P:K) com a inoculação do fungo *Metahizium anisopliae* em toletes de cana-de-açúcar no desenvolvimento inicial da planta. O experimento foi conduzido em vasos, colocados a céu aberto, em esquema fatorial 2 x 5, assim especificado: com e sem inoculação do fungo nos toletes e cinco doses de adubação NPK (0%, 50%, 75%; 100% e 125% da adubação recomendada). Foi realizada a mensuração da altura de planta aos 53, 77 e 91 dias após o plantio (DAP) e quantificada da matéria seca (MS) da raiz e parte aérea aos 91 DAP. Para todos as vaiáveis analisadas, não houve diferença significativa para interação inoculação do fungo com as doses de adubação NPK (*P>0,05*). Entretanto, foi observada diferença significativa para a inoculação (*P<0,05*) e as doses de adubos NPK *<0,05*). Sendo que a inoculação de *M. anisopliae* resultou em maior altura de plantas, maior MS de raiz e da parte aérea em comparação com as plantas não inoculadas, independente das doses de adubação. Neste sentido, novos estudos devem ser realizados para viabilizar uma forma de redução de utilização de adubação NPK na cana-de-açúcar por meio da inoculação de *M. anisopliae.*

Palavras-chave: Fungos entomopatogênicos, Fertilizantes, Microorganismos promotores de crescimento de plantas.

1. Introduction

Sugarcane yield is influenced by biotic and abiotic factors, including the low availability of nutrients in the soil (Elser et al., 2007). Therefore, adding nutrients to the soil is necessary to meet the crop's needs and enable good yield, which considerably increases the production cost. Plant growth-promoting microorganisms are one possible option for increasing agricultural yield sustainably and cheaply.

In recent years, new uses for entomopathogens have been discovered beyond pest control, such as the promotion of plant growth by entomopathogenic fungi improving the absorption of nutrients such as N and P (Behie et al., 2012; Behie and Bidochka, 2014), and the production of growth regulating hormones such as cytokinins, auxins, and gibberellins (Bose and Keharia, 2013). It also stimulates branching and root hair production, increases root and shoot biomass, and improves plant resistance to adverse conditions such as water and salt stress and phosphate solubilization (Barrow and Osuna, 2002; Chaudhary et al., 2023).

Entomopathogenic fungi can live part of their life cycle endophytically in plants without causing any disease in the host plant (Vega, 2018). Species of the genus *Metarhizium* are commonly found in the soil, presenting a multifunctional ecology, living as saprophytes, endophytes, insect pathogens, and in the plant rhizosphere. It has a symbiotic relationship with the host plant and can benefit from its photosimilates and, in return can transfer N from the colonized dead insect to the host plant, resulting in better plant development (Stone and Bidochka, 2020).

In this context, taking into account that the fungus M. anisopliae is applied annually to more than 2 million hectares of sugarcane to control sugarcane spittlebug in Brazil (Bettiol, 2011), making its use viable by exploiting its multi functionalities, such as better use of soil nutrients, could lead to a reduction in the number of fertilizers applied to the crop, both through the transfer of soil nutrients mediated by the fungus and through greater growth of the root system. This study aimed to evaluate the interaction between fertilizer (N:P:K) doses at planting and inoculation of the *Metarhizium anisopliae* fungus in sugarcane billets during initial plant development.

2. Material and Methods

The study was carried out in pots, under full sun, from March 2022 to July 2022, in Nova Andradina, Mato Grosso do Sul (22°14'6" S and 53°19'54" W). The *Metarhizium anisopliae* (ESALQ-E9) fungus was used, and before using the conidia in the experiment, viability was tested according to the methodology proposed by Oliveira et al. (2015) to adjust the concentration in viable conidia. The fungal suspensions were prepared using a 0.01% (v/v) Tween 80® solution and added to the fungal multiplication plates to detach the conidia. The concentration was adjusted to 1 x 10^7 viable conidia. mL^{-1} using a Neubauer chamber.

The experimental design used was entirely randomized and arranged in a 2 x 5 factorial scheme, as follows: with and without inoculation of the *M. anisopliae* fungus in the seedlings and five doses of NPK fertilizer (0%, 50%, 75%, 100%, and 125% of the recommended fertilizer), with four replications. Each treatment consisted of one 6 L pot filled with 5.5 kg of soil, classified as Latossolo Vermelho, with the following chemical properties: pH = 5.8 ($CaCl_2$); organic matter = 8 $g.dm^{-2}$; P (Resin) = 9 $mg.dm^{2}$; K = 2.8 $mmol.dm^{-3}$; Ca = 22 $mmol.dm^{-3}$; Mg = 10 $mmol.dm^{-3}$; H + Al = 9 $mmol.dm^{-3}$; effective CEC = 44 $mmol.dm^{-3}$; base saturation (%) = 80%; Al saturation = 0%.

Fertilizer was applied at planting using NPK fertilizer (08-30-10) at a dose of 500 kg ha^{-1}, as described in Table 1. The recommendation followed the one adopted by the Bioenergética Santa Helena company (Nova Andradina, MS), where the soil was collected, and the sugarcane variety used in the experiment was grown. The variety studied was RB92579 because it is commonly planted in the region.

The buds were disinfected before use to avoid contamination by other fungi or bacteria. A 4% sodium hypochlorite solution was used, and the buds were washed thrice with running water directly from the tap. Immediately after planting, the fungus was inoculated by applying the suspension directly to the soil (50 mL $vase^{-1}$), with the soil damp. The same amount was used for the treatments without injection, with only a 0.01% (v/v) Tween 80® solution.

Plant length assessments were carried out using a ruler at 53, 77, and 91 days after planting (DAP). At the end of the experiment (91 days after planting), the shoot and root were separated to calculate the dry matter. To avoid damaging the roots, the adhered soil was loosened with running water using a hose. Subsequently, the shoot and root were separated. After being weighed on a precision scale, they were placed in paper bags and dried in a drying oven at 60 °C for 72 hours to obtain the dry matter of the root and shoot.

Table 1. Amounts ($kg.ha^{-1}$) of nutrients per treatment according to the technical recommendation of 500 $kg.ha^{-1}$.

Treatments (NPK doses - %)	Nitrogen (N)	Phosphorus (P)	Potassium (K)
125	50	187.5	62.5
100	40	150	50
75	30	112.5	37.5
50	20	75	25
0	0	0	0

The experimental data was submitted for analysis of variance, and when significant, regression analysis was carried out using the "R" statistical software, version 4.3.1 (R Development Core Team, 2023).

3. Results and Discussion

The height of sugarcane plants was analyzed independently at 53, 77, and 91 days after planting (DAP). In all evaluations, there was no influence of the interaction between M. anisopliae inoculation and NPK doses (P>0.05). However, there was a significant influence of the inoculation (P<0.05) and NPK fertilizer doses (<0.05), fitting linear regression models. With increasing fertilizer doses, greater plant height was observed, which was higher in the treatments with fungusinoculation (Figure 1). Thus, regardless of the dose of fertilizer, inoculating the fungus at planting led to greater plant growth in height, which could result in greater biomass at the end of the crop cycle.

There was no influence of the interaction between *M. anisopliae* inoculation and the NPK doses (*P*>0.05) on the shoot and root dry matter of the sugarcane plants at the end of the experiment (91 DAP). However, a significant difference was found between inoculation (*P*<0.05) and the doses of NPK fertilizer (*P*<0.05), fitting linear regression models, with an increase in the doses of fertilizer, a greater shoot and root dry matter of the plants was observed (Figure 2), with the highest values being observed in the treatments with inoculation at all doses of fertilizer.

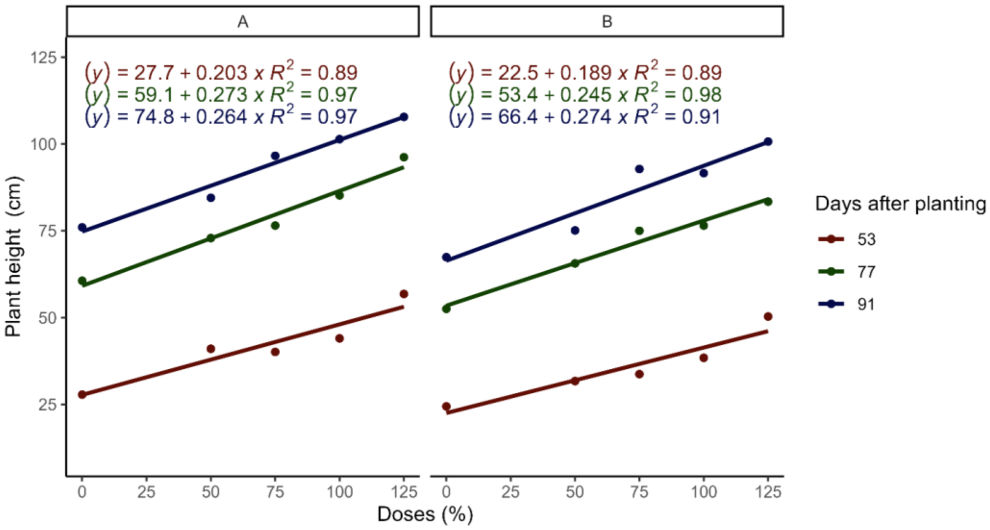

Figure 1. Plant height (cm) of sugarcane with (A) and without (B) *Metarhizium anisopliae* (ESALQ-E9) inoculation and fertilized with different NPK doses at planting at 53, 77, and 91 days after planting (DAP) in Nova Andradina-MS. *Doses=percentage of NPK fertilizer (8:30:10) concerning the recommended dose (500 kg.ha^{-1}).

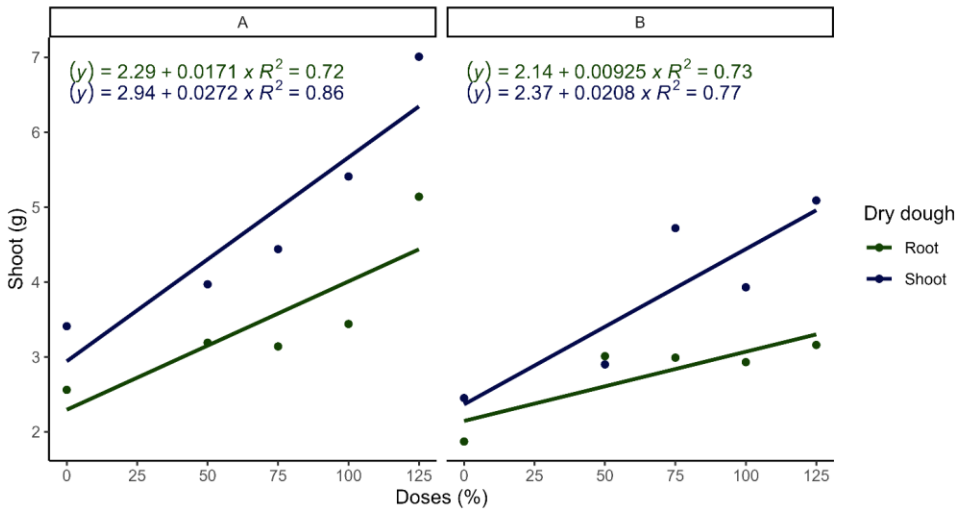

Figure 2. Shoot and root dry matter of sugarcane plants with (A) and without (B) *Metarhizium anisopliae* (ESALQ-E9) inoculation and fertilized with different NPK doses at planting at 91 days after planting (DAP) in Nova Andradina-MS. *Doses=percentage of NPK fertilizer (8:30:10) concerning the recommended dose (500 kg.ha^{-1}).

The results obtained are similar to those observed by other authors who have demonstrated the potential of species of the *Metarhizium* genus to promote plant growth (Jaber and Enkerli, 2017; Sasan and Bidochka, 2012). The greater accumulation of dry matter is directly related to the greater development of the plants in the treatments with inoculation of the fungus. Plants inoculated with *M. anisopliae* accumulated approximately 1.3 times more root and shoot dry matter than non-inoculated plants, regardless of the NPK doses. *M. anisopliae* promoted the growth of sugarcane plants. However, it did not allow a reduction in the amount of NPK fertilizers used in the crop.

In this context, Bose and Keharia (2013) state that entomopathogenic fungi can promote various mechanisms for plant growth, such as the production of growth regulating hormones like cytokinins, auxins, and gibberellins. In addition, they can stimulate branching and root hair production and increase the biomass of the root and shoot (Barrow and Osuna, 2002). The initial hypothesis of this study was the reduction of NPK fertilizer doses by the interaction with *M. anisopliae* inoculation, however, no significant interaction was observed. On the other hand, Tall and Meyling (2018) observed different results when corn plants were inoculated with *Beauveria bassiana* with low doses of fertilizers, which did not result in plant growth promotion, resulting in lower growth.

Further studies should be carried out to reduce the use of sugarcane fertilizer through the *M. anisopliae* inoculation. Thus, inoculation with *M. anisopliae* at planting, with the aim of improving plant development, may also result in a reduction in the pest population due to contamination with the fungus accumulated in the soil. In this sense, Lacey et al. (2015) demonstrated that species of *Metarhizium* sp. persisted for one month in plant roots after seed inoculation by immersion in conidial suspension. *M. robertsii* and *M. anisopliae* species were recovered from rhizospheric soil even 12 months after its application in an area of strawberry cultivation in Minas Gerais, demonstrating the high persistence of the fungus in the soil (Castro et al., 2016).

4. Conclusions

The *M. anisopliae* (ESALQ-E9) inoculation promoted the growth of sugarcane plants and increased the shoot and root dry matter of the plants compared to the non-inoculated plans, regardless of the NPK doses. The plants inoculated and non-inoculated with *M. anisopliae* (ESALQ-E9) showed a linear increase in height and shoot and root dry matter concerning fertilizer doses.

Authors' Contribution

Lucas Xavier de Souza Oba and Lucrecia Mattos: Experiment execution, data collection and tabulation, and manuscript writing. Nancy Farfan Carrasco and Gabriel Ferreira Paiva: Data analysis and interpretation, drawing graphs, and revising the manuscript. Elcio Ferreira dos Santos: Experimental design and writing and revising the manuscript, mainly in the part involving fertilization and plant nutrition. Luiz Henrique Costa Mota: Setting up and conducting the experiment, data interpretation, and writing and revising the manuscript.

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