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Enhanced soil fertility, plant growth promotion and microbial enzymatic activities of vermicomposted fly ash

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It is reported that coal consumption in the Asia-Pacific region is going to increase to about 87.2 percent by 2035. Management of coal combustion residues (CCRs) generated by industries is a major bottleneck towards handling the repercussions of coal usage. The present study investigates a management technique for these potentially hazardous wastes by means of vermicomposting. In the present investigation, studies were made on the effects of various concentrations of vermicomposted fly ash (VCF) added to agricultural soil, on the growth and yield of tomato (*Lycopersicon esculentum* Mill.) and brinjal (*Solanum melongena* L.) plants. The toxicity of trace elements in VCF were estimated using coefficient of pollution and potential ecological risk index, which revealed no apparent risks to the environment. A gradual increase in VCF concentrations in the agricultural soil improved the physico-chemical properties, enzymatic activities, microbial biomass, carbon and microbial population upto 90 days after sowing of seeds. The VCF amendments significantly ($p < 0.05$) improved the soil quality (2.86% nitrogen and 1.05% Phosphorous) and germination percentage (82.22%) of seeds in *L. esculentum* and also in *S. melongena*. The results of this study reveal that, CCRs can be effectively managed in agriculture specially in developing economies.

The demand for electricity is increasing throughout the world and the trend is expected to continue in the years to come. About 70% of the electricity in India is generated through coal based thermal power plants, which produce approximately 65 million tons of fly ash (FA) in a year as a by-product¹. The production of FA majorly depends on the coal quality, which comprises a fairly high proportion of ash that leads to 10–30% of FA formation². In recent times, disposal of FA has become a chief concern globally. Moreover, this problem has become a serious apprehension in the developing countries and is generally carried out in landfills nearby the thermal power plants.

Utilization of FA in revegetating the landfill regions is an alternative for FA management, which serves both for stabilization and delivering an amiable landscape^{3–6}. Additionally, this management technique possibly conveals the physico-chemical properties of soil like pH, texture and water holding capacity (WHC). Supplement of alkaline FA, which has a pH above 9.0⁷, can decrease soil acidity to a level suitable for agriculture⁸, and can increase the accessibility of trace metals, SO₂ and other nutrients⁹. However, direct application of FA to agricultural ground would not be quite advantageous to crops, due to little availability of most of the essential nutrient elements viz. nitrogen (N) and phosphorous (P), and a lower rate of FA degradation after its application in soil. Moreover, FA has a prevalence of heavy metals in the material and soluble forms¹⁰. FA comprises a high concentration of toxic heavy metals like Cr, Pb, Cd, Ni, Cu, Zn, etc.^{11–13}.

Utilization of FA through vermicomposting is a crucial step towards environmental sustainability and retaining soil quality to reduce the dependency on agrochemical fertilizers. It is also an effective method for extenuation of metals from FA¹⁰. Earthworm species exhibiting vermicomposting (*Eisenia fetida*, *Eudrilus eugeniae* and *Lumbricus rubellus*) have an ability to increase the availability of key nutrient elements like phosphorous and nitrogen in FA, whilst reducing the solubility of heavy metals. Application of vermicomposted fly ash (VCF) to enhance crop productivity would not only be a resolution to the problem of FA disposal, but might also decline the use of chemical non-nitrogen fertilizers^{14,15}.

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| Parameters | Days | T1 | T2 | T3 | T4 | T5 | T6 |
|-----------------------------------|----------|---------------|---------------|---------------|---------------|---------------|---------------|
| Bulk density (g/cm ³) | 0 | 1.61 ± 0.05b | 1.46 ± 0.02b | 1.37 ± 0.01c | 1.23 ± 0.02a | 1.06 ± 0.02d | 0.97 ± 0.01e |
| | 120 (Le) | 1.30 ± 0.01a | 1.31 ± 0.01a | 1.26 ± 0.02b | 0.93 ± 0.07d | 0.98 ± 0.01c | 0.92 ± 0.006c |
| | 120 (Sm) | 1.32 ± 0.05a | 1.34 ± 0.02a | 1.28 ± 0.04b | 0.96 ± 0.002c | 0.94 ± 0.01c | 0.89 ± 0.02c |
| Porosity (%) | 0 | 41.71 ± 1.65d | 44.26 ± 1.21c | 46.38 ± 0.20b | 47.96 ± 0.50b | 48.71 ± 0.36b | 50.86 ± 0.71a |
| | 120 (Le) | 48.11 ± 0.52b | 48.89 ± 0.14b | 47.38 ± 0.35c | 48.52 ± 0.04b | 50.41 ± 0.08a | 50.67 ± 0.91a |
| | 120 (Sm) | 46.24 ± 0.66b | 47.66 ± 0.28a | 46.54 ± 1.05b | 46.28 ± 0.55b | 45.27 ± 1.12c | 44.89 ± 0.06d |
| WHC (%) | 0 | 40.51 ± 0.22e | 42.88 ± 0.58d | 45.37 ± 0.23c | 47.13 ± 1.32b | 48.05 ± 0.67b | 54.50 ± 0.84a |
| | 120 (Le) | 44.51 ± 0.87f | 45.25 ± 0.44e | 46.86 ± 0.20d | 48.32 ± 0.10c | 49.04 ± 0.57b | 55.50 ± 1.10a |
| | 120 (Sm) | 43.75 ± 0.02e | 45.78 ± 0.55d | 45.42 ± 0.87d | 47.76 ± 0.02b | 46.84 ± 0.06c | 48.32 ± 1.72a |
| CEC (meq/100g) | 0 | 3.88 ± 0.01d | 4.03 ± 0.02d | 4.10 ± 0.02c | 4.15 ± 0.02c | 4.61 ± 0.01b | 4.88 ± 0.01a |
| | 120 (Le) | 4.67 ± 0.09c | 4.81 ± 0.03b | 4.12 ± 0.03e | 4.21 ± 0.04d | 4.72 ± 0.06b | 5.14 ± 0.03a |
| | 120 (Sm) | 3.75 ± 0.05b | 3.81 ± 0.02a | 3.12 ± 0.002d | 3.25 ± 0.06c | 3.72 ± 0.02b | 3.14 ± 0.08d |
| pH | 0 | 6.56 ± 0.02e | 6.78 ± 0.02e | 7.01 ± 0.10d | 7.14 ± 0.02c | 7.36 ± 0.02b | 7.52 ± 0.02a |
| | 120 (Le) | 6.96 ± 0.02c | 7.47 ± 0.07d | 7.82 ± 0.02b | 7.83 ± 0.02b | 7.95 ± 0.03a | 7.96 ± 0.02a |
| | 120 (Sm) | 6.86 ± 0.01b | 6.78 ± 0.02b | 6.95 ± 0.05b | 7.04 ± 0.02a | 7.06 ± 0.04a | 7.09 ± 0.05a |
| OC (%) | 0 | 1.35 ± 0.03d | 1.54 ± 0.02c | 1.78 ± 0.02b | 1.94 ± 0.02b | 2.05 ± 0.03a | 2.09 ± 0.02a |
| | 120 (Le) | 1.49 ± 0.06f | 1.56 ± 0.02e | 1.99 ± 0.06d | 2.08 ± 0.02c | 2.17 ± 0.02b | 2.34 ± 0.03a |
| | 120 (Sm) | 1.55 ± 0.07c | 1.57 ± 0.03c | 1.98 ± 0.002b | 2.12 ± 0.05b | 2.34 ± 0.06a | 2.47 ± 0.02a |
| EC (dS/m) | 0 | 3.15 ± 0.06d | 3.69 ± 0.03c | 3.80 ± 0.05b | 3.85 ± 0.01b | 3.97 ± 0.02a | 4.07 ± 0.02a |
| | 120 (Le) | 3.70 ± 0.15d | 3.92 ± 0.04c | 4.00 ± 0.02bc | 4.03 ± 0.03b | 4.09 ± 0.02b | 4.80 ± 0.05a |
| | 120 (Sm) | 3.85 ± 0.05c | 3.97 ± 0.04c | 4.02 ± 0.02b | 4.05 ± 0.06b | 4.10 ± 0.60a | 4.12 ± 0.57a |
| Total N (%) | 0 | 1.26 ± 0.01e | 1.38 ± 0.02d | 1.44 ± 0.03d | 2.04 ± 0.03c | 2.17 ± 0.02b | 2.29 ± 0.03a |
| | 120 (Le) | 1.26 ± 0.01f | 2.52 ± 0.23c | 2.06 ± 0.02e | 2.44 ± 0.09d | 3.51 ± 0.06a | 2.86 ± 0.09b |
| | 120 (Sm) | 1.46 ± 0.01d | 2.66 ± 0.33c | 2.65 ± 0.15c | 2.54 ± 0.02b | 2.58 ± 0.05b | 2.72 ± 0.03a |
| Available P (%) | 0 | 0.31 ± 0.02c | 0.37 ± 0.02c | 0.39 ± 0.02c | 0.47 ± 0.03b | 0.58 ± 0.01b | 0.88 ± 0.01a |
| | 120 (Le) | 0.42 ± 0.02e | 0.40 ± 0.02e | 0.54 ± 0.02d | 0.59 ± 0.03c | 0.73 ± 0.06b | 1.05 ± 0.03a |
| | 120 (Sm) | 0.54 ± 0.002e | 0.57 ± 0.01d | 0.64 ± 0.03c | 0.66 ± 0.002c | 0.68 ± 0.02ab | 0.70 ± 0.05a |
| Exc. Potassium (%) | 0 | 0.21 ± 0.05f | 0.24 ± 0.02e | 0.27 ± 0.02d | 0.29 ± 0.05c | 0.32 ± 0.06b | 0.36 ± 0.05a |
| | 120 (Le) | 0.25 ± 0.02d | 0.34 ± 0.02c | 0.25 ± 0.02d | 0.28 ± 0.03d | 0.37 ± 0.02b | 0.51 ± 0.03a |
| | 120 (Sm) | 0.28 ± 0.003e | 0.31 ± 0.01d | 0.33 ± 0.002d | 0.36 ± 0.002c | 0.39 ± 0.06b | 0.41 ± 0.001a |
| Sulphate (%) | 0 | 2.96 ± 0.14e | 3.15 ± 0.03d | 3.67 ± 0.02c | 3.79 ± 0.03b | 4.12 ± 0.04a | 4.18 ± 0.01a |
| | 120 (Le) | 3.08 ± 0.02e | 3.49 ± 0.05d | 3.93 ± 0.03b | 3.87 ± 0.02c | 4.64 ± 0.05a | 4.40 ± 0.03a |
| | 120 (Sm) | 3.25 ± 0.05f | 3.46 ± 0.02e | 3.58 ± 0.09d | 3.64 ± 0.03c | 3.75 ± 0.01b | 3.81 ± 0.02a |

Table 1. Physico-chemical properties of vermicomposted fly ash amended soil at the time of sowing and harvesting of *Lycopersicon esculentum* and *Solanum melongena*. Le: *Lycopersicon esculentum*; Sm: *Solanum melongena*. Values are in Mean ± SD; (n = 3). Different letters in the same row represent significant differences in the physico-chemical parameters of different treatments comprising *L. esculentum* and *S. melongena* at $p < 0.05$ according to Duncan's Multiple Range Test (One-way ANOVA followed by Tukey's test).

There are almost no studies performed on the incorporation of VCF to the agricultural soil to determine the growth and yield of vegetable crops. In view of the above and to attain an efficient utilization of FA in agriculture, the present study deals with two main objectives: (i) to determine the variations in the physico-chemical properties of VCF amended soil at different rates of FA incorporation and (ii) to assess the growth and yield of two vegetable plants (*Lycopersicon esculentum* Mill. and *Solanum melongena* L.). The study also includes the quantification of photosynthetic pigments, shoot nitrogen and boron from both the plants. Photosynthesis and respiration rates were also estimated during the growth period of the plants.

Results

Physico-chemical properties of treated soil. The physico-chemical characteristics of treatments, before and at harvesting of *L. esculentum* are presented in Table 1. The bulk density of the treatments at the time of sowing was found to be lower as compared to the time of harvesting. The maximum cation exchange capacity (CEC) at the time of harvesting was observed for T6 i.e. 5.14 meq/100 g and minimal value of CEC was observed for T1 (4.67 meq/100 g). CEC values, total N and available P showed an increasing trend from the time of sowing to the time of harvest. The concentration of Mg was found to be higher at the time of harvesting compared to sowing. Mn concentration was maximum for T6 (25.35 mg/kg) and the concentration at the time of harvesting was higher compared to that of sowing for all the treatments (Table 2).

In the case of *S. melongena*, bulk density was higher in the treatments at the time of sowing compared to that of harvesting. The bulk density of the treatments ranged from 0.89–1.34 g/cm³ and maximum bulk density was observed for T2 (Table 1). The metal concentrations at 90 days after sowing (DAS) showed the following trend:

| Metals | Days | T1 | T2 | T3 | T4 | T5 | T6 |
|--------|----------|-----------------|-----------------|-----------------|----------------|------------------|-----------------|
| Cu | 0 | 1.50 ± 0.13d | 1.86 ± 0.04c | 1.86 ± 0.02c | 1.96 ± 0.02b | 2.16 ± 0.03b | 2.57 ± 0.01a |
| | 120 (Le) | 1.78 ± 0.04f | 1.95 ± 0.01e | 2.09 ± 0.01d | 2.15 ± 0.02c | 2.57 ± 0.03b | 3.17 ± 0.05a |
| | 120 (Sm) | 1.45 ± 0.07e | 1.52 ± 0.04d | 1.66 ± 0.04c | 1.68 ± 0.04c | 1.71 ± 0.02b | 2.10 ± 0.05a |
| Zn | 0 | 3.27 ± 0.07c | 4.57 ± 0.09c | 4.84 ± 0.02b | 4.89 ± 0.03b | 5.00 ± 0.03b | 5.28 ± 0.03a |
| | 120 (Le) | 3.44 ± 0.07d | 4.85 ± 0.03c | 4.94 ± 0.02b | 4.99 ± 0.04b | 4.87 ± 0.01c | 5.99 ± 0.08a |
| | 120 (Sm) | 2.88 ± 0.50d | 2.97 ± 0.02c | 3.04 ± 0.06c | 3.15 ± 0.52b | 3.26 ± 0.64b | 3.34 ± 0.60a |
| Cd | 0 | 0.30 ± 0.021d | 0.34 ± 0.02d | 0.58 ± 0.12c | 0.54 ± 0.05c | 0.66 ± 0.02b | 0.77 ± 0.01a |
| | 120 (Le) | 0.53 ± 0.04d | 0.45 ± 0.04e | 0.58 ± 0.02d | 0.69 ± 0.02c | 0.76 ± 0.02b | 0.91 ± 0.05a |
| | 120 (Sm) | 0.62 ± 0.004c | 0.57 ± 0.002d | 0.62 ± 0.001c | 0.75 ± 0.02b | 0.78 ± 0.02b | 0.84 ± 0.01a |
| Pb | 0 | 1.91 ± 0.05d | 2.04 ± 0.01d | 3.10 ± 0.02c | 3.12 ± 0.03c | 3.19 ± 0.01b | 3.79 ± 0.06a |
| | 120 (Le) | 2.13 ± 0.04e | 2.33 ± 0.04d | 3.14 ± 0.02c | 3.16 ± 0.01c | 3.26 ± 0.02b | 3.95 ± 0.03a |
| | 120 (Sm) | 1.97 ± 0.02e | 2.10 ± 0.05d | 2.13 ± 0.03d | 2.24 ± 0.02c | 2.45 ± 0.06b | 2.52 ± 0.03a |
| Ni | 0 | 0.75 ± 0.03e | 0.87 ± 0.02d | 1.05 ± 0.02c | 1.08 ± 0.02c | 1.16 ± 0.01b | 1.21 ± 0.11a |
| | 120 (Le) | 1.05 ± 0.01d | 0.93 ± 0.02e | 1.15 ± 0.02c | 1.12 ± 0.02c | 1.32 ± 0.07b | 1.44 ± 0.02a |
| | 120 (Sm) | 0.92 ± 0.02e | 0.98 ± 0.01e | 1.05 ± 0.002d | 1.15 ± 0.07c | 1.22 ± 0.30b | 1.34 ± 0.04a |
| Fe | 0 | 258.33 ± 0.58e | 272 ± 3.60d | 287.33 ± 2.08c | 282.84 ± 9.04c | 292.51 ± 1.99b | 315.37 ± 3.14a |
| | 120 (Le) | 265.66 ± 12.01e | 278.67 ± 16.20d | 297.07 ± 1.86c | 303.96 ± 7.78b | 311.72 ± 6.44b | 325.88 ± 1.46a |
| | 120 (Sm) | 267.45 ± 15.74e | 275.42 ± 23.65d | 282.58 ± 16.43c | 284.66 ± 8.45c | 295.33 ± 11.67 b | 312.57 ± 10.56a |
| Cr | 0 | 0.38 ± 0.04e | 0.55 ± 0.01d | 0.62 ± 0.06c | 0.67 ± 0.02c | 0.78 ± 0.02b | 0.93 ± 0.03a |
| | 120 (Le) | 0.47 ± 0.02d | 0.75 ± 0.03c | 0.74 ± 0.02c | 0.74 ± 0.02c | 0.94 ± 0.04b | 1.03 ± 0.02a |
| | 120 (Sm) | 0.52 ± 0.03e | 0.68 ± 0.02d | 0.75 ± 0.01c | 0.78 ± 0.004c | 0.85 ± 0.05b | 0.94 ± 0.01a |
| Mn | 0 | 13.74 ± 0.05d | 17.55 ± 0.10c | 17.87 ± 0.08c | 20.17 ± 0.05b | 21.08 ± 0.04b | 24.68 ± 1.00a |
| | 120 (Le) | 16.46 ± 0.75d | 18.78 ± 0.17c | 18.51 ± 0.06c | 21.08 ± 0.74b | 28.25 ± 0.53a | 25.35 ± 0.49a |
| | 120 (Sm) | 15.42 ± 0.66d | 16.34 ± 0.58c | 17.33 ± 0.47c | 18.73 ± 0.44b | 18.85 ± 0.36b | 19.27 ± 0.05a |
| Ca* | 0 | 0.10 ± 0.015e | 0.24 ± 0.02d | 0.3 ± 0.02c | 0.34 ± 0.02c | 0.45 ± 0.01b | 0.67 ± 0.02a |
| | 120 (Le) | 0.14 ± 0.03d | 0.47 ± 0.03c | 0.47 ± 0.01c | 0.45 ± 0.02c | 0.58 ± 0.02b | 0.83 ± 0.02a |
| | 120 (Sm) | 0.18 ± 0.02d | 0.24 ± 0.02c | 0.27 ± 0.05c | 0.32 ± 0.04b | 0.35 ± 0.02b | 0.38 ± 0.02a |
| Mg* | 0 | 0.083 ± 0.002e | 0.09 ± 0.003d | 0.15 ± 0.01c | 0.18 ± 0.01c | 0.27 ± 0.02b | 0.41 ± 0.04a |
| | 120 (Le) | 0.10 ± 0.00e | 0.11 ± 0.002e | 0.18 ± 0.01d | 0.27 ± 0.01c | 0.36 ± 0.03b | 0.43 ± 0.02a |
| | 120 (Sm) | 0.12 ± 0.004c | 0.16 ± 0.005c | 0.24 ± 0.03b | 0.31 ± 0.002a | 0.34 ± 0.001a | 0.37 ± 0.01a |

Table 2. Metal concentrations (mg/kg) of vermicomposted fly ash amended soil during sowing and harvesting of *Lycopersicon esculentum* and *Solanum melongena*. Le: *Lycopersicon esculentum*; Sm: *Solanum melongena*; Ca* and Mg*: Values of Ca and Mg are in percentage (%). Values are in Mean ± SD; (n = 3). Different letters in the same row represent significant differences in the mean of the metal concentrations of different treatments comprising *L. esculentum* and *S. melongena* at $p < 0.05$ according to Duncan's Multiple Range Test (One-way ANOVA followed by Tukey's test).

T6 > T5 > T4 > T3 > T2 > T1 (Table 2) Concentrations of Ca and Mg were found to be higher at 90th DAS compared to the 0th day of sowing of seeds. The maximum concentration of Mg was observed for treatment T6 (0.37) while, the minimum concentration was observed for T1 (0.12) in the case of *S. melongena* (Table 2).

A volcano plot depicting the relationship between various physico-chemical parameters after harvesting of both the crops are depicted in Fig. 1(a,b). In *L. esculentum*, parameters such as Available P, WHC, Zn, Pb, Fe, Sulphate and Mn were observed to be very significant ($p < 0.01$) points of interest which displays both high-level statistical significance ($-\log_{10}$ of p values, y-axis) and great magnitude fold changes (x-axis) (Fig. 1a). The parameters such as CEC, pH and EC were obtained with values having $p < 0.05$ (statistically significant).

In the case of *S. melongena*, parameters such as P, WHC, EC, CEC and pH were observed to have strong statistical differences ($p < 0.01$), while Zn and Fe were found to be moderately significant with values having $p \leq 0.05$ (Fig. 1b).

Dehydrogenase and Alkaline Phosphatase activities. Variations in the dehydrogenase activity were observed during harvesting of *L. esculentum* and *S. melongena*. Dehydrogenase activity was higher in treatments comprising *L. esculentum* compared to *S. melongena*. The trend of the dehydrogenase action in the treatments was as follows: T6 > T5 > T4 > T3 > T2 > T1 both in the case of *L. esculentum* and *S. melongena*. Maximum dehydrogenase activity was observed for T6 in both *L. esculentum* (ranging from 6.01–6.4 $\mu\text{g TPF/g/h}$) and *S. melongena* (6.17–6.24 $\mu\text{g TPF/g/h}$) (Figs. 2a,b).

Alkaline phosphatase activity displayed an increase in trend with an increase in concentration of VCF in both the treatments comprising *L. esculentum* and *S. melongena* (Fig. 2c,d). The activity was found to be lower in treatments comprising soil alone. The phosphatase activity in *L. esculentum* was higher for treatments T5 and T6 while, the lower enzyme activity was observed for T1 and T2 (Fig. 2c). In *S. melongena*, maximum phosphatase activity was observed for T6 (7.89 $\mu\text{mol PNP/g/h}$) while minimum activity was observed for T1 (6.37 $\mu\text{mol PNP/g/h}$) (Fig. 2d).

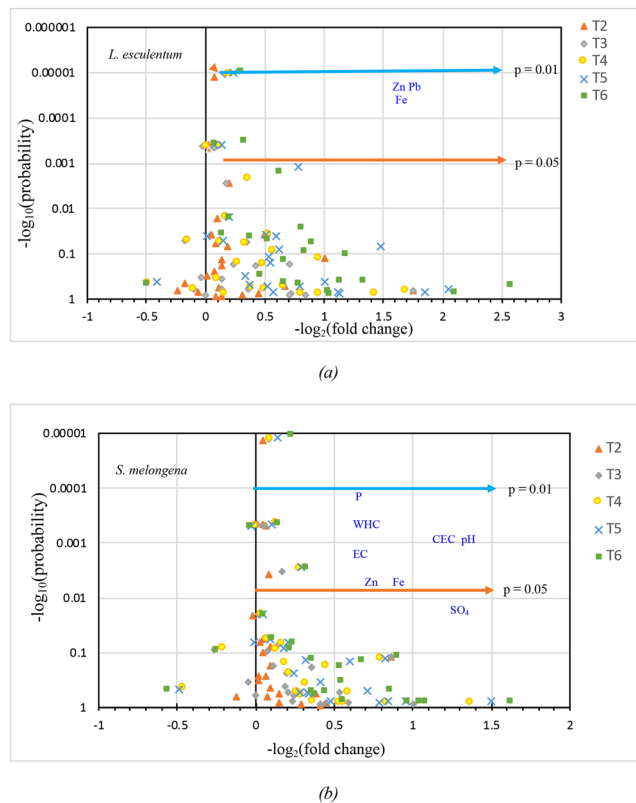


Figure 1. A volcano plot representing the expression of vermicomposted fly ash amended soil parameters under five different treatments scheme. Illustration: The effects of vermicomposted fly ash amended with soil before and after harvesting of *Lycopersicon esculentum* and *Solanum melongena* were enumerated by computing the average of treatment T1 (Control) and treatments: T2, T3, T4, T5, and T6. The X-axis represents logarithmic changes in soil physicochemical and nutritional attributes over basic soil, and the Y-axis represents the statistical significance of the difference based on one-way ANOVA considering the effects of a nutrient management scheme. Every individual attribute is displayed as a point. The compounds for which strong statistical differences ($p < 0.01$) were found are presented with names. Av: Available; P: porosity; WHC: water holding capacity; EC: Electrical conductivity; CEC: Cation exchange capacity.

Variation in bacterial population among treatments. The trend for phosphate solubilizing bacteria (PSB) population was as follows: $T6 > T5 > T4 > T3 > T2 > T1$ at 90 DAS of *L. esculentum* (Table 3). In *S. melongena*, the maximum PSB population was observed for T6 (116×10^4 cfu g^{-1}) while, minimum was obtained for T1 (14×10^4 cfu g^{-1}). Significant differences ($p < 0.05$) were detected in populations of *Azotobacter* among the several treatments. The maximum *Azotobacter* population was observed for T6 (104×10^4 cfu g^{-1}) in the case of *L. esculentum*.

The population of potash mobilizing bacteria was observed to be significantly ($p < 0.05$) lesser than PSB and *Azotobacter* at the time of harvesting of *L. esculentum* and *S. melongena*. The population of potash mobilizing bacteria was observed to be lower for T1 at the time of sowing and harvesting of *L. esculentum* and *S. melongena* at 90 days after sowing of *L. esculentum* (Table 3). For Treatment T6, the trend for Potash mobilizing bacteria was higher at the time of harvesting of *L. esculentum* compared to *S. melongena* at the 0th day of sowing.

Evaluation of PGP traits. All bacterial strains tested were positive to produce indole acetic acid (IAA) (Table 4). In *L. esculentum*, maximum siderophores production was observed for PSB (28.57), followed by *Azotobacter* (12.67) and potash mobilizing bacteria (3.55) (Table 4). In the case of *S. melongena*, the isolated strains of PSB, showed maximum siderophores production (25.45). All the isolated bacterial strains tested positive to produce ammonia. The details on PGP characteristics of the isolates are listed in Table 4.

Furthermore, PSB showed almost 100% phosphate solubilization during harvesting of *L. esculentum* and *S. melongena* while, *Azotobacter* and potash mobilizing bacteria showed lower rates of phosphorous solubilization (Table 4). Thus, the bacteria present in treatment T6 after harvesting of *L. esculentum* and *S. melongena* displayed a wide variety of activities which are essential for plant growth such as production of IAA, solubilisation of phosphates and production of ammonia and siderophores.

Microbial biomass carbon. Microbial biomass carbon (MBC) showed a direct relation with the concentration of VCF. Higher MBC values were observed for T5 and T6 in both *L. esculentum* and *S. melongena* (Fig. 3a,b). MBC values were found to be lower for T1. The trend for MBC among the treatments was $T6 > T5 > T4 > T3 > T2 > T1$.

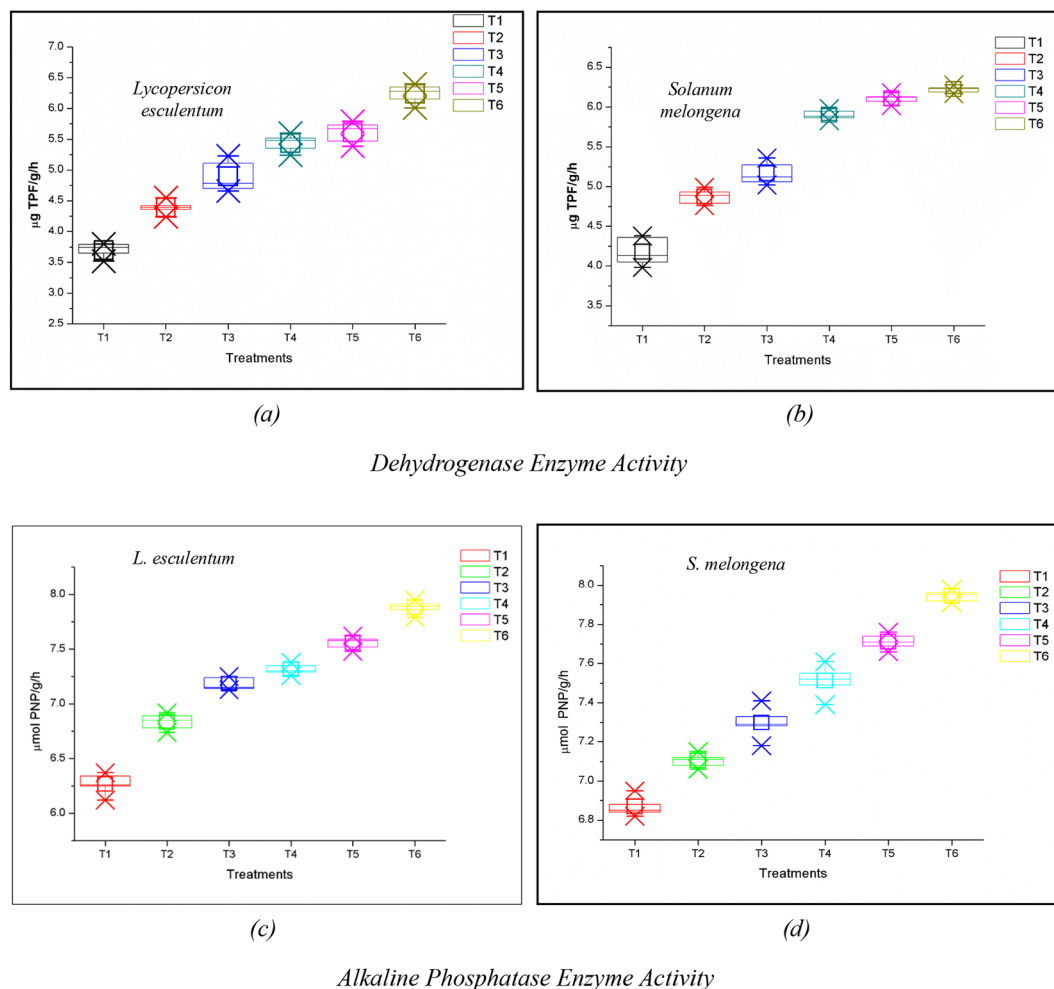


Figure 2. Variations in the dehydrogenase enzyme and alkaline phosphatase enzyme activity of treatments at the time of harvesting of *Lycopersicon esculentum* and *Solanum melongena*.

Effects of application of vermicomposted fly ash on the plant growth. Seed germination. *L. esculentum* plants showed positive response towards VCF soil amendment thus, exhibiting luxuriant growth. No visual symptoms related to toxicity of the FA, or to deficit of a particular nutrient had effects on the rate of seed germination. The results showed that the rate of seed germination significantly ($p < 0.05$) increased with the increase in rates of application of VCF (Table 5). The maximum increase in seed germination was found for the treatment, T6 (i.e. 8.56). The rate of seed germination of *S. melongena* was found to significantly increase ($p < 0.05$) with the increase in concentration of VCF showing the following trend T6 (5.56) > T5 (4.02) > T4 (3.48) > T3 (2.13) > T2 (1.89) > T1 (1.75) (Table 5).

Effects on shoot and root length and weight and number of leaves. The data on shoot and root length of *L. esculentum* at different growth stages as influenced by bio-formulations are presented in Fig. 4a. The shoot length displayed an upsurge in trend as per the duration of sowing of seeds. At the time of harvesting (90 DAS), the shoot length depicted an increase with an increase in the rates of VCF amendment to the agricultural soil. An increase in the trend of root length was also observed with increase in the rates of VCF (Fig. 4b).

The increase in the weight of shoots with increased rates of application of VCF and duration of sowing was observed (Fig. 4c–f). The dry and fresh weight of root and shoot of *L. esculentum* were found to increase with the duration of sowing. The maximum increase in shoot fresh weight was observed for T6 (35.50 g) at 90 days after sowing and maximum increase in shoot dry weight was also observed for T6 (3.51 g). The root fresh and dry weight of *L. esculentum* were also observed to be maximized for treatment T6 at 90 DAS with values 5.61 g and 0.66 g respectively (Fig. 4c,e).

The number of leaves increased with the increase in rates of application of VCF to the treatments (Fig. 5a,b). A maximum number of leaves were observed for the treatments comprising 15% VCF added to agricultural soil, while, minimum in treatments comprising 3% VCF.

Effects on the number of flowers and fruits in *Lycopersicon esculentum* and *Solanum melongena*. Significant differences were observed in the flower count among various treatments (Table 5) and maximum number of flowers were

| Days/Crop | Unit | T1 | T2 | T3 | T4 | T5 | T6 |
|--|-------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| <i>Phosphate solubilizing bacteria</i> | | | | | | | |
| 0 th day (<i>Le/Sm</i>) | cfu = --- ×10 ⁴ | 03 ± 0.002Cc | 04 ± 0.001Cb | 04 ± 0.002Cb | 04 ± 0.05Cb | 05 ± 0.003Ca | 05 ± 0.006Ca |
| 90 th day (<i>Le</i>) | | 16 ± 0.05Af | 78 ± 0.54Ae | 88 ± 0.25Ad | 102 ± 1.25Ac | 112 ± 0.58Ab | 123 ± 1.54Aa |
| 90 th day (<i>Sm</i>) | | 14 ± 0.02Bf | 74 ± 0.36Be | 85 ± 0.57Bd | 97 ± 0.75Bc | 106 ± 1.12Bb | 116 ± 0.82Ba |
| <i>Azotobacter</i> | | | | | | | |
| 0 th day (<i>Le/Sm</i>) | cfu = --- ×10 ³ | 07 ± 0.001Cc | 08 ± 0.005Cb | 08 ± 0.03Cb | 08 ± 0.002Cb | 09 ± 0.05Ca | 09 ± 0.02Ca |
| 90 th day (<i>Le</i>) | | 12 ± 0.20Af | 69 ± 0.38Ae | 75 ± 0.55Ad | 86 ± 0.45Ac | 95 ± 0.27Ab | 104 ± 1.02Aa |
| 90 th day (<i>Sm</i>) | | 10 ± 0.08Bf | 65 ± 0.54Be | 73 ± 0.65Bd | 84 ± 0.52Bc | 92 ± 0.25Bb | 101 ± 1.46Ba |
| <i>Potash mobilizing bacteria</i> | | | | | | | |
| 0 th day (<i>Le/Sm</i>) | cfu = --- ×10 ² | 04 ± 0.001Cd | 06 ± 0.003Cc | 08 ± 0.04Cb | 08 ± 0.002Cb | 9 ± 0.04Ca | 9 ± 0.05Ca |
| 90 th day (<i>Le</i>) | | 09 ± 0.25Af | 18 ± 0.42Ae | 21 ± 0.50Ad | 24 ± 0.52Ac | 28 ± 0.09Ab | 38 ± 0.45Aa |
| 90 th day (<i>Sm</i>) | | 07 ± 0.03Be | 15 ± 0.55Bd | 19 ± 0.68Bc | 21 ± 0.87Bc | 23 ± 0.51Bb | 31 ± 0.17Ba |

Table 3. Bacterial Population in soil amended with vermicomposted fly ash before (0th day) and after (120th day) harvesting of *Lycopersicon esculentum* and *Solanum melongena*. *Le*: *Lycopersicon esculentum*; *Sm*: *Solanum melongena*; cfu: colony forming unit. Values are in Mean ± SD; (n = 3). Different small letters along the row represents significant differences in the bacterial count of different treatments of *Le* and *Sm* at a particular time interval (sowing and harvesting) according to Duncan's Multiple Range Test (One-way ANOVA) at $p < 0.05$. Different capital letters along the column represents significant differences in the count of phosphate solubilizing bacteria, *Azotobacter* and Potash mobilizing bacteria in soil of *Le* and *Sm* before and after plantation within the same treatment, respectively, according to Duncan's Multiple Range Test (ANOVA) at $p < 0.05$.

| Bacteria | Number of strains (%) | IAA Production (%) | Siderophores (%) | HCN test (%) | Ammonia production (%) | P-Solubilization (%) |
|--------------------------------|-----------------------|--------------------|------------------|--------------|------------------------|----------------------|
| <i>Lycopersicon esculentum</i> | | | | | | |
| PSB | 23 | 100 | 28.57 | 0 | 57.14 | 100 |
| <i>Azotobacter</i> | 18 | 100 | 12.67 | 0 | 47.28 | 15 |
| Potash mobilizing | 09 | 100 | 3.55 | 0 | 63.65 | 2 |
| <i>Solanum melongena</i> | | | | | | |
| PSB | 21 | 100 | 25.45 | 0 | 52.65 | 98 |
| <i>Azotobacter</i> | 15 | 100 | 10.28 | 0 | 40.53 | 12 |
| Potash mobilizing | 06 | 100 | 2.36 | 0 | 58.20 | 2 |

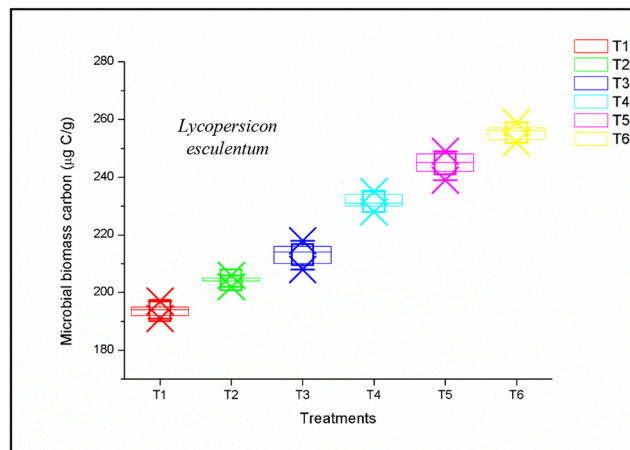
Table 4. Plant Growth Promoting (PGP) properties of selected isolates during harvesting of *L. esculentum* and *S. melongena* for treatment comprising 15% vermicomposted fly ash (T6). PSB: Phosphate solubilisation bacteria; IAA: Indole acetic acid; HCN: Hydrogen cyanide.

found in case of treatment, T6. The number of fruits per pot was maximum for T6 (23) while, minimum for T1 (5). The weight of the fruits showed an increase with an increase in the rates of application of VCF proving FA to have good fertilizing activity. Significant ($p < 0.05$) variances were found in the yield of fruit per plant along the treatments. The yield of fruits per *L. esculentum* plant was high for treatments T5 (922.48 g/plant) and T6 (952.29 g/plant).

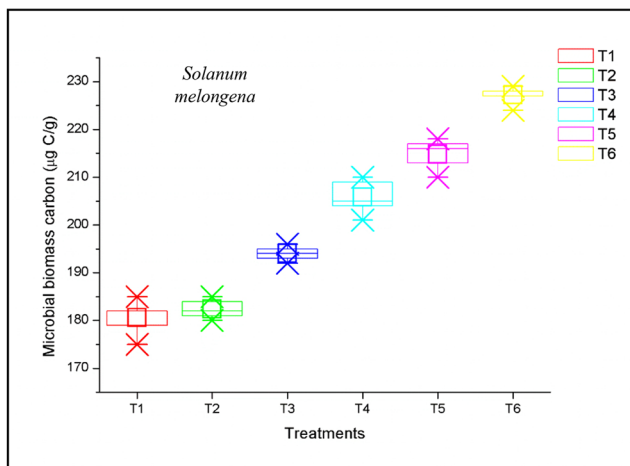
The number of *S. melongena* fruits per pot was found to be maximum in T6 and minimum in T1 (Table 5). Maximum yield in fruits per plant was observed for T6 (1293.13 g/plant) followed by T5 (1249 g/plant) and T4 (1293.13 g/plant). Regression equations depicting the relationship between VCF concentration and fruit yield were derived for both the crops and are depicted in Fig. 6(a,b).

Effects on photosynthetic pigments, boron, shoot nitrogen and total phenols. In *L. esculentum*, the maximum concentration of chlorophyll a (749.37 µg/g) and chlorophyll b (462.55 µg/g) were found for T6 (Table 6). The concentration of carotenoids showed the following trend: T6 > T5 > T4 > T3 > T2 > T1. Carotenoid concentration was found to be minimum for T1 (5.85 µg/g) and maximum for T6 (7.83 µg/g). The VCF on application to the agricultural soil at the rate of 15% by weight showed a maximum concentration of carotenoids, thus verifying it to have good fertilizing ability. The concentration of boron in the treatments comprising VCF as the amendment was found to be maximum for treatment T6 (447.98 µg/g). Shoot nitrogen was found to vary significantly ($p < 0.05$) along the treatments. Total phenols and boron showed a direct relationship with the increase in the concentration of VCF.

In *S. melongena*, the concentration of chlorophyll a was observed to increase significantly ($p < 0.05$) with an increase in the concentration of VCF. The maximum concentration of carotenoid was observed in the treatment T6 (376.37 µg/g) while, minimum concentration in T1 (822.84 µg/g) (Table 6). The trend in boron concentration had direct relations with the application rates of VCF of agricultural soil. Total phenols also showed a significant ($p < 0.05$) increase in trend with concentrations varying among the treatments as T1 (336.46 mg/100 g), T2 (415.53 mg/100 g), T3 (439.29 mg/100 g), T4 (469.29 mg/100 g), T5 (481.04 mg/100 g), T6 (447.61 mg/100 g).



(a)



(b)

Figure 3. Variations in the microbial biomass carbon of treatments at the time of harvesting of *Lycopersicon esculentum* and *Solanum melongena*.

Effects on photosynthesis and respiration rates. VCF deposition augmented the apparent rate of photosynthesis in both the crops, approaching a maximum of $15.5 \text{ mg CO}_2 \text{ dm}^{-2} \text{ h}^{-1}$ in *L. esculentum* (Fig. 7a) and $19.5 \text{ mg CO}_2 \text{ dm}^{-2} \text{ h}^{-1}$ in *S. melongena* (Fig. 7b) at 90 DAS. The increase in the rate of photosynthesis was attributed to increased foliar temperatures, that might have hastened photosynthetic activity. In both crops, rate of respiration increased with upsurge in concentration of VCF amended soil. Due to the maximum increase in plant growth and several greener leaves in T6, respiration rates in leaves of *S. melongena* were high (Fig. 7d). In *L. esculentum*, maximum respiration rate was observed for T6 ($20 \mu\text{l g}^{-1}$ dry wt) and minimum for T1 ($17 \mu\text{l g}^{-1}$ dry wt) (Fig. 7c).

Discussion

The outcomes of the current study reveal that vermicomposted fly ash on addition to soil enhanced the soil quality, improved the microbial and enzymatic activities and showed substantial increase in the growth and yield of tomato and brinjal. Perez-Murcia *et al.*¹⁶ and Iglesias and Jimenez¹⁷ stated that when composted materials are used as fertilizers, they should be completely stabilized to prevent negative growth effects caused due to oxygen depletion and nitrogen mineralization. The proportion of the compost added to soil is also important for preventing potential hazards. In the present investigation, the optimum concentration of VCF added to soil showing maximum growth was 15%. The experiments were also performed with 18% and 21% VCF however, above 15% of VCF, the plant growth and yield were observed to decline. The leaves synthesized more photosynthetic pigments and plants yielded more flowers and fruits. Leaves acquired a dark green colour because of increase in chlorophyll and carotenoid content. The plant pigments result in higher photosynthetic activity leading to enhanced growth and yield. Better growth and yield may also be owed to the improved nutrient content (N, P, K) in VCF. Mishra and Shukla¹⁸ reported about the existence of essential plant nutrients in fly ash.

The bulk density of the treatments was observed to be higher during sowing compared to harvesting. Pandey *et al.*¹⁹ and Goswami *et al.*²⁰ observed a decline in bulk density and a rise in porosity and WHC on the application

| Treatments | Increase in Seed Germination over Control (%) | Rate of Seed Germination | Number of Flowers | No. of fruits per pot | Weight of fruits (g) | Yield of fruit per plant (g/plant) |
|--------------------------------|---|--------------------------|-------------------|-----------------------|----------------------|------------------------------------|
| <i>Lycopersicon esculentum</i> | | | | | | |
| T1 | — | 6.75 ± 0.05f | 3.00 ± 0.58e | 5.00 ± 1.00e | 161.98 ± 36.42f | 126.14 ± 5.99f |
| T2 | 37.78 | 6.89 ± 0.42e | 7.00 ± 1.00d | 11.00 ± 1.00d | 260.47 ± 44.52e | 326.17 ± 22.50e |
| T3 | 53.30 | 7.13 ± 0.66d | 13.00 ± 2.08c | 14.00 ± 3.00c | 323.82 ± 12.11d | 668.54 ± 16.31d |
| T4 | 60.00 | 7.48 ± 0.54c | 14.00 ± 2b | 20.00 ± 4.04b | 632.84 ± 53.86c | 859.00 ± 5.99c |
| T5 | 73.33 | 8.02 ± 0.18b | 15.00 ± 3.51b | 21.00 ± 2.65b | 885.59 ± 66.89b | 922.48 ± 13.14b |
| T6 | 82.22 | 8.56 ± 0.53a | 25.00 ± 2.52a | 23.00 ± 3.06a | 1334.91 ± 9.26a | 952.29 ± 10.60a |
| <i>Solanum melongena</i> | | | | | | |
| T1 | — | 1.75 ± 0.05f | 1.00 ± 0.58 f | 7.00 ± 1.00d | 724.21 ± 35.23f | 618.35 ± 42.84f |
| T2 | 16.92 | 1.89 ± 0.42e | 2.00 ± 1.00e | 7.00 ± 0.53d | 827.29 ± 16.83e | 836.12 ± 44.42e |
| T3 | 21.54 | 2.13 ± 0.66d | 4.00 ± 1.53d | 8.00 ± 1.00c | 1266.39 ± 16.67d | 1078.56 ± 8.08d |
| T4 | 26.15 | 3.48 ± 0.54c | 5.67 ± 1.53c | 8.00 ± 0.58b | 1646.68 ± 10.35c | 1142.59 ± 43.39c |
| T5 | 29.2 | 4.02 ± 0.18b | 6.00 ± 2.00b | 9.00 ± 1.53b | 1800.61 ± 45.38b | 1249.07 ± 48.55b |
| T6 | 32.30 | 5.56 ± 0.53a | 8.00 ± 1.00a | 11.00 ± 0.58a | 1984.81 ± 61.48a | 1293.13 ± 43.97a |
| Results of two-way ANOVA test | | | | | | |
| A | — | — | 4.72* | 10.47** | 7.58** | 6.43* |
| F | — | — | 32.29*** | 23.51** | 14.25*** | 23.57** |
| A x F | — | — | 3.15* | 0.92 ^{NS} | 0.48 ^{NS} | 0.74 ^{NS} |

Table 5. Effects of vermicomposted fly ash on the rate of seed germination, number of flowers, fruits per pot, weight of fruits, yield of fruits per plant and percent increase of yield over control in *L. esculentum* and *S. melongena*. Values are in Mean ± SD, n = 3 Levels of significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS = not significant (Two-way ANOVA); (—): Two-way ANOVA not applied. Different letters in the same column denote significant differences ($p < 0.05$) in the rate of seed germination, number of flowers, number of fruits per pot, weight of fruits, yield of fruits per plant of *L. esculentum* and *S. melongena*, respectively in different treatments (One-way ANOVA; Tukey's test).

of FA to the soil. These results were consistent with the current study. Goswami *et al.*²⁰ reported that vermicompost amendment improved soil structure by reduction of bulk density.

Porosity refers to the air space amongst the soil particles that is generally subjugated by water on availability²¹. Thus, increase in WHC on addition of VCF occurred because of greater space amongst the soil particles. EC shows positive correlation with pH thus representing the overall concentration of soluble anions and cations²². The EC values fall within the range desirable for the growth of crops²³.

There were no significant differences between the metal concentration at the time of sowing and harvesting. The metal concentrations were within the permissible limits of soil for plant growth²⁴ and were also lower than the critical limit of soil prescribed by²⁵.

Dehydrogenase enzyme is extracellular in nature and mediates the oxidative phosphorylation process²⁶ and is responsible for the microbial activity in biological environments. It is generally concerned with microbial energy metabolism in the gut of earthworms. High dehydrogenase activity in treatments comprising 15% VCF in both the plants might be due to huge quantities of readily degradable organic substrates in T6 accessible for the proliferation of microorganisms, that lead to augmented microbial activity and therefore to enhanced dehydrogenase activity. However, at later stages, the activity reduced because of substrate loss leading to diminished activity²⁷. The variances in alkaline phosphatase activity of the treatments may be due to the changes in organo-phosphate complexes and variances in activity of microorganisms in each treatment. Phosphatase enzyme enhances agricultural attributes and can be changed to diverse forms of inorganic phosphorous (PNP), that can be assimilated by plants. Alkaline phosphate is linked along with the phosphorous (P) cycles and aids in the breakdown of organic phosphate esters²⁸. High activity of alkaline phosphatase enzyme was maintained by the range of pH of VCF in which enzyme stayed active.

The bacterial populations were observed to be lower at the time of sowing compared to that of harvesting of *L. esculentum* and *S. melongena*. Maximum population of *Azotobacter*, Potash mobilizing bacteria and Phosphate solubilizing bacteria were observed for T6 in both the crops. *Azotobacter* shows a direct relation to the increase in the VCF, as it has higher population of bacteria²⁹.

Siderophores are iron chelating compounds produced by bacteria and can act as biopesticide by preventing insect attacks on crops and plants³⁰. HCN production by bacteria can be used as a pesticide for plants³¹. None of the bacterial strain in treatment comprising *L. esculentum* and *S. melongena* tested positive for HCN production. Plants cannot directly uptake the nitrate present in the substrate, hence the production of ammonia is an important PGP characteristic³².

Plants are unable to utilize phosphate present in the soil in its natural form. Phosphate solubilization makes the soil fertile and provides nutrients to the plants for agricultural effects³⁰. Phosphorus is an essential micronutrient

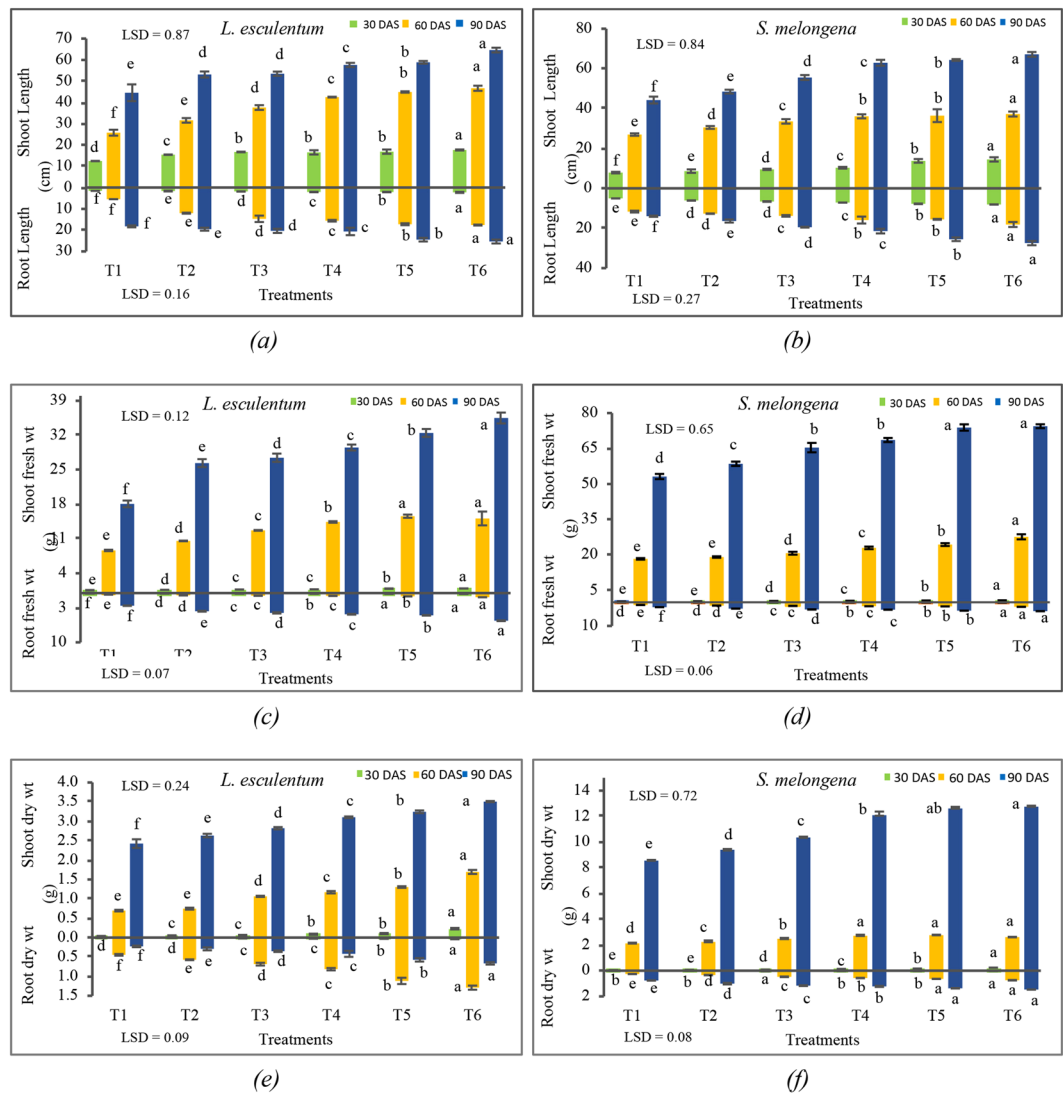


Figure 4. Effects of vermicomposted fly ash on the shoot and root length, shoot and root fresh weight and shoot and root dry weight of *Lycopersicon esculentum* and *Solanum melongena* at different durations after sowing in different treatments. Illustration: Values are in Mean \pm SD; (n = 3). Means with bars of different colours followed by the same letter are not significantly different at $p < 0.05$ (One-way ANOVA followed by Least significance difference test). The normality and homogeneity of variances were verified, respectively, by Shapiro-Wilk and Levene values at $p > 0.05$.

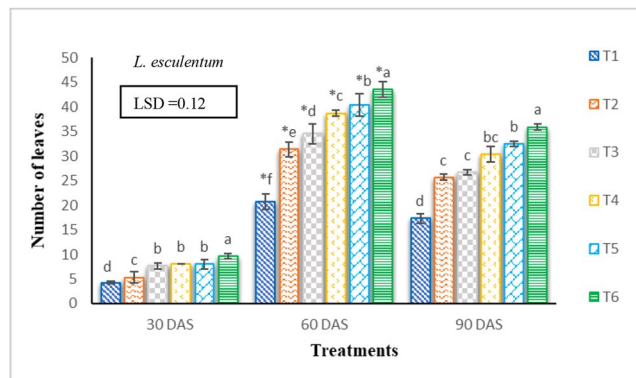
and is present in insoluble forms, thus converting them into soluble forms. This holds great significance for the plants.

MBC takes an efficient part in evaluating the microbial condition of the treatments and is perceptible to management systems and pollution²⁷. Substrate health can be determined by MBC as it regulates nutrient cycling posing as a labile source of plant availability. The trend for MBC among the treatments was T6 > T5 > T4 > T3 > T2 > T1. This may be attributed to high organic matter and enhanced physico-chemical properties in treatments comprising VCF³³. Moreover, microbial biomass carbon and respiratory activities are more in treatments comprising a higher concentration of VCF²⁷.

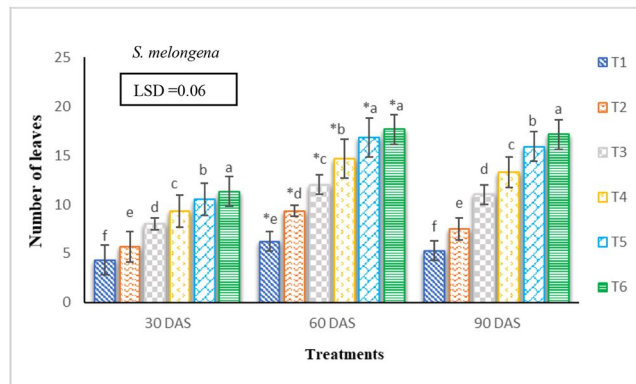
Enhancement of seed germination with increased applications of VCF might be due to the good fertilizing ability of VCF applied at the rate of 15% to agricultural soil. Mishra *et al.*³⁴ also reported that FA amendments caused significant improvement in the quality of the soil and germination percentage of crops.

The root length of *L. esculentum* was maximum for the treatment T6 during harvesting. Khan and Wajid³⁵ reported that plant growth parameters such as root length and shoot length were found to increase with an increase in the concentration of FA to the soil. Significant ($p < 0.05$) differences were obtained between the root length and shoot length of *S. melongena* in different treatments.

The number of leaves exhibited significant ($p < 0.05$) differences between the various treatments at the different duration of sowing. Leaf production was high during the early stages of growth (30–60 DAS) but it decreased during later stages (30–90 DAS). This may be attributed to the fact that senescence occurs during later stages of



(a)



(b)

Figure 5. Effects of vermicomposted fly ash applied at different rates on the number of leaves in *Lycopersicon esculentum* and *Solanum melongena* at different durations. Illustration: Values are in Mean \pm SD; (n = 3). Means with bars of different patterns followed by the same letter are not significantly different at $p < 0.05$ (One-way ANOVA followed by LSD test). The normality and homogeneity of variances were verified, respectively, by Shapiro-Wilk and Levene values at $p > 0.05$. *Repeated measures ANOVA was applied to determine significant differences between the number of leaves in different treatments at different durations.

growth²⁰. Khan³⁶ also observed that growing the tomato plants in the ash-soil mixture exhibited dense growth having more greener leaves.

An increase in fruit yield over control was observed throughout the treatments. The previous studies have also reported that augmentation of 40% FA to agricultural soil was useful for higher crop harvest, exceeding which had a hostile impact on crop yield^{2,35}.

Conclusion

This paper deals with the implications for the safe utilization of VCF in agricultural sectors. The rate of seed germination and plant growth were found to enhance with an increase in the application of VCF to the treatments in both *L. esculentum* and *S. melongena*. Fruit yield showed direct relation with VCF concentration and was maximum for the treatment comprising 15% VCF added to soil. The photosynthetic pigments (chlorophyll a and b, and carotenoids), levels of boron and total phenols were observed to reach a maximum in case of T6 while, they were minimum in case of T1. Thus, the VCF was observed to be a potent fertilizer when applied at the rate of 12–15% by weight to the agricultural soil leading to good crop growth and yield. Moreover, VCF is a biological fertilizer with reduced metal concentrations and enhanced N, P, K contents. It is thus necessary to utilize FA more effectively in the agricultural sector to reduce the burden of its disposal and exploit its physical and chemical properties completely, which are quite beneficial for soil and crop health.

Materials and Methods

Experimental setup and bio efficacy study. The VCF used in this study was obtained from a prior vermicomposting experiment carried out by Usmani *et al.*¹⁰, that involved mixing of coal FA (collected from Chandrapura Thermal Power Station) and cow-dung (collected from local area of Dhanbad) in the ratio of 1:3. The mixture was then subjected to vermicomposting using *Eudrilus eugeniae* species of earthworm for a duration of 90 days. The vermicompost (FA + CD; 1:3) attained by the above process was used in the current study as it was observed to be the best in terms of plant nutrient contents such as N, P and K and reduced metal concentrations compared to other tested mixtures. Seeds of *Lycopersicon esculentum* Mill. (Tomato) and *Solanum melongena* L. (Brinjal) were obtained from authorized vender of the local market. Soil sample was collected from the research field of IIT (ISM) Dhanbad, India.

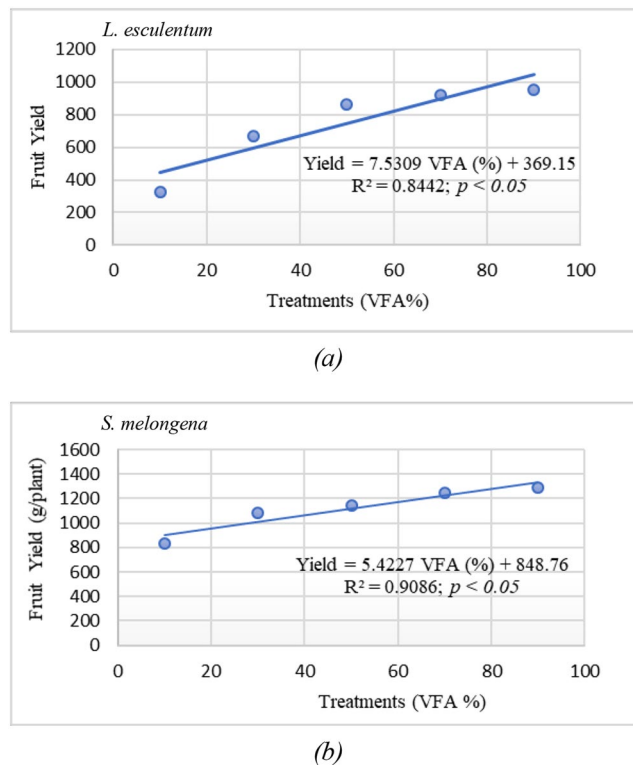


Figure 6. The relationship between vermicomposted fly ash concentrations incorporated in soil and yield of *Lycopersicon esculentum* and *Solanum melongena* respectively.

| Treatment | Chlorophyll a (µg/g) | Chlorophyll b (µg/g) | Carotenoids (µg/g) | Boron (µg/g) | Shoot Nitrogen (µg/g) | Total Phenols (mg/100 g dry weight) |
|--------------------------------|----------------------|----------------------|--------------------|--------------------|-----------------------|-------------------------------------|
| <i>Lycopersicon esculentum</i> | | | | | | |
| T1 | 655.22 ± 2.56f | 321.71 ± 3.67f | 5.85 ± 0.19f | 204.14 ± 1.72f | 1.55 ± 0.03a | 325.95 ± 7.24f |
| T2 | 664.28 ± 1.37e | 360.73 ± 7.44e | 6.22 ± 0.03e | 222.17 ± 3.97e | 1.43 ± 0.03b | 408.82 ± 3.65e |
| T3 | 683.42 ± 4.11d | 374.60 ± 6.93d | 6.94 ± 0.04d | 274.57 ± 3.50d | 1.32 ± 0.04c | 423.94 ± 4.01d |
| T4 | 704.16 ± 9.30c | 397.44 ± 0.95c | 7.27 ± 0.08c | 312.32 ± 7.54c | 1.20 ± 0.04d | 434.11 ± 7.25c |
| T5 | 729.94 ± 4.45b | 404.33 ± 7.69b | 7.53 ± 0.08b | 412.35 ± 6.09b | 1.05 ± 0.03e | 457.11 ± 1.08b |
| T6 | 749.37 ± 6.32a | 462.55 ± 17.57a | 7.83 ± 0.04a | 447.98 ± 7.66a | 0.87 ± 0.04f | 513.98 ± 1.23a |
| <i>Solanum melongena</i> | | | | | | |
| T1 | 691.21 ± 2.41e | 679.48 ± 18.75e | 6.36 ± 0.26d | 246.44 ± 11.15e | 2.76 ± 0.10a | 336.46 ± 7.04f |
| T2 | 715.21 ± 18.3d | 691.99 ± 57.83d | 6.60 ± 0.64d | 276.25 ± 8.89d | 2.46 ± 0.11b | 415.53 ± 5.04e |
| T3 | 744.50 ± 30.28c | 726.02 ± 87.18c | 7.38 ± 0.22c | 304.63 ± 7.30c | 2.15 ± 0.03d | 439.29 ± 11.71d |
| T4 | 757.26 ± 51.96c | 756.74 ± 32.29b | 7.63 ± 0.45c | 330.66 ± 10.51b | 2.22 ± 0.32c | 469.36 ± 10.80c |
| T5 | 791.95 ± 30.44b | 763.34 ± 85.17b | 8.45 ± 0.28b | 332.14 ± 11.90b | 1.80 ± 0.14e | 481.04 ± 10.80b |
| T6 | 811.59 ± 14.02a | 822.84 ± 18.25a | 9.24 ± 0.36a | 376.37 ± 7.48a | 1.67 ± 0.015f | 497.61 ± 77.11a |
| Results of two-way ANOVA test | | | | | | |
| A | 225*** | 247.82*** | 138.65** | 210.57*** | 176.44** | 95.88** |
| F | 135.11** | 87.56** | 75.42** | 63.08** | 82.59** | 48.36** |
| A x F | 12.08** | 5.82** | 7.63** | 1.95 ^{NS} | 3.77** | 7.63** |

Table 6. Effects of vermicomposted fly ash amendment on photosynthetic pigments, boron, shoot nitrogen and total phenol in leaves of *Lycopersicon esculentum* and *Solanum melongena* plant during harvesting. DAS: Days after sowing. Values are in Mean ± SD, n = 3. Levels of significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS = not significant (Two-way Anova). Different letters in the same column denote significant differences ($p < 0.05$) in the concentrations of chlorophyll a, chlorophyll b, carotenoids, boron, shoot nitrogen and total phenol in *Lycopersicon esculentum* and *Solanum melongena* plants respectively (One-way ANOVA; Tukey's test).

The VCF used in this experiment was also compared with the prescribed guidelines of vermicompost provided by the Fertilizer Control Order, India (FCO) (Supplementary Table 1). The ecological risk assessment of metals in VCF was further determined by using the potential ecological risk index (PERI)³⁷. The formulas used

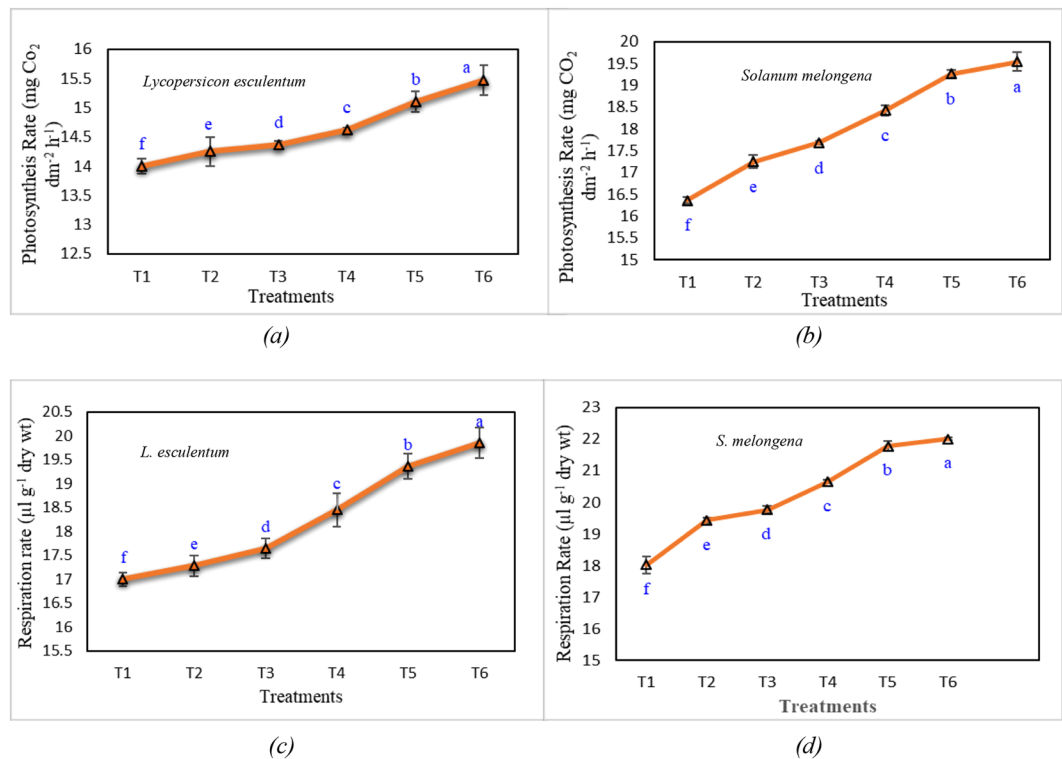


Figure 7. Photosynthesis and Respiration rate during harvesting of *Lycopersicon esculentum* and *Solanum melongena* dusted at different rates of vermicomposted fly ash. Illustration: Values are in Mean \pm SD; (n = 3). Different letters at each data point of the line graph depict significant differences in the photosynthetic and respiration rates respectively at $p < 0.05$ (One-Way ANOVA). The normality and homogeneity of variances were verified, respectively, by Shapiro-Wilk and Levene values at $p > 0.05$.

for the estimation of the coefficient of pollution (C^f), potential ecological risk factor (Er^i), and finally the risk index (PERI) are elaborated in Table 7. The trace element concentration in the VCF showed no obvious risks towards the environment based upon the C^f , Er^i and PERI values (Table 8).

The pilot experiment was performed in the research field of IIT (ISM) Dhanbad, Jharkhand. The VCF was mixed with agricultural soil at the rates of 3, 6, 9, 12 and 15% (w/w) (Supplementary Table 2). The treatment codes comprising different concentrations of VCF as amendments for pot experiments were as follows: T1 (Agricultural soil alone); T2 (Soil + 3% VCF); T3 (Soil + 6% VCF); T4 (Soil + 9% VCF); T5 (Soil + 12% VCF); T6 (Soil + 15% VCF). These soil samples were placed in earthen pots of 5 kg capacity (25 cm diameter). Control pot constituted only agricultural soil. To maintain drainage, a small perforation was made at the bottom of each pot. The study was carried out for a duration of 90 days (September 2016 to December 2016) and the plants were harvested after fruiting. During the growth period of crops, the temperature varied from 10–36 °C, humidity from 21–100% and air pressure showed variations from 996–1019 mbar. The detailed information about the growing environment of the crops over a duration of four months are presented in Supplementary Table 3. The experiment was performed in an entirely randomized block design with three replicates for every treatment.

Estimation of physico-chemical characteristics of vermicomposted fly ash. The bulk density of VCF was evaluated by the soil core method³⁸. Porosity was determined by dividing the volume of void spaces in the soil by the total volume of soil in the core and WHC by Keen-Raczowski box method. pH (1:2.5 fly-ash: water) was determined using a digital pH meter (EI Model 101E). EC 1:2 (Fly-ash: water) was determined by digital conductivity meter (EI Model 612). Cation exchange capacity (CEC) was assessed through titration on switching the complex with ammonium ions and further titrating it using hydrochloric acid³⁹. Total organic carbon was determined by the rapid dichromate oxidation method⁴⁰. Total nitrogen by the CHNS elemental analyzer and total phosphorous by Phosphomolybdic blue colorimeter⁴¹. Exchangeable Ca and Mg by Ammonium acetate extractable method⁴² and estimated using Flame Atomic Absorption Spectrophotometer (FAAS, GBC AVANTA 3000). Metals like Cu, Zn, Cd, Ni, Fe and Cr were extracted by acid digestion and estimated using FAAS. Potassium, calcium, magnesium and manganese were determined by analytical methods suggested by⁴³.

Enzyme activity analysis. The assays of dehydrogenase and alkaline phosphatase enzymes were determined. Dehydrogenase activity was assessed by the procedure given by⁴⁴. The dehydrogenase activity was measured by a UV-Spectrophotometer (UV-1800, Shimadzu, Japan) at wavelength of 485 nm. Alkaline phosphatase activity was measured by samples incubation with *p*-nitrophenyl phosphate at 37 °C for 1 h in an incubator⁴⁵ and was measured at 480 nm in a spectrophotometer.

| Factor/Index | Formulas | Annotations | Threshold values |
|---|----------------------------|---|---|
| Coefficient of pollution (C_f^i) | $C_f^i = \frac{C_M}{C_B}$ | C_f^i : coefficient of pollution C_M : concentration of individual metal in Vermicomposted fly ash (FA + CD; 1:3) C_B : background concentration of the trace metal ⁶² for Cr, Cu, Zn, Pb, As, Cd, Ni, and Co were 90, 45, 95, 20, 13, 0.3, 68, and 19 mg/kg | $C_f^i = 0$: none; $C_f^i = 1$: none to medium; $C_f^i = 2$: moderate; $C_f^i = 3$: moderate to strong; $C_f^i = 4$: strongly polluted; $C_f^i = 5$: strong to very strong; $C_f^i = 6$: very strong |
| Potential ecological risk factor (Er^i) | $Er^i = C_f^i \times Tr^i$ | Er^i : potential ecological risk factor of trace metal; Tr^i : toxic metal response factor of trace metals. Tr^i for metals such as Zn, Cr, Cd, As, Cu, Pb and Ni are 1, 2, 30, 10, 5, 5, 5 as per ⁶³ . | $Er^i < 40$: Low risk; $80 < Er^i < 160$: considerable risk; $160 < Er^i < 320$: high risk; $Er^i > 320$: very high risk ⁶⁴ |
| Potential ecological risk index (PERI) | $PERI = \sum Er^i$ | $\sum Er^i$: Sum of potential ecological risk indices of all the heavy metals | PERI < 150: low risk; $150 < PERI < 300$: moderate risk; $300 < PERI < 600$: considerable risk; PERI > 600: very high risk ⁶⁵ |

Table 7. Factors/Indices to determine potential ecological risk of vermicomposted fly ash to the environment.

| Metals | Coefficient of Pollution (C_f^i) | Risk Evaluation as per C_f^i | Potential ecological risk factor (Er^i) | Risk Evaluation as per Er^i | Potential ecological risk index (PERI) ($\sum Er^i$) |
|--------|--------------------------------------|--------------------------------|---|-------------------------------|---|
| Cu | 0.05 | No risk; $C_f^i = 1$ | 0.25 | very low risk; $Er^i < 40$ | $\sum Er^i = Cu Er^i + Zn Er^i + Cd Er^i + Pb Er^i + Ni Er^i + Cr Er^i + As Er^i$ $\sum Er^i = 34.61$ Indicating low risk towards environment |
| Zn | 0.03 | No risk | 0.03 | very low risk | |
| Cd | 0.97 | No risk | 28.13 | low risk | |
| Pb | 0.60 | No risk | 3.00 | very low risk | |
| Ni | 0.18 | No risk | 0.90 | very low risk | |
| Cr | 0.05 | No risk | 0.10 | very low risk | |
| As | 0.22 | No risk | 2.2 | very low risk | |

Table 8. Potential ecological risk assessment of trace elements in vermicomposted fly ash to environment.

Microbial biomass carbon determination. Soil microbial biomass carbon (MBC) was evaluated by sieving treatment sub-samples by the chloroform fumigation extraction (CFE) process as pronounced by⁴⁶. The extracts obtained were examined for dissolved organic C by a Shimadzu TOC-L CSH with an OCT-L sampler (Shimadzu Corp., Kyoto, Japan) having 5X dilution as designated by⁴⁷. Soil microbial biomass C was evaluated using the formula described by⁴⁸.

$$MBC = (C_{\text{fumigated}} - C_{\text{control}}) / kEC \quad (1)$$

where, MBC: microbial biomass carbon; kEC: extraction coefficient

The extraction coefficients (kEC) used for carbon to determine MBC was 0.45 as per Potthoff *et al.*⁴⁹ and Joergensen *et al.*⁵⁰.

Isolation of PGP bacterial strains. The different PGP bacterial strains were isolated in their respective selective medium by soil dilution pour plate technique at the time of sowing and harvesting of experimental crops. For PSB strain, the soil solution was grown in Pikovaskaya agar medium⁵¹, the colonies showing halo zone were initially considered as PSB strain. *Azotobacter* sp. was isolated on Ashby's mannitol agar media (Himedia[®], Mumbai, India), Potash mobilizing bacteria was isolated in Glucose yeast agar media (Himedia[®], Mumbai, India) and the colonies showing potassium releasing zone were considered as potash mobilizing strain.

Determination of PGP traits. Different PGP traits were determined for the treatments after harvesting of *L. esculentum* and *S. melongena* using standardized methods. IAA production was determined using the method employed by Gordon and Weber⁵². Siderophores production of the selected isolates were performed using Meyer and Abdallah⁵³. Standard methods for hydrogen cyanide (HCN) and urea production were as per Lorck⁵⁴ and Cappuccino and Sherman⁵⁵, respectively. Phosphate solubilization was determined by Watanabe and Olisen⁵⁶ method.

Plant growth analysis. The bioefficacy study was grounded on germination of seeds, shoot and root length, dry and fresh weight of root and shoot, and the number of leaves at 30, 60 and 90 days after sowing (DAS). For treatment of seeds, collected seeds were superficially sterilized with 2% sodium hypochlorite for 3 mins and further washed 5 times with deionized water (1:1) under sterilized conditions⁵⁷.

The rate of germination (R_G) was calculated using the formula:

$$R_G = \sum N_i / D_i \quad (2)$$

where N_i is the number of germinated seeds in each time and D_i is the time unit (day)⁵⁸. To evaluate the growth parameters, plants were taken randomly and separated into the shoot and root. Shoot and root were rinsed to eliminate all soil particles and further dried in an oven at 70 °C for 3 days till constant weight was achieved⁵⁹ for biomass analysis.

Fruit and yield. Fruits were generally harvested weekly after attaining a mature stage. Picking was done 2–3 times as per the requirement. Fruit yield was assessed by counting and weighing all the fruits on individual plant.

Photosynthetic pigments. The photosynthetic pigments like chlorophyll a, chlorophyll b and carotenoids were analysed from the leaves of *L. esculentum* and *S. melongena*. Fresh leaves weighing 0.5 g were homogenized in 20 mL of 80% acetone (Acetone: water v/v) in a pre-chilled mortar and pestle. The filtrate was centrifuged at 3000 rpm for 15 min in Janetzki refrigerated centrifuge Model K - 24 at 4 °C. The supernatant was decanted, and the volume was made up to 25 mL with 80% acetone. Care was taken to shield the chlorophyll extract from bright light. The optical density was measured at 480, 510, 645, and 663 nm wavelength using the spectrophotometer (UV-1800, Shimadzu, Japan). The amount of chlorophyll a, chlorophyll b and carotenoids were assessed using the formula described by⁶⁰.

$$\text{Chlorophyll a} = 12.3D_{663} - 0.86D_{645} \times V \times d \times 1000 \times W \quad (3)$$

$$\text{Chlorophyll b} = 19.3D_{645} - 3.6D_{663} \times V \times d \times 1000 \times W \quad (4)$$

$$\text{Carotenoid} = 7.6D_{480} - 1.49D_{510} \times V \times d \times 1000 \times W \quad (5)$$

where, D = optical density at 480, 510, 645, 663 nm, respectively. V = volume of the chlorophyll extract in acetone (mL). d = light path length (cm). W = leaves fresh weight (g).

Response parameters. The leaves were removed from the plants and leaf area was determined for all the leaves per plant. Fruit weights were measured and recorded. Fresh weights of plant shoot and root were documented. The plant parts were kept at 70 °C for 72 h and dry weights were also noted. Total Phenols in leaves were evaluated as per the methods explained by⁶¹. Data obtained were verified by statistical analysis.

Photosynthesis and respiration rates. Photosynthesis and Respiration rate were determined for a distinct leaf bounded in a perspex chamber consisting of a leaf base fastened amid rubber gaskets to impart hermetic seals. The conditions were maintained as per the studies done by¹⁸. The removal rate of CO₂ was assessed by a Grubb Parsons infrared gas analyzer and photosynthesis rate per unit leaf area was determined. Respiration rate was assessed using the volumetric method.

Statistical analyses. The data on physico-chemical properties of the FA-soil mixtures were validated by Analysis of variance (One-way ANOVA followed by the *Tukey's HSD Test*). The statistical strength of the data was determined by a volcano plot representing the expression of various FA amended soil parameters (MS Excel 16.0 v). Plant growth and yield were analyzed using Analysis of variance (One-way ANOVA) and least significant differences (L.S.D.) at $p < 0.05$ were estimated. The mean values of these parameters were compared by means of Duncan's multiple range test (DMRT) at $p \leq 0.05$ level of significance for the column factor. Pre- and post-plantation soil study was determined by paired-sample *t* test using SPSS software package 20.0 version. Raw data on yield of plant was assessed by curvilinear regression to examine responses relating to the FA concentration.

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Author Contributions

V.K. designed experiments, Z.U. performed bioefficacy experiments and wrote the manuscript. G.G. performed bacterial experiments. P.G. and R.R. helped with the enzymatic estimations. A.C. analyzed data and helped with the manuscript.

Additional Information

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