revista Ceres
https://doi.org/10.71252/2177-34912025720035
ISSN: 2177-3491

Interaction between systemic insecticide and humic substance applied to Conilon coffee seedlings

Gabriel Barbieri Rampinelli[1], *Ronyson Ruy Zucoloto*[1], *Cátia Aparecida Simon*[1], *Sebastião Ferreira de Lima*[2*], *Rodrigo Fraga Jegeski*[1], *Bliane Morozini Bacheti*[1]

[1] FAESA Centro Universitário, Linhares, ES, Brazil. gabrielrampinelli@outlook.com; ronysonruy@hotmail.com; catiasimonsimon@gmail.com; rjegeski@gmail.com; blianebachete@gmail.com

[2] Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, MS, Brazil. sebastiao.lima@ufms.br

*Corresponding author: sebastiao.lima@ufms.br

Editors:
Maribela Pestana
Marihus Baldotto

Submitted: April 15th, 2025.
Accepted: October 16th, 2025.

ABSTRACT

The objective of the study was to evaluate Conilon coffee seedlings treated with a systemic insecticide and a humic substance, both of which have growth-promoting functions. The experiment was conducted in a commercial nursery for the production of Conilon coffee seedlings in the municipality of Linhares, Espírito Santo, Brazil. The experimental design was a randomized block in a 2 x 3 factorial scheme with four replicates. Two clones, A1 and K61, were used, and treatments included the use of insecticides alone or in combination with humic substances. Characteristics of roots, shoots, leaves, and cuttings of Conilon coffee seedlings were evaluated. The combined use of insecticides and humic substances resulted in increases of 39.0%, 47.8%, and 20.5% in shoot length, leaf area, and root volume, respectively, compared to the control. Superior production of leaf and aerial biomass was also observed in clone A1 due to the use of the insecticide in combination with the humic substance. It was concluded that clone A1 has better seedling growth compared to clone K61 and that the use of insecticide in combination with humic substances is recommended for the production of Conilon coffee seedlings.

Keywords: Neonicotinoid, anthranilamide, *Coffea canefora*, biostimulants.

INTRODUCTION

Brazil is the largest coffee producer in the world, followed by Vietnam, Colombia, Indonesia, and Ethiopia. Together, these countries produce more than 75% of the world's coffee, with a forecast of 127.15 million bags for the 2023/2024 harvest.(1) Brazil alone produced 66.4 million bags in the 2022/2023 harvest, corresponding to 38.1% of the total coffee market share.(1,2)

Coffee cultivation is of great economic importance, but good field productivity depends on producing healthy, high-quality seedlings with high vigor and suitable origin, which leads to better plant development and productivity.(3) In Conilon coffee, seedlings are produced from cuttings collected from mother plants, unlike Arabica coffee, where seedlings are formed from seeds.(4)

In coffee seedling production, synthetic chemical pesticides are necessary to control pests such as the coffee leaf miner (*Leucoptera coffeella*).(5) This pest attacks coffee seedling leaves, and its larvae feed on the mesophyll, causing necrosis and loss of photosynthetic capacity, which leads to defoliation and negatively affects seedling growth and quality.(6,7)

Systemic contact and ingestion insecticides such as thiamethoxam and chlorantraniliprole (a neonicotinoid and an anthranilamide, respectively) are commonly used to prevent infestation by this pest. Thiamethoxam has been reported to have bioactivating effects on plant metabolism,(8) which have also reported by Leite et al.(9) in Arabica coffee at low doses. This suggests the potential use of this insecticide for its biostimulatory effect, although further research is needed to confirm this. However, more vigorous plant growth can be achieved by using growth-promoting bioinputs.

In coffee seedling production in nurseries, various bioinputs can be used to promote plant growth and development. Among these, the application of organic molecules based on humic substances stands out as an alternative to improve the performance of both the root system and the aerial parts of the seedlings.(10,11)

Humic substances can be divided into humic acids, fulvic acids, and humin, depending on their fraction. Each fraction has different properties, with humic acids having a higher molecular weight than fulvic acids. Fulvic acids are composed of molecules with low molecular mass and a higher content of acidic functional groups. The substances not extracted from the soil make up the humified fraction, which is closely associated with the soil's mineral fraction and is called humin.(12,13)

The mechanism by which humic substances associate with thiamethoxam and chlorantraniliprole is not yet known, but their combined use during coffee seedling production in nurseries may increase the production of root and aerial parts of the plants. Therefore, the hypothesis was established that the combined use of systemic insecticides and humic substances may improve the growth characteristics of Conilon coffee seedlings. The objective of the study was to evaluate Conilon coffee seedlings treated with a systemic insecticide and a humic substance, both of which have growth-promoting functions.

MATERIAL AND METHODS

The experiment was conducted only in 2023, between May and August, at a commercial nursery certified for clonal coffee seedling production, located in the municipality of Linhares, Espírito Santo, Brazil, at geographical coordinates 19º31'47.3"S and 40º10'23.4"W. Seedling production was performed by vegetative propagation of the Conilon coffee plant using the cuttings cloning method.(14) The cuttings were taken from the grower's clonal garden, and two clonal varieties were used: A1 and K61.

There is no description of the characteristics of these clones in the literature or scientific records. However, it is known that clone A1 is tall, has medium maturity, high productivity per plant and is resistant to coffee rust, while clone K61 is tall, has early maturity, high productivity per plant and is resistant to coffee rust, but is more susceptible to fusariosis.

The cuttings were obtained from the terminal part of the plagiotropic branches located in the upper third of the matrix. After removal from the matrix, a straight cut was made at the base and an oblique cut at the apex. Cuttings with a basal length between 5.0 and 6.0 cm, an apical length between 1.5 and 1.6 cm, and a remaining plagiotropic branch length between 1.5 and 2.0 cm were used. In addition, the leaves were cut to remove 40% to 60% of the length of the central vein.

After the cuttings have produced, they were placed in plastic bags measuring 0.20 x 0.10 x 0.05 m containing soil (red-yellow Latosol, medium texture, good drainage) as a substrate. For each m^3 of soil, a mixture of 0.25 m^3 of clay litter, 5 kg of simple superphosphate, and 5 kg of dolomitic limestone was used. Nutritional, water, and phytosanitary management was carried out according to the recommendations ofreferences.[15,16]

A randomized block design in a 2 x 3 factorial scheme with four replications was used. The first factor was the use of two conilon coffee clones, A1 and K61; the second factor was the application of an insecticide, the combined use of the insecticide with humic substances, and a control treatment. Each plot consisted of 16 plants, each placed individually in a plastic bag.

The insecticide used for the treatment is commercially known as Durivo®. It is a systemic contact and ingestion product from the chemical groups of neonicotinoids (thiamethoxam) and anthranilamides (chlorantraniliprole), with concentrations of 200 g L^{-1} and 100 g L^{-1} of the active ingredient, respectively. In Conilon coffee plants, this insecticide is used to control the pests leaf miner (*Leucoptera coffeella*), coffee plant pincer (*Quesada gigas*), and cochineal (*Planococcus minor*). This product is often used in the production of seedlings in tree nurseries and in the care of coffee plants in the field.

The other product used as treatment is a humic substance, classified as a biofertilizer, with a peat-based composition, commercially called Turfa Gel®. It contains 1% nitrogen, 5% potassium, 8.5% total organic carbon, a density of 1.13 kg L^{-1}, 10% humic acid, 17% fulvic acid, water solubility at 20°C of 100 g L^{-1}, electrical conductivity of 1.78 mS cm^{-1}, saline index of 16.31%, and pH of 9.12.

The treatments were applied 40 and 64 days after the cuttings. For the isolated application of the insecticide, a dose of 140 mL of commercial product, corresponding to 29.0 + 14.0 g of the active ingredients thiamethoxam + chlorantraniliprole, was diluted in 200 L of water. For the treatment with the insecticide in combination with humic substances, 140 mL of the insecticide and 200 mL of humic substances were diluted in 200 L of water, with 5 mL of this solution applied to each seedling. The control treatment did not include the insecticide or humic substances.

After pricking out, the seedlings were grown for 120 days under commercial nursery conditions for seedling production. After this period, they were taken to the INCAPER (Capixaba Institute of Research, Technical Assistance and Rural Extension) laboratory for evaluation, located in the municipality of Linhares, Espírito Santo, Brazil.

The parameters evaluated were number of roots (NR), length of the largest root (LR), root volume (RV), root dry mass (RDM), shoot length (SL), shoot diameter (SD), cutting length (CL), cutting diameter (CD), stem dry mass (SDM), number of leaves (NL), leaf area (LA), leaf dry mass (LDM), and shoot dry mass (SHDM).

NR was measured by counting the vigorous roots. LR was measured with a ruler, considering the main root. RV was determined by water displacement in a graduated cylinder: the roots were placed in a cylinder with a known volume of water (90 mL), and the displaced volume was measured. The result was calculated by the difference in volume, using the equivalence 1 mL = 1 cm^3.

SL was measured as the distance from the base (branch at the cut) to the apex using a millimeter ruler. SD was measured in the region of the branch on the cutting with a digital caliper. CL was determined by measuring the height from the stem to the apical bud using a ruler. CD was determined in the region of the stem of the cutting using a digital caliper.

The dry masses RDM, LDM, SDM, and SHDM were obtained after drying in an oven with forced air circulation at 65 °C until reaching constant mass. The LA was measured in cm^2 using the Scanner Area Meter LI-3100C (Biosciences, USA).

The assumptions of normal distribution and homogeneity of variances for the data were checked. The data were then subjected to analysis of variance, and the mean values were compared using the Tukey test with Sisvar 5.1 software[17] at a 5% probability level.

RESULTS AND DISCUSSION

No influence of the treatments on the variables NR, RDM, and NL was observed (Table 1). The variables CL, CD, and SDM were influenced only by the clones, while the variables SD, LA, and LDM were influenced only by the product solution used. The variables LR, SL, and SDM were individually influenced by both factors studied, and only the variable VR showed an interaction between the treatments (Table 1).

For coffee seedling growth traits, clone A1 showed higher values for SL, CL, LR, and CD, which were 4.5%, 11.2%, 3.6%, and 7.8% higher, respectively, than those observed for clone K61. The simultaneous use of the insecticide and the humic substance led to increases in shoot length and leaf area of 39.0% and 47.8%, respectively, compared to the control. For the length of the largest root, the isolated use of the insecticide or its mixture with the humic substance were equally favorable and promoted an average increase of 5.3% compared to the control (Table 2).

Table 1. Analysis of variance for number of roots (NR), length of the largest root (LR), root volume (RV), root dry mass (RDM), shoot length (SL), shoot diameter (SD), cutting length (CL), cutting diameter (CD), number of leaves (NL), leaf area (LA), leaf dry mass (LDM), leaf dry mass (SHDM) and stem dry mass (SDM), as a function of the use of insecticides and humic substance in the production of conilon coffee seedlings. Linhares, ES, 2024

SV	DF	NR	LR	RV	RDM	SL	SD	CL
Block	3	0.200	0.050	0.034	0,594	0,055	0,176	0,000
Clone	1	0.872^{ns}	0.015*	0.002*	0.846 ^{ns}	0.009*	0.323 ^{ns}	0.000*
Prod	2	0.402^{ns}	0.004*	0.148 ^{ns}	0.823 ^{ns}	0.000*	0.022*	0.387 ^{ns}
Clone*Prod	2	0.099^{ns}	0.315^{ns}	0.000*	0.841 ^{ns}	0.622 ^{ns}	0.655 ^{ns}	0.658 ^{ns}
CV(%)		24.33	14.3	32.11	47.23	16	12.07	14.46
Average		6.54	18.07	6.03	1.23	17.96	3.94	6.03

SV	DF	CD	NL	LA	LDM	SHDM	SDM
Block	3	0.000	0.826	0.986	0.741	0.553	0.360
Clone	1	0.000*	0.215 ^{ns}	0.322 ^{ns}	0.101 ^{ns}	0.023*	0.035*
Prod	2	0.569 ^{ns}	0.081 ^{ns}	0.000*	0.000*	0.002*	0.903 ^{ns}
Clone*Prod	2	0.631 ^{ns}	0.143 ^{ns}	0.211 ^{ns}	0.378 ^{ns}	0.640 ^{ns}	0.685 ^{ns}
CV(%)		16.90	28.04	27.78	35.15	28.71	33.11
Average		5.76	8.94	268.62	1.48	3.36	1.88

* Significant by Tukey test at 5% probability. ^{NS} = not significant. SV – Sources of variation; DF – Degrae of freedom; Prod-Products. CV – coefficient of variation

Table 2. Number of roots (NR), length of the largest root (LR), root volume (RV), root dry mass (RDM), shoot length (SL), shoot diameter (SD), cutting length (CL), cutting diameter (CD), stem dry mass (SDM), number of leaves (NL), leaf area (LA), leaf dry mass (LDM) and aerial part dry mass (SHDM), as a function of the use of insecticide and humic substance in the production of conilon coffee seedlings. Linhares, ES, 2024

Clones	SL	CL	NR	LR	SD	CD	NL	LA
	(cm)			(cm)	(mm)			(cm^2)
A1	18.35 a	6.35 a	6.53 a	18.39 a	3.91 a	5.97 a	8.78 a	272.39 a
K61	17.56 b	5.71 b	6.55 a	17.75 b	3.96 a	5.54 b	9.09 a	264.85 a

Prod	SL	CL	NR	LR	SD	CD	NL	LA
	(cm)			(cm)	(mm)			(cm^2)
Control	14.83 c	6.02 a	6.69 a	17.45 b	4.01 a	5.81 a	8.78 a	219.82 c
INS	18.42 b	6.11 a	6.43 a	18.31 a	3.8 5b	5.69 a	8.69 a	261.14 b
INS + HS	20.62 a	5.96 a	6.5 a	18.45 a	3.96 ab	5.77 a	9.34 a	324.90 a

A longer shoot length (Table 2) implies more buds that can form leaves. The greater number of leaves increases the leaf area of seedlings (Table 2), and leaf area is related to the photosynthetic potential of the species.[18] Thus, plant height or shoot length and leaf area are important morphological characteristics of seedlings that are closely related to their growth.[19] According to Fascella et al.[20], humic substances improve plant growth and leaf production, in this case due to the higher relative content of chlorophyll and the net photosynthesis achieved by the plants.

Another fundamental parameter in the study of seedlings is root growth (Table 2). Root length is crucial for the formation and establishment of seedlings, as it influences the onset of shoot growth, water and nutrient uptake, and the architecture of the root system,[21] and also enables greater resistance to abiotic stress.[22] Root growth is also correlated with morphological parameters such as height, stem diameter, and biomass.[23] This effect on roots occurs because humic substances influence the development of plant roots[24] by acting as effective growth promoters and being involved in hormonal pathways and genes/enzymes involved in nitrogen assimilation and cell division.[25]

Stem diameter is often used in seedling experiments as a reliable indicator of plant performance and seedling robustness. Higher values indicate better plant performance in the field, and a larger diameter can reduce damage caused by drought, heat, and competing vegetation.[26,27] In this experiment (Table 2), the treatments did not increase shoot or cutting diameter.

For traits related to plant biomass formation, clone A1 had higher average values of SDM and SHDM compared to clone K61 (Figure 1A). Among the solutions used, the combination of insecticide and humic substance resulted in higher dry mass of leaves and aerial parts (Figure 1B).

Clones A1 accumulated greater dry mass in stems and shoots, suggesting that seedling genetic material behaves differently under similar conditions, although no effect of the clone on mass accumulation in leaves and roots of Conilon coffee seedlings was observed (Figure 1A). The concomitant application of the insecticide with humic substance resulted in greater accumulation of dry matter in leaves and shoots of Conilon coffee seedlings compared to the control (Figure 1B).

The insecticide used in this study contains thiamethoxan and chlorantraniliprole. Studies have shown that thiamethoxan, in addition to its role in insect control, has a bioactivating effect.[28] Thiamethoxan can increase seedling vigor and favor initial plant growth,[29] but it can also have negative effects and impact the morphophysiological characteristics of coffee seedlings.[9]

In addition to the possible effects of the insecticide on biomass accumulation, humic substances may have also contributed to the increases in dry matter (Figure 1B), as they are considered biostimulants and bioactivators that promote plant growth and development due to their complexity and high physiological activity.[30,31] Humic substances are characterized by stimulating and adaptogenic effects at the cellular and subcellular levels.[30] They can promote seedling growth and increase plant biomass.[32,33]

Regarding root volume, the use of insecticides alone or in combination with humic substances resulted in a 20.5% increase in clone A1 compared to the control (Table 3). Humic substances can increase the number, diameter, and length of roots,[34] raise the concentration of indole-3-acetic acid,[35] stimulate energy metabolism and protein synthesis, and regulate enzymes involved in redox homeostasis.[36]

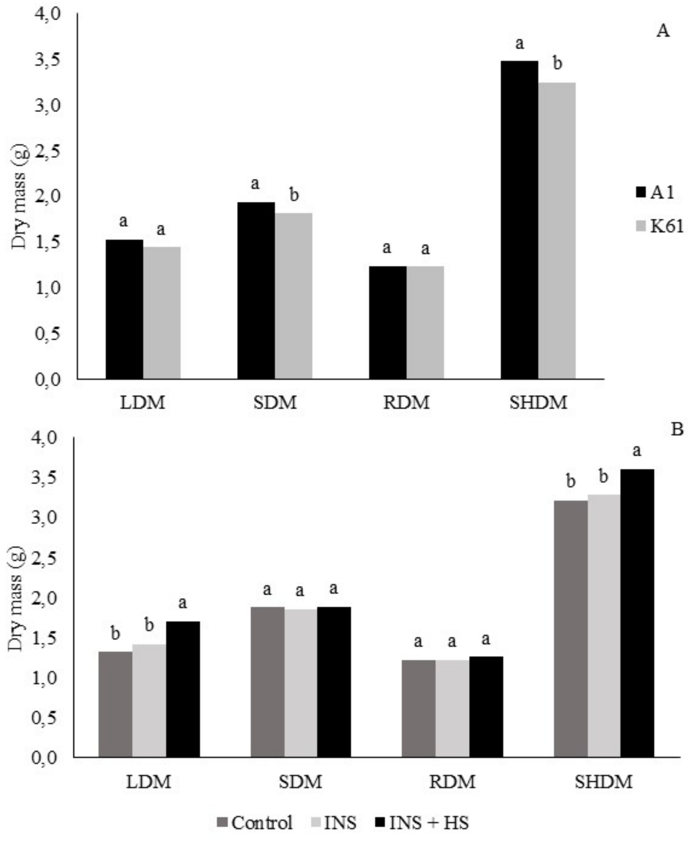

Figure 1. Leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and shoot dry mass (SHDM) for clones A1 and K61 (A). Evaluation of isolated application (INS) or not (INS+HS) for the parameters LDM, SDM, RDM and SHDM in Conilon coffee seedlings.

Table 3. Analysis of root volume (cm^3) in seedlings of Conilon coffee clones A1 and K61 subjected to insecticide application in combination (INS+HS) or without (INS) with humic substances

Clones	Root volume (cm^3)		
	Control	INS	INS + HS
A1	5.04 Bb	5.75 Aab	6.40 Aa
K61	7.26 Aa	5.76 Ab	5.95 Ab

The same uppercase letter in the column and the same lowercase letter in the row do not differ statistically from each other.

The results of this study indicate that the clones respond differently to the same treatments, and clone A1 being more sensitive and showing potential to increase both the root system and aerial part during seedling production in the nursery when the insecticide is used in combination with humic substances. Vigorous seedlings with a well-developed root system, large leaf area, and greater stem diameter ensure good establishment in the field, reduce transplanting costs, and promote rapid initial growth, thereby increasing the longevity and productivity of the coffee crop.[37]

CONCLUSIONS

Clone A1 exhibits stronger seedling growth than clone K61. The use of insecticides combined with humic substances is recommended as a biostimulant for producing Conilon coffee seedlings.

ACKNOWLEDGMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

To INCAPER for the infrastructure provided and to the students of the Plant Physiology Laboratory who helped evaluate the quality of the seedlings.

The authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

All datasets supporting the results of this study were used in this article

AUTHOR CONTRIBUTIONS

Conceptualization: Cátia Aparecida Simon, Cátia Aparecida Simon, Rodrigo Fraga Jegeski, Ronyson Ruy Zucoloto.

Data curation: Rodrigo Fraga Jegeski, Ronyson Ruy Zucoloto.

Formal analysis: Cátia Aparecida Simon, Cátia Aparecida Simon, Sebastião Ferreira de Lima.

Investigation: Bliane Morozini Bacheti, Cátia Aparecida Simon, Cátia Aparecida Simon, Gabriel Barbieri Rampinelli, Ronyson Ruy Zucoloto.

Methodology: Cátia Aparecida Simon, Cátia Aparecida Simon, Gabriel Barbieri Rampinelli, Ronyson Ruy Zucoloto.

Resources: Gabriel Barbieri Rampinelli, Rodrigo Fraga Jegeski.

Validation: Bliane Morozini Bacheti, Gabriel Barbieri Rampinelli.

Writing – original draft: Bliane Morozini Bacheti, Cátia Aparecida Simon, Gabriel Barbieri Rampinelli, Sebastião Ferreira de Lima.

Writing – review & editing: Cátia Aparecida Simon, Rodrigo Fraga Jegeski, Ronyson Ruy Zucoloto, Sebastião Ferreira de Lima.

REFERENCES

1. USDA, United States Department of Agriculture. Coffee: World Markets and Trade. [Internet] [cited 2025 Jan. 26]. Available from: https://apps.fas.usda.gov/psdonline/circulars/coffee.pdf.
2. CONAB, Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de café, v.1, n.1 [Internet]. Brasília: Conab; 2025 [cited 2025 Apr. 3]. Available from: http://www.conab.gov.br.
3. Verdin AC Filho, Mauri AL, Volpi PS, Fonseca AF, Ferrão RG, Ferrão MA, et al. Growth and quality of clonal plantlets of conilon coffee (*Coffea canephora* Pierre ex A. Froehner) influenced by types of cuttings. Am. J. Plant Sci. 2014;5(14):2148-53.
4. Lima AE, Castro ED, Cunha SH, Guimarães RJ, Chalfun NN, Carvalho AM, et al. Production of Coffea canephora seedlings through cuttings in a nursery and hydroponics using different containers. Coffee Sci.. 2023;18:e182084.
5. Dantas J, Motta IO, Vidal LA, Nascimento EF, Bilio J, Pupe JM, et al. A Comprehensive review of the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)- a major pest for the coffee crop in Brazil and others neotropical countries. Insects. 2021;12(12):1130.
6. Almeida JD, Motta IO, Vidal LA, Bílio JF, Pupe JM, Veiga AD, et al. Bicho mineiro (*Leucoptera coffeella*): uma revisão sobre o inseto e perspectivas para o manejo da praga. Brasilia: Embrapa Recursos Genéticos e Biotecnologia; 2020. 39 p.
7. Koutouleas A, Collinge DB, Raebild A. Alternative plant protection strategies for tomorrow's coffee. Plant Pathol. 2023;72(3):409-29.
8. Godoi CT, Campos SO, Monteiro SH, Ronchi CP, Silva AA, Guedes RN. Thiamethoxam in soybean seed treatment: Plant bioactivation and hormesis, besides whitefly control? Sci. Total Environ. 2023;857:159443.
9. Leite SA, Guedes RN, Costa DR, Colmenarez YC, Matsumoto SN, Santos MP, et al. The effects of thiamethoxam on coffee seedling morphophysiology and Neotropical leaf miner (*Leucoptera coffeella*) infestations. Pest Manag. Sci. 2022;78(6): 2581-7.
10. Moura OV, Barbara RL, Torchia DF, Silva HF, Castro TA, Tavares OC, et al. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops. A review. J. Saudi Soc. Agric. Sci. 2023;22(8):493-513.
11. Azevedo HP, Carvalho AM, Rodrigues JD, Vidal DA, Santos HO. Rooting biostimulants for *Coffea arabica* L. cuttings. Coffee Sci. 2020;15:e151635.

12. Schnitzer M. Humic Substances: Chemistry and Reactions. In: Schnitzer M, Khan SU, editors. Developments in Soil Science, Elsevier; 1978. p. 1-64.

13. Lamar RT, Monda H. Quantification of humic and fulvic acids in humate ores, doc, humified materials and humic substance-containing commercial products. J. Vis. Exp. 2022;181:e61233.

14. Verdin AC Filho, Volpi PS, Ferrão RG, Ferrão MA, Fonseca AF, Freitas SJ, et al. Produção de mudas clonais de cafeeiro: Avanços na padronização dos cortes e dimensões de estacas. Vitória: Incaper; 2022.

15. Ferrão RG, Fonseca AF, Ferrão MA. Conilon coffee. 3. ed. Vitória: Incaper; 2019.

16. Fonseca AF, Verdin AC Filho, Volpi PS, Mauri AL, Ferrão MA, Ferrão RG, et al. Clonal gardens, seed production and conilon coffee seedling. In: Ferrão RG, Fonseca AF, Ferrão MA, Muner LH. Conilon Coffee. Vitória: Incaper; 2019. p. 289-325.

17. Ferreira DF. SISVAR: a computer analysis system to fixed effects Split plot type designs. Braz J Biom. 2019; 37(4): 529-35.

18. Jo W, Shin J. Effect of leaf-area management on tomato plant growth in greenhouses. Hortic. Environ. Biotechnol. 2020; 61(6):981-8.

19. Yang S, Zheng L, Gao W, Wang B, Hao X, Mi J, et al. An efficient processing approach for colored point cloud-based high-throughput seedling phenotyping. Remote Sens. 2020;12(10):1540.

20. Fascella G, Montoneri E, Rouphael Y. Biowaste-derived humic-like substances improve growth and quality of orange jasmine (*Murraya paniculata* L. Jacq.) plants in soilless potted culture. Resour. 2021;10(8):80.

21. Pflugfelder D, Kochs J, Koller R, Jahnke S, Mohl C, Pariyar S, et al. The root system architecture of wheat establishing in soil is associated with varying elongation rates of seminal roots: quantification using 4D magnetic resonance imaging. J. Exp. Bot. 2022;73(7):2050–60.

22. Chen H, Zhao C, Yang Z, Zeng Z, Li W, Liu Y, et al. Identification and validation of a locus for wheat maximum root length independent of parental reproductive environment. Front. Plant Sci, 2022;13:999414.

23. Burcer P, Carandag W, Gascon A, Tiburan CL Junior. Effect of Root Pruning on the Root Growth Potential (RGP) of Three Philippine Native Tree Species. Philipp. J. Sci. 2021;150(2):429-44.

24. Kleczek A. Agricultural Use of Natural Biostimulants – Humic Substances: a review. Rocz. Ochr. Śr. 2022;24:1-14.

25. Nardi S, Schiavon M, Francioso O. Chemical structure and biological activity of humic substances define their role as plant growth promoters. Molecules. 2021;26(8):2256.

26. Gallegos-Cedilo VM, Diánez F, Nájera C, Santos M. Plant agronomic features can predict quality and field performance: a bibliometric analysis. Agron. 2021;11(11):2305.

27. Grossnickle SC, Macdonald JE. Why seedlings grow: influence of plant attributes. New Forests. 2018;49:1–34.

28. Macedo W, Castro P. Avanços tecnológicos aplicados à pesquisa na produção vegetal. Viçosa: Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo; 2015. Biorreguladores, bioestimulantes e bioativadores na agricultura tropical: 505-530.

29. Macedo W, Castro P. Thiamethoxam: molecule moderator of growth, metabolism and production of spring wheat. Pestic. Biochem. Physiol. 2011;100(3): 299-304.

30. Bezuglova O, Klimenko A. Application of Humic Substances in Agricultural Industry. Agron. 2022;12(3):584.

31. Castro TA, Berbara RL, Tavares OC, Mello DF, Pereira EG, Souza BC, et al. Humic acids induce a eustress state via photosynthesis and nitrogen metabolism leading to a root growth improvement in rice plants. Plant. Physiol. Biochem. 2021;162:171–84.

32. Zandonadi D, Matos C, Castro R, Spaccini R, Olivares F, Canellas L. Alkamides: a new class of plant growth regulators linked to humic acid bioactivity. Chem. Biol. Tecnol. Agric. 2019;6:1-12.

33. Rahmi EE, Satriawan H, Suwardi. Soil amelioration using several types of humic substances extracted from andisol, spodosol, peat and lignite to increase the growth of corn plants (*Zea mays*). IOP Conf. Ser.: Earth Environ. Sci. 2022;1115:012089.

34. Nunes R, Dominiciano G, Alves W, Melo A, Nogueira F, Canellas L, et al. Evaluation of the effects of humic acids on maize root architecture by label-free proteomics analysis. Sci. Rep. 2019;9(1):12019.

35. Hita D, Fuentes M. Fernández V, Zamarreño A, Olaetxea M, Garcia-Mina J. Discriminating the short-term action of root and foliar application of humic acids on plant growth: emerging role of jasmonic acid. Front. Plant Sci. 2020;11:493.

36. Roomi S, Mais A, Conselvan G, Trevisan S, Quaggiotti S, Pivato M, et al. Protein profiling of arabidopsis roots treated with humic substances: insights into the metabolic and interactome networks. Front. Plant Sci. 2018;9:1812.

37. Monteiro RS, Oliveira VE, Malta AO, Pereira WE, Silva JÁ. Produção de mudas de cafeeiro em função da época e da adubação fosfatada. PesquisAgro. 2018;1(1):28-8.