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Metabolomic exploration of CTC tea manufacturing waste validates its potentiality as organic fertilizer

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ABSTRACT

Valorization of agro-industrial waste resources is today's main focus for agribiotechnologists. This research work was designed to valorise tea industrial waste, i.e., manufactured by-products from crush-tear-curl (CTC) tea factory. Physicochemical analysis has been carried out to characterize tea waste treated soil. Pot experiment with cowpea [*Vigna unguiculata* (L.) Walp.] was considered to study the impact of tea waste on plant growth. Morphological parameters such as length of plants and pods, and girth diameter were considered for growth study. Effect of tea factory waste on soil nutrition was found remarkable with increased organic carbon, organic matter, nitrogen, phosphorus, potassium and sulphur content. Pot culture revealed impact of tea waste composted soil on boosted plant growth. GC-MS based metabolite profiling revealed xanthosine and caffeine as major compounds in tea waste extract. A possible pathway has been proposed to explain the role of xanthosine and caffeine breakdown in fertilization of soil and plant growth. Disposal of tea wastes produced during tea manufacturing can be managed in a sustainable manner if this research is implemented industrially. This research portrays a notable nutrient richness in tea waste treated soil. Detection of purine metabolites revealed remarkable fertilizing and plant growth promoting properties of CTC tea waste.

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1. Introduction

Fertilization of soil is an essential factor that provides nutrients for boosting crop productivity and quality. Substantial employment of chemical fertilizer has caused depletion in soil health by affecting the function of soil microorganisms. Excessive use of such chemicals has given rise to serious problems on soil fertility, crop production, environment and human health which have stressed scientists, biotechnologists, activists, and policy makers. So, presently, organic manure and bio fertilizers have emerged as alternative and effective options for fertilizing soils without damaging the environment (Mekki et al., 2017; Ngan and Riddech, 2021). Green waste management and organic farming are two sides of the same coin because organic or biodegradable waste exhibits its necessity as far as organic agriculture is concerned. Compost manuring is an interesting agronomic practice as well as an attractive waste management strategy. Crop residues, manures and compost from organic wastes not only fertilize the soil but also improve its physical properties that have been affected due to overuse of inorganic substances since ages (Annabi et al., 2017). Proper utilization would have rather portrayed waste as a solution, not a problem. Recycling and effective valorization of different agro-industrial waste resources are reported by researchers around the world. Recovery of value-added compounds and conversion of wastes into various processed materials are such economic and environmental approaches, which can reduce the problems regarding conventional disposal of waste.

From producers to consumers, plantation workers to tea stalls, the whole tea industry connecting chain produces vast number of bio-wastes, i.e., pruning litters from gardens, post-processing fiber rich waste tea from factories and spent or leftover tea or remainders from tea stall and households. Tea factory waste is a resource of stalks, hard leaves, stems, and other fiber rich parts of the tea plant (Majumder et al., 2022). Extraction of caffeine from industrial tea waste is referred to as the most popular and effective method so far which is used by multiple tea factories to make profit (Shalmashi et al., 2010; Khan et al., 2018). Moreover, it was reported that tea factory waste could be utilized as a source of renewable energy to produce biochar and bio methane (Majumder et al., 2022). Being a novel adsorbent of toxic pollutants, industrial tea wastes are often used to remove toxic substances like heavy metal ions, dyes, phenols, antibiotics, benzene etc. from wastewater (Hussain et., 2018; Kabir et al., 2021; Majumder et al., 2022). Feeding livestock i.e., pigs, poultry and ruminants (Jayasuriya et al., 1978; Angga et al., 2018) recovery of tannin and antioxidant polyphenolic compounds (Abdeltaif et al., 2018; Rajapaksha et al., 2020) and production of bioactive probiotic beverage kombucha (Majumder et al., 2022) are various other ways to recycle the fiber rich tea factory waste as demonstrated by agribiotechnologists and food scientists. As organic fertilizer and priming agent, scientists (Gurav and Sinalkar, 2013; Gammoudi et al., 2021) proved functionality of leftover tea (spent tea waste). People around the world who have been using this kitchen waste as compost for houseplants and gardens since ages. According to Tea Board of India, "tea waste" means tea sweepings, tea fluff, tea fiber or stalks or any article purporting to be tea which does not conform to the specification for tea laid down under Prevention of Food Adulteration Act. 1954 (37 of 1954) but does not include green tea or green tea stalks and minimum volume of tea waste and made tea should be at the ratio of 2:100 kilograms (Anonymous, 2022a).

In this context, the aim of this research was to study the effect of tea factory waste on enhancement of soil nutrition and its impact on plant growth. To achieve these goals, physicochemical analysis on tea waste treated soil were conducted along with a simple pot assay. GC-MS analysis of CTC (crush-tear-curl, a type of processed black tea) waste extract has also been carried to detect the metabolites responsible for soil fertilizing and promoting plant growth. Furthermore, results of soil analysis and pot assay have also been elucidated with a metabolomic discussion.

2. Materials and methods

2.1. Collection of tea waste, soil preparation and composting of tea waste for pot experiment

Post-manufacturing tea waste was collected from CTC tea factory of Jayantika Tea Estate, Darjeeling (26°32'06.0"N, 88°16'18.0"E). For pot experiment, garden soil was taken from a barren land and sun-dried for three days. The soil was ground, sieved through 2 mm filter and homogenously mixed (Ngan and Riddech, 2021). Cylindrical plastic pots having 15 cm depth and 5 cm diameter were taken. One and half kilogram of dried soil was placed in each pot.

A total of twenty pots were prepared with dried soil and out of which ten were treated with 5% (w/w) tea waste, i.e., seventy-five grams of dried tea waste in each pot, for composting tea waste to prepare tea waste treated soil (TWS). Other ten replicas were considered as control soil or CS. All the CS and TWS pots were irrigated on a regular basis with equal amount of tap water and tilled or mixed daily up to three months using a tilling fork to avoid any weed growth. Just before sowing seeds, soil samples were collected from all the pots to examine nutritional values as described below.

Table 1. Physicochemical characters of control soil (CS) and tea waste treated soil (TWS)

Physicochemical parameters	Sample	Results
pH	CS	6.14 ± 1.02^{b}
	TWS	4.5 ± 0.87^{a}
EC (mS/m)	CS	78 ± 14^{a}
	TWS	196 ± 6^{b}
Moisture content (%)	CS	18.66 ± 3.91^{a}
	TWS	31.23 ± 2.17^{b}
Organic carbon (%)	CS	0.98 ± 0.26^{a}
	TWS	5.06 ± 0.6^{b}
Organic matter (%)	CS	1.69 ± 0.31^{a}
	TWS	8.71 ± 0.87^{b}
Total nitrogen (%)	CS	0.13 ± 0.02^{a}
	TWS	0.52 ± 0.1^{b}
Available phosphorus (ppm)	CS	51 ± 4^{a}
	TWS	72 ± 2^{b}
Available potassium (ppm)	CS	25 ± 6^{a}
	TWS	201 ± 24^{b}
Available sulphur (ppm)	CS	10 ± 2^{a}
	TWS	29 ± 1^{b}

The superscript letters in the same column represent statistically ($p \leq 0.05$) different groups for each parameter.

2.2. Analysis of soil nutrients with selected physicochemical parameters

CS and TWS samples from all twenty pots (ten replicates for each sample) were air dried and sieved through 2 mm filter. For organic carbon determination, the samples were further grind and sieved through a 0.5 mm filter. pH, electrical conductivity (EC), moisture content, organic carbon (OC), total nitrogen (N), available phosphorus (P), available potassium (K) and available sulphur (S) were assessed on both samples. The pH and EC were measured in water extracts (20 g soil in 100 mL double distilled water) using a pre-calibrated pH meter and EC meter respectively (Ghosh et al., 2022). Moisture content percentage of soil samples were determined following protocol of Ghosh et al. (2022). OM (%) and OC (%) were assessed following the titration method developed by Walkley and Black (Walkley and Black, 1934). Total N (%) was measured by Kjeldahl method (Kirk, 1950). Available P was extracted and measured using the colorimetric method with molybdenum (Bray and Kurtz, 1945). Estimation of available K was done by using a flame photometer (Jackson and Smith, 1956). Available S was figured out following the protocol of Williams and Steinbergs (1962). Values of available P, K and S were calculated based on respective standard curves and results have been expressed as parts per million or ppm.

2.3. Pot experiment

Cowpea [*Vigna unguiculata* (L.) Walp.] seeds were bought from a local market of Siliguri (Darjeeling district of West Bengal, India). Seeds were dipped in warm tap water (60 °C) for 1 h (sinker-floater test) to determine healthy seeds. Twenty of those healthy seeds were taken up and sown in CS and TWS pots which were being prepared for three months as described earlier. The incubation was conducted for seventy days and during that period, every pot was irrigated with equal amount of tap water. From vegetative growth to reproductive development- a detailed plant growth study was done through morphological characterization. Germination period, foliation and flowering stages, leaf count, plant height, girth diameter, fruit setting period and fruit length were recorded regularly on every fifth alternate day. Single or only one fruit bearing plants were considered standard for this comparative study. So, data of three of such pots (for each CS and TWS) were taken from the records for statistical analysis. After one month of pot culture, plants were harvested and post-harvest morphological characters like weight (both fresh and dry) and length of a whole plant and its parts (stem, root, and the fruit); number of seeds inside the fruit; and number and weight of nodules present in root were recorded.

2.4. GC-MS analysis on tea waste extract

Tea waste (1% w/v) was soaked in freshly boiled hot water for fifteen minutes. After this hot-water extraction, 1 mL of the extract was dried and dissolved in 1 mL of methanol to prepare the sample for GC-MS analysis. GC-MS analysis was done following the protocol of Majumder et al. (2021). One microliter of sample (in 20:1 split ratio) was injected in GCMS-QP2010 Plus (Shimadzu Co., Japan). DB-5 fused-silica capillary column (0.25 µm x 0.25 mm x 30 m) was used. Interface and source temperature was set to 270°C and 230°C, respectively. Helium was used as carrier gas. Total flow rate was 16.3 mL/min and column flow rate stood at 1.21 mL/min. Mass spectra were recorded at 5 scan/sec with a scanning rate of 40-650 m/z. Compounds were identified after comparing the spectral configurations obtained with that of available mass spectral databases (NIST08s.LIB and WILEY8.LIB). The chromatogram (TIC or Total Ion Chromatogram) is based on the intensity of fragments produced by the ionization. Quantification of the amount (area %) of each compound was done based on peak areas (Majumder et al., 2020; Acharyya et al., 2021). The data obtained from GCMS analysis were further studied from available reported scientific literature to find out responsible growth promoting components or priming agents present in tea waste.

2.5. Statistical analysis

Data obtained from the various experiments during this research were analyzed statistically using Microsoft Excel. Results have been expressed as mean ± SD (n = 3). The test for statistical difference was performed using the Student's t-test to compare the means between two treatments (CS and TWS). Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of tea waste on soil nutrients

Results of soil nutrient analysis represented in Table 1, exhibits a noticeable variation of physicochemical parameters between CS and TWS. Soil was turned into more acidic due to tea waste treatment as the pH value of TWS was found 4.5±0.87 while for CS it was recorded 6.14±1.02. Moisture content in TWS was as high as 31.23±1.17% and for CS it was only 18.66±0.91%, despite the same amount of irrigation. Composting of CTC tea waste in soil has enriched other nutrient values too. Amounts of N, P, K and S for CS were 0.13±0.02%, 51±4 ppm, 25±6 ppm and 10±2 ppm respectively while after composting, the same soil showed very much high values i.e., 0.52±0.1%, 72±2 ppm, 201±24 ppm and 29±1 ppm for N, P, K and S respectively.

3.2. Effect of tea waste on growth and development of cowpea plant

Experimental data collected during pot-culture and postharvest periods have been studied to evaluate the effect of tea waste on plant growth and development which have been discussed below.

Various stages such as seed germination period, sprouting, flowering, fruit-setting, and fruit maturation phases were noted since sowing of seeds to determine time taken by each plant to accomplish each event. Table 2 has been provided to express the results that shows, from germination to fruit maturation, plants grown on tea waste compost (TWS) took lesser time to succeed every growth level compared to the plants grown on control soil (CS). On TWS, germination was happened after 7.57±0.76 d while for CS, it took 9.5±0.36 d. Similarly, sprouting of leaf on CS plants was observed on 12±0.4 d where TWS plants took only 10±0.9 d. Moreover, in CS plants, no flowering was there till thirty days of pot experiment while on TWS, the first flower was already bloomed on 28.2 d. Both fruit-setting and fruit maturation stages were faster in TWS plants with 33.87±0.21 d and 49.60±1.31 d accordingly compared to CS plants where maturation process was detected even after sixty days of pot culture (Table 2, Figure 1). Maturation or ripening was determined by the change in pod's color (turning into whitish yellow from green) and raise in pod's length which was immobilized after a certain period (Figure 1).

Being deciduous, leaves of bean plants were seen to be shed during growth resulting an irregular foliage which was contradictory in this relative study. However, leaves produced by each plant during the whole pot culture period were counted. TWSP recorded high with a total of 47.17±2.33 leaves production in each plant while each CSP produced only 29.29±1.07 leaves. After seventy days of pot culture, plant height development was found higher in plants grown in TWS (from 1.8±0.36 cm to 23.13±3.53 cm) compared to the CS plants (from 1±0.12 cm to 16.33±0.25 cm). Results of girth diameter did not differ significantly CS and TWS plants, but the graphical representation (Figure 1) never failed to keep TWS ahead of CS. In case of TWS fruits, the rate of pod enlargement was lofty upto fifteen days from fruit set before attaining maturity. Meanwhile, CS fruits took about twenty-five days to achieve the same and the growth was also nowhere near to TWS as shown in the graph (Figure 1). Fresh and dry weight, moisture content and length of different parts i.e., stem, root and fruit from harvested plants were recorded and the results have been given in Table 3. A lower content of moisture signifies a higher mass or dry matter content, which was seen in TWS plants. Moreover, not only growth and development but also production was influenced by tea waste. In TWS plants, 16-18 seeds or beans were found in each pod while the numbers were lower in CS plants with 8-10 beans only.

Table 2. Effect of tea waste treated soil on germination period, sprouting, flowering, fruit-setting, and fruit maturation phases compared to control soil.

	Germination		Sprouting		Flowering		Fruit-Setting		Fruit Maturation	
	CS	TWS	CS	TWS	CS	TWS	CS	TWS	CS	TWS
Mean	9.5±0.4^{b}	7.6±0.7^{a}	12±0.4^{d}	10±0.9^{c}	31.2±1.6^{e}	29.5±1.5^{e}	35.8±1.6^{g}	33.9±0.2^{f}	58.2±2.7^{i}	49.6±1.3^{h}
Min.	9.1	6.7	11.6	9.1	30.3	28.2	34.9	33.7	55.2	48.2
Max.	9.8	8.1	12.4	10.9	33.1	31.1	37.7	34.1	60.3	50.8

The test for statistical difference was performed using ANOVA (analysis of variance). Values are means ± standard deviation, means with different superscripts in are significantly different (P < 0.05).

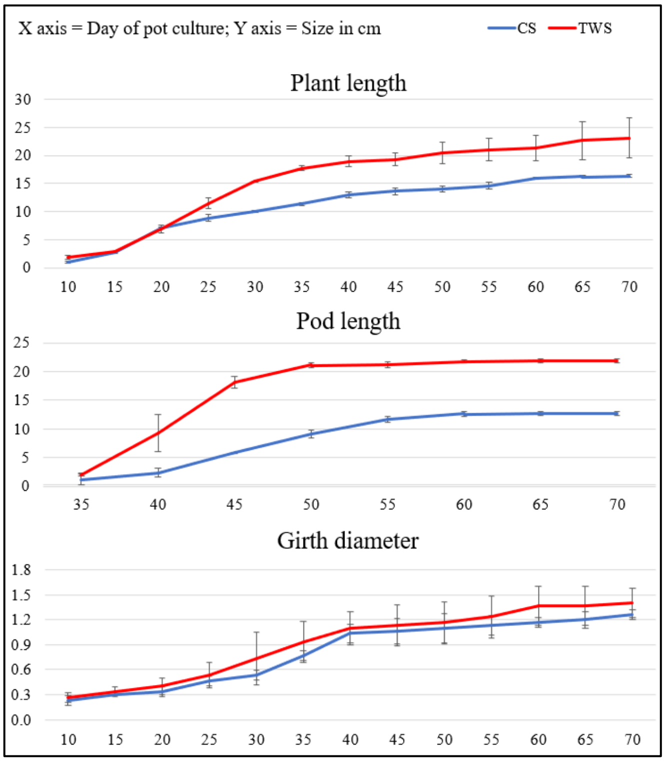

Figure 1. Impact of tea waste compost (TWS) on plant growth i.e., plant length, pod length and girth diameter. Values are means ± SD (n = 3).

13-18 nodules (158.33±49.74 mg of total weight) were obtained from each CS plant while in TWS, the count was ranged between 8 to 11 (73.33±11.72 mg of total weight) for each plant. Most certainly, tea waste composting left an effect on soil nitrogen status as described earlier. Moreover, not only soil nitrogen availability but also accumulation of that nitrogen was high in TWS plants which was clearly reflected by other results of this pot experiment. Additionally, GC-MS based metabolomics was considered to evaluate the results mentioned above.

3.3. *Responsible plant growth promoting compounds in tea waste*

Metabolite profiling of the waste extract revealed twelve different components (Table 4) dominated by xanthosine with a share of 68.89% peak area and caffeine (5.19%) (Figure 2). Detection and isolation of caffeine from tea waste were already recounted in various research papers (Shalmashi et al., 2010; Khan et al., 2018; Majumder et al., 2022). Xanthosine is just another metabolite of the same pathway by which caffeine is biosynthesized, i.e., tea's purine metabolic pathway. Xanthosine is the precursor of caffeine as well (Majumder et al., 2022). Presence of these two substantial nitrogenous components in tea waste extracts surely gives an account of TWS's fertilizing properties. Moreover, by freeing ribose (five-carbon sugar), xanthosine certainly degrades into xanthine which is reported as a plant growth promoting agent. Perhaps soil microbes helped this purine degradation process during composting of tea waste.

Table 3. Effect of tea waste treated soil (TWS) on selected parameters considered to evaluate harvested plants in comparison with control soil (CS).

	Stem		Root		Fruit	
	CS	TWS	CS	TWS	CS	TWS
Fresh weight (g)	6.87±0.23^{a}	9.76±1.34^{b}	3.20±0.65^{c}	4.61±1.32^{d}	5.9±0.36^{e}	9.86±0.47^{f}
Dry weight (g)	1.98±0.02^{e}	3.01±0.8^{f}	0.79±0.02^{a}	1.38±0.52^{c}	0.91±0.02^{b}	1.83±0.05^{d}
Moisture content (%)	71.15±1.2^{a}	69.41±2.44^{a}	75.17±0.77^{c}	70.02±2.21^{b}	84.37±0.8^{e}	81.54±0.66^{d}
Length (cm)	16.33±0.25^{b}	23.13±3.52^{e}	16.01±0.39^{b}	20.1±2.36^{c}	12.47±0.38^{a}	21.6±0.36^{d}

Values are means ± standard deviation, means with different superscripts in each row are significantly different (P < 0.05).

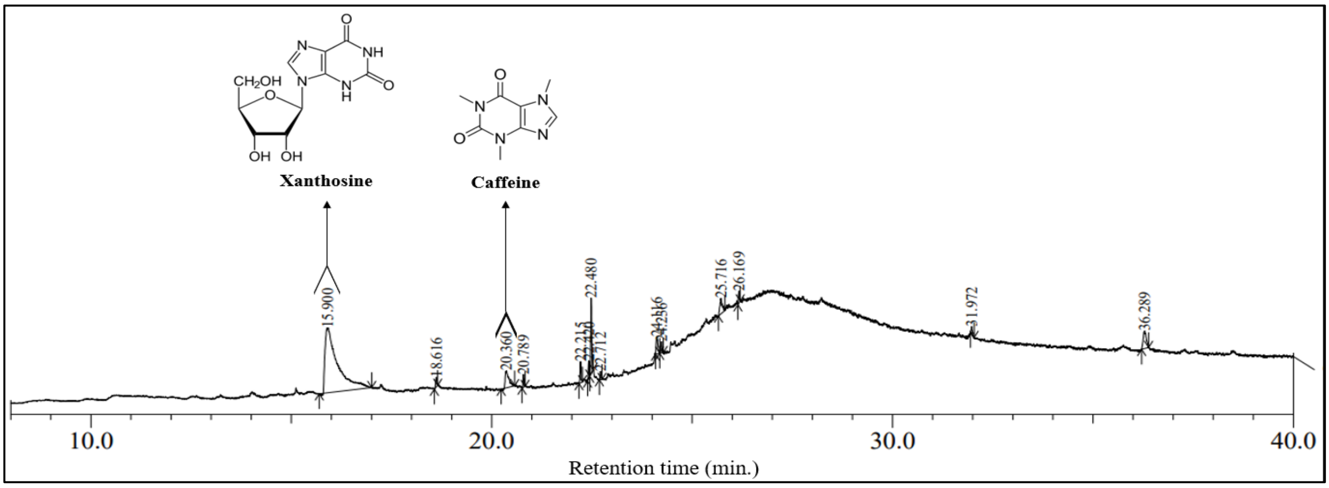

Figure 2. GC-MS total ion chromatogram of tea waste extract indicating peaks of different compounds listed in Table 4. Peaks representing major compounds xanthosine and caffeine are labelled.

Table 4. Components of tea waste extract revealed through GC-MS analysis.

Peak index	Retention time	Area%	Name of compound
1	15.9	68.89	Xanthosine
2	18.616	1.04	Octadecanal
3	20.36	5.19	Caffeine
4	20.789	1.09	Stearic acid methyl ester
5	22.215	2	13-Hexyloxacyclotridec-10-en-2-one
6	22.42	1.28	Linolelaidic acid, methyl ester
7	22.48	6.41	Petroselinic acid methyl ester
8	22.712	0.73	Stearic acid methyl ester
9	24.116	2.41	(Z,Z)-3,9-cis-6,7-epoxy-nonadecadiene
10	24.236	1.19	Lauric acid, chloride
11	25.716	3.35	1-oleoylglycerol
12	26.169	1.1	Dinonyl Phthalate
13	31.972	0.81	beta-Sitosterol
14	36.289	4.53	beta-Sitosterol

3.4. Possible metabolic breakdown of xanthosine and caffeine in TWS

A set of probable pathways directing breakdown of tea waste metabolites have been proposed through a pictorial demonstration in Figure 3. Metabolic breakdowns of major tea waste compounds xanthosine and caffeine are proportional to increased nitrogen availability in soil and nitrogen accumulation by TWS plants as reflected through soil fertility status and faster growth of plants on TWS. During composting of tea waste, xanthosine and caffeine have produced xanthine which further bio-transformed into the final product of purine catabolism i.e., urea. During this conversion, compounds such as uric acid, hydroxyisourate, OHCU (2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline), allantoin, allantoic acid etc. are also produced as intermediates (see Figure 3). Urea then enters nitrogen metabolism pathway by producing ammonia using the enzyme urease (EC 3.5.1.5). Moreover, ammonia is transformed to absorbable forms (NH_4^+ and NO_3^- ions) that can be uptaken by plants to accomplish their nitrogen needs. From production of urea to nitrification of ammonia, a series of enzymatic reactions are involved where different soil microbes take their parts.

Certainly, results of this research have shown effect tea waste on soil nutrient profile. Naturally, tea is found to be acidic (Das et al., 2020) due to presence of various organic acids and phenolics, which can be a reason behind the low pH of TWS. Moreover composting, especially food waste composting obviously lowers the pH of soil, which indicates an increased soil fertility (Sundberg et al., 2013). According to Ritchie and Dolling (Ritchie and Dolling, 1985) soil acidification is a sign of degradation of organic matter. Previously, Porter (1980) described how added organic matter lowers soil pH. According to the study, organic matter releases hydrogen ions that were associated with organic anions and helps to improve the process of nitrification (Porter, 1980). So, results of OC and OM were quite satisfying as those were observed boosted (Table 1) after composting of tea waste in soil and the negative relation with the trend of pH was also there. Soil EC, much like pH, is also a good overall indicator of soil fertility which does not directly affect plant growth but indicates the amount of nutrients available for plant uptake (Anonymous, 2022b).

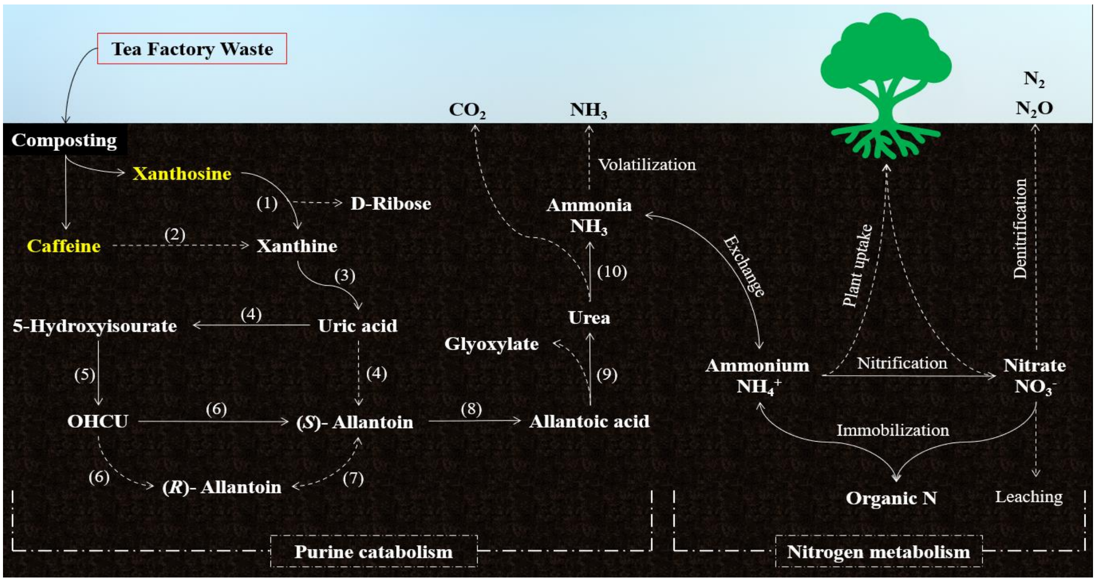

Figure 3. Possible metabolic pathways of xanthosine and caffeine catabolism and nitrogen metabolism in tea waste treated soil (TWS). Numbers indicate the reaction steps catalysed by the enzymes mentioned below:

(1) Purine (xanthosine) nucleosidase (EC 3.2.2.1); (2) xanthine oxidase; (3) xanthine dehydrogenase (EC 1.1.1.204); (4) uricase or urate oxidase (EC 1.7.3.3); (5) HIU (5-hydroxyisourate) hydrolase (EC 3.5.2.17); (6) OHCU (2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline) decarboxylase (EC 4.1.1.97); (7) allantoin racemase (EC 5.1.99.3); (8) allantoinase (EC 3.5.2.5); (9) allantoicase (EC 3.5.3.4); (10) urease (EC 3.5.1.5).

It is reported that non-saline soils having a higher EC value have more available nutrients than those that have a lower EC value (Anonymous, 2022c) and here, EC of TWS (196±6) was detected to be higher than the EC of CS (78±14). Beside acidification, organic matter can improve the water-holding capacity of soil (Hudson, 1994) that was also clearly reflected in the results. Interestingly, promising amount of OC, N, P and K have been reported in left over green tea waste (Iqbal et al., 2007) and left-over black tea composted soil (Gurav and Sinalkar, 2013). In this research, tea factory waste composted soil also exhibited remarkable results.

Multiple growth parameters and plant development attributes were considered in this research to evaluate tea waste's effect on plant growth and development. Post-harvest characterization also helped to evaluate the effect of tea waste on plant's growth and development. On the other hand, parameters linked to nodule characterization indicated the soil fertility status indirectly by conveying the nitrogen demand and availability (Xia et al., 2017). Certainly, results were higher for TWS plants compared to CS plants, with only moisture percentage as exception.

Root nodules are found primarily in legumes because of symbiosis with nitrogen-fixing bacteria. Under nitrogen-limiting conditions, plants create a symbiotic relationship with a nitrogen fixing bacteria called rhizobia through nodulation (Bordeleau and Prévost, 1994; Xia et al., 2017). Interestingly, the nodulation process in a plant is controlled by nitrogen availability in soil and nitrogen demand of that plant. A limiting nitrogen status in soil increases the rate of nodulation (Xia et al., 2017). Low pH soil also inhibits nodulation process (Ferguson et al., 2013) and it has been reflected in results of research as plants grown in lower pH of TWS had less amount and weight of nodules compared to plants of higher pH- i.e., CS plants. Likewise, in this research, available nitrogen in CS samples was lower than TWS (Table 1) where significant nodulations have occurred. The objective of GC-MS analysis was to find out any nitrogen rich fertilizing agent or priming agent or any other growth-promoting factor among the tea waste metabolome, which may be responsible for TWS's positive outcome. Result of this analysis have demonstrated presence of nitrogen rich xanthosine and caffeine, which were introduced as abundant components of tea waste (Table 4). Previously, Brychkova et al. (2008) applied xanthine and some other purines exogenously as a nitrogen source and reported an improved rate of seed germination and plant growth while, here, the tea waste extract itself is rich in such components. Yi et al. (2021) reported the effect of xanthine on both seedling growth and early senescence of cotyledons and that too under a nitrogen deficient condition. According to their study, seedlings treated with xanthine as the only nitrogen source grew faster and more cotyledon chlorophyll was broken down, compared to seedlings without xanthine. Moreover, xanthine oxidase catalyzes the oxidation of xanthine to produce ureides, urea and other metabolically active intermediates which are effective soil fertilizing components (Brychkova et al., 2008; Kostić et al., 2015). Therefore, exposures of materials in soil that source xanthine may help plants to grow healthy (Nakagawa et al., 2007). Caffeine, the other key component of the tea waste extract, can also degrade into xanthine and further urea (Mazzafera, 2002) just like xanthosine. Caffeine has already been recognized as a potential priming agent for seed germination (Ransom, 1912) and plant growth promoter as well, but there are mixed opinions (Montes, 2014).

Breakdown of tea waste metabolites as shown in Figure 3 was based on two reference pathways i.e., purine catabolism pathway (Anonymous 2022d) and nitrogen metabolism pathway (Powlson, 1993; Ashihara, 2012). There are some pioneer works which established microbial metabolic pathways involved in purine catabolism. Triplett et al. (1980) reported synthesis of allantoic acid in root nodule cytosol of legume plants via enzyme xanthine dehydrogenase. Activity of uricase in root nodules of cowpea (plant used in this pot experiment) was demonstrated by cytochemical methods, which confirmed the ureide biogenesis in cowpea (Webb and Newcomb, 1987). High allantoinase activity was also reported inside the nodules of this plant (Webb and Newcomb, 1987). Sun et al. (2021) have stated that exogenous application of xanthine promotes not only the plant growth but also the root elongation which has been observed in this research as well. Sun et al. (2021) and Cunliffe et al. (2016) have reported microbial purine catabolism pathway in the environment where production of urea and ammonia by utilizing xanthine has clearly been mentioned.

5. Conclusion

Disposal of huge quantity of solid tea wastes produced during tea manufacturing is considered as a problem without looking into its potential application in enhancing soil fertility. This research portrays a notable nutrient richness in tea waste treated compost (TWS) over control soil (CS). Organic carbon, nitrogen and other nutrient content were remarkably highly in TWS which was also reflected in the results of pot assessment. Detection of major peaks of purine metabolites like xanthosine and caffeine in tea waste extract has validated results of soil analysis and pot experiment where TWS revealed remarkable fertilizing properties, seed germination property, plant growth and beans production. These two metabolites were reported as plant growth promoting factors whose possible breakdown pathways have been graphically explained in this research. Conclusively, soil physicochemical analysis, pot experiments and GC-MS based metabolomics together established the fertilizer potentiality of CTC tea waste. An integrated approach to process design is therefore recommended for the utilization of CTC tea factory waste, which will help tea industry to shift towards a carbon neutral, sustainable and zero waste industry.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Sahadeb Sarkar: Conceptualization, Participated in material collection, soil analysis and pot experiment, Review and editing. **Soumya Majumder:** Methodology, Conceptualization, Formal analysis, Metabolomics, Writing - original draft, **Arindam Ghosh:** Formal analysis, Review and editing. **Sumedha Saha:** Participated in material collection, Formal analysis **Sukanya Acharyya:** Review and editing. **Sourav Chakraborty:** Data curation and statistical analysis. **Malay Bhattacharya:** Participated in supervision of the work starting from the proposal up to final draft, Conceptualization, Validation, Review and editing.

Ethical approval

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Data availability

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Consent for publication

Not applicable.

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