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Defective Coffee Bean Extract Enhances Coffee Tree Growth and Physiological, Biochemical, and Nutritional Responses in the Presence of *Meloidogyne paranaensis*

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Abstract

Meloidogyne paranaensis is widespread in Brazil's main coffee-growing regions, reducing productivity and potentially causing plant death. Defective black-green-sour (BGS) coffee beans are an abundant source of bioactive compounds capable of activating plant defense mechanisms. This study evaluated the potential of aqueous BGS-bean extracts for their ability to induce physiological and biochemical defenses in susceptible *Coffea arabica* cv. 'Catuaí Vermelho IAC 144'. Plants were inoculated with 5000 *M. paranaensis* eggs, and BGS-bean extracts diluted to different chlorogenic acid (CGA) concentrations (0.075, 0.150, 0.250, and 0.300 mg mL^{-1}) were applied one day before inoculation, 45 days after, and 15 days before harvest. We assessed nematode population density, shoot biomass, bioactive compounds, foliar nutrients, antioxidant enzyme activities (Superoxide dismutase (SOD), catalase (CAT)), lipid peroxidation (MDA), and photosynthetic pigments. BGS-bean extracts containing 0.25 and 0.30 mg mL^{-1} CGA increased root CGA content and preserved chlorophyll *a* and carotenoids under nematode challenge. Treatments reduced lipid peroxidation through enhanced CAT activity in roots and SOD activity in leaves, while increasing foliar concentrations of potassium (K), magnesium (Mg), nitrogen (N), and manganese (Mn). Shoot dry mass increased regardless of nematode presence. Overall, BGS bean extracts enhanced coffee tree growth and induced physiological, biochemical, and nutritional responses commonly associated with plant defense mechanisms in the presence of *Meloidogyne paranaensis*.

Keywords: bioactive compounds; *Coffea arabica*; nematode; defective coffee beans (BGS); defense response

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1. Introduction

Coffee is the second most valuable commodity worldwide, making it one of the most economically important crops globally [1]. However, the economic losses caused by nematodes have been widely reported, rendering coffee cultivation unviable in several infested regions [2,3]. Among these pathogens, *Meloidogyne paranaensis* is one of the most aggressive and damaging species due to its high destructive potential and wide distribution [4–6]. It severely compromises the plant's health by inducing significant physiological and morphological changes in the root system [5,7], beginning as early as the first biological cycle with root swellings and longitudinal cracks that progress into deep fissures, frequently leading to a sharp decline in vigor and plant death [3,8].

Concurrently, the use of chemical products remains the primary approach for nematode control. However, their intensive use threatens ecological balance and biodiversity, human health and the environment [9]. Consequently, plant genetic resistance, crop rotation and plant resistance inducers have emerged as sustainable alternatives for managing this pest. The production and alteration of plant metabolites in response to this pathogen [10] can prevent parasite development and reproduction [11]. Plant defense responses include effector-triggered immunity [12–15] and metabolic alterations involving bioactive compounds [16,17], such as chlorogenic acid (CGA), caffeine, and trigonelline [18,19].

CGA, an abundant antioxidant phenolic compound in coffee [20–22], is produced via secondary metabolism during the defense response against nematode infection [23]. In a recent study conducted by our team on coffee plant resistance to nematodes, it was found that resistant genotypes showed a significant increase in CGA content following inoculation, whereas the susceptible cultivar 'Catuaí Vermelho IAC 144' did not exhibit this increase [19]. Simultaneously, the induction of antioxidant system enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was observed; an activation that may be associated with CGA accumulation [24]. These enzymes play an essential role in neutralizing reactive oxygen species (ROS) produced in response to various biotic and abiotic stresses, thereby contributing to the protection of plant tissues [25].

Trigonelline is associated with stress adaptation, osmoprotection, and antioxidant regulation, contributing to plant tolerance under adverse environmental conditions. Its accumulation under stress suggests a role in maintaining cellular stability and defense readiness. Caffeine also contributes to plant defense, acting as both a protective metabolite and a signaling molecule involved in resistance responses [26].

In this regard, the application of exogenous bioactive molecules can trigger plant defense responses [15]. Furthermore, ref. [9] highlighted the potential of plant extracts to enhance plant immunity and contribute to nematode management, offering a sustainable approach that aligns agricultural production with environmental preservation. Plant extracts can stimulate the production of defense-related metabolites, reinforce cell walls, enhance antioxidant capacity, and modulate defense-related signaling pathways. Consequently, plant extracts have increasingly been explored as environmentally friendly tools for crop protection and for enhancing plant resilience.

Defective black, green, and sour (BGS) coffee beans constitute an abundant and low-cost source of these bioactive compounds, such as chlorogenic acids, trigonelline and caffeine [27,28]. Although these beans have little commercial value for beverage production, their chemical composition suggests considerable potential for agricultural applications aimed at inducing defense responses and improving crop resilience. However, the physiological, biochemical, and nutritional effects associated with the application of BGS-bean extracts in coffee plants challenged by *M. paranaensis* remain poorly understood.

Therefore, this study evaluated the potential of aqueous BGS bean extracts to promote physiological, biochemical, and nutritional responses associated with plant defense in

susceptible *Coffea arabica* plants during infection by *Meloidogyne paranaensis*, by assessing antioxidant metabolism, nutritional status, and bioactive compound accumulation.

2. Materials and Methods

2.1. Experimental Setup, Meloidogyne Paranaensis Identification, Inoculation and Plant Collections

The experiment was conducted from March to September 2025 in the greenhouse of the EPAMIG experimental campus in Lavras (CELA), located in the substation area of the Universidade Federal de Lavras, Lavras-MG, geographic coordinates (−21°13′36.59″ S, −44°58′41.69″ W) and altitude 918 m. During the experimental period, greenhouse temperatures were maintained between 24 and 27 °C, and relative humidity ranged from 60 to 80%, which is considered suitable for coffee cultivation. Plants were grown under natural photoperiod conditions. *C. arabica* seedlings of the 'Catuaí Vermelho IAC 144' susceptible to *M. paranaensis*, and six months old, were transplanted into pots (1L capacity) containing a soil–sand mixture (2:1 *v*/*v*). The substrate was then sterilized by autoclaving twice consecutively at 120 °C for 1 h. Before pot filling, the soil was chemically characterized and corrected according to the nutritional requirements recommended for coffee cultivation, including pH adjustment and nutrient supplementation to provide adequate conditions for plant establishment and growth. The pots were kept with a drip irrigation system to maintain soil moisture at field capacity.

The *M. paranaensis* population was confirmed by molecular analysis using DNA extracted with a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) [29] and identified via the SCAR-Multiplex PCR method [30]. Inoculum was propagated on coffee trees grown in a greenhouse, and eggs were extracted as described by [31]. An egg suspension was calibrated to 5.000 eggs per plant using an inverted objective microscope and inoculated into holes around the root zone 30 days after transplanting.

Leaves were collected six months after nematode inoculation, then cleaned to remove any residual BGS-bean extract from the foliar application. The leaves were gently wiped with cotton moistened with distilled water to avoid damage to the epidermal tissues and to preserve sample integrity. Excess water was subsequently removed with paper towels. This procedure was adopted to reduce potential contamination from surface residues and to minimize their interference with the quantification of leaf metabolites and mineral nutrients. Consequently, the measurements were intended to represent the physiological and biochemical responses of the plant tissues rather than residual extract components remaining on the leaf surface.

2.2. Extraction and Characterization of Bioactive Compounds from BGS Coffee Beans

Defective coffee beans (black, green, and sour; BGS) discarded during processing were ground in liquid nitrogen using an analytical mill (IKA A11 basic, (IKA-Werke GmbH & Co. KG, Staufen, Germany)). For analytical characterization, 0.5 g of ground material was extracted with 50 mL of distilled water at ~95 °C for 3 min in a water bath (model SL-151/10, SOLAB Científica Equipamentos para Laboratórios Ltda., Piracicaba, SP, Brazil)). The extract was filtered (0.45 µm polyethylene membrane) and analyzed by HPLC according to [28] to quantify chlorogenic acid (CGA), trigonelline, and caffeine. The BGS beans contained 3.00 g 100 g^{-1} CGA, 1.00 g 100 g^{-1} trigonelline, and 1.11 g 100 g^{-1} caffeine. To prepare the stock extract for plant assays, 4 g of ground BGS beans were extracted in 400 mL of distilled water, maintaining the same solid-to-liquid ratio (*w*/*v*). This yielded a stock extract containing 0.300 mg mL^{-1} CGA, 0.100 mg mL^{-1} trigonelline, and 0.111 mg mL^{-1} caffeine (Supplementary Materials, Figure S1). Since CGA was the primary compound, its concentration served as the baseline for the experimental treatments. Treatment solutions

(0.075, 0.150, 0.250, and 0.300 mg mL^{-1} CGA) were prepared by diluting the stock extract with distilled water using the dilution equation:

$$C_1V_1 = C_2V_2$$

where C_1 is the stock CGA concentration (0.300 mg mL^{-1}), V_1 is the stock extract volume, C_2 is the target CGA concentration, and V_2 is the final solution volume.

2.3. Application of Treatments

Treatments were applied to coffee trees at three time points: 1 day before inoculation, 45 days after inoculation, and 15 days prior to the final evaluation (conducted 6 months post-*M. paranaensis* inoculation). The study evaluated five treatments: four BGS extract concentrations (0.075, 0.150, 0.250, and 0.300 mg mL^{-1} CGA) and a control (distilled water, 0.00 mg mL^{-1} CGA).

During application, each plant was isolated in an acrylic chamber to prevent spray drift. A calibrated manual hand sprayer delivered 10 mL of solution per plant—a volume previously determined to ensure complete leaf coverage without runoff, eliminating the need for surfactants or adjuvants. Pots and soil surfaces were sealed with aluminum foil to protect the roots and rhizosphere from extract contact. Plants were maintained in a greenhouse under drip irrigation at field capacity.

2.4. Bioactive Compound Analyses

Immediately after collection, the leaves were gently wiped with cotton moistened with distilled water to remove potential surface residues without damaging the leaf surface. Excess water was removed with paper towels, and the leaves were frozen until analysis. Leaves and roots from treated plants were collected separately, ground in an analytical mill (IKA A11 basic), and 0.5 g of each sample was weighed for further analysis. Each sample was extracted with 50 mL of hot distilled water (~95 °C) for 3 min in a water bath (model SL-151/10). After extraction, the samples were filtered through 0.45 µm polyethylene membranes and analyzed by HPLC as described by [28]. The analyzed compounds were trigonelline, CGA, and caffeine (Supplementary Materials, Figure S1). The stock solution of all standards (1 mg mL^{-1}, Sigma-Aldrich® Brasil Ltda., Cotia, SP, Brazil.) was prepared in deionized water and diluted to concentrations ranging from 0.01 to 0.7 mg mL^{-1} to build the calibration curve [28].

2.5. Biochemical Analysis

2.5.1. Catalase (CAT) and Superoxide Dismutase (SOD) Activity

Leaves and roots from treated plants were collected separately, frozen in liquid nitrogen and kept at −80 °C until needed. The enzyme extract was obtained from 0.2 g of leaf and root tissues macerated in liquid nitrogen; we then added polyvinylpolypyrolidone (PVPP) and 1.5 mL of extraction buffer composed of potassium phosphate 400 mM (pH 7.8), EDTA 10 mM, and ascorbic acid 200 mM. The extract was centrifuged at 13,000 rpm for 10 min at 4 °C to obtain the supernatant. SOD activity was estimated by the enzyme's ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) [32]. The reaction mixture for SOD activity determination consisted of 50 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 0.1 µM EDTA, 75 µM nitro blue tetrazolium (NBT), 2 µM riboflavin, and distilled water. Absorbance was measured at 560 nm after 10 min of incubation under illumination. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction. CAT activity was determined according to [33] by monitoring the decomposition of H_2O_2 at 240 nm at 15-second intervals for 3 min. The CAT activity assay mixture contained 50 mM potassium phosphate buffer (pH 7.0)

and 12.5 mM H_2O_2. For each biological replicate, three technical replicates were analyzed. Blanks and control reactions without enzyme extract were included to correct for non-enzymatic reactions, and only measurements within the linear range of each assay were used for activity calculations. SOD activity was expressed as U SOD min^{-1} g^{-1} fresh mass (FM), whereas CAT activity was expressed as mmol H_2O_2 min^{-1} g^{-1} FM.

2.5.2. Lipid Peroxidation Assessment by Malondialdehyde (MDA) Quantification

Samples were ground in liquid nitrogen in the presence of PVPP to minimize oxidation. Subsequently, 0.2 g of tissue was homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 15 min at 4 °C. An aliquot of the resulting supernatant (125 µL) was mixed with 250 µL of the reaction mixture containing 0.5% thiobarbituric acid (TBA) and 10% TCA. The mixture was incubated at 95 °C for 30 min, rapidly cooled on ice, and the absorbance was measured spectrophotometrically at 535 nm and 600 nm. Lipid peroxidation was expressed as ηmol of MDA g^{-1} fresh weight (FW), according to the method described by [34].

2.6. Photosynthetic Pigment Quantification and Plant Growth Assessment

Photosynthetic pigments chlorophyll *a*, chlorophyll *b* and carotenoids were quantified as adapted by [35]. The extract was obtained from 20 mg of leaf tissue, macerated in three stages using 100%, 80%, and 50% ethanol. The three fractions were combined to yield a final volume of approximately 1 mL. A 50 µL aliquot of the combined extract was mixed with 120 µL of 100% ethanol in a 96-well ELISA plate (total volume 170 µL per well). Absorbance was recorded at 470, 649, and 664 nm using a microplate spectrophotometer, with 170 µL of 100% ethanol as a blank. Pigment concentrations were calculated according to [35], accounting for the microplate path length. The results, expressed in µg/mL, were calculated as chlorophyll *a* (Chl a) (µg/mL) = 13.36 × A664 − 5.19 × A649; chlorophyll *b* (Chl b) (µg/mL) = 27.43 × A649 − 8.12 × A664 and carotenoids (µg/mL) = (1.000 × A470 − 2.13 × Chl a − 97.63 × Chl b)/209.

Plant development was assessed six months after inoculation by evaluating shoot (leaves and stems) dry mass following oven-drying at 70 °C to constant weight.

2.7. Foliar Nutrients Analysis and Nematode Population Quantification

For foliar macro- and micronutrient analysis (K, Ca, Mg, N, Mn, Fe, B, and Na), leaves were dried in a forced-air oven at 70 °C for 48 h until reaching constant weight and processed for nutrient quantification following the methodologies described by [36].

The population density of *M. paranaensis* eggs and second-stage juveniles (J2) per gram of root was determined following extraction according to [31]. Nematodes were quantified in duplicate using a counting slide under an inverted microscope. A limitation of this study is that nematode populations were quantified only in the root fraction remaining after sampling for biochemical analyses. Therefore, total nematode population per root system and reproduction factor could not be accurately determined. To avoid biased estimates, nematode abundance was expressed as the number of nematodes per gram of root tissue.

2.8. Experimental Design and Statistical Analysis

The experiment was conducted in a randomized complete block design (RCBD) arranged in a 5 × 2 factorial scheme, consisting of five extract treatments (water control and four CGA concentrations: 0.075, 0.150, 0.250, and 0.300 mg mL^{-1}) and two inoculation conditions (inoculated and non-inoculated), with four blocks. Each experimental unit consisted of one individual plant, resulting in four independent biological replicates per treatment combination and a total of 40 experimental units. For each evaluated variable,

tissue samples were collected individually from each plant, without pooling samples from different plants. Each biological sample was analyzed in triplicate (three technical replicates), and the mean of the three technical measurements was used as a single observation for statistical analysis, thereby avoiding pseudoreplication.

Data normality and homogeneity of variance were confirmed using the Shapiro–Wilk and Bartlett tests ($p > 0.05$), respectively. Data were subjected to factorial analysis of variance (ANOVA), considering extract treatment, inoculation, their interaction (extract treatment × inoculation), and block effects. The statistical model was expressed as

$$Yijk = \mu + Ti + Ij + (TI)ij + Bk + \varepsilon ijk$$

where Yijk represents the observed response, μ is the overall mean, Ti is the effect of the ith extract treatment, Ij is the effect of the jth inoculation condition, (TI)ij is the interaction effect between extract treatment and inoculation, Bk is the effect of the kth block, and εijk is the random experimental error.

When a significant extract treatment × inoculation interaction was detected ($p < 0.05$), the interaction was further examined through simple-effect analyses, and means were compared using Tukey's test at the 5% significance level. For variables showing no significant interaction ($p \geq 0.05$), the main effects of extract treatment and inoculation were evaluated separately, with Tukey's test applied to treatment means when appropriate.

Nematode population density was analyzed separately because this variable was assessed only in inoculated plants. Therefore, the 5 × 2 factorial arrangement was not applicable to this response variable. Data from inoculated plants were analyzed according to a randomized complete block design (RCBD), considering five extract treatments and four blocks, with one plant per experimental unit (n = 4 biological replicates per treatment; 20 experimental units in total). The statistical model included extract treatment and block effects. Following verification of the ANOVA assumptions, treatment means were compared using Tukey's test at the 5% significance level when a significant treatment effect was detected ($p < 0.05$). Statistical analyses were conducted in R software (v4.2.1) using the ExpDes and ggplot2 packages.

3. Results

3.1. Bioactive Compounds: Chlorogenic Acid, Trigonelline, and Caffeine

The coffee bean extract treatments significantly influenced the concentration of bioactive compounds, particularly in the roots. Overall, plants accumulated higher contents of chlorogenic acid (CGA) and trigonelline in the leaves (Figure 1a,c) compared to the roots (Figure 1b,d).

In the leaves, CGA content did not differ significantly among treatments or inoculation conditions (Figure 1a). For leaf trigonelline, non-inoculated plants exhibited a sharp reduction at the 0.30 mg mL^{-1} CGA treatment compared to other treatments, whereas inoculated plants maintained stable trigonelline levels across all extract concentrations (Figure 1c). Consequently, at the 0.30 mg mL^{-1} concentration, inoculated plants had significantly higher leaf trigonelline than non-inoculated plants. Leaf caffeine content remained consistent across most treatments (~0.90 − 1.10 g 100 g^{-1}), except at the 0.300 mg mL^{-1} concentration, where non-inoculated plants showed a marked decline, while inoculated plants maintained higher values (Figure 1e).

In the roots, CGA levels generally increased following extract application (Figure 1b). In non-inoculated plants, the highest root CGA content was observed at the 0.150 mg mL^{-1} treatment, significantly exceeding the control. Among inoculated plants, the 0.150 mg mL^{-1} treatment led to the greatest root CGA accumulation. For root trigonelline (Figure 1d), a significant difference between inoculation states occurred only at the 0.075 mg mL^{-1}

treatment, where pathogen inoculation significantly reduced trigonelline content compared to non-inoculated plants. In non-inoculated plants, the 0.075 mg mL^{-1} treatment resulted in the highest root trigonelline concentration compared to all other treatments. Caffeine was not detected in the roots.

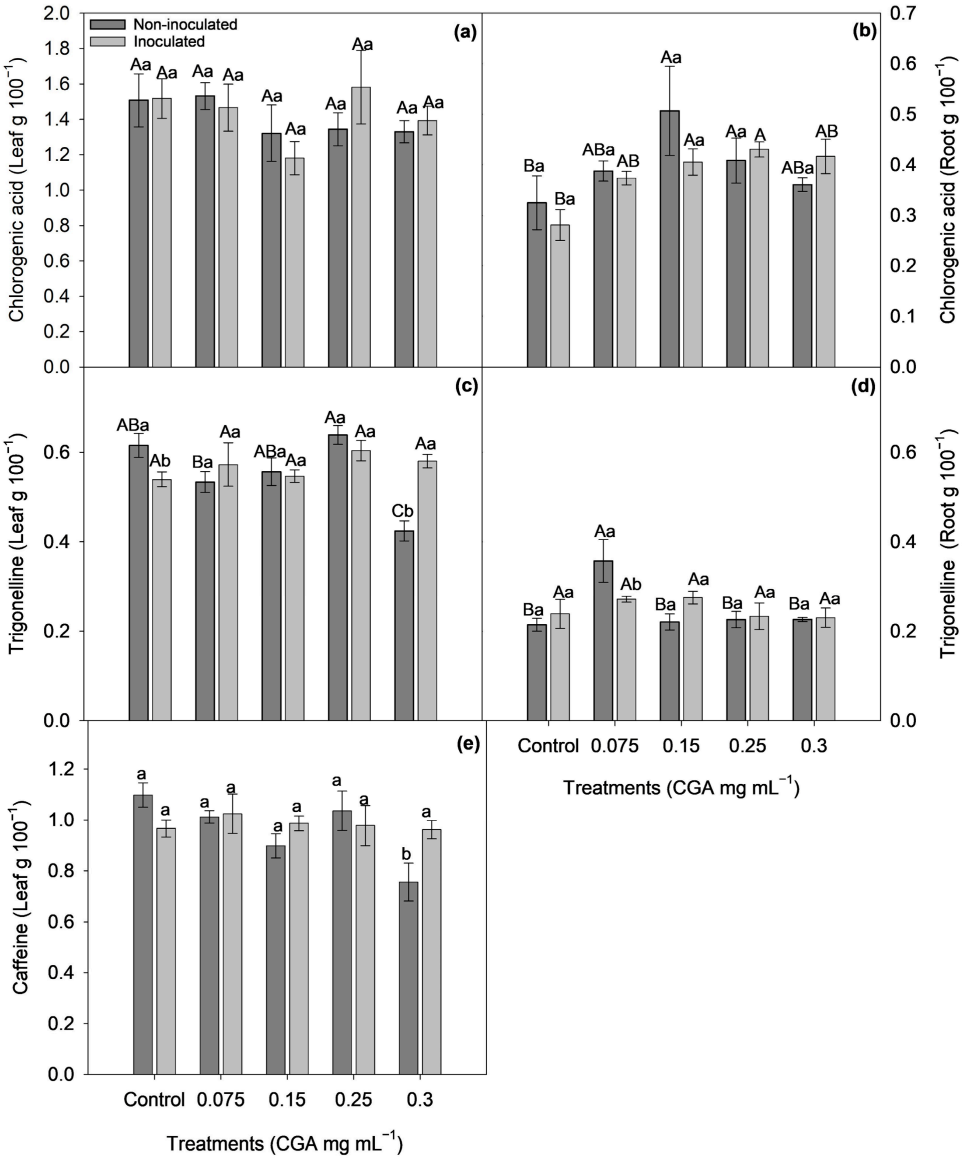


Figure 1. Chlorogenic acid content (**a**,**b**), trigonelline (**c**,**d**) and caffeine (**e**) in the leaves and roots of Arabica coffee plants subjected to treatments with BGS extract containing different concentrations of chlorogenic acid (0.075–0.30 mg mL^{-1}). Means followed by different uppercase letters are significantly different ($p < 0.05$) among extract concentrations within the same inoculation condition. Means followed by different lowercase letters significantly different ($p < 0.05$) between the inoculation conditions (inoculated and non-inoculated) within the same treatment. Error bars represent standard error. Each experimental unit consisted of one individual plant, resulting in four independent biological replicates per treatment combination and a total of 40 experimental units.

3.2. Biochemical Analysis

The application of the extract significantly modulated the activity of antioxidant enzymes, Superoxide dismutase (SOD) (Figure 2a,b) and catalase (CAT) (Figure 2c,d), and attenuated lipid peroxidation (MDA; Figure 2e,f) under pathogen challenge.

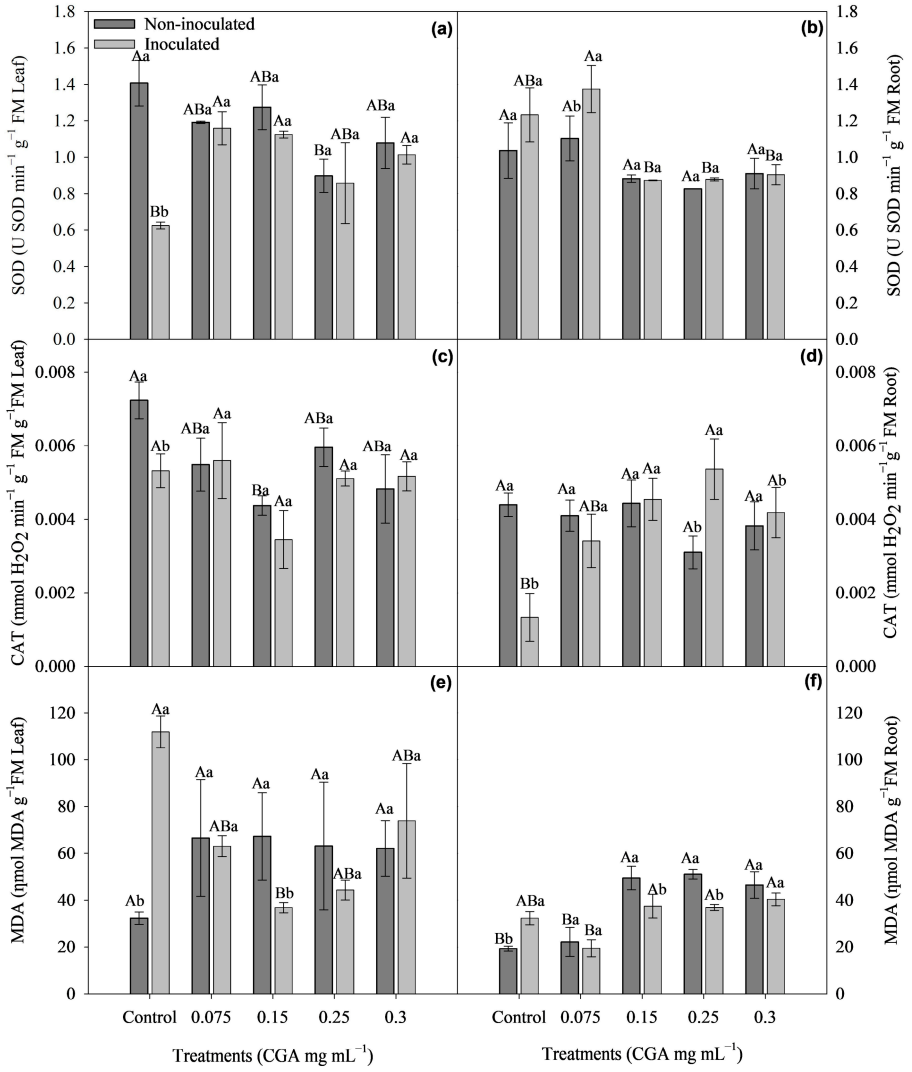


Figure 2. Superoxide dismutase (SOD) (**a**,**b**) and catalase (CAT) (**c**,**d**) activities, and lipid peroxidation (MDA) (**e**,**f**) in the leaves and roots of coffee plants subjected to treatments with BGS coffee bean extract containing different concentrations of chlorogenic acid (0.075–0.30 mg mL^{-1}). Means followed by different uppercase letters are significantly different ($p < 0.05$) among extract concentrations within the same inoculation condition. Means followed by different lowercase letters significantly different ($p < 0.05$) between the inoculation conditions (inoculated and non-inoculated) within the same treatment. Error bars represent standard error. Each experimental unit consisted of one individual plant, resulting in four independent biological replicates per treatment combination and a total of 40 experimental units.

In leaves, untreated inoculated control plants exhibited a sharp reduction in SOD activity compared to non-inoculated plants (Figure 2a). However, extract application restored SOD activity in inoculated leaves to levels comparable to non-inoculated controls (Figure 2a). In roots, SOD activity in inoculated plants reached a maximum at 0.075 mg mL^{-1} CGA concentration, significantly exceeding the non-inoculated group, before returning to baseline levels at higher concentrations (Figure 2b).

For CAT activity, untreated inoculated plants with the extract showed significantly lower enzyme activity in both leaves and roots (Figure 2c,d) compared to non-inoculated controls. Extract treatments effectively rescued leaf CAT activity in inoculated plants back to non-inoculated levels (Figure 2c). In roots, extract application markedly boosted CAT activity in inoculated plants, with the 0.250 mg mL^{-1} CGA treatment resulting

in significantly higher CAT activity in inoculated roots compared to non-inoculated ones (Figure 2d).

Lipid peroxidation (MDA content) was strongly elevated in non-treated inoculated plants in both leaves and roots (Figure 2e,f). In leaves, extract application substantially mitigated this oxidative stress, with the 0.150 mg mL^{-1} CGA concentration significantly reducing leaf MDA content in inoculated plants compared to non-inoculated ones (Figure 2e). In roots, significant reductions in MDA levels of inoculated plants were observed at 0.150 mg mL^{-1} and 0.250 mg mL^{-1} CGA extract concentrations relative to non-inoculated plants (Figure 2f).

3.3. Photosynthetic Pigment Quantification and Plant Growth Assessment

In control plants, nematode inoculation caused a ~37% reduction in chlorophyll *a* content compared to non-inoculated plants, resulting in significantly lower levels than in extract-treated plants (Figure 3a). The extract application successfully prevented this pigment loss, maintaining high chlorophyll *a* levels in inoculated plants, with values reaching parity with non-inoculated controls (Figure 3a). Carotenoid contents were similarly modulated by extract application (Figure 3b). Inoculated plants treated with the highest extract concentration (0.300 mg mL^{-1} CGA) accumulated significantly greater carotenoid levels compared to untreated inoculated controls (Figure 3b). No significant differences were observed for chlorophyll *b* (Supplementary Materials, Figure S2).

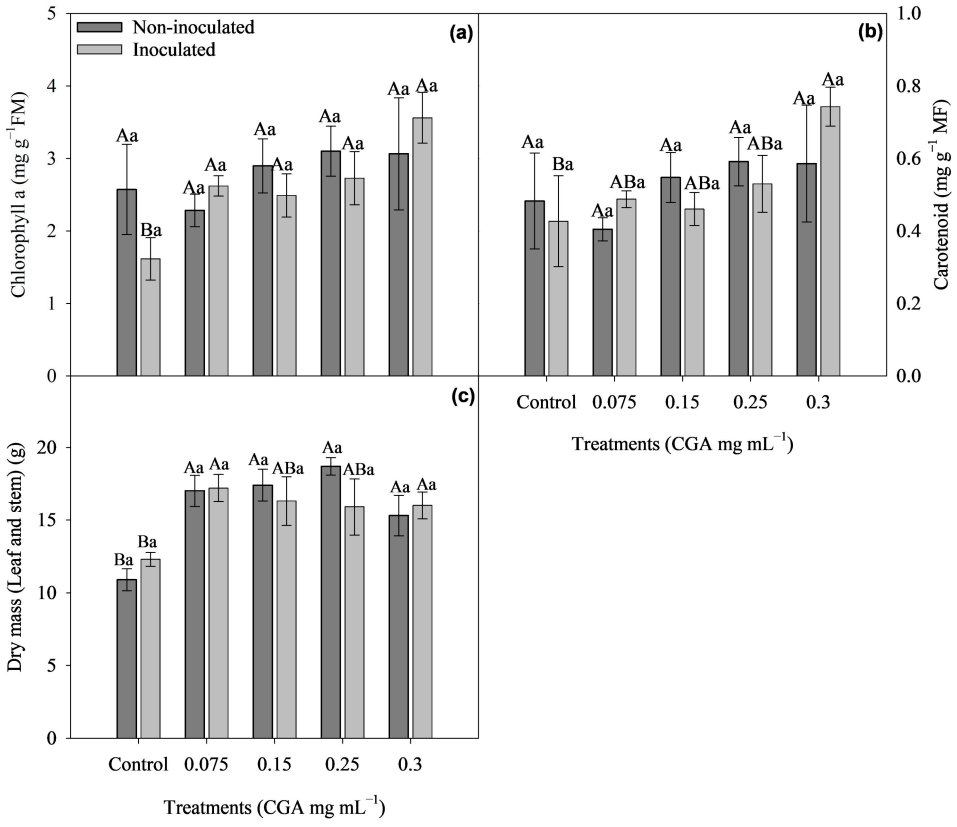


Figure 3. Chlorophyll *a* (**a**) and carotenoid (**b**) content in the leaves and Plant development (**c**) of coffee plants subjected to treatments with BGS coffee bean extract containing different concentrations of chlorogenic acid (0.075–0.30 mg mL^{-1}). Means followed by different uppercase letters are significantly different ($p < 0.05$) among extract concentrations within the same inoculation condition. Means followed by different lowercase letters significantly different ($p < 0.05$) between the inoculation conditions (inoculated and non-inoculated) within the same treatment. Error bars represent standard error. Each experimental unit consisted of one individual plant, resulting in four independent biological replicates per treatment combination and a total of 40 experimental units.

Regarding plant growth, extract treatment exerted marked growth on shoot dry mass (leaves and stems; Figure 3c). Regardless of nematode inoculation, plants treated with extract exhibited significantly higher shoot dry mass (around 15.5–18.5 g) compared to untreated control plants (around 11.0–12.3 g). These quantitative gains in biomass are clearly mirrored in the visual development of the plants (Supplementary Materials, Figure S3).

3.4. Foliar Macro- and Micronutrient Analyses

Foliar macronutrient analysis revealed significant differences among treatments and inoculation states (Table 1). Potassium (K) content increased in inoculated plants treated with the extract compared to untreated controls. The lowest K concentrations were observed in untreated control plants regardless of inoculation status. Inoculated plants receiving the 0.250 mg mL^{-1} and 0.300 mg mL^{-1} extract treatments showed higher foliar K concentration levels than their non-inoculated counterparts, achieving the highest K values among all inoculated groups. In contrast, for non-inoculated plants, the peak K concentration occurred at the 0.150 mg mL^{-1} CGA extract treatment (Table 1). Calcium (Ca) concentration among inoculated plants reached its maximum at the 0.250 mg mL^{-1} CGA treatment, whereas in non-inoculated plants, the highest Ca level was observed at the 0.300 mg mL^{-1} concentration. Nematode inoculation significantly reduced leaf magnesium (Mg) content in untreated control plants compared to non-inoculated controls (Table 1). However, among inoculated plants, extract concentrations of 0.250 mg mL^{-1} and 0.300 mg mL^{-1} CGA restored and enhanced Mg foliar concentration to the highest levels. In non-inoculated plants, Mg content increased significantly at the 0.300 mg mL^{-1} treatment. Furthermore, at the 0.250 mg mL^{-1} CGA concentration, inoculated plants maintained higher Mg levels than non-inoculated ones. For nitrogen (N), inoculated plants treated with 0.300 mg mL^{-1} CGA foliar concentration had significantly higher content than non-inoculated plants (Table 1).

Table 1. Macronutrients (g kg^{-1}) and micronutrients (mg kg^{-1}) concentrations in the leaves of coffee plants subjected to treatments with BGS coffee bean extract with different concentrations of chlorogenic acid (0.075 mg mL^{-1}–0.300 mg mL^{-1}).

Treatments	**Control**		**0.075**		**0.150**		**0.250**		**0.300**	
Nutrients	**Non-IN**	**IN**	**Non-IN**	**IN**	**Non-IN**	**IN**	**Non-IN**	**IN**	**Non-IN**	**IN**
K^+	10.55 ^{Ba}	10.68 ^{Ca}	13.85 ^{ABa}	13.70 ^{BCa}	16.40 ^{Aa}	12.65 ^{BCb}	11.70 ^{Bb}	18.60 ^{Aa}	13.08 ^{ABb}	16.70 ^{ABa}
Ca^{2+}	9.80 ^{ABa}	8.50 ^{Ba}	10.25 ^{ABa}	9.20 ^{Ba}	9.98 ^{ABa}	9.97 ^{Ba}	9.38 ^{ABb}	12.93 ^{Aa}	13.60 ^{Aa}	11.13 ^{ABa}
Mg^{2+}	2.95 ^{ABa}	2.10 ^{Cb}	2.90 ^{ABa}	2.85 ^{Ba}	2.57 ^{Ba}	2.80 ^{Ba}	3.12 ^{ABb}	3.90 ^{Aa}	3.77 ^{Aa}	3.35 ^{ABa}
N	21.53 ^{Aa}	21.95 ^{Aa}	19.78 ^{ABa}	17.83 ^{Ba}	19.10 ^{ABa}	20.55 ^{ABa}	21.98 ^{Aa}	19.98 ^{ABa}	17.62 ^{Bb}	21.95 ^{Aa}
Mn	153.28 ^{Ba}	114.65 ^{Ca}	193.55 ^{ABa}	182.40 ^{CBa}	166.53 ^{Ba}	191.13 ^{CBa}	173.00 ^{ABb}	304.68 ^{Aa}	255.25 ^{Aa}	234.93 ^{ABa}
Fe^{2+}	158.57 ^{Aa}	159.57 ^{Aa}	195.65 ^{Aa}	147.30 ^{Aa}	209.40 ^{Aa}	189.15 ^{Aa}	176.05 ^{Ab}	297.275 ^{Aa}	240.00 ^{Aa}	234.375 ^{Aa}
B	44.35 ^{Ab}	82.60 ^{Aa}	33.63 ^{Aa}	35.30 ^{Ba}	35.83 ^{Aa}	32.18 ^{Ba}	27.48 ^{Aa}	42.28 ^{Ba}	39.10 ^{Aa}	35.45 ^{Ba}
Na^+	373.00 ^{Aa}	320.95 ^{Aa}	240.38 ^{Ba}	226.13 ^{Aa}	285.95 ^{ABa}	265.93 ^{Aa}	286.40 ^{ABa}	346.68 ^{Aa}	265.68 ^{ABa}	331.93 ^{Aa}

Means followed by different uppercase letters within the same row indicate significant differences ($p < 0.05$) among extract concentrations for the same inoculation state according to Tukey's test. Means followed by different lowercase letters within the same row indicate significant differences ($p < 0.05$) between the inoculation conditions: non-inoculated (non-IN) vs. inoculated (IN), for a given extract concentration.

Extract application also significantly influenced foliar micronutrient—higher foliar concentration (Table 1). Inoculated plants treated with the extract at a 0.250 mg mL^{-1} CGA concentration exhibited a higher foliar concentration of manganese (Mn) than non-inoculated plants. Untreated inoculated plants showed the lowest leaf Mn levels, whereas the 0.250 mg mL^{-1} and 0.300 mg mL^{-1} CGA in the extract treatments yielded the highest Mn foliar concentration in inoculated foliage. For iron (Fe), inoculated plants treated with 0.250 mg mL^{-1} CGA showed a significant increase compared to all other treatments. Conversely, boron (B) presented higher levels in untreated inoculated plants, with no

significant differences observed among non-inoculated treatments. Lastly, sodium (Na) foliar concentration was lower in non-inoculated plants receiving extract treatments relative to controls (Table 1).

3.5. Nematode Population Quantification

Evaluation of *M. paranaensis* population density (eggs and J_2 juveniles per gram of root) revealed only numerical reductions in extract-treated coffee plants relative to untreated controls (Figure 4), with no statistically significant differences observed among the treatments.

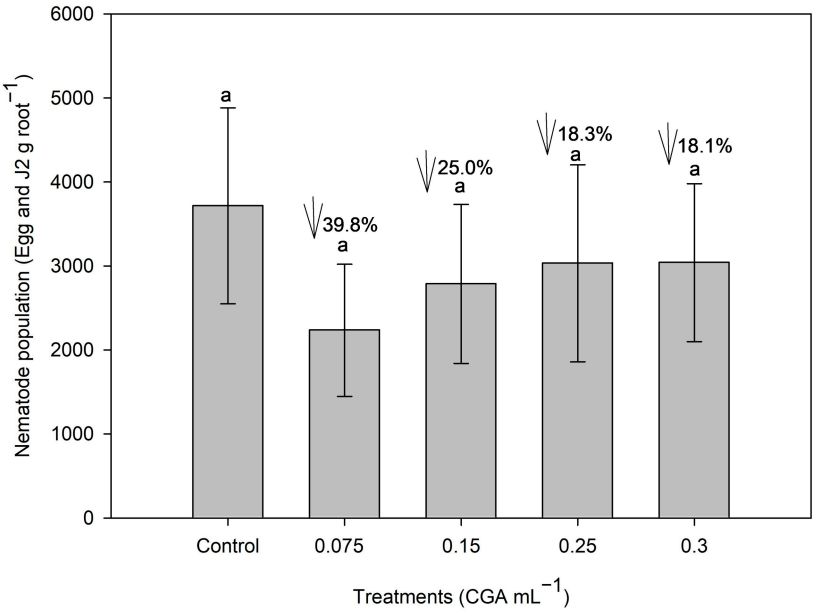


Figure 4. Population density of Meloidogyne paranaensis (eggs and J2 juveniles per gram of root) in coffee plants subjected to treatments with BGS coffee bean extract containing different concentrations of chlorogenic acid (0.075–0.30 mg mL^{-1}). Values above bars represent percentage reductions relative to the control and the arrow indicates the reduction. Means followed by the same letter do not differ significantly according to Tukey's test ($p < 0.05$). Error bars represent standard error. Each experimental unit consisted of one individual plant, resulting in four independent biological replicates per treatment combination and a total of 40 experimental units. ⩛ Indicates the reduction.

4. Discussion

This research elucidated the primary biochemical, physiological, nutritional, and growth mechanisms of coffee plants susceptible to *M. paranaensis* through exogenous application of plant extracts derived from defective coffee beans. Analysis of bioactive compounds revealed significant changes in root chlorogenic acid (CGA) content following extract application (Figure 1b). In addition, higher foliar concentrations of trigonelline and caffeine at the highest extract concentration (0.30 mg mL^{-1} of CGA; Figure 1c,e) demonstrate a response that enhances plant defense against *M. paranaensis*. These physiological adjustments promoted the growth of parasitized coffee trees, as evidenced by an increase in shoot dry mass (Figure 3c). The application of the extract also influenced the for greater foliar concentration of potassium (K), magnesium (Mg) and Mn (Table 1), induced antioxidant defense enzymes (catalase (CAT) and Superoxide dismutase (SOD)) and increased photosynthetic pigment concentrations.

The defense responses elicited by the application of bioactive compounds present in the extract, particularly chlorogenic acid, trigonelline, and caffeine, are associated with both their direct antioxidant activity and their capacity to activate plant defense mechanisms

against phytopathogens [19–24]. These compounds trigger a cascade of physiological and biochemical responses, including the activation of antioxidant systems and other defense-related pathways, thereby enhancing plant tolerance to pathogen infection [19,37]. A key component of this response is the upregulation of antioxidant enzymes involved in reactive oxygen species (ROS) scavenging, which reduces membrane lipid peroxidation and mitigates cellular oxidative stress [37].

The coordinated modulation of the antioxidant enzymes SOD and CAT shows a clear defense mechanism in susceptible coffee plants treated with BGS extract. Notably, leaf SOD activity was increased in extract-treated, infected plants (Figure 2a), a response absent in untreated controls. While SOD acted predominantly in the leaves, CAT acted primarily in the roots (Figure 2d) to eliminate ROS and reduce lipid peroxidation caused by nematode infection. As a primary antioxidant enzyme, SOD catalyzes the dismutation of the superoxide radicals (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2) [38]. CAT then breaks down H_2O_2 into water and oxygen, preventing excessive peroxide accumulation in plant cells [39]. Together, these enzymes maintain redox homeostasis, balancing defense signaling with cell protection during biotic and abiotic stresses [39].

Biochemical changes in response to phytopathogen infection alter plant physiology [40], including alterations in chloroplast structure and depletion of photosynthetic pigments [41,42]. This reduction is driven by oxidative stress, lipid peroxidation, and subsequent chlorophyll degradation [40]. Other results highlight the susceptibility of Catuai IAC 144 to *M. paranaensis* [19,43,44]. Our results show that parasitized plants left untreated showed a 37% reduction in chlorophyll *a* compared with non-parasitized controls (Figure 3a). However, parasitized plants treated with BGS extract exhibited significantly higher chlorophyll *a* (Figure 3a). This recovery is likely linked to enhanced Mg foliar concentration (Table 1), as this element is the central structural atom of the chlorophyll molecule. Carotenoid content also increased in infected plants treated with 0.300 mg mL^{-1} extract (Figure 3b), as did overall plant growth (Figure 3c). The enhancement of photosynthetic pigment levels can mitigate the effects of phytopathogen damage [45].

Both chlorophylls and carotenoids are vital for light harvesting in photosystems I and II (PI and PII) degradation [40]. Carotenoids quench singlet oxygen to protect the photosynthetic machinery [46–48] and help preserve chloroplast integrity, thereby sustaining chlorophyll levels and PII quantum efficiency [47,49]. The increase in photosynthetic pigment content is associated with enhanced photosynthetic efficiency, favoring carbon assimilation and promoting carbohydrate accumulation in plant tissues [50]. Consequently, the BGS extract-induced increase in photosynthetic pigments minimizes nematode damage and drives the increase in shoot dry mass observed here (Figure 3c).

Furthermore, the more concentrated extract increased foliar nutrient concentrations, mainly K, Mg and manganese (Mn) (Table 1). Potassium is essential for cellular osmotic balance, stomatal regulation, photosynthetic enzyme activation, sugar and nutrient transport, and general disease resistance [51,52]. Magnesium plays a central role in chlorophyll structure, carbohydrate partitioning, protein synthesis, and the activation of over 300 enzymes [53]. Although they have distinct physiological functions, K and Mg synergistically regulate foliar photosynthetic capacity. Their interaction may have synergistic effects on the regulation of foliar photosynthetic capacity [54]. Meanwhile, Mn plays an important role in the initial defense reactions, serving as a required cofactor for Mn-SOD in chloroplasts [55]. The concurrent increase in leaf Mn content and SOD activity in parasitized plants underscores the importance of this micronutrient in reducing pathogen-induced oxidative stress. The elevated levels of K, Mg, and Mn observed under the highest CGA concentration treatments (0.250 and 0.300 mg mL^{-1}) (Table 1) may have contributed to maintaining photosynthetic performance and supporting antioxidant defense mechanisms.

Although changes in foliar nutrient concentrations were observed following extract application, the mineral composition of the extract was not determined. Therefore, the relative contributions of direct nutrient supply and extract-induced physiological responses cannot be distinguished under the conditions of the present study.

The activation of plant defense via exogenous application of phenolic antioxidants can exert dose-dependent effects on host enzyme activity, and nematode infection, suggesting that bioactive compounds present in the extract-mediated redox shifts may either suppress or promote pathogen establishment depending on the applied dose [56]. In the present study, although extracts promoted vegetative growth and enhanced defense-related responses, their effects on nematode population levels were not statistically significant (Figure 4). Therefore, the observed reductions in nematode numbers should be interpreted only as numerical differences among treatments rather than evidence of nematode control. Moreover, because nematode populations were quantified only in the root fraction remaining after sampling for biochemical analyses, total nematode population per root system and reproduction factor could not be accurately determined, which represents an important limitation in the interpretation of the nematode data.

Given the multiple response variables and statistical comparisons evaluated in this study, some significant findings may reflect chance associations rather than consistent biological effects. Therefore, these results should be regarded as preliminary evidence of the potential benefits of extracts.

5. Conclusions

This study demonstrates that waste-derived black-green-sour (BGS) coffee bean extracts rich in bioactive compounds showed growth-promoting activity and modulated biochemical, physiological, and nutritional traits associated with defense responses in Coffea arabica plants challenged by Meloidogyne paranaensis under the greenhouse conditions evaluated in this study. Exogenous application of the extract mitigated pathogen-induced oxidative stress by modulating the activity of Superoxide dismutase (SOD) and catalase (CAT), thereby reducing lipid peroxidation. Furthermore, the extract protected leaf photosynthetic pigments (chlorophyll *a* and carotenoids) and improved host nutritional status by enhancing the foliar concentrations of key nutrients (K, Mg, N, and Mn). Consequently, treated plants maintained higher shoot dry mass despite nematode infestation. These findings highlight the potential of CGA-rich extracts as plant defense modulators and growth-promoting amendments, while further studies are required to determine whether they can provide significant nematode suppression under different experimental conditions.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy16212166/s1, Figure S1: Chromatographic profile of the standard solution of 0,1 mg mL^{-1} (a), Extract from defective coffee beans (b), inoculated plant leaf (c) and inoculated plant Root (d). T- Trigonelline, CGA -chlorogenic acid and C – caffeine. Quantification of bioactive compounds in extract from defective coffee beans: CGA -chlorogenic acid and (0.30 mg mL^{-1}), T- Trigonelline: 0.10 mg mL^{-1}), caffeine (0.11 mg mL^{-1}); Figure S2: Chlorophyll *b* content in the leaves of coffee plants subjected to treatments with BGS coffee bean extract containing different concentrations of chlorogenic acid (0.075 – 0.30 mg mL^{-1}); Figure S3: Leaf and stem images of coffee plants subjected to treatments with BGS coffee bean extract containing different concentrations of chlorogenic acid.

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