









Production of clonal seedlings of black pepper cv. Bragantina under doses of controlled-release fertilizer

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Abstract

Commercial substrates and their fertilization are essential for the morphophysiological, nutritional, and sanitary quality of seedlings. Seedlings of the species *P. nigrum* cv. Bragantina requires standardization in commercial substrates due to soil infestation by pathogens that affect diseases such as fusariosis and nematosis, where their fertilization allows seedlings to be more adapted to commercial planting. In this perspective, this study aimed to indicate the best dosage of the fertilizer Osmocote® 19-06-10 Mini Prill for the production of clonal seedlings of black pepper cv. Bragantina. The treatments were constituted by five doses of the controlled-release fertilizers (CRF) Osmocote® 19-06-10 Mini Prill (3-4 months) (0, 2, 4, 6, and 8 kg m⁻³), with four replications of 16 cuttings. The CRF Osmocote®, especially in doses close to 4.16 kg m⁻³, showed to be promising for the production of clonal seedlings of the black pepper cultivar Bragantina.

Keywords: cutting, osmocote®, *Piper nigrum* L., vegetative propagation

Introduction

Black pepper is a perennial vine grown for its berries, it is one of the oldest known spices in the Piperaceae family. Although native to the tropical rainforest of Western Ghats of southern India, it is cultivated in many countries such as Indonesia, Malaysia, Brazil, Sri Lanka, Vietnam, China (Somashekar et al., 2021). The species (*Piper nigrum* L.) is one of the most appreciated spices with high added value in the world, known as the “king of spices” and “black gold” (Wang et al., 2020). Its striking aroma and spicy flavor combined with the bioactive compounds present in the fruits that confer thermogenic, antioxidant, anticancer, antidiabetic, antimicrobial action and ability to improve digestion, make the interest in this species increasing (Cancian et al., 2018; Sen et al., 2019; Shityakov et al., 2019; Sousa et al., 2019).

It is widely used for culinary purposes, especially for the growing business of pre-treatment foods, and

processed foods such as fried foods, sweets and baked goods. According to the global market research of black pepper, the world production was recorded at its maximum volume at 752 thousand tons in the year 2018 with a value of 4.1 billion dollars (Khew et al., 2022). In 2020, Brazil ranked second among the largest black pepper exporters in the world, representing 15% of global trade, behind only Vietnam (41.5%). In Brazil, Espírito Santo is the state that stands out as an exporter of black pepper, accounting for almost 55% of total exports of the product in 2021.

The role of black pepper in agribusiness is one of the most important in the agricultural trade balance. However, some factors have decreased the advancement of this activity, among them the occurrence of pests and diseases, significantly decreasing the yield and useful life of crops. Of the primary preventions for soil-borne diseases is the planting of seedlings of ensured health,

obtained with propagules (orthotropic branches) and substrates free of phytopathogenic microorganisms. The commercial substrate composed of ground Pinus bark and vermiculite can be used in the production of clonal black pepper seedlings, since, besides being a preventive measure for the control of fusariosis and nematode infection (Secundino et al., 2018), when associated with controlled-release fertilizers it can produce seedlings with higher dry matter accumulation (Serrano et al., 2012).

Controlled-release fertilizers are the future of precision agriculture since they aim to avoid the basic problem of traditional fertilizers, which is the low release rate of mineral nutrients. The release rate causes the plants not to trespass 50% of absorption of the nutrients applied, causing the rest to penetrate deeply into the soil, resulting in the eutrophication of water reservoirs if there is accumulation or leaching into underground waters (Mikula et al., 2020).

Controlled-release fertilizers also act against soil impoverishment caused by intense plant cultivation, making nutrients available in a gradual manner, trying to coincide with the nutrient demand of the plant, and causing losses to be minimized when compared to the use of mineral fertilizers (Chen et al., 2018; Mikula et al., 2020). This is possible because these fertilizers are available in the form of granules covered by a pellicle, usually an elastic polymer with the release of its nutrients occurring through a diffusion process (Chen et al., 2018).

Osmocote® (19-06-10) Mini Prills is a fertilizer with a release time of around 3 to 4 months which, in addition to containing 19% nitrogen, 6% phosphorus and 10% potassium, also has 3.5% sulfur formulation. The size of the granules is a great option for propagation and production of seedlings in small packages, providing greater homogeneity in the substrate.

Furthermore, these characteristics confer economic advantages for reducing production and environmental costs, by avoiding the leaching of these materials into effluents, decreasing the emission of greenhouse gases into the atmosphere (Xiao et al., 2019). In this way, the objective was to produce and analyze the quality of seedlings, and to indicate the best dosage of the fertilizer Osmocote® 19-06-10 Mini Prill for the production of clonal seedlings of black pepper cv. Bragantina.

Material and Methods

The cuttings used were produced from orthotropic herbaceous branches of the black pepper cultivar Bragantina cultivated in the field, obtained in the municipality of São Mateus - ES, located in the geographic coordinates 18° 39' 51.599" S latitude, 40° 5' 36.687" W longitude, and with an elevation of 80.2 m. The climate of the region is Aw-type, with a wet tropical climate, dry winter, and rainy summer, according to the classification by Köppen. The mean annual rainfall is 1,200 mm, concentrated from November to January. The mean annual temperature is 23 °C, with a maximum of 29 °C and a minimum of 18 °C (Alvares et al., 2013).

The cuttings were standardized in size (10 cm) and diameter (7 mm), and the leaves of the cuttings were divided into two thirds of their size, and a simple bevel cut was made at the base of each cutting. Afterward, the cuttings were treated with indole-3-butyric acid (IBA) at the concentration of 4,000 mg kg⁻¹ in inert talcum (Secundino et al., 2014), and afterward planted in Bioplant® substrate (Pinus bark and coconut fiber) fertilized with five doses (0, 2, 4, 6, and 8 kg m⁻³) of Osmocote® 19-06-10 Mini Prill (3-4 months), in polystyrene trays with 200 cm³ cells. The chemical and physical analyses of the Bioplant® substrate can be seen in Table 1 (MAPA, 2007).

Table 1. Chemical and physical analyses of the Bioplant® commercial substrate used in the rooting of cuttings of *P. nigrum* cv. Bragantina.

Chemical characteristics								
pH H ₂ O	P	K ⁺ (mg dm ⁻³)	Na ⁺	Ca ²⁺	Mg ²⁺	Mg ²⁺ + Ca ²⁺ (cmol _c dm ⁻³)	Al ³⁺	H ⁺ +Al ³⁺
5.2	198.0	990.0	510.0	7.8	3.2	11.0	0.4	4.7
OM (dag kg ⁻¹)	CEC (t) (mg dm ⁻³)	SB %	Zn	Fe	Mn	Cu	B	
8.5	13.9	74.2	15.2	206.0	45.0	2.4	1.2	
Physical characteristics								
Dry density (kg m ⁻³)			Porosity (%)		Particle size (mm)		Distribution (%)	
303.59			64.09		> 4.75		0.07	
-			-		2.00 a 4.75		34.56	
-			-		1.00 a 2.00		43.36	
-			-		0.5 a 1.00		18.78	
-			-		0.25 a 0.5		2.31	
-			-		< 0.25		0.92	

The cuttings were kept in a plant nursery covered with a double layer of transparent polyethylene with 150 micra, equipped with an irrigation system by intermittent misting, with automatic activation at every three minutes, for thirty seconds, presenting mean values of temperature and air relative humidity of 30.2 °C and 84%, respectively.

The experimental design was in randomized blocks, with plots arranged in five treatments, with four replications of 16 cuttings. The rooted cuttings produced seedlings that were cultivated for 120 days, analyzing the following variables: sprouting (%); number of bifurcations in the bud; bud length (cm), with the aid of a ruler; number of leaves on the bud; bud diameter (mm), with the aid of a caliper; shoot dry mass (g), dried in an oven at 70 °C until constant mass is obtained; chlorophyll a, b and total index obtained clorofiloLOG Falker®; rooting (roots emitted in the basal or nodal region, or in both regions) (%); basal rooting (%); nodal rooting (%); number of roots; longest root length (longest basal or nodal root length) (cm); longest basal root length (cm); length of the longest nodal root (cm), the lengths were obtained with the aid of a ruler; root volume (volume of basal + nodal roots) (cm³); basal root volume (cm³); nodal root volume (cm³), performed by measuring the displacement of the column of water in a measuring cylinder, that is, placing the roots, after washing, in a graduated cylinder tube containing a volume known amount of water (100 mL); root dry mass (basal root + nodal dry mass) (g); basal root dry mass (g) and nodal root dry mass (g), the masses were obtained by drying the material in an oven at 70 °C until constant mass was obtained. The data were subjected to analysis of variance and the means were analyzed by regression, using the Genes software (Cruz, 2016). The maximum points were obtained with the derivation of the quadratic regression equation.

Results and Discussion

The maximum percentage of sprouts (68%), number of bifurcations (0.24), length (9.64 cm), number of leaves (2.7), diameter (3.49 mm), and sprout dry mass (0.62 g) of the seedlings of the black pepper cultivar Bragantina were obtained with the doses of 4.54, 5.58, 4.71, 4.89, 4.63, and 4.69 kg m⁻³ of Osmocote® 19-06-10 Mini Prill, respectively (Figure 1). The number of sprouts within the genus *Piper* is variable according to the species: for *P. arboreum* it is 2.23 sprouts, whereas, for *P. amplum* and *Piper* sp., it does not reach 0.27 sprouts (Magevski et al., 2011). In addition, this variation between species may occur due to the nutritional and hormonal status of the plant that donated the stake, the genotype, the planting season and climatic conditions, and cultural

management, and variation may even occur within the species itself. Considering that all cuttings with shoots rooted in the present study, a higher average of shoots will favor the formation of the aerial part of the adult plant. For the total dry mass of seedlings, the Osmocote® doses (NPK 15-09-12) of 4.4, 6.4, and 5.3 kg m⁻³ promoted the highest values for the seedlings of the black pepper cultivars Guajarina (8.4 g), laçar (5.72 g), and Cingapura (3.46 g), respectively (Serrano et al., 2012).

Chlorophyll a, b and total indices increased linearly up to the highest dose of CRF (Figures 2a, b, c, respectively). This behavior is usually justified by the correlation between the chlorophyll index and the nitrogen content, which is an important indirect way of assessing the nutritional status of seedlings, in which the relative chlorophyll index was an indicator of the nutritional nitrogen status (Haim et al., 2012). In other studies, the chlorophyll index (SPAD), total nitrogen levels and the potential quantum yield of photosystem II (Fv/Fm) reached higher mean values in plants fertilized with CRF Osmocote® 18-05-09 at the dose of 5 kg m⁻³ (Machado et al., 2011). For most species, the approximate value of the Fv/Fm ratio is 0.832±0.004 in typical and non-stressful situations (Bjorkman & Demmig, 1987). However, Critchley (1998) verified that values below 0.725 certainly characterize the inhibition of photosynthesis.

The adventitious rooting of the cuttings of the black pepper cultivar Bragantina resulted in a quadratic regression, with a maximum point at the Osmocote® dose of 2.99 kg m⁻³, with 68.17% (Figure 3a), corresponding to an increase of 9.89% in relation to the control (non-fertilized), indicating a low effect of the fertilizer on the induction of rhizogenesis. Secundino et al. (2014, 2018) obtained mean rooting values of 91% and 40.75%, respectively, with the cultivar Bragantina without the CRF in the substrate. This significant variation may be related to the genetic material employed and the nutritional status of the stock plant, despite belonging to the same cultivar.

Other Piperaceae species feature an easy rhizogenic process, such as *P. hispidum*, which, when grown in the washed sand and commercial substrates, presented the highest rooting percentages of 81.56 and 81.33%, respectively (Cunha et al., 2015), suggesting that the nutritional quality of the substrates does not influence rhizogenesis. Another example of a Piperaceae species with easy rhizogenesis is *P. xylostoides*, for possessing ramified shoot structures (stolons) that grow on the surface of the substrate in the forest, presenting adventitious roots and becoming an independent structure from the original plant (Souza et al., 2009).

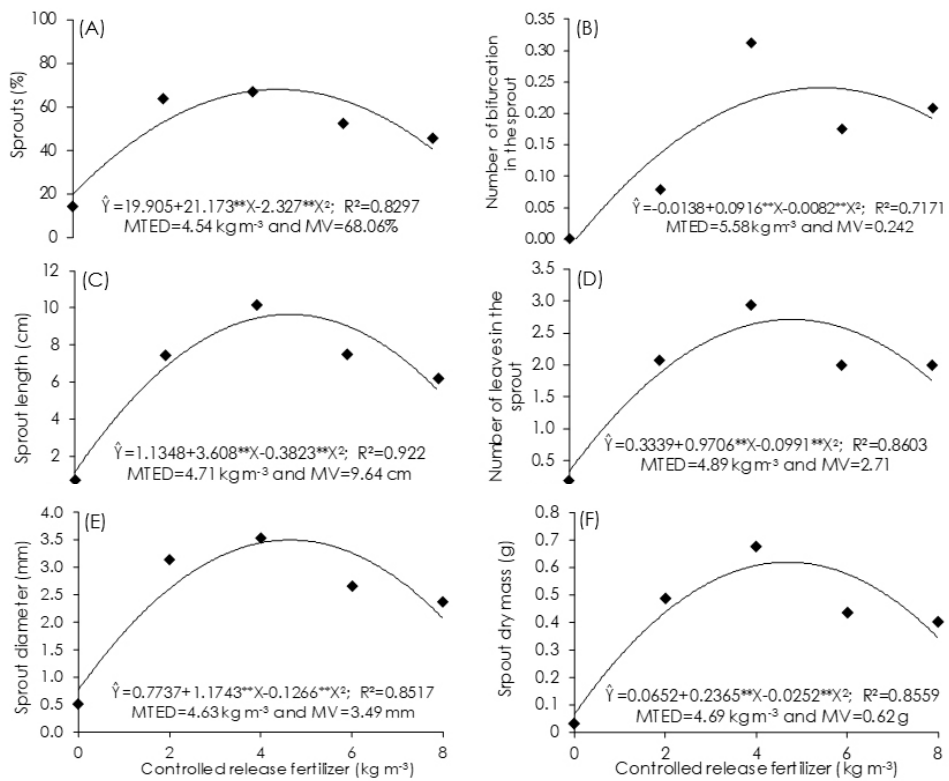


Figure 1. Sprouts (a), number of bifurcations in the sprout (b), sprout length (c), number of leaves in the sprout (d), sprout diameter (e), and sprout dry mass (f) of cuttings of the black pepper (*P. nigrum*) cultivar Bragantina grown in Bioplant® substrate fertilized with doses of Osmocote®. **Significant at 1% of probability. MTED. Dose of maximum technical efficiency. MV. Maximum value.

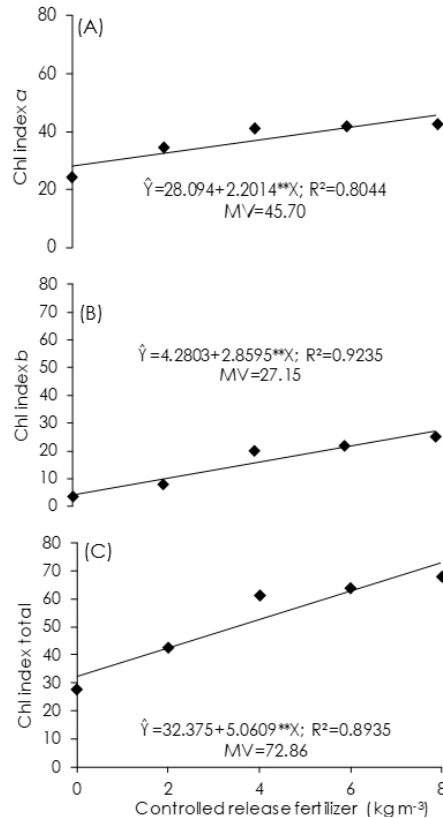


Figure 2. Index of chlorophyll a (a); index of chlorophyll b (b), and index of total chlorophyll (c) in leaves of cuttings of the black pepper (*P. nigrum*) cultivar Bragantina grown in Bioplant® substrate fertilized with different doses of Osmocote®. **Significant at 1% of probability. MV. Maximum value

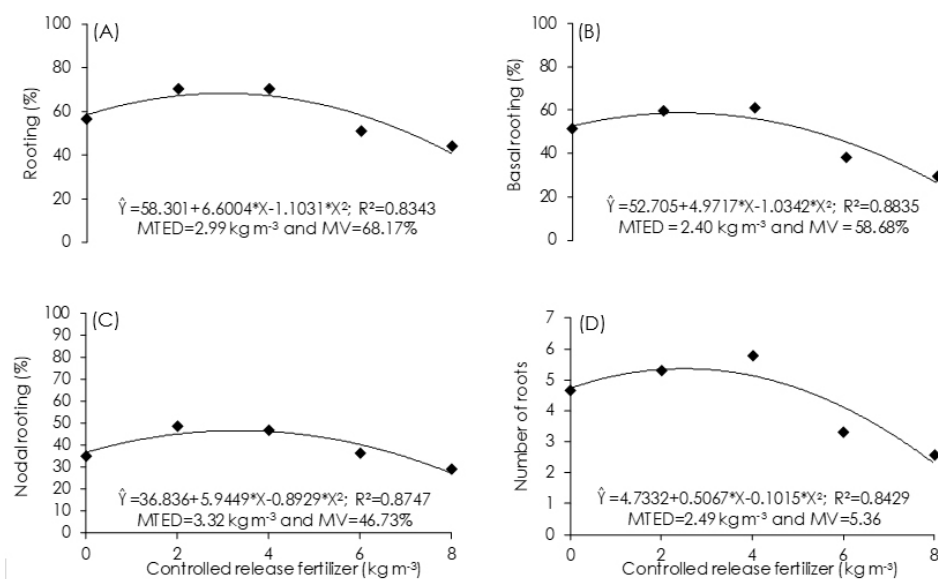


Figure 3. Rooting (a), basal rooting (b), nodal rooting (c), and number of roots (d) of the black pepper (*P. nigrum*) cultivar Bragantina grown in Bioplant® substrate fertilized with different doses of Osmocote®. *Significant at 5% of probability. MTED. Dose of maximum technical efficiency. MV. Maximum value.

The highest number of roots (5.4) of the cuttings of *P. nigrum* cv. Bragantina was verified with the Osmocote® dose of 2.50 kg m⁻³ (Figure 3d), corresponding to a 12.4% increase in relation to the non-use of the CRF (4.7). Leaf cuttings of the species *P. longum* produced 13.5 roots, indicating a new way to replace traditional propagules since the number of roots was high (Basak et al., 2014). According to Xiao et al. (2019), the important thing is that the root system presents a higher number of thin roots, which are more efficient in nitrogen absorption.

The highest root volume (1.1 cm³) was obtained with the Osmocote® dose of 4.21 kg m⁻³ (Figure 4d), which provided a 178.48% increase in relation to the non-fertilized treatment. The volume of the root system is directly related to the plant's capacity in exploring the substrate, strongly correlating with shoot quality. The longest lengths of basal (5.91 cm) and nodal roots (5.84 cm) were obtained with the application of the doses of 2.72 (Figure 4b) and 3.74 kg m⁻³ (Figure 4c) of the CRF. These values are superior to the control treatment and those found by Secundino et al. (2018) (2.55 and 2.79 g, respectively) in the absence of fertilization for the cultivar Bragantina, an increment of 131.76% (basal) and 109.31% (nodal). For the obtainment of these values, higher values were necessary for the roots of nodal origin, probably because these roots originate from the upper part of the cutting, and when deepening into the substrate, this one is already being exploited/impoverished by the roots of basal origin.

The highest root dry mass (0.1210 g) was obtained with the CRF dose of 4.11 kg m⁻³, and, in its

absence (0.043 g), there was a difference of 0.078 g, representing an increment of 181.4% with the use of Osmocote® (Figure 5a). The development of the root system of the black pepper cultivar the use of the CRF also favored the black pepper cultivar Bragantina, as observed by Secundino et al. (2014, 2018), in which the absence of this fertilizer limited in 0.0756 and 0.0741 g the root dry matter of the black pepper cultivar Bragantina, a difference of approximately 0.046 g for the same cultivar as in the present study. Most of this difference is justified by the higher rooting (91%) verified by these authors. This is probably related to the genetic material and good endogenous balance of hormones, more adequate at the moment of collection of the cuttings.

In general, Osmocote® 19-06-10 Mini Prill (3-4 months), at doses of approximately 4.16 kg m⁻³, provides superior results compared with the remaining concentrations tested in this study. With doses above this value, the results of all characteristics analyzed decrease, to which one of the explanations is the probable decrease of pH in the substrate, which before was 5.2 (Table 1). According to the study performed by Freitas et al. (2011), as the doses of Osmocote® are increased, the pH of the substrate is consequently decreased, and the concentration of Al³⁺ increases. Values of pH below 4.5 make nutrient solubility difficult and, consequently, their availability to plants, making the plants unable to absorb water in sufficient quantities. In this perspective, one of its visual symptoms is the decrease in root length (Hasanuzzaman et al., 2019).

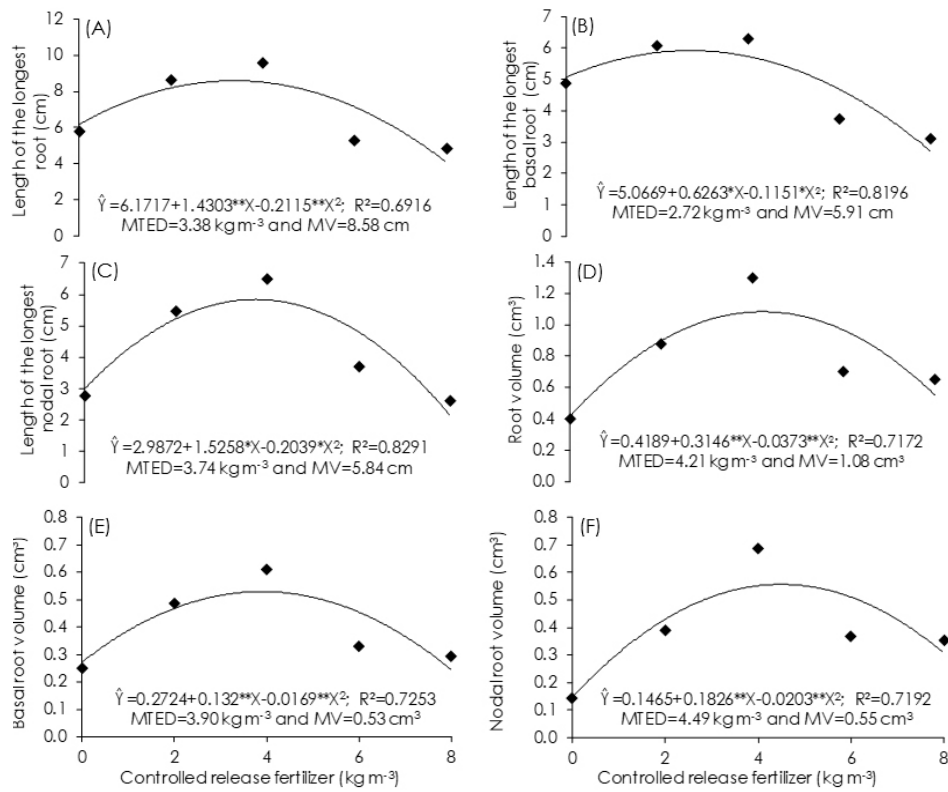


Figure 4. Length of the longest root (a), length of the longest basal root (b), length of the longest nodal root (c), root volume (d), basal root volume (e), and nodal root volume (f) of the black pepper (*P. nigrum*) cultivar Bragantina grown in Bioplant® substrate fertilized with doses of Osmocote®. ** and *Significant at 1 and 5% of probability, respectively. MTED. Dose of maximum technical efficiency. MV. Maximum value.

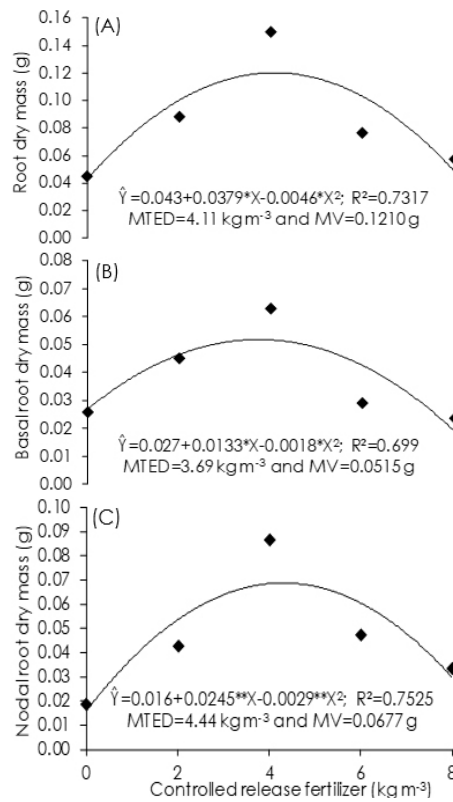


Figure 5. Root dry mass (a), basal root dry mass (b), and nodal root dry mass (c) of the black pepper (*P. nigrum*) cultivar Bragantina grown in Bioplant® substrate fertilized with doses of Osmocote®. ** and *Significant at 1 and 5% of probability, respectively. MTED. Dose of maximum technical efficiency. MV. Maximum value.

The content of Al^{3+} in the substrate ($0.4 \text{ cmol}_c \text{ dm}^{-3}$) of the control treatment (Table 1) may have increased with higher concentrations of Osmocote®. With a neutral pH, the Al^{3+} in a chemical reaction forms non-soluble compounds, but with the decrease in the pH, these compounds break, and Al^{3+} becomes available, causing toxicity to the plants. Aluminum restricts the nutrient removal capacity of the roots, causing a nutritional deficiency, and also limits the depth of penetration, causing the disappearance of root hairs and reducing root growth (Hasanuzzaman et al., 2019), as observed in Figure 3.

Therefore, doses of 4.16 kg m^{-3} showed to be promising in the propagation via cuttings of *P. nigrum*, for favoring the necessary conditions for rooting, which in its 19-06-10 formulation, observed in the highest nutrient concentration, probably provides the increase of carbohydrate metabolism, an energy source for the formation of root primordia (Picolotto et al., 2015). Furthermore, it also promotes a proper formation of the shoot part, compared with the control treatment.

Conclusions

For the production of clonal seedlings of the black pepper cultivar Bragantina, the fertilizer Osmocote® 19-06-10 Mini Prill (3-4 months) in a single dose of 4.16 kg m^{-3} is recommended.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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