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Biocontrol potential of Trichoderma erinaceum It-58 and T. afroharzianum P-8 against Neoscytalidium dimidiatum, pathogen responsible of root rot in cucumber (Cucumis sativus)

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Research Article

Keywords: Trichoderma, Neoscytalidium dimidiatum, plant growth promotion, biochemical resistance, Cucumis sativus

Posted Date: January 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8500337/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at International Microbiology on June 29th, 2026. See the published version at https://doi.org/10.1007/s10123-026-00834-z.

Abstract

Green agricultural development requires safe, effective, and environmentally friendly plant disease control measures. This study evaluated the antagonistic properties of *Trichoderma erinaceum* It-58 and *Trichoderma afroharzianum* P-8 against *Neoscytalidium dimidiatum*, as well as their ability to promote growth and induce biochemical resistance in cucumber seedlings. The pathogen responsible for both cucumber fruit rot and seeds damping off was isolated and identified morphologically. The antagonistic potential of the *Trichoderma* strains was assessed using dual culture, volatile, and non-volatile organic compound methods. For pot experiments, seeds coated with antagonists were pre-germinated and transplanted. Twenty-one days later, roots were infected with the pathogen. Fifteen days post-infection, seedlings were harvested to evaluate growth parameters (height, biomass, diameter) and biochemical parameters (polyphenols, proteins, peroxidases, polyphenol oxidases). The pathogen was identified as *Neoscytalidium dimidiatum*. In dual culture, *T. erinaceum* and *T. afroharzanium* inhibited the pathogen by 74.36% and 68.20% respectively. Diffusible non-volatile substances showed even greater inhibition of mycelial growth at 71% (*T. erinaceum*) and 97% (*T. afroharzanium*). No inhibition was observed with volatile organic compounds. In pot experiments, both antagonists significantly stimulated plant growth parameters and reduced disease incidence (16.67% for *T. afroharzianum*, 33.33% for *T. erinaceum*). They induced a significant increase in polyphenol content while significantly decreasing peroxidase and polyphenol oxidase activities in treated and infected plants compared to controls. There was a significant positive correlation between disease incidence, polyphenol content, and peroxidase activity, and a negative correlation with polyphenol oxidase. Thus, *T. erinaceum* and *T. afroharzanium* can effectively promote cucumber growth and prevent seedling wilt caused by *N. dimidiatum*.

Introduction

Cucumber (*Cucumis sativus* L.) is an herbaceous, creeping, and climbing vegetable crop of the Cucurbitaceae family (Pandey and Kujur 2022). It is recognized as one of the most economically important vegetable crops worldwide. In Cameroon, cucumber cultivation is concentrated in subtropical and tropical regions, particularly in the Northwest, Southwest, West, and Littoral areas. According to the Food and Agriculture Organization of the United Nations, global cucumber production reached 94,718,396.55 tons in 2022, with Cameroon contributing an estimated 262,120 tons (FAO 2022). Cucumbers play a vital role in food security by providing essential nutrients that support a healthy diet. They are an excellent source of dietary fiber, vitamins (notably vitamin C and vitamin B6), and minerals such as magnesium, calcium, potassium, and iron (Murray and Horswill 2022). In addition to their nutritional value, cucumbers possess medicinal importance due to their rich composition of phenolic compounds and flavonoids, which are well known for their antioxidant properties (Gelaye 2023). These bioactive compounds contribute to the prevention of oxidative stress-related disorders, thereby enhancing the functional food value of cucumbers.

Cucumber cultivation is increasingly impacted by climate change, which heightens crop vulnerability to a broad spectrum of pathogenic microorganisms, including viruses, bacteria, nematodes, and filamentous

pathogens such as fungi and Oomycetes. The pressure exerted by these phytopathogens results in reduced yields and compromises both the nutritional and commercial quality of cucumbers. Among them, soilborne fungi are regarded as the most destructive, as they can cause severe yield losses ranging from 15% to 60%, leading to substantial economic damage for farmers (Shi et al. 2019). Numerous fungal phytopathogens infect diverse host species and are responsible for emerging and re-emerging diseases across different geographical regions (Dean et al. 2012; Cacciola and Gullino 2019; Morris and Moury 2019; Fones et al. 2020). *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, a tropical and subtropical fungus belonging to the Botryosphaeriaceae family of ascomycetes, has recently emerged as a serious threat to economically important crops, including members of the Cucurbitaceae family such as cucumber (Crous et al. 2006; Mirtalebi et al. 2019; Derviş and Göksel 2023). This phytopathogen infects host tissues either through natural openings and wounds or by secreting cell wall-degrading enzymes such as mannanases, α-glucosidases, and pectate lyases, which facilitate colonization of fruits and soft tissues. *Neoscytalidium* species can spread via air, soil, seeds, and fruit, ultimately causing root and fruit rot in cucumber plants (Peja et al. 2025).

Management of this pathogen has traditionally relied on the application of synthetic fungicides such as methyl bromide, Ridomil, and Pencozeb. However, the prolonged and indiscriminate use of these chemicals can accelerate the emergence of resistant pathogen strains and poses significant risks to both environmental sustainability and human health (Satapute et al. 2019). In the context of the current climate crisis, it is imperative to reconsider conventional agricultural practices and adopt strategies that safeguard ecological integrity while ensuring consumer safety.

One of the most promising approaches is the use of beneficial microorganisms in biological control. Several bacterial genera, including *Agrobacterium*, *Pseudomonas*, *Streptomyces*, and *Bacillus*, as well as fungal genera such as *Trichoderma*, *Gliocladium*, *Ampelomyces*, *Candida*, and *Coniothyrium*, have demonstrated strong antagonistic potential against plant pathogens (Vinale et al. 2008). Among these, fungi of the genus *Trichoderma* stand out as particularly effective biocontrol agents due to their multifaceted mechanisms of action and consistent performance in diverse cropping systems (Yao et al. 2023).

Trichoderma species employ multiple modes of action in suppressing plant pathogens, including competition for nutrients and space, secretion of hydrolytic enzymes (e.g., chitinases, cellulases, proteases, lipases), production of volatile and non-volatile antifungal metabolites, direct mycoparasitism, and stimulation of host resistance mechanisms (Yao et al. 2023; Sarma et al. 2014). Induced resistance in plants is typically characterized by the synthesis of defense-related biochemical compounds such as phenolics, soluble proteins, oxidative stress-regulating enzymes, and enzymes associated with pathogenicity responses (Rai et al. 2024). Collectively, these complementary mechanisms contribute to a significant reduction in disease incidence. Beyond their biopesticidal properties, *Trichoderma* spp. also exhibits biofertilizing effects by enhancing nutrient solubilization and uptake, thereby improving plant growth and vigor (Nishad et al. 2020). For effective disease management, *Trichoderma* is commonly applied through seed treatment, biopriming, seedling soaking, soil incorporation, or wound treatment.

Among these, seed coating represents a particularly simple and efficient strategy for controlling soil-borne diseases, as propagules of the antagonist colonize the seed surface and subsequently establish in the rhizosphere and root tissues of germinating seedlings (Sanjeev et al. 2009).

The use of *Trichoderma* not only promotes healthy plant development but also contributes to environmental sustainability by reducing dependence on chemical pesticides (Yao et al. 2023). Within this genus, *T. afroharzianum* and *T. erinaceum* have demonstrated strong antagonistic potential, producing volatile and non-volatile metabolites that inhibit the mycelial growth of several common bean pathogens, including *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*, and *Pythium ultimum* (Bedine et al. 2020). However, their efficacy against *Neoscytalidium dimidiatum* in cucumber crops remains unexplored.

Accordingly, the present study aims to evaluate the antagonistic potential of *T. erinaceum* It-58 and *T. afroharzianum* P-8, assess their ability to promote cucumber plant growth, and determine their effectiveness as seed-coating agents in preventing seedling root rot and wilt through the induction of biochemical resistance markers.

Materials and methods

Trichoderma strains

Two *Trichoderma* species used in this study were obtained from the core culture collection of the Laboratory of Biochemistry (University of Douala). These antagonist agents were previously isolated from rhizosphere bean plants taken in center region in Cameroon and identified by Bedine et al (2020) as *Trichoderma erinaceum* It-58 (CTCC-SJ-G-HB40350) and *Trichoderma afroharzanium* P-8 (NBAII TN-9). Theses strains of *Trichoderma* were maintained on PDA slants at 4°C and sub-cultured bimonthly until use.

Seeds damping off pathogen Isolation

The pathogens responsible for cucumber rot were isolated and then evaluated for their ability to inhibit cucumber seed germination.

Pathogens were isolated from cucumber fruits harvested in the Littoral Region of Cameroon displaying symptoms of disease. The tissue fragments (1 to 2 cm) taken from the edges of the lesions were superficially disinfected in ethanol 70% for 60 seconds, rinsed three times with sterile distilled water, and then inoculated into Petri dishes containing Potato Dextrose Agar (PDA) medium (10 ml) supplemented with penicillin and ampicillin (250 g/L). After three days of incubation at a temperature of 28 ± 2°C, the emerging fungal morphotypes from the tissue fragments were purified through successive subculturing. The pathogenicity test on the fruits, as described by Sameza et al (2014), was used to assess the ability of the obtained isolates to induce disease. Isolates that induced necrosis were re-isolated, purified

through successive subculturing, and their spore solutions were used for the selection of pathogens responsible for inhibiting cucumber seed germination.

The inoculum was obtained by adding 5 ml of sterile distilled water to a 7-day-old pre-culture of the pathogens. Subsequently, the surface of the Petri dishes containing these pathogens was scraped using a sterile loop. The resulting solution was filtered using Whatman No. 4 paper. The filtrate was adjusted to $2x10^4$ spores/ml with a colloidal carboxymethylcellulose (CMC) solution (2%).

Pathogens that induced pre-germination inhibition of cucumber seeds were isolated according to the method described by Dorrance et al (2004), with some modifications. Indeed, the apparently healthy cucumber seeds (Variety F_1 Classico) were washed with running water, disinfected with 70% alcohol for one minute, rinsed three times with sterile distilled water, and then dried using sterile paper towels. These seeds (10 seeds per batch) were introduced into 5 ml of a pathogen inoculum solution separately for one minute. In the control seed lot, the inoculum was replaced with the aqueous CMC 2%. All treated seeds were dried under a hood at room temperature (28 ± 2° C) for two hours and then placed in Petri dishes containing sterile filter paper soaked in sterile distilled water and incubated for 7 days. The pathogens that induced predamping off were selected and subjected to morphological identification based on the identification key of Chabasse et al (2002) and Botton et al (1990).

In vitroantagonism test

In vitro antagonist assay was done by dual culture and antibiosis.

Dual culture test

The dual culture assay was performed following the method used by Ntah et al (2023). Pathogen and antagonist plugs (5 mm in diameter), excised from the margin of actively growing mycelia (2 days old), were inoculated on opposite sides of potato dextrose agar (PDA) plates. The cultures were incubated at 28 ± 2°C for seven days. Control plates were inoculated only with pathogen plugs. Each treatment was replicated three times to ensure reproducibility. Radial growth of both microorganisms was measured, and the time required for *Trichoderma* to establish contact with the pathogen was recorded. The percentage inhibition of pathogen mycelial growth was calculated using the following formula:

$$\mathrm{I\%} = \frac{100(\mathrm{Do} - \mathrm{Dx})}{\mathrm{Do}} (1)$$

where I%: percentage of inhibition of radial mycelial growth; Dx: radial mycelial growth of the pathogen in the presence of Trichoderma; Do: radial mycelial growth of the pathogen in the absence of *Trichoderma*.

Volatile’s organics compounds test

The antagonist and the pathogen were co-inoculated at the center of 9 cm Petri dishes containing 10 mL of potato dextrose agar (PDA). The dishes, without lids, were stacked and hermetically sealed with two layers of parafilm, then incubated at 28 ± 2°C for seven days. For the control treatment, the *Trichoderma* plug was replaced with a sterile agar disc. Pathogen growth inhibition was quantified using the previously described formula (**1**).

Non-volatiles organics compounds test

Cellophane discs were sterilized and aseptically placed over the entire surface of 10 mL of potato dextrose agar (PDA) medium. A *Trichoderma* mycelial explant (5 mm in diameter, 2 days old) was positioned at the center of the dish and incubated at 28 ± 2°C for two days. Following incubation, the membrane containing the *Trichoderma* mycelium was carefully removed, and a pathogen mycelial disc (5 mm) was subsequently inoculated at the center of the Petri dish. Plates were then incubated at 28 ± 2°C for three days. Control treatments were prepared by replacing the pathogen disc with a sterile agar plug. The percentage of pathogen growth inhibition was calculated using the previously described formula (**1**).

Experimental pots

Spores production of Trichoderma strains and pathogenic fungi

Spore production was achieved using liquid culture multiplication in potato dextrose broth (PDB). Conical flasks (1 L) containing 200 mL of sterile PDB medium were inoculated separately with six explants derived from three-day-old precultures of *Trichoderma* strains and the pathogen. The flasks were incubated at 28 ± 2°C under stationary conditions for 14 days. Following incubation, the cultures were homogenized using a blender, and the resulting suspensions were standardized with a hemacytometer to concentrations of 1×10^8 conidia·mL^{-1} for the antagonists and 5×10^6 conidia·mL^{-1} for the pathogen.

Control suspensions were prepared by replacing microbial explants with sterile agar discs. To improve adhesion and uniform distribution, 2% carboxymethylcellulose (CMC) was added to the spore suspensions of the *Trichoderma* strains and to the antagonist control.

Experimental design

Seeds of the F_1 *Classico* cucumber variety, known to be susceptible to the pathogen, were used in this study. The seeds were first washed with running water, disinfected with 70% ethanol for 10 minutes, and rinsed three times with sterile distilled water. Subsequently, five grams of disinfected seeds were immersed in separate suspensions of antagonist spores for 24 hours, drained, air-dried under a laminar flow hood, and subjected to pre-germination in pots (10 cm in diameter) for three days. Each pot contained 300 g of a sterilized substrate composed of agricultural topsoil and quartz mixed at a 3:1

(*w/w*) ratio. Sterilization was performed twice at 121°C for 1 hour at two-day intervals, followed by cooling for 48 hours. Control seeds were immersed in a sterile *Trichoderma* solution.

Seedlings obtained from pre-treatment and pre-germination, displaying uniform morphological characteristics, were transplanted into holes 7 cm deep in polyethylene pots (18 × 18.5 cm) containing the same sterile soil mixture. One seedling was placed per pot, maintained at room temperature, and irrigated daily with 200 mL of sterile distilled water.

Twenty-one days after transplanting, each seedling was infected at the rhizosphere with 20 ml of *N. dimidiatum* spore suspension. Uninfected plants received 20 ml of sterile distilled water or benomyl at the root level and served as negative controls or positive control respectively. The pots were arranged in a completely randomized block design comprising 8 treatments as follow: negative control (T0), inoculated with *T. afroharzianum* (T1), inoculated with *T. erinaceum* (T2), positive control (B0), inoculated with *N. dimidiatum* (Ti), inoculated with *T. afroharzanium* and infected with *N. dimidiatum* (T1i), inoculated with *T. erinaceum* and infected with *N. dimidiatum* (T2i), treated with Benomyl and infected with *N. dimidiatum* (B0i). Each treatment consisted of 4 plants repeated twice.

Evaluation of disease incidence and plant growth

Fifteen days after infection of the plants, symptoms (on leaves and roots) were observed and disease incidence (%I) was calculated using the following formula:

$$\mathrm{I}\% = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ x } 100$$

The plants were harvested, washed with tap water to remove soil particles and the growth parameters were evaluated. The height (cm) was measured using a tape measure, determining the distance from the base of the stem to the growth tip of each plant. After separating the aerial part (leaves and stems) from the root system, the biomass of both root and aerial parts was obtained by measuring the fresh mass (g) of each part with a balance. The diameter of the collar was measured with a tape measure at the transition zone between the root system and the petiole. Aerial parts were stored at -20° C for the assessment of biochemical parameters.

Biochemical Parameters assay

Polyphenol compounds assay

Phenolic compounds were quantified following the method of Singleton and Rossi (1965), with minor modifications. Briefly, one gram (1g) of fresh cucumber leaf tissue was crushed and macerated at 4°C for 1 hour in 10 mL of 70% (v/v) methanol. The homogenate was centrifuged at 3,000 rpm for 20 minutes, and the resulting supernatant was collected for analysis. For the assay, 100 µL of the extract

was mixed with 2 mL of Folin–Ciocalteu reagent and 2.5 mL of 20% aqueous sodium carbonate (Na_2CO_3). The reaction mixture was incubated in the dark for 60 minutes, after which absorbance was measured at 760 nm using a spectrophotometer. A blank was prepared by replacing the extract with 70% methanol. Catechol (100 µg/mL) was used as the standard reference compound. Results were expressed as catechol equivalents, calculated in micrograms (µg) of total phenols per gram of dry weight of cucumber leaves.

Soluble Proteins content

The soluble protein assay was performed according to the colorimetric method of Bradford (1976). One gram of leaf material was crushed with 1 mL of sodium phosphate buffer, 0.05 M, pH 7 and centrifuged at 3000 prm for 10 minutes. Twenty microliter of supernatant was added at 80 µl of sodium phosphate buffer pH 7 (0.05 M) followed by 2 ml of Bradford reagent (Coomassie G250 + orthophosphoric acid + ethanol 95%). The mixture was incubated in the dark during 10 minutes and the optical density at 595 nm was measured with a spectrophotometer against a blank tube where the supernatant was replaced with the buffer. The standard curve was obtained from an aqueous solution of bovine serum albumin (BSA) 0.1%. Protein content was expressed in µg/g of fresh matter.

Enzymes assay

For enzyme extraction, one gram (1 g) of fresh leaf material was homogenized at 4°C with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) for the study of polyphenol oxidase (PPO) activity, and with 2 mL of 1/15 M sodium phosphate buffer (pH 6.0) for peroxidase (PO) activity. The homogenates were centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatants were collected as crude enzyme extracts.

The specific activity of PPO was determined spectrophotometrically following the method of Ambata et al (2025), with slight modifications. Briefly, 20 µL of enzyme extract was added to a reaction mixture containing 500 µL of phosphate buffer (pH 7.0; 100 mM) and 700 µL of catechol (0.01 M). The reaction was initiated by incubation at 30°C for 2 minutes, then terminated by placing the tubes in an ice bath. Absorbance was measured at 420 nm against a blank in which the enzyme extract was replaced by buffer. PPO activity was expressed as ΔOD/min/mg protein per gram of fresh leaf tissue.

PO activity was assayed according to Ambata et al. (2025). The reaction mixture consisted of 2% H_2O_2 (1 volume), 1% guaiacol (2 volumes), and 1/15 M phosphate buffer (5 volumes, pH 6.0). Two milliliters of this mixture were placed in test tubes, and the reaction was initiated by adding 10 µL of enzyme extract, followed by incubation at 25°C for 3 minutes. Reaction progress was monitored with a stopwatch, and absorbance at 420 nm was recorded every minute against a blank (buffer replacing enzyme extract). The change in absorbance (ΔOD) between the third and first minute was used to calculate specific activity, expressed as ΔOD/min/mg protein per gram of fresh weight.

Statistical Analysis

The data analyzed was performed using SPSS software version 22 for Windows (SPSS, Inc., Chicago, IL, USA). Quantitative variables were expressed as means ± standard error, while qualitative variables were summarized descriptively. Differences among groups were assessed using one-way analysis of variance (ANOVA). When significant differences were detected, Duncan's multiple range post hoc test was applied to determine pairwise comparisons. Pearson's correlation analysis was conducted to evaluate the relationships among the studied parameters. Statistical significance was established at a threshold of $p < 0.05$.

Results

Identification of the Pathogen

Isolation from rotting cucumber fruits yielded several morphotypes of fungal colonies emerging from tissue fragments. Among these, only a single isolate was capable of reproducing fruit rot symptoms (Fig. 1A) and inhibiting cucumber seed germination (Fig. 1B).

Macroscopic characterization of this isolate on potato dextrose agar (PDA) revealed a fluffy, woolly colony texture with a gray coloration on the first day, which progressively darkened to a blackish shade by day three. The reverse side of the Petri dish exhibited a distinct black pigmentation. The isolate displayed a rapid growth rate of approximately 2 cm per day (Fig. 1C1).

Microscopic examination showed a branched mycelium composed of septate, hyaline hyphae (Fig. 1C2). The hyphae fragmented into arthrospores, either solitary or arranged in chains, which were ellipsoidal in shape and contained zero to one septum (Fig. 1C3). These morphological characteristics were compared with the identification keys of Chabasse et al. (2002) and were consistent with the diagnostic features of *N. dimidiatum*.

Antagonism activity of Trichoderma against N. dimidiatum

In paired culture assays, *T. erinaceum* and *T. afroharzianum* not only invaded the pathogen (Fig. 2) but also significantly inhibited ($p \leq 0.05$) its radial mycelial growth by 74.36% and 68.20%, respectively.

The non-volatile metabolites produced by both *Trichoderma* strains also exerted strong inhibitory effects on pathogen growth, reducing mycelial development by 71.11% for *T. erinaceum* and 97.04% for *T. afroharzianum* (Fig. 3A). In contrast, volatile compounds produced by these strains did not induce measurable inhibition of *N. dimidiatum* mycelial growth (Fig. 3B).

Effect of the antagonists on the disease incidence and on growth parameters of cucumber plants

The incidence of disease was significantly reduced ($p \leq 0.05$) in pots treated with both *Trichoderma* strains and subsequently infected with *N. dimidiatum*. Disease incidence values were 41.66% for T. afroharzianum and 25.00% for *T. erinaceum*, corresponding to reductions of 16.67% and 33.33%,

respectively. In contrast, plants treated with benomyl exhibited a significantly higher disease incidence (50%) compared to those treated with biological control agents (Fig. 4).

Beyond disease suppression, plants treated with *T. afroharzianum* and *T. erinaceum* showed significant improvements ($p \leq 0.05$) in key growth parameters, including seedling length, collar diameter, root biomass, and shoot biomass, when compared to untreated controls. Notably, these two Trichoderma strains were more effective than benomyl in promoting plant growth under non-infected conditions. However, infection with *N. dimidiatum* led to a reduction in all measured parameters, although values in treated plants remained consistently higher than those observed in infected controls (Table 2; Fig. 4).

Biochemical results

Polyphenol content

The results showed, after statistical analysis, that plants inoculated with both strains of *Trichoderma* significantly increased ($p \leq 0.05$) polyphenol content in both healthy (260.00 and 194.67 mg/g of fresh matter respectively for *T. afroharzianum* and *T. erinaceum*) and infected plants (371.67 and 220.00 mg/g of fresh matter respectively for *T. afroharzianum* and *T. erinaceum*) compared to controls (118.67 and 180.33 mg/g of fresh matter respectively for healthy and infected plants). However, polyphenol content decreased with infection in plants treated with *Trichoderma*. Similar results were observed in healthy and infected plants treated with benomyl. Conversely, in control plants, the polyphenol content increased with infection (Fig. 6). There appears to be a significant positive correlation ($P = 0.00$; $R^2 = + 0.88$) between phenolic compound content and disease incidence.

Soluble proteins content

Results showed that treated plants (healthy and infected) did not exhibit any significant difference ($p \leq 0.05$) in their soluble protein content. However, soluble protein content increased significantly with infection in control plants (untreated plants) (Fig. 7). There was a significant positive correlation ($P = 0.00$; $R^2 = + 0.69$) between soluble protein and disease incidence.

Enzymes activity

The results of PO and PPO activities are shown in Figs. 8 and 9, respectively.

No significant difference in PO activity was observed in healthy plants. However, infection significantly ($p \leq 0.05$) increased PO activity. Furthermore, in treated plants, this activity was significantly ($p \leq 0.05$) reduced. There was a significant positive correlation ($P = 0.00$; $R^2 = + 0.68$) between PO activity and disease incidence.

A significant ($p \leq 0.05$) increase in PPO activity was observed in healthy plants treated with both strains of *Trichoderma* and benomyl. However, infection significantly ($p \leq 0.05$) decreased PPO activity in the treated plants and significantly increased it in the control plants. Statistical analyses showed a significant and negative correlation ($P = 0.01$; $R^2 = -0.50$) between PPO activity and diseases.

Discussion

Soil-borne fungal diseases represent one of the most significant challenges for global crops, as they pose numerous difficulties due to the long survival of pathogens, their resting structures, and their wide range of hosts. Among the crops affected by soil-borne pathogens are cucumber plants. In present study, morphological characterization of the fungal phytopathogen isolated from rotten cucumber fruits that can inhibit seed germination has linked this phytopathogen to the probable species *Neoscytalidium dimidiatum*. *N. dimidiatum* is a well-documented pathogen of Cucurbitaceae crops, including melons (Mirtalebi et al. 2019). However, to the best of our knowledge, this study reports the first isolation of *N. dimidiatum* from cucumber.

The use of antagonistic fungi from the *Trichoderma* genus represents a promising alternative for sustainable crop protection, owing to their capacity to suppress plant pathogens while enhancing yields, conserving biodiversity, and maintaining soil fertility (Woo et al. 2014; Tchameni et al. 2020). *In vitro* evaluation of the antagonistic potential of *T. harzianum* and *T. erinaceum* strains demonstrated their capability to significantly inhibit the growth of *N. dimidiatum*. Dual method assays revealed that these strains significantly ($p < 0.05$) suppressed pathogen growth at varying rates. Numerous studies have shown that the efficacy of biological control agents varies not only among antagonists but also across different pathogens (Tchameni et al. 2017; Lin-Qi et al. 2020; Bedine et al. 2020). Indeed, the degree of antagonism is influenced by the genetic profile of the antagonist isolate (Anand and Reddy 2009; Sanjeev Kumar et al. 2009; Anees 2010). Furthermore, the biocontrol activity of *Trichoderma* species is typically mediated by multiple mechanisms, which differ among strains (Jogaiah et al. 2018; Cai et al. 2021; Ruangwong et al. 2021). One of the most prominent mechanisms is mycoparasitism, whereby *Trichoderma* directly attacks the pathogen. This process is morphologically expressed by the antagonist's ability to overgrow and sporulate on the pathogen during dual culture assays, as observed in the present study (Abo-Elyousr et al. 2014). The antagonist strains used have grown on the pathogen and have sporulated on them. Mycoparasitism is one of the mechanisms of action of biological agents of the genus *Trichoderma* aimed at destroying the pathogen. This mechanism is complemented by *Trichoderma*'s ability to produce lytic enzymes that degrade pathogens cells walls and secretes secondary metabolites whose action could prevent the formation of the cell membrane (Gajera et al. 2012).

Antibiosis test using the cellophane membrane method revealed significant ($p < 0.05$) a significant inhibition of pathogen growth, with inhibition rates of 71.11 ± 2.22% and 97.04 ± 1.28% depending on *the* strain of the antagonistic tested. In contrast, antibiosis assays conducted under micro-atmosphere conditions did not result in inhibition of radial mycelial growth. This suggests volatile compound production by *Trichoderma* species is highly variable and dependent on the strain, consistent with previous reports (Benitez et al. 2004; Ntah et al. 2018; Anees et al. 2010; Tchameni et al. 2020). The absence of inhibition observed against *N. dimidiatum* may be attributed either to the production of antagonistic substances at concentrations insufficient to exert a measurable effect, or to the intrinsic insensitivity of *N. dimidiatum* to these metabolites. This lack of direct inhibition underscores the

complexity of biocontrol interactions, where efficacy is not solely dependent on metabolite toxicity but rather on the interplay of multiple mechanisms. Accordingly, the overall antagonistic effect observed *in vitro* is likely the result of a synergistic combination of mechanisms, including mycoparasitism, antibiosis, and competition for nutrients and ecological niches (Yao et al. 2023).

Trichoderma species are well recognized not only for their ability to reduce the incidence of diseases caused by plant pathogens but also for their contribution to soil fertility and the production of natural fertilizers (Yao et al. 2023, Ambata et al. 2025). The application of these biological agents to cucumber seeds enabled the evaluation of their effects on plant growth parameters. Overall, the *Trichoderma* strains used significantly ($p < 0.05$) enhanced the morphological traits, including plant height, collar diameter, root and shoot biomass. Mei et al. (2019) demonstrated that *T. asperellum* 525, T. *harzianum* 610 and *T. pseudokoningii* 886 significantly improved plant height, stem diameter, leaf area, shoot and root fresh weight. Furthermore, Satyendra and Rekha (2021) highlight the dual role of *T. harzianum* in stimulating cucumber growth parameters while counteracting the negative impacts of soil-borne pathogens such as *Rhizoctonia solani* and *Fusarium oxysporum*. The ability of *Trichoderma* to improve agronomic characteristics of seedlings can be attributed to its dual colonization strategy: the fungus not only colonizes the root surface but also penetrates and resides within root tissues, thereby facilitating the efficient assimilation of essential mineral elements required for plant growth (Contreras-Cornejo et al. 2024). Indeed, mushrooms of the genus *Trichoderma* can produce plant growth stimulators (such as indoleacetic acid, abscisic acid, ethylene, gibberellic acid, and cytokinins and harzianolide) to promote root development and overall plant growth (Degani et al. 2021; Wang et al. 2021). They can also secrete phytase, ferritin, gluconic, citric, and fumaric acids, which decrease soil pH to assist plants in absorbing phosphorus, magnesium, and iron, decompose soil organic matter, increase nutrient availability, and enhance photosynthetic efficiency (Lombardi et al. 2020; Samuelian 2016).

In addition, this ability of *Trichoderma* to improve agronomic characteristics is also attributed to the secretion of bioactive substances, including diverse secondary metabolites and cell wall-degrading enzymes (Jaiswal et al. 2020; Tseng et al. 2020; Viriyasuthee et al. 2019; Domínguez et al. 2016). By contrast, soil-borne pathogens occupy the same ecological niche in the rhizosphere, where they disrupt the vascular system of the host plant and interfere with critical physiological processes involved in water and nutrient uptake. Such interference directly explains the reduction in agronomic parameters observed in infected plants (Ayala-Donas et al. 2020).

Biological control agents that promote plant growth would also contribute to induce plants resistance. Induced resistance refers to the enhanced defensive capacity of plants triggered by external biotic or abiotic factors. Colonization of plant roots by *Trichoderma* spp. initiates profound physiological and metabolic changes, leading to the production of diverse secondary metabolites that cat as elicitors of defense responses (Al-Hazmi and TariqJaveed, 2016). The induction of resistance in plants by *Trichoderma* is typically manifested as a visible reduction in disease incidence. In the present study, cucumber plants treated with *T. afroharzianum* and *T. erinaceum* and subsequently infected with *N. dimidiatum* exhibited reductions in disease incidence of 16.67% and 33.33%, respectively. Similary,

Satyendra and Rekha (2021) demonstrated that the application of *T. harzianum* on cucumber plants reduced the severity of the disease caused by *R. solani* and *F. oxysporum*.

The *Trichoderma* strains used in this study demonstrated the ability to stimulate the polyphenol biosynthetic pathways. These findings are consistent with those of Nawrocka et al. (2018), who reported that *Trichoderma atroviride* enhances the synthesis of phenolic compounds in cucumber plants. The accumulation of these compounds in plant tissues following infection is closely associated with their linked to their dual role: exerting direct antimicrobial activity and reinforcing cell wall structure. Slatnar et al (2016) further demonstrated that polyphenol content increases significantly during plant-microorganism interactions. Beyond their role in stimulating plant defense, polyphenols also contribute to plant growth and reproduction microorganisms (Slatnar et al. 2016). As secondary metabolites, they serve as substrates for several key enzymes involved in plant responses to both biotic and abiotic stress. Moreover, during plant–microorganism interactions, plants synthesize a wide array of structural proteins and defense-related enzymes that safeguard cellular integrity and enhance resilience (Chakraborty et al. 2023).

The results regarding peroxidase (PO) activity demonstrate the ability of *Trichoderma* strains to stimulate both the synthesis and activity of this enzyme, even in the absence of infection. The observed increase in PO activity is closely linked to its role in plant defense. Specifically, PO catalyzes the degradation of hydrogen peroxide, leading to the generation of reactive oxygen species (ROS) that can interact with pathogen cell wall components, ultimately causing structural damage and pathogen death. In addition, PO contributes to the accumulation of toxic molecules that inhibit pathogen growth, as well as to the regulation of key metabolic processes, such as protein modification mediated by nitric oxide (Chakraborty et al. 2017;). Ngadze et al. (2012) demonstrated that PO activity increases during interactions with compatible host-pathogen systems, while Almaghasla et al. (2023) showed a significant rise in PO activity in cucumber plants infected by *R. solani* and treated with *T. asperellum* KSATC or *T. asperellum* KSAT1E. These data are similar to those of the present study which showed that PO activity further increases with infection, but in treated plants, it is significantly lower compared to the untreated control. This result may be attributed to the actions of *Trichoderma* strains that could have modulated the plant's response to stress, as the plant had already been sensitized beforehand. Moreover, the decrease in PO activity observed in treated and infected plants could also be explained by the limited availability of its substrate, hydrogen peroxide (H_2O_2). This molecule may have been redirected toward other metabolic processes, such as diffusion into uninfected cells to activate enzymes involved in salicylic acid biosynthesis, a key signaling molecule in systemic acquired resistance. H_2O_2 can also act directly as a toxic species against pathogens (Parent et al. 2008), or function as a signaling messenger in the activation of defense-related genes and pathways during plant–pathogen interactions.

PPO plays a pivotal role in plant defense, primarily through its ability to oxidize phenolic compounds into quinones, which generally exhibit greater antimicrobial activity than their precursor phenols (Taranto et al. 2017). In this study, PPO activity displayed distinct patterns depending on infection status and treatment with antagonistic agents. Uninfected plants treated with *Trichoderma* spp. showed a

significant elevation in PPO activity compared to untreated controls, suggesting that antagonistic fungi can prime host tissues by enhancing basal defense enzyme activity. Interestingly, when treated plants were subsequently challenged with *N. dimidiatum*, PPO activity declined, whereas untreated control plants exhibited a marked increase in PPO activity upon infection. This contrasting response highlights a nuanced regulatory mechanism: *Trichoderma* treatment may initially stimulate PPO activity in healthy tissues, thereby activating defense pathways, but subsequently modulate enzymatic activity to prevent excessive stress responses. Such regulation could mitigate the detrimental effects of uncontrolled PPO overactivity, including oxidative damage and disruption of cellular homeostasis. The variability in PPO activity may also be explained by biochemical and structural factors. Certain PPO isoforms require activation by detergents or proteases to achieve full catalytic potential (Constabel et al. 2000; Gandía - Herrero et al. 2005). Moreover, PPO activity is highly pH-dependent; slightly acidic conditions induce conformational changes in the active site that enhance substrate accessibility (Kanade et al., 2006). Microbial colonization whether pathogenic or antagonistic can alter rhizosphere and apoplastic pH through the secretion of organic acids or alkalinizing metabolites, thereby influencing PPO activity. Taken together, these findings suggest that PPO activity is not merely a static defense marker but a dynamic component of the plant's biochemical arsenal. Its modulation by *Trichoderma* treatment underscores the complexity of plant–microbe interactions, where antagonistic fungi act both as inducers and regulators of host defense responses. This dual role positions PPO as a valuable indicator of induced resistance and highlights the potential of *Trichoderma*-based biocontrol strategies to fine-tune enzymatic defense pathways for sustainable disease management.

Conclusion

T. erinaceum and *T. afroharzianum* exhibit strong antagonistic activity against *N. dimidiatum*, the causal agent of cucumber root rot. Seed coating with these biocontrol fungi not only enhanced cucumber growth parameters, such as germination rate, shoot and root development but also stimulated key biochemical responses, including elevated antioxidant enzyme activity and accumulation of defense-related metabolites. These physiological and biochemical changes collectively indicate the induction of systemic resistance in cucumber plants against *N. dimidiatum*. Given their dual role in promoting plant growth and suppressing pathogen infection, the bioagents used represent promising candidates for incorporation into biopesticide formulations aimed at sustainable management of cucumber root rot. Future investigations will focus on evaluating the synergistic effects of combining these antagonists, thereby assessing their integrated bioprotective potential and optimizing their application in integrated pest management strategies.

Declarations

Disclosure statement

No conflict of interest was reported by the author(s).

Funding Declaration

This work received no funding

Author Contribution

Moïse A Eyong Ntah: designed the study, performed research.Marie Ampère Boat Bedine: formal analysis, methodology and designed the studyVictor Davy Mussango conceptualization, formal analysis.Geroges Junior Chakoua and Henri Tibo Ambata Ambata: formal analysis, methodology. Séverin Nguemezi Tchamen: performed research, formal analysis, methodology, writing the original draft paper. Modeste Lambert Sameza and Jean Duplex Wansi: supervised the research and writing-review editing.All authors reviewed the manuscript.

Acknowledgement

The author are grateful to the Laboratory of Biochemistry, Faculty of Science, University of Douala, for providing reasearch facilities.

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Tables

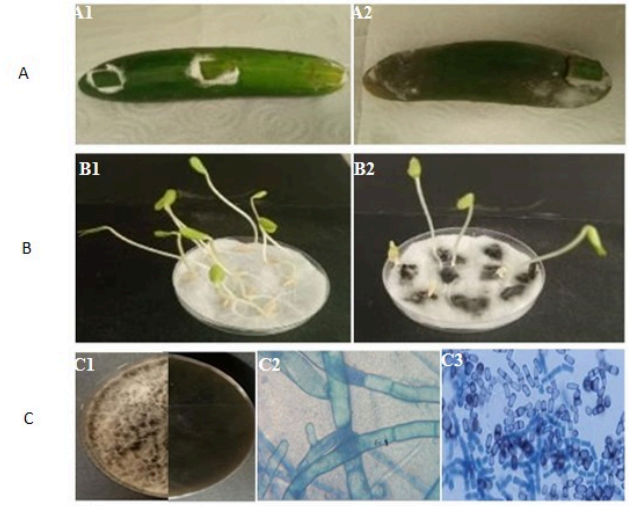
Table 1

Effects of Trichoderma strains on growth morphological parameters on healthy and infected cucumber.

Treatment	Plant height (cm)	Stem diameter (cm)	Shoot Biomass (g)	Root Biomass (g)
T0	61.67 ± 4.68^{b}	1.78 ± 0.25^{e}	13.93 ± 1.31^{e}	0.73 ± 0.10^{d}
T1	91.67 ± 5.24^{a} (+ 48.65%)	3.07 ± 0.52^{a} (+ 72.01%)	20.17 ± 2.01^{b} (+ 44.74%)	1.98 ± 0.23^{a} (+ 170.53%)
T2	89.17 ± 4.49^{a} (+ 44.59%)	3.33 ± 0.26^{a} (+ 86.93%)	26.47 ± 2.75^{a} (89.96%)	2.48 ± 0.26^{a} (+ 238.74%)
B0	69.17 ± 3.43^{b} (+ 12.16%)	2.25 ± 0.14^{d} (+ 26.19%)	16.00 ± 0.95^{d} (+ 14.84%)	1.05 ± 0.15^{b} (+ 43.25%)
T0i	49.17 ± 4.22^{d}	1,07 ± 0,16^{g}	9,92 ± 1.15^{f}	0.28 ± 0.08^{e}
T1i	64.67 ± 4.46^{b} (+ 51.52%)	1,83 ± 0,15^{e} (+ 71.79%)	16.67 ± 1.36^{d} (+ 68.06%)	0,95 ± 0.14^{c} (+ 235,69%)
T2i	56.50 ± 3.67^{c} (+ 14.91)	1,80 ± 0,19^{e} (+ 68.70)	17.23 ± 1.18^{c} (+ 73.77%)	0,93 ± 0,15^{c} (+ 229.68%)
B0i	61.33 ± 3.50^{bc} (+ 24.74%)	2,17 ± 0,21^{d} (+ 103.09%)	15.20 ± 1.28^{d} (+ 53.27%)	0,90 ± 0,18^{c} (+ 218.02%)

T0: Contro, T1: plant inoculated with T. afroharzianum, T2 : plant inoculated with T. erinaceum, B0 : plant inoculated with Benomyl, Ti: inoculated with N. dimidiatum, T1i: plant inoculated with T. afroharzianum and infected with N. dimidiatum, T2i: plant inoculated with T. erinaceum and infected with N. dimidiatum, B0i: plant inoculated with Benomyl and infected with N. dimidiatum. Value presented in parentheses [ ] and bold are percent increase (+) over control. In the same growth parameter, according to Duncan's test, values ± standard deviation with the same letters are not significantly different at $p \leq 0.05$

Figures

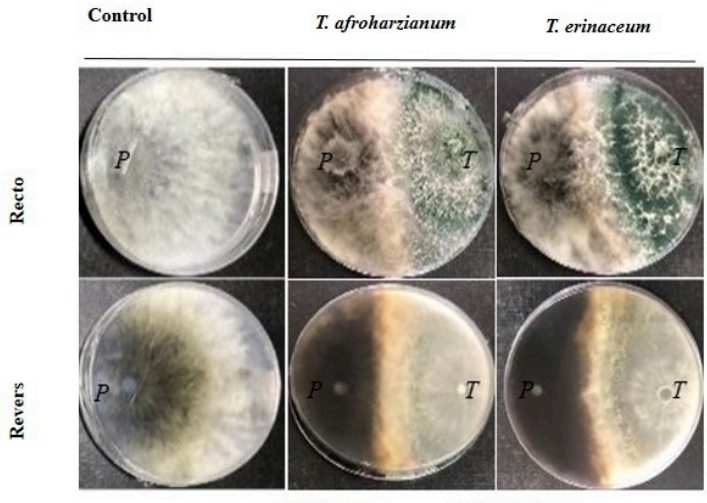


A) Pathogenicity test, B) inhibition of cucumber seed germination, C) macroscopy and microscopy characterization of pathogen
A1: control, A2: cucumber infected, B1: control seeds, B2: cucumber seeds infected, C1: macroscopic appearance of the isolate on PDA medium after 5 days of incubation, C2: microscopic appearance of the mycelium, C3: microscopic appearance of the spores

Figure 1. isolation and morphological characterization of pathogen: A) pathogenicity test, B) inhibition of cucumber seed germination, C) macroscopy and microscopy characterization of pathogen

Figure 1

See image above for figure legend.



P: Pathogen; T: *Trichoderma*

Figure 2. *Trichoderma* strains versus *S. dimidiatum* in dual culture on PDA medium

Figure 2

See image above for figure legend.

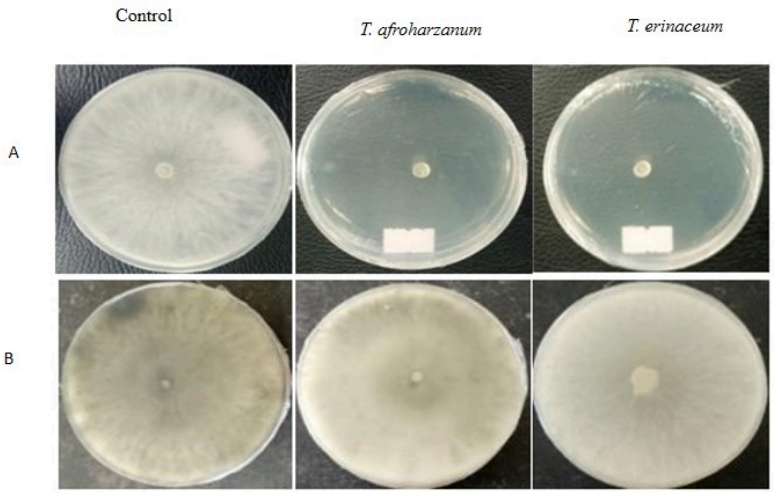
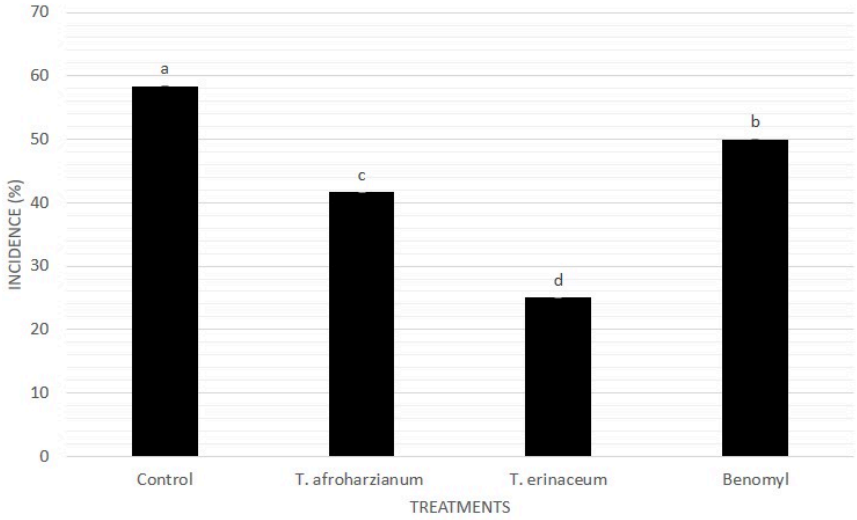


Figure 3. Antibiosis test: A) non-volatile compounds test, B) volatile compounds test

Figure 3

See image above for figure legend.



The histograms bearing the same letter are not significantly different according to the Duncan test at 5%.

Figure 4. Effect of treatments (Trichoderma strains and benomyl) on the incidence of cucumber diseases.

Figure 4

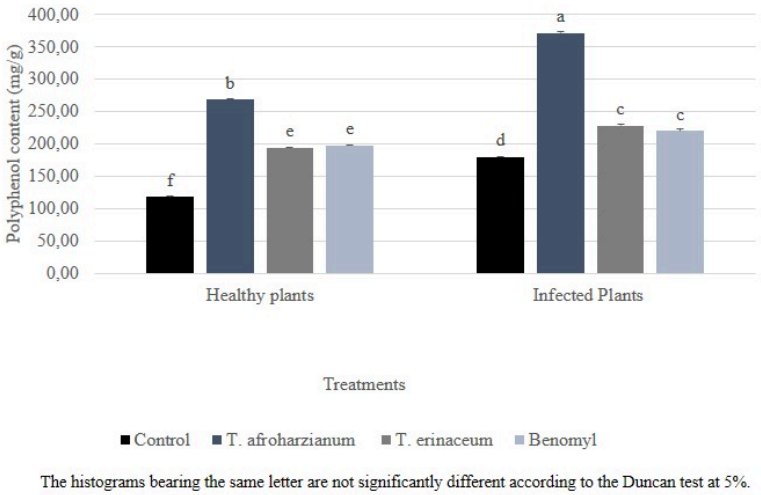
See image above for figure legend.



Figure 5. Effect of *Trichoderma* strains on morphological growth parameters of cucumber plants

Figure 5

See image above for figure legend.

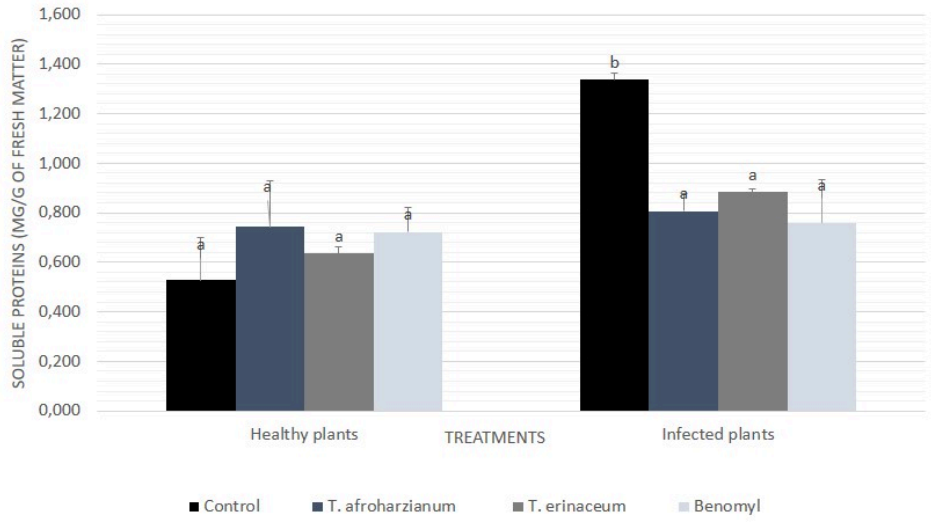


The histograms bearing the same letter are not significantly different according to the Duncan test at 5%.

Figure 6. Polyphenol Content

Figure 6

See image above for figure legend.

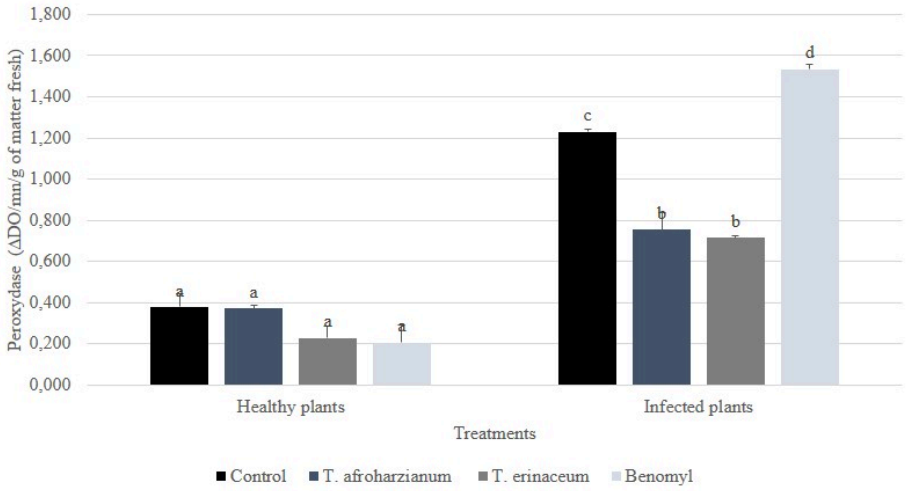


The histograms bearing the same letter are not significantly different according to the Duncan test at 5%.

Figure 7. Soluble Proteins content

Figure 7

See image above for figure legend.

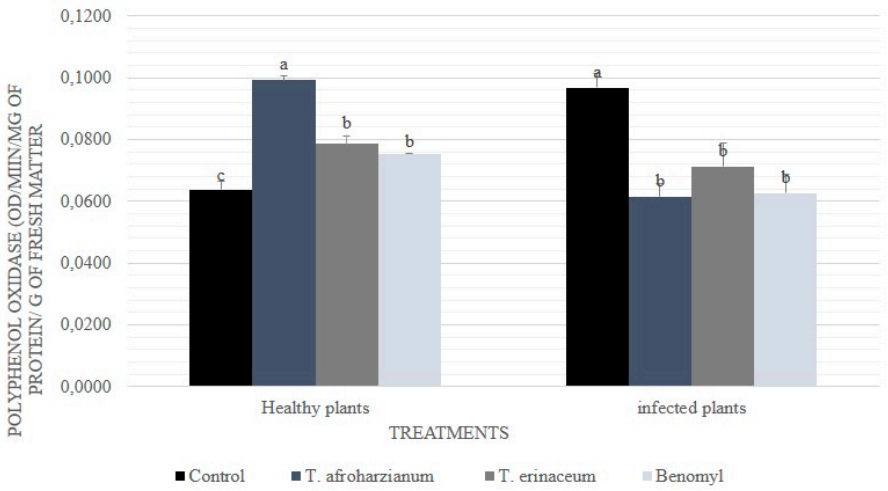


The histograms bearing the same letter are not significantly different according to the Duncan test at 5%.

Figure 8. Peroxidase activity.

Figure 8

See image above for figure legend.



The histograms bearing the same letter are not significantly different according to the Duncan test at 5%.

Figure 9. Polyphenol oxidase activity

Figure 9

See image above for figure legend.