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POTENTIAL INDUCER OF PHYTOALEXINS IN BEANS, SOYBEANS AND SORGHUM BY COPPER, ZINC, MANGANESE AND CALCIUM CHELATES

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ABTRACT: The activation of plant defense mechanisms is a method alternative to the use of pesticides to control plant diseases. Several products, including some metals, may act as defense elicitors. The aim of this study was to use chelated copper, zinc, manganese and calcium amino acids to induce the synthesis of phytoalexins in beans, soybeans and sorghum. Chelates were used at concentrations of 0.1%, 0.5%, 1%, 2.5% and 5% (volume/volume) from commercial products and distilled water was used as control. The test for the production of phytoalexin phaseolin was carried out on etiolated bean hypocotyls; the test for the production of phytoalexin glyceollin was carried out in soybean cotyledons, and the test for the production phytoalexin 3-deoxyanthocyanidins was carried out in elongated sorghum mesocotyls. The amount of phytoalexins was measured on spectrophotometer at 280 nm, 285 nm and 480 nm, respectively for beans, soybeans and sorghum. Data were submitted to analysis of variance and regression. An inducing effect by activating the synthesis of phytoalexins in the three crops was observed. For beans, all chelates had a dose-dependent inducing effect. For soybeans, manganese chelate presented maximum inducing effect at calculated dose of 3.4%, while calcium chelate also presented a dose-dependent inducing effect, but with no induction from copper and zinc chelates. For sorghum, copper, zinc, manganese and calcium chelates had inducing effect, with the highest induction at calculated doses of 3.69%, 3.47%, 3.49% and 3.68%, respectively.

KEYWORDS: resistance induction, elicitor, alternative control, chelates.

POTENCIAL INDUTOR DE FITOALEXINAS EM FEIJÃO, SOJA E SORGO POR QUELATOS DE COBRE, ZINCO, MANGANÊS E CÁLCIO

RESUMO: A ativação de mecanismos de defesa vegetal é um método alternativo ao uso de pesticidas para o controle de doenças em plantas. Vários produtos, incluindo alguns metais, podem agir como eliciadores dessa defesa. O objetivo deste estudo foi utilizar aminoácidos quelatados à base de cobre, zinco, manganês e cálcio para induzir a síntese de fitoalexinas em feijão, soja e sorgo. Os quelatos foram utilizados nas concentrações de 0,1%, 0,5%, 1%, 2,5% e 5% (volume/volume) a partir de produto comercial e para a testemunha foi utilizada água destilada. O ensaio para produção da fitoalexina faseolina foi realizado em hipocótilos estiolados de feijão, o ensaio para produção da fitoalexina gliceolina foi em cotilédones de soja e o ensaio para a produção das fitoalexinas 3-deoxiantocianidinas foi em mesocótilos estiolados de sorgo. As fitoalexinas produzidas foram mensuradas em espectrofotômetro a 280 nm, 285 nm e 480 nm, respectivamente para feijão, soja e sorgo. Os dados foram submetidos à análise de variância e regressão. Houve efeito indutor de resistência pela ativação da síntese das fitoalexinas nas três culturas. Para feijão, todos os quelatos apresentaram efeito indutor de maneira dose-depende. Para soja, o quelato de manganês apresentou maior efeito indutor na dose calculada de 3,4%, o quelato de cálcio apresentou efeito indutor de maneira dose-dependente, não havendo indução a partir dos quelatos de cobre e zinco. Para sorgo, os quelatos de cobre, zinco, manganês e cálcio apresentaram efeito indutor, sendo a maior indução nas doses calculadas de 3,69%, 3,47%, 3,49% e 3,68%, respectivamente.

PALAVRAS CHAVE: indução de resistência, eliciador, controle alternativo, quelatos.

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.), soybean [*Glycine max* (L.) Merrill] and sorghum [*Sorghum bicolor* (L.) Moench] crops are of great importance for the Brazilian agriculture and are widely used for human and/or animal nutrition. Beans are widely used in the Brazilian diet, being present on most tables every day, due to their high nutritional value, combined with their low cost, being part of the Brazilian gastronomic culture. In addition to their importance as food, they also contribute economically and socially due to the labor required during their production cycle (Rossetti, Winnie and Silva, 2021).

Soybean cultivation plays an important role in Brazilian agriculture, ranking first in national production both in terms of productivity and cultivated area. Economically, it makes Brazil a prominent export country. Soybean is not a product widely consumed directly by Brazilians, despite having high nutritional value, but it is widely used for animal nutrition (DERAL, 2021; Hirakuri and Lazzarotto, 2014).

Sorghum, like soybeans, is also widely used in animal nutrition, having good nutritional properties; however, with a not so significant cultivation area. It has also been researched and used as a nutritious food for human diet, being rich in iron, zinc, proteins, fiber and vitamins (DERAL, 2021; Martino et al., 2014).

The appearance of diseases during the cultivation of these crops can be one of the determining factors for the low crop performance, being responsible for damages, reducing the physiological and nutritional quality of plants. The chemical method is the most used to control diseases in these crops; however, the need for the use of products alternative to chemicals is increasing, especially when considering the resistance that pathogens have developed to chemical products applied to monocultures, in addition to the fact that these products contaminate the environment and waters, as well as humans (Cota et al. 2015; Embrapa, 2013; Wendland et al., 2016; Xavier et al., 2013).

Resistance induction is a tool that can be used in the context of alternative control of plant diseases, because, through treatment with biotic or abiotic agents, latent defense mechanisms in plants are activated, preventing and/or delaying infection and pathogen colonization. Phytoalexins are among these defense mechanisms, which have antimicrobial activity (Stangarlin et al., 2011; Teixeira, 2011).

The most important phytoalexin in the pathogen-plant interaction in bean crops is phaseolin, a compound with antimicrobial activity, which was first detected by Müller (1958), and some studies have shown its induction in plants (Rissato et al., 2017).

Soybean has glyceollin as phytoalexin, which is a pterocarpanoid (flavonoid), which causes inhibition of the activation of fungal enzymes, cytoplasmic granulation, disorganization of cellular contents and rupture of the plasma membrane of pathogens (Gouvea et al., 2011).

In sorghum, four flavonoid phytoalexins of the 3-deoxyanthocyanidin class are known: luteolinidin, 5-methoxyluteolinidin, apigeninidin and the arabinisole caffeic acid ester 5-o-apigeninidin (Nicholson et al., 1988). It has been shown that sorghum phytoalexins initially accumulate in subcellular inclusions within the epidermal cell that is being attacked by the pathogen, and not simply as a response from cells surrounding the infection site (Teixeira, 2011).

Several substances can activate plant defense mechanisms, acting as resistance inducers against pathogens (Schwan-Estrada and Stangarlin, 2005). It has been hypothesized that chelated amino acids can activate the production of phytoalexins, contributing to plant protection (Lorenzetti et al., 2021).

Copper, zinc, manganese and calcium chelates from the commercial product mimic chelates found in plants and are in their most bioavailable form, and can be used in smaller amounts when compared to the conventional use of these minerals. Chelates and complexes are prepared from isolated amino acids, and have the same substances and chemical structures as molecules naturally synthesized by plants (NPA, 2023).

Considering the potential of these chelates, the aim of this work was to verify their resistance-inducing effect on bean, soybean and sorghum plants by activating the synthesis of phytoalexins phaseolin, glyceolin and 3-deoxyanthocyanidin, respectively, using commercial doses of copper, zinc, manganese and calcium chelates.

MATERIAL AND METHODS

Experiments were carried out at the Laboratory of Phytopathology and Nematology of the State University of Western Paraná – UNIOESTE, Marechal Cândido Rondon campus, state of Paraná. Commercial products based on copper, zinc, manganese and calcium from LINHA BIOMETAL® were used: Chelated Minerals with Amino Acids with excellent bioavailability (NPA, 2021).

Treatments

Treatments that elicited the synthesis of the phytoalexins phaseolin in beans, glyceolin in soybeans and deoxyanthocyanidins in sorghum were copper, zinc, manganese and calcium chelates, all used at concentrations of 0.1%, 0.5%, 1%, 2.5 % and 5% (volume/volume) from the commercial product diluted in distilled water. Control treatment used only distilled water.

Bioassay for phytoalexin production in bean hypocotyls

For the phytoalexin phaseolin production assay, bean seeds of “Carioca” variety were superficially disinfected in 98% ethanol for 2 minutes, 1:3 sodium hypochlorite (1 part of hypochlorite to 3 parts of distilled water) for 3 minutes and washed in distilled water until all hypochlorite was removed. After superficial disinfestation, seeds were sown in plastic trays containing sand sterilized in autoclave at 120 °C and 1 atm for 1 hour. After seven days, when hypocotyls were etiolated, they were cut into 5-cm segments, washed in distilled water to remove impurities and kept on absorbent paper for 10 min to remove excess water. Three hypocotyl segments were transferred to each test tube, so that each one received 1 mL of treatment (Bailey and Burden, 1983).

After being kept for 48 hours in the absence of light, hypocotyls from each replicate were weighed on analytical scale and transferred to 5mL test tubes. Then, 98% methanol (approximately 4 mL) was added to test tubes and kept at 4 ºC for 48 hours to extract the phytoalexin formed.

Subsequently, the phaseolin content was measured in spectrophotometer at 280 nm. Sterile distilled water was used as control. Values were expressed as absorbance per gram of fresh mass (ABS gpf^{-1}) (Bailey and Burden, 1983).

Bioassay for phytoalexin production in soybean cotyledons

Soybean seeds of “Syn 13561IPRO” cultivar were superficially disinfected in 98% ethanol for 2 minutes, 1:3 sodium hypochlorite for 3 minutes and washed in distilled water until all hypochlorite was removed. Then, they were sown in expanded polystyrene trays containing autoclaved sand (1 hour at temperature of 120 ºC and 1 atm). Trays were placed on a bench and exposed to fluorescent light with 12-hour photoperiod. After 10 days, the cotyledons of seedlings were detached to set up bioassays. The detached cotyledons were placed in Petri dishes (six cotyledons/dish) containing three sheets of sterilized filter paper moistened in sterilized distilled water. In each cotyledon, a small cut was made on the abaxial surface with the aid of a stylet (scalpel) and, in each of these, 20 µL of treatments were added (Ziegler and Pontzen, 1982).

Plates were kept in BOD at temperature of 25 ºC, in the dark, for 20 hours. After this period, cotyledons were transferred to Erlenmeyer flasks with 15 mL of sterilized distilled water, which were shaken in orbital shaker (150 rpm) for 1 hour to extract the phytoalexin. Subsequently, cotyledons were removed and the supernatant was read in spectrophotometer at absorbance of 285 nm. Sterile distilled water was used as control. Values were expressed as absorbance per gram of fresh mass (ABS gpf^{-1}) (Ziegler and Pontzen, 1982).

Bioassay for phytoalexin production in sorghum mesocotyls

Sorghum seeds of “Brandes” cultivar were superficially disinfected in 98% ethanol for 2 minutes, 1:3 sodium hypochlorite for 3 minutes and washed in distilled water. After this period, they were rolled into moistened germination paper sheets and incubated in BOD in the dark at 28 °C for four days. Then, formed seedlings were initially exposed to light for 4 hours to stop the elongation of mesocotyls. For the phytoalexin production test, mesocotyls were excised 0.5 centimeters above the scutellar node and placed in microcentrifuge tubes (three mesocotyls per tube), containing a 1.5 mL aliquot of treatments (Nicholson et al., 1988).

Tubes were kept in humid chamber at 25 °C under fluorescent light, for 60 hours. After this period, mesocotyls were removed from tubes and the basal 0.5 mm of each mesocotyl was discarded. The upper portion (2.5 cm) was weighed, cut into small segments and placed in new tubes containing 1.4 mL of 80% acidified methanol (0.1% HCl; v/v). Mesocotyls were kept at temperature of 4 °C in methanol for 96 hours to extract pigments. Absorbance was determined at 480 nm. Values were expressed as absorbance per gram of fresh mass (ABS gpf^{-1}) (Nicholson et al., 1988).

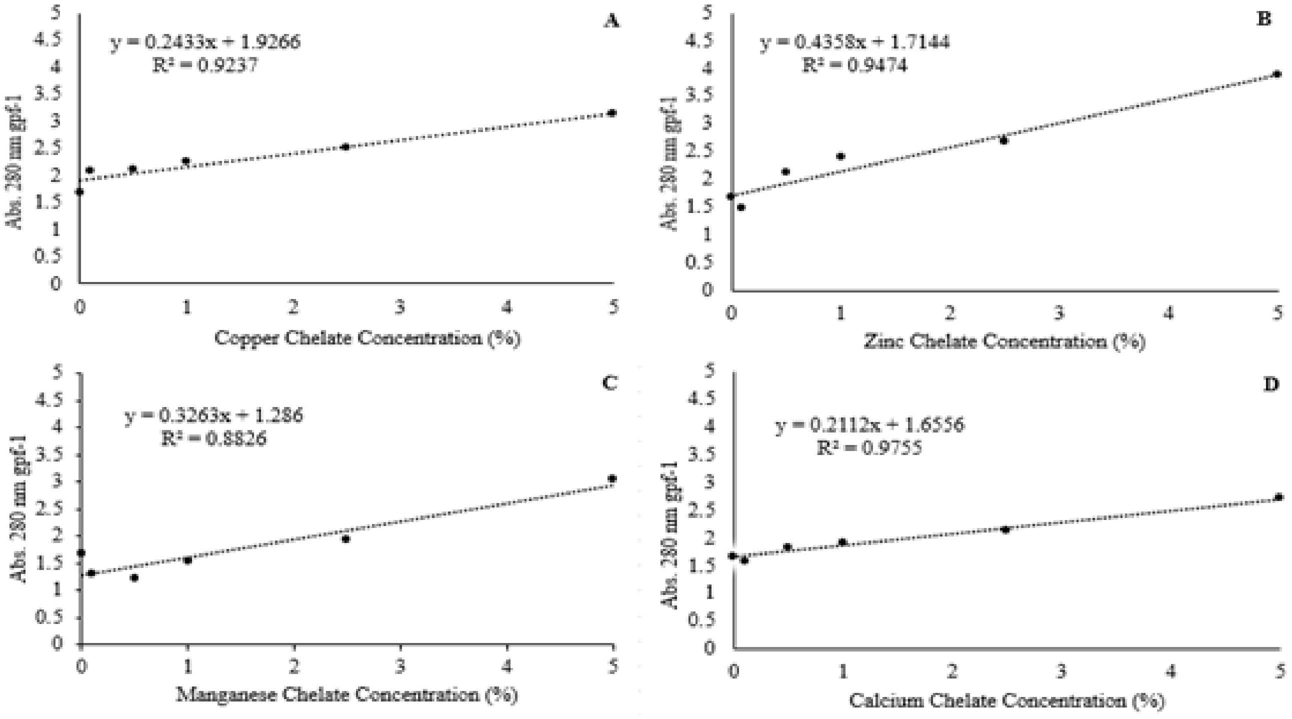
Experimental design and statistical analysis

The experimental design for bioassays carried out with beans, soybeans and sorghum was completely randomized with four replicates, carrying out individual tests for each chelate. The results obtained were submitted to analysis of variance and regression at 5% error probability. The free GENES software was used (Cruz, 2016).

RESULTS AND DISCUSSION

For the phytoalexin phaseolin induction test in beans, it was observed that for all chelates used, the higher the chelate concentration, the greater the amount of formed phytoalexins, that is, the activation of the phytoalexin phaseolin synthesis occurred in a dose-dependent manner (Figure 1). In comparison to control, the highest dose (5% v/v) found from the positive linear regression, showed increase in the production of phytoalexins 146% higher for copper chelate, 221% higher for zinc chelate, 124% higher for manganese chelate and 103% higher for calcium chelate.

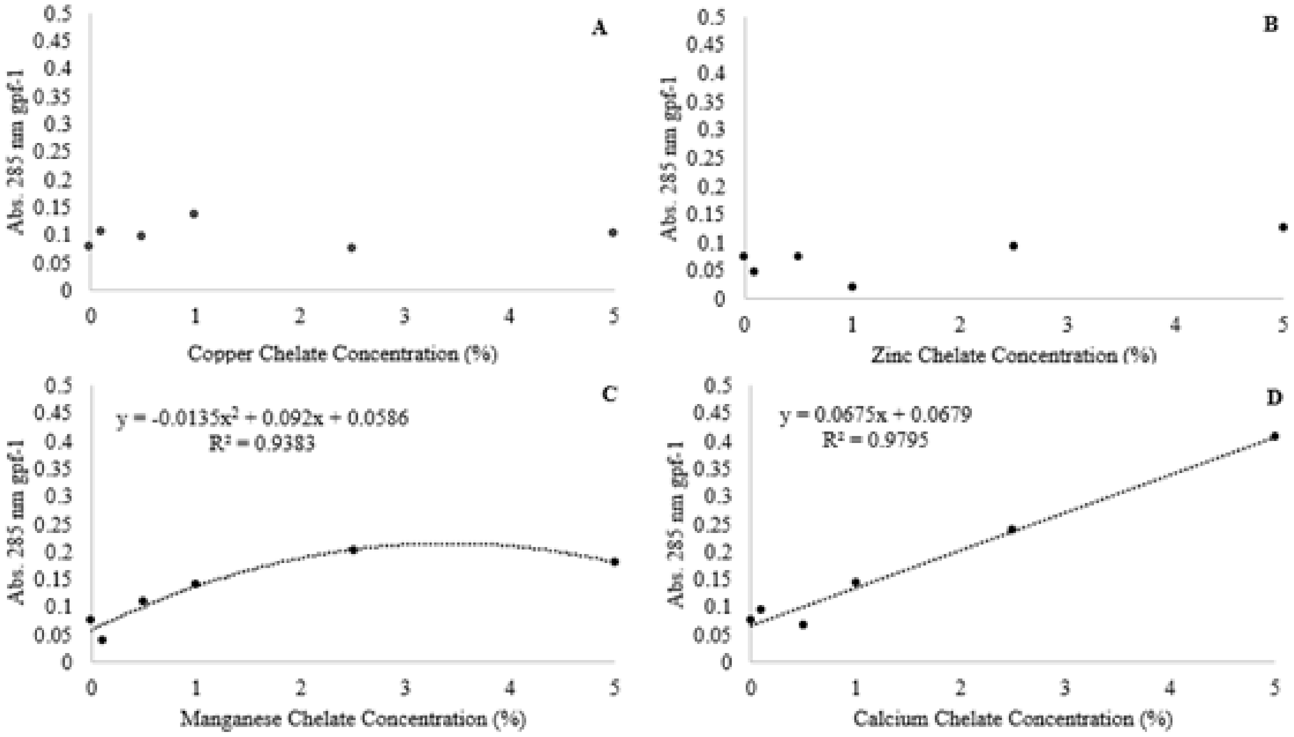
Figure 1. Production of phytoalexin phaseolin (absorbance per gram of fresh mass - ABS gpf^{-1}) in bean hypocotyls ("Carioca" cultivar) treated with concentrations of copper, zinc, manganese and calcium chelates (0%, 0.1%, 0.5%, 1%, 2.5% and 5%).



These results corroborate those obtained in similar studies. The same copper (2 mL L^{-1}), manganese (2 g L^{-1}), calcium (3 g L^{-1}) and zinc chelates (3 mL L^{-1}) (doses recommended by the manufacturer) have been proven to induce phaseolin resistance to infection by *Xanthomonas axonopodis* pv. *phaseoli* in bean plants (Queiroz, 2019).

In results obtained from the induction of phytoalexin glyceolin in soybeans, it was observed that there was no induction by two of the tested chelates, copper (Figure 2A) and zinc (Figure 2B), appearing statistically similar in comparison to control using the applied regression technique. Manganese chelate, based on quadratic regression, showed greater induction when at calculated product concentration of 3.4% (v/v), with increase in phytoalexin production 14% higher compared to control (Figure 2C). Regarding calcium chelate, it was observed that as the product concentration increased, the production of phytoalexin glyceolin also increased, that is, the increase is dose-dependent, showing positive linear regression, and at the highest dose (5% v/ v), the increase in phytoalexins in relation to control was 33% (Figure 2D).

Figure 2. Production of phytoalexin glyceolin (absorbance per gram of fresh mass - ABS gpf^{-1}) in soybean cotyledons ("Syn 13561IPRO" cultivar) treated with concentrations of copper, zinc, manganese and calcium chelates (0%, 0.1 %, 0.5%, 1%, 2.5% and 5%).



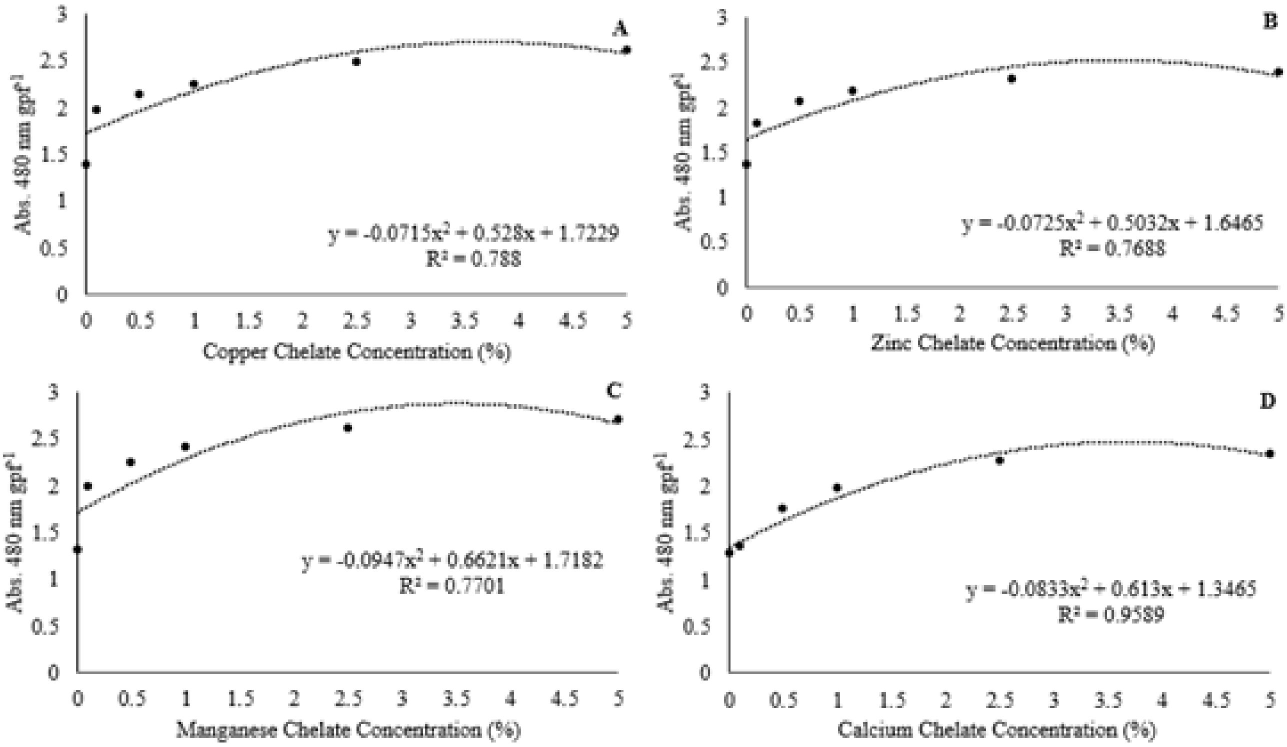
Similar studies on resistance induction in soybeans have shown the inducing effect of phytoalexin glyceolin by potassium phosphite (0.1; 1; 3 and 9 g L^{-1}) through commercial product Phosphilux Super® (54% phosphorus and 36 % potassium), with water as control and commercial product Bion® (0.1 g L^{-1}) as reference inducer (Castanho et al., 2014). Copper, zinc, manganese, calcium and potassium phosphite are nutrients necessary for plants and can act as phytoalexin inducers depending on the form of supply and amount absorbed by plants.

Regarding results obtained from the induction test of phytoalexins 3-deoxyanthocyanidins in sorghum, maximum inductions from copper, zinc, manganese and calcium chelates occurred, respectively, at doses calculated from quadratic regression of 3.69 % (v/v), with phytoalexin production 120% higher than control (Figure 3A); 3.47% (v/v), with a 142% higher production (Figure 3B); 3.49% (v/v), with a 287% higher production (Figure 3C); and 3.68% (v/v), with a 251% higher production (Figure 3D). Thus, all chelates showed induction for these phytoalexins in sorghum.

Copper, zinc, manganese and calcium have important functions in plant metabolism. Copper is the main catalyst for molecular oxygen reduction reactions in plant respiration, being an important component of several enzymes (Costa, 2014). Zinc is an essential micronutrient as enzyme activator, actively participating in the synthesis of growth regulators (auxins), also playing an important structural and functional role. Manganese is an essential microelement in enzyme activation and chlorophyll synthesis, while calcium is a fundamental structural and regulatory nutrient (NPA, 2021). These functions probably have the ability to stimulate the production of phytoalexins at some point in the physiological process through the activation of different metabolic pathways in plants (Stangarlin et al., 2010).

In field experiments carried out with maize, it was found that products based on calcium, copper, manganese and zinc had an effect on the agronomic traits of thousand-grain weight and productivity and on defense against diseases in first-crop maize. This effect may have occurred because chelates help plants maintain greater health, activating plant defense mechanisms through the induction of resistance or strengthening defense mechanisms, for example, by supplying calcium and manganese, or for having direct fungitoxic action on certain pathogens, as in the case of copper (Lorenzetti et al., 2020).

Figure 3. Production of phytoalexins 3-deoxyanthocyanidins (absorbance per gram of fresh mass - ABS gpf^{-1}) in sorghum mesocotyls ("Brandes" cultivar) treated with concentrations of copper, zinc, manganese and calcium chelates (0%, 0, 1%, 0.5%, 1%, 2.5% and 5%)



These functions performed by such nutrients show their importance in plant metabolism. Therefore, when in correct concentrations and in a more bioavailable form to plants, they can also contribute to the production of phytoalexins, as observed in this study, with the formation of phytoalexins phaseolin, glyceolin and 3-deoxyanthocyanidins in beans, soybeans and sorghum, respectively. However, it is important to carry out further in-depth field studies.

In conclusion, copper, zinc, manganese and calcium chelates have an inducing effect on phytoalexins in beans "Carioca" variety, by activating phaseolin synthesis, which occurred in a dose-dependent manner.

Manganese and calcium chelates have an inducing effect on phytoalexin in soybeans, by activating glyceolin synthesis, with the greatest induction by manganese occurring at calculated product concentration of 3.4% (v/v) and for calcium, the synthesis occurred in a dose-dependent manner. There was no induction of glyceolin synthesis from copper and zinc.

Copper, zinc, manganese and calcium chelates have an inducing effect of phytoalexins in sorghum by activating the synthesis of 3-deoxyanthocyanidins. Maximum inductions occurred at calculated doses of 3.69%, 3.47%, 3.49% and 3.68% (v/v), respectively.

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