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**VS Bhagde**
Research Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

**CV Ambadkar**
Associate Professor, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

**MA Gaikwad**
Research Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

**SR Itahpe**
Research Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

**DK Saini**
Research Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

**Corresponding Author:**
**VS Bhagde**
Research Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

# Biodegradation efficiency and soil nutrient enhancement by *Trichoderma harzianum* and *Pseudomonas fluorescens* in agricultural bio-waste management

**VS Bhagde, CV Ambadkar, MA Gaikwad, SR Itahpe and DK Saini**

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**Abstract**
Improper management of agricultural bio-waste such as sugarcane trash, soybean straw, and cow dung leads to environmental degradation, including greenhouse gas emissions and soil quality loss. The present study was conducted to evaluate the decomposition potential of efficient microbes isolated from the rhizosphere of safflower (*Carthamus tinctorius* L) on various agricultural bio-wastes. A total of eight microbial isolates, including six fungal strains (*Trichoderma harzianum, T. asperellum, T. koningii, T. virens, Aspergillus niger,* and *A. flavus*) and two bacterial strains (*Bacillus subtilis* and *Pseudomonas fluorescens*), were identified based on morphological and cultural characteristics. Based on growth efficiency, *T. harzianum* and *P. fluorescens* were selected and applied individually and in consortia to three different organic substrates sugarcane trash, cow dung, and soybean straw and monitored over a 120-day, during which parameters such as pH, EC, C:N ratio and macro/micro-nutrient content (N, P, K, Zn, Cu, Mn, Fe) were evaluated.
The combination of *T. harzianum* and *P. fluorescens* demonstrated superior biodegradation efficiency across all substrates. Among the treatments, soybean straw treated with both microbes ($T_9$) showed the highest degradation rate (50%), fastest decomposition (81 days), and significant improvements in compost quality, including reductions in C:N ratio (24.35) and increases in nitrogen (1.04%), phosphorus (0.48%), potassium (1.38%), copper (284 ppm), and iron (3420 ppm) contents.
These findings suggest that microbial consortia enhance compost maturity and nutrient enrichment, offering a sustainable and eco-friendly solution for agricultural waste management and soil fertility improvement.

**Keywords:** Agricultural bio-waste, microbial consortia, biodegradation, decomposition, nutrient enrichment, C:N ratio

## Introduction
Agricultural activities produce significant amounts of organic waste, including crop residues and plant biomass, which, if not properly managed, lead to soil degradation, water pollution, air contamination, and greenhouse gas emissions. In many developing countries, these wastes are often burned or dumped indiscriminately, posing serious environmental and health risks. Agricultural residues are primarily composed of complex organic materials such as cellulose, lignin, and pectin, which decompose slowly under natural conditions. However, composting has emerged as an effective, eco-friendly method for managing this waste. Through composting, these materials can be transformed into nutrient-rich compost, improving soil fertility, enhancing crop nutrition and reducing reliance on chemical fertilizers.
Microorganisms, particularly fungi like *Trichoderma*, *Aspergillus*, and *Pseudomonas*, play a crucial role in breaking down lignocellulosic components by producing enzymes such as cellulase and ligninase. Using microbial consortia can accelerate the composting process and improve efficiency. On-farm composting with locally available residues such as sugarcane trash, rice straw, and weeds offers a sustainable, low-cost solution. It not only recycles waste but also boosts soil microbial activity, enhances water retention, and contributes to climate change mitigation. Promoting awareness and adoption of microbial-based composting is essential for sustainable agriculture and environmental protection.

**Material and Methodology**

Eight microorganisms were isolated from rhizhosphere of safflower crop. Among the eight isolated microbes, *Pseudomonas fluorescens* and *Trichoderma harzianum* were selected for further study**.** For the experiment three types of agricultural bio-waste were selected including sugarcane trash, soybean straw and cow dung. The selected agricultural bio-waste was added to composting pits with dimensions of (5×4×1) feet at the experimental site, with 50 kg of agriculture bio-waste (sugarcane trash, cow dung and soybean straw) per pit. The isolated microbial inoculants applied individually and in consortium at a rate of 5 percent. (w/w) of the bio-waste. The inoculants were thoroughly mixed with the bio-waste to ensure even distribution. Moisture content in each pit was maintained above 60 percent throughout the composting period to support microbial activity.

Different parameters were observed over a time period of 120 days, including change in colour, texture and odour. Physiochemical parameter such as change in C:N ratio, degradation rate, loss in weight, macro and micro nutrient were analyzed both at the beginning and at the end of the 120-day composting period. All above parameters analysed using different methods as mentioned below:

**C:N Ratio**

$$\text{C:N ratio} = \frac{\text{Organic carbon}}{\text{Nitrogen}}$$

Organic Carbon

Total carbon was determined by dry combustion method (Nelson and Sommers, 1982) [10].

$$\%\text{ of ash} = \frac{\text{Weight of ash}}{\text{Weight of compost}} \times 100$$

$$\%\text{ of organic carbon} = \frac{100 - \%\text{ ash}}{1.724} \times 100$$

**Total nitrogen**

Total nitrogen was estimated by Kjeldahl's method (Bremner, 1965) [4].

$$\%\text{ of Nitrogen} = \frac{\text{N } 0.28\,(\text{S} - \text{B})}{\text{Weight of compostable sample (mg)}} \times 100$$

Where, S = mL of 0.02N HCl used for compostable sample
B = mL of 0.02N HCl used for blank

**Total phosphorus**

The total phosphorus content in the compostable sample was determined by the method of John (1970) [7].

$$\text{mg/kg compostable sample} = \frac{\text{g}\frac{\text{P}}{\text{mL}}\text{ corresponding to absorbance} \times 50 \times 100}{\text{mL of aliquot taken} \times \text{weight of compost}}$$

**Total Potassium**

Total Potassium in the samples was estimated using Flame photometer by direct feeding method (Jankowski *et al.* 1961) [6].

$$\text{Total K (\%)} = \frac{\text{Concentration of K in ppm} \times \text{dilution factor} \times 100}{\text{Weight of compost sample (g)} \times 1000}$$

**Total micronutrient content**

The micronutrient Zn, Fe, Mn and Cu content in compost were determined by using Atomic Absorption Spectrophotometer, as described by Lindsay and Norvell (1978) [8].

$$\text{Micronutrient (mg/kg)} = \frac{\text{Concentration of in ppm} \times \text{dilution factor}}{\text{Weight of compost sample (g)}}$$

**Statistical analysis**

The data related to total organic carbon (C), nitrogen (N), phosphorus (P) and potassium (K) micro-nutrient (Zn, Fe, Mn and Cu) in the compost, as obtained during the present investigation, were subjected to statistical analysis. The significance of treatment effects was tested using Analysis of Variance (ANOVA), following the standard procedures outlined by Panse and Sukhatme (1967) [11] and Gomez and Gomez (1984) [5]. Statistical computations were carried out using the OPSTAT online software and additional analysis and tabulations were performed using customized Excel spreadsheets.

**Result and Discussion**

During decomposition under different treatments there was a considerable change in the organic carbon, nitrogen, phosphorous, potassium and C:N ratio as shown in Table 1.

A significant decrease in total carbon content was recorded in $T_8$, where values declined from 40.89 percent to 22.88 percent, indicating effective carbon mineralization through microbial decomposition. This observation is in agreement with the findings of Srikanth *et al.* (2000) [15], who reported reduction in carbon content during composting of crop residues using cellulolytic fungi, supporting that carbon depletion is a desirable outcome indicating organic matter degradation. Similarly, Maheshwari (2002) [9] and Bhoyar *et al*. (2007) [3] observed significant carbon loss during decomposition of soybean stover and wheat straw using *T. harzianum*, *T. ressei* and *T. viride*, further corroborating the effectiveness of fungal and bacterial inoculants in enhancing compost maturity. The current results are thus consistent with these previous findings, reinforcing the role of microbial inoculants in accelerating carbon mineralization during composting.

Nitrogen content increased substantially in $T_9$ (Soybean straw + *P. fluorescens* + *T. harzianum*), rising from 0.48 percent to 1.04 percent, which may be attributed to microbial nitrogen fixation and reduced nitrogen losses.

**Table 1:** Effect of *T. harzianum* and *P. fluorescens* on carbon content (%), nitrogen content (%), Phosphorus content (%), potassium content (%) and C:N ratio of different agricultural bio-waste during decomposition

| Tr. No | C | | N | | P | | K | | C:N Ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| $T_1$ | 40 | 28.14 | 0.56 | 0.75 | 0.24 | 0.36 | 0.98 | 1.22 | 95.23 | 37.52 |
| $T_2$ | 40.89 | 29.45 | 0.54 | 0.96 | 0.21 | 0.29 | 0.86 | 1.08 | 75.72 | 30.67 |
| $T_3$ | 40.8 | 30.11 | 0.48 | 0.95 | 0.22 | 0.28 | 0.94 | 1.17 | 72.85 | 31.7 |
| $T_4$ | 40 | 26 | 0.56 | 0.73 | 0.24 | 0.32 | 0.98 | 1.26 | 95.23 | 35.61 |
| $T_5$ | 40.89 | 30 | 0.54 | 0.98 | 0.21 | 0.3 | 0.86 | 1.14 | 75.72 | 30.61 |
| $T_6$ | 40.8 | 27.89 | 0.48 | 0.9 | 0.22 | 0.38 | 0.94 | 1.28 | 72.85 | 30.99 |
| $T_7$ | 40 | 24.3 | 0.56 | 0.82 | 0.24 | 0.46 | 0.98 | 1.56 | 95.23 | 29.63 |
| $T_8$ | 40.89 | 22.88 | 0.54 | 1.02 | 0.21 | 0.52 | 0.86 | 1.43 | 75.72 | 22.43 |
| $T_9$ | 40.8 | 25.33 | 0.48 | 1.04 | 0.22 | 0.48 | 0.94 | 1.38 | 72.85 | 24.35 |
| $T_{10}$ | 40 | 36.8 | 0.56 | 0.45 | 0.24 | 0.24 | 0.98 | 1.01 | 95.23 | 80.88 |
| $T_{11}$ | 40.89 | 38.33 | 0.54 | 0.62 | 0.21 | 0.23 | 0.84 | 0.86 | 75.72 | 61.82 |
| $T_{12}$ | 40.8 | 40.6 | 0.48 | 0.70 | 0.22 | 0.22 | 0.94 | 0.97 | 72.85 | 67.66 |
| S.E.(m) ± | | 1.5 | | 0.04 | | 0.01 | | 0.06 | | 1.42 |
| C.D. at 5% | | 4.41 | | 0.12 | | 0.05 | | 0.17 | | 4.18 |

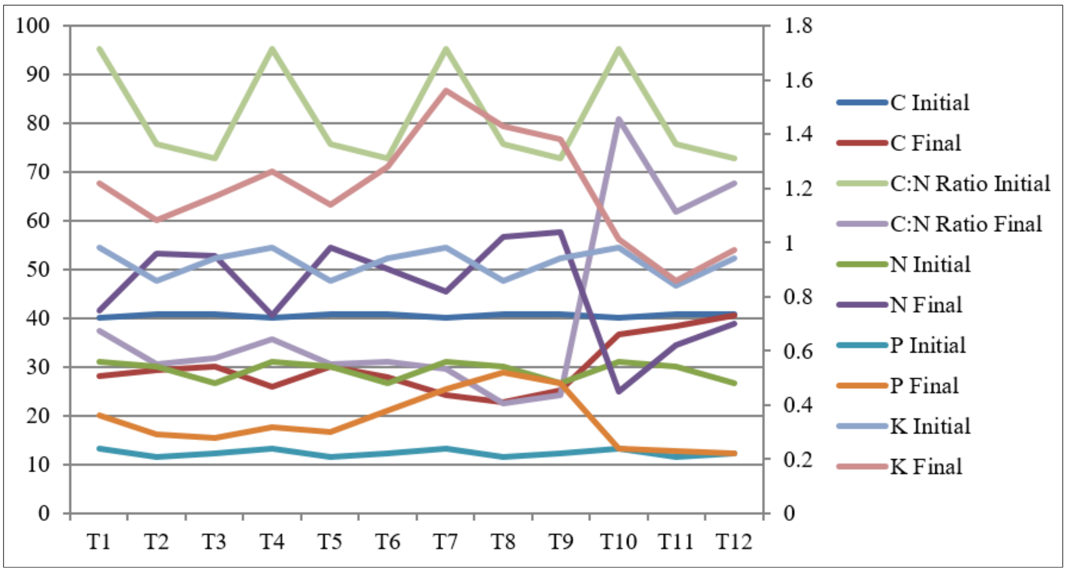


**Fig 1:** Effect of *T. harzianum* and *P. fluorescens* on carbon content (%), nitrogen content (%), Phosphorus content (%), potassium content (%) and C:N ratio of different agricultural bio-waste during decomposition

Phosphorus content showed the highest improvement in $T_8$, increasing from 0.21 percent to 0.52 percent, reflecting microbial solubilization of phosphate compounds during composting. Enhanced phosphorus availability through microbial action has been documented by.

Potassium content was found to be highest in $T_7$ (Sugarcane trash + *P. fluorescens* + *T. harzianum*), which increased from 0.56 to 1.56. The enrichment of potassium during composting is often associated with microbial degradation of lignocellulosic matter and release of bound nutrient.

Similarly for N, P and K, Patil (1994) [12], while working on wheat straw composting, recorded total nutrient contents of 0.54 percent nitrogen, 0.12 percent phosphorus and 1.45 percent potassium. Likewise, Sarker *et al.* (2013) [14] assessed the nutrient profile of compost prepared from sugarcane press mud using microbial consortia and found that by the end of the composting period, nitrogen content had reached 2.34 percent, while phosphorus and potassium contents were 1.15 percent and 1.37 percent, respectively. These values were considerably higher than those found in the uninoculated controls, reflecting the positive role of microbial inoculants. Additionally, Yadav and Tripathi (2011) [17] reported that composting crop residues with *Trichoderma* species significantly increased nutrient levels, with nitrogen reaching up to 2.10 percent, phosphorus 1.20 percent, and potassium 1.40 percent by the end of the process. Similarly, Zhang *et al.* (2023) [18] found that microbial inoculation with *Pseudomonas fluorescens* and *Trichoderma harzianum* enhanced the nutrient content of compost made from various organic wastes, resulting in nitrogen, phosphorus, and potassium concentrations higher than uninoculated controls. Additionally, Babu *et al.* (2009) [1] observed that farm waste composts enriched with microbial activity showed substantial increases in available potassium up to 1.34 percent and nitrogen content, confirming the beneficial effects of microbial inoculants on nutrient enrichment during composting.

C:N ratio notably decrease from 75.72 to 22.43 in treatment $T_8$ (Cow dung + *P. fluorescens* + *T. harzianum*), followed by $T_9$ from 72.85 to 24.35, in which soybean straw was treated with *P. fluorescens* + *T. harzianum*. These observations are in agreement with the findings of Bhattacharyya *et al.* (2009) [2] and Sundararajan *et al.* (2011) [16], who reported enhanced compost maturity and reduced C:N ratios when agricultural residues were inoculated with lignocellulolytic fungi and nitrogen-transforming bacteria such as *T. harzianum* and *P. fluorescens*. Similarly the changes in micronutrient content during decomposition were given in Table 2. Copper content was found to be highest in $T_9$ (Soybean straw + *Pseudomonas fluorescens* + *Trichoderma harzianum*), increasing from 154 ppm to 284 ppm. This indicates enhanced microbial action that made copper more available during composting.

Iron content reached its peak in $T_7$ (Sugarcane trash + *P. fluorescens* + *T. harzianum*), rising from 2842 ppm to 3457 ppm. The microbial consortium likely improved mineralization and mobilization of iron.

Zinc was most enriched in $T_8$ (Cow dung + *P. fluorescens* + *T. harzianum*), where levels rose from 26.2 ppm to 190 ppm. The cow dung likely supported strong microbial activity, helping release zinc from organic bindings.

Manganese content was highest in $T_7$ (Soybean straw + *P. fluorescens* + *T. harzianum*), increasing from 292 ppm to 1018 ppm. The decomposition of soybean straw by microbes appears to have released significant quantities of manganese into the compost.

**Table 2:** Effect of *T. harzianum* and *P. fluorescens* on copper, iron, zinc and manganese content (ppm) of different agricultural bio-waste during decomposition

| Tr. No | Cu | | Fe | | Zn | | Mn | |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| $T_1$ | 151 | 229 | 2842 | 3372 | 28.61 | 145 | 292 | 937 |
| $T_2$ | 164 | 261 | 2832 | 3362 | 26.2 | 131 | 312 | 908 |
| $T_3$ | 154 | 241 | 2442 | 3374 | 39.8 | 155 | 239 | 940 |
| $T_4$ | 151 | 244 | 2842 | 3296 | 28.61 | 169.8 | 292 | 852 |
| $T_5$ | 164 | 220 | 2832 | 3320 | 26.2 | 137.8 | 312 | 655 |
| $T_6$ | 154 | 262 | 2442 | 3324 | 39.8 | 140.2 | 239 | 624 |
| $T_7$ | 151 | 230 | 2842 | 3457 | 28.61 | 185 | 292 | 1018 |
| $T_8$ | 164 | 276 | 2832 | 3440 | 26.2 | 190 | 312 | 994 |
| $T_9$ | 154 | 284 | 2442 | 3420 | 39.8 | 170.8 | 239 | 968 |
| $T_{10}$ | 151 | 174 | 2842 | 2848 | 28.61 | 29 | 292 | 344 |
| $T_{11}$ | 164 | 168 | 2832 | 2918 | 26.2 | 31 | 312 | 355 |
| $T_{12}$ | 154 | 160 | 2442 | 2520 | 39.8 | 40.2 | 239 | 256 |
| S.E.(m) ± | | 1.43 | | 1.14 | | 1.17 | | 1.52 |
| C.D. at 5% | | 4.19 | | 3.33 | | 3.44 | | 4.46 |

Similar result related to micronutrient (Cu, Fe, Zn and Mn) were observed by Zhang *et al*. (2023) [18], who observed significant enrichment in composted organic wastes, particularly iron (Fe) and manganese (Mn), with Fe increasing up to 28.7 percent and copper (Cu) rising by as much as 235 percent in certain compost piles. Supporting this, Raut *et al*. (2008) [13] found that the use of microbial consortia containing efficient fungi and bacteria enhanced the availability of zinc (Zn) by 28 to35 percent, iron by 22 to 30 percent and manganese by 25 to 32 percent, and copper by 18 to 26 percent compared to uninoculated controls. This improvement was attributed to microbial production of organic acids, chelating agents, and enzymes that facilitated the solubilization and mineralization of nutrients. These studies reinforce the current findings by demonstrating the effectiveness of microbial inoculants in promoting micronutrient release, accelerating decomposition, and enhancing the overall nutrient quality of compost.

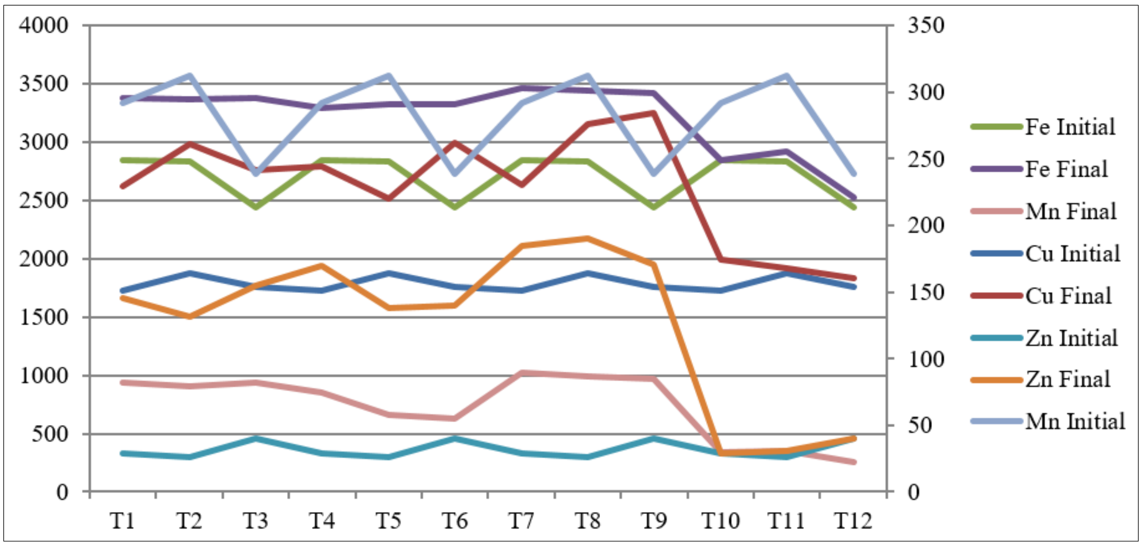


**Fig 2:** Effect of *T. harzianum* and *P. fluorescens* on copper, iron, zinc and manganese content (ppm) of different agricultural bio-waste during decomposition

## Conclusion

The study demonstrated that the use of microbial inoculants significantly enhanced the decomposition process and nutrient enrichment of compost. Among the treatments, $T_8$ (Cow dung + *P. fluorescens* + *T. harzianum*) showed the most effective carbon mineralization, with a notable decrease in organic carbon content and C:N ratio, indicating advanced compost maturity. $T_9$ (Soybean straw + *P.*

*fluorescens* + *T. harzianum*) recorded the highest nitrogen and copper content, suggesting improved nitrogen retention and micronutrient solubilization. $T_8$ also resulted in maximum phosphorus and zinc enrichment, while $T_7$ (Sugarcane trash + *P. fluorescens* + *T. harzianum*) was superior in potassium and iron content. The combined microbial action significantly accelerated the degradation of lignocellulosic material and improved the availability of both macro- and micronutrients. These findings reinforce the potential of microbial consortia in producing nutrient-rich compost from agricultural residues, contributing to sustainable soil fertility management.

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