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RESEARCH PAPER

Differential watering and organic amendment effects on the yield, quality and metabolite constituents of pepper (*Capsicum chinense* Jacq.)

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Key Message: The combined application of biochar and *Trichoderma harzianum* significantly improved the growth, yield, fruit quality, nutritional composition, and soil properties of *Capsicum chinense* under varying watering regimes. The combined treatment under full irrigation (100% field capacity) produced the highest growth and yield, while its application under moderate water deficit (60% field capacity) maintained relatively high productivity.

Abstract

Water deficit remains a major constraint to pepper production in tropical agro-ecosystems, necessitating sustainable soil and microbial management strategies. This study evaluated the effects of biochar and *Trichoderma harzianum* on the growth, yield, fruit quality, soil properties, and stress physiology of pepper (*Capsicum chinense* Jacq.) under varying watering regimes. A screenhouse experiment was conducted using a Completely Randomized Design (CRD) with fourteen treatment combinations, including sole and combined applications of biochar and *T. harzianum* under 100, 60, and 40% field capacity, replicated three times. Growth, yield, physiological, and biochemical parameters, including antioxidant enzymes (SOD, CAT, POD, APX) and proline, were assessed alongside fruit quality and soil properties. Results showed that the combined application of biochar and *T. harzianum* under full irrigation (B+T+100% FC) produced the highest growth and yield, with plant height (90.27 cm), leaf area (166.69 cm²), and fruit yield (36.93 fruits plant^{-1}; 150.00 g fresh weight). The B+T+60% FC treatment also significantly enhanced growth and yield (35.23 fruits plant^{-1}), indicating improved tolerance to moderate water deficit, whereas 40% FC markedly reduced performance. Water stress significantly increased proline (60 µmol g^{-1} DW), total phenolics (820 mg GAE/100 g DW), and antioxidant enzyme activities, while amendment treatments, particularly B+T, reduced these stress indicators (proline: 6–11 µmol g^{-1}). Chlorophyll content was highest under combined treatments at 60–100% FC (~73 mg g^{-1} FW). Biochar improved ash (5.88%), crude fibre (38.11%), and protein (2.43%), whereas *T. harzianum* enhanced carbohydrate (65.44%) and vitamin content (155.22 mg/100 g). Mineral composition was highest under B+T+100% FC, notably K (224.19 mg/100 g), Ca (15.64 mg/100 g), and Mg (39.87 mg/100 g). Soil properties were also improved, with reduced bulk density (1.03 g cm^{-3}) and increased water-holding capacity (34.10%) and organic matter (14.25%). Overall, co-application of biochar and *T. harzianum* enhanced pepper productivity, stress tolerance, and soil quality under water-limited conditions. © 2026 The Author(s)

Keywords: Antioxidant enzymes, Biochar, *Capsicum chinense*, Organic amendment, *Trichoderma harzianum*, Water stress

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Introduction

Pepper (*Capsicum chinense* Jacq.) is one of the most widely cultivated vegetable and spice crops worldwide, valued for its economic importance, culinary versatility, and nutritional and medicinal properties. In Nigeria and other tropical regions, pepper occupies an important place in local diets and markets due to its pungency, high vitamin content, and strong consumer demand (FAOSTAT, 2012; Mustapha et al., 2021). Nigeria is recognized as the largest producer of pepper among the sub-Saharan African countries (Akpan et al., 2023) and ranks among the leading producers globally, contributing about 50% of Africa's total pepper production. The crop is predominantly cultivated in the northern region of the country (Ogunbo, 2015). In 2020, Nigeria produced 762,174 tonnes of green chilies and peppers, representing a slight increase of 0.27% compared to the production recorded in 2019 (FAO, 2020).

Drought has been reported to affect several physiological and biochemical processes in plants, including photosynthesis, nitrogen assimilation, and metabolic activities (Nadeem et al., 2019; Kim et al., 2023; Pei et al., 2023). In response to drought stress, plants undergo a range of physiological adjustments aimed at maximizing water-use efficiency while maintaining essential metabolic pathways (Qi et al., 2021; Sato et al., 2024). These adaptations include stomatal closure to reduce water loss through transpiration and modifications in root architecture to enhance water uptake from the soil (Gupta et al., 2020). In southwestern Nigeria, particularly

within the Akoko region of Ondo State, pepper cultivation is strongly influenced by seasonal water availability. Farmers in communities such as Etioro-Supare Akoko, Akungba Akoko, and Ayegunle Akoko communities often experience reduced production during the dry season due to limited soil moisture and inadequate irrigation facilities. This seasonal decline in production frequently results in supply shortages and noticeable increases in market prices toward the end of the year and during the early months of January and February. Water plays a fundamental role in plant growth and development, constituting between 80 and 95% of the biomass of fresh plant tissues (Lisar et al., 2012). Under drought conditions, plants experience metabolic disruptions and oxidative stress resulting from excessive production of reactive oxygen species (ROS), which can damage cellular membranes, proteins, and nucleic acids (Hasanuzzaman et al., 2022).

Although plants accumulate compatible solutes such as sugars, proline, and sugar alcohols to stabilize proteins and cellular structures under stress conditions (Ghosh et al., 2021), however, prolonged water scarcity can nevertheless lead to a substantial decline in crop productivity. Thus, sustainable agronomic strategies capable of improving soil moisture retention and enhancing plant stress tolerance are increasingly being explored. Biochar is a carbon-dense material obtained by thermally decomposing organic biomass through pyrolysis under oxygen-limited conditions. It has emerged as a promising climate-smart amendment for improving soil quality. Its application can enhance soil aggregation, pore characteristics, cation exchange capacity, and water-holding ability, which collectively contribute to greater soil moisture conservation and improved nutrient accessibility, particularly during periods of drought (Blanco-Canqui, 2017; Bohara et al., 2019). In addition, the porous structure of biochar provides favorable habitats for beneficial soil microorganisms, promoting microbial colonization and activity in the rhizosphere (Wu et al., 2022).

Trichoderma harzianum is a widely studied beneficial fungus known for its myco-parasitic and plant growth–promoting abilities (Mukherjee et al., 2013). Its root colonization enhances plant tolerance to stress through physiological and biochemical adjustments, improving root growth, nutrient uptake, and overall plant development (Ji et al., 2012). The application of different *Trichoderma* treatments significantly enhanced the accumulation of essential nutrients, including N, P, S, Ca, Mg, and K. These treatments also resulted in a pronounced decline in the Na/K ratio, which was primarily associated with improved potassium acquisition by the plants (Saha et al., 2024). Druzhinina et al. (2011) explained that *Trichoderma harzianum*, a prominent species of *Trichoderma*, produces various growth-promoting substances, including ethylene and indole-3-acetic acid (IAA), which stimulate root development. They further noted that hydro-phobins and swollenin produced by *Trichoderma* facilitate effective colonization of plant roots. Zhang et al. (2019) demonstrated that *T. harzianum* induces increased expression of antioxidant enzyme genes and enhances the activity of key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), thereby reducing reactive oxygen species (ROS) levels in plant cells. Similarly, Saha et al. (2024) observed increased activities of POD and ascorbate peroxidase (APX) in *Trichoderma*-treated Indian mustard under saline conditions, which were associated with improved yield and enhanced stress tolerance. Although the individual roles of biochar and *Trichoderma* in improving crop performance under abiotic stress have been widely reported, information on their combined effects under varying moisture regimes remains limited, particularly for pepper. Therefore, this study evaluated the effects of biochar and *Trichoderma harzianum*, applied individually and in combination, on the growth, yield, fruit quality, soil properties, and stress-related metabolites of pepper under different watering regimes (100%, 60%, and 40% field capacity).

Materials and Methods

Experimental site

The study was conducted in the screenhouse of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Biochar preparation

Biochar was produced from cow dung, poultry droppings, *Pennisetum purpureum* (Elephant grass), *Chromolaena odorata*, and *Tithonia diversifolia*. Raw materials were cleaned of debris, chopped into 2–5 cm pieces, air-dried for 5–7 days, oven-dried at 60–70 °C for 24–48 h to reduce moisture, ground, and sieved through a 2 mm mesh (Downie, 2011). Equal proportions of the biomass were mixed thoroughly. Two hundred grams of the mixed biomass was placed into sealed stainless-steel crucibles to limit oxygen and pyrolyzed in a muffle furnace at 400 °C for 1–2 h After cooling to <100 °C, biochar was stored in clean airtight containers labeled with feedstock composition, pyrolysis temperature, and production date pending use (Narzari et al., 2017)

Isolation and culture of *Trichoderma harzianum*

Soil samples were collected from the rhizosphere of healthy plants. Serial dilutions (10^{-1}–10^{-6}) were prepared in sterile distilled water and aliquots plated on Potato Dextrose Agar (PDA) amended with streptomycin to inhibit bacterial growth (Elad et al., 1981). Plates were incubated at 25 ± 2 °C for 5–7 days. Distinct *Trichoderma* colonies were sub-cultured via hyphal tip transfer to obtain pure isolates. Identification was based on colony morphology and microscopic features of conidiophores and conidia (Sharma & Singh, 2014). For mass culture, 5 mm mycelial discs from pure *T. harzianum* cultures were inoculated into Potato Dextrose Broth (PDB) and incubated on a rotary shaker at 25 ± 2 °C for 7–10 days (Asghar et al., 2024). The resulting fungal biomass was harvested by filtration, washed with sterile distilled water, and prepared as a suspension containing ≥10^6–10^8 CFU mL^{-1} for experimental application.

Seed and soil sources

The local variety of *Capsicum chinense* (Atarodo) seeds used were obtained from a local market in Akungba-Akoko, Ondo State, Nigeria. Topsoil was collected from the experimental field of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Experimental set-up

Four-week-old seedlings were transplanted into pots containing 5 kg of the collected topsoil. Biochar (150 g) was incorporated into designated pots two weeks before transplanting. One week after transplanting, *T. harzianum* suspension (30 mL; $\geq 10^6$–10^8 CFU mL^{-1}) was applied near the root zone of plants assigned to *Trichoderma* treatments. The experiment was arranged in a Completely Randomized Design (CRD) with fourteen treatments, each replicated three times:

T1 = 100% Field Capacity
T2 = 60% Field Capacity
T3 = 40% Field Capacity
T4 = Biochar alone
T5 = *Trichoderma* alone
T6 = Biochar + 100% Field Capacity
T7 = Biochar + 60% Field Capacity
T8 = Biochar + 40% Field Capacity
T9 = *Trichoderma* + 100% Field Capacity
T10 = *Trichoderma* + 60% Field Capacity
T11 = *Trichoderma* + 40% Field Capacity
T12 = Biochar + *Trichoderma* + 100% Field Capacity
T13 = Biochar + *Trichoderma* + 60% Field Capacity
T14 = Biochar + *Trichoderma* + 40% Field Capacity

Data collection

Plant height was determined by measuring the distance from the soil surface to the apical bud with a meter rule. Stem girth was recorded 2 cm above the plant base using a digital vernier caliper (0–200 mm). Leaf dimensions, including length and breadth, were measured with a meter rule, and these measurements were used to determine leaf area. The total number of leaves per plant was obtained through direct manual counting. Following plant uprooting, root length was measured with a meter rule, while the number of roots was determined by manual counting. Fresh and dry weights of the individual plant components were recorded using an electronic weighing balance.

Chlorophyll content analysis

Total leaf chlorophyll content was estimated following the procedure described by Arnon (1949). The chlorophyll concentration was calculated using the following equation:

$$\text{Total chlorophyll} = [(20.2 \times D645) + (8.02 \times D663)] \times (50/1000) \times (100/5) \times ½$$

Where D645 represents the absorbance of the chlorophyll extract measured at 645 nm, while D663 denotes the absorbance recorded at 663 nm.

Fruit quality analyses

Dried fruit material was subjected to digestion with 10 mL of 20% sulfuric acid prior to mineral analysis. Sodium (Na^+) and potassium (K^+) concentrations were quantified using a flame photometer, whereas magnesium (Mg^{2+}) and calcium (Ca^{2+}) were estimated through EDTA titration following the procedures described by AOAC (1990). Proximate composition was determined in accordance with the standard analytical methods recommended by AOAC (1990).

Determination of antioxidant enzyme activities

Freshly collected leaf tissues were rapidly frozen in liquid nitrogen, subsequently freeze-dried, and maintained at −80 °C until biochemical analyses were performed. For enzyme extraction, approximately 25 mg of the dried tissue was homogenized in 50 mM sodium phosphate buffer (pH 7.8). The homogenate was centrifuged at 15,000 × g for 20 min at 4 °C, and the clear supernatant was collected and used for subsequent enzyme assays. Superoxide dismutase (SOD) activity was quantified according to Giannopolitis & Ries (1977), based on the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Absorbance was recorded at 560 nm, and one unit of SOD activity was defined as the amount of enzyme producing 50% inhibition of NBT reduction. Peroxidase (POD) and catalase (CAT) activities were assayed following Zhang & Kirkham (1994). POD activity was estimated by monitoring the increase in absorbance at 470 nm associated with guaiacol oxidation, whereas CAT activity was determined by measuring the decline in absorbance at 240 nm caused by the decomposition of hydrogen peroxide (H_2O_2). Ascorbate peroxidase (APX) activity was measured according to Nakano & Asada (1981) by recording the decrease in absorbance at 290 nm resulting from the oxidation of ascorbate.

Determination of proline and total phenolics content

Proline content was quantified according to the procedure of Bates et al. (1973), with minor modifications. Approximately 25 mg of freeze-dried plant tissue was homogenized in 10 mL of 3% sulfosalicylic acid and subsequently filtered through Whatman filter paper. An aliquot of 2 mL of the resulting extract was combined with 2 mL each of acid-ninhydrin reagent and glacial acetic acid. The reaction mixture was incubated at 100 °C for 1 h and then rapidly cooled in an ice bath. Proline was extracted by adding 4 mL of toluene and thoroughly mixing the contents. The upper chromophore-containing phase was carefully separated and allowed to reach room temperature. Absorbance was recorded at 520 nm using a UV–Vis spectrophotometer. Proline concentration was estimated from a standard curve prepared using known concentrations of proline. Total phenolic content (TPC)

was estimated using the Folin–Ciocalteu colorimetric assay following Rizvi et al. (2023). For the assay, 1 mL of plant extract was mixed with 200 µL of 1 N Folin–Ciocalteu reagent and 1.8 mL of distilled water. After allowing the mixture to react for 3 min, 400 µL of 10% Na_2CO_3 was added, followed by 600 µL of distilled water to obtain a final reaction volume of 4 mL. The reaction mixture was maintained at room temperature for 1 h before measuring its absorbance at 725 nm. A calibration curve prepared with gallic acid was used to determine the phenolic concentration, and the results were expressed as µg g^{-1} dry weight.

Soil analysis

The physicochemical characteristics of the soil samples were evaluated using established analytical procedures. Soil pH was measured with a Metro pH meter (Model E250). Particle-size distribution was assessed by the hydrometer technique following Bouyoucos (1962). Cation exchange capacity (CEC) was determined using the ammonium saturation procedure described by Jackson (1968), whereas soil organic matter was estimated through the wet oxidation method of Walkley & Black (1934). Exchangeable potassium (K) and sodium (Na) concentrations were quantified by flame photometry. Calcium (Ca) and magnesium (Mg) contents were measured using the versenate (EDTA) titration method. Available soil phosphorus was assessed using the Olsen and Mehlich extraction procedures as described by Iatrou et al. (2014). Soil microbial load was estimated by the standard serial dilution and spread-plate technique following Seeley & Vandemark (1981).

Statistical analysis

All data were analyzed using SPSS software. Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at a 5% level of significance.

Results

Physicochemical properties of biochar produced

Table 1 shows the physicochemical properties of the biochar produced. The biochar had a pH of 9.50, indicating that it is alkaline. The cation exchange capacity (CEC) was 35.54 cmol kg^{-1}, while the carbon content was 33.18 g kg^{-1} with a carbon-to-nitrogen ratio of 150:1. The concentrations of macronutrients recorded were phosphorus (0.25 g kg^{-1}), potassium (2.597 g kg^{-1}), calcium (0.43 g kg^{-1}), and magnesium (0.188 g kg^{-1}). Other elements present included sodium (0.0248 g kg^{-1}), silicon (0.0385 g kg^{-1}), iron (0.0267 g kg^{-1}), copper (0.0398 g kg^{-1}), manganese (0.1264 g kg^{-1}), zinc (0.277 g kg^{-1}), and boron (0.048 g kg^{-1}). The ash content of the biochar was 46.08 g kg^{-1}. The biochar exhibited alkaline properties, high cation exchange capacity, and appreciable carbon and mineral nutrient contents, suggesting its potential to improve soil fertility and nutrient availability.

Table 1 Physical and chemical properties of biochar used for the study

Properties	Values
pH	9.50
CEC (cmol/kg)	35.54
Carbon (g/kg)	33.18
Carbon/Nitrogen ratio	150:1
P (g/kg)	0.25
K (g/kg)	2.597
Ca (g/kg)	0.43
Mg (g/kg)	0.188
Na (g/kg)	0.0248
Si (g/kg)	0.0385
Fe (g/kg)	0.0267
Cu (g/kg)	0.0398
Mn (g/kg)	0.1264
Zn (g/kg)	0.277
B (g/kg)	0.048
Ash content (g/Kg)	46.08

Effects of amendments on pepper growth variables of pepper under differential watering

Table 2 shows that both biochar and *Trichoderma harzianum* individually improved pepper growth, but their combined application (B+T) had the greatest effect. For example, plant height reached 90.27 cm under B+T at 100% FC, compared to 76.13 cm with biochar alone and 68.10 cm with *Trichoderma* alone. Leaf number was highest (169.67 leaves) under B+T at 60% FC, while leaf area reached 166.69 cm^2 under B+T at 100% FC. Root development was also enhanced, with the number of roots increasing to 125 and root length to 19.00 cm under B+T at 100% FC. These values show that the combination of biochar and *Trichoderma,* especially under higher watering regimes, significantly promotes pepper growth.

Effects on pepper biomass yields

Table 3 shows that both biochar and *Trichoderma harzianum,* individually and in combination, increased fresh and dry weights of pepper roots, leaves, and stems compared to untreated plants. For example, root fresh weight reached 14.93 g under biochar alone and 14.31 g under B+T at 100% FC, while leaf fresh weight peaked at 24.06 g under T+40% FC. Stem fresh weight was highest (26.97 g) under B+T at 60% FC. Overall, the combination of biochar and *Trichoderma,* especially under higher watering regimes, consistently enhanced biomass accumulation across all plant parts.

Effects on fruit yield variables

Table 4 shows that the combination of biochar and *Trichoderma harzianum* produced the highest pepper yield. B+T at 100% FC had the most fruits per plant (36.93), highest fruit fresh weight (150.00 g), and greatest fruit dry weight (25.00 g). Single applications of biochar or *Trichoderma* improved yield compared to untreated plants, while low water availability (40% FC) reduced fruit number and weight. These results highlight that B+T under adequate or moderate watering maximizes pepper yield.

Table 2 Effects of application of biochar and *Trichoderma harzianum* on growth parameters of pepper subjected to differential watering regimes

Treatments	Pepper growth variables at 12th WAT					
	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Stem girth (cm)	Number of roots	Root length (cm)
Biochar	76.13^{c}	114.00^{cd}	74.60^{b}	8.23^{ab}	58.00^{b}	21.53^{d}
Trichoderma	68.10^{abc}	100.33^{bc}	71.59^{ab}	8.21^{ab}	82.33^{cde}	18.33^{abc}
40% FC	43.83^{a}	74.67^{a}	64.27^{a}	7.97^{a}	48.67^{a}	14.73^{ab}
60% FC	49.50^{a}	89.00^{ab}	69.82^{ab}	7.07^{a}	51.67^{ab}	15.40^{ab}
100% FC	75.40^{c}	135.67^{a}	86.33^{c}	8.20^{ab}	78.33^{cd}	12.73^{a}
B+40% FC	67.40^{abc}	102.67^{cd}	76.69^{ab}	9.43^{bc}	68.00^{bc}	16.50^{b}
B+60% FC	55.93^{ab}	102.67^{c}	101.02^{d}	9.83^{bc}	80.00^{cd}	18.73^{ab}
B+100% FC	74.29^{c}	120.67^{b}	71.74a^{b}	8.93^{b}	86.33^{cde}	14.13^{ab}
T+40% FC	68.70^{abc}	96.33^{a}	87.44^{bc}	8.83^{b}	71.67^{c}	14.40^{ab}
T+60% FC	55.70^{ab}	115.00^{cd}	96.06^{c}	8.43^{b}	95.00^{de}	14.73^{a}
T+100% FC	76.13^{c}	113.00^{b}	79.60^{ab}	8.33^{ab}	87.00^{d}	15.70^{ab}
B+T+40% FC	51.40^{ab}	79.67^{ab}	91.34^{c}	8.83^{b}	70.33^{cd}	15.83^{ab}
B+T+60% FC	78.70^{c}	169.67^{f}	101.02^{d}	10.77^{c}	90.67^{d}	15.10^{ab}
B+T+100%FC	90.27^{d}	126.00^{de}	166.69^{e}	10.83^{c}	125.00^{f}	19.00^{c}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*, WAT= Weeks after treatment application

Table 3 Effects of Biochar and *Trichoderma* and*Trichoderma harzianum* on pepper biomass yields under differential watering

Treatments	Pepper biomass yields					
	Root fresh weight (g)	Leaf fresh weight (g)	Stem fresh weight (g)	Root dry weight (g)	Leaf dry weight (g)	Stem dry weight (g)
Biochar	14.93^{e}	20.76^{e}	25.17^{c}	4.77^{c}	5.41^{c}	6.44^{c}
Trichoderma	12.20^{cd}	19.72^{de}	24.56^{c}	3.68^{bc}	3.16^{a}	4.25^{b}
40% FC	9.86^{b}	7.92^{a}	14.03^{a}	2.24^{a}	3.56^{a}	4.10^{b}
60% FC	9.82^{b}	12.13^{b}	18.65^{b}	2.74^{a}	3.09^{a}	4.30^{b}
100% FC	9.82^{b}	20.04^{e}	24.66^{c}	2.25^{a}	4.05^{ab}	5.06^{bc}
B+40% FC	11.25^{c}	18.37^{d}	23.83^{c}	3.58^{bc}	3.09^{a}	2.23^{a}
B+60% FC	12.36^{cd}	21.29^{ef}	25.88^{c}	3.12^{bc}	5.61^{ab}	6.56^{c}
B+100% FC	13.58^{a}	21.48^{ef}	25.89^{c}	3.89^{bc}	5.42^{ab}	6.74^{c}
T+40% FC	12.99^{a}	24.06^{fg}	20.46^{bc}	3.88^{bc}	5.07^{b}	6.90^{c}
T+60% FC	10.15^{bc}	23.76^{fg}	26.87^{a}	2.93^{a}	5.99^{ab}	6.73^{c}
T+100% FC	11.44^{c}	15.16^{c}	19.47^{b}	3.59^{bc}	3.95^{a}	4.69^{b}
B+T+40% FC	7.31^{a}	17.52^{cd}	22.22^{bc}	2.31^{a}	4.06^{ab}	5.79^{a}
B+T+60% FC	13.10^{d}	21.05^{ef}	26.97^{c}	3.95^{bc}	5.66^{bc}	6.54^{c}
B+T+100% FC	14.31^{e}	20.74^{a}	25.49^{a}	4.02^{a}	5.30^{c}	6.44^{c}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Table 4 Effects of application of Biochar and *Trichoderma harzianum* on yield of pepper subjected to varied watering regimes

Treatments	Pepper fruit yield characters		
	Number of fruit/plants	Fruit Fresh weight/plant (g)	Fruit Dry weight/plant (g)
Biochar	25.70^{cd}	130.00^{d}	23.07^{c}
Trichoderma	24.34^{c}	125.33^{cd}	23.39^{c}
40% FC	18.50^{a}	81.00^{a}	16.99^{a}
60% FC	19.72^{ab}	84.00^{a}	17.33^{ab}
100% FC	25.34^{cd}	120.67^{cd}	21.05^{bc}
B+40% FC	20.48^{abc}	124.00^{cd}	23.37^{c}
B+60% FC	24.30^{c}	119.33^{c}	20.71^{abc}
B+100% FC	28.03^{d}	129.33^{d}	21.73^{a}
T+40% FC	22.74^{c}	120.67^{cd}	21.03^{a}
T+60% FC	25.83^{cd}	122.33^{cd}	22.59^{c}
T+100% FC	27.32^{d}	135.33^{e}	20.55^{abc}
B+T+40% FC	25.12^{cd}	120.33^{cd}	21.83^{bc}
B+T+60% FC	35.23^{e}	146.67^{f}	23.17^{c}
B+T+100% FC	36.93^{f}	150.00^{fg}	25.00^{d}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Effects of amendments on phytochemical constituents of pepper under differential watering

Effects on chlorophyll content

Biochar and *Trichoderma harzianum* increased total chlorophyll content in pepper leaves, with the highest values observed under their combination (B+T) at 60–100% FC, around 73 mg g^{-1} FW (Fig. 1). Lower water regimes (40% FC) had reduced chlorophyll, while higher water availability enhanced chlorophyll accumulation. The results indicate that B+T treatments improve leaf chlorophyll, especially under adequate or moderate watering.

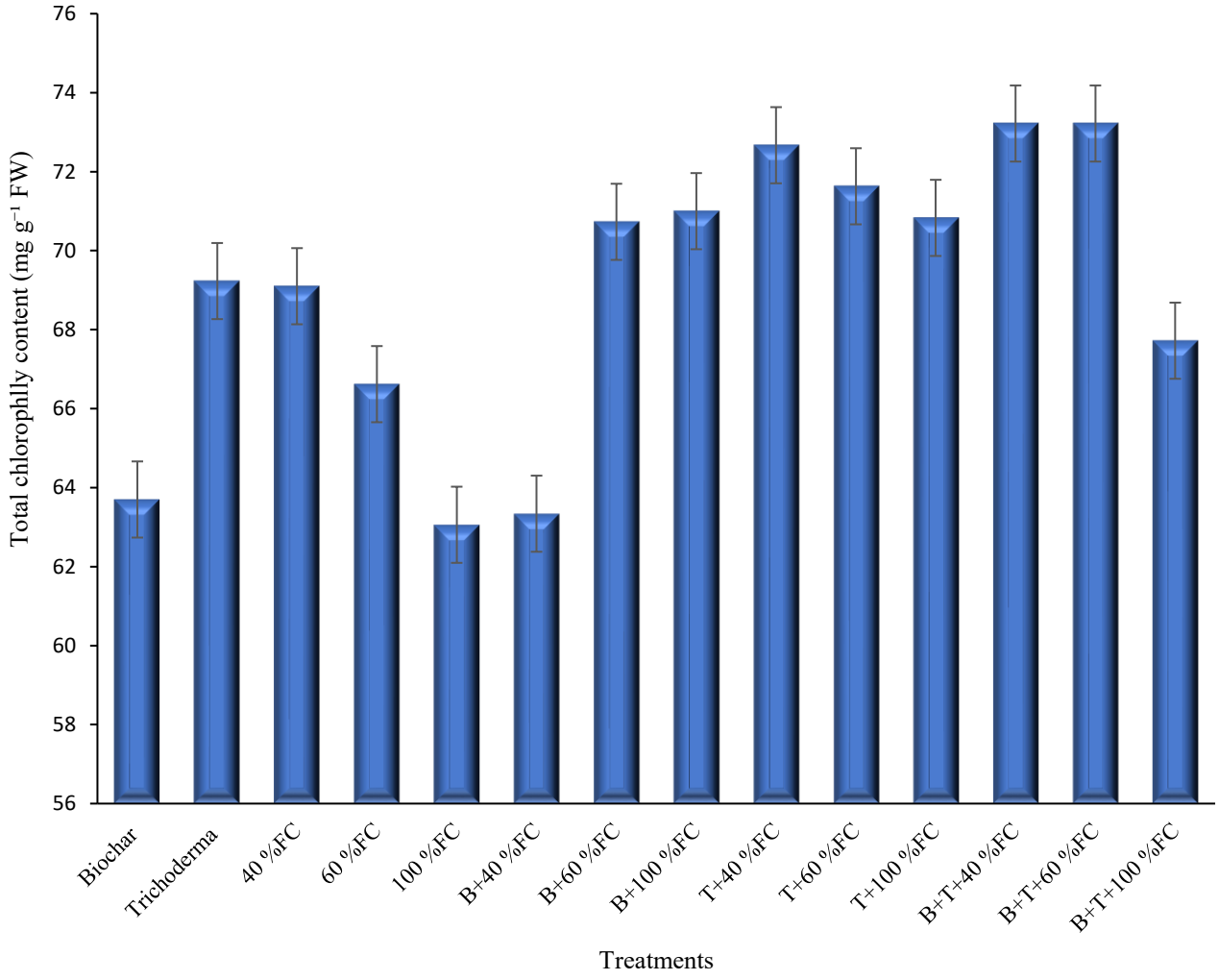


Fig. 1 Effects of application of Biochar and *Trichoderma harzianum* on the total chlorophyll content (mg g^{-1} FW) of pepper subjected to varied watering regimes

Effects on proximate compositions

The co-application of biochar (B) and *Trichoderma harzianum* (T) under varied soil moisture regimes affected the proximate composition of pepper fruits. Moisture content (7.11–7.16%) and total soluble solids (92.85–92.89%) were largely unchanged. Biochar, particularly at 100% FC, increased ash (5.88%), crude fiber (38.11%), and protein (2.43%), while *Trichoderma* alone at 40% FC enhanced carbohydrates (65.44%) and vitamins (155.22 mg/100 g). Combined treatments (B+T) generally moderated these effects, with protein ranging from 1.52–1.59% and carbohydrates from 57.79–61.02% (Table 5).

Effects on mineral composition

The co-application of biochar (B) and *Trichoderma harzianum* (T) under varied moisture regimes significantly influenced mineral composition in pepper fruits. Biochar at 100% FC and B+T+100% FC recorded the highest mineral levels: Na (0.64 mg/100 g), Ca (15.64 mg/100 g), Mg (39.87 mg/100 g), Cl (0.41 mg/100 g), N (0.39 mg/100 g), P (21.24 mg/100 g), and K (224.19 mg/100 g) (Table 6). *Trichoderma* alone under low moisture (T+40% FC) had the lowest mineral contents, e.g., Na (0.24 mg/100 g) and K (176.12 mg/100 g). The combined application under full irrigation maximized fruit mineral accumulation.

Table 5 Effect of co-application of biochar and *Trichoderma harzianum* on the proximate compositions of pepper subjected to varied moisture content

Treatment	Moisture content (%)	Ash content (%)	Crude fiber (%)	Fat content (%)	Crude protein (%)	Carbohydrate (%)	Vitamin (mg/100 g)	Total soluble solid (%)
Biochar	7.14^{bcd}	4.02^{d}	25.57^{d}	0.39^{c}	1.78^{d}	61.09^{h}	102.76^{d}	92.86^{a}
Trichoderma	7.16^{d}	4.17^{e}	29.20^{g}	0.41^{c}	1.84^{e}	61.39^{i}	132.73^{f}	92.84^{a}
40% FC	7.13^{abc}	4.33^{f}	32.41j	0.42^{c}	1.92^{f}	53.81^{c}	155.22^{g}	92.86^{a}
60% FC	7.13^{bc}	4.61^{g}	38.13	0.42^{c}	2.00^{g}	47.73^{b}	162.05^{hi}	92.87^{a}
100% FC	7.12^{ab}	5.36^{h}	28.71^{f}	0.41^{c}	1.98^{g}	56.42^{e}	97.11^{cd}	92.88^{a}
B+40% FC	7.11^{a}	5.46^{i}	30.17^{i}	0.36^{b}	2.06^{h}	54.81^{d}	125.27^{e}	92.89^{a}
B+60% FC	7.15^{bcd}	5.52j	34.22k	0.43^{c}	2.23^{i}	46.01^{a}	158.29^{gh}	92.86^{a}
B+100% FC	7.13^{abcd}	5.88k	38.11	0.44^{c}	2.43j	46.02^{a}	165.58^{i}	92.87^{a}
T+40% FC	7.14^{abcd}	3.67^{a}	22.05^{a}	0.26^{a}	1.46^{a}	65.44	86.60^{a}	92.87^{a}
T+60% FC	7.15^{cd}	3.84^{b}	24.18^{b}	0.29^{ab}	1.48^{a}	63.08k	91.08^{abc}	92.86^{a}
T+100% FC	7.15^{bcd}	3.91^{c}	25.22^{c}	0.30^{ab}	1.57^{c}	61.91j	93.63^{bc}	92.85^{a}
B+T+40% FC	7.13^{abcd}	3.87^{b}	26.20^{e}	0.28^{ab}	1.52^{b}	61.02^{g}	89.16^{ab}	92.87^{a}
B+T+60% FC	7.12^{ab}	3.91^{c}	29.29^{h}	0.31^{ab}	1.59^{c}	57.79^{f}	93.23^{bc}	92.88^{a}
B+T+100% FC	7.13^{abcd}	3.87^{b}	26.20^{e}	0.28^{ab}	1.52^{b}	61.02^{g}	89.16^{ab}	92.87^{a}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Effects of amendments on the metabolites and antioxidant enzymes of pepper under differential watering

Watering at 40% FC strongly increased stress metabolites and antioxidant enzyme activities in pepper fruits, with proline reaching 60 µmol g^{-1}, total phenolics 820 mg GAE/100 g DW, SOD 150 U mg^{-1}, CAT 60 U mg^{-1}, POD 90 U mg^{-1}, and APX 70 U mg^{-1} (Table 7). Biochar and *Trichoderma*, alone or combined, mitigated these stress responses, reducing proline to 28–45 µmol g^{-1} and moderating enzyme activities under moderate stress. Under optimal moisture (100% FC), stress metabolites and antioxidant activities were lowest (proline 6–10 µmol g^{-1}, SOD 68–75 U mg^{-1}), indicating minimal oxidative stress. Hence, biochar and *Trichoderma* alleviated oxidative stress in pepper under water deficit.

Organic amendment and watering regime effects on soil physical properties

Application of biochar and *Trichoderma harzianum* improved soil properties under differential watering (Table 8). Bulk density ranged from 1.03–1.28 g cm^{-3} and was generally lower in biochar-amended soils, indicating better structure. Porosity (31.50–37.50%) and water holding capacity (26.40–34.10%) increased, especially under combined treatments and higher field capacity, enhancing moisture retention. Soil moisture content also rose with increasing watering levels (Table 8). Soil texture remained sandy loam across treatments. The results indicated that sand was the dominant soil fraction across all treatments, ranging from 74.20% to 78.80%, followed by silt (12.90–17.20%) and clay (8.30–9.50%). The highest sand content (78.80%) was recorded under 40% FC, followed by 60% FC (77.90%),. Clay content showed no significant differences among treatments, ranging from 8.30% to 9.50%. However, the highest silt content (17.20%) was recorded under biochar, followed by *Trichoderma* (17.10%), while the lowest value (12.90%) was observed under 40% FC (Table 9).

Table 6 Effect of co-application of biochar and *Trichoderma harzianum* on the mineral composition of pepper subjected to varied moisture content

Treatments	Na (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Cl (mg/100 g)	N (mg/100 g)	P (mg/100 g)	K (mg/100 g)
Biochar	0.38^{b}	12.64^{abc}	32.12^{abc}	0.32^{bc}	0.28^{abc}	13.12^{ab}	189.12^{ab}
Trichoderma	0.42^{bc}	12.98^{abc}	34.64^{abc}	0.33^{bc}	0.29^{abc}	14.11^{ab}	194.02^{ab}
40% FC	0.48^{c}	13.04^{bc}	36.87^{bc}	0.36^{c}	0.31^{bc}	15.16^{abc}	200.12^{b}
60% FC	0.56^{d}	13.56^{bc}	38.12^{c}	0.38^{cd}	0.32^{bc}	17.23^{c}	209.17^{bc}
100% FC	0.42^{bc}	14.13^{c}	33.37^{abc}	0.34^{c}	0.32^{bc}	14.17^{ab}	192.23^{ab}
B+40% FC	0.48^{c}	14.48^{c}	34.87^{abc}	0.36^{c}	0.33^{bc}	17.24^{c}	199.11^{abc}
B+60% FC	0.56^{d}	14.79^{c}	37.12^{c}	0.38^{cd}	0.36^{c}	19.18^{cd}	214.15^{c}
B+100% FC	0.64^{de}	15.64^{cd}	39.87^{c}	0.40^{e}	0.39^{cd}	21.24^{cde}	224.19^{d}
T+40% FC	0.24^{a}	10.21^{a}	29.12^{a}	0.26^{a}	0.23^{a}	11.12^{a}	176.12^{a}
T+60% FC	0.29^{ab}	10.48^{a}	29.68^{a}	0.29^{ab}	0.24^{a}	13.07^{ab}	196.07^{ab}
T+100% FC	0.34^{b}	10.59^{a}	30.48^{ab}	0.30^{abc}	0.24^{a}	16.27^{c}	206.43^{bc}
B+T+40% FC	0.27^{ab}	11.07^{ab}	31.12^{ab}	0.31^{abc}	0.25^{ab}	19.27^{cd}	214.34^{c}
B+T+60% FC	0.42^{bc}	11.46^{ab}	31.42^{ab}	0.32^{bc}	0.25^{ab}	21.04^{cde}	220.34^{d}
B+T+100% FC	0.64^{de}	15.64^{cd}	39.87^{c}	0.41^{e}	0.39^{cd}	21.24^{de}	224.19^{d}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Table 7 Levels of stress metabolites (proline, total phenolics) and antioxidant enzyme activities (SOD, CAT, POD, and APX) in pepper (*Capsicum chinense*) fruits subjected to biochar, *Trichoderma* inoculation, and varying watering regimes

Treatment	Proline (µmol g^{-1} DW)	SOD (U mg^{-1} protein)	Total phenolics (mg GAE/ 100 g DW)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	APX (U mg^{-1} protein)
Biochar	9.00^{a}	70.00^{a}	480.00^{a}	22.00^{a}	35.00^{a}	26.00^{a}
Trichoderma	8.00^{a}	75.00^{a}	520.00^{b}	23.00^{a}	38.00^{a}	28.00^{a}
40% FC	60.00^{g}	150.00^{e}	820.00^{d}	60.00^{e}	90.00^{d}	70.00^{e}
60% FC	25.00^{abc}	110.00^{c}	620.00^{bc}	40.00^{d}	65.00^{b}	45.00^{cd}
100% FC	10.00^{a}	60.00^{a}	420.00^{a}	20.00^{a}	30.00^{a}	24.00^{a}
B + 40% FC	45.00^{cd}	110.00^{c}	780.00c	45.00^{d}	80.00^{d}	52.00^{c}
B + 60% FC	16.00^{a}	85.00^{b}	580.00^{b}	30.00^{ab}	55.00^{b}	35.00^{ab}
B + 100% FC	8.00^{a}	68.00^{a}	500.00^{b}	22.00^{a}	36.00^{a}	27.00^{a}
T + 40% FC	38.00^{c}	105.00^{c}	800.00^{d}	44.00^{d}	78.00^{c}	50.00^{c}
T + 60% FC	14.00^{ab}	82.00^{ab}	600.00^{bc}	28.00^{ab}	53.00^{b}	33.00^{abc}
T + 100% FC	7.00^{a}	72.00^{a}	540.00^{b}	23.00^{a}	39.00^{a}	29.00^{a}
B + T + 40% FC	28.00^{abc}	95.00^{b}	760.00^{c}	38.00^{b}	72.00^{c}	45.00^{cd}
B + T + 60% FC	11.00^{a}	78.00^{ab}	570.00^{b}	26.00^{a}	50.00^{b}	32.00^{abc}
B + T + 100% FC	6.00^{a}	70.00^{a}	550.00^{b}	22.00^{a}	37.00^{a}	28.00^{a}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*; SOD = Superoxide dismutase; CAT = Catalase; POD = Peroxidase; APX = Ascorbate peroxidase

Table 8 Watering regime and biochar effects on the physical properties of experimental soil

Treatments	Bulk density (g cm^{-3})	Porosity (%)	Water holding capacity (%)	Moisture contents (%)
Biochar	1.08^{c}	36.20^{a}	32.10^{a}	9.85^{a}
Trichoderma	1.12^{bc}	34.10^{ab}	29.85^{ab}	9.40^{a}
40% FC	1.28^{ab}	31.50^{bc}	26.40^{c}	6.30^{c}
60% FC	1.22^{abc}	32.80^{bc}	28.75^{bc}	7.10^{bc}
100% FC	1.18^{bc}	33.60^{b}	30.20^{ab}	8.05^{b}
B + 40% FC	1.15^{bc}	34.80^{ab}	30.50^{ab}	7.25^{bc}
B + 60% FC	1.10^{c}	35.90^{a}	31.80^{a}	8.20^{b}
B + 100% FC	1.05^{c}	37.10^{a}	33.40^{a}	9.10^{a}
T + 40% FC	1.20^{abc}	33.00^{bc}	28.10^{bc}	6.95^{bc}
T + 60% FC	1.16^{bc}	34.20^{ab}	29.60^{ab}	7.85^{b}
T + 100% FC	1.13^{bc}	35.10^{ab}	31.00^{a}	8.60^{ab}
B + T + 40% FC	1.14^{bc}	34.60^{ab}	30.80^{ab}	7.40^{bc}
B + T + 60% FC	1.09^{c}	36.00^{a}	32.20^{a}	8.35^{b}
B + T + 100% FC	1.03^{c}	37.50^{a}	34.10^{a}	9.30^{a}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Table 9 Soil physical properties at termination of experiment

Treatments	Sand (%)	Clay (%)	Silt (%)	Textural class
Biochar	74.20^{b}	8.60^{a}	17.20^{a}	Sandy loam
Trichoderma	74.50^{b}	8.40^{a}	17.10^{a}	Sandy loam
40% FC	78.80^{a}	8.30^{a}	12.90^{c}	Sandy loam
60% FC	77.90^{a}	8.90^{a}	13.20^{bc}	Sandy loam
100% FC	77.20^{a}	9.10^{a}	13.70^{bc}	Sandy loam
B + 40% FC	77.50^{a}	9.00^{a}	13.50^{bc}	Sandy loam
B + 60% FC	76.80^{ab}	9.20^{a}	14.00^{b}	Sandy loam
B + 100% FC	76.00^{ab}	9.40^{a}	14.60^{b}	Sandy loam
T + 40% FC	77.60^{a}	8.80^{a}	13.60^{bc}	Sandy loam
T + 60% FC	76.90^{ab}	9.10^{a}	14.00^{b}	Sandy loam
T + 100% FC	76.30^{ab}	9.30^{a}	14.40^{b}	Sandy loam
B + T + 40% FC	77.10^{a}	9.00^{a}	13.90^{b}	Sandy loam
B + T + 60% FC	76.40^{ab}	9.30^{a}	14.30^{b}	Sandy loam
B + T + 100% FC	75.80^{ab}	9.50^{a}	14.70^{b}	Sandy loam

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Organic amendment and watering regime effects on soil chemical properties

In this study, soil pH ranged from 7.42 to 7.78, with no significant differences among treatments. The highest pH (7.78) was recorded under 100% FC and B + T + 100% FC, whereas the lowest (7.42) was observed under biochar application. Electrical conductivity varied significantly among treatments. The highest EC (470 µS cm^{-1}) was recorded under B + T + 60% FC, followed by B + T + 100% FC (460 µS cm^{-1}), while the lowest value (300 µS cm^{-1}) was observed under 100% FC. CEC ranged from 13.30 to 13.85 cmol kg^{-1}, with the highest cation exchange capacity (CEC) (13.85 cmol kg^{-1}) was recorded under biochar application, while the lowest CEC (13.30 cmol kg^{-1}) obtained under 60% FC. Organic matter and organic carbon were significantly influenced by the treatments. The highest organic matter content (14.25%) was recorded under B + T + 100% FC, followed by B + 100% FC (14.20%), whereas the lowest (13.05%) was observed under 100% FC. Similarly, the highest organic carbon content (3.12%) was recorded under B + T + 100% FC, followed by B + 100% FC (3.10%), while the lowest (2.55%) was observed under 100% FC (Table 10). Total N content varied significantly among treatments. The highest N content (0.20%) was recorded under 40% FC and B + T + 40% FC, followed by T + 40% FC (0.19%), while the lowest (0.14%) was observed under 100% FC. The highest Available P (1.85 mg kg^{-1}) was recorded under B + T + 40% FC, followed by 40% FC (1.80 mg kg^{-1}), while the lowest (1.35 mg kg^{-1}) was observed under 100% FC (Table 11). The highest potassium content (9.05 cmol kg^{-1}) was recorded under B + T + 60% FC, followed by B + T + 40% FC (9.00 cmol kg^{-1}), while the lowest (8.30 cmol kg^{-1}) was observed under 100% FC. Similarly, the highest Ca content (2.70 cmol kg^{-1}) was recorded under 40% FC and the lowest (2.10 cmol kg^{-1}) under 100% FC. The highest Mg content (1.45 cmol kg^{-1}) was recorded under 40% FC and B + T + 40% FC, while the lowest value (1.10 cmol kg^{-1}) was observed under 100% FC. In contrast, Na content showed an increasing trend with increasing watering levels in the absence of organic amendments, reaching its highest value (0.45 cmol kg^{-1}) under 100% FC, whereas the lowest value (0.22 cmol kg^{-1}) was recorded under biochar application (Table 11).

Table 10 Soil chemical properties at termination of experiment

Treatments	pH (1:2 H_2O)	EC (µS cm^{-1})	CEC (cmol kg^{-1})	Organic matter (%)	Organic carbon (%)
Biochar	7.42^{a}	420^{ab}	13.85^{a}	14.10^{a}	3.05^{a}
Trichoderma	7.48^{a}	360^{bc}	13.70^{ab}	13.85^{a}	2.85^{ab}
40% FC	7.60^{a}	390^{bc}	13.45^{bc}	13.20^{b}	2.65^{bc}
60% FC	7.72^{a}	410^{ab}	13.30^{c}	13.10^{b}	2.60^{bc}
100% FC	7.78^{a}	300^{c}	13.55^{bc}	13.05^{b}	2.55^{c}
B + 40% FC	7.65^{a}	430^{ab}	13.60^{b}	13.75^{a}	2.90^{ab}
B + 60% FC	7.70^{a}	460^{a}	13.55^{bc}	13.95^{a}	3.00^{a}
B + 100% FC	7.75^{a}	440^{ab}	13.65^{ab}	14.20^{a}	3.10^{a}
T + 40% FC	7.68^{a}	420^{ab}	13.50^{bc}	13.60^{ab}	2.80^{ab}
T + 60% FC	7.72^{a}	440^{ab}	13.45^{bc}	13.70^{a}	2.85^{ab}
T + 100% FC	7.76^{a}	410^{ab}	13.50^{bc}	13.80^{a}	2.90^{ab}
B + T + 40% FC	7.70^{a}	450^{a}	13.55^{bc}	13.90^{a}	2.95^{a}
B + T + 60% FC	7.74^{a}	470^{a}	13.50^{bc}	14.05^{a}	3.00^{a}
B + T + 100% FC	7.78^{a}	460^{a}	13.60^{b}	14.25^{a}	3.12^{a}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*; EC = Electrical conductivity; CEC = Cation exchange capacity

Table 11 Soil chemical properties at termination of experiment

Treatments	Total N (%)	P (mg kg^{-1})	K (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	Na (cmol kg^{-1})
Biochar	0.16^{ab}	1.55^{ab}	8.70^{a}	2.30^{ab}	1.35^{a}	0.22^{c}
Trichoderma	0.17^{a}	1.60^{a}	8.60^{a}	2.20^{ab}	1.32^{a}	0.23^{c}
40% FC	0.20^{a}	1.80^{a}	8.40^{ab}	2.70^{a}	1.45^{a}	0.25^{bc}
60% FC	0.15^{b}	1.45^{ab}	8.55^{a}	2.40^{ab}	1.20^{ab}	0.30^{ab}
100% FC	0.14^{b}	1.35^{b}	8.30^{b}	2.10^{b}	1.10^{b}	0.45^{a}
B + 40% FC	0.18^{a}	1.70^{a}	8.85^{a}	2.50^{a}	1.40^{a}	0.24^{bc}
B + 60% FC	0.17^{a}	1.65^{a}	8.90^{a}	2.45^{a}	1.38^{a}	0.26^{bc}
B + 100% FC	0.16^{ab}	1.60^{a}	8.80^{a}	2.35^{ab}	1.30^{a}	0.32^{ab}
T + 40% FC	0.19^{a}	1.75^{a}	8.70^{a}	2.45^{a}	1.35^{a}	0.25^{bc}
T + 60% FC	0.17^{a}	1.65^{a}	8.75^{a}	2.40^{ab}	1.30^{a}	0.28^{ab}
T + 100% FC	0.16^{ab}	1.55^{ab}	8.65^{a}	2.30^{ab}	1.25^{ab}	0.34^{ab}
B + T + 40% FC	0.20^{a}	1.85^{a}	9.00^{a}	2.60^{a}	1.45^{a}	0.24^{bc}
B + T + 60% FC	0.18^{a}	1.75^{a}	9.05^{a}	2.55^{a}	1.42^{a}	0.27^{bc}
B + T + 100% FC	0.17^{a}	1.70^{a}	8.95^{a}	2.50^{a}	1.38^{a}	0.30^{ab}

FC = Field capacity; B = Biochar; T = *Trichoderma harzianum*

Discussion

The produced biochar exhibits physicochemical properties that support its suitability as a soil amendment for improving soil quality and mitigating drought stress. Its alkaline pH (9.50) indicates a strong potential to ameliorate soil acidity and enhance nutrient availability. Additionally, the relatively high cation exchange capacity (35.54 cmol kg^{-1}) reflects an improved capacity for nutrient retention and soil moisture conservation under water-limited conditions. Our findings are supported by Mao et al. (2024); Kumar et al. (2025). The carbon content (33.18 g kg^{-1}) and high C:N ratio (150:1) indicate a stable, recalcitrant carbon matrix capable of improving soil structure and water-holding capacity, thereby supporting plant performance under drought stress, in accordance with the earlier report of Jeffery et al. (2017). Recent studies further confirm the role of biochar in enhancing soil water dynamics and carbon stability (Acharya et al., 2024; Ernest et al., 2024). Furthermore, the presence of essential nutrients, particularly the relatively high potassium content (2.597 g kg^{-1}), may enhance plant drought tolerance through improved osmotic regulation and physiological functioning, consistent with earlier and recent reports (Agegnehu et al., 2019; Hossain et al., 2020; Adekiya et al., 2024; Malik et al., 2024). Our findings highlight the potential of the biochar to enhance soil fertility, improve moisture retention, and increase crop resilience under drought conditions, in accordance with both earlier and recent studies (Kumar et al., 2024; Su et al., 2024).

According to a previous research study, the use of biochar and *Trichoderma harzianum* together had a major impact on pepper development under various watering regimes. While *Trichoderma* encourages plant growth through nutrient solubilization, hormone production, and improved root development (Yao et al., 2023), biochar's synergistic effects on soil water-holding capacity and nutrient retention are probably responsible for the increased growth under combined treatments (Zulfiqar et al., 2022; Sharma et al., 2025). Additionally, there was an improvement in the development of the leaves and stems, indicating greater photosynthetic capacity and structural strength that assist drought resilience. Improved soil exploration for water and nutrients, a crucial adaptation under low moisture, as indicated by the root growth seen in plants treated with biochar and *Trichoderma* (Khan et al., 2024). These findings support recent reports on biochar–microbial synergism in crop stress tolerance (Khan et al., 2024) and suggest that biochar and *Trichoderma harzianum*, particularly in combination, can reduce drought stress in pepper by improving soil physical characteristics, nutrient availability, and plant physiological responses.

Our findings show that biomass accumulation in the roots, leaves, and stems was greatly impacted by both amendments and a sufficient supply of moisture. This improvement could be explained by biochar's ability to improve soil structure, nutrient retention, and water-holding capacity, all of which support biomass buildup and plant growth. According to a number of studies, biochar enhances nutrient uptake and root development, which increases vegetative growth in vegetable crops under both ideal and water-limited circumstances (Lehmann et al., 2021). Compared to watering regime alone, *Trichoderma* inoculation increased plant biomass. According to Harman et al. (2021), *Trichoderma* species are well known for their ability to stimulate root growth, promote nutrient solubilization, and increase plant resistance to abiotic stresses like drought. The higher root and shoot biomass seen in this study was probably caused by these mechanisms. Plant growth was also significantly impacted by the availability of water. Fresh weights of leaves and stems were often higher in plants kept at 100% field capacity than in those kept at 40% or 60% field capacity. On the other hand, the lowest biomass values were primarily found at 40% field capacity, especially for stem fresh weight (14.03 g) and leaf fresh weight (7.92 g), suggesting that a severe water deficit severely limited biomass production. It is well established that water stress reduces plant growth and dry matter buildup by limiting cell expansion, photosynthesis, and nutrient uptake (Naik et al., 2022). The highest biomass values were often obtained from the combination treatments of biochar and *Trichoderma* under suitable moisture conditions. Significantly, high root fresh weight (14.31 g) and root dry weight (4.02 g) were produced when biochar and *Trichoderma* were combined at 100% field capacity, suggesting a strong synergistic effect between the amendments under ideal moisture conditions. Similarly, treatments like *Trichoderma* + 60% FC and biochar + *Trichoderma* + 60% FC produced high stem fresh weights (26.87 g and 26.97 g, respectively), indicating that by enhancing soil microbial activity and root function, these amendments may lessen moderate water stress. Additionally, prior research has shown that beneficial microbes and biochar work in concert to improve plant growth, nitrogen uptake, and stress tolerance (Dissanayake et al., 2022).

Our results indicate that the application of biochar and *Trichoderma harzianum* significantly improved the yield of pepper under different watering regimes. Water stress markedly reduced yield parameters, as plants grown at 40% field capacity recorded the lowest number of fruits per plant (18.50), fruit fresh weight (81.00 g), and fruit dry weight (16.99 g), while higher values were observed at 100% field capacity. This reduction in yield under moisture deficit conditions has been attributed to impaired photosynthesis, reduced nutrient uptake, and limited assimilate translocation to developing fruits (Barnabá et al., 2008; Hasanuzzaman et al., 2020). The sole effects of biochar and *Trichoderma* moderately enhanced pepper yield compared with the water-stressed treatments, with biochar producing 25.70 fruits per plant and 130.00 g fruit fresh weight, while *Trichoderma* produced 24.34 fruits per plant and 125.33 fresh weight fruits. Biochar is known to improve soil water retention, nutrient availability, and soil structure, thereby enhancing crop productivity (Sun & Lu, 2014; Jien et al., 2021). Similarly, *Trichoderma* species promote plant growth and yield by improving root development, nutrient uptake, and stress tolerance (Basirat et al., 2019; Li et al., 2022). Harman et al. (2021) reported that species of *Trichoderma* have a strong capacity to establish and proliferate along plant roots, thereby increasing the extent of root–soil contact and effectively enhancing the root surface area exposed to the surrounding

soil. The co-application of biochar and *Trichoderma* produced the highest yield responses, particularly under adequate and moderate moisture conditions. The treatment B + T + 100% FC recorded the highest number of fruits (36.93 fruits/plant), fruit fresh weight (150.00 g), and fruit dry weight (25.00 g), followed by B + T + 60% FC. This synergistic effect may be attributed to improved soil physicochemical properties and enhanced microbial activity in the rhizosphere, which collectively promote plant growth and fruit production. According Alkharabsheh et al. (2021), biochar serves as an effective soil amendment capable of enhancing soil fertility, improving its physical properties, and promoting beneficial interactions with the soil microbial community. Its porous structure and high specific surface area provide favorable sites for plant growth, nutrient retention and acquisition, and the establishment of beneficial fungi. In particular, the extensive surface area of biochar can facilitate fungal hyphal proliferation and elongation, thereby supporting root colonization and overall plant development (Hussain et al., 2021).

The proximate and mineral composition of pepper fruits was significantly influenced by the co-application of biochar and *Trichoderma harzianum* under varying moisture regimes. Moisture content varied slightly among treatments (7.11–7.16%), indicating that the amendments had minimal effect on fruit moisture. However, notable variations were observed in other nutritional parameters. Ash, crude fibre, crude protein, carbohydrate, and vitamin contents were generally enhanced under treatments involving biochar and adequate moisture levels. For instance, the combination of biochar with 100% field capacity (B+100% FC) produced higher ash (5.88%) and crude protein (2.43%) contents compared with most other treatments, suggesting improved mineral accumulation and nutrient assimilation. Biochar is known to improve soil nutrient availability and retention, which can enhance the nutritional quality of crops (Lehmann & Joseph, 2015). Water deficit treatments (40% and 60% FC) showed relatively higher crude fibre and vitamin contents, which may reflect physiological adjustments of plants under stress conditions. Similar increases in certain nutritional compounds under moderate stress have been reported as part of plant adaptive responses (Hasanuzzaman et al., 2020). Treatments involving *Trichoderma* alone or in combination also influenced fruit composition, although values for crude protein and ash were comparatively lower in some cases. Nevertheless, *Trichoderma* is widely reported to enhance plant metabolism and nutrient uptake, thereby influencing crop nutritional quality (Harman et al., 2021; Poveda, 2021; Jiménez Bremont et al., 2026). The results indicate that the integration of biochar and *Trichoderma harzianum*, particularly under optimal moisture conditions, can improve the proximate and nutritional quality of pepper fruits, while water stress may induce variations in certain biochemical constituents.

Co-application of biochar and *Trichoderma harzianum* significantly influenced the mineral composition of pepper fruits under different soil moisture regimes. Overall, treatments combining biochar and *Trichoderma* under higher field capacities (60% and 100% FC) generally produced the highest concentrations of Na, Ca, Mg, Cl, N, P, and K. For example, the B+T+100% FC treatment consistently recorded the highest values for Na (0.64 mg/100 g), Ca (15.64 mg/100 g), Mg (39.87 mg/100 g), Cl (0.41 mg/100 g), N (0.39 mg/100 g), P (21.24 mg/100 g), and K (224.19 mg/100 g). Individually, biochar or *Trichoderma* alone also enhanced mineral content relative to the control at moderate field capacities, but the magnitude was lower compared to combined applications. Notably, *Trichoderma* applied at 40% FC resulted in the lowest mineral concentrations, suggesting that microbial inoculation alone may be insufficient under water-limited conditions. Conversely, biochar appears to buffer nutrient availability, particularly when combined with adequate moisture, likely due to its high cation exchange capacity and improvement of soil physicochemical properties (Lehmann & Joseph, 2015). These results indicate synergistic interactions between biochar, *Trichoderma*, and optimal soil moisture in enhancing pepper fruit mineral nutrition. Such improvements in nutrient content could contribute to better fruit quality and yield under both controlled and field conditions, aligning with recent reports on biochar–microbe interactions improving crop nutrient uptake (Ighalo et al., 2025).

Total chlorophyll content of pepper was influenced by biochar and *Trichoderma harzianum* under different watering regimes. Lower chlorophyll values were observed under water stress, particularly at reduced field capacity, indicating that moisture deficit negatively affects chlorophyll synthesis and photosynthetic activity. Blum (2017) inferred that a decline in the production of photosynthetic assimilates can hinder osmotic adjustment and reduce the ability of plants to withstand drought stress. The co-application of biochar or *Trichoderma* improved chlorophyll content, likely due to enhanced soil moisture retention and improved nutrient uptake. It has been reported Biochar (BC) application can enhance plant chlorophyll content and growth rate by indirectly improving soil physicochemical properties, such as water availability, while also promoting the activity of native soil microbial communities (Aubertin et al., 2021; Batool et al., 2022). Similarly, Soliman et al. (2020) reported that the application of *T. harzianum* to *Cucurbita pepo* significantly increased photosynthetic pigments and metabolite accumulation under salinity stress. Highest chlorophyll contents recorded suggest the synergistic effect of watering and biochar on pepper physiological functions and mitigation of soil moisture deficit. Similar improvements in chlorophyll content and overall plant physiological performance under the combined application of biochar and *Trichoderma* have been widely reported (Danish & Zafar-ul-Hye, 2020; Jabborova et al., 2022; Amanullah & Khan, 2023).

Elevated proline and phenolic contents under drought stress are consistent with well-established osmotic adjustment and reactive oxygen species (ROS) scavenging mechanisms in plants. Proline accumulation plays a central role in maintaining cellular osmotic balance, stabilizing proteins and membranes, and protecting metabolic processes under water deficit conditions. Many experimental studies in *Capsicum frutescens* and other crops have confirmed that drought stress significantly increases proline levels as part of adaptive osmotic

regulation while simultaneously enhancing antioxidant defense responses (Szabados & Savouré, 2010; Sharma et al., 2012). Similarly, phenolic compounds function as key non-enzymatic antioxidants involved in ROS detoxification and protection of cellular structures during abiotic stress. Their accumulation under drought conditions has been widely associated with improved oxidative stress tolerance and membrane stability, further reinforcing plant defense capacity under water limitation (Dixon & Paiva, 1995; Sharma et al., 2012). Biochar and *Trichoderma* co-application mitigated drought-induced stress responses relative to the untreated 40% field capacity (FC) treatment, indicating improved physiological resilience and enhanced ROS detoxification. This response is likely attributed to improved soil physical properties, increased water retention capacity, and microbially mediated regulation of plant metabolic activity. Synergistic effects of biochar and *Trichoderma* on antioxidant defense systems have been widely reported, with combined applications enhancing the activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), while reducing oxidative stress markers and increasing phenolic accumulation compared with single amendments (Zhao et al., 2026). In addition, biochar alone has been shown to modulate plant stress physiology by improving soil moisture availability and nutrient retention, thereby reducing the metabolic demand for excessive osmolyte synthesis under drought stress (Wu et al., 2023). Biochar also enhances rhizosphere microbial activity and nutrient cycling, which indirectly supports antioxidant defense mechanisms and overall plant stress adaptation (Ighalo et al., 2025). Collectively, the integrated application of biochar and *Trichoderma* enhances drought tolerance in pepper by promoting osmotic adjustment, improving nutrient uptake efficiency, and strengthening ROS scavenging capacity. These findings align with recent reports demonstrating that combined soil amendment strategies significantly improve physiological performance and stress tolerance in horticultural crops under water-limited conditions (Ighalo et al., 2025).

Our findings show that biochar and *Trichoderma harzianum* significantly influenced soil physical properties, particularly bulk density, porosity, water-holding capacity (WHC), and moisture content. Soils amended with biochar or *Trichoderma* generally exhibited lower bulk density and higher porosity compared with drought-stressed soils, indicating improved soil structure and aeration. Water-holding capacity was also enhanced by biochar and microbial inoculation, suggesting greater soil water retention, which is critical for mitigating drought stress in crops (Lehmann & Joseph, 2015). While moisture content varied across treatments, biochar- and *Trichoderma*-amended soils maintained relatively higher moisture levels, consistent with their known ability to improve soil water retention (Bui et al., 2024; Khan et al., 2024). The textural analysis showed that all soils remained sandy loam, indicating that amendment applications did not alter soil texture but improved its functional physical properties. Treatment effects on soil chemical properties showed that application of biochar and *Trichoderma* slightly increased soil and cation exchange capacity, enhancing the soil's ability to retain essential nutrients. Organic matter and organic carbon levels were slightly higher in amended soils, reflecting the contribution of biochar as a stable carbon source (Hossain et al., 2020). Macronutrient analysis showed moderate improvements in total nitrogen, phosphorus, potassium, calcium, and magnesium in treated soils, particularly in biochar- or *Trichoderma*-amended soils, while sodium remained low. These results suggest that amendments improved nutrient availability without causing salinity issues. The enhanced CEC and nutrient retention are consistent with previous reports that biochar and beneficial microbes improve soil fertility and facilitate sustainable crop production under water-limited conditions (Bui et al., 2024). Conclusively, this study indicates that biochar and *Trichoderma harzianum* improve soil physical and chemical properties, enhancing water-holding capacity, nutrient retention, and soil structural quality, thereby providing a more favorable environment for plant growth under drought stress.

Conclusion

This study demonstrated that the application of biochar and *Trichoderma harzianum* significantly improved the growth, biomass production, and yield of *Capsicum chinense* under different watering regimes. The combined application of biochar and *Trichoderma* produced the best overall performance, particularly under 100% and 60% field capacity. Plants subjected to severe water deficit (40% field capacity) showed reduced growth and productivity; however, the presence of biochar and *Trichoderma* mitigated some of the negative effects of water stress. The improved performance of pepper plants under combined treatments may be attributed to enhanced soil physicochemical properties, improved water retention, better nutrient availability, and the plant growth-promoting effects of *Trichoderma*. These results highlight the potential of integrating biochar and beneficial microbial inoculants as a sustainable strategy for improving pepper production under water-limited conditions.

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Declarations

i. Ethics approval and consent to participate: This study did not involve human participants, human data, or animal studies; therefore, ethical approval was not required.

ii. Competing interests: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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