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Role of biodegradable substrates in improving productivity and quality of Sweet Pepper (Capsicum annuum L.)

Muhammad Usman Tariq
University of the Punjab
Mubeen Sarwar
mubeensarwar4@yahoo.com

University of the Punjab
Sumreen Anjum
University of the Punjab
Adnan Mukhtar
University of Agriculture Faisalabad
M Irfan Ashraf
University of Agriculture Faisalabad
Tanveer Hussain
Pir Mehr Ali Shah Arid Agriculture University

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Abstract

Sweet pepper (*Capsicum annuum* L.) is a nutritionally and economically important crop; however, identifying sustainable and efficient substrate options for its cultivation remains a major challenge in horticultural production. This study evaluated biodegradable substrates silt, perlite, cocopeat, farmyard manure (FYM), compost, and vermicompost, applied individually or in mixtures under controlled pot culture, with farm soil as the control. Nine treatments (T_0–T_8) were arranged in a completely randomized design. Morphological traits (leaf area, stem length, root biomass), yield attributes (fruit number, fruit weight, total yield), and physiological indicators (chlorophyll content, relative water content, electrolyte leakage, proline accumulation, and antioxidant enzyme activities) were assessed. The silt + FYM (1:1) treatment significantly increased leaf area (189.5 cm²), root fresh weight (29.99 g), average fruit weight (109 g), and total yield (1300.75 g), representing increases of 29%, 47%, 28%, and 156% over the control, respectively. Silt + compost (1:1) achieved the highest relative water content (53%), while mixed substrates enhanced chlorophyll and proline accumulation, indicating improved physiological performance and stress tolerance. Overall, integrating organic substrates such as FYM or compost with silt enhanced substrate functionality, plant resilience, and sweet pepper productivity, offering practical insights for sustainable horticultural systems.

1. Introduction

Sweet pepper (*Capsicum annuum* L.) is a widely cultivated vegetable crop of considerable nutritional and economic importance. Its non-pungent fruits are rich in vitamins A, B, and C, as well as essential minerals such as calcium, potassium, and iron, making it a valuable component of human diets and a source of bioactive compounds with recognized health benefits (Aboyeji et al., 2021; Brezeanu et al., 2022). Despite its significance, global sweet pepper production is increasingly constrained by declining soil fertility, salinity, and water scarcity, which collectively reduce crop productivity and fruit quality (Homma et al., 2022; Abdelaal et al., 2019). Although chemical fertilizers can enhance yields, their continuous use has been associated with soil degradation, nutrient imbalances, and environmental pollution (Nisar et al., 2021). Organic amendments such as compost and farmyard manure (FYM) improve soil structure and microbial activity, yet when applied alone they often fail to meet the complete nutrient requirements of high-value crops, resulting in inconsistent performance (Jindo et al., 2016; Matisic et al., 2024).

To address these limitations, soilless and substrate-based cultivation systems have emerged as sustainable alternatives, offering improved water and nutrient use efficiency in protected horticulture. Substrates such as perlite, cocopeat, compost, and vermicompost have demonstrated benefits in aeration, water retention, and root development (Wan Fazilah & Ahmad, 2017; Mejía et al., 2022). Recent studies highlight that integrating fruit-waste compost with perlite increased pepper yield by more than 30% compared with cocopeat alone (Kartal et al., 2025), while date-palm waste substrates performed comparably to rockwool, reducing unmarketable fruits by 15% (Qaryouti et al., 2024). Furthermore, drought resilience assessments indicate that organic and hybrid sweet pepper cultivars exhibit similar

responses under water-limited conditions, reinforcing the potential of sustainable production approaches (Ficiciyan et al., 2021).

Despite these advances, most research has focused on substrates applied individually, with limited exploration of the synergistic effects of combining multiple biodegradable media. Integrated substrate systems may enhance nutrient buffering, root-zone microbiology, plant vigor, and stress tolerance, thereby supporting sustainable substrate management in horticultural production. In this context, optimizing substrate combinations is critical for resilient and climate-smart agriculture. Therefore, this study investigates the effects of various substrate mixtures, including silt combined with perlite, cocopeat, FYM, compost, and vermicompost, on the morphological, physiological, and yield-related traits of sweet pepper under controlled conditions, aiming to provide practical insights for sustainable horticultural practices.

2. Materials and Methods

2.1. Experimental Site and Design

A pot experiment was conducted at the Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan, to assess the effects of biodegradable growing substrates on the growth and productivity of sweet pepper (*Capsicum annuum* L.). The experiment was carried out under ambient environmental conditions in a net house at the Field Laboratory of Plant Stress Management. A completely randomized design (CRD) was employed, comprising nine substrate treatments with three replicates each, resulting in a total of 27 experimental pots.

2.2. Substrate Treatments and Pot Preparation

The substrate treatments were as follows: T_0, farm soil (control); T_1, silt; T_2, silt + perlite (1:1, v/v); T_3, silt + cocopeat (1:1, v/v); T_4, silt + farmyard manure (FYM) (1:1, v/v); T_5, silt + compost (1:1, v/v); T_6, silt + vermicompost (1:1, v/v); T_7, silt + FYM + compost (1:1:1, v/v); and T_8, silt + perlite + cocopeat (1:1:1, v/v). Farm soil used as the control substrate was collected from the departmental research field, air-dried, and sieved to remove stones and plant debris. The soil was classified as sandy clay loam with a pH of 6.2 and an electrical conductivity (EC) of 2.0 dS m^{-1}. All substrate components were thoroughly mixed manually in the specified volumetric ratios and filled into plastic pots (30 cm diameter × 25 cm height). The prepared pots were placed in the net house and allowed to equilibrate for seven days prior to transplanting.

2.3. Seedling Raising and Transplanting

Sweet pepper (*Capsicum annuum* L.) cultivar KS-2201 was used in this study. Seeds were obtained from Krishibid Upokoron Nursery and treated with Vitavex-200 at 5 g kg^{-1} seed prior to sowing to prevent seed-borne diseases. Seedlings were raised in a 1 m × 1 m nursery bed amended with well-decomposed cow dung at 10 t ha^{-1}. For insect pest management in the nursery, Sevin 50 WP was applied at a rate of 5 kg ha^{-1}. Seeds were sown at a spacing of 5 cm and a depth of approximately 2 cm, lightly irrigated, and

covered with polyethylene sheets during germination to maintain moisture and temperature. No synthetic fertilizers were applied during the nursery stage.

Uniform and healthy seedlings, 25 days old and bearing 5–6 true leaves, were transplanted in the afternoon to minimize transplanting stress. Two seedlings were initially transplanted into each pot and irrigated immediately after transplanting. The pots were covered with transparent polyethylene sheets to provide partial shading for 5–7 days to facilitate establishment. After acclimatization, one vigorous seedling was retained per pot by thinning.

2.4. Fertilizer Application and Crop Management

Fertilizer application was carried out according to recommended nutrient requirements for sweet pepper (Kartal et al., 2014). Each pot received 9 g urea, 12 g triple superphosphate (TSP), and 9 g muriate of potash (MOP). One-third of the urea along with the full doses of TSP and MOP were incorporated into the substrates prior to transplanting, while the remaining urea was applied as side dressing at 30 and 60 days after transplanting, following the method described by Anas et al. (2020). Irrigation was applied as required to maintain optimal moisture conditions, and weeds were controlled manually. No significant incidence of insect pests or diseases was observed during the experimental period.

2.5. Growth and Yield Measurements

Growth and yield parameters were recorded at physiological maturity unless otherwise stated. Plant height (cm) was measured from the substrate surface to the tip of the main stem using a graduated ruler. Stem diameter (mm) was measured at 5 cm above the substrate surface using a digital vernier caliper. The number of leaves per plant was counted manually. At final harvest, plants were carefully uprooted, and roots were gently washed under running water to remove adhering substrate particles. Root length (cm) was measured from the crown to the tip of the longest root using a ruler. Root fresh weight (g) was determined immediately after washing and blotting the roots dry using an electronic balance. Root dry weight (g) was recorded after oven-drying the root samples at 70°C until a constant weight was achieved.

Fruit yield attributes were recorded throughout the harvesting period. The total number of fruits per plant was counted, individual fruit weight (g) was measured using an electronic balance, and total yield per plant (g) was calculated as the cumulative weight of all harvested fruits.

2.6. Physiological and Biochemical Measurements

Chlorophyll content was measured on fully expanded, healthy leaves using a SPAD-502 chlorophyll meter (Konica Minolta, Japan), and readings were averaged per plant. Leaf relative water content (LRWC) was determined following standard procedures using fresh leaf samples. Fresh weight (FW) was recorded immediately after sampling, after which leaves were immersed in distilled water for 4 h under dark conditions to obtain turgid weight (TW). Samples were then oven-dried at 70°C to a constant weight to obtain dry weight (DW). LRWC was calculated using Eq. 1:

$$\text{Electrolyte leakage (\%)} = \frac{C_1}{C_2} \times 100 \quad (2)$$

Electrolyte leakage (%) was assessed using the method of membrane stability analysis. Leaf discs were rinsed with deionized water and incubated in test tubes containing deionized water at room temperature for 24 h, after which initial electrical conductivity (C_1) was measured using a conductivity meter. The samples were then autoclaved to release total electrolytes, cooled to room temperature, and final conductivity (C_2) was recorded. Electrolyte leakage was calculated by using Eq. 2:

$$\text{Electrolyte leakage (\%)} = \frac{C_1}{C_2} \times 100 \quad (2)$$

Proline content was quantified following the method of Bates et al. (1973). Fresh leaf tissue was homogenized in sulfosalicylic acid, reacted with acid ninhydrin reagent, and the chromophore was extracted with toluene. Absorbance was measured at 520 nm using a UV–Vis spectrophotometer, and proline concentration was calculated using a standard curve.

Antioxidant enzyme activities were determined using fresh leaf extracts. Fully expanded leaves were collected from each treatment and immediately processed to minimize enzyme degradation. Approximately 0.5–1.0 g of leaf tissue was homogenized in 5 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 2% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 × *g* for 15 min at 4°C, and the resulting supernatant was used as the crude enzyme extract. Enzyme activities were expressed units per gram of fresh leaf tissue (U g^{-1} FW).

Superoxide dismutase (SOD) activity was assayed based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following Giannopolitis and Ries (1977). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 mM riboflavin, 50 µM NBT, and 100 µL of enzyme extract in a final volume of 3 mL. The mixture was illuminated under fluorescent light for 15 min, and absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction per gram of fresh leaf tissue (U g^{-1} FW).

Peroxidase (POD) activity was determined by monitoring the oxidation of guaiacol (Chance & Maehly, 1955). The assay mixture comprised 50 mM potassium phosphate buffer (pH 6.0), 20 mM guaiacol, 40 mM H_2O_2, and 0.1 mL of enzyme extract in a total volume of 3 mL. The formation of tetraguaiacol was followed as an increase in absorbance at 470 nm over 1 min. POD activity was expressed as the change in absorbance per minute per gram of fresh leaf tissue (U g^{-1} FW).

Catalase (CAT) activity was measured by monitoring the decomposition of hydrogen peroxide at 240 nm (Aebi, 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2, initiated by the addition of 0.1 mL enzyme extract. The decrease in absorbance at 240 nm over 1 min was used to calculate CAT activity, expressed as µmol H_2O_2 decomposed per minute per gram of fresh leaf tissue (µmol min^{-1} g^{-1} FW).

2.7. Soil and Substrate Analysis

Substrate samples were collected after harvest for physicochemical analysis. Organic matter content was determined using the Walkley–Black method. Total nitrogen (N) was analyzed by the Kjeldahl digestion method, available phosphorus (P) was determined using the Olsen method, and available potassium (K) was measured by flame photometry following standard analytical procedures.

2.8. Harvesting

Fruits were harvested manually at marketable maturity, beginning 85 days after transplanting and continued at regular intervals until the final harvest. Harvested fruits were counted and weighed immediately to determine yield-related parameters.

2.9. Statistical Analysis

Data for morphological, physiological, and yield parameters were analyzed using analysis of variance (ANOVA) appropriate for a completely randomized design (CRD). Treatment means were compared using Tukey's honestly significant difference (HSD) test at a 5% probability level ($p < 0.05$) to determine statistically significant differences among treatments. All analyses were conducted using SPSS v. 27 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Growth and Morphological Traits

The composition of organic substrates exerted a pronounced influence on vegetative growth, root development, and yield attributes of sweet pepper. These responses reflect differences in nutrient availability, water-holding capacity, aeration, and organic matter dynamics among substrate formulations. Treatment-wise comparisons of individual traits are presented in Fig. 1A–I, while an integrated multivariate overview is illustrated in Fig. 1J and 1K.

Leaf Area

Leaf area differed significantly among treatments (Fig. 1A). Silt + FYM (T_4) produced the largest leaves (189.5 cm²), representing a 29% increase over the control (T_0, 147.25 cm²). Silt + compost (T_5; 178 cm²) and silt + vermicompost (T_6; 168.75 cm²) also markedly enhanced leaf expansion, whereas silt + perlite (T_2; 154.75 cm²) resulted in comparatively reduced leaf area. The superior leaf development under silt + FYM and silt + compost substrates can be attributed to higher organic matter content and nitrogen availability, which stimulate chlorophyll synthesis and lamina expansion. Enhanced leaf area increases photosynthetically active surface, thereby improving assimilate production, consistent with earlier reports in pepper and tomato under organic fertilization (Rajapaksha et al., 2024; Ralebhat et al., 2021; Bălăiţă et al., 2024; Jankauskienė et al., 2025).

Stem Length

Stem elongation was significantly influenced by substrate composition (Fig. 1B). The longest stems were recorded in ternary mixtures, particularly silt + perlite + cocopeat (T_8; 31.7 cm) and silt + compost + vermicompost (T_7; 30.55 cm), followed by silt + FYM (T_4; 26.55 cm). In contrast, silt + perlite (T_2; 18.78 cm) and the control (T_0; 22.2 cm) produced the shortest stems. Enhanced stem growth in ternary mixtures reflects improved substrate aeration and balanced physical structure, facilitating internodal elongation and vascular development. Comparable improvements in stem elongation under optimized substrate blends have been reported in greenhouse-grown peppers (Watabe et al., 2021; Bălăiţă et al., 2024).

Root Length, Fresh Weight, and Dry Weight

Root growth parameters responded strongly to organic substrate incorporation (Figs. 1C–E). Silt + FYM (T_4) consistently produced the most vigorous root system, with the greatest root length (15.73 cm), fresh weight (29.99 g), and dry weight (24.05 g), followed by silt + compost (T_5). Conversely, silt alone (T_1) and silt + perlite (T_2) resulted in weaker root systems. Enhanced root development under organic substrates is associated with improved porosity, microbial activity, and nutrient buffering capacity, which collectively enhance water and nutrient uptake efficiency. Vermicompost-based substrates have similarly been shown to stimulate rhizosphere microbial communities and root biomass in solanaceous crops (Rehman et al., 2023; Carricondo Martínez et al., 2022; Erdal & Aktaş, 2025).

Number of Leaves

Leaf production varied significantly among treatments (Fig. 1F). The highest number of leaves was observed in silt + FYM (T_4; 239 leaves), followed by silt + cocopeat (T_3; 210.75 leaves). Silt + perlite (T_2; 192 leaves) recorded the lowest leaf number. Increased leaf production under silt + FYM and silt + cocopeat substrates reflects enhanced nutrient availability and water retention, supporting sustained canopy development. In contrast, mineral substrates exhibited limited nutrient buffering capacity, restricting vegetative proliferation (Akande et al., 2020; Carricondo Martínez et al., 2022; Ralebhat et al., 2021).

Fruit Number, Fruit Weight, and Yield per Plant

Reproductive performance was markedly improved by organic substrate incorporation (Figs. 1G–I). Silt + FYM (T_4) produced the highest number of fruits (12 per plant), maximum mean fruit weight (109 g), and the greatest yield per plant (1300.75 g), corresponding to a 156% yield increase over the control (508.5 g). Silt + compost (T_5; 916 g) and silt + vermicompost (T_6; 935 g) also significantly enhanced yield, whereas silt + perlite (T_2) and ternary mixtures (T_7, T_8) resulted in comparatively lower yields (678–797 g). The superior yield performance of silt + FYM is closely linked to its higher organic matter content (2.48%; Fig. 4D), which improved nutrient retention, root development, and water availability. Enhanced vegetative vigor consequently promoted greater assimilate partitioning toward reproductive sinks,

consistent with observations in pepper and eggplant systems (Qaryouti et al., 2023; Kartal et al., 2025; Jankauskienė et al., 2025).

The radar diagrams (Fig. 1J–K) provide an integrated visualization of treatment performance across multiple growth and yield traits. This multivariate representation clearly illustrates the overall superiority of silt + FYM (T_4), which consistently ranked highest across most parameters, followed by silt + compost and silt + vermicompost substrates. While ternary mixtures improved specific traits such as stem length, their overall performance was less balanced compared with FYM-based substrates. The present results strongly support the use of organic substrates as sustainable and productive alternatives to inert media in sweet pepper cultivation, corroborating recent findings in protected horticultural systems (Rajapaksha et al., 2024; Watabe et al., 2021; Ralebhat et al., 2021; Bălăiţă et al., 2024; Jankauskienė et al., 2025).

3.2. Physiological Traits

Leaf Relative Water Content (LRWC)

Leaf relative water content differed significantly among treatments (Fig. 2A). The highest LRWC was recorded in silt + compost (T_5; 53%), followed by silt + FYM (T_4; 49%) and silt + perlite + cocopeat (T_8; 47%). Intermediate values were observed in silt + cocopeat (T_3; 43%), silt + vermicompost (T_6; 42%), and silt + FYM + compost (T_7; 43%). The control (T_0; 40%) and silt alone (T_1; 38%) exhibited comparatively lower LRWC, while silt + perlite (T_2; 35%) recorded the minimum value.

Higher LRWC in organic-amended substrates reflects improved water-holding capacity, osmotic adjustment, and maintenance of cell turgor, which collectively mitigate drought stress and sustain photosynthetic activity. The superior performance of silt + compost and silt + FYM can be attributed to their higher organic matter content, which enhances substrate porosity, water retention, and nutrient buffering. These findings are consistent with earlier reports in pepper and cucumber, where organic amendments improved leaf hydration status and stress tolerance (Ntanasi et al., 2025; Ficiciyan et al., 2021; Carricondo Martínez et al., 2022).

Electrolyte Leakage (EL)

Electrolyte leakage varied significantly among treatments (Fig. 2B). The highest leakage was observed in silt alone (T_1; 32%), representing a ~ 33% increase compared with the control (T_0; 24%). Elevated leakage was also recorded in silt + cocopeat (T_3; 28%) and silt + perlite (T_2; 25%). In contrast, the lowest leakage occurred in silt + FYM (T_4; 9%), followed by silt + compost (T_5; 22%), silt + vermicompost (T_6; 23%), silt + FYM + compost (T_7; 22%), and silt + perlite + cocopeat (T_8; 25%).

Lower electrolyte leakage in organic-amended substrates indicates enhanced membrane stability and reduced oxidative damage, reflecting improved water retention, nutrient buffering, and antioxidant activity. The superior performance of silt + FYM is consistent with its higher organic matter content, which supports cell membrane integrity under stress conditions. Conversely, the high leakage in silt

alone highlights the vulnerability of mineral substrates, where limited organic matter and poor water-holding capacity exacerbate membrane injury.

These results align with previous findings in solanaceous crops, where organic amendments reduced electrolyte leakage and improved stress tolerance by stabilizing cell membranes and enhancing antioxidant enzyme activity (Ntanasi et al., 2025; Ficiciyan et al., 2021; Erdal & Aktaş, 2025). The contrasting responses between mineral and organic substrates underscore the importance of organic matter in mitigating abiotic stress and maintaining physiological resilience in sweet pepper.

Chlorophyll Content

Total chlorophyll content varied significantly among treatments (Fig. 2C). The highest chlorophyll content was recorded in silt + FYM (T_4; 30.3 SPAD units), which was markedly greater than all other treatments and the control. Intermediate values were observed in silt + cocopeat (T_3; 26.6), silt + compost (T_5; 24.1), silt + vermicompost (T_6; 21.0), silt + FYM + compost (T_7; 20.3), and silt + perlite + cocopeat (T_8; 20.5). The control (T_0; 18.5) and silt + perlite (T_2; 21.9) exhibited comparatively lower chlorophyll levels.

The superior chlorophyll accumulation in silt + FYM reflects enhanced nitrogen availability and organic matter content, which promote chlorophyll biosynthesis and sustain photosynthetic activity. Organic substrates improve nutrient buffering and water retention, thereby supporting pigment stability and leaf greenness. Conversely, the reduced chlorophyll content in mineral substrates such as silt alone or silt + perlite highlights their limited nutrient supply and poor water-holding capacity, which restrict photosynthetic efficiency.

These findings are consistent with earlier studies in pepper and tomato, where organic amendments significantly increased chlorophyll content and photosynthetic performance under greenhouse conditions (Rajapaksha et al., 2024; Ficiciyan et al., 2021; Ralebhat et al., 2021; Bălăiţă et al., 2024). Enhanced chlorophyll levels under FYM-based substrates further corroborate the role of organic matter in improving physiological resilience and productivity in solanaceous crops.

Proline Content

Proline content varied significantly among treatments (Fig. 2D). The highest accumulation was observed in silt + FYM (T_4; 48.2 µmol g^{-1} FW), representing a ~ 93% increase compared with the control (T_0; 25 µmol g^{-1} FW). Elevated proline levels were also recorded in silt + perlite + cocopeat (T_8; 44 µmol g^{-1} FW) and silt + compost + FYM (T_7; 38 µmol g^{-1} FW). Intermediate values were found in silt + compost (T_5; 33 µmol g^{-1} FW) and silt + cocopeat (T_3; 31 µmol g^{-1} FW). Lower proline accumulation was observed in silt + vermicompost (T_6; 25 µmol g^{-1} FW) and silt + perlite (T_2; 26 µmol g^{-1} FW), while the control (T_0) recorded the minimum value.

The pronounced increase in proline under silt + FYM reflects enhanced osmotic adjustment and stress tolerance, as proline functions as a compatible solute that stabilizes proteins, membranes, and cellular

redox balance. Elevated proline levels in organic-amended substrates indicate improved resilience to water deficit and oxidative stress, consistent with their superior water-holding capacity and nutrient buffering. Conversely, the low proline accumulation in mineral substrates such as silt alone or silt + perlite suggests limited osmotic regulation and reduced stress adaptation capacity.

These findings corroborate earlier reports in solanaceous crops, where organic amendments promoted proline accumulation and improved tolerance to abiotic stress conditions (Ntanasi et al., 2025; Ficiciyan et al., 2021; Erdal & Aktaş, 2025). The results highlight the role of FYM-based substrates in enhancing physiological resilience, complementing their positive effects on growth and yield traits.

The radar diagram (Fig. 2E) provides an integrated visualization of physiological responses across treatments. It clearly illustrates the superior performance of silt + FYM (T_4), which consistently ranked highest in chlorophyll content, proline accumulation, and reduced electrolyte leakage, while also maintaining high LRWC. Silt + compost (T_5) and silt + perlite + cocopeat (T_8) showed balanced improvements across multiple parameters, whereas silt alone (T_1) exhibited poor performance, with high electrolyte leakage and low LRWC, chlorophyll, and proline. This multivariate synthesis reinforces the conclusion that organic-amended substrates, particularly FYM-based combinations, provide holistic physiological resilience compared with mineral substrates, corroborating recent findings in solanaceous crops (Rajapaksha et al., 2024; Carricondo Martínez et al., 2022; Erdal & Aktaş, 2025).

3.3. Biochemical Traits

Superoxide Dismutase (SOD) Activity

SOD activity differed significantly among treatments (Fig. 3A). The highest activity was recorded in silt + FYM (T_4; 52.17 U g^{-1} FW), representing a ~ 49% increase compared with the control (T_0; 35 U g^{-1} FW). Elevated SOD levels were also observed in silt + compost (T_5; 47.6 U g^{-1} FW), followed by silt + vermicompost (T_6; 39.35 U g^{-1} FW), silt + FYM + compost (T_7; 38.79 U g^{-1} FW), and silt + perlite + cocopeat (T_8; 38.23 U g^{-1} FW). Intermediate activity was recorded in silt + cocopeat (T_3; 36 U g^{-1} FW), while lower values were found in silt alone (T_1; 30 U g^{-1} FW) and silt + perlite (T_2; 27 U g^{-1} FW), which showed the minimum SOD activity.

Enhanced SOD activity in organic-amended substrates reflects improved antioxidant defense mechanisms, which detoxify superoxide radicals and protect cellular components from oxidative damage. The superior performance of silt + FYM and silt + compost is attributed to their higher organic matter content and microbial activity, which stimulate enzymatic antioxidant responses under stress conditions. These findings are consistent with previous studies in solanaceous crops, where organic substrates significantly increased SOD activity and improved oxidative stress tolerance (Ficiciyan et al., 2021; Erdal & Aktaş, 2025; Jankauskienė et al., 2025). The comparatively low activity in mineral substrates underscores their limited capacity to support biochemical resilience, reinforcing the importance of organic amendments in enhancing physiological and biochemical performance in sweet pepper.

Peroxidase (POD) Activity

POD activity differed significantly among treatments (Fig. 3B). The highest activity was recorded in silt + cocopeat (T_3; 2.8 U g^{-1} FW), representing a ~ 27% increase compared with the control (T_0; 2.2 U g^{-1} FW). Elevated POD levels were also observed in silt + FYM (T_4; 2.25 U g^{-1} FW), silt + compost (T_5; 1.88 U g^{-1} FW), silt + vermicompost (T_6; 2.38 U g^{-1} FW), silt + FYM + compost (T_7; 1.90 U g^{-1} FW), and silt + perlite + cocopeat (T_8; 2.38 U g^{-1} FW). The lowest activity was found in silt alone (T_1; 1.7 U g^{-1} FW), while silt + perlite (T_2; 1.88 U g^{-1} FW) showed slightly higher values but remained among the weaker treatments.

Enhanced POD activity in organic-amended substrates, particularly silt + cocopeat, reflects improved hydrogen peroxide scavenging and lignification processes, which strengthen cell walls and enhance stress tolerance. The superior performance of cocopeat-based substrates can be attributed to their high water-holding capacity and aeration, which stimulate enzymatic activity and support oxidative stress regulation. Conversely, the low POD activity in silt alone highlights the limited biochemical resilience of mineral substrates, consistent with their poor nutrient buffering and water retention. These results corroborate earlier findings in solanaceous crops, where organic amendments significantly enhanced POD activity and improved tolerance to abiotic stress conditions (Rajapaksha et al., 2024; Ficiciyan et al., 2021; Erdal & Aktaş, 2025). The data emphasize the role of organic substrates in activating antioxidant defense pathways, complementing their positive effects on growth and physiological traits.

Catalase (CAT) Activity

CAT activity was significantly influenced by substrate composition (Fig. 3C). The highest activity was recorded in silt + cocopeat (T_3; 0.068 µmol min^{-1} g^{-1} FW), which was markedly greater than the control (T_0; 0.033 µmol min^{-1} g^{-1} FW) and all other treatments. Elevated CAT levels were also observed in silt + vermicompost (T_6; 0.060 µmol min^{-1} g^{-1} FW) and silt + FYM + compost (T_7; 0.053 µmol min^{-1} g^{-1} FW). Intermediate values were found in silt + perlite (T_2; 0.040 µmol min^{-1} g^{-1} FW) and silt + FYM (T_4; 0.040 µmol min^{-1} g^{-1} FW). The lowest activity was observed in silt + compost (T_5; 0.013 µmol min^{-1} g^{-1} FW) and silt + perlite + cocopeat (T_8; 0.015 µmol min^{-1} g^{-1} FW), indicating limited catalase induction under these treatments.

Enhanced CAT activity in cocopeat- and vermicompost-based substrates reflects improved hydrogen peroxide detoxification, which protects cells from oxidative stress and maintains redox homeostasis. The superior performance of silt + cocopeat can be attributed to its high water-holding capacity and aeration, which stimulate enzymatic antioxidant responses. Conversely, the low activity in silt + compost highlights the limited biochemical resilience of this substrate, despite its moderate nutrient contribution. These findings are consistent with earlier reports in solanaceous crops, where organic amendments enhanced CAT activity and improved tolerance to oxidative stress (Rajapaksha et al., 2024; Ficiciyan et al., 2021; Erdal & Aktaş, 2025). Together with SOD and POD responses, the results emphasize the role of organic substrates in activating antioxidant defense pathways, thereby complementing their positive effects on growth, physiology, and yield.

The radar diagram (Fig. 3D) provides an integrated visualization of antioxidant enzyme activities across treatments. It highlights the superior biochemical resilience of silt + FYM (T_4) and silt + cocopeat (T_3), which consistently exhibited high SOD, POD, and CAT activities. Silt + vermicompost (T_6) and silt + FYM + compost (T_7) also showed balanced enzyme induction, whereas mineral substrates such as silt alone (T_1) and silt + perlite (T_2) displayed weak antioxidant responses. This multivariate synthesis reinforces the conclusion that organic-amended substrates activate multiple antioxidant defense pathways simultaneously, thereby enhancing stress tolerance and complementing their positive effects on growth and physiological traits.

3.4. Soil Nutrient Content and Organic Matter

Nitrogen Content

Nitrogen content varied significantly among treatments (Fig. 4B). The highest value was recorded in silt + FYM (T_4; 2.3%), followed by silt + perlite + cocopeat (T_8; 2.1%) and silt + cocopeat (T_3; 1.97%). Intermediate levels were observed in silt + vermicompost (T_6; 1.90%), silt + FYM + compost (T_7; 1.88%), and silt + compost (T_5; 1.63%). Lower nitrogen content was found in silt + perlite (T_2; 1.60%) and silt alone (T_1; 0.29%), while the control soil (T_0; 0.10%) exhibited the minimum value.

The superior nitrogen accumulation in FYM-based substrates reflects enhanced mineralization and microbial activity, which increase nitrogen availability for plant uptake. Elevated nitrogen levels in cocopeat- and vermicompost-amended substrates further highlight the role of organic matter in sustaining nutrient cycling and chlorophyll biosynthesis. Conversely, the low nitrogen content in mineral substrates such as silt alone or silt + perlite underscores their limited capacity to support vegetative growth and photosynthetic efficiency.

These findings are consistent with earlier reports in solanaceous crops, where organic amendments significantly improved nitrogen availability and enhanced plant performance under greenhouse conditions (Rajapaksha et al., 2024; Ficiciyan et al., 2021lăiţă et al., 2024). The results emphasize the importance of organic substrates in maintaining soil fertility and ensuring sustainable nutrient supply.

Phosphorus Content

Phosphorus content varied significantly among treatments (Fig. 4C). The highest value was recorded in silt + FYM (T_4; 21.3 mg L^{-1}), followed closely by silt + FYM + compost (T_7; 20.4 mg L^{-1}) and silt + cocopeat (T_3; 17.22 mg L^{-1}). Intermediate levels were observed in silt + perlite + cocopeat (T_8; 14.5 mg L^{-1}), silt + vermicompost (T_6; 13.1 mg L^{-1}), and silt + perlite (T_2; 12.4 mg L^{-1}). Lower values were found in silt + compost (T_5; 10.13 mg L^{-1}) and silt alone (T_1; 3.04 mg L^{-1}), while the control soil (T_0; 2.7 mg L^{-1}) exhibited the minimum phosphorus content.

The superior phosphorus accumulation in FYM- and compost-based substrates reflects their ability to enhance phosphorus solubility, reduce fixation, and stimulate microbial-mediated release. Elevated phosphorus levels in cocopeat- and vermicompost-amended substrates further highlight the role of

organic matter in improving nutrient availability and root development. Conversely, the low phosphorus content in mineral substrates such as silt alone or silt + perlite underscores their limited capacity to sustain phosphorus supply under greenhouse conditions. These findings are consistent with earlier reports in solanaceous crops, where organic amendments significantly increased phosphorus availability and improved plant growth and yield (Rajapaksha et al., 2024; Carricondo Martínez et al., 2022lăiţă et al., 2024).

Potassium Content

Potassium content showed marked variation among treatments (Fig. 4D). The highest value was recorded in silt + perlite + cocopeat (T_8; 381 mg L^{-1}), followed by silt + FYM + compost (T_7; 359 mg L^{-1}). Elevated potassium levels were also observed in silt + FYM (T_4; 289 mg L^{-1}) and silt + cocopeat (T_3; 271 mg L^{-1}). Intermediate values were found in silt + compost (T_5; 217 mg L^{-1}), silt + vermicompost (T_6; 190 mg L^{-1}), and silt + perlite (T_2; 189 mg L^{-1}). Lower potassium content was observed in silt alone (T_1; 109 mg L^{-1}), while the control soil (T_0; 107 mg L^{-1}) exhibited the minimum value.

The superior potassium accumulation in perlite + cocopeat + silt and FYM + compost-based substrates reflects their ability to enhance mineralization, improve cation exchange capacity, and stimulate microbial activity, thereby increasing potassium availability. Adequate potassium supply is essential for osmotic regulation, enzyme activation, and stress tolerance, which complements the physiological and biochemical improvements observed in earlier sections. Conversely, the low potassium content in mineral substrates such as silt alone and the control highlights their limited capacity to sustain nutrient availability under greenhouse conditions. These findings are consistent with earlier reports in vegetable crops, where organic and mixed substrates significantly improved potassium availability and enhanced plant resilience (Rajapaksha et al., 2024; Ficiciyan et al., 2021lăiţă et al., 2024). The results emphasize the importance of substrate composition in maintaining potassium fertility, thereby supporting growth, yield, and stress tolerance in sweet pepper.

Organic Matter Content

Organic matter content varied significantly among treatments (Fig. 4A). The highest value was recorded in silt + FYM (T_4; 2.47%), followed by silt + perlite (T_2; 2.10%) and silt + compost + FYM (T_8; 1.90%). Intermediate levels were observed in silt + cocopeat (T_3; 1.39%), silt + compost (T_5; 1.20%), and silt + FYM + compost (T_7; 1.20%). Lower values were found in silt + vermicompost (T_6; 0.90%) and silt alone (T_1; 0.54%), while the control soil (T_0; 0.22%) exhibited the minimum organic matter content.

The superior organic matter accumulation in FYM- and compost-based substrates reflects their high humic content, which enhances soil fertility, cation exchange capacity, and water-holding ability. Organic matter also improves microbial activity and nutrient cycling, thereby supporting plant growth and resilience under greenhouse conditions. Conversely, the low organic matter in mineral substrates such as silt alone or silt + perlite underscores their limited capacity to sustain long-term fertility.

These findings are consistent with earlier reports, which demonstrated that organic amendments improve substrate quality, nutrient buffering, and sustainability in intensive horticultural systems (Khalaj et al., 2011; Alsanius et al., 2016; Carricondo-Martínez et al., 2022). The results highlight the critical role of organic matter as the foundation of soil fertility, directly influencing nitrogen, phosphorus, and potassium availability discussed in subsequent sections.

The radar diagram (Fig. 4E) provides an integrated visualization of substrate fertility parameters (N, P, K, and organic matter). It highlights the superior fertility of silt + FYM (T_4), which consistently ranked highest across most parameters, followed by silt + FYM + compost (T_7) and silt + perlite + cocopeat (T_8). In contrast, silt alone (T_1) and the control soil (T_0) exhibited poor fertility profiles, with minimal nutrient and organic matter content. This multivariate synthesis reinforces the conclusion that organic-amended substrates, particularly FYM-based combinations, provide balanced nutrient availability and long-term fertility, corroborating earlier findings in intensive horticultural systems (Khalaj et al., 2011; Carricondo Martínez et al., 2022).

4. Conclusion

This study demonstrates that substrate composition strongly influences sweet pepper performance, with organic amendments, particularly silt + FYM, silt + compost, and silt + cocopeat enhancing leaf water status, chlorophyll content, antioxidant enzyme activity, and soil fertility (N, P, K), thereby improving growth, stress tolerance, and yield. Among treatments, silt + FYM proved most effective, while mixed substrates such as silt + perlite + cocopeat and silt + FYM + compost offered synergistic benefits, especially for potassium dynamics. In contrast, mineral substrates showed poor physiological and biochemical responses, underscoring their limited capacity to sustain productivity. Overall, the findings highlight that integrating organic amendments into substrate management not only enriches soil fertility but also activates defense mechanisms, supporting resilient and sustainable sweet pepper cultivation in intensive horticultural systems.

Declarations

Funding

This research was conducted as part of the MSc degree requirements of the first author. No external funding was received for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

Ethics declaration: Not applicable. This study did not involve human participants or animals. The research was conducted on plant material (*Capsicum annuum* L.) under standard institutional and

agronomic practices.

Ethics and Consent to Participate

The plant material used in this study was commercially cultivated and obtained from authorized sources. The cultivation and experimental procedures complied with all relevant local and national agricultural guidelines and regulations. No wild plant collection was involved, and no specific permits or licenses were required for conducting this study.

Consent to Participate

Consent to Participate declaration: Not applicable.

Consent to Publish

Consent to Publish declaration: Not applicable.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

MUT, MS Conceptualization, methodology, experimentation, data collection, formal analysis, writing – original draft. SA, AD, MIA, and TH validation, review and editing, statistical analysis, review and editing. (All authors read and approved the final manuscript.)

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Figures

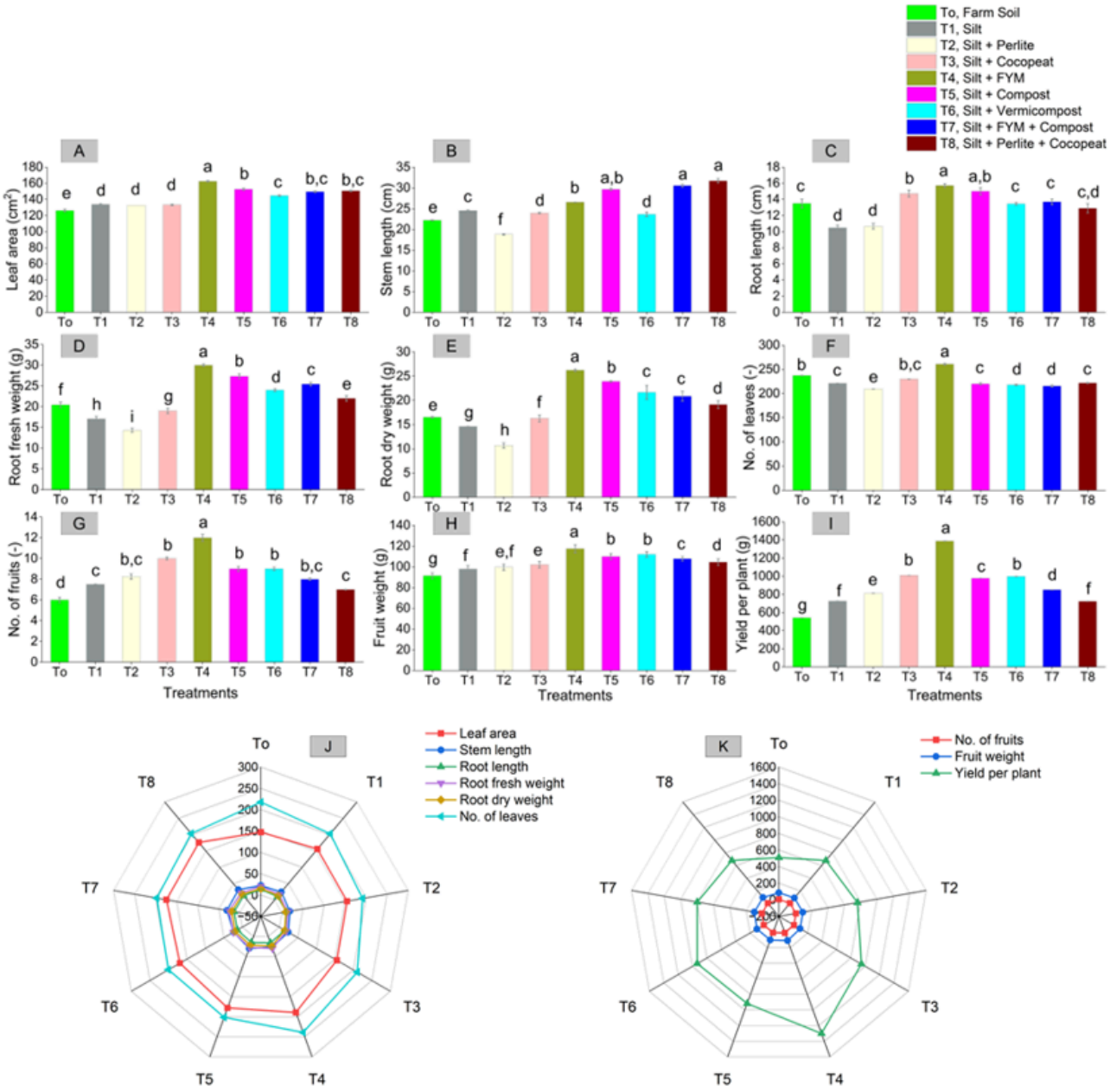

Figure 1

Growth and yield traits of sweet pepper under different substrate treatments (T_0–T_8). (A) Leaf area (cm²), (B) stem length (cm), (C) root length (cm), (D) root fresh weight (g), (E) root dry weight (g), (F) number of leaves, (G) number of fruits, (H) fruit weight (g), (I) yield per plant (g). (J) Radar diagram summarizing vegetative traits (leaf area, stem length, root length, root fresh weight, root dry weight, number of leaves). (K) Radar diagram summarizing reproductive traits (number of fruits, fruit weight, yield per plant). Treatments: T_0 = farm soil (control), T_1 = silt, T_2 = silt + perlite, T_3 = silt + cocopeat, T_4 = silt + FYM, T_5 = silt + compost, T_6 = silt + vermicompost, T_7 = silt + FYM + compost, T_8 = silt + perlite + cocopeat. Error bars represent standard deviation (SD) of the mean. Different letters above bars indicate significant

differences at $p < 0.05$. Larger polygons in radar diagrams indicate superior overall performance across traits.

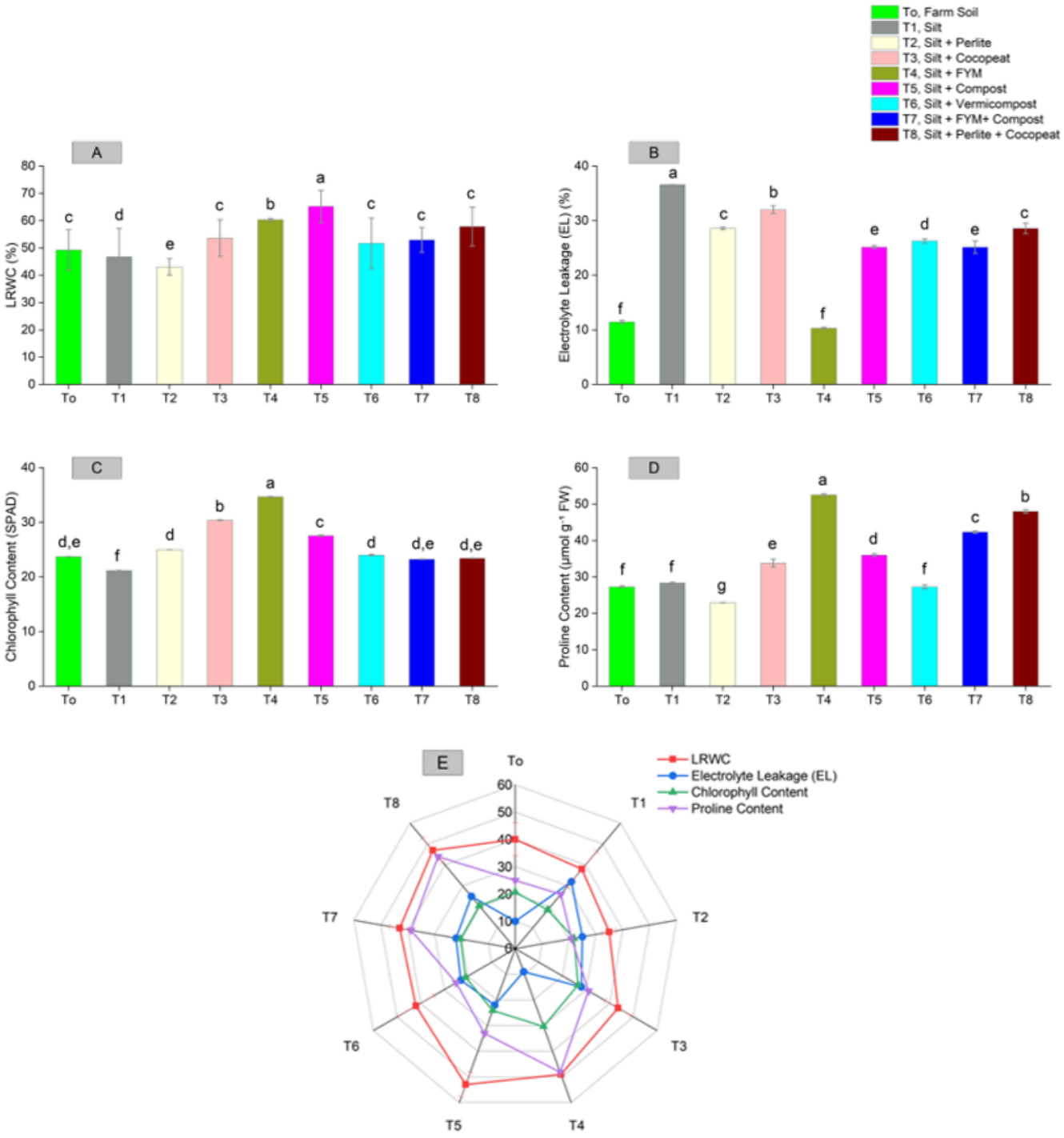

Figure 2

Physiological traits of sweet pepper under different substrate treatments (T_0–T_8). (A) Leaf relative water content (LRWC, %), (B) electrolyte leakage (EL, %), (C) chlorophyll content (SPAD units), (D) proline content (μmol g^{-1} FW), and (E) radar diagram summarizing physiological traits. Treatments: T_0 = farm soil (control), T_1 = silt, T_2 = silt + perlite, T_3 = silt + cocopeat, T_4 = silt + FYM, T_5 = silt + compost, T_6 = silt +

vermicompost, T_7 = silt + FYM + compost, T_8 = silt + perlite + cocopeat. Error bars represent standard deviation (SD) of the mean. Different letters above bars indicate significant differences at $p < 0.05$.

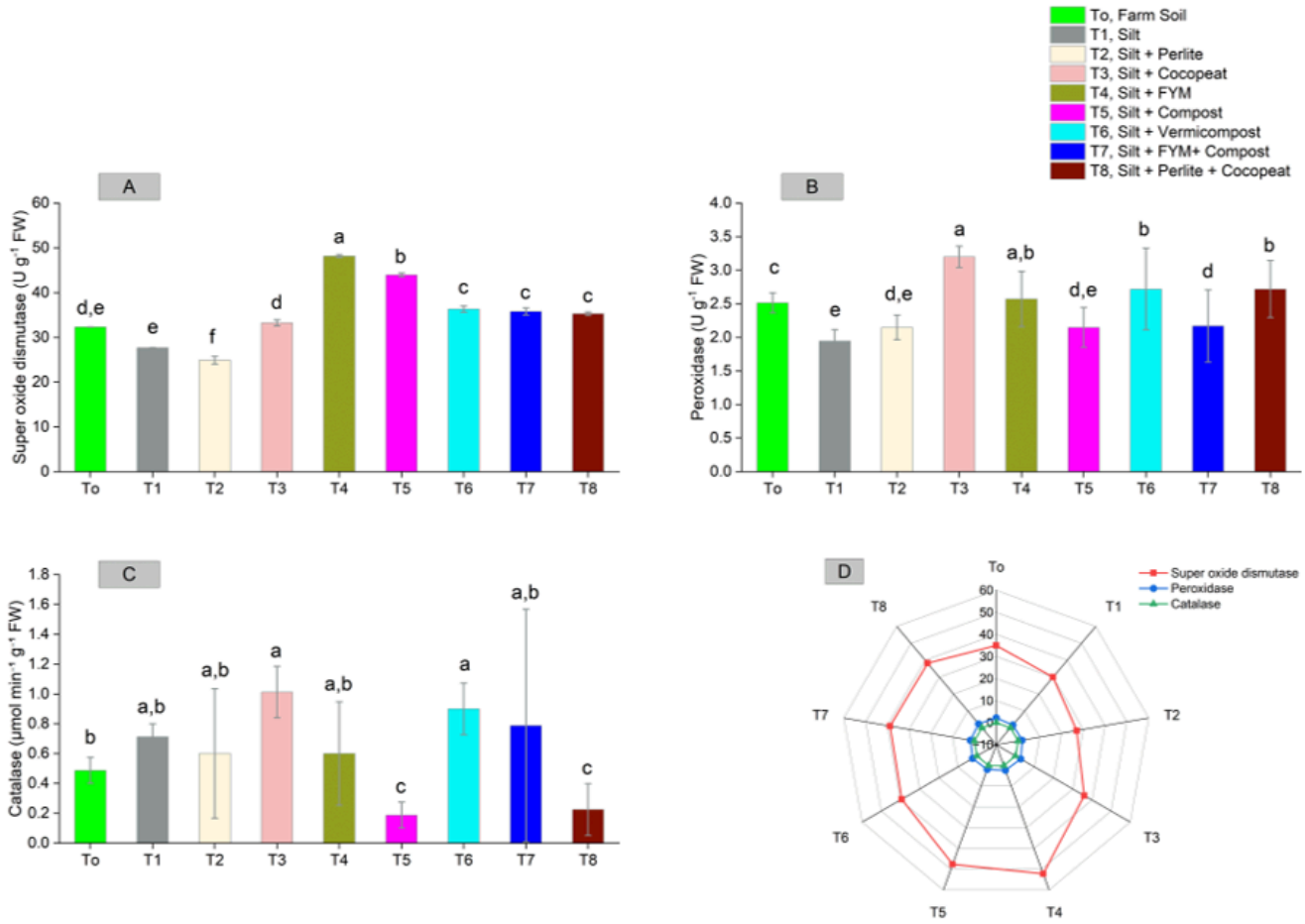

Figure 3

Antioxidant enzyme activities of sweet pepper under different substrate treatments (T_0–T_8). (A) Superoxide dismutase (SOD, U g^{-1} FW), (B) peroxidase (POD, U g^{-1} FW), (C) catalase (CAT, µmol H_2O_2 min^{-1} g^{-1} FW), and (D) radar diagram summarizing antioxidant enzyme activities. Treatments: T_0 = farm soil (control), T_1 = silt, T_2 = silt + perlite, T_3 = silt + cocopeat, T_4 = silt + FYM, T_5 = silt + compost, T_6 = silt + vermicompost, T_7 = silt + FYM + compost, T_8 = silt + perlite + cocopeat. Error bars represent standard deviation (SD) of the mean. Different letters above bars indicate significant differences at $p < 0.05$. Larger polygons in radar diagrams indicate superior overall antioxidant performance across treatments.

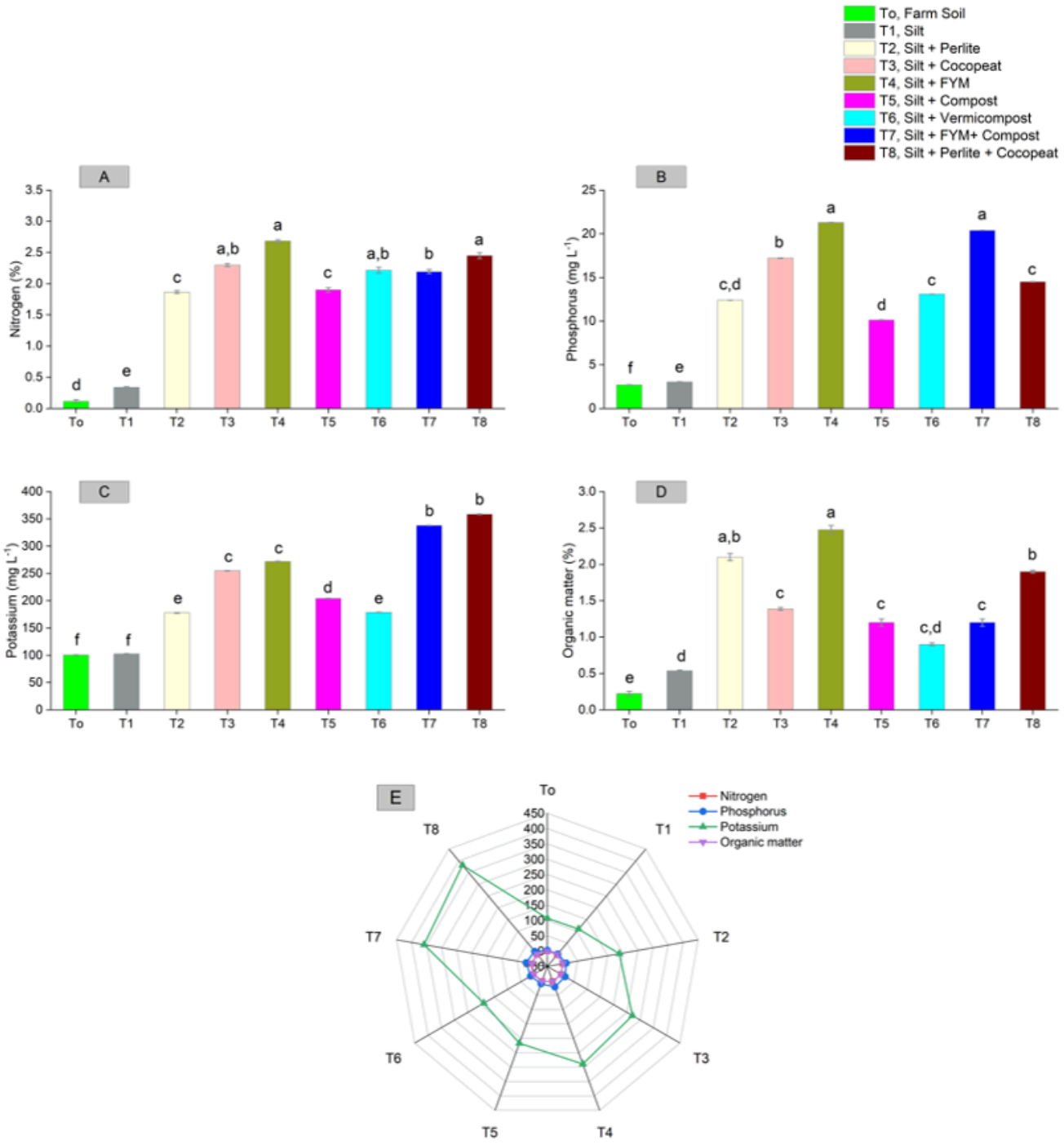

Figure 4

Soil and substrate fertility traits of sweet pepper under different substrate treatments (T_0–T_8). (A) nitrogen content (%), (B) phosphorus content (mg L^{-1}), (C) potassium content (mg L^{-1}), (D) Organic matter content (%), and (E) radar diagram summarizing fertility parameters (N, P, K, organic matter). Treatments: T_0 = farm soil (control), T_1 = silt, T_2 = silt + perlite, T_3 = silt + cocopeat, T_4 = silt + FYM, T_5 = silt + compost, T_6 = silt + vermicompost, T_7 = silt + FYM + compost, T_8 = silt + perlite + cocopeat. Error bars represent standard deviation (SD) of the mean. Different letters above bars indicate significant

differences at $p < 0.05$. Larger polygons in radar diagrams indicate superior overall fertility performance across traits.