



The Impact of Organic Fertilizers on Growth, Yield, and Rhizospheric Bacterial Diversity in Black Turmeric (*Curcuma caesia*)

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Abstract

Background: Agricultural applications of organic manures are critical for enhancing soil health and producing higher-quality black turmeric, a plant used in various traditional medicines. Identifying and analyzing the biochemical activities of rhizospheric bacteria in different soil conditions can provide insights into sustainable agricultural practices. **Method:** A field study was conducted over three to four months by planting black turmeric rhizome samples in two different soil conditions: organic and conventional. Morphological features were extensively documented. UV-Vis spectroscopy was performed on organic rhizome methanolic extracts, while both organic and conventional aqueous extracts underwent XRF analysis. Antibacterial activity against eight pathogenic bacteria was tested using methanolic and aqueous rhizome extracts. Rhizobacteria were isolated from organic soil using a serial dilution procedure and identified through pure culture techniques. Various biochemical activities, including catalase production, starch hydrolysis, antibiotic sensitivity, pH tolerance, temperature tolerance, and gram staining, were performed to characterize the bacterial species. **Results:**

The study showed distinct inhibitory zones for pathogenic bacteria in both methanol and aqueous extracts. UV-Vis spectroscopy identified a peak in the 200-250 nm range. XRF analysis revealed the presence of various elements in both soil and leaves, with differing concentrations. Gram-positive bacteria were identified, and these bacteria exhibited catalase activity, starch hydrolysis, and significant antibiotic sensitivity. Soil bacteria demonstrated pH tolerance and resistance to high temperatures. Rhizomes grown in organic soil exhibited better growth and development compared to those in conventional soil. **Conclusion:** Organic fertilizers positively influence the growth and quality of black turmeric. The role of soil bacteria in sustainable agriculture offers promising new techniques for managing plant diseases and enhancing soil health.

Keywords: Organic farming, Black turmeric, Rhizosphere bacteria, Organic fertilizers, Medicinal plants

Significance | Organic fertilizers boost growth, yield, and bacterial diversity in black turmeric, supporting medicinal plant preservation and drug development.

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1. Introduction

Organic farming is a creative framework that stays away from or dramatically avoids the utilization of artificially intensified manures, pesticides, development controllers, hereditarily adjusted organic entities, and domesticated animal food-added substances (Das et al. 2020). Organic farming lessens the use of pesticides, herbicides, and other destructive synthetic substances that wash off the significant soil verdure. By empowering natural cultivation, regular plants, bugs, birds, and creatures will make due and be bountiful in the typical habitat, thereby keeping up with the

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biological equilibrium (Behera et al., 2022). The rhizosphere comprises various plant-related organisms essential to the plants' digestive processes. Rhizosphere microorganisms live near plant roots and help each other (Ren et al., 2020). Soil fertility and ecosystem function depend heavily on microorganisms. When it comes to a plant's roots, it's all about the soil around them. Roots, soil, and microorganisms all interact strongly in the rhizosphere. Rhizosphere bacteria are crucial to cycling nutrients and water below ground (Xun et al., 2021). In agricultural production, fertilizer is expected to be used as a management tool, promoting crop growth, increasing yield, and affecting soil microbes. The widespread use of chemical fertilizers has decreased soil fertility and numerous environmental issues (Wan et al., 2020). At the same time, the increased use of bioorganic fertilizer has increased soil fertility and prevented many ecological problems caused by chemical fertilizers. Bioorganic fertilizer can be applied to the rhizosphere microbes to improve farmland soil fertility (Liang et al., 2020).

Curcuma caesia Roxb. is the scientific name for black turmeric. Black Zedoary is another name for it. For medicinal and religious purposes, black turmeric is generally used. The rhizome is bluish-black. Black turmeric is a priceless factory extensively employed in traditional Chinese medicines. Rhizomes are lower, raying out, and two elevations long. Black turmeric rhizome has medicinal properties (Singh et al., 2021; Shahinozzaman et al., 2013). In ancient periods, growers employed their leaves to restore rice seed germination. Its leaves are used as energy. Black turmeric-dried rhizomes and leaves treat fever, asthma, wounds, malignant growth, heaps, sensitivities, and toothache. Using rhizome glue can mend rheumatic joint pain (Mahato & Sharma, 2018; Venugopal et al., 2017). The concentrate of the rhizome is utilized as a smooth muscle relaxant and cell reinforcement against the tumour. The rhizomes heal diseases, haemorrhoids, fever, asthma, regurgitating, wounds, anthelmintic, gonorrhoeal releases, sexual enhancers, and irritation in old age. The rhizome glue is likewise utilised for snake chomps, scorpion nibbles, and bug bites. The new rhizome can recuperate the injury and give speedy help (Gaikwad et al., 2023; Sanodiya et al., 2022). Rhizomes are being used to fix stomach agony and are hostile to diarrhoea. It resolves acid reflux and helps legitimate absorption and smooth liver and kidney function by biting a piece of a rhizome. Rhizomes are utilized for wounds, pox, and cancers in Asia. Rhizomes are blended with mustard oil to fix steer looseness of the bowels in Assam (Arya et al., 2022; Adrianta & Wardani, 2022).

Curcuma caesia is an essential species of *Curcuma*, but it has received less attention than other species. The rhizome is the primary research subject. There is a lack of additional research on the different parts of the plant, such as the leaves and flowers, which may have helpful bioactive substances and medicinal benefits. To

demonstrate its usefulness, researchers recommend conducting additional studies such as clinical trials, phytochemical analysis, and human toxicity testing (Haida et al., 2022). Extensive research has been conducted on *C. caesia* bioactivities. Still, nothing has been done to investigate the possible volatile elements responsible for these effects in a virtual environment, i.e., in silico analysis (Khuntia et al., 2023). Plant products are preferred over synthetic compounds in treating many diseases since they have no adverse side effects on humans or animals. This plant's phytochemical content can potentially develop into a therapeutically beneficial active principle (Gaikwad et al., 2023). To better understand how organic manures, such as farmyard manure (FYM), vermicompost (verm), and poultry manure (PM), and their different combinations affect the growth of black turmeric, a field study was conducted (Deka & Swami, 2022).

To the best of our knowledge, not much research has been done to improve the yield, nutrient content, and other aspects of organically grown black turmeric. Not much had been done to investigate how rhizosphere bacteria might function along with black turmeric to improve growth, yield, nutrient content, and other characteristics. My study aims to examine organic fertilizers' effect on the black turmeric variety of Odisha. This study focuses on the use of organic fertilizer on black turmeric plantation and to determine its growth and different activities such as antibacterial activity, starch hydrolysis, rhizospheric bacterial isolation and identification, biochemical activities like catalase, pH, temperature tolerance, antibiotic sensitivity, and XRF analysis of both soil and rhizome components. This farming technique, or the employment of helpful microorganisms, can potentially prevent the extinction of the threatened plant and make essential contributions to the medical community and the drug development process.

2. Materials and methods

2.1 Area of Study

This research was conducted in Bhubaneswar, a historic city in the eastern Indian state of Odisha, at the Centurion University of Technology and Management medicinal garden. The town is located in Khurda, between 20°15' N and 85°52' E in latitude and longitude. Bhubaneswar has a tropical climate. Bhubaneswar receives far less precipitation in the winter than in the summer. The average annual temperature in Bhubaneswar is 26.6 degrees Celsius (79.9 degrees Fahrenheit). Annual precipitation averages 64.1 inches (1,628 mm). The soil type is rich, red, and fertile. The majority of the soil in Bhubaneswar is made up of laterite, with smaller amounts of alluvial and sandstone (Latlong.net; Swain et al., 2019).

2.2 Collection and plantation of sample

The sample of the Black turmeric variety was gathered from Sambalpur local cultivars, an old city of Odisha. This city is located

in a tropical region. Sambalpur is situated at a latitude of 21°46' and a longitude of 83°97'. During the summer months in Sambalpur, the weather is much wetter than in the winter. It is usually 27.0 °C/80.5 °F. Summers are hot and dry, followed by a humid monsoon, and winters are bitterly cold in this region. Between 10 and 46 degrees, a centigrade is a typical temperature. The southwest monsoon brings rain to this region. The district experiences an average of 1495.7 mm of precipitation per year. This district has a wide variety of soils, including red forest soil, brown forest soil, and sandy soil (Latlong.net; Das et al., 2022). Both organic soil (Soil + Vermicompost in a 1:1 ratio) and regular soil were used to grow the Black turmeric species. Both conditions involved the cultivation of black turmeric in a polyhouse. The sample was allowed to mature for around three to four months.

2.3 Data recording

When the plants from each growth condition fully matured in February, they were harvested by hand. The morphological analysis was performed on fully developed plants. Three plantlets from each growth parameter were randomly chosen to collect information on eight yield-related plant characteristics. Height of the plant (in cm), tiller no. Per plant, total no. of leaves per plant, number of primary leaves, number of secondary leaves, preliminary leaf length (in cm), secondary leaf length (in cm), and total rhizome weight (in gm) were the eight plant traits on which the data were recorded. The harvested rhizome was carefully measured before being placed in a clean, sterile area for future study.

2.4 Statistical analysis

The statistical data analysis of the reported plant attributes included means, standard deviations, standard errors, analysis of variance (ANOVA) and correlation coefficient. The Statistical Analysis Software, SPSS 17.0. (SPSS Inc., Chicago, IL, USA) was used for the evaluation.

2.5 Authentication of sample

Plant samples were collected and identified based on their morphological characteristics. This information was recorded for future reference. Each observation was cross-referenced against several taxonomy references. The obtained data was identified and characterized using searches of multiple internet databases, including PubMed, Google Scholar, etc. Finally, the data was examined, and Dr. Gyanranjan Mahalik, Associate Professor at CUTM in Bhubaneswar, verified the plant. The plants were subsequently preserved as herbarium in the University Botany lab.

2.6 Extraction of plant rhizome extracts using different solvents

Fresh rhizome was harvested from both soil types to compare the effects of organic and conventional soils. It was decided to let the rhizome samples dry out. The powdered form of the dried rhizome sample was then extracted using a Soxhlet apparatus and two solvents (methanol and distilled water). The rotary extractor was

used to collect the samples. The methanolic and aqueous extracts were preserved for later testing (Abubakar & Haque, 2020).

2.7 UV Visible Spectroscopic analysis

Ultraviolet (UV)- visible spectroscopy is a sort of ingestion spectroscopy where the particle consumes UV-apparent light. Ingestion of the UV-apparent radiations brings about the excitation of the electrons from lower to higher energy levels. The methanolic extract was prepared by taking the organic black turmeric rhizome, and a reading was taken using a UV spectrophotometer. The UV analysis was performed according to the documented principles. The absorbance peak was noted down (Sharma et al., 2018).

2.8 XRF analysis

The XRF elemental analysis of an aqueous organic and conventional grown rhizome extract and a fine organic soil sample from the Bhubaneswar and Sambalpur regions was conducted at the ATC lab at Centurion University of Technology and Management, Bhubaneswar (Mallick et al., 2021).

2.9 Antibacterial activity

Salmonella typhi (MTCC 735), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Serratia marcescens* (MTCC 4822), *Staphylococcus coagulase-negative* (MTCC 5856), *Staphylococcus aureus* (MTCC 96), *Vibrio cholerae* (MTCC 3904), *Klebsiella pneumoniae* (MTCC 4032) bacterial strains were used to test the antibacterial efficacy. The agar-well diffusion test was used to analyze the antibacterial activity of methanolic and aqueous extracts of both conventional and organic soil rhizomes toward eight pathogenic bacterial strains. Bacteria were spread across the entire plate once the agar had set. An agar well diffusion assay was performed using the established procedure. The inhibition zones were measured after incubating the plates for 24 hours (Mahalik, 2017).

2.10 Isolation and identification of bacteria

Bacteria were isolated using a standard procedure that involved a serial dilution step. One gram of the finely dried soil was weighed after air-drying. The bacteria were equally distributed by shaking a mixture of nine ml of distilled water and one gram of fine soil for a few seconds in a test tube. The volume of distilled water added to each test tube was 9ml. The first test tube contained 9 millilitres of distilled water, and one millilitre of suspension was transferred to it to make 10 millilitres of volume, and the mixture was shaken vigorously. 1 ml of suspension was transferred from tube 1 to tube 2, and the process was repeated for tubes 3, 4, 5, and 6 to complete the dilution. One millilitre of the mixture was then poured onto a Petri dish. The plates were inverted and incubated for 24 to 36 hours. Following incubation, bacterial counts and morphological analyses were performed (Jyotirmayee et al., 2021). One strain of bacteria was isolated by performing a pure culture. The loop inoculation technique selected a single bacterial colony from the mother culture plate and streaked it on the Petri plate with agar

media. Finally, the plate was observed in an incubator after 24-36 hours (Jyotirmayee et al., 2021).

Bacteria were identified using the standard protocol of gram staining. In the gram staining method, a clean, grease-free slide was taken. The bacteria were smeared from the pure culture with the help of an inoculation loop. The smear was allowed to air dry and fixed by heat. Crystal violet was poured, kept for about 30 secs-1min and rinsed with distilled water. After waiting 1 minute with a gram of iodine, it was rinsed with distilled water. Then, it was put through a decolourizer for 10-20 seconds before being cleaned thoroughly with distilled water. After 1 minute of adding safranin, the area was rinsed with distilled water once more. After that, it was air-dried and blotted dry. Under a microscope, the slide was examined. Gram-positive bacteria are purple, while gram-negative bacteria are pink (Tripathi & Sapra, 2022).

2.11 Biochemical activities of isolated bacteria

2.11.1 Catalase test

A clean, grease-free glass slide was taken, and the smear was ready by taking the bacterial sample from the pure culture. Hydrogen peroxide was poured over the streak. The bubble formation shows the presence of catalase (Shah et al., 2021).

2.11.2 Antibiotic sensitivity test

The antibiotic susceptibility of a particular soil strain of bacteria was tested using the agar well diffusion technique. An agar well diffusion test was performed, in which soil microorganisms were streaked throughout the surface. The antibiotic named Amikacin was used for the antibiotic sensitivity test. The plates were incubated for 24 hours at 28°C. The zone of inhibition was measured after 24 hours (Rahman et al., 2019).

2.11.3 pH analysis

The prepared nutrient broth was poured into the test tubes. Cultured soil bacteria were inoculated into it. For 24 hours, the inoculated broth medium was kept at 37°C in an incubator. 1N HCL, 1N NaOH, and a computerized pH meter were used to raise the pH of the cultured broth medium from 5 to 9. After that, the CFU/ml was counted (Mohapatra et al., 2015).

$CFU/ml = \text{number of colonies} \times \text{dilution factor} / \text{volume of the culture plate}$

2.11.4 Temperature tolerance

Different test tubes were filled with nutritious broth. For the duration of the experiment, it was incubated at four different temperatures. It was decided to test temperature resistance by allowing bacterial growth to develop at varying temperatures. It takes 24 hrs for bacteria to disengage at temperatures ranging from 23 °C to 44°C. The CFU/ml was then counted (Mohapatra et al., 2015).

$CFU/ml = \text{number of colonies} \times \text{dilution factor} / \text{volume of the culture plate}$

2.11.5 Starch hydrolysis

The starch analysis was performed using a newly sub-cultured bacterial plate. Sub-culture bacteria plates were inundated with iodine solution and left to sit for 10 minutes while the results were monitored (Sigmon, 2008).

3. Results

3.1 Morphological studies

Plants planted in two distinct soil types were observed under controlled settings at various points throughout the growing season to study their numerous features. The harvesting phase was when the complete data were obtained. A variety of plant properties were estimated. Triplicate plants were selected from each soil condition to estimate plant features. In-plant tests revealed a difference in evaluated plant traits between the two soil types. The following data was collected under organic soil parameters: plant height (84, 92, 100 cm), tiller number per plant (4, 3, 4), total leaf number (4, 3, 5), number of primary leaves (1, 2, 0), number of secondary leaves (4, 5, 4), length of preliminary leaves (28, 20, 26 cm), length of secondary leaves (40, 33, 42 cm), and a yield (153, 138, 146 g). For plants grown in the conventional growing medium, the following parameters were measured: plant height (96, 82, 83 cm), number of tillers per plant (3, 4, 6), total leaves (4, 5, 6), number of primary leaves (2, 1, 1), number of secondary leaves (4, 4, 3), length of preliminary leaves (27, 25, 29 cm), length of secondary leaves (39, 50, 37 cm), and yield (141, 129, 134 g) Table 1.

A comparison of statistical metrics (mean, standard error, etc.) for plants cultivated in two distinct soil types is illustrated in (Figure. 1) (Mohanty et al., 2021). According to the results of the ANOVA test, $p > 0.05$ indicates that none of the data are statistically significant. Table 2 shows the Pearson correlation coefficient about the morphological traits of black turmeric plants cultivated in two distinct soil types. The findings of the correlation analysis indicated that primary leaf length and total rhizome weight had the highest correlation.

3.2 UV- Spectroscopy analysis

The Methanolic sample of organic black turmeric shows an absorbance between 200-250 nm, but the highest peak is 230 nm (Figure. 2) (Murthi et al., 2019).

3.3 XRF analysis

Rhizomes from plants growing under two different environments were tested for numerous elements and found to have concentrations significantly greater than those in typical plant soils. For instance, in the case of organic soil, various components can be found in varying concentrations, such as Silicon (541.2 ppm), Phosphorus (904.4 ppm), Sulphur (300.4 ppm), Chlorine (323.2 ppm), Potassium (0.120 per cent), Calcium (228.0 ppm), Manganese (0.0 ppm), Iron (15.7 ppm), Tin (44.7 ppm), Europium (21.3 ppm), and Erbium (63.5 ppm). The presence of elements like Si (0.214 %), P (0.119 %), S (316.5 ppm), Cl (284.3 ppm), K (0.233

%, Ca (232.5 ppm), Ti (16.4 ppm), Mn (19.9 ppm), Fe (63.8 ppm), Rb (4.5 ppm), and Sn (55.4 ppm) in standard soil-grown rhizome samples also indicates the presence of different elements (Figure. 3) (Shwe & Khin, 2019).

In the case of the organic soil in Bhubaneswar, several of the chemicals that influence plant development and nutritional content were present which includes Al_2O_3 (16.571 %), SiO_2 (66.721 %), P_2O_5 (1.465 %), SO_3 (0.433 %), Cl (0.241 %), K_2O (2.660 %), CaO (2.269 %), TiO_2 (1.310 %), V_2O_5 (265.7 ppm), Cr_2O_3 (216.5 ppm), MnO (0.182 %), Fe_2O_3 (7.738 %), NiO (88.9 ppm), CuO (113.5 ppm), ZnO (201.7 ppm), Ga_2O_3 (33.1 ppm), As_2O_3 (4.9 ppm), Rb_2O (157.0 ppm), SrO (125.8 ppm), Y_2O_3 (43.5 ppm), ZrO_2 (0.126 %), Nb_2O_5 (31.7 ppm), SnO_2 (135.7 ppm), BaO (503.3 ppm), Eu_2O_3 (710.6 ppm), Yb_2O_3 (59.5 ppm), IrO_2 (9.1 ppm), PbO (80.8 ppm), ThO_2 (45.1 ppm), CO_2 (0.0 ppm), and Re (3.0 ppm) (Figure. 3).

Compounds that make up the organic soil of Sambalpur includes Al_2O_3 (12.723 %), SiO_2 (73.342 %), P_2O_5 (0.771 %), SO_3 (0.163 %), Cl (0.183 %), K_2O (2.975 %), CaO (1.448 %), TiO_2 (1.339 %), V_2O_5 (167.4 ppm), Cr_2O_3 (355.9 ppm), MnO (527.6 ppm), Fe_2O_3 (6.637 %), NiO (134.2 ppm), CuO (100.9 ppm), ZnO (170.7 ppm), Ga_2O_3 (32.9 ppm), As_2O_3 (8.1 ppm), Rb_2O (137.6 ppm), SrO (173.3 ppm), Y_2O_3 (65.0 ppm), ZrO_2 (0.102 %), Nb_2O_5 (44.6 ppm), SnO_2 (113.8 ppm), TeO_2 (67.3 ppm), BaO (541.1 ppm), Eu_2O_3 (384.4 ppm), Yb_2O_3 (56.2 ppm), IrO_2 (3.4 ppm), PbO (46.0 ppm), ThO_2 (43.1 ppm), CO_2 (0.0 ppm), Re (4.1 ppm) (Figure. 3) (Mallick et al., 2021).

3.4 Antibacterial activity

The Aqueous and methanolic rhizome extract of black turmeric shows antibacterial action against various human pathogenic microorganisms, for example, *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Serratia marcescens* (MTCC 4822), *Staphylococcus coagulase - ve* (MTCC 5856), *Staphylococcus aureus* (MTCC 96), *Vibrio cholerae* (MTCC 3906), *Klebsiella pneumonia* (MTCC 3906). Antibacterial effects were examined for eight different bacteria, two of which were gram-positive (*Staphylococcus aureus* and *Staphylococcus coagulase-ve*), and six of which were gram-negative. The aqueous and methanolic rhizome extract from two different soil conditions was taken for antibacterial analysis, showing a decent distinction in the two cases.

In contrast to aqueous plant extract, *Escherichia coli* and *Serratia marcescens* exhibit a large inhibition zone in organic soil, followed by *Pseudomonas aeruginosa* and *Salmonella typhi*. At the same time, *Staphylococcus aureus* has the largest inhibition zone in conventional soil, followed by *Salmonella typhi* and *Pseudomonas aeruginosa*. The highest inhibition zone was found for *Pseudomonas aeruginosa* and *Klebsiella pneumonia* in the methanolic extract from organic soil, followed by *Staphylococcus coagulase - ve* and *Vibrio cholerae*. *Vibrio cholerae* exhibited the highest inhibition zone in aqueous extract, followed by

Pseudomonas aeruginosa and *Klebsiella pneumonia* (Figure. 4) (Niteeshkumar et al., 2022).

3.5 Bacterial isolation and identification

Serial dilution was done by repeating the transformation of suspension from one test tube to another, which showed a decrease in the number of microorganisms in the soil sample. In isolation, two different types of bacterial colonies are found: a white colony of bacteria and a yellow colony. The two colonies found during the isolation process were grown in an agar medium for pure culture and showed good bacterial colony growth. In the gram staining process, the type of bacteria was investigated. Gram staining revealed the white colony as gram-positive and the yellow colony as gram-positive. It was found that the presence of rod-shaped bacteria was found, which indicates the presence of bacillus bacteria Figure. 5 and Table 3 (Shaheen et al., 2022).

3.6 Biochemical activity of isolated soil bacteria

3.6.1 Catalase test

Hydrogen peroxide was poured over slides containing smears of bacterial colonies, and bubbles revealed the presence of the Catalase enzyme in both the white and yellow colonies, which had been collected from pure culture bacteria Figure. 5 and Table 3 (Andy et al., 2020).

3.6.2 Antibiotic sensitivity test

In the antibiotic sensitivity test, soil bacteria were exposed to a synthetic antibiotic, Amikacin, which demonstrated a significant diameter of inhibition zone, i.e., > 30 mm. This proved that the bacteria isolated from the soil responded favourably to the antibiotic amikacin Figure. 5 and Table 3 (Nath et al., 2019).

3.6.3 Starch hydrolysis

The starch hydrolysis test spread the iodine solution over the pure culture. After some time, the bacterial culture showed a deep brown-red colour in the presence of iodine solution, which concluded the presence of starch Figure. 5 and Table 3 (Nimisha et al., 2019).

3.6.4 pH tolerance test

In the case of pH tests, different concentration levels were used to determine the tolerance level of soil bacteria. The CFU count was 9.8×10^7 and 7.9×10^6 , the optimum pH for the growth of white and yellow bacterial isolates. pH 5 had 2.9×10^5 CFUs in the white colony and 2.1×10^5 CFUs in the yellow colony, while pH 9 had 9.2×10^3 CFUs in the white colony and 5.7×10^5 CFUs in the yellow colony. For the development of isolates, the ideal pH is 7, close to the rhizosphere pH Figure. 5 and Table 4 (Paul et al., 2020).

3.6.5 Temperature tolerance

The optimal growth temperature for the white and yellow bacterial strains was 37°C. The growth rate for the identified white bacterial strain was moderate at 44°C and lower at 23°C and 30°C. At 23°C, 30°C, 37°C, and 44°C, the CFUs per isolate were 3.5×10^4 , 2.1×10^5 , 8.9×10^6 , and 6.9×10^5 , respectively. Yellow bacterial isolates grew

moderately at 30 and 44 degrees Celsius, whereas at 23 degrees Celsius, they grew at a lower rate. At different temperatures (23°C, 30°C, 37°C, and 44°C), 1.8×10^5 , 4.1×10^5 , 6.1×10^6 , and 5.3×10^5 CFU/isolate were found in Figure. 5 and Table 4 (Paul et al., 2020).

4. Discussion

One of several therapeutic potentials contributing to new drug development is found in black turmeric, an endangered species. So, to combat endangered species and satisfy clinical research needs, there has been a rapid rise in the demand for organically farmed black turmeric. In the current research, the impact of organic fertilizers on black turmeric production, antibacterial activity, and microbial diversity was investigated in a field trial. The experiment was carried out over three to four months on a permanently placed plot of land. Documentation of the morphological analysis was made. When conducting experiments, the available nutritional status of the soil was considered. The microbiological method enhances plant growth in several direct and indirect ways. A minimal phytomedicine that could benefit medical systems is plant growth promoting rhizo bacteria-driven improvement in herbal goods. Several processes, including induced plant defence, rhizosphere competency, and improved nutrition, allow them to increase plant health and eradicate diseases.

Research on 20 different black turmeric genotypes found that one genotype, BTG 12, from Mekkarai, Tenkasi, Tamil Nadu, performed significantly better across all growth, yield, and quality metrics. These metrics included plant height, leaf count, tiller count, chlorophyll content, rhizome count, primary finger count, fresh rhizome harvest and many more. The genotype BTG 13, sourced from Panjagutta, Hyderabad, had the worst performance, whereas genotype BTG 6, sourced from Ariyankavu, Kerala, demonstrated the second best (Narendhiran et al., 2023). An effective procedure for micropropagation of this species was developed to obtain *C. caesia* raw resources to conserve natural populations quickly. The best composition for inducing shoot induction and multiplying was MSB5 medium combined with BAP and IBA. Therefore, *C. caesia* might be mass-produced using the method established (Haida et al., 2022).

The morphological traits of black turmeric germplasm revealed subtle differences in the rhizome and differences in leaf colour. Due to the challenges inherent in conventional breeding, cluster analysis has shown to be a valuable tool for classifying accessions, which could lead to their more efficient use in crop development programs involving selection (Sahu et al., 2022). Plants sprayed with 3% Panchakavya increased height, leaf count, tiller count, leaf length, and leaf breadth. The expected fresh rhizome output differed considerably from other treatments when treated with 3% Panchagavya (Chitra et al., 2020).

C. aromatica had the most potent antibacterial action, whereas *C. caesia* demonstrated the most vital antidiabetic activity. Curcuma species have the potential to be a valuable bioresource for the development of antibiotic and antidiabetic drugs (Jain et al., 2019). *Curcuma caesia* methanol extracts were more effective against bacteria than *Curcuma amada* methanol extracts and *Curcuma angustifolia* chloroform extracts (Yadav & Kaliyaperumal, 2021). Black turmeric extract may reduce nosocomial infections by acting as an antibacterial agent against gram-positive and gram-negative bacteria. Against *Staphylococcus aureus* bacteria, the maximum effectiveness was achieved at an 80% concentration (Juariah et al., 2023). *Klebsiella pneumonia* dropped, and the cell walls appeared wrinkled and disintegrated after treatment with combined ethanol extracts, red ginger, and black turmeric. Thus, red ginger and black turmeric ethanol extracts can be suggested as an alternate natural antibiotic that inhibits the growth of *K. pneumonia*—the causative agent of pneumonia (Juariah et al., 2023).

Compared to other treatments, a mixture of FYM, Vermicompost, and poultry manure had the most significant effect on the development and production of black turmeric. To increase the output of black turmeric in acidic soils, applying all three organic manures together may be recommended as an appropriate adequate nutrient treatment strategy (Deka & Swami, 2022). Utilizing organic manuring techniques with kashuri turmeric, such as FYM, organic manure, and neem cake, as well as microbiological microbes such as Azospirillum, AMF, Trichoderma, and Pseudomonas, significantly improved soil physiological, biochemical, and ecological qualities can be accomplished (Shamrao et al., 2013). *With the help of socialization, seed collection, organic fertilizer fabrication, shoot transfer to pots, and e-commerce services, women can find a way to spend their free time productively growing black turmeric and become agropreneurs by selling the crop's produce, raising their standard of living and decreasing their dependency on seafood* (Handayani et al., 2022).

The combination of NPK, FYM, and Trichokavach substantially impacted the essential oil yield of black turmeric. The treatments that included biofertilizers and organic and inorganic fertilizers worked well for improved secondary metabolites. T10 found the highest concentration of camphene in the essential oil obtained from freshly harvested rhizomes (Vidya et al., 2023). One fungal pathogen of turmeric, *Pythium aphanidermatum*, is inhibited by the *Pseudomonas plecoglossicida* strain. Compared to the control group, turmeric rhizomes treated with either strain alone or a mix of the two significantly improved in all growth categories. Utilizing various strains of bacteria in a single composition is an emerging trend in sustainable agriculture (Jagtap et al., 2023). Soil microbial populations were shown to have increased in treatments using organic nutrient management practices, including biofertilizers and green manure. Applying fermenting cow manure mixture resulted

Table 1. Morphological analysis of black turmeric under organic and conventional soil conditions.

Sl. no.	Characters	Values (Mean ± SD) of plants (n=3)	
		Organic	Conventional
1.	Plant height (cm)	92 ± 6.53	87 ± 6.37
2.	Tiller no. per plant	3.66 ± 0.47	4.33 ± 1.24
3.	Total no. of leaves per plant	4 ± 0.81	5 ± 0.81
4.	Number of primary leaves	1 ± 0.81	1.33 ± 0.47
5.	Number of secondary leaves	4.33 ± 0.47	3.66 ± 0.47
6.	Primary leaf length (cm)	24.66 ± 3.39	27 ± 1.63
7.	Secondary leaf length (cm)	38.33 ± 3.85	42 ± 5.71
8.	Total rhizome weight (gm)	145.66 ± 6.12	134.66 ± 4.92

Table 2. Correlation analysis of the relationships between several plant characteristics cultivated in organic and conventional soils.

Sample Traits	Correlation	Analysis
Plant height	-0.83224	Both variables move in the opposite direction
Tiller no. per plant	0.188982	Poor Correlation
Total no. of leaves per plant	0.5	Moderately correlated
Number of primary leaves	0	No linear relationship exists between two continuous variables, i.e., no correlation.
Number of secondary leaves	0.5	Moderately correlated
Primary leaf length (cm)	0.720577	Good correlation
Secondary leaf length (cm)	-0.99756	Strong opposite correlated
Total rhizome weight (gm)	0.990982	Strong correlation

Table 3. Morphological properties of isolated soil bacteria.

Sl. No.	Plant name	Form	Bacteria colony colour	Gram staining	Gram Bacteria	Bacteria shape	Catalase	Bubble formation	Antibiotic amikacin	Starch
1.	<i>C. caesia</i> (Black turmeric)	Round	White	Purple	Positive	Rod-shaped bacillus	Present	Yes	Sensitive	Present
		Round	Yellow	Purple	Positive	Rod-shaped bacillus	Present	Yes	Sensitive	Present

Table 4. pH tolerance test of isolated soil bacteria.

Sl. No.	Isolated bacteria	pH	CFU/ml	Rate of growth
1.	White colony	5	2.9×10 ⁵	Less
		7	9.8×10 ⁷	More
		9	9.2×10 ³	More
2.	Yellow colony	5	2.1×10 ⁵	Less
		7	7.9×10 ⁶	More
		9	5.7×10 ⁵	Less

Table 5. Temperature tolerance of isolated soil bacteria.

Sl. No.	Isolated bacteria	Temperature	CFU/ml	Rate of growth
1.	White colony	23°	3.5×10 ⁴	Less
		30°	2.1×10 ⁵	Less
		37°	8.9×10 ⁶	More
		44°	6.9×10 ⁵	Moderate
2.	Yellow colony	23°	1.8×10 ⁵	Less
		30°	4.1×10 ⁵	Moderate
		37°	6.1×10 ⁶	More
		44°	5.3×10 ⁵	Moderate

Table 6. The elements reported in black turmeric aqueous rhizome extract from two soil types.

Sl. No.	Elements	Symbol	Amount in different Soil	
			Organic	Conventional
1.	Silicon	Si	0.05412 %	0.214 %
2.	Phosphorus	P	0.09044 %	0.119 %
3.	Sulphur	S	0.03004 %	0.03165 %
4.	Chlorine	Cl	0.03232 %	0.02843 %
5.	Potassium	K	0.120 %	0.233 %
6.	Calcium	Ca	0.02280 %	0.02325 %
7.	Manganese	Mn	0 %	0.00199 %
8.	Iron	Fe	0.00157 %	0.00638 %
9.	Rubidium	Ru	-	0.00045 %
10.	Tin	Sn	0.00447 %	0.00554 %
11.	Europium	Eu	0.00213 %	-
12.	Erbium	Er	0.00635 %	-

Table 7. The compounds are found in organic soil conditions of two separate locations.

Sl. No.	Compound	Symbol	Amount in Organic Soil	
			Bhubaneswar	Sambalpur
1.	Aluminium oxide	Al ₂ O ₃	16.571 %	12.723 %
2.	Silicon dioxide	SiO ₂	66.721 %	73.342 %
3.	Phosphorus pentoxide	P ₂ O ₅	1.465 %	0.771 %
4.	Sulfur trioxide	SO ₃	0.433 %	0.163 %
5.	Chlorine	Cl	0.241 %	0.183 %
6.	Potassium oxide	K ₂ O	2.660 %	2.975 %
7.	Calcium oxide	CaO	2.269 %	1.448 %
8.	Titanium dioxide	TiO ₂	1.310 %	1.339 %
9.	Vanadium oxide	V ₂ O ₅	0.02657 %	0.01674 %
10.	Chromic oxide	Cr ₂ O ₃	0.02165 %	0.03559 %
11.	Manganese (II) oxide	MnO	0.182 %	0.05276 %
12.	Iron (III) oxide	Fe ₂ O ₃	7.738 %	6.637 %
13.	Nickel oxide	NiO	0.00889 %	0.01342 %
14.	Copper (II) oxide	CuO	0.01135 %	0.01009 %
15.	Zinc oxide	ZnO	0.02017 %	0.01707 %
16.	Gallium oxide	Ga ₂ O ₃	0.00331 %	0.00329 %
17.	Arsenic trioxide	As ₂ O ₃	0.00049 %	0.00081 %
18.	Rubidium oxide	Rb ₂ O	0.01570 %	0.01376 %
19.	Strontium oxide	SrO	0.01258 %	0.01733 %
20.	Yttrium oxide	Y ₂ O ₃	0.00435 %	0.00065 %
21.	Zirconium dioxide	ZrO ₂	0.126 %	0.102 %
22.	Niobium pentoxide	Nb ₂ O ₅	0.0317 %	0.00446 %
23.	Tin (IV) oxide	SnO ₂	0.01357 %	0.01138 %
24.	Tellurium dioxide	TeO ₂	-	0.00673 %
25.	Barium oxide	BaO	0.05033 %	0.05411 %
26.	Europium oxide	Eu ₂ O ₃	0.07106 %	0.03844 %
27.	Yttrium (III) oxide	Yb ₂ O ₃	0.00595 %	0.00562 %
28.	Iridium dioxide	IrO ₂	0.00091 %	0.00034 %
29.	Lead (II) oxide	PbO	0.00808 %	0.00460 %
30.	Thorium oxide	ThO ₂	0.00451 %	0.00431 %
31.	Carbon dioxide	CO ₂	0 %	0 %
32.	Rhenium	Re	0.00003 %	0.00041 %

Figure 1. Assessment of statistically significant variations in plant characteristics grown under two distinct conditions

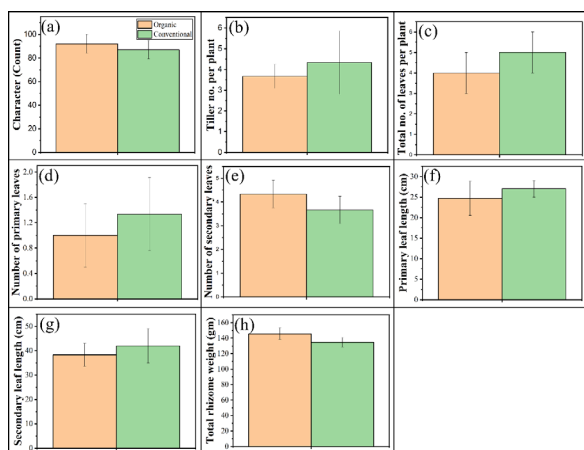


Figure 2. Analysis of the UV Spectroscopy of black turmeric methanol extract.

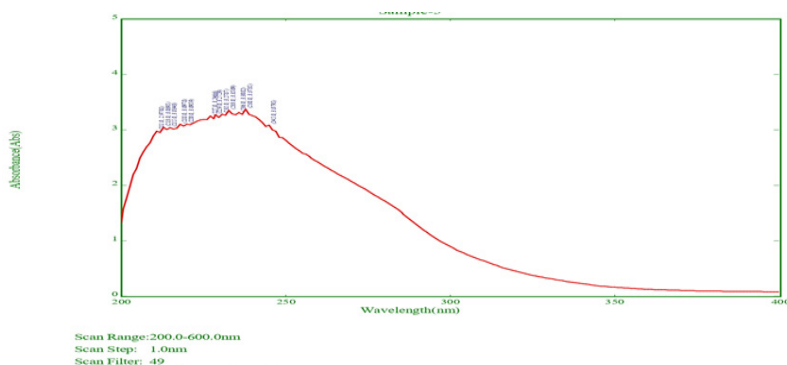


Figure 3. Elemental analysis of rhizome and soil sample using XRF. a) Organic aqueous rhizome extract. b) Conventional aqueous rhizome extract. c) Bhubaneswar soil. d) Sambalpur soil.

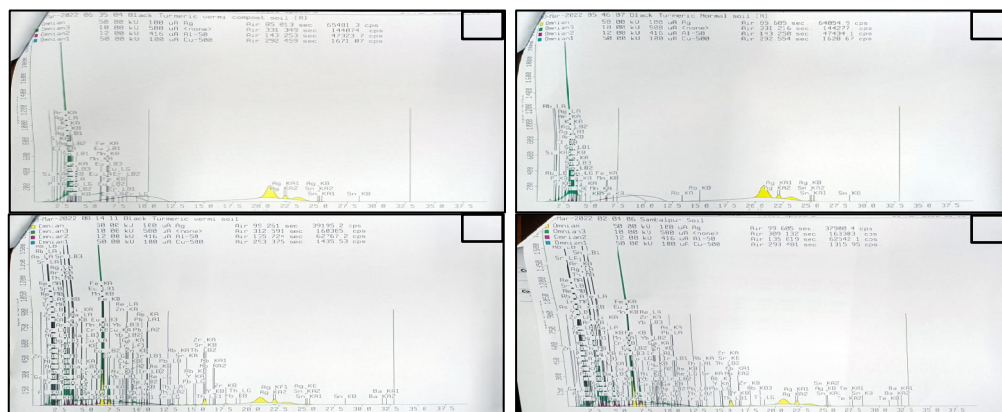
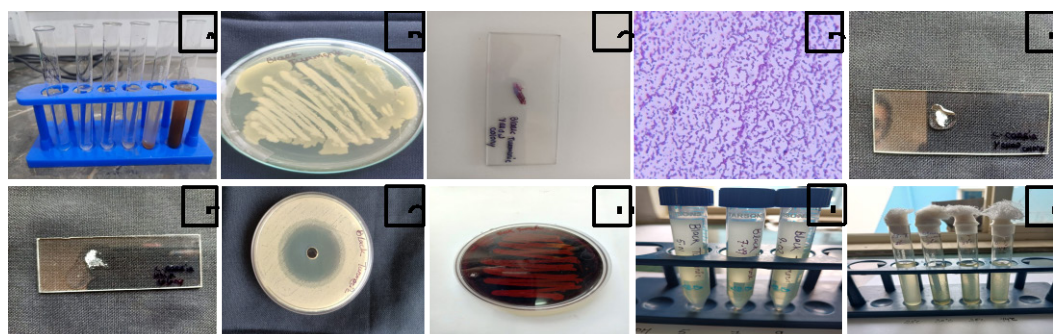


Figure 5. Isolated soil bacteria's isolation, identification, and biochemical activities. a) Serial dilution method. b) Pure culture. c) Gram staining. d) Gram-positive. e, f) Catalase activity. (g) Antibiotic sensitivity activity. h) Starch hydrolysis. i) pH tolerance test. j) Temperature tolerance test.



in the highest population of fungus, bacteria, and actinomycetes (Bendre et al., 2019). Potassium was the most abundant element in the aqueous rhizome extract of black turmeric in organic and conventional soils (0.120 and 0.233 per cent, respectively), with Silicon and phosphorus following in varying concentrations (Table 6). Bhubaneswar and Sambalpur organic soil show the highest concentration of Silicon dioxide (SiO_2) (66.721 % and 73.342 %), followed by Aluminium oxide (Al_2O_3) (16.571 % and 12.723 %) (Table 7). In the case of nitrogen, Potassium (K) plays a vital role in overall plant growth because it regulates various essential processes in plant advancement. It aids plants in dealing with environmental abiotic stress factors and serves an essential regulatory role (Johnson et al., 2022). As a basic agronomic technique, applying K fertilizer to plants increases crop tolerance to short-term water shortages. The availability of water and other mineral components, such as nitrogen, is enhanced due to this development, increasing plant growth and productivity (Pandey & Mahiwal, 2020). Numerous plant species benefit from Silicon (Si) and its compounds, particularly when subjected to biotic and abiotic stressors. When applied to wheat plants, SiO_2 Nano Particles significantly reduce the negative impacts of drought conditions (Behboudi et al., 2018).

When nano silica penetrates a bean seed, it changes specific physical and chemical reactions, improving the germination rate (Alsaeedi et al., 2017). The physiological and developmental processes of the crop are both impacted by Al_2O_3 NPs. It is possible to propose priming crops with dosages of Al_2O_3 NPs to bring about beneficial modifications in roselle and other agricultural plants (Abdel et al., 2020). Innovative metal oxide nanoparticles have recently been developed as nano fertilizers, biosensors, pesticides, and antimicrobial agents. These developments have essential effects on the future of environmentally friendly agricultural nanotechnology, including improved growth of plants and pest/disease monitoring, as well as long-term crop management and preservation (Maity et al., 2022).

5. Conclusion

In the face of declining soil quality, agroecosystem conservation is essential for preserving ecological functions for future generations. As soil qualities and agricultural yields continue to deteriorate, microbiota has the potential to enhance soil's inherent suppressiveness against soil-borne disease vectors. When compared to conventional farming methods, organic farming is considerably superior. These have the potential to significantly improve sustainable agricultural output, lessen the use of pesticides in agriculture, and ultimately benefit both humans and the environment. Soil types and microbial populations maintained organically may increase resistance in plants, reducing the attractiveness of plants to pests. Organic fertilizer used after

treatment can quickly boost the soil's microbial community, leading to better nutrition, ecosystem services, and improved plant yield and quality. Because current antibiotics have been ineffective in combating the rise of resistant microbes, searching for new strains of bacteria that produce antimicrobials is an urgent matter.

The selected plant cultivated in organic soil significantly affects human pathogenic bacteria. There was a notable difference between the rhizomes grown in conventional soil and those planted in organic soil conditions concerning growth and measurement. The rhizome grown in organic soil outperformed its conventionally grown counterparts regarding growth, development, nutrient uptake, antibacterial activity, and overall yield. Isolates demonstrating antimicrobial activity against various test pathogens should be investigated for further antimicrobial component searches.

Author contributions

B.J., S.T. wrote, collected data, arranged data, and interpreted; G.M. conceptualized the work; G.M., H.B.S. critically revised the manuscript; B.J., S.T., G.M., H.B.S. read and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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