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Is Tea Waste A Promising Co-substrate for Optimizing The Cultivation, Growth, and Yield of Charleston Pepper (*Capsicum annuum* **L.)?**

Çay Atığı, Çarliston Biberi (*Capsicum annuum* L.) Yetiştiriciliğinde, Büyüme ve Verimi Optimize Etmek İçin Umut Verici Yardımcı Bir Substrat mıdır?

ABSTRACT

To address growing concerns about sustainable agriculture and waste management, this study aimed to explore the viability of tea waste as an eco-friendly alternative substrate for cultivating Charleston peppers (*Capsicum annuum*), with the goal of optimizing plant growth and yield while reducing soil dependence, lowering cultivation costs, and repurposing agro-industrial waste. Six different substrate combinations were evaluated: 1) Tea waste, 2) Tea waste + Manure, 3) Tea waste + Soil, 4) Manure + Soil, 5) Tea waste + Manure + Soil, and 6) Tea waste + Manure + Soil + Perlite. Data were analyzed using both multivariate and univariate analyses to assess significant differences among treatments. Notably, significant differences in stem diameter were observed among plants grown on different substrates (one-way MANOVA, p <.05). However, plant height and chlorophyll content remained unaffected by substrate type. Although leaf structure exhibited considerable variation across treatments, no significant difference in dry matter content was observed. These results demonstrate that tea waste, especially when combined with other materials, is a promising sustainable substrate for Charleston pepper cultivation, potentially reducing soil dependence and agro-industrial waste.

Keywords: Pepper, Organic substrate, Manure, Tea waste substrate, Perlite

ÖZ

Sürdürülebilir tarım ve atık yönetimi konusundaki artan endişelere yanıt olarak, bu çalışma, Çarliston biberi (*Capsicum annuum*) yetiştiriciliğinde çay atığının çevre dostu alternatif bir substrat olarak kullanılabilirliğini araştırmayı ve bu sayede bitki büyümesini ve verimini optimize ederek toprağa bağımlılığı azaltmayı, yetiştirme maliyetlerini düşürmeyi ve tarımsal sanayi atıklarını yeniden değerlendirmeyi amaçlamıştır. Bu amaçla, altı farklı substrat kombinasyonu değerlendirilmiştir: 1) Çay atığı, 2) Çay atığı + Gübre, 3) Çay atığı + Toprak, 4) Gübre + Toprak, 5) Çay atığı + Gübre + Toprak ve 6) Çay atığı + Gübre + Toprak + Perlit. Elde edilen veriler, uygulamalar arasında anlamlı farklılıkları değerlendirmek amacıyla hem çok değişkenli hem de tek değişkenli analizler kullanılarak incelenmiştir. Farklı substratlarda yetiştirilen bitkiler arasında gövde çapında anlamlı farklılıklar gözlenmiştir (tek yönlü MANOVA, *p* <.05). Ancak, bitki boyu ve klorofil içeriği substrat tipinden etkilenmemiştir. Yaprak yapısı uygulamalar arasında önemli farklılıklar göstermesine rağmen, kuru madde içeriğinde anlamlı bir fark gözlenmemiştir. Bu sonuçlar, özellikle diğer materyallerle kombine edildiğinde çay atığının, Çarliston biberi yetiştiriciliği için toprak bağımlılığını ve tarımsal sanayi atıklarını potansiyel olarak azaltabilecek sürdürülebilir bir substrat olduğunu göstermektedir.

Anahtar Kelimeler: Biber, Organik substrat, Gübre, Çay atığı substratı, Perlit

Introduction

Tea (*Camellia sinensis*), a globally cultivated perennial crop with a worldwide production of 27.2 million tons in 2020 and 28.2 million tons in 2021, is projected to experience significant market growth of 54.4%, increasing from USD \degree 96 billion in 2020 to USD \degree 148 billion by 2027 (Debnath et al., 2021; Industry Research, 2022; FAOSTAT, 2023). According to FAOSTAT (2023) China was the largest tea producer with 13.7 million tonnes in 2021, followed by India (5.4 million tonnes), Kenya (2.3 million tonnes), and Türkiye (1.4 million tonnes). Tea leaves are processed into different types (i.e., green, white, yellow, oolong, black, and dark tea) based on the degree of fermentation, ranging from nonfermented to post-fermented (Ho et al., 2008; Wong et al., 2022; Shi et al., 2023). Black and green teas are the most popular, accounting for approximately 75% and 15% of global tea consumption, respectively (Debnath et al., 2021; Shi et al., 2023).

As global tea consumption rises, so does the amount of biomass waste generated during harvesting and processing. Tea consumption was estimated at 6.3 million tons in 2020 and is expected to reach 7.4 million tons by 2025 (Duarah et al., 2024).The increase in tea consumption has resulted in a corresponding growth in tea waste, including discarded leaves, buds and stems. This waste disposal poses environmental risks if not properly managed (Debnath et al., 2021; Duarah et al., 2024). India, the second-largest tea producer, reported producing approximately 0.2 million tons of tea waste, representing 22.2% of its total production of 0.9 million tons (Wasewar et al., 2009; Debnath et al., 2021). Improper disposal of tea waste can contribute to environmental pollution, affecting water, soil, and air quality (Debnath et al., 2021). In response to these environmental concerns, various studies have explored the potential and sustainable utilization of tea waste in diverse fields, such as environmental remediation (e.g., bioremediation and soil amendment) (Kaliaperumal et al., 2023; Zou et al., 2023), energy generation (e.g., biogas production) (Seth et al., 2023), fabrication of polymer composites (Prabhu et al., 2021), development of electrical devices for energy storage (e.g., supercapacitors) (Ratnaji & Kennedy, 2020), and its application as a bio-manure (Karataş, 2022; Seth et al., 2023). Tea waste contains similar components and quantities to regular tea, with notable levels of nitrogen (4.5%), potassium (4.6%), and phosphorus (0.6%), and it has been proven pathogen-free and non-phytotoxic (Manyuchi et al., 2018; Sui et al., 2019; Debnath et al., 2021; Seth et al., 2023). Several studies have shown that the use of tea waste as a co-substrate can significantly improve the growth and yields of both tomato and oyster mushrooms compared with control treatments (Pane et al., 2016; Karataş, 2022).

Peppers (*Capsicum annum* L.), an exotic vegetable with a unique flavor, are a valuable source of vitamins and bioactive compounds (Anaya-Esparza et al., 2021). These compounds, including provitamins A, E, and C, phenolic compounds, and carotenoids, offer several health benefits, including anti-inflammatory, antidiabetic, antimicrobial, and immunomodulatory properties (Sagar et al., 2018; Coman et al., 2020; Samtiya et al., 2021). Peppers, which range in color from red to yellow depending on ripeness and pigments (chlorophylls or carotenoids), are categorized as either hot or sweet and are cultivated in subtropical climates worldwide (Anaya-Esparza et al., 2021).

This study conducted the first comprehensive evaluation of tea waste as a substrate for Charleston pepper cultivation, investigating its effects on growth, yield, and optimization potential in various combinations: 1) Tea waste alone, 2) Tea waste + manure, 3) Tea waste + soil, 4) Tea waste + manure + soil, and 5) Tea waste + manure + soil + perlite. Additionally, a control substrate of 6) Manure + soil, commonly used for Charleston pepper cultivation, was included for comparison. This study aims to provide complementary data on the potential of tea waste as a sustainable alternative substrate, with the goal of reducing reliance on soil, lowering cultivation costs, and promoting the valorization of agro-industrial waste.

Material and Methods

The study was conducted at the Recep Tayyip Erdoğan University Faculty of Agriculture research greenhouse in 2016. The experimental setup used organic tea waste sourced from a tea factory in Rize as the primary growing medium. Composted barn manure was obtained from a local producer in Rize. The manure used was derived from year-old, composted cattle manure. The perlite used in the mixture was coarse agricultural perlite with a particle size of 3-6 mm. The peat used was a fine-textured sphagnum moss peat with a pH of 6 and was sterilized to be free of pathogens such as nematodes and fungi (Klasmann TS1). Both perlite and peat were acquired from the "Tartes" company.

The soil characteristics analyzed in this research were as follows: $pH = 4.7$ (indicating acidity), $EC = 0.73$ dS/m (non-saline), organic matter content = 1.93% (low), lime content = 0.21% (low), and phosphorus content = 2.19 mg/kg (very low). The soil was classified as clayey.

The plant material used in this study consisted of the "Charliston 341" "Yalova Charleston" variety obtained from the Torun Seed Company. This variety is characterized by its yellow-green color, thick fruit wall, and sweet taste, and it is classified as a Charleston-type pepper.

Compositions of different substrates applied to Charleston pepper (Capsicum annuum L.) cultivation.

The experiment involved the preparation of five different media using tea waste (T), perlite (P), soil (S), and composted barn manure (M) at varying volume ratios (v/v) (Table 1). A mixture of manure and soil $(M + S)$ was used as the control. The experiment was designed as a randomized block trial with three replications, each containing five plants.

- Seed sowing occurred on March 24, 2016, in black plastic pots (18x16 cm), with 3-4 seeds planted per pot.
- After germination, two seedlings were left in each pot, and one was used for seedling measurements. The time at which 50% of the seeds germinated was recorded as the germination time.
- Measurements were taken when the seedlings had 3-4 true leaves and included cotyledon width, cotyledon length, leaf width, leaf length, leaf area, leaf chlorophyll content, hypocotyl length, seedling height, seedling stem diameter, and dry matter content. These measurements were conducted on five samples.
- Flowering dates were recorded when 50% of the plants had flowered.
- Plant and leaf measurements were taken on August 9, 2016, and fruit harvesting was conducted on August 10, 2016. For green peppers that had reached harvest maturity, measurements included fruit width, fruit length, peduncle length, average fruit weight, and dry matter content. These measurements were taken from five fruits. Additional mature Charleston peppers were harvested once and categorized into three groups: red, orange, and green. For each group, measurements of fruit width, length, fruit count, total fruit weight, and average fruit weight were taken.
- Leaf measurements were performed on 10 fully grown leaves to assess leaf blade width, length, area, leaf chlorophyll content, and dry matter content. At the end of the experiment, plant height and stem diameter were recorded for each of the five plants.

Irrigation was carried out using a filtered bucket, based on the drying of the soil in the pots. To more precisely determine the impact of environmental conditions on plant development, no commercial manure was used.

- The dry matter content was determined by drying the leaves in an oven at 70°C until the weight of the fresh leaves stabilized.
- Leaf area measurements were taken on fully developed leaves randomly selected from each plant. The leaf area was measured using the WinDIAS image analysis system (Delta-T Devices, UK) and an HP Scanjet G2410 scanner (Hewlett-Packard, Palo Alto, California, USA).
- Chlorophyll levels were measured using a Konica Minolta SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). Width and length measurements were performed using a digital caliper and millimeter ruler.

Statistical analysis

The data from three replications are presented as Mean \pm SD, are displayed in descending order in the figures to clearly illustrate the decrease in observed values across different substrate treatments. To evaluate significant variations in the cultivation, growth, and yield characteristics of Charleston pepper across various substrates, one-way ANOVA was used if the data followed a normal distribution, as verified using the Shapiro-Wilk test. For data that did not meet the normality assumption, the Kruskal-Wallis test was applied. Prior to conducting principal component analysis and dendrogram analysis, the data were standardized using the log + 0.1 transformation (Alkan et al., 2019). All statistical analyses were performed using R software.

Results

Duration to T₅₀

Seed germination

The mean number of days required for T_{50} germination of control seeds was 20.3 \pm 0.6 days, which was significantly higher than the Tea Waste + Manure + Soil treatment and Tea Waste + Manure + Soil + Perlite treatment (one-way ANOVA, $F_{(5,12)} = 17.6$, $p < 0.05$). The overall maximum number of days was observed for tea waste treatment, with 8.4% more time required for *T⁵⁰* germination (Figure 1).

Flowering

The control treatment, Manure + Soil, produced T_{50} flowering in 55.0 \pm 1.7 days, which was significantly shorter than that of all other treatments (one-way ANOVA, $F_{(5,12)} = 32.1$, $p < 0.05$). The control treatment was followed by Tea Waste + Soil treatment, which took 58.0 \pm 1.0 days to T_{50} flowering (Figure 1).

Figure 1.

Mean (± s.d.) time taken for 50% (T50) of seeds/seedlings to achieve germination, flowering, and fruiting of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Figure 2.

Physical properties Seedling leaf of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Figure 3.

Physical properties of Cotyledons of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Fruiting

Similarly, flowering, the control treatment of Manure + Soil took a shorter time to provide T_{50} fruiting, which was 60.0 \pm 1.0 days, followed by Tea Waste + Manure + Soil treatment with 62.0 \pm 1.0 days) and Tea Waste + Soil treatment 62.7 \pm 1.5 days (Figure 1). The *T*⁵⁰ fruiting was significantly different between treatments (one-way ANOVA, *F*(5,12) = 21.3, *p <*.05).

Physical properties Seedling leaf and Cotyledon

The mean $(\pm s. d.)$ of the seedling leaf and cotyledon leaves under different treatments are provided in Table 1. The control treatments, Manure + Soil and Tea Waste + Soil, provided the seedling leaves with the highest length and width sizes, which differed significantly from the other treatments (Figure 2). On the other hand, on the other hand, the smallest length and width of the seedling leaves were treated with Tea Waste, followed by Tea Waste + Manure. The highest chlorophyll contents were observed in the control treatment (Manure + Soil) followed by Tea Waste + Manure + Soil treatment, while the Tea Waste treatment had the lowest chlorophyll contents (Figure 2). The highest dry matter content of seedling leaves was provided by the Tea Waste + Manure treatment, followed by the Tea Waste + Manure + Soil and Tea Waste + Manure + Soil + Perlite treatments, which were significantly similar (oneway ANOVA, $P > 0.05$). Likewise, the Tea Waste treatment provided the poorest results and the lowest dry matter content among the treatments (Figure 2).

The Cotyledon response to different treatments was consistent with seedling leaf, providing better results in the control, Manure + Soil, and Tea Waste + Soil treatments. Furthermore, the lowest chlorophyll was observed in Charleston pepper cultivated under Tea Waste treatment. However, the smallest Cotyledon length and width were provided by Tea Waste + Manure + Soil + Perlite followed by Tea Waste (Figure 3).

Physical properties of plants and leaves

The plant size of the Charleston pepper also showed significant variations between different treatments, with the highest height of the plant being achieved by the Tea Waste + Manure treatment, followed by the Tea Waste + Soil. The smallest plant size was observed for Tea Waste + Manure + Soil + Perlite followed by the control treatment, Manure + Soil treatment (Figure 4). The highest chlorophyll contents were recorded in leaves treated with Tea Waste + Manure and Tea Waste + soil. On the other hand, the control treatment (Manure + Soil treatment) provided the leaves with the lowest chlorophyll content (Figure 4).

The length, width, and dry matte of leaves did not significantly differ between treatments ($P > 0.05$). The highest length and width of leaves were recorded for Tea Waste + Soil followed by Tea Waste + Manure + Soil and then the control treatment, Manure + Soil (Figure 5). However, the highest dry matter content of leaves was recorded in the Tea Waste + Manure + Soil + Perlite treatment, followed by the Tea Waste + Manure + Soil treatment and then the Control treatment, Manure + Soil.

Figure 4.

Physical properties, plant height, and leaf chlorophyll content of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Figure 5.

Physical properties of leaves of the Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite.

Fruit number per plant

At 139 days, the fruits were harvested and categorized (as green, yellow, or red), and counted for each substrate. The highest mean number of total fruits (green, yellow, and red) per plant was observed in the Tea Waste + Soil substrate, with an average of 18 fruits per plant. This was followed by Tea Waste + Manure + Soil + Perlite substrate, with an average of 15 fruits per plant. The lowest mean count of total fruits per plant was recorded for the Tea Waste + Manure substrate, with an average of 11 fruits per plant.

The mean counts of fruits (green, yellow, or red) among different substrates showed significant differences. However, in the subsequent analysis using the Tukey test, the differences among the different substrates were not significant. Nonetheless, significant differences were observed for the green fruits, with the Tea Waste substrate exhibiting a significantly higher number of green fruits (F(5,12) = 12.59, *p <*.001; Figure 6). The mean weight of a single Charleston pepper did not differ significantly among the different substrates (Green: one-way ANOVA, *F*(3,6) = 1.78, *p* =.251, Yellow Charleston pepper: Kruskal-Wallis One-Way ANOVA, *H* = 4.02, *p* =.547; and Red: one-way ANOVA, *F*(5,12) = 0.578, *p* =.717).

Physical characteristics of the Charleston pepper

The physical properties of the Charleston pepper differed greatly between treatments. The highest-width Charleston pepper was produced using the Manure + Soil treatment, followed by Tea Waste + Manure + Soil and Tea Waste + Soil. The Charleston pepper was the smallest width in the Tea Waste treatment. The dry matter of the Charleston pepper also exhibited significant variations. The highest dry matter content was provided by Tea Waste + Manure + Soil + Perlite, followed by Tea Waste and Tea Waste + Soil. The dry matter of Charleston peppers obtained using the Manure + Soil treatment was significantly smaller than that of other types (Table 2).

PCA and dendrogram analysis

The first and second principal components explained up to 60% of the variation in the data (Figure7). The number of significant correlations was 17, and six were negatively correlated. Negative correlations were found between fruit weight (green) and Fruit width with a Pearson correlation of -0.65. A significant positive correlation between Fruit width and red Charleston pepper count was recorded (a Pearson correlation of 0.49. However, the green fruit and red Charleston pepper counts were negatively correlated, with a Pearson correlation of -0.82 (Figure 7).

The cluster analysis dendrogram identified three clusters. The first cluster was created by Tea Waste treatment and Tea Waste + Manure treatment, which contributed up to

97.1%. This cluster exhibited the highest dissimilarity with the others. The second cluster was created by the control group, Manure + Soil (Figure 8). This second cluster exhibited the highest similarities with the third cluster compared to the first cluster. In the third cluster, the highest similarity was recorded between the Tea Waste treatment and the Tea Waste + Manure + Soil, at 98.2%.

Figure 6.

Fruit count per plant and mean (± s. d.) weight of individual Charleston pepper (Capsicum annuum L.) cultivated on different substrates. T, tea waste; M, manure; S, soil; P, perlite. The green, yellow, and red colors indicate the fruit colour stage.

Table 2.

Effects of different substrates on the physical properties (Mean ± s. d.) of Charleston pepper (Capsicum annuum L.) harvested at T⁵⁰ fruiting stage.

TSS: total soluble solid content

Substrates with the same letter are not significantly *different from each other.*

Standardise Variables by Total Resemblance: S17 Bray Curtis similarity

Figure 8.

A dendrogram based on data provided in Figure 7 revealing the similarities and dissimilarities among Charleston pepper (Capsicum annuum L.) plants cultivated on various substrates. In the dendrogram, T, tea waste; M, manure; S, soil; P, perlite.

Figure 7.

Principal component analysis revealed a correlation between the different types of Charleston pepper cultivated on different substrates. The green, yellow, and red colors indicate the fruit colour stage and values in italic belong to the fruiting stage of T50. TSS stands for total soluble solid content (%).

Discussion

The findings of this study highlight the potential of tea waste as a sustainable co-substrate for Charleston pepper cultivation, especially when combined with other materials such as soil and manure. This is consistent with the results reported by Duarah et al. (2024), who also demonstrated the effectiveness of tea waste in enhancing soil fertility and plant growth when used as part of an integrated substrate mix. In this study, it was observed that high concentrations of tea waste (e.g., tea waste treatment) initially hindered seed germination and early plant growth. This can be attributed to the high polyphenol and tannin content in tea waste (Sökmen et al., 2018; Duarah et al., 2024; Wang et al., 2024), which might have allelopathic effects and potential phytotoxicity in young seedlings. These compounds are known to delay germination and inhibit root elongation, a phenomenon previously documented in studies on organic waste materials in agriculture (De Almeida et al., 2014; Nahed et al., 2015; Wang et al., 2024). However, when used as part of a balanced co-substrate, tea waste has significant benefits for early-stage pepper growth, likely due to the synergistic interactions between tea waste and other organic materials (Debnath et al., 2021; Kumar et al., 2023). The combination of soil, manure, and perlite in the growing medium not only dilutes the phytotoxic effects of polyphenols but also enhances the physical properties of the substrate (Kumar et al., 2023). This balanced mixture may improve aeration, water-holding capacity, and nutrient availability, promoting more vigorous early growth, as

evidenced by increased seedling height and leaf number. In this study, the inclusion of perlite, for example, could contribute to better root oxygenation and drainage, mitigating potential waterlogging issues often associated with the use of organic waste as a substrate (Pane et al., 2016; Karataş, 2022; Kumar et al., 2023).

Tea leaves, which are rich in nitrogenous compounds (Wang et al., 2020; Debnath et al., 2021), release nitrogen into the soil as they decompose, which can improve leaf color and plant health over time, even without the use of commercial manure (Peksen & Yakupoglu, 2009). Nitrogen is a vital macronutrient for pepper plants, particularly during the vegetative stage, as it is essential for chlorophyll synthesis, leaf development, and biomass accumulation (Hunde, 2020; da Silva Magalhães et al., 2023).

Tea waste enhances soil structure by improving porosity, water retention, and cation exchange capacity (CEC), which are crucial for plant growth, while also helping to retain moisture, reduce irrigation needs, and form stable soil aggregates that facilitate root penetration and nutrient access (Debnath et al., 2021; Kumar et al., 2023). The presence of lignocellulosic compounds in tea waste further supports its use as a co-substrate (Barathi et al., 2017). These compounds enhance the structural integrity of substrates, allowing for better root anchorage and water infiltration (Sial et al., 2019). Thus, combining tea waste with faster-decomposing materials like manure might offer a balanced nutrient supply throughout the growth cycle, making it a valuable resource for sustainable agriculture, particularly where high-quality organic amendments are limited.

Conclusion and Recommendations

This study highlights the potential of tea waste as a sustainable alternative substrate for Charleston pepper cultivation, particularly when combined with components like soil, organic manure, and perlite. While high tea waste concentrations initially inhibited seed germination and early vegetative growth—probably due to its limited immediate nutrient availability and the presence of inhibitory compounds—its use as part of a balanced mixture showed significant benefits. The combination of tea waste with soil and manure promoted early seedling development, enhanced leaf coloration, and improved overall plant vigor, suggesting a gradual release of nutrients, particularly nitrogen, as tea waste decomposes over time.

Based on these findings, the use of tea waste as a cosubstrate is recommended, rather than as the sole medium for Charleston pepper cultivation. Further research is needed to optimize tea waste as a sustainable substrate by exploring pre-treatment methods like composting or microbial inoculation to enhance nutrient release. Additionally, testing different substrate compositions with materials such as biochar or compost could help identify optimal mixtures for various crops. Investigating the longterm effects of tea waste on soil health, including nutrient cycling and microbial activity, is also crucial, as is studying crop-specific responses to ensure broader agricultural applicability. Addressing these areas will further validate tea waste as an eco-friendly alternative to synthetic fertilizers and promote more sustainable agricultural practices.

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