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Morphological characteristics of cacao seedlings treated with the bioactivator thiamethoxam

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ABSTRACT

This work aimed to investigate the effect of thiamethoxam on the growth and quality of seedlings obtained through orthotropic cocoa cuttings. A completely randomized design, in a 4x4 factorial, was used, where the treatments were composed of concentrations of thiamethoxam diluted in water (0, 1, 2 and 4 g L^{-1}) and immersion times of the cuttings (0, 15, 30 and 60 min), with six replications and one plant per experimental plot. The following characteristics were evaluated: shoot length, shoot diameter, number of leaves, number of roots, root length, leaf dry mass, shoot dry mass, cutting dry mass, aboveground dry mass, total root dry mass, main root dry mass, secondary root dry mass and Dickson's quality index. The results indicated that, except for the number and length of roots, thiamethoxam improved the other morphological characteristics of the seedlings, with doses between 2.0 and 2.2 g L^{-1} promoted the best seedling growth under the experimental conditions evaluated. To obtain greater shoot dry mass, the best dose of thiamethoxam was 1.71 g L^{-1}. It is concluded that thiamethoxam can be an effective bioactivator in the propagation of cocoa by cuttings, favoring the growth of seedlings.

Keywords: Theobroma cacao L., cuttings, orthotropic braches, rooting.

INTRODUCTION

The cacao tree (Theobroma cacao L.) is a species belonging to the Malvaceae family, originating from regions of rain forests located in Central and South America. The plants are cultivated in more than 60 countries around the world, vegetating at different altitudes, humidity and light levels.[1,2] In Brazil, most of the cocoa cultivation areas, in production, were implanted with seminal seedlings.[3] However, in new plantings, the use of high-production genetic materials has been recommended,[4] in addition to propagative techniques, such as cuttings and grafting,[5] to obtain uniform and productive plants.[6]

Cocoa branches show orthotropic and plagiotropic dimorphic growth with different habits and growth, which influence the choice of material to be used in vegetative propagation.[7] The orthotropic branches form plants with a structure similar to that of a cocoa tree of seminal origin, with compact crowns and requiring less pruning for formation and maintenance.[8] In general, orthotropic branches also root more easily compared to plagiotropic branches, even in the absence of the growth regulator. This is because they are younger and more vigorous, because they have characteristics associated with reduced ontogenetic, physiological, and chronological age, and a lower amount of secondary metabolites.[9]

The proper choice of plant material and the use of complementary strategies can significantly influence the propagation success and performance of seedlings. In this context, the use of technologies such as bioactivators, including agrochemicals with specific physiological properties, becomes a promising practice. Agrochemicals are products that, in general, seek effectiveness in controlling pests and diseases in plants. However, it has been found that some molecules cause physiological effects capable of modifying metabolism and morphology, influencing growth and production.[10,11]

When an agrochemical has this activity, it can be classified as a bioactivator.[10] Among these bioactivating molecules is thiamethoxam [3-(2-chloro-1,3-thiazole-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine], a systemic insecticide of the neonicotinoid group with a broad spectrum of action at low concentrations.[12-14] In addition to its insecticidal action, thiamethoxam is capable of increasing agricultural production.[10,14,15] Calafiori and Barbieri[16] demonstrated the efficacy of thiamethoxam on bean crops, revealing an increase in grain germination and the number of viable nodules counted from plant roots, especially when associated with fertilizers containing N, P and K.[16]

Considering that the bioactivator thiamethoxam can improve the growth and quality of seedlings, the objective of this work was to evaluate the effect of thiamethoxam and the time of exposure in cocoa seedlings obtained by cutting orthotropic branches.

MATERIAL AND METHODS

The research was conducted in a nebulization chamber and in the laboratory of the Plant Physiology Section, located at the Cocoa Research Center (CEPEC), the main research unit of the Executive Committee of the Cocoa Crop Plan (CEPLAC), located in the municipality of Ilhéus-BA (14º 45' S and 39º 40' W), between October 2023 and February 2024.

The experimental design was completely randomized in a 4x4 factorial scheme, with six replications, each experimental plot consisting of one cutting, totaling 96 experimental units and the treatments formed by concentrations of thiamethoxam diluted in water (0, 1, 2 and 4 g L^{-1}) and immersion times of the cuttings (0, 15, 30 and 60 min).

Orthotropic cuttings of the PS 13.19 clone with a diameter ranging from 4 to 8 mm and an average length of 4 cm were used, containing 3 buds each and collected in the subapical position in cocoa mother plants, which were previously arched to stimulate the growth of orthotropic shoots according to the methodology described by Sodré and Gomes.[17] This clone was chosen because it has good productivity and is widely cultivated in the cocoa region of the state of Bahia.

Polypropylene tubes with 18 cm height and 5 cm top opening were used, with a volume of 288 mL. The components of the substrate and the respective fertilizer doses were: - Carolina Standard® Substrate (Sphagnum Peat, Expanded Vermiculite, Dolomitic Limestone, Gypsum and NPK Fertilizer) 12.5 kg - Simple Superphosphate 200 g - Fertilizer PGMIX® N, P, K 14-16-18 + micros 41.5 g - Osmocote® Fertilizer N, P, K 19-06-10 45.5 g. The tubes were initially filled with the substrate and arranged in rigid polyethylene trays with a capacity for 54 tubes. Then, the trays were taken to a wet chamber in order to standardize the humidity. After three days of wetting in a wet chamber, the cuttings were prepared.

Initially, a straight cut was made at the base of a leaf bud and they were immediately immersed in water for 10 minutes for hydration and removal of excess mucilage exuded after cutting. The commercial product Actara® 250 WG (Syngenta Crop Protection), a water-dispersible granule (WG) formulation containing 250 g kg^{-1} of thiamethoxam as the active ingredient, was used to prepare the treatment solutions. The tested concentrations (1, 2 and 4 g L^{-1}) corresponded to the commercial product diluted in water, while distilled water was used as the control treatment. The basal 2 cm of each cutting was immersed in the corresponding thiamethoxam solution. For the 0-min treatment, the cutting base was brought into contact with the solution and immediately withdrawn, whereas the remaining cuttings were maintained in the respective solutions for 15, 30, or 60 min. After each time, the cuttings were removed from the solution and subjected to 3-second contact with the rooting solution prepared with indolebutyric acid (IBA) with hydroalcoholic dilution in the proportion 1:1 (v/v).

The dilution of IBA was performed using 480 mg of solute to 40 mL of water and 40 mL of absolute alcohol to reach a concentration of 6000 mg L^{-1}. After the thiamethoxam treatments and the IBA protocol, the cuttings were inserted into the substrate (staking) at a depth of 2 to 3 cm and conducted for rooting. Every 10 minutes, the humid chamber environment received 15 seconds of nebulization, which was maintained daily from 07:30 h to 17:00 h. During this period, the internal temperature varied between 27 and 32 °C.

In the first 90 days after staking, the trays were relocated every 7 days on the benches of the nebulization chamber, as well as the tubes of each tray, in order to ensure that all experimental units (cuttings) received the same amount of water. In addition, fertilization was carried out every 21 days in the substrate with PG Mix fertilizer, applying 10 mL of the solution made with 5 g L^{-1} diluted in distilled water on the surface of each tube. At 90 days, nitrogen fertilization was performed, applying 10 mL of urea solution 5 g L^{-1} per tube.

At the end of the experiment, which took place at 145 days, the following morphological characteristics or growth variables in the seedlings were evaluated: shoot length (SL), shoot diameter (SD), number of leaves (NL) greater than 5 cm, number of roots (NR), root length (RL), considering the size of the main root measured manually with a ruler and with the use of a caliper, leaf dry mass (LDM), shoot dry mass (SDM), cutting dry mass (CDM) (material that gave rise to the seedlings), aboveground dry mass (ADM), total root dry mass (TRDM), main root dry mass (MRDM), secondary root dry mass (SRDM) and Dickson quality index (DQI), according to the methodology proposed by Dickson et al.[18] The dry mass content was obtained by drying the material in an oven at 65 °C for 72 hours, followed by weighing on a semi-analytical scale with a precision of 0.01 g.

The data were initially analyzed to verify the assumptions of the analysis of variance and, later, to the analysis of variance ($p \leq 0.05$). When significance was found, regression models were obtained that were adjusted according to the concentrations of thiamethoxam diluted in water (0, 1, 2 and 4 g L^{-1}) and the immersion times of the cuttings (0, 15, 30 and 60 min). Due to the absence of normal distribution, for some variables, the analysis was carried out with the transformation of the data (square root transformation $\sqrt{x}$). Statistical analyses were performed using the SISVAR program.[19] The models that presented significant coefficients of up to 5% by the F test and the highest coefficient of determination (R^2) were accepted.

RESULTS

There was an interaction between thiamethoxam doses and immersion time for secondary root dry matter (SRDM) and shoot dry matter (SDM). A difference was also observed between thiamethoxam doses for shoot length (SL), shoot diameter (SD), number of leaves (NL), root length (RL), root number (RN), leaf dry mass (LDS), aboveground dry mass (ADM), root dry mass (RDM) and Dickson quality index (DQI). Cutting dry mass (CDM) and primary root dry mass (PRDM) differed statistically for thiamethoxam doses and immersion time alone (Table 1).

Figure 1 shows the quadratic effect for shoot length (SL) and shoot diameter (SD) with the maximum thiamethoxam doses of 1.65 g L^{-1} and 1.15 g L^{-1}, resulting in values of 17.92 cm and 4.88 mm, respectively, which corresponds to an increase of 20.3% and 4.1% in relation to control.

When the number of leaves (NL) (Figure 2A), the number of roots (NR) (Figure 2B) and the root length (RL) (Figure 2C) were evaluated, the quadratic adjustment of thiamethoxam occurred only for the number of leaves at the dose of 1.61 g L^{-1}, with a maximum value of 3.35 leaves. The variables number of roots and root length showed linear adjustments, showing a reduction as a direct effect of thiamethoxam doses.

Table 1. Mean squares from the analysis of variance for shoot length (SL), shoot diameter (SD), number of leaves (NL), root length (RL), root number (RN), shoot dry mass (SDM), leaf dry mass (LDM), cutting dry mass (CDM), aboveground dry mass (ADM), main root dry mass (MRDM), secondary root dry mass (SRDM), total root dry mass (TRDM) and Dickson quality index (DQI) of orthotropic seedlings of cocoa clone PS 13.19 submitted to thiamethoxam doses and immersion times

SV	DF	SL	SD	NL[1]	RL[1]	RN[1]	SDM[1]	LDM[1]	DF	CDM[1]	ADM[1]	MRDM[1]	SRDM[1]	TMSR[1]	DQI[1]
Doses (D)	3	184.78**	8.08**	1.76**	1.98**	1.12**	0.05**	0.50**	3	0.09**	0.74**	0.10**	0.12**	0.19**	0.20**
Times (T)	3	32.10	2.63	0.14	0.35	0.63	0.01	0.09	3	0.23**	0.25	0.04*	0.01	0.05	0.07
D x T	9	44.89	2.10	0.55	0.37	0.35	0.02*	0.10	9	0.03	0.17	0.02	0.04*	0.04	0.04
Residue	80	35.39	1.68	0.35	0.24	0.25	0.01	0.09	80	0.03	0.11	0.01	0.02	0.02	0.03
Average		15.48	4.51	3.12	3.91	1.67	1.17	1.04		0.71	1.40	0.49	0.36	0.62	0.75
CV (%)		38.4	28.7	19.1	12.6	29.8	8.1	28.5		24.4	24.3	24.2	37.4	25.6	22.7

** Significant F at 1% and * Significant F at 5%. [1] Data transformed into √x.

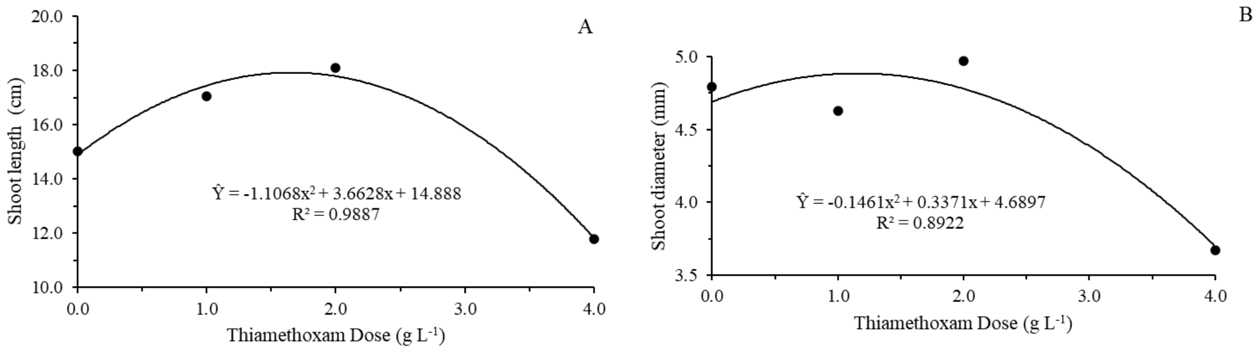

Figure 1. Shoot length (A) and shoot diameter (B) of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses.

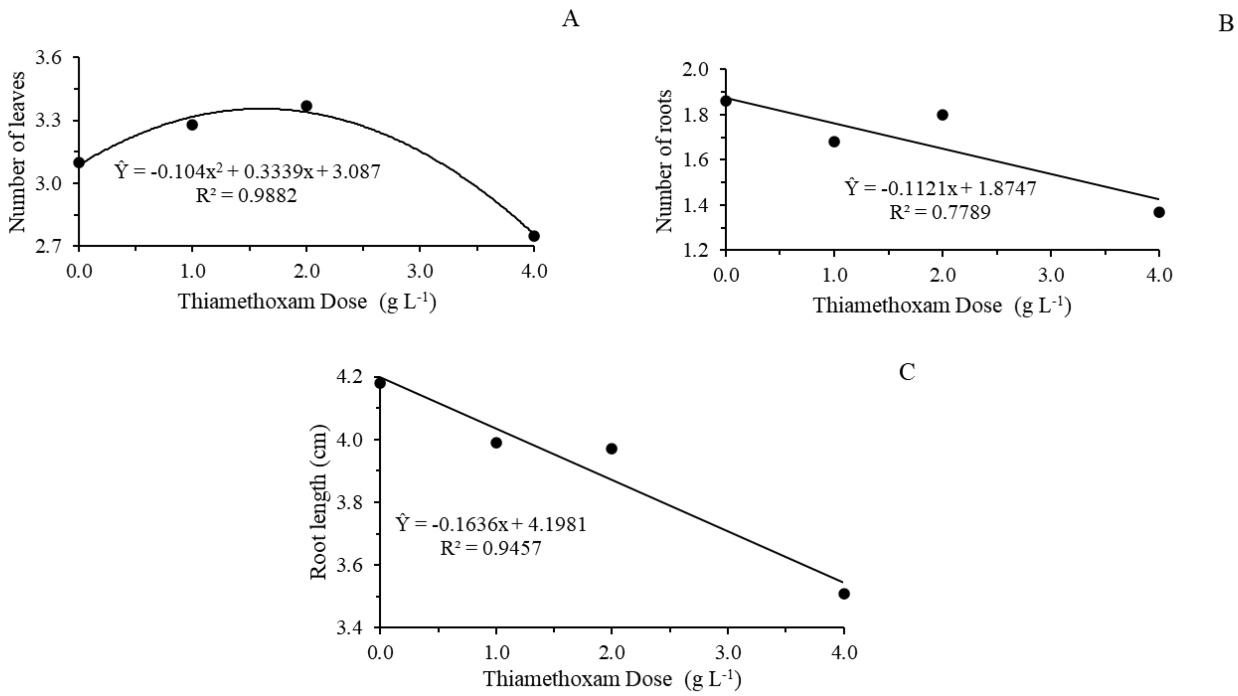

Figure 2. Number of leaves (A), number of roots (B) and root length (C) of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses. Data transformed into √x.

For leaf dry mass (LDM) (Figure 3A), aboveground dry mass (ADM) (Figure 3B) and root dry mass (RDM) (Figure 3C), maximum values of 1.17 g, 1.56 g and 0.68 g were observed with thiamethoxam doses corresponding to 1.65 g L^{-1}, 1.71 g L^{-1} and 1.31 g L^{-1}, respectively.

For the cutting dry mass (CDM), a significant effect was found for thiamethoxam doses and immersion time alone. Figure 4A shows the concentration of thiamethoxam (1.85 g L^{-1}) responsible for the highest observed CDM (0.78 g), while Figure 4B shows that 23.5 min was the immersion time of the cutting in thiamethoxam corresponding to the highest dry mass (0.80 g).

The main root dry mass (MRDM), only the thiamethoxam dose demonstrated quadratic effect (Figure 5A), while the time of exposure of the cutting led to a reduction in MRDM, with a decline of 18.3% from time zero (Figure 5B).

The shoot dry mass (SDM) showed maximum values of 1.0 g and 0.98 g in the curves corresponding to 30 and 60 min, whose thiamethoxam concentrations were 2.02 and 2.20 g L^{-1}, respectively (Figure 6A). For the dry mass of secondary roots, only in 30 min was the maximum value of 0.52 g observed, corresponding to 1.91 g L^{-1} of thiamethoxam (Figure 6B).

For the Dickson quality index (DQI), a quadratic fit was observed for thiamethoxam doses, with the highest DQI (0.81) obtained at a dose of 1.27 g L^{-1} (Figure 7).

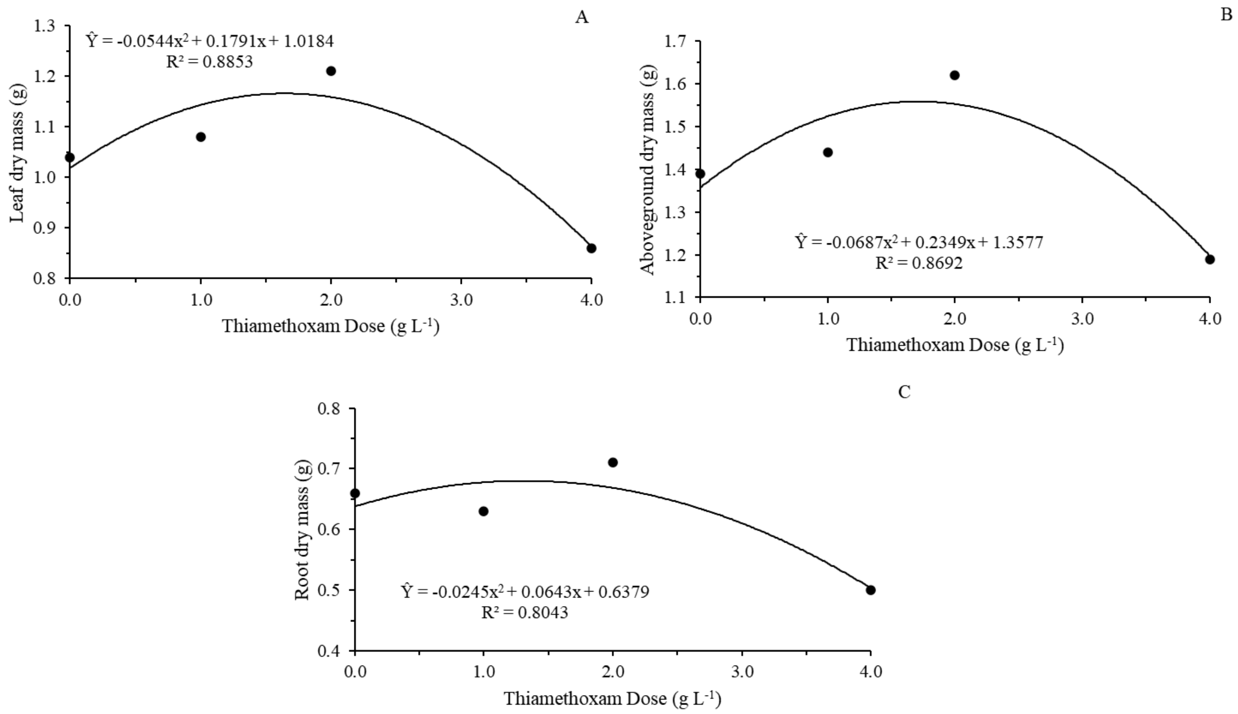

Figure 3. Leaf dry mass (A), aboveground dry mass (B) and root dry mass (C) of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses.

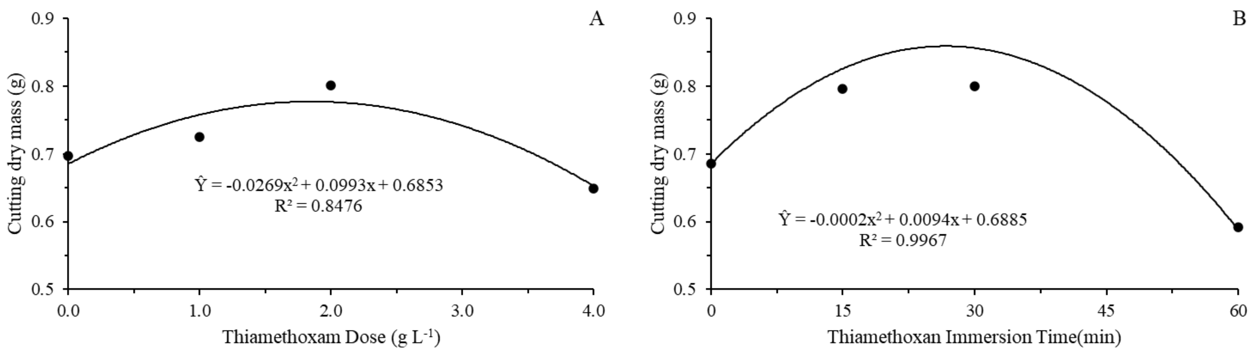

Figure 4. Cutting dry mass of seedlings of orthotropic cocoa cuttings PS. 13.19 submitted to thiamethoxam doses (A) and different immersion times (B). Data transformed into √x.

DISCUSSION

It was observed that the SL presented a better fit of the regression model compared to the SD. This result can be explained because plants grow from apical meristems with the bud showing significant growth in relation to diameter when evaluated at the same time. According to Sleigh et al.[20] and Waldburger et al.,[21] the growth of shoots and roots in cocoa seedlings has a rhythmic cycle, controlled by the level of N, P, K, soluble carbohydrates and starch in the mature leaves.[20,21]

The increase in the number of leaves can be explained because thiamethoxam increases the concentration of chlorophyll in the leaves and the photosynthetic rate, favoring the accumulation of biomass as already observed in rice[22] and lettuce,[23] cotton and okra crops.[24] Li et al.[25] also demonstrated, in pak choi cabbage, that thiamethoxam is readily transferred to the leaves, whose transfer rate is relatively higher than the plant's own capacity to absorb the molecule by the roots.

It is highlighted that aboveground dry mass is the most effective variable and it is suggested that the maximum dose identified for this variable resulted in greater aboveground dry mass under the conditions evaluated, indicating the bioactivating potential of thiamethoxam for cocoa

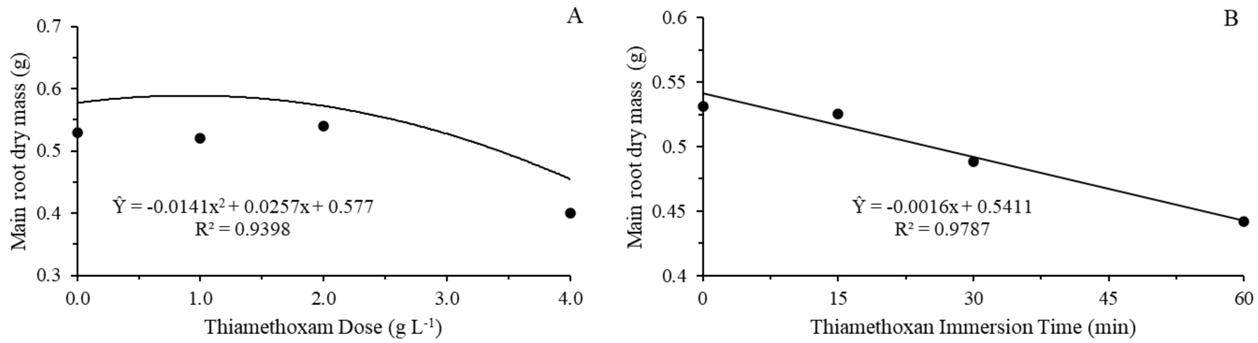

Figure 5. Main root dry mass of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses (A) and different immersion times (B). Data transformed into √x.

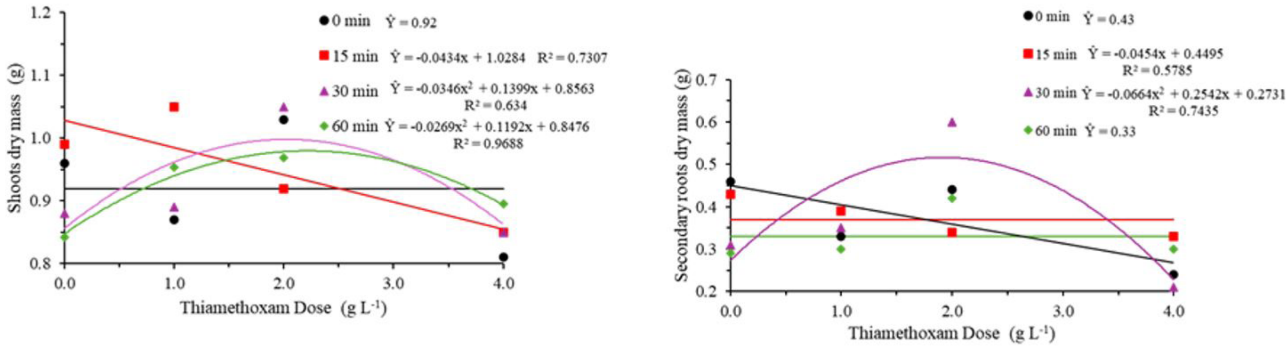

Figure 6. Shoots dry mass of (A) and secondary roots dry mass (B) of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses for different immersion times. Data transformed into √x.

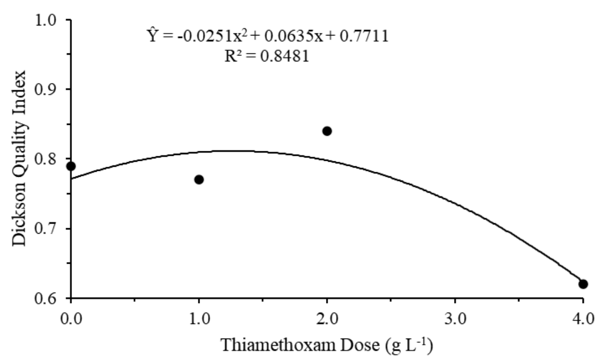

Figure 7. Dickson quality index (DQI) of orthotropic seedlings of cocoa clone PS. 13.19 submitted to thiamethoxam doses. Data transformed into √x.

seedlings. However, additional studies involving different genotypes and cultivation conditions are necessary before broader recommendations can be made. In the context of the use of thiamethoxam in seedlings, Assis et al.[26] found good development of the aerial part of coffee seedlings when submitted to application with thiamethoxam and according to Cruz et al.,[27] thiamethoxam has a bioactivating effect, causing an increase in the volume and root area of coffee seedlings (Coffeea arabica L.), which corroborates the results of this research, where an increase in the total root dry mass was observed after treatment of cocoa cuttings with thiamethoxam.

According to Macedo and Castro,[28] thiamethoxam stimulates important physiological and metabolic changes in the plant, influencing initial growth, through the increase of roots, the alteration of the distribution of photoassimilates, the increase in the concentration of total soluble protein, the reduction in the activity of the enzyme nitrate reductase and the increase in the activity of phenylalanine ammonia-lyase. In this work, a decline in the growth of cocoa seedlings was observed in the immersion times and higher doses of thiamethoxam, suggesting a possible growth retarding effect. In this context, Costa et al.[29] highlighted that the physiological effect of thiamethoxam is dependent on the type of cultivar and the dose applied.

Considering that high doses resulted in a decline in some morphological characteristics, it is possible that thiamethoxam has a hormesis effect, in which low or even very low doses of the substance modify plant metabolism, and can stimulate: growth, nutrient absorption, biomass accumulation, leaf area, protein content, sugar levels and increase or reduction in crop production.[30-34] According to Barreto et al.[35] and Jalal et al.,[35,36] high doses of chemicals applied to plants would be potentially toxic by inducing an inhibitory phase for plant development.

The highest values found for the times of 30 and 60 min in the evaluation of the SDM are similar, this result suggests that thiamethoxam doses between 2.0 and 2.20 g L^{-1} would be adequate and, therefore, recommended for the production of cocoa seedlings by cutting orthotropic branches. According to Macedo and Castro,[28] thiamethoxam, in addition to being an efficient insecticide, can promote linear increases in root development, protein content and shoot dry matter, which are considered relevant aspects of its bioactivating effect.

With regard to DQI, and according to Pereira et al.,[37] a good reference value of cocoa seedlings is 0.33, on the other hand, Souza and Peres[38] and Caldeira et al.[39] highlighted that for eucalyptus seedlings, the higher the DQI, the higher the quality of the seedlings produced. Thiamethoxam promoted an increase in the DQI, suggesting a better quality of cocoa seedlings treated with the bioactivator.

According to Lauxen et al.,[40] the definition of thiamethoxam doses to promote an increase in growth variables, for example, will depend on each species, in addition to, in the case of cocoa, the type of seedling (seminal or grafting) and the management conditions in the nursery. The doses of thiamethoxam tested in this work were apparently high, which may have affected the growth of seedlings, verifying, for example, an increase of only 6% in the total root dry mass, comparing the treated cuttings with and the control. However, the results also indicated positive effects of thiamethoxam on growth with an increase in the quality, expressed in mass of roots and shoots of seedlings that received treatment, compared to those not treated.

CONCLUSIONS

Cocoa seedlings originated from orthotropic cuttings and treated with thiamethoxam at ideal concentrations showed a significant increase in shoot length, number of leaves, shoot dry mass, cutting dry mass, aboveground dry mass and secondary root dry mass.

The dose of thiamethoxam that allowed the highest aboveground dry mass was 1.71 g L^{-1}.

High doses of thiamethoxam interfered with the growth of orthotropic seedlings and, in general, doses between 2.0 and 2.20 g L^{-1} promoted the best growth responses under the experimental conditions evaluated.

Thiamethoxam soaking time above 30 minutes was detrimental to the main root dry matter, aboveground dry mass and secondary root dry matter.

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DATA AVAILABILITY STATEMENT

The entire data set supporting the results of this study was used in this article.

AUTHOR CONTRIBUTIONS

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