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The effect of novel biotechnological vermicompost on tea yield, plant nutrient content, antioxidants, amino acids, and organic acids as an alternative to chemical fertilizers for sustainability

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Abstract
In this study, the performance of a novel organic tea compost developed for the first time in the world from raw tea waste from tea processing factories and enriched with worms, beneficial microorganisms, and enzymes was tested in comparison to chemical fertilizers in tea plantations in Rize and Artvin provinces, where the most intensive tea cultivation is carried out in Turkey. In the field trials, the developed organic tea vermicompost was incorporated into the root zones of the plants in the tea plantations in amounts of 1000 (OVT1), 2000 (OVT2) and 4000 (OVT4) (kg ha^{-1}). The experimental design included a control group without OVT applications and positive controls with chemical fertilizers (N: P: K 25:5:10, (CF) 1200 kg ha^{-1}) commonly used by local growers. The evaluation included field trials over two years. The average yields obtained in two-year field trials in five different areas were: Control (6326), OVT1 (7082), OVT2 (7408), OVT4 (7910), and CF (8028) kg ha^{-1}. Notably, there was no significant statistical difference in yields between the organic (at 4000 kg ha^{-1}) and chemical fertilizers (at 1200 kg ha^{-1}). The highest nutrient contents were obtained when CF and OVT4 were applied. According to the average values across all regions, the application of OVT4 increased the uptake of 63% N, 18% K, 75% P, 21% Mg, 19% Na, 29% Ca, 28% Zn, 11% Cu and 24% Mn compared to the control group. The application of chemical fertilizers increased the uptake of 75% N, 21% K, 75% P, 21% Mg, 28% Na, 27% Ca, 30% Zn, 18% Cu and 31% Mn compared to the control group. The organic fertilizer treatment had the lowest levels of antioxidants compared to the control groups and the chemical fertilizers. It was also found that the organic fertilizer increased the levels of amino acids, organic acids and chlorophyll in the tea plant. Its low antioxidant activity and proline content prepared them for or protected them from stress conditions. With these properties, the biotechnologically developed organic tea compost fertilizer has proven to be very promising for tea cultivation and organic plant production.

Keywords Tea waste, Biotechnology, Organic fertilizer, Tea cultivation, Yield, Amino acids

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Introduction

The use of chemical fertilizers disturbs various natural cycles found in nature, including those related to water and air circulation, nutrient recycling, automatic disease and pathogen control, and ion exchange. Consequently, when soils lose their biodiversity, they also lose their ability to function effectively, resulting in decreased productivity and efficiency [1]. Commercially cultivated tea plants are typically harvested in the form of shoots and young leaves, which leads to an increased need for fertilizer during their growth phase [2]. As a result, the annual application of chemical fertilizers in tea cultivation often exceeds twice the recommended dosage. However, the irresponsible use of chemical fertilizers does not lead to a proportional increase in tea yield [3, 4] and quality. Furthermore, this practice exacerbates soil acidification [5, 6], soil compaction [7, 8], and problems related to nutrient runoff [9–11]. Numerous recent studies have investigated the effects of fertilization not only on soil conditions but also on tea yield and quality [12–15]. For example, a study of 70 tea fields in Japan found that about 77% of the total soil area had a pH below 4.0, with excessive nitrogen fertilization endangering both the normal growth of tea plants and the quality of surface water [16]. Similarly, an analysis of soil pH and its impact on tea yield and quality in 145 tea fields in Nanjing County, Fujian Province, China, found that 82.1% of these fields had a pH below 4.5 [17], while in Anxi County, about 37.67% of tea plantation soils had a pH below 4.5 [18]. In addition, soil acidification had a significant positive correlation with tea yield and quality, with both aspects decreasing significantly [19]. Tea fields with soil pH below 4.5 rely primarily on chemical fertilizers, while those utilizing organic fertilizers have higher soil pH values. A study conducted on 5285 tea plantations in Anxi County, China, found that about 68.44% of tea fields had soil pH below 4.5, depending on the predominant use of chemical fertilizers. In contrast, fields that used organic fertilizers had higher soil pH values, suggesting the role of organic fertilizers in mitigating soil acidification [20]. After the erosion of the ecological system became noticeable due to the use of chemicals for increased production and profit, the development of organic farming became necessary. This alternative system aims to preserve the ecological balance without compromising production and efficiency. Organic fertilizers can regulate soil acidification, improve the survival environment of soil microorganisms, increase microbial and enzymatic activities in the soil, and thus promote nutrient uptake for utilization by plants and effectively improve crop yield and quality [21–23]. The quality of tea is strongly influenced by organic and inorganic compounds that transform into flavor quality in the harvested shoots. Therefore, improving tea quality is of great importance for increasing the economic benefits of tea [24, 25]. Amino acids, the primary metabolites of tea plants, also play a crucial role in the formation of the sweet taste of tea [26–29]. Many researchers emphasize that high amino acid content in tea is often an indicator of good quality when evaluating the effects of biotic or abiotic stress on tea quality [30–32]. However, in practical agricultural production, studies often focus on directly reducing the use of chemical fertilizers by combining them with organic fertilizers [16, 33–39]. An estimated 1 billion tons of agricultural waste is generated worldwide every year. China, the United States, and India are among the largest producers of agricultural waste in the world [40, 41]. Furthermore, this number is predicted to increase rapidly due to the increasing demand for agricultural products [42, 43]. Therefore, the utilization of agricultural waste not only benefits soil health but also provides an effective approach to sustainable agricultural models.

In developing countries, significant quantities of vegetable waste are frequently incinerated, leading to environmental pollution through this incineration process [44]. Nevertheless, these residues from the incinerated products contain a substantial amount of nutrients, comprising 30–35% nitrogen and phosphorus minerals and 70–80% potassium, which can be returned to the soil [45]. Vermicompost, produced by decomposing organic material with the help of worms and microorganisms at non-thermophilic temperatures, is considered a sustainable method or environmentally friendly technology for dealing with plant residues [46]. Therefore, the development of vermicompost technologies is a good alternative to incineration because vermicompost technologies enhance soil micronutrients [47], physical properties [48], salinity [49], and pH balance, which contributes to soil recovery [50]. They also impact the activity of important soil enzymes such as acid phosphatase, acid invertase, and catalase [51]. Soil enzymes, essential for ecosystem function, are central to nutrient cycling [52]. In addition, active soil enzymes' composition, quantity, and microbial community determine nutrient availability and thus influence soil health at a given time [53]. Microbial communities and their activities have various effects on soil fertility and structure and contribute to the mineralization and availability of nutrients. In particular, microbes involved in the degradation and decomposition of soil organic matter contribute positively to critical aspects of soil fertility. They influence the cation exchange capacity, the reserves of nitrogen (N), sulfur (S), and phosphorus (P), the acidity and toxicity of the soil, as well as the water retention capacity of the soil [54]. This transformation has led to significant changes in agricultural systems worldwide. An urgent need is now to adopt regenerative farming practices to promote soil health, increase soil carbon, improve soil physical properties, and preserve soil biodiversity. Sustainable agricultural approaches include using vermicompost, which contains various beneficial microbial groups such as cellulose and lignin decomposers, phosphate solubilizers, nitrogen fixers, and antibiotic producers. These agricultural methods lead to higher crop production while maintaining soil quality. They also ensure food availability without chemicals that are safe for the population and thus protect the environment [55].

The discovery of tea's anti-inflammatory, antioxidant, and weight-reducing effects is expected to lead to an increase in global tea consumption and production over the next decade, particularly in developing countries with high demand [56]. However, meeting this demand has led tea producers to resort to intensive chemical inputs in conventional agriculture to achieve rapid yield increases, leading to ecological imbalances. In addition, processing fresh tea leaves into black tea in tea factories produces solid waste such as garbage, fibers, and dust. For example, about 22 kg of black tea in Turkey is produced from 100 kg of harvested green shoots [1]. Tea factories produce, on average, 4% of the green shoot waste generated by commercial tea production [57]. The amount of waste produced by tea factories is constantly increasing due to rising demand. However, the areas in which this waste can be used effectively in sustainable agriculture are insufficient. In a two-year study, enzymes (including protease, lipase, dehydrogenase, hydrolase, urease, nitrogenase, cellulase) and beneficial microorganisms (such as *Aspergillus flavus*, *Bifidobacterium* spp., *Bacillus* subtilis, *Rhodotorula* spp., *Lactobacillus*, *Rhodopseudomas* spp.) in addition to worms were used to mineralize raw tea waste from processing plants. The aim of this study is to transform tea waste into nature-friendly organic fertilizer with low C footprint and low input costs by transforming it from nature to nature with biotechnological systems containing worms, microorganisms and enzymes. This innovative approach was tested for the first time globally and served as an alternative to the use of chemical fertilizers in tea-growing areas in Turkey. The study was supported by the Ministry of Agriculture and Forestry of the Republic of Turkey, in particular by its Directorate General of Agricultural Research and Policy.

Materials and methods

"In the context of waste disposal, a specialized organic vermi-tea (OVT) fertilizer, consisting of 60% raw tea waste, 40% waste from mushroom cultivation, and a mixture of microorganisms was obtained from Kiana Agriculture B.V. from Netherland (*Aspergillus flavus*, *Bifidobacterium* spp., *Bacillus subtilis*, *Rhodotorula* spp., *Lactobacillus*, *Rhodopseudomas spp.)* and enzymes (Protease, Lipase, Dehydrogenase, Hydrolase, Urease, Nitrogenase, Cellulase), was developed using a rapid biotechnological method " [1]. This method is achieved by adding special enzymes, organisms and biological compounds that serve to separate plant wastes into all their components in a short time. OVT chemical composition; organic matter, total nitrogen (N), available phosphorus (P_2O_5), total potassium (K_2O), pH, organic C were 75.26%, 2.7%, 1.29%, 2.17%, 7.57, 42.28%, respectively.

It was then employed in field trials conducted in April 2019 and 2020 within tea plantations located in the Ardeşen (Sesli kaya *41.146923*, *41.029996)*, Çayeli (Yeşiltepe 40.96905705742561, 40.80714515884804), Hopa (Pınarlı 41.361765, 41.423920), Guneysu (Taşpınar 41.015520, 40.603630), and Fındıklı (Yeşildere 40.894871, 40.519566) districts of Rize province, known for intensive tea cultivation in Turkey. In the field trials, OVT was incorporated into the root zones of plants in the tea plantations at rates of 1000 (OVT1), 2000 (OVT2), and 4000 (OVT4) kg ha^{-1}. The experimental setup also included a control group with no OVT applications and was also added positive controls with chemical fertilizer (N: P: K 25:5:10, (CF) 1200 kg ha^{-1}) commonly used by local growers. This study was conducted in 5 different regions with 5 different applications and 3 replication, according to the fully randomized trial design. Each parcel used in the experiment consists of 500 m2, (50mx10 m) in size. In the places where the trial was conducted between 2019 and 2020, the average highest temperature was 38, the lowest average low temperature was minus 6 degrees, total annual precipitation was 2300 ml, and the average number of rainy days was 172 days. The physicochemical properties of the soils in the experimental fields were analyzed before the application of OVTs and CF (Table 1).

Method for extracting, purifying, and analyzing hormones from Plant leaves

The extraction of the leaves of plant and purification procedures followed the methods outlined in [58, 59] and were conducted in triplicate. After isolating phenolic compounds and dyes using the techniques described by [60, 61], 1 g of insoluble polyvinylpolypyrrolidone (PVPP, Sigma) was prepared for each sample. It was added to the beaker containing the supernatant and thoroughly mixed [62, 63]. Hormones adsorbed by the cartridges were then transferred to small vials by dissolving in 80% methanol (3 ml for 1 g of fresh sample). The collected samples in the vials were utilized for HPLC analyses [64]. For the analysis of indoleacetic acid and abscisic acid, HPLC measurements were conducted according to the methods proposed by [65–67].

Determination of organic acids from plant leaves

To accurately analyze the leaves organic acids (OA) of plant, a solution was prepared containing specific

Table 1 The physicochemical properties of the soils of the experimental fields (*n* = 3)

Physicochemical properties of the soils	Ardeşen	Hopa	Fındıklı	Güneysu	Yeşiltepe
pH (1:2.5)	4,360 ± 0.13	4,65 ± 0.29	4,80 ± 0.29	5,20 ± 0.15	5,05 ± 0.20
OM, %	1,30 ± 0.25	1,60 ± 0.35	1,75 ± 0.30	1,90 ± 0.22	1,35 ± 0.19
Total- N, %	0.11 ± 0.03	0,11 ± 0.04	0,12 ± 0.02	0,14 ± 0.01	1,10 ± 0.14
K, (cmol kg^{-1})	2.32 ± 0.11	2,56 ± 0.32	2,34 ± 0.41	2,17 ± 0.18	2,77 ± 0.19
Ca, (cmol kg^{-1})	3.48 ± 0.40	4,18 ± 0.18	3,96 ± 0.20	3,65 ± 0.31	3,79 ± 0.21
Mg, (cmol kg^{-1})	1.12 ± 0.13	1,28 ± 0.22	1,34 ± 0.21	1,27 ± 0.22	1,43 ± 0.23
Na, (cmol kg^{-1})	0.15 ± 0.08	0,20 ± 0.11	0,15 ± 0.03	0,13 ± 0.03	0,10 ± 0.00
readily available P, (mg kg^{-1})	3,20 ± 0.08	4,70 ± 0.25	4,10 ± 0.28	5,30 ± 0.19	3,55 ± 0.35
readily available Fe, (mg kg^{-1})	3.40 ± 0.35	3,70 ± 0.35	2,50 ± 0.35	2,90 ± 0.21	2,25 ± 0.15
readily available Zn, (mg kg^{-1})	2.54 ± 0.29	1,90 ± 0.33	1,25 ± 0.23	2,15 ± 0.30	1,35 ± 0.19
readily available Cu, (mg kg^{-1})	1.14 ± 0.12	1,56 ± 0.39	1,48 ± 0.29	1,45 ± 0.05	1,80 ± 0.17
readily available Mn, (mg kg^{-1})	5.48 ± 0.20	6,68 ± 0.45	4,68 ± 0.18	5,65 ± 0.30	4,40 ± 0.17
Sand, %	20.70 ± 0.15	35,78 ± 0.50	29,60 ± 0.30	38,55 ± 0.35	42,50 ± 0.33
Loam, %	35.90 ± 2.93	27,48 ± 0.28	39,5 ± 0.50	29,40 ± 0.30	35,80 ± 0.64
Clay, %	43.40 ± 0.35	36,74 ± 0.31	30,90 ± 2.94	32,05 ± 0.50	21,70 ± 0.24
Soil Texture Group	Clay	Clay Loam	Clay Loam	Clay Loam	Loam

concentrations of various acids: 15.6 µM oxalic acid, 66.6 µM tartaric acid, 74.6 µM malic acid, 339 µM succinic acid, 96 µM malonic acid, 5.7 µM L-ascorbic acid, 1.7 µM maleic acid, 95.1 µM citric acid and 1.7 µM fumaric acid [68]. After the preparation of the standards, each mixture was subjected to HPLC analysis to detect individual peaks.

Determination of amino acids from Plant leaves

The method for the determination of amino acids (AA) involves column separation with phenyl isothiocyanate (PITC) [69]. First, standard solutions are prepared. Then 10 µl samples are dissolved in a 100 µl buffer solution and dried under high pressure for one hour. The dried samples are then redissolved in 100 µl buffer solution with the addition of 5 µl PITC. After a reaction time of 5 min at room temperature [70], the PITC derivatives are dissolved a second time under high pressure. These amino acid derivatives are formed in 0.05 M sodium acetate at pH 6.8. They are then dissolved in a 9:1 (v/v) mixture of 0.1 M sodium acetate and 10% methanol in 40% acetonitrile [68]. Finally, 10–20 µl of the solution is analyzed by HPLC.

Extraction of antioxidant enzymes (peroxidase, Catalase, Superoxide dismutase) in plants

All operations were conducted at 4 °C. Plant leaf cells (0.5 g) were homogenized in a mortar with 3 ml of 50 mM phosphate buffer at pH 7. The homogenates were filtered through two layers of Miracloth and centrifuged at 15,000 g for 15 min at 4 °C. The supernatant obtained was stored at −80 °C. For antioxidant enzyme assays, frozen cell samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 0.5% polyvinylpolypyrrolidone (PVP). The activities of CAT, POD, and SOD enzymes in the apoplastic fractions were measured spectrophotometrically [71].

CAT activity was determined by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H2O2. One unit of CAT activity was defined as the amount of enzyme that decomposes 1 µmol H2O2 per minute [72]. POD activity was assessed by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H2O2 [71]. SOD activity in the apoplastic fractions was measured by recording the reduction in optical density of nitro-blue tetrazolium dye due to the enzyme. Absorbance was recorded at 560 nm. One unit of SOD activity was defined as the enzyme amount required to reduce the absorbance reading by 50% compared to tubes lacking enzyme [73, 74].

Chlorophyll content

The chlorophyll content of the plant leaves and the total chlorophyll values were determined using the SPA-502 chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan).

Determination of plant mineral content

The contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in the plant leaf samples were analyzed in a three-step procedure with nitric acid-hydrogen peroxide (2:3) acid. First, the samples were wet-baked for 5 h at 145 °C and 75% microwave power in a pressure-resistant 40-bar microwave oven (Speedwave MWS-2, Berghof Products + Instruments, Harresstr.1, 72800 Enien, Germany) [75]. The subsequent steps included a 10-minute microwave treatment at 90% power and 180 °C (second step) and 10 min at 40% power and 100 °C (third step). After wet combustion, the concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined using an ICP OES spectrophotometer (Inductively Coupled Plasma Spectrophotometer) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484–4794, USA) [75].

Statistical analyzes

The data were analyzed using ANOVA analysis of variance to compare the effects of the treatments. Duncan's Multiple Range Test was used to determine differences in means ($p<0.05$).

Results

The field trials conducted in the first and second years found that the application of OVT and chemical fertilizers to tea plants significantly influenced the yield during the first, second, and third sprout periods compared to the control application ($p<0.05$). According to the first-year field trial results, the highest yield was achieved with OVT4 and the application of CF. Yields increased compared to the control groups in the first sprout period (23.79%, 26.39%), second sprout period (23.3%, 25.7%), and third sprout period (24.8%, 25.6%). The highest yield was obtained with OVT4 and CF applications in the second-year field trial. The yield increases compared to the control groups were (25.90%, 27.90%) in the first sprout period, (26.2%, 28.08%) in the second sprout period, and (25.25%, 27.27%) in the third sprout period. (Fig. 1).

Figure 1. Yield values of the first, second, and third sprout periods of the tea plant.

Depending on the applications at different locations (OVT1, OVT2, OVT4, and CF), the yield increases of the tea plant during the three sprout periods in the first year were compared with the control groups in each case. Ardeşen (11%, 15%, 24%, and 27%), Hopa (8%, 14%, 22%, 21%), Fındıklı (10%, 16%, 25%, and 26%), Yeşiltepe (7%, 13%, 24%, and 28%). Yield increases in the second year were determined in Ardeşen (17%, 25%, 34%, and 25%), Hopa (19%, 18%, 24%, and 29%), Fındıklı (18%, 22%, 26%, and 34%), Guneysuyu (9%, 17%, 24%, and 31%), Yeşiltepe (11%, 15%, 22%, and 22%) compared to the control application (Fig. 2). The yield increase of OVT is 9% higher than that of CF in this place, and for many years, organic amendment has already been used in the region.

Figure 2. Two-Year Average Yields of Tea Plants with OVT and CF Applications Across Various Plantation Sites.

Depending on the tea shoot periods in various tea plantation locations, the yield increases compared to the control groups in the first sprout period were as follows: Ardeşen (14%, 22%, 30%, and 24%), Hopa (11%, 15%, 24%, and 26%), Fındıklı (13%, 18%, 24%, and 32%), Guneysuyu (8%, 16%, 25%, and 25%), Yeşiltepe (8%, 15%, 26%, and 25%). In the second tea sprout period: Ardeşen (14%, 20%, 29%, and 29%), Hopa (14%, 16%, 23%, and 26%), Fındıklı (15%, 20%, 26%, and 31%), Guneysuyu (9%, 16%, 24%, and 28%), Yeşiltepe (9%, 14%, 23%, and 22%). In the third shoot period: Ardeşen (14%, 20%, 30%, and 24%), Hopa (11%, 15%, 24%, and 26%), Fındıklı (13%, 18%, 24%, and 32%), Guneysuyu (8%, 16%, 25%, and 25%), Yeşiltepe (8%, 15%, 26%, and 25%).

The highest values of the two-year field trial regarding the nutrient content of the tea plants at the first, second, and third sprouting stages were achieved by applying OVT4 and CF. It was also found that the effects of OVT

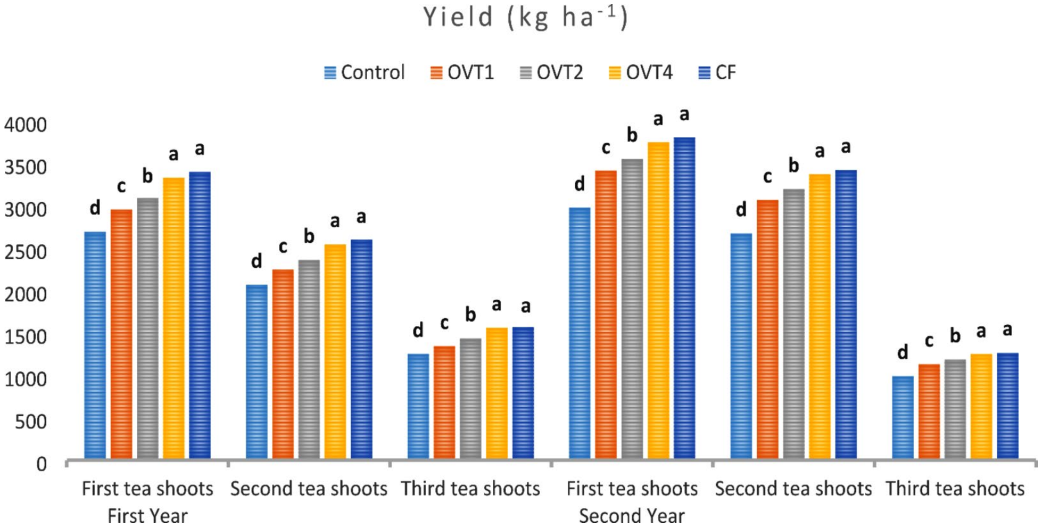
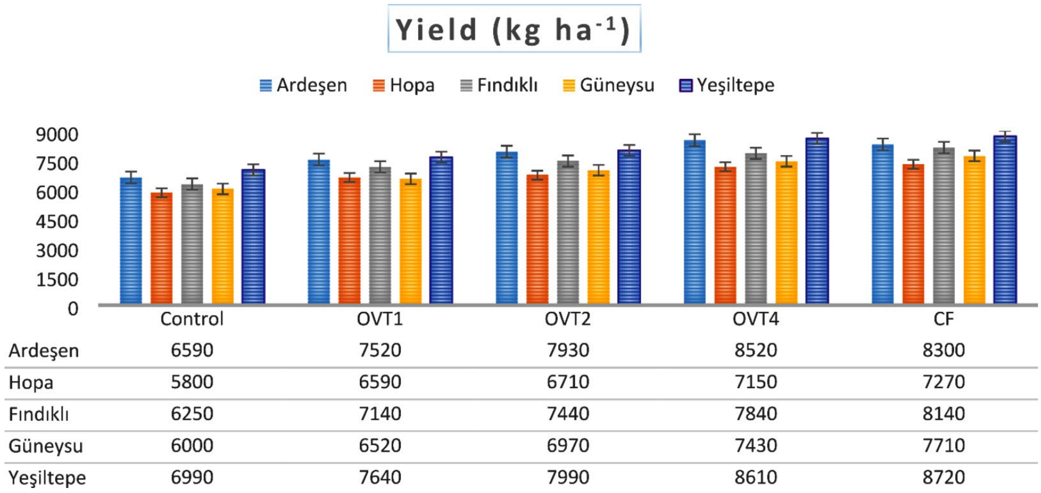


Fig. 1 Yield values of the first, second, and third sprout periods of the tea plant



	Control	OVT1	OVT2	OVT4	CF
Ardeşen	6590	7520	7930	8520	8300
Hopa	5800	6590	6710	7150	7270
Fındıklı	6250	7140	7440	7840	8140
Güneysu	6000	6520	6970	7430	7710
Yeşiltepe	6990	7640	7990	8610	8720

Fig. 2 Two-year average yields of tea plants with OVT and CF applications across various plantation sites

Table 2 The average amino acid content of tea sprouts harvested during three germination periods in the first and second year in relation to OVTs and CF applications

	First year					Second year				
Amino acid	**control**	**OVT1**	**OVT2**	**OVT4**	**CF**	**control**	**OVT1**	**OVT2**	**OVT4**	**CF**
Asp	271.20b	412.00a	414.40a	458.80a	422.40a	280.60b	425.40a	433.60a	495.40a	442.60a
Glu	159.60b	235.80a	245.60a	216.20a	280.80a	163.80b	241.60a	255.40a	235.60a	243.20a
Asn	296.60b	489.8a	447.80a	423.40a	481.60a	315.00c	528.80a	470.00ba	437.20b	495.20ba
Ser	270.00b	506.60a	480.80a	443.60a	498.00a	278.20b	524.00a	509.40a	481.60a	523.40a
Gln	132.20b	182.00a	187.00a	169.00a	186.00a	136.20b	185.80a	193.80a	184.20a	195.80a
His	166.00b	213.80a	242.00a	205.20a	216.80a	175.80c	230.60ba	254.20a	213.60b	223.40ba
Gly	157.00b	222.00a	248.20a	226.40a	229.40a	162.60b	236.40a	262.80a	245.60a	243.20a
Thr	140.20b	294.40a	267.20a	259.80a	307.00a	143.40b	299.80a	277.40a	282.40a	325.40a
Arg	362.20a	439.60a	451.60a	427.40a	433.20a	382.60a	474.20a	473.20a	445.00a	446.00a
Ala	111.20b	127.00b	174.20a	218.20a	205.00a	115.20b	131.40b	186.00a	238.60a	219.00a
Tyr	117.00b	397.60a	374.40a	347.40a	381.60a	119.80b	406.40a	388.80a	377.60a	404.80a
Cys	128.40d	148.00c	177.00b	203.80a	202.40a	135.60d	160.40c	186.40b	209.80a	208.60a
Val	224.80b	352.80a	375.20a	375.80a	370.80a	231.6b	365.60a	399.60a	409.60a	392.20a
Met	139.60b	252.60a	289.20a	276.20a	281.00a	143.6b	259.4a	299.60a	301.20a	298.40a
Try	145.40b	307.40a	322.60a	363.00a	368.40a	154.00b	334.40a	341.00a	375.00a	379.20a
Phe	674.20b	819.00ba	807.20ba	831.80ba	867.00a	693.40b	849.00a	852.60a	895.60a	908.00a
Ile	140.00a	218.20a	232.60a	216.20a	229.00a	145.00a	224.80a	244.20a	237.00a	235.80a
Leu	184.80b	187.40b	243.60ba	270.00a	277.20a	195.80c	202.80bc	254.20ba	276.80a	284.80a
Lys	186.20d	230.20c	302.00b	356.60a	352.40a	193.40d	237.60c	319.40b	386.20a	370.80a
Hydroxproline	181.00c	214.00c	291.60b	363.20a	339.40a	186.60c	219.20c	302.80b	395.80a	358.40a
Sarcosine	294.80d	348.60c	377.00cb	434.80a	404.80ba	311.60c	376.00b	394.20b	444.80a	416.20ba
Pro (pmol/ul)	261.80a	217.00b	175.60c	155.60c	215.00b	269.40a	224.60b	186.20c	168.60c	226.80b

Data followed by a different letter were significantly different according to Duncan's Multiple Range Test.($p < 0.05$)

and chemical fertilizer on nutrient content during the first, second, and third sprouting stages were statistically significant ($p < 0.05$). In the two-year average, the highest nutrient contents were obtained by applying CF and OVT4. According to the average values across all regions, the application of OVT4 increased the uptake of 63% N, 18% K, 75% P, 21% Mg, 19% Na, 29% Ca, 28% Zn, 11% Cu, and 24% Mn compared to the control group. The application of chemical fertilizers increased the uptake of 75% N, 21% K, 75% P, 21% Mg, 28% Na, 27% Ca, 30% Zn, 18% Cu, and 31% Mn compared to the control group (SI 1, 2).

The effect of OVTs and CF on the amino acid content of tea sprouts during the two-year three-sprout periods of the tea plant was found to be statistically significant ($p < 0.05$) (Table 2). Over the two-year period, an increase in the amino acid content of the tea sprouts was observed compared to the control group, except for proline. These increases are as follows: Aspartate (Asp) (51.76, 53.68,

Table 3 The average organic acid content of tea sprouts harvested during three germination periods in the first and second year in relation to OVTs and CF applications

	First Year					Second Year				
Organic acids	**control**	**OVT1**	**OVT2**	**OVT4**	**CF**	**control**	**OVT1**	**OVT2**	**OVT4**	**CF**
Oxalic	17.16d	28.90bc	34.67b	40.85a	22.67 cd	18.11d	31.14cb	36.47b	42.20a	21.21dc
Propionic	37.16d	48.86cb	57.06ba	65.99a	41.10dc	39.13c	52.83b	60.11cb	68.07a	41.31c
Tartaric	18.30c	27.66b	34.05b	51.93a	27.28b	18.90c	28.36b	36.07b	56.21a	28.89b
Butyric	15.37d	30.26c	44.15b	57.18a	27.21c	16.32d	32.72c	46.41b	58.97a	28.14c
Malonic	16.80c	27.08b	30.96b	40.37a	30.38b	17.77c	29.44b	32.38ba	41.72a	31.42b
Malic	9.68d	21.97c	30.87b	44.85a	18.48c	10.00d	22.80c	32.68b	48.72a	19.42c
Lactic	19.36d	35.08c	46.29b	64.27a	33.71c	20.44d	37.88c	48.84b	66.43a	34.79c
Citric	19.03c	27.24cb	33.31b	55.14a	31.53b	20.14c	29.71cb	35.33b	56.71a	32.41b
Maleic	5.32c	14.60cb	18.75b	33.21a	11.29cb	5.43c	15.34cb	“9.67b	36.09a	11.95cb
Fumaric	8.81d	28.46cb	36.47b	53.32a	25.36c	9.29d	30.79cb	38.12b	55.30a	26.14c
Succinic	7.80d	32.45c	40.12b	52.95a	32.72c	8.11d	35.06c	42.12b	54.64a	33.85c

Data followed by a different letter were significantly different according to Duncan’s Multiple Range Test.($p<0.05$)

Table 4 The average Chlorophyll content and antioxidant enzyme activities of tea sprouts harvested during three germination periods in the two years in relation to OVTs and CF applications

	First Year					Second Year				
	control	**OVT1**	**OVT2**	**OVT4**	**CF**	**control**	**OVT1**	**OVT2**	**OVT4**	**CF**
CAT (EU gr/Leaf)	61.00a	40.4b	41.20ba	36.80b	53.80ba	63.80a	42.80b	43.00b	37.40b	55.20ba
POD (EU gr/Leaf)	1351.20a	1186.00ba	917.00cb	710.00c	1268.20a	1392.00a	1228.60ba	976.80cb	773.20c	1338.80ba
SOD (EU gr/Leaf)	173.40a	135.20ba	87.80cb	66.80c	137.20ba	181.20a	144.20a	91.8b	69.00b	142.00a
Chlorophyll (SPAD)	18.20d	129.60cb	152.00b	208.60a	99.00c	19.4d	139.4cb	159.00b	214.40a	102.00c

Data followed by a different letter were significantly different according to Duncan’s Multiple Range Test. ($p<0.05$)

72.92, 56.76%), glutamate (Glu) (47.62, 54.92, 39.70, 62.03%), asparagine (Asn) (66.55, 50.07, 40.71, 59.71%), serine (Ser) (88.00, 80.63, 68.77, 86.32%), glutamine (Gln) (37.03, 41.88, 31.59, 42.25%), histidine (His) (30.02, 45.17, 22.53, 28.79%), glycine (Gly) (43.43, 59.89, 47.68, 47.87%), threonine (Thr) (109.52, 92.03, 91.18, 122.99%), arginine (Arg) (22.69, 24.17, 17.13, 18.05%), alanine (Ala) (14.13, 59.10, 101.77, 87.28%), tyrosine (Tyr) (239.53, 222.30, 206.17, 232.09%), cysteine (Cys) (16.82, 37.65, 56.67, 55.68%), valine (Val) (57%) 0.41, 69.76, 72.09, 67.18%), methionine (Met) (80.79, 107.91, 103.88, 104.59%), tryptophan (Trp) (114.36, 121.64, 146.49, 149.70%), phenylalanine (Phe) (21.97, 21.37, 26.31, 29.79%), isoluecine (Ile) (55.44, 67.30, 59.02, 63.09%), leucine (Leu) (2.52, 30.79, 43.67, 47.66%), sarcosine (19.49, 27.18, 45.05, 35.39%), lysine (Lys) (23.23, 63.70, 95.68, 90.52%), hydroxyproline (17.85, 61.70, 106.47%, 89.83%) and proline (Pro) (-16.87, -31.89, -38.97, -16.83%), respectively.

The changes in organic acid content in the shoots of the tea plant due to OVT and CF of the tea plant were statistically significant ($p<0.05$) (Table 3). Over a two-year average, the highest organic acid contents were obtained with CF and an application of OVT4. Compared to the control groups, the content of organic acids increased in all regions by an average of (134.60%, 32.10%) for oxalic acid, (77.58%, 10.60%) for propionic acid, (183.33%, 50%) for tartaric acid, (272%, 80%) for butyric acid, (150%, 87.5%) for malonic acid, (236%, 74) for malic acid, (236%, 74%) for lactic acid, (190%, 63%) for citric acid, (560%, 120%) for maleic acid, (562%, 213%) for fumaric acid, and (578%, 357%) for succinic acid (Table 3).

The impact of varying OVT doses and CF on the chlorophyll content and enzyme activity of tea sprouts in the first, second, and third harvest periods over a total of two years was found to be statistically significant ($p<0.05$) (Table 4).

As a result of the 2-year field trial, the antioxidant enzyme activities in the three-season shoots of the tea plant decreased with the application of the OVTs and chemical fertilizers compared to the control groups. The reductions after the treatments (OVT1, OVT2, OVT4, and CF) were respectively CAT (33.33, 32.53, 40.54, and 12.66%), POD (11.98, 30.96, 45.93, and 4.97%), SOD (21.21, 49.35, 61.70, and 21.26%). In contrast, chlorophyll levels increased compared to the control groups, with increases of (615.43, 727.13, 1025.00, and 434.57%) respectively. Additionally, the effects of OVTs and CF applications on the chlorophyll contents and enzyme activities of tea shoots grown in tea plantations across different locations during the first, second, and third shoot periods were found to be statistically significant ($p<0.05$).

The study investigated the effects of OVTs and CF applications on yield, plant nutrients, amino acid and organic acid content, chlorophyll content, and antioxidant content involved in stress mechanisms in tea plants. The results indicated statistically significant differences among OVT1, OVT2, OVT4, and CF applications compared to the control group. However, no significant difference was observed between OVT4 and CF applications. The data were subjected to cluster analysis, revealing three distinct groups in the characteristics of tea plants resulting from these different applications. This analysis also highlighted similarities between OVT1 and OVT2 applications, and between OVT4 and CF applications. Additionally, the control group did not show similarities with the others. However, it exhibited close similarities with the OVT1 application. The findings indicate that as the quantity of OVT application rises, the outcomes tend to converge towards those obtained from CF application (Fig. 3).

Discussion

As a result of field trials conducted at different locations, the highest average yield of tea plants over two years was achieved with a CF application in conventional cultivation in the region (8030±498.2 kg ha^{-1}). In the biotechnologically developed OVT4, proposed as an alternative to chemical fertilizers, the yield was (7910±578.8 kg ha^{-1}). The average tea yield in the control groups was (6320 kg ha^{-1}). However, there was no statistically significant difference between the yields obtained with the highest-yielding CF and OVT4 application. While tea shoot yields obtained with OVT4 treatments in the Hopa, Fındıklı, Güneysu, and Yeşiltepe regions were close to those obtained with CF application, the highest yield was obtained in the Ardeşen region with an application of OVT4. Although other OVT doses produced significant yield increases compared to the control, the yield was lower compared to OVT4 and CF.

Composting tea waste is not only an environmentally friendly disposal method, but it is also expected to promote plant growth due to its nutrient and tannic acid content, creating a more productive environment [76]. Vermicompost produced from tea leaves using *Eisenia fetida* worms has been reported to significantly influence the growth of water spinach and shows promising potential as a soil fertilizer precursor [77]. Previous studies on pineapple [78], tomato [79–81], oil rose [82], and pepper [83] indicate that vermicompost applications led to yield increases. In some studies, the highest yield increases were observed when vermicompost applications were combined with chemical fertilizer applications [81, 84–86]. However, certain studies suggest that increasing doses of vermicompost applications may not always correlate positively with enhanced crop productivity, emphasizing the importance of applying it at optimal rates [83, 87–90]. The present study found

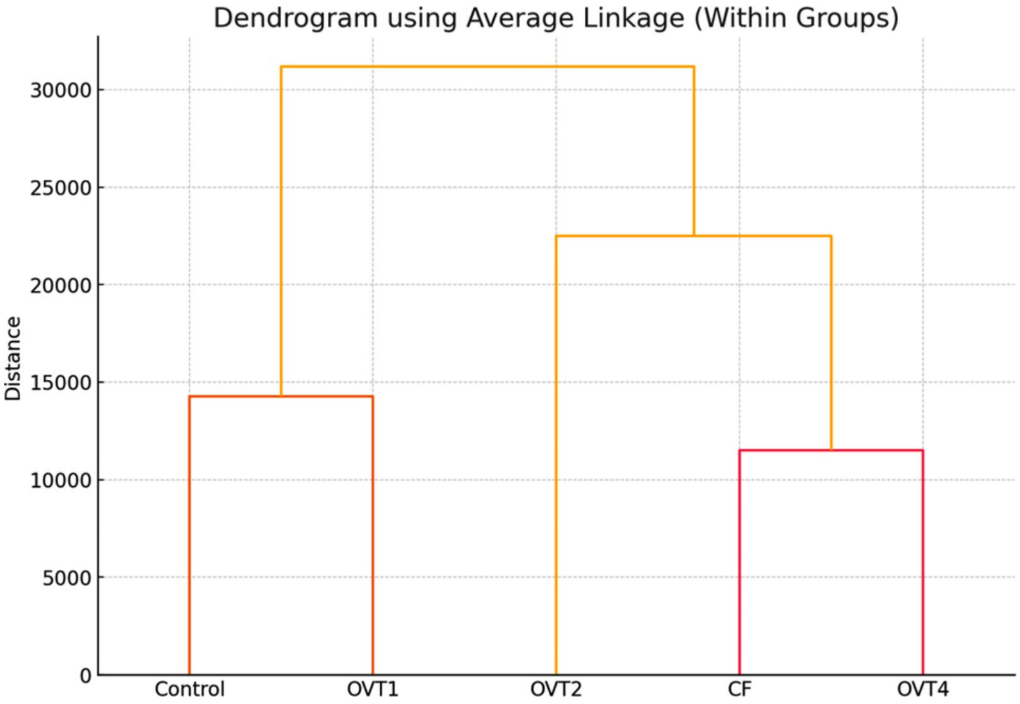


Fig. 3 Decnt yield, plant nutrients, amino acid and organic acid content, chlorophyll content, and antioxidant content as a function of OVT and CF applications

a direct correlation between the amount of OVT application and the average yield increase over two years. However, previous studies have reported that increased compost application rates do not always result in higher yields [83, 87–90]. Therefore, based on the results of the two-year field trials, the optimal application rates were determined using a linear relationship. These estimates aim to serve as a guideline for future studies and provide practical recommendations for achieving the highest possible yield. According to these estimates, the application of 4122.30 kg ha^{-1} of OVT in Ardeşen could potentially yield 8505.60 kg ha^{-1} of tea ($y= -0.0011\times^2+0.9069x+6627.30$, $R^2=0.9919$) In Hopa, a yield of 7828.50 kg ha^{-1} could also be expected at an application rate of 4097.50 kg ha^{-1} ($y= -0.0008\times^2+0.6556x+5856.40$, $R^2=0.9539$), while in Fındıklı a yield of 7511.40 kg ha^{-1} is predicted at an application rate of 5014.20 kg ha^{-1} ($y= -0.0012\times^2+0.8574x+6280.80$, $R^2=0.9832$). In Guneysuyu, an estimated tea yield of 5210.00 kg ha^{-1} is expected at an application rate of 4097.50 kg ha^{-1} ($y= -0.0006\times^2+0.6017x+5996.80$, $R^2=0.9996$), and in Yeşiltepe, a yield of 8645.10 kg ha^{-1} could be achieved at an application rate of 5210.00 kg ha^{-1} ($y=-0.0006\times^2+0.6252x+7010.20$, $R^2=0.9950$).

The levels of essential plant nutrients in leaves, referred to as critical concentrations, play a crucial role in assessing the nutritional status of plants [89, 91]. The two-year experiment results indicated that the application of OVTs and CF statistically increased nutrient concentrations in the tea plant. However, the most significant difference was observed between OVT4 and CF. It can be affirmed that this finding supports nitrogen nutrition in all regions, where the application of OVT4 provides nitrogen nutrition similarly to chemical fertilizers, with no statistical difference. Indeed, numerous studies using vermicompost have reported a significant increase in nitrogen concentration in plants [1, 85, 92]. Literature research [93–97] has indicated that soils in the tea-growing areas of the Artvin region have very low nutrient content. According to the study results, OVT applications were found to significantly increase the content of both macro and micronutrients in the plant. The application of OVT4 and CF significantly increased the uptake of various nutrients (N, K, P, Mg, Na, Ca, Zn, Cu, Mn) compared to the control group. This is due to the improved availability of these nutrients in the soil. The organic matter in OVT helps in chelating nutrients, making them more accessible to the plant roots, whereas CF directly supplies these nutrients in a readily available form. Previous studies have indicated that vermicompost application enhances P mineralization in the soil [98, 99], and it serves as a fertilizer that enriches the soil with N, P, K, and micronutrients [78, 100–103]. The increase in yield and nutritional value of the tea plant with the application of OVT4 was not statistically different from the application of chemical fertilizers. The similar increases provided by OVTs and CF can be attributed to the effect of OVT on the physicochemical structure of the soil in tea plantations and its support of soil reactions with its microbial and enzymatic richness [104, 105].

Amino acids play a crucial role in the accumulation of osmolytes, forming a part of the stress tolerance mechanism [106]. They serve various functions, including promoting plant growth [107], regulating intracellular pH, detoxifying reactive oxygen species (ROS) and xenobiotics [108], facilitating mineral uptake and participating in signaling [109–112]. The increase in amino acid content (except for proline) suggests that OVT and CF applications boost protein synthesis and overall metabolic activity in tea plants. Amino acids like aspartate, glutamate, and others are precursors for various metabolic pathways and are supposed to contribute to plant growth and stress response. The significant increase in amino acids such as threonine, alanine, tyrosine, and tryptophan was considered to indicate an enhanced capacity for protein synthesis and stress adaptation. AAs, considered biologically active compounds [113], enhance growth or transformation rates, particularly under challenging stress conditions during the developmental phase of plants [114]. To accomplish this, plants require amino acids present in their physiological structure, serving as fundamental components in protein processes, especially during critical growth phases, with approximately 20 essential AAs involved in each process [89]. However, plants expend substantial energy for amino acid production during this phase. According to the findings of this study, the AA contents of tea sprouts treated with OVTs were significantly higher compared to the control group, indicating that the OVT application primed the tea plants to respond to stress. Moreover, the elevated AA content in the shoots contributed to increased yield by reducing the stress on the tea plants. Organic acids play a critical role in plant metabolism, including respiration, photosynthesis, and stress tolerance. The increase in organic acids (oxalic acid, propionic acid, tartaric acid, etc.) in plants treated with OVT and CF suggests enhanced metabolic activity and improved stress response mechanisms. Organic acids are suspected to contribute to nutrient solubilization and uptake, further supporting plant growth. The metabolism of organic acids (OA) is of fundamental importance at the cellular level throughout the plant. OAs constitute a significant portion of root exudates and serve as intermediate products in the tricarboxylic acid cycle of cellular metabolism. Many environmental stresses promote the biosynthesis of OA and their release from the roots. However, research on the mineral nutrition of OA metabolism in tea is limited. Tea is primarily cultivated in acidic soils with a pH ranging from 4.5

to 5.5, across humid, semi-humid, tropical, subtropical, and temperate regions [115]. In acidic soils, the high P deficiency results in yield and quality losses, as soil phosphorus converts to insoluble forms with Fe and Al. The negative charge of the carboxyl groups of OAs enables them to react with positively charged cations such as Al and Mg, forming insoluble complexes with inorganic phosphorus in the soil [116]. This property is more pronounced as the number of carboxyl groups increases, making it most effective in binding low molecular weight OAs like citrate and malate to metal cations, separating them from inorganic phosphate-bound complexes in the soil [117]. Other OAs, such as oxalate and gluconic acid, have demonstrated utility in increasing the availability of inorganic phosphate in soil for certain plants [118, 119]. Various OAs have also been reported to be excreted in response to Fe, Mn, Zn, and P deficiency [120–125]. According to the study results, the application of OVT 4000 kg ha^{-1} maintained the organic acid content in tea plants at the highest level, consequently enhancing nutrient uptake. This can be attributed to the increased tea yield, serving as an indicator of the well-nourished state of tea plants.

The balance of the microbial flora in agricultural soils plays a fundamental role in productivity, forming the foundation for effective organic matter mineralization. Consequently, AAs serve as precursors or enhancers of phytohormones and growth factors. Methionine, for instance, is a crucial growth factor stabilizing the cell walls of the microbial flora. Glutamic acid and aspartic acid generate other AAs through transamination. Proline uniquely influences the plant's hydrogen balance, reinforcing cell walls, and enhancing resistance to adverse climatic conditions. Alanine, valine, and leucine contribute to improved fruit quality, while histidine aids in proper fruit ripening. The increased levels of all amino and organic acids in the tea plants developed and treated with OVT4 led to elevated nutrient content and positively impacted fruit quality and the activity of antioxidant enzymes. Plants employ strategies to counteract the negative effects of biotic and abiotic stress conditions. Under stresses, plants may excessively produce ROS, leading to peroxidation of vital cellular components. To counter this, plants deploy an effective defense system comprising enzymatic and non-enzymatic antioxidants like CAT, POD, and SOD [126]. These defense mechanisms efficiently convert superoxide radicals into H_2O_2 and directly detoxify ROS into water and oxygen [127], involving non-enzymatic low molecular weight antioxidants such as proline, ascorbic acid, and glutathione [54, 128, 129]. Numerous studies indicate that abiotic stress can increase MDA and H_2O_2 levels in plants, leading to elevated membrane lipid peroxidation and cell damage [113, 130–135].

The significant increase in chlorophyll content in the OVT and CF treatments indicates improved photosynthetic capacity, and chlorophyll is necessary to capture light energy. According to the study results, these increased levels indicate better energy uptake and utilization for growth. In addition, OVT4 is thought to provide the plant with micronutrients such as magnesium (an important component of chlorophyll) in a more bioavailable form, which should improve chlorophyll synthesis. Comparatively higher levels of OAs and chlorophyll were obtained by applying OVTs to tea plantations, as opposed to control plants and those treated with CF. The significant increase in chlorophyll content in the OVT and CF treatments indicates improved photosynthetic capacity, and chlorophyll is necessary to capture light energy. According to the study results, these increased levels indicate better energy uptake and utilization for growth. In addition, OVT4 is thought to provide the plant with micronutrients such as magnesium (an important component of chlorophyll) in a more bioavailable form, which should improve chlorophyll synthesis. Additionally, in the current study, lower antioxidant enzyme activities and proline contents were observed depending on OVT treatments. Antioxidant enzymes such as CAT, POD, and SOD are crucial for the reduction of oxidative stress in plants. It can be explained by lower enzyme activity in the treated plants, which could indicate lower oxidative stress due to better nutrient availability and overall better plant health. It is thought that OVT4 treatment likely increases plant stress tolerance by maintaining a balance between the production of reactive oxygen species and the scavenging of these species, thus reducing the need for high antioxidant enzyme activity. These findings suggest that tea plants experience less stress when OVT is applied, indicating good nutrition, tea quality, and a current increase in productivity. It is established that the activity of SOD and CAT increases under stress conditions with high ROS production in plants [136]. Research indicates that the activity of SOD and CAT enzymes decreases in *Withania somnifera* (L.) treated with vermicompost, reflecting low/balanced ROS production and improved plant health [137]. Increased CAT helps mitigate the hazardous effects of H_2O_2 and restores tissue metabolism equilibrium [138]. Upregulated SOD is crucial in countering oxidative stress induced by various abiotic stressors, ensuring plant survival [89, 131, 136, 139]. Numerous studies affirm that increased activities of these antioxidant enzymes are directly linked to free radical detoxification, protecting plants from oxidative bursts and reducing oxidative damage [140, 141].

Conclusion

Raw organic tea vermicompost, a nutrient-rich organic material produced by worms and bacteria's controlled decomposition of raw tea waste and enriched with various enzymes, significantly increased productivity and closely approached that of chemical fertilizers when 4000 kg ha^{-1} was applied. It is expected that the yields obtained with this organic tea-worm compost will reach a level comparable to that of conventional chemical fertilizers in the long term, as it has a health-promoting effect by improving the biological and physicochemical structure of the soil. In addition, the current study found that applying organic tea worm compost significantly increased the nutrient content of tea plants, including essential macro- and micronutrients, amino acids, and organic acids. This nutrient availability and uptake improvement contributed to a higher tea yield and positively affected plant health and stress tolerance mechanisms. In addition, the study showed that the application of organic fertilizers, especially OVT4, reduced stress indicators such as antioxidant enzyme activity and proline content. The tea plants were thus protected from oxidative damage. This indicates that organic fertilizers provide important nutrients and help to improve plant resilience to biotic and abiotic stress factors, leading to sustainable tea production. Organic tea worm compost was also a good alternative for sustainable tea cultivation. In addition, the current study determined the optimal application rates of organic worm manure to maximize tea yields in different locations. This underlines the importance of precise fertilizer application to achieve the desired results.

Overall, the results show the potential of raw tea compost, produced using new biotechnological methods, as an alternative to chemical fertilizers in tea cultivation. Using raw tea compost can improve the yield and quality of tea and contribute to environmental sustainability by promoting the recycling of organic waste and reducing the use of chemicals in agriculture. Therefore, integrating organic fertilizers in tea cultivation can lead to more resilient and environmentally friendly tea-growing systems.

Supplementary Information

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Supplementary Material 1

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by A.K, Y.İ, N.K, M.T, S.A, E.Y, G.G, N.E, A.G, H.K, B.G, A.B, Ö.A, and M.A. The first draft of the manuscript was written and interpreted whole parameters by AK. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript and supplementary information files.

Declarations

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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