

1 **Comparative Impact of Bio-Organic and Inorganic Fertilizer Application on Soil Health,**  
2 **Grain Quality and Yield Stability in Nutrient Deficient Regions**

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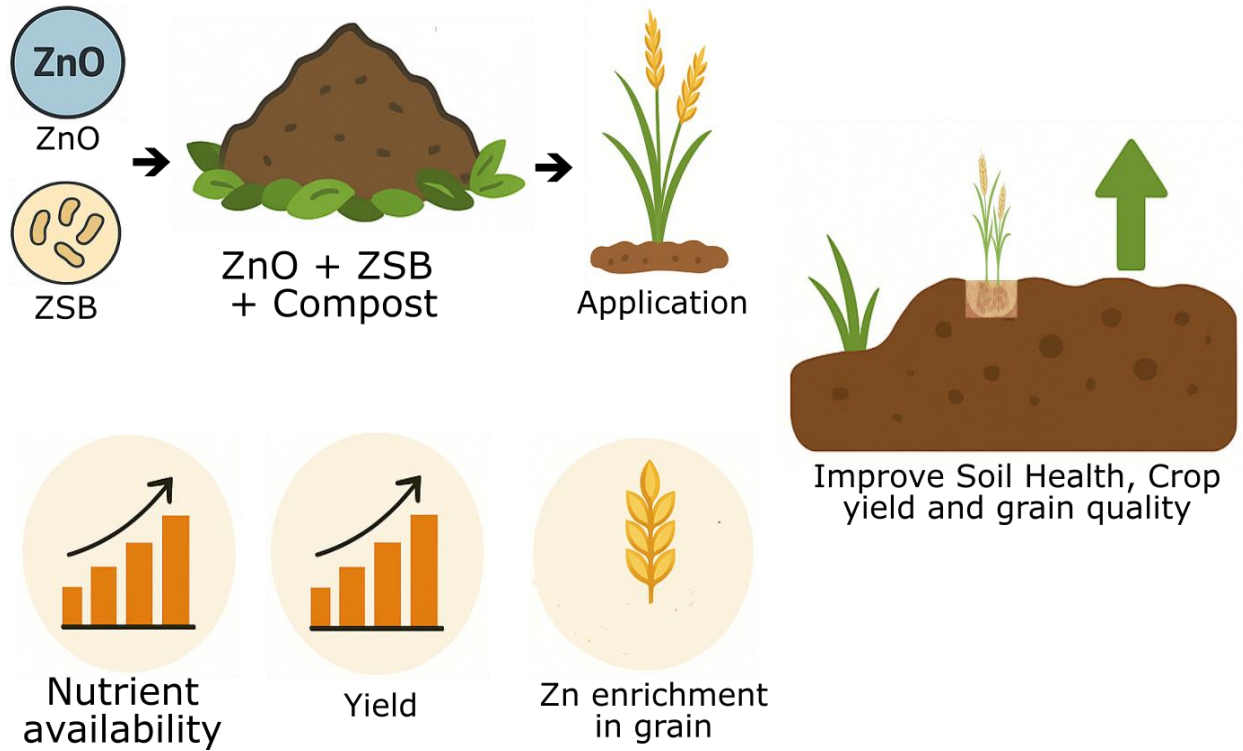
12 **Abstract**

13 Soil fertility limitations in arid regions restrict wheat productivity and grain nutritional quality,  
14 with zinc (Zn) deficiency being a major concern. Sustainable soil amendments combining organic  
15 and microbial inputs offer potential to address these constraints. This study aimed to evaluate the  
16 effectiveness of bio-organic fertilization in enhancing wheat growth, yield, grain Zn  
17 biofortification, and soil fertility under deficient arid field conditions. Two field trials were  
18 conducted in Bahawalpur and Bahawalnagar, Pakistan, using a randomized complete block design.  
19 Treatments included compost, ZnO (2%), ZnSO<sub>4</sub>, zinc-solubilizing bacteria (ZSB), and their  
20 combinations. Wheat growth, yield, grain nutrient concentrations, and soil fertility indicators  
21 (organic matter, microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), and  
22 nutrient availability) were measured. Microbial populations were determined through colony-  
23 forming units. Correlation and principal component analysis (PCA) were applied to explore  
24 associations among variables. The integrated application of compost + ZnO + ZSB significantly  
25 improved wheat height (19%), biomass (20%), yield attributes (10%), and grain Zn concentration  
26 (39%) compared with the control. Soil fertility parameters also increased (organic matter, 39%;  
27 MBN, 32%; MBC, 27%). Correlation and PCA highlighted strong positive relationships among  
28 microbial populations, soil fertility, and crop performance. Bio-organic fertilization provides an  
29 eco-friendly and effective strategy to improve wheat yield, Zn biofortification, and soil fertility in  
30 arid agroecosystems.

31 **Keywords:** Eco-friendly; Microbial biomass; Organic matter; Soil health; Zinc solubilizing  
32 bacteria

33

34 **Graphical Abstract**



35

36 **1. Introduction**

37 Soil fertility degradation and micronutrient deficiency, particularly of zinc (Zn), are increasingly  
38 critical concerns in arid and semi-arid agroecosystems, where climatic stressors and anthropogenic  
39 pressures have exacerbated soil quality (Lal, 2024). In these fragile environments, characterized  
40 by low organic matter, poor water retention, and nutrient depletion, the production of staple crops,  
41 such as wheat (*Triticum aestivum* L.), is consistently threatened (Hossain et al., 2021). Zinc  
42 deficiency is among the most widespread micronutrient disorders affecting cereal crops globally,  
43 with particularly high prevalence in calcareous and coarse-textured soils typical of arid zones  
44 (Dhaliwal et al., 2022). It is estimated that over 50% of soils cultivated for cereals in arid and semi-  
45 arid regions suffer from Zn insufficiency, which not only reduces crop productivity but also  
46 compromises grain Zn concentration, a factor that directly affects human nutrition in regions where  
47 wheat is a dietary staple (Younas et al., 2023).

48 To address such multifaceted challenges, integrated soil fertility management approaches have  
49 emerged, combining organic and inorganic strategies to enhance soil health, crop productivity, and  
50 environmental sustainability (Imran, 2024). Among these, composting has garnered attention due  
51 to its capacity to recycle organic waste into a valuable soil amendment that can improve physical  
52 structure, chemical fertility, and biological activity (Sharma et al., 2024). Compost application  
53 increases soil organic carbon (SOC), stabilizes soil aggregates, enhances microbial biomass carbon  
54 (MBC) and microbial biomass nitrogen (MBN), and provides a slow-release source of macro- and  
55 micronutrients (Khan et al., 2024; Zhao et al., 2025). However, standard composts are often limited  
56 in their ability to address specific micronutrient deficiencies such as Zn, particularly in soils with  
57 high pH, where Zn becomes poorly available to plants due to adsorption and precipitation  
58 processes (Qian et al., 2023).

59 In this context, the concept of "bioactivation" of compost, which involves enriching compost with  
60 bioavailable forms of essential nutrients such as zinc (Zn), along with the inclusion of beneficial  
61 microorganisms, represents an innovative and promising solution (Manea and Bumbac, 2024).  
62 Bioactivated compost not only supplies nutrients but also enhances microbial functions, potentially  
63 improving nutrient cycling, enhancing soil enzymatic activity, and mitigating abiotic stress in  
64 plants (Clagnan et al., 2023). The microbial solubilization of Zn compounds, for example, through  
65 the action of zinc-solubilizing bacteria (ZSB), can significantly increase the bioavailability of Zn  
66 in the rhizosphere, promoting better root development and nutrient uptake (Singh et al., 2024).  
67 When applied to nutrient-deficient arid soils, bioactivated Zn-enriched compost may thus serve as  
68 a multifaceted amendment to restore soil fertility, stimulate microbial activity, and ultimately  
69 improve crop yield and quality (Maitra et al., 2024).

70 Despite its potential, the use of bioactivated Zn-enriched compost in arid regions remains  
71 underexplored, particularly in terms of its comparative effects on key soil health indicators such  
72 as organic matter content, SOC, MBC, MBN, and plant-available nutrients. Moreover,  
73 understanding the interaction between compost-borne microbial communities and Zn dynamics in  
74 the soil–plant system is essential for optimizing compost formulations and application strategies  
75 (Wang et al., 2024). The wheat crop, as a high-input cereal with substantial nutrient demand, serves  
76 as an ideal model for evaluating the effectiveness of such integrated nutrient management  
77 approaches under stress-prone environmental conditions.

78 Despite growing interest in Zn-enriched composts and microbial inoculants, most existing studies  
79 have been conducted under controlled or single-site experimental conditions, which limits their  
80 applicability in farmer-managed arid agroecosystems. Moreover, the combined role of organic  
81 matter, zinc oxide, and zinc-solubilizing bacteria (ZSB) in regulating soil biological health,  
82 nutrient availability, yield stability, and grain Zn biofortification under field-scale conditions  
83 remains poorly understood. In particular, comparative multi-location assessments that integrate  
84 soil biochemical indicators with agronomic and economic outcomes are scarce. Addressing these  
85 gaps is critical for developing scalable, cost-effective, and environmentally sustainable zinc  
86 management strategies for nutrient-deficient regions.

87 Recently, our research group reported the development of a bio-activated Zn-enriched compost  
88 from organic waste and its application in a field experiment on wheat in Zn-deficient soils (Naeem  
89 et al., 2025), where significant improvements in soil biochemical properties, Zn availability and  
90 wheat grain yield were observed. However, that study was conducted at a single research site a  
91 research area where all recommended practices were carried out and monitored by researchers. In  
92 contrast, the present study expands upon that work by conducting field trials at two distinct  
93 farmer-field locations under real-life agronomic conditions. The current research evaluates the  
94 integrated application of Zn-enriched compost, combined with zinc-solubilizing bacteria, to  
95 provide a broader assessment of the approach's agronomic performance, adaptability, and  
96 scalability. This study, therefore, contributes new knowledge by validating the field-scale efficacy  
97 of the bio-activated compost under variable environmental conditions, examining soil-crop  
98 interactions across sites, and supporting the practical adoption of sustainable Zn fertilization  
99 strategies in nutrient-deficient regions.

100 Specifically, the research aims to: assess changes in soil organic matter, SOC, macro- and  
101 micronutrient content, microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN)  
102 following compost application; determine the effectiveness of Zn enrichment and microbial  
103 activation in enhancing Zn availability and uptake; and quantify the response of wheat growth,  
104 yield, and Zn content under field conditions typical of arid agroecosystems. The working  
105 hypothesis is that microbial-assisted Zn-enriched compost leads to significant improvements in  
106 soil fertility and microbial health, resulting in higher wheat productivity and nutritional quality  
107 compared to conventional practices.

108 From an environmental perspective, the utilization of compost, particularly when derived from  
109 agricultural, municipal, or agro-industrial residues, contributes to sustainable waste management  
110 by diverting organic matter from landfills and mitigating greenhouse gas emissions. Composting  
111 stabilizes organic residues, reducing methane generation and leachate formation, while also  
112 sequestering carbon in soils—an important consideration in climate change mitigation.  
113 Furthermore, the inclusion of beneficial microbes and micronutrient enrichment in compost  
114 contributes to soil biodiversity and resilience, supporting the ecological functions that underpin  
115 sustainable

## 116 **2. Materials and Methods**

### 117 **2.1. Compost Preparation**

118 To prepare zinc-enriched compost from domestic organic waste (Fruit and vegetable waste), a  
119 systematic approach comprising composting, zinc fortification, and microbial bioactivation was  
120 used. Initially, segregated domestic organic waste (vegetable peels, fruit residues, kitchen scraps)  
121 is collected and pre-processed by shredding to reduce particle size for faster decomposition. A  
122 composting drum was used, where the organic material was layered with urea (1% w/w) to speed  
123 up the composting process. Moisture is maintained at 40–50%, and the pile is turned periodically  
124 to ensure aerobic conditions. After the thermophilic phase (21 days), during the mesophilic stage,  
125 zinc was introduced in the form of zinc oxide (ZnO) at a predetermined concentration (2% w/w).  
126 This timing ensures optimal microbial assimilation and mineral retention within the compost  
127 matrix. The composting continues for 28 days until maturation, characterized by a dark brown  
128 color and crumbly texture. For bioactivation, a microbial consortium of Zinc solubilizing bacteria  
129 (ZSB) is prepared by culturing strains such as *Bacillus subtilis* (IUB2; accession No. MN696212  
130 and IUB6; accession No. MN696214), *Bacillus velezensis* (IUB3; accession No. MN696213),  
131 *Bacillus vallismortis* (IUB10; accession No. MN696215) and *Bacillus megaterium* (IUB11;  
132 accession No. MN696216) in a nutrient broth for 24–48 hours at 28–30°C until reaching an optical  
133 density of 0.8–1.0 at 600 nm. The mature compost is inoculated with the ZSB consortium (0.1%  
134 v/w) by spraying the bacterial suspension uniformly over the compost during final turning.  
135 Moisture is adjusted to around 40% to support microbial activity, and the compost is incubated for  
136 an additional 2 days under shaded conditions to stabilize the bioactivation process. The final  
137 microbial-assisted zinc-enriched compost is then dried, sieved, and stored for agronomic use. This

138 dual enrichment process enhances the compost's micronutrient content and microbial efficiency  
139 for sustainable soil fertility management.

140 The bacterial strains used in the present study had already been characterized for zinc and  
141 phosphorus solubilization, catalase and urease activity, exopolysaccharide, siderophore and indole  
142 3 acetic acid production and plant growth promotion (Naseem et al., 2022). The elevated levels of  
143 nitrogen (3.36%), phosphorus (23 mg kg<sup>-1</sup>), potassium (221 mg kg<sup>-1</sup>), iron (12.43 mg kg<sup>-1</sup>) and  
144 zinc (23.14 mg kg<sup>-1</sup>) observed in the Zn-enriched compost, as compared to the unenriched  
145 compost, are primarily due to incorporation of nutrient-rich materials during the enrichment  
146 procedure. Zinc oxide was added, and beneficial rhizobacteria were introduced to enhance the  
147 solubilization and availability of nutrients. The increased Zn and Fe concentrations are a direct  
148 result of these inputs, while the rise in N, P and K levels can be attributed to enhanced microbial  
149 activity. This microbial stimulation promotes the decomposition of organic matter and accelerates  
150 nutrient cycling. Additionally, the introduced microbes contribute by mobilizing phosphorus and  
151 micronutrients through the secretion of organic acids and chelating agents. As a result, the  
152 enrichment process not only enhances the compost's nutrient content but also improves its  
153 microbial efficacy, resulting in improved nutrient availability.

## 154 **2.2. Field trial**

155 Two field experiments were conducted at separate arid locations, Bahawalpur (29.3544° N,  
156 71.6911° E) and Bahawalnagar (29.1903° N, 72.6343° E), to evaluate the effects of ten (10)  
157 different treatments on crop performance. The weather data, including monthly average  
158 temperature and rainfall (mm) during the cropping season (2021-22), were presented in fig. 1. The  
159 treatments included the sole and combined application of a market source of zinc (ZnSO<sub>4</sub>), a cheap  
160 source of zinc (ZnO), compost, zinc solubilizing rhizobacteria, and microbial-assisted zinc-  
161 enriched compost. The experiment consists of ten treatments; T0: Control; T1: ZnSO<sub>4</sub>; T2: ZnO;  
162 T3: Compost; T4: ZSB; T5: compost + 2% ZnO; T6: ZnSO<sub>4</sub> + ZSB; T7: ZnO + ZSB; T8: compost  
163 + ZSB; T9: compost + 2% ZnO + ZSB. A randomized complete block design (RCBD) was used  
164 with three replications per treatment at each site to ensure statistical reliability.

165 Before sowing, composite soil samples were collected from the 0–15 cm depth across each site  
166 and analyzed for baseline fertility status. The soils at both locations were characterized as deficient  
167 arid soils with the average properties: pH (7.69 and 8.1), electrical conductivity (EC) (1.27 and  
168 1.42 dS m<sup>-1</sup>), organic matter (0.43 and 0.51%), total nitrogen (0.032 and 0.035%), available

169 phosphorus (8.8 and 9.8 mg kg<sup>-1</sup>), available potassium (73 and 85 mg kg<sup>-1</sup>), DTPA-extracted zinc  
170 (0.63 and 0.71 mg kg<sup>-1</sup>) and DTPA extracted iron (0.56 and 0.51 mg kg<sup>-1</sup>) in trial I (Bahawalpur)  
171 and II (Bahawalnagar), respectively. Based on soil test results and crop nutrient requirements,  
172 recommended doses of fertilizers (e.g., 120 kg of N ha<sup>-1</sup>, 90 kg of P ha<sup>-1</sup>, and 60 kg of K ha<sup>-1</sup>)  
173 were applied. Phosphorus and potassium were incorporated at the time of seedbed preparation  
174 using diammonium phosphate (DAP) and sulfate of potash (SOP), respectively, while nitrogen  
175 was split-applied: one-third as a basal dose using urea at sowing, and the remaining two-thirds in  
176 two equal splits at tiller formation and early flowering stages to minimize losses and enhance  
177 nutrient use efficiency. A basal dose of ZnSO<sub>4</sub> (33% Zn) and microbial-assisted zinc-enriched  
178 compost were applied @ 12 kg ha<sup>-1</sup> and 250 kg ha<sup>-1</sup>, respectively. Chemical fertilizer application  
179 was reduced by 5% in the treatment where Microbial-assisted zinc-enriched compost was used.  
180 All agronomic practices were kept uniform across treatments.

### 181 **2.3. Soil and plant samples collection and analysis**

182 The growth parameters, such as plant height, number of tillers and biomass, were recorded at  
183 harvest. Yield attributes, including 1000-grain weight, grain yield per hectare, and harvest index,  
184 were measured post-harvest. For nutrient analysis, plant tissues (e.g., grain) were collected at  
185 harvest, dried, ground, and analyzed for macro- and micronutrient content using standard  
186 procedures. Rhizosphere soil samples were carefully collected at harvest by gently shaking the soil  
187 adhered to the root zone, then analyzed for microbial population (CFU g<sup>-1</sup> soil), total organic  
188 carbon, available NPK, DTPA extracted Fe and Zn, and microbial biomass carbon and nitrogen.

### 189 **2.4. Plant analysis**

190 The determination of nitrogen (N), phosphorus (P), and potassium (K) in grain samples was  
191 conducted using a wet digestion method involving sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide  
192 (H<sub>2</sub>O<sub>2</sub>). Oven-dried grain samples (0.5 g) were placed in digestion tubes and initially treated with  
193 concentrated H<sub>2</sub>SO<sub>4</sub> (6 mL) to break down organic matter. Hydrogen peroxide (2 mL) was added  
194 dropwise to enhance oxidation and complete the digestion. The mixture was heated until the  
195 solution became clear, indicating complete digestion. The digested samples were allowed to cool,  
196 diluted with distilled water, and filtered. The clear filtrate was analyzed for total N using the  
197 Kjeldahl method, P content was determined colorimetrically using the molybdenum blue method,  
198 and K was quantified using a flame photometer. This method ensures efficient mineralization of

199 organic components and accurate estimation of macronutrients essential for grain quality  
200 assessment (Ryan et al., 2001).

201 The analysis of zinc (Zn) and iron (Fe) concentrations in grain samples was conducted using the  
202 diacid digestion method, employing a mixture of nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>).  
203 Oven-dried and ground grain sample (0.5 g) was accurately weighed into a digestion flask. A 10  
204 mL aliquot of concentrated HNO<sub>3</sub> was added, and the mixture was allowed to pre-digest overnight  
205 at room temperature to initiate breakdown of organic matter. The following day, 4 mL of  
206 concentrated HClO<sub>4</sub> was added, and the sample was subjected to controlled heating on a digestion  
207 block, gradually increasing the temperature up to 350 °C until white fumes appeared, indicating  
208 the completion of organic matrix oxidation. The digestion was continued until a clear solution was  
209 obtained. After cooling, the digested sample was diluted with deionized water and filtered. The  
210 final volume was made up to a known volume (50 mL) with deionized water. The concentrations  
211 of Zn and Fe in the digest were then determined using an atomic absorption spectrophotometer  
212 (AAS). All glassware was acid-washed to minimize contamination, and analytical-grade reagents  
213 were used throughout the procedure. The instrument was standardized by using respective standard  
214 solutions of Fe and Zn (Antreich, 2012).

## 215 **2.5. Soil chemical analysis**

216 For the soil biochemical analysis, soil samples were systematically collected from designated study  
217 sites using a clean stainless-steel auger to minimize contamination. Samples were air-dried, sieved  
218 through a 2 mm mesh to remove debris and stones, and stored in polythene bags for laboratory  
219 analysis. Chemical properties were assessed by measuring soil organic matter content through the  
220 Walkley-Black method (Jha et al., 2014). Available nutrients, including nitrogen (N), phosphorus  
221 (P), and potassium (K), were quantified using Kjeldahl digestion for N (Estefan et al., 2013), Olsen  
222 methods for P (Olsen, 1954), and flame photometry for K after ammonium acetate extraction  
223 (Shuman and Duncan, 1990).

## 224 **2.6. Soil biochemical analysis**

225 For the analysis of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN),  
226 soil samples were collected from the study area and air-dried before sieving through a 2 mm mesh.  
227 The fumigation-extraction method was employed, where one set of samples was fumigated with  
228 chloroform vapor for 24 hours to lyse microbial cells, while the other set served as non-fumigated  
229 controls (Brookes et al., 1985). Following fumigation, both sets were extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>

230 solution, and the extracts were analyzed for dissolved organic carbon and nitrogen using a total  
231 organic carbon analysis and the Kjeldahl method, respectively. Microbial biomass carbon and  
232 nitrogen were calculated by subtracting the values of non-fumigated samples from fumigated ones.  
233 All analyses were conducted in triplicate to ensure accuracy and reproducibility, and standard  
234 quality control procedures were followed throughout the process.

235 For the analysis of soil ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) nitrogen, soil extracts were prepared  
236 by shaking 10 g of soil with 100 mL of 2 M potassium chloride (KCl) solution for 30 minutes to  
237 displace exchangeable ammonium and nitrate ions. The suspension was then filtered through a  
238 Whatman No. 42 filter paper. Ammonium nitrogen concentration in the filtrate was determined  
239 colorimetrically using the indophenol blue method, while nitrate nitrogen was measured by the  
240 phenol disulfonic acid method and readings were taken on UV spectrophotometer at 220 nm  
241 (Kachurina et al., 2000). All analyses were performed in triplicate to ensure accuracy, and results  
242 were expressed in  $\text{mg kg}^{-1}$  of soil. Quality control included calibration curves using standard  $\text{NH}_4^+$   
243 and  $\text{NO}_3^-$  solutions, reagent blanks, and periodic checks with known reference materials.

## 244 **2.7. Economic analysis**

245 The economic analysis was estimated, and subsequently, the costs and returns of various items  
246 used in this study for wheat cultivation, as normally practiced by farmers (control), and the  
247 application of a market source of zinc and microbial-assisted zinc-enriched compost (Khan et al.,  
248 2012) were apportioned. Gross margin was calculated by the following formula to make  
249 comparisons.

$$250 \quad \text{Gross Margin (GM)} = \text{Total Revenue (TR)} - \text{Total Variable Cost (TVC)}$$

## 251 **2.8. Statistical Analysis**

252 The statistical analysis in this study was conducted using a combination of multivariate techniques.  
253 Analysis of Variance (ANOVA) was employed to test for significant differences between group  
254 means. Principal Component Analysis (PCA) was done to reduce dimensionality and identify the  
255 gradients of variation in the dataset, facilitating visualization of patterns and clustering. Pearson  
256 correlation coefficients were calculated to quantify the strength and direction of linear associations  
257 between pairs of continuous variables. All analyses were performed using OriginPro 2021b and  
258 Statistix 8.1 software, with significance levels set at  $\alpha = 0.05$ .

## 259 **3. Results**

260 The results from both field trials consistently demonstrated the positive role of microbial-assisted  
261 zinc-enriched compost in improving wheat growth, nutrient accumulation, and soil health.  
262 Treatments integrating compost, zinc sources, and zinc-solubilizing bacteria (ZSB) performed  
263 better than sole or dual applications, highlighting the synergistic effect of organic matter and  
264 microbial activity. The results demonstrated the influence of microbial-assisted zinc-enriched  
265 compost on plant performance, grain nutritional quality, and soil fertility parameters.

### 266 **3.1. Comparative effect of bio-organic and organic fertilizers on soil biochemical properties**

267 In both Trial I (Bahawalpur) and Trial II (Bahawalnagar), the application of microbial-assisted  
268 zinc-enriched compost significantly improved soil fertility parameters compared to the control and  
269 sole application of inorganic fertilizer (Table 1). The highest enhancement in organic matter (OM),  
270 total organic carbon (TOC), and macro- and micronutrient contents (N, P, K, Fe, and Zn) was  
271 observed in the treatment combining compost with 2% ZnO and zinc-solubilizing bacteria (ZSB).  
272 In Trial I, the treatment Compost + 2% ZnO + ZSB (T9) resulted in a remarkable increase in soil  
273 OM and TOC by 38.6% over the control. Nitrogen (N) content increased by 17.1%, phosphorus  
274 (P) by 23.2%, and potassium (K) by 17.9%. Micronutrient concentrations were also significantly  
275 enhanced, with iron (Fe) increasing by 22.6% and zinc (Zn) by an impressive 22.0% relative to  
276 the control. Similarly, in Trial II, the same treatment (Compost + 2% ZnO + ZSB) demonstrated  
277 the most effective results, improving OM by 38.3%, TOC by 31.8%, N by 20.4%, P by 20.2%,  
278 and K by 22.0%. Increases in Fe and Zn were recorded at 25.8% and 20.6%, respectively,  
279 compared to untreated soil.

280 In Trial I, the treatment (compost + 2% ZnO + ZSB) led to a maximum CFU count ( $34.1 \times 10^6$   
281 CFU g<sup>-1</sup> soil), showing an increase of 50.3% over the control. Microbial biomass carbon (MBC)  
282 and microbial biomass nitrogen (MBN) were significantly elevated, with increases of 21% and  
283 26%, respectively, compared to the untreated control. Additionally, ammonium-N and nitrate-N  
284 concentrations increased by 25% and 23%, respectively. Similarly, in Trial II, the Compost + 2%  
285 ZnO + ZSB treatment again resulted in the highest values, with CFU increasing by 39%, MBC by  
286 27%, MBN by 32%, ammonium-N by 28.2%, and nitrate-N by 27.7% relative to the control.

287 Among individual and dual component treatments, Compost + ZSB and compost + 2% ZnO  
288 showed superior performance compared to sole applications. Compost + ZSB increased MBC by  
289 16% in Trial I and 22% in Trial II, whereas compost + 2% ZnO enhanced nitrate-N by 12% in  
290 both trials. The integration of compost, zinc (in the form of ZnO), and zinc-solubilizing bacteria

291 (ZSB) synergistically improved soil biochemical properties, underscoring the potential of  
292 microbial-assisted zinc-enriched compost as a sustainable soil amendment in enhancing soil  
293 fertility and microbial function.

### 294 **3.2. Multivariate analysis of the studied parameters compares the effectiveness of bio-** 295 **organic and inorganic fertilizers to improve soil health**

296 The significance of the results was further confirmed through multivariate analysis. Principal  
297 component analysis (PCA) revealed clear separation among treatments, indicating distinct  
298 influences of different zinc and organic amendments on soil biochemical properties (Fig. 2). In  
299 trial I, the two principal components (PC1 and PC2) accounted for 95.58% of the total variance,  
300 with PC1 alone contributing 92.55%. Treatments involving combinations of compost and zinc  
301 sources, especially Compost + 2% ZnO + ZSB and Compost + ZSB, were closely associated with  
302 higher scores on PC1, primarily driven by elevated levels of soil organic matter (OM), total organic  
303 carbon (TOC), nitrogen (N), and available phosphorus (P). These treatments clustered in the  
304 positive quadrant of the biplot, indicating strong synergistic effects on soil fertility indicators. On  
305 the other hand, the control and mineral ZnSO<sub>4</sub>-alone treatment showed negative associations with  
306 PC1 and PC2, reflecting minimal improvements in soil nutrient status. Zinc oxide in combination  
307 with ZSB (ZnO + ZSB) showed a moderate effect, clustering near the origin, suggesting limited  
308 but positive contributions to zinc availability and other nutrients.

309 In Trial II, PCA highlighted similar patterns with the two principal components explaining 96.12%  
310 of the total variance, where PC1 accounted for 91.52% and PC2 for 4.60%. Compost-based  
311 treatments again formed a distinct cluster, particularly Compost + 2% ZnO + ZSB and Compost +  
312 ZSB, indicating their significant influence on soil OM, TOC, and micronutrient (Fe and Zn)  
313 concentrations. Control and ZnSO<sub>4</sub> alone consistently showed the lowest contributions across both  
314 principal components, reinforcing their limited efficacy in improving soil fertility. Overall, the  
315 PCA confirmed that treatments integrating organic matter (compost) and biological agents (ZSB)  
316 with zinc sources (ZnO) led to more comprehensive improvements in soil nutrient status compared  
317 to inorganic treatments.

### 318 **3.3. Effectiveness of bio-organic fertilizer to improve plant growth and yield**

319 In both Trial I (Bahawalpur) and Trial II (Bahawalnagar), the application of microbial-assisted  
320 zinc-enriched compost demonstrated a significant improvement in wheat growth, particularly in  
321 terms of plant height, shoot dry biomass, 1000-grain weight and grain yield (Fig. 3). Among the

322 treatments, the combined application of Compost + 2% ZnO + ZSB (T9) yielded the most  
323 pronounced effects. In Trial I, the Compost + 2% ZnO + ZSB treatment increased plant height by  
324 16.7% and shoot dry biomass by 17.6% compared to the control. Similarly, in Trial II, this  
325 treatment resulted in a 19.4% increase in plant height and a 19.8% increase in shoot dry biomass  
326 over the control. The next most effective treatments were Compost + ZSB (T8) and Compost +  
327 ZnO (T5). Compost + ZSB enhanced plant height by 12.5% and 12.1% and shoot biomass by  
328 15.1% and 12.8% in Trials I and II, respectively. Compost + ZnO led to increases of 9.7% and  
329 13.6% in plant height, and 4.7% and 10.5% in biomass for Trials I and II, respectively.

330 In Trial I, the highest 1000-grain weight was recorded in the compost + 2% ZnO + ZSB (T9)  
331 treatment, showing an 8.7% increase over the control. This was followed by compost + ZSB  
332 (8.2%) and ZnO + ZSB (7.7%). In terms of grain yield per hectare, the compost + 2% ZnO + ZSB  
333 treatment led to a significant 9.5% increase compared to the control. Other notable increases in  
334 yield included compost + ZSB (7.3%), ZnSO<sub>4</sub> + ZSB (6.3%), and ZnO + ZSB (7.7%). Trial II  
335 confirmed the trends observed in Trial I. The compost + 2% ZnO + ZSB treatment again yielded  
336 the highest 1000-grain weight, with a 9.6% increase over the control. Compost + ZSB and ZnSO<sub>4</sub>  
337 + ZSB treatments improved 1000-grain weight by 7.3% and 5.9%, respectively. Grain yield was  
338 similarly enhanced, with the compost + 2% ZnO + ZSB treatment resulting in a 8.5% increase  
339 over the control. The compost + ZSB (6.7%), ZnSO<sub>4</sub> + ZSB (5.0%), and ZnO + ZSB (6.6%)  
340 treatments also showed considerable improvements.

341 In contrast, sole applications of ZnO, ZnSO<sub>4</sub>, or compost alone had moderate effects on both  
342 parameters, with increases ranging between 1%–3% depending on the trial and variable measured.  
343 The ZSB treatment alone showed a limited but positive effect, suggesting that microbial inoculants  
344 are more effective when combined with zinc sources and organic amendments.

#### 345 **3.4. Comparative improvement in grain quality by the application of bioorganic and** 346 **inorganic fertilizers**

347 Based on the results obtained from both Trial I (Bahawalpur) and Trial II (Bahawalnagar), the  
348 application of microbial-assisted zinc-enriched compost significantly improved the concentration  
349 of macronutrients (N, P, K) and micronutrients (Fe and Zn) in wheat grains compared to the control  
350 and individual application of inorganic fertilizers (Fig. 4). In Trial I, the combined treatment of  
351 Compost + 2% ZnO + ZSB (T9) demonstrated the highest nutrient enhancement across all  
352 measured parameters. This treatment led to an increase of 16.5% in nitrogen (N), 13.9% in

353 phosphorus (P), and 11.3% in potassium (K) contents compared to the control. In terms of  
354 micronutrients, grain iron (Fe) content ( $30.4 \text{ mg kg}^{-1}$ ) and zinc (Zn) content ( $38.5 \text{ mg kg}^{-1}$ ) were  
355 recorded, which were increased by 27.6% and 37.3%, respectively, relative to the control.  
356 Similarly, in Trial II, the Compost + 2% ZnO + ZSB treatment consistently outperformed all other  
357 treatments. Compared to the control, it increased grain nitrogen by 15.9%, phosphorus by 15.0%,  
358 potassium by 13.7%, iron by 27.9%, and zinc by 39.4%. The treatments ZnO + ZSB and ZnSO<sub>4</sub> +  
359 ZSB also showed considerable improvements over the sole applications of ZnO or ZnSO<sub>4</sub>,  
360 suggesting the synergistic effect of zinc-solubilizing bacteria (ZSB) in mobilizing native and  
361 supplemented zinc sources.

362 The Compost + ZSB treatment also enhanced nutrient accumulation in grains, albeit to a lesser  
363 extent than the Compost + 2% ZnO + ZSB. Sole compost and ZSB treatments moderately  
364 increased nutrient content compared to the control, while the sole application of ZnSO<sub>4</sub> and ZnO  
365 resulted in lower improvements than when these sources were bioactivated. Overall, the combined  
366 application of compost, ZnO, and ZSB (Compost + 2% ZnO + ZSB) was the most effective  
367 strategy for promoting the biofortification of wheat grains with essential nutrients in both trials.

### 368 **3.5. Correlation analysis demonstrates the interactive effect of improved soil fertility** 369 **parameters on yield**

370 Correlation analysis revealed significant positive relationships among soil biochemical  
371 parameters, bacterial population in term of colony forming units (CFU g<sup>-1</sup> soil), microbial biomass  
372 carbon (MBC), and microbial biomass nitrogen (MBN) and key soil nutrients (N, P, K, Fe, and  
373 Zn) with crop yield (Fig. 5). Specifically, CFU exhibited a strong positive correlation with MBC  
374 ( $r = 0.82$ ,  $p < 0.01$ ) and MBN ( $r = 0.76$ ,  $p < 0.01$ ), indicating that enhanced microbial abundance  
375 supports higher microbial biomass. MBC and MBN were significantly correlated with available  
376 nitrogen ( $r = 0.79$  and  $0.74$ , respectively;  $p < 0.01$ ), suggesting active microbial involvement in  
377 nutrient mineralization and cycling. Available phosphorus and potassium were also positively  
378 correlated with microbial indicators (MBC–P:  $r = 0.68$ ; MBN–K:  $r = 0.63$ ;  $p < 0.05$ ), underscoring  
379 the role of microbial processes in improving nutrient availability. Moreover, micronutrients such  
380 as Fe and Zn showed moderate but significant correlations with MBC ( $r = 0.59$  and  $0.57$ ,  
381 respectively;  $p < 0.05$ ), suggesting microbial-mediated enhancement of micronutrient solubility.  
382 Importantly, crop yield demonstrated strong positive correlations with MBC ( $r = 0.85$ ), MBN ( $r =$   
383  $0.80$ ), and CFU ( $r = 0.78$ ), as well as with macronutrients N ( $r = 0.83$ ), P ( $r = 0.77$ ), and K ( $r =$

384 0.75). These findings collectively indicate that microbial activity and biomass are closely linked  
385 to nutrient availability and are reliable predictors of soil fertility and crop productivity.

### 386 **3.6. Comparison of bio-organic and inorganic (ZnSO<sub>4</sub>) fertilizers for cost of production and** 387 **net returns for one hectare of wheat**

388 In the study area, the total cost of cultivating one hectare of control (untreated), ZnSO<sub>4</sub> (market  
389 source of zinc) and microbial-assisted zinc-enriched compost (bio-organic fertilizer) of wheat  
390 comprised several components, including land preparation, sowing, seed, fertilizer application,  
391 plant protection (weedicide and pesticide), manual labor, irrigation, and harvesting and threshing  
392 operations (Table 2). The production cost per hectare was calculated as Rs. 230,371 for control  
393 wheat, Rs. 235,353 for ZnSO<sub>4</sub>-treated wheat, and Rs. 239,438 for the microbial-assisted zinc-  
394 enriched compost-treated wheat. The treated wheat incurred higher costs than the control  
395 (untreated) wheat, mainly due to the application of zinc fertilizers. For wheat cultivation, the total  
396 production cost also accounted for opportunity costs, calculated as a 12% markup from land  
397 preparation to fertilizer application. These amounted to Rs. 14,529 for control, Rs. 15,063 for  
398 ZnSO<sub>4</sub>, and Rs. 15,446 for the microbial-assisted zinc-enriched compost. Manual labor costs were  
399 Rs. 2965, Rs. 3,033, and Rs. 3,478, respectively. As detailed in Table 3, the total cost (TC) was  
400 broken down into Total Variable Cost (TVC) and Total Fixed Cost (TFC). The TVC included  
401 expenses related to land preparation, seed, fertilizers, labor, markup, and harvesting and threshing.  
402 The estimated TVC per hectare was Rs. 167,484 for control, Rs. 172,534 for ZnSO<sub>4</sub>, and Rs.  
403 176,551 for the microbial-assisted zinc-enriched compost. TFC, on the other hand, included fixed  
404 expenses such as land rent and *abiana* (government water charges), which collectively amounted  
405 to Rs. 62,887 per hectare. Land rent was treated as an opportunity cost, as most farmers in the  
406 region owned their land. The total cost of production (TC) was derived by summing the TVC and  
407 TFC.

408 The gross margin, net return, and revenue-to-cost (R/C) ratio were calculated for the cultivation of  
409 one hectare of wheat treated as control, ZnSO<sub>4</sub>, and microbial-assisted zinc-enriched compost  
410 (Table 3). Based on Equation 1, the gross margins were estimated at Rs. 111,997 for control, Rs.  
411 112,304 for ZnSO<sub>4</sub> and Rs. 130,578 for the microbial-assisted zinc-enriched compost. Net returns  
412 were determined by deducting the total costs (TC) from total revenue (TR). When land rent (LR)  
413 was included, the net returns amounted to Rs. 49,110 for control, Rs. 49,417 for ZnSO<sub>4</sub>, and Rs.  
414 67,691 for the microbial-assisted zinc-enriched compost. Excluding land rent, the net returns

415 increased to Rs. 110,885, Rs. 111,192, and Rs. 129,466, respectively. Likewise, the revenue-to-  
416 cost ratios, including land rent, were 1.21 for control and ZnSO<sub>4</sub>, and 1.28 for the microbial-  
417 assisted zin-enriched compost. Without considering land rent, these ratios improved to 1.67, 1.65,  
418 and 1.74, respectively. Overall, the economic analysis revealed that the application of the market  
419 source of zinc (ZnSO<sub>4</sub>) has a negligible impact on farmers' net income. Whereas the application  
420 of microbial-assisted zinc-enriched compost showed a significant increase in net income.

#### 421 **4. Discussion**

422 The results of both trials distinctly highlight the agronomic and soil-enhancing potential of  
423 bioorganic fertilizer, particularly when integrated with zinc oxide (ZnO) and zinc-solubilizing  
424 bacteria (ZSB). The treatment combination of Compost + 2% ZnO + ZSB consistently  
425 outperformed all other treatments across all growth, yield, nutrient uptake, and soil fertility  
426 parameters. These findings underscore the efficacy of an integrated nutrient management approach  
427 combining organic, inorganic, and biological amendments in enhancing wheat productivity and  
428 soil health.

429 Soil chemical properties were significantly influenced by the bio-organic fertilizer application.  
430 The observed increase in zinc (Zn) availability can be attributed to two complementary  
431 mechanisms: the gradual and sustainable release of Zn from ZnO particles and the active microbial  
432 solubilization of Zn compounds facilitated by Zn-solubilizing bacteria (ZSB) (Huang et al., 2022).  
433 These processes ensure a continuous supply of bioavailable Zn, reducing the risk of nutrient  
434 fixation in soil. In addition, the enhanced availability of nitrogen (N), phosphorus (P), and  
435 potassium (K) is primarily linked to accelerated microbial mineralization of organic matter,  
436 enzymatic hydrolysis of complex organic compounds, and the decomposition of compost materials  
437 (Reimer et al., 2023). Collectively, these mechanisms enhance soil nutrient cycling and ensure the  
438 synchronized release of nutrients that match plant demand.

439 Biological indicators of soil fertility, including colony-forming units (CFU), microbial biomass  
440 carbon (MBC), and microbial biomass nitrogen (MBN), were improved by the Compost + 2%  
441 ZnO + ZSB treatment. This demonstrates not only the proliferative effect of compost as a microbial  
442 substrate but also the synergistic interactions between microbial inoculants and native soil  
443 microbiota, which enhance microbial colonization and community stability (Dincă et al., 2022; Lu  
444 et al., 2025). ZSB plays a critical mechanistic role by releasing organic acids (Sethi et al., 2025),  
445 siderophores (Zhu et al., 2025), and hydrolytic enzymes that mobilize nutrients (Mujumdar et al.,

446 2024), improve rhizosphere activity, and stimulate beneficial plant–microbe interactions (Jalal et  
447 al., 2024; Feng et al., 2024). Furthermore, the observed increases in nitrate-N and ammonium-N  
448 concentrations indicate enhanced nitrogen transformation pathways, including ammonification,  
449 nitrification, and mineralization processes, actively mediated by microbial communities (Duan et  
450 al., 2023; Chen et al., 2025). These transformations improve nitrogen turnover and ensure steady  
451 nutrient availability to crops, thereby supporting higher productivity and nutrient use efficiency.

452 The pronounced improvement in soil biochemical properties under the integrated application of  
453 compost + ZnO + ZSB can be attributed to synergistic interactions between organic matter inputs,  
454 microbial activity, and zinc chemistry in alkaline arid soils. Compost provides a stable carbon  
455 source that stimulates microbial proliferation and enzymatic activity, thereby accelerating organic  
456 matter decomposition and nutrient mineralization (Gao et al., 2024; Meng et al., 2025). The  
457 concurrent increase in microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)  
458 observed in this study reflects enhanced microbial turnover and nutrient immobilization-  
459 mineralization dynamics, which are critical indicators of soil functional fertility (Edrisi et al.,  
460 2019).

461 Zinc oxide, when applied alone, is often poorly soluble in calcareous soils due to precipitation and  
462 adsorption reactions; however, the presence of zinc-solubilizing bacteria markedly alters this  
463 behavior. ZSB are known to release low-molecular-weight organic acids, protons, and chelating  
464 compounds that lower rhizosphere pH and convert insoluble Zn compounds into plant-available  
465 forms (Sethi et al., 2025). The higher DTPA-extractable Zn and Fe concentrations observed in  
466 compost + ZnO + ZSB treatments therefore reflect biologically mediated micronutrient  
467 mobilization rather than simple fertilizer addition. Similar synergistic effects of organic  
468 amendments and microbial inoculants on micronutrient availability have been reported in other  
469 arid and semi-arid systems (Kumar et al., 2025).

470 Plant growth, measured in terms of plant height and shoot dry biomass, was significantly improved  
471 by the Compost + 2% ZnO + ZSB treatment. These improvements are attributable to enhanced  
472 nutrient availability, particularly zinc and nitrogen, resulting from the synergistic effect of the  
473 organic matrix, micronutrient enrichment, and microbial solubilization. Zinc plays a critical role  
474 in auxin synthesis and enzyme activation (Wang et al., 2023b), and its increased availability in a  
475 bioavailable form likely stimulated vegetative growth. Moreover, the compost component  
476 improved soil physical structure and provided a steady nutrient supply (Kelbesa, 2021), while ZSB

477 enhanced micronutrient solubility through the production of organic acids and siderophores  
478 (Asghar et al., 2024).

479 The sole application of ZnSO<sub>4</sub> or ZnO resulted in comparatively modest gains, indicating the  
480 limitations of mineral zinc sources in calcareous or alkaline soils where Zn availability is  
481 inherently low due to fixation (Gupta et al., 2024). In contrast, microbial inoculation with ZSB in  
482 conjunction with ZnO improved plant growth, suggesting the pivotal role of ZSB in solubilizing  
483 ZnO particles (Sethi et al., 2025). Compost + ZSB treatment also performed well, reaffirming the  
484 benefits of incorporating biological inputs into organic nutrient management systems.

485 In terms of yield, the Compost + 2% ZnO + ZSB treatment consistently showed the highest grain  
486 yield. Enhanced grain filling and development can be attributed to the continuous availability of  
487 zinc, phosphorus, and nitrogen, facilitated by microbial mineralization and compost-mediated  
488 nutrient retention (Campana et al., 2025). Furthermore, the observed increase in 1000-grain weight  
489 in treatments involving ZSB aligns with previous research, which reported that ZSB not only  
490 solubilizes native zinc but also stimulates root growth and enhances nutrient uptake efficiency  
491 (Singh et al., 2024).

492 Nutrient accumulation in grains, particularly of nitrogen (N), phosphorus (P), potassium (K), iron  
493 (Fe), and zinc (Zn), was significantly higher in the Compost + 2% ZnO + ZSB treatment. The  
494 improvement in nutrient uptake is primarily linked to enhanced microbial activity, which mobilizes  
495 native and added micronutrients and the organic matter that prevents nutrient leaching and  
496 enhances cation exchange capacity (Dhaliwal et al., 2024). These results are crucial in the context  
497 of human nutrition, particularly in zinc-deficient regions, and support the promotion of agronomic  
498 biofortification strategies.

499 The superior wheat growth and yield response observed under compost + ZnO + ZSB treatment  
500 can be mechanistically linked to improved soil biological functioning and micronutrient  
501 availability. Zinc plays a pivotal role in enzyme activation, protein synthesis, and auxin  
502 metabolism, directly influencing tillering, biomass accumulation, and grain development (Gupta  
503 et al., 2025; Sohail et al., 2025). The sustained availability of Zn through microbial solubilization,  
504 rather than transient availability from soluble ZnSO<sub>4</sub>, explains the superior performance of  
505 integrated treatments over mineral fertilization alone.

506 Additionally, compost-mediated improvements in soil structure and water-holding capacity likely  
507 alleviated abiotic stress typical of arid soils, indirectly supporting plant growth and nutrient uptake

508 (Rehman et al., 2023; Wang et al., 2023a). The observed enhancement in 1000-grain weight and  
509 grain yield under ZSB-based treatments aligns with earlier studies demonstrating that microbial  
510 inoculants improve root architecture, nutrient acquisition efficiency, and assimilate partitioning  
511 during grain filling (Galindo-Castañeda et al., 2022).

512 The findings of the study validate the hypothesis that integrating organic matter (compost),  
513 micronutrient supplementation (ZnO), and microbial inoculants (ZSB) offers a sustainable,  
514 efficient, and environmentally friendly strategy to improve crop growth, yield, nutrient density,  
515 and soil health. This integrated approach is promising in zinc-deficient soils and can serve as a  
516 cornerstone of sustainable agricultural and biofortification efforts in developing regions. Future  
517 studies should explore the scalability of the strategy under different agroecological zones and  
518 cropping systems, as well as its long-term impacts on soil microbial ecology and plant health.

519 The economic analysis of wheat cultivation under different zinc fertilization treatments  
520 demonstrated that although input costs were higher for treated plots, especially for microbial-  
521 assisted zinc-enriched compost, the overall profitability was enhanced, particularly under bio-  
522 organic fertilization. This aligns with research, which reported that the addition of external nutrient  
523 sources in wheat production generally increases variable costs, especially when micronutrient  
524 fortification is involved (Ali and Tsou, 1997). Despite the increased costs, the economic benefits  
525 in terms of gross margins and net returns were significantly higher for the microbial-assisted zinc-  
526 compost treatment. This improvement may be attributed to enhanced nutrient use efficiency and  
527 improved plant growth parameters due to the synergistic effects of compost and beneficial  
528 microbes. It is evident from the literature that microbial inoculants can promote nutrient  
529 solubilization and uptake, translating into higher yields (Sammauria et al., 2020) and better  
530 financial returns. The higher net income in the microbial-assisted zinc-enriched compost treatment  
531 relative to the control suggests that bio-organic-assisted biofortification not only improves soil  
532 health and crop yield but also enhances farm-level economic resilience. This strategy offers a  
533 sustainable solution for nutrient-deficient soils, supporting the transition towards low-input, high-  
534 efficiency farming systems (Akbar et al., 2020).

535 Although initial input costs were higher with microbial-assisted zinc-enriched compost, the  
536 significant gains in yield and net return justify its adoption. The data strongly support integrating  
537 microbial-assisted composts into conventional fertilization regimes to improve agronomic  
538 performance and economic returns under resource-constrained conditions. The present study was

539 conducted under specific soil and climatic conditions and over limited cropping seasons, which  
540 may restrict the generalization of results across diverse agroecological zones. Long-term effects  
541 on soil microbial diversity, nutrient cycling, and micronutrient buildup were not assessed, and the  
542 findings were restricted to wheat without testing in other cropping systems. Future research should  
543 therefore focus on multi-location and multi-season trials, long-term soil health monitoring, and  
544 extending the approach to different crops. Additionally, developing cost-effective microbial  
545 inoculant formulations and delivery mechanisms will be crucial to enhance adoption, particularly  
546 by resource-constrained farmers.

## 547 **5. Conclusion**

548 The integrated application of microbial-assisted zinc-enriched compost (Compost + 2% ZnO +  
549 ZSB) enhances wheat growth, yield, grain nutrient biofortification, and soil fertility compared to  
550 sole amendments. This combined treatment consistently outperformed others across both trials,  
551 markedly increasing plant height, biomass, grain weight, and yield, while significantly elevating  
552 macronutrient (N, P, K) and micronutrient (Fe, Zn) concentrations in grains. Soil chemical and  
553 biological parameters also showed pronounced improvements, with enhanced organic matter,  
554 microbial biomass, and nutrient availability, underscoring the synergistic effects of organic,  
555 microbial, and zinc amendments. These findings highlight the efficacy of integrated nutrient  
556 management strategies involving microbial-assisted zinc-enriched compost for sustainable wheat  
557 production and soil health restoration, offering promising avenues for addressing micronutrient  
558 deficiencies in cereal crops.

## 559 **Data Availability Statement**

560 The authors confirm that the data, figures, and tables included in this manuscript are original and  
561 have not been previously published. The primary data are accessible and can be provided upon  
562 request. Relevant data is submitted on the NCBI website, and accession numbers MN696212,  
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## 564 **Author Contributions**

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### 573 **Competing interest**

574 The authors declared no potential conflict/competing interest relevant to this work.

575

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Table 1: Effect of Microbial-assisted zinc-enriched compost on soil biochemical properties

Treatment	Organic matter (%)		Total organic carbon (g kg <sup>-1</sup> )		Bacterial population (CFU × 10 <sup>6</sup> )		Total nitrogen (%)	
	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
T0	0.47 ± 0.01 d	0.54 ± 0.01 e	5.8 ± 0.3 d	6.3 ± 0.3 c	22.7 ± 0.7 f	13.1 ± 0.3 f	0.029 ± 0.0004 f	0.033 ± 0.0004 f
T1	0.47 ± 0.00 d	0.56 ± 0.00 de	6.0 ± 0.4 cd	6.5 ± 0.3 bc	23.7 ± 0.5 ef	14.4 ± 0.7 def	0.029 ± 0.0002 ef	0.033 ± 0.0001 ef
T2	0.48 ± 0.00 d	0.55 ± 0.01 de	6.0 ± 0.2 cd	6.5 ± 0.2 c	25.4 ± 0.7 de	13.5 ± 0.3 ef	0.029 ± 0.0001 ef	0.033 ± 0.0004 ef
T3	0.61 ± 0.01 b	0.70 ± 0.01 b	7.0 ± 0.2 bc	7.5 ± 0.3 abc	29.1 ± 0.8 b	14.9 ± 0.4 cde	0.031 ± 0.0004 cd	0.036 ± 0.0004 cd
T4	0.48 ± 0.01 cd	0.57 ± 0.00 cd	6.2 ± 0.2 cd	7.0 ± 0.4 bc	26.8 ± 1.0 cd	14.0 ± 0.2 def	0.029 ± 0.0003 ef	0.034 ± 0.0002 e
T5	0.62 ± 0.01 b	0.71 ± 0.00 b	6.9 ± 0.5 bc	7.3 ± 0.6 abc	29.9 ± 0.7 b	16.3 ± 0.7 bc	0.032 ± 0.0006 bc	0.036 ± 0.0002 bc
T6	0.49 ± 0.00 cd	0.57 ± 0.00 cd	6.4 ± 0.2 cd	6.9 ± 0.5 bc	28.0 ± 0.3 bc	15.4 ± 0.4 cd	0.030 ± 0.0002 de	0.034 ± 0.0001 e
T7	0.50 ± 0.01 c	0.58 ± 0.00 c	6.4 ± 0.2 bcd	6.9 ± 0.5 bc	26.9 ± 0.2 cd	16.2 ± 0.7 bc	0.031 ± 0.0003 cd	0.035 ± 0.0002 d
T8	0.63 ± 0.01 ab	0.72 ± 0.01 b	7.5 ± 0.7 ab	7.9 ± 0.4 ab	29.5 ± 0.6 b	17.1 ± 0.2 ab	0.032 ± 0.0006 ab	0.037 ± 0.0003 bc
T9	0.65 ± 0.01 a	0.75 ± 0.01 a	8.0 ± 0.6 a	8.3 ± 0.7 a	34.1 ± 0.9 a	18.2 ± 0.3 a	0.034 ± 0.0006 a	0.039 ± 0.0007 a
<b>LSD (<i>p</i> ≤ 0.05)</b>	<b>0.0249 ****</b>	<b>0.0207 ****</b>	<b>1.0147 **</b>	<b>1.3130 ns</b>	<b>2.0018 ****</b>	<b>1.4673 ****</b>	<b>1.244 ****</b>	<b>1.034 ****</b>
Treatment	Ammonium nitrogen (mg kg <sup>-1</sup> )		Nitrate nitrogen (mg kg <sup>-1</sup> )		Microbial biomass nitrogen (mg kg <sup>-1</sup> )		Microbial biomass carbon (mg kg <sup>-1</sup> )	
T0	9.9 ± 0.2 e	10.2 ± 0.2 f	13.6 ± 0.5 c	14.7 ± 0.5 d	133 ± 4.6 c	134 ± 2.0 f	651 ± 3.8 e	654 ± 3.8 e
T1	10.2 ± 0.2 de	10.4 ± 0.2 ef	14.0 ± 0.9 bc	15.4 ± 0.3 cd	135 ± 2.6 c	139 ± 1.8 ef	659 ± 4.1 e	665 ± 3.5 e
T2	10.3 ± 0.1 de	10.7 ± 0.2 def	14.0 ± 1.2 bc	15.5 ± 0.3 cd	136 ± 3.1 c	138 ± 1.8 ef	664 ± 4.3 e	664 ± 2.9 e
T3	10.8 ± 0.2 bc	11.4 ± 0.2 c	15.0 ± 0.9 abc	16.3 ± 0.3 bcd	147 ± 3.2 b	151 ± 3.2 cd	701 ± 7.6 d	734 ± 5.2 d
T4	11.2 ± 0.2 b	11.4 ± 0.2 c	15.1 ± 0.4 abc	16.5 ± 0.9 bcd	147 ± 4.9 b	153 ± 3.2 c	705 ± 3.2 d	724 ± 4.5 d
T5	11.3 ± 0.1 b	11.3 ± 0.2 c	15.2 ± 0.9 abc	16.6 ± 0.4 bcd	149 ± 2.6 b	151 ± 2.3 cd	720 ± 5.2 c	754 ± 6.8 c
T6	10.4 ± 0.2 cde	10.8 ± 0.1 de	14.2 ± 0.9 bc	15.6 ± 0.9 cd	142 ± 2.3 bc	144 ± 2.1 de	696 ± 5.1 d	738 ± 5.2 d
T7	10.5 ± 0.1 cd	11.1 ± 0.1 cd	14.6 ± 0.4 abc	16.0 ± 1.0 cd	142 ± 1.9 bc	145 ± 2.0 cde	692 ± 4.2 d	735 ± 5.0 d
T8	11.9 ± 0.2 a	12.4 ± 0.3 b	16.2 ± 0.9 ab	18.0 ± 0.6 ab	161 ± 3.4 a	168 ± 3.8 b	755 ± 5.2 b	798 ± 4.9 b
T9	12.4 ± 0.1 a	13.1 ± 0.2 a	16.7 ± 0.9 a	18.7 ± 0.9 a	168 ± 3.8 a	178 ± 3.2 a	787 ± 5.8 a	830 ± 4.6 a
<b>LSD (<i>p</i> ≤ 0.05)</b>	<b>0.4843 ****</b>	<b>0.4855 ****</b>	<b>2.3988 ns</b>	<b>1.9418 *</b>	<b>9.9833 ****</b>	<b>7.9141 ****</b>	<b>14.602 ****</b>	<b>14.073 ****</b>
Treatment	Olsen P (mg kg <sup>-1</sup> )		Extractable K (mg kg <sup>-1</sup> )		DTPA extracted Fe (mg kg <sup>-1</sup> )		DTPA extracted Zn (mg kg <sup>-1</sup> )	
T0	5.17 ± 0.11 f	5.92 ± 0.10 c	65.8 ± 0.90 f	66.2 ± 1.00 f	0.52 ± 0.002 e	0.53 ± 0.004 d	0.56 ± 0.004 f	0.58 ± 0.003 e
T1	5.20 ± 0.05 f	5.96 ± 0.07 c	67.3 ± 1.56 ef	67.9 ± 1.26 fg	0.52 ± 0.004 e	0.54 ± 0.004 d	0.57 ± 0.006 f	0.61 ± 0.009 de
T2	5.29 ± 0.06 f	5.99 ± 0.03 c	67.0 ± 1.99 ef	67.5 ± 1.15 fg	0.53 ± 0.002 e	0.54 ± 0.006 d	0.58 ± 0.008 ef	0.61 ± 0.009 de
T3	5.97 ± 0.07 bc	6.65 ± 0.05 b	71.5 ± 1.19 cd	74.8 ± 1.36 bc	0.56 ± 0.006 d	0.58 ± 0.009 c	0.59 ± 0.003 def	0.61 ± 0.014 de
T4	5.57 ± 0.04 c	6.49 ± 0.03 bc	72.3 ± 1.07 bcd	74.4 ± 0.50 cd	0.56 ± 0.008 d	0.58 ± 0.007 c	0.58 ± 0.002 ef	0.62 ± 0.015 cd
T5	5.87 ± 0.04 cd	6.67 ± 0.06 b	73.5 ± 0.83 abc	73.3 ± 1.71 cde	0.58 ± 0.007 c	0.59 ± 0.007 c	0.65 ± 0.013 b	0.67 ± 0.019 ab
T6	5.67 ± 0.03 e	6.27 ± 0.06 d	70.0 ± 0.67 cde	70.1 ± 1.68 ef	0.58 ± 0.006 cd	0.58 ± 0.003 c	0.61 ± 0.011 cde	0.65 ± 0.018 bc
T7	5.70 ± 0.06 de	6.34 ± 0.05 cd	69.2 ± 1.70 def	70.6 ± 1.09 def	0.57 ± 0.007 cd	0.58 ± 0.010 c	0.62 ± 0.012 cde	0.66 ± 0.018 bc
T8	6.12 ± 0.07 b	6.94 ± 0.07 a	76.2 ± 1.26 ab	78.6 ± 1.67 ab	0.61 ± 0.008 b	0.62 ± 0.029 b	0.62 ± 0.016 bc	0.65 ± 0.019 bc
T9	6.36 ± 0.06 a	7.11 ± 0.07 a	77.6 ± 1.63 a	80.8 ± 1.77 a	0.63 ± 0.005 a	0.66 ± 0.011 a	0.69 ± 0.008 a	0.70 ± 0.015 a
<b>LSD (<i>p</i> ≤ 0.05)</b>	<b>0.1788 ****</b>	<b>0.1918 ****</b>	<b>4.1686 ***</b>	<b>3.8615 ****</b>	<b>0.0155 ****</b>	<b>0.0335 ****</b>	<b>0.0280 ****</b>	<b>0.0330 ****</b>

Data is presented as mean of three replicates ± standard error. Means sharing same letter(s) within a column are statistically non-significant at 5 % probability. \*: *P* < 0.05; \*\*: *P* < 0.01; \*\*\*: *P* < 0.001; \*\*\*\*: *P* < 0.0001; ns: non-significant

**Table 2:** Comparison of the per-hectare cost of production of wheat treated as a control (no application of zinc), ZnSO<sub>4</sub> and microbial-assisted zinc-enriched compost

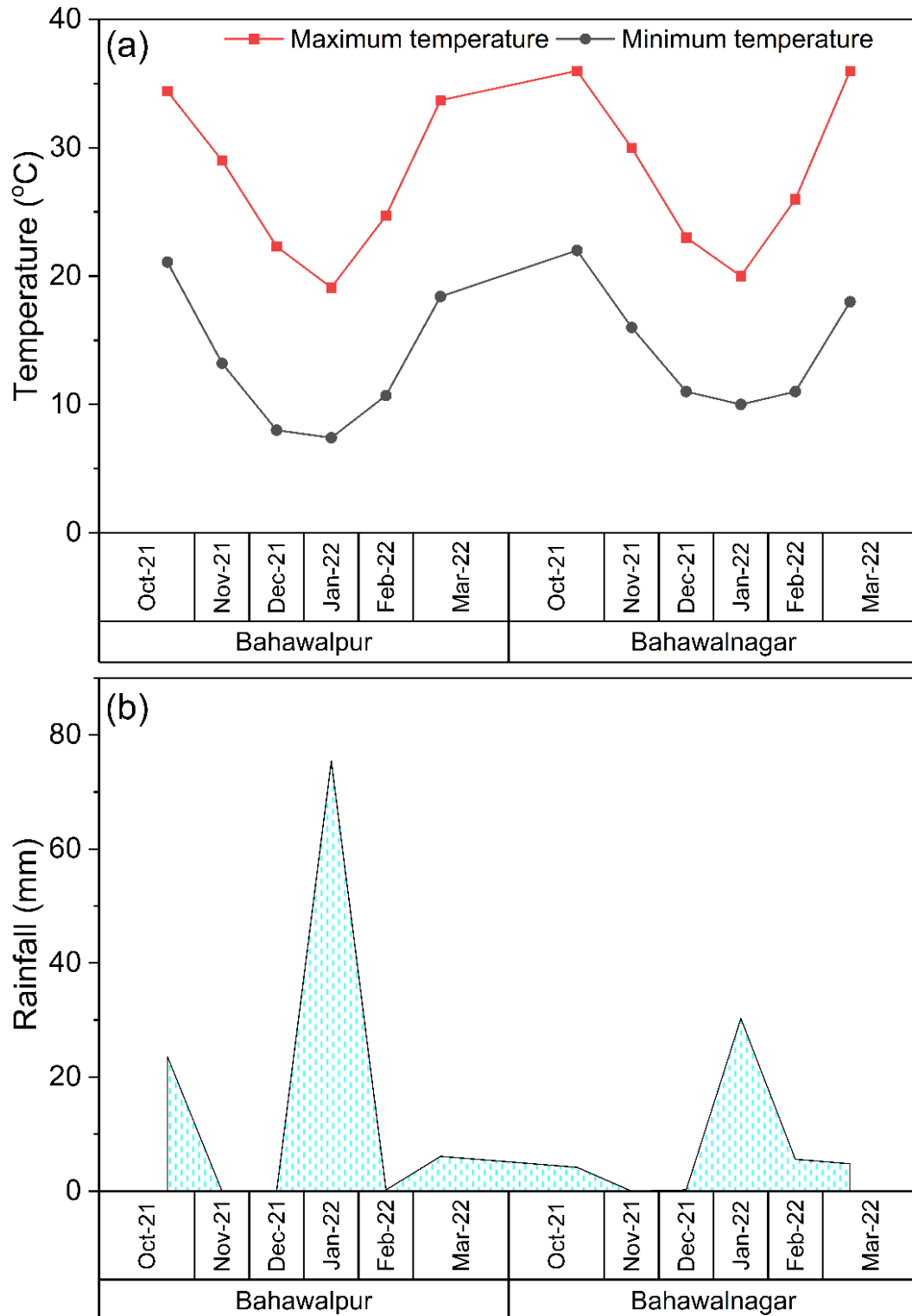
Activity	Costs of production per hectare (Rs.)		
	Control	Market Source of Zinc (ZnSO <sub>4</sub> )	Microbial-assisted zinc-enriched compost
<b>Land preparation</b>	17,297	17,297	17,297
<b>Seed</b>	14,826	14,826	14,826
<b>Fertilizers</b>	88,956	88,956	84,508 *
<b>Zinc application</b>	0	4,448 **	12,085 ***
<b>Manual Labor charges</b>	2,965	2,965	2,965
<b>Plant protection (weedicide application)</b>	2,965	3,033	3,478
<b>Mark up @ <i>i</i>=12% (land prep. to Fert. application)</b>	14,529	15,063	15,446
<b>Harvesting</b>	12,355	12,355	12,355
<b>Threshing</b>	13,591	13,591	13,591
<b>Total variable cost</b>	167,484	172,534	176,551
<b>Land Rent</b>	61775	61775	61775
<b>Abiana (Fixed water charges)</b>	1112	1112	1112
<b>Total fixed cost (Rs)</b>	62887	62887	62887
<b>Total cost (Rs.)</b>	230,371	235,421	239,438

The cost of production was calculated using the current rates of commodities in the local market.  
 \*: Reduced application of chemical fertilizers by 5%; \*\*: Price of ZnSO<sub>4</sub>; \*\*\*: Price of microbial-assisted zinc-enriched compost

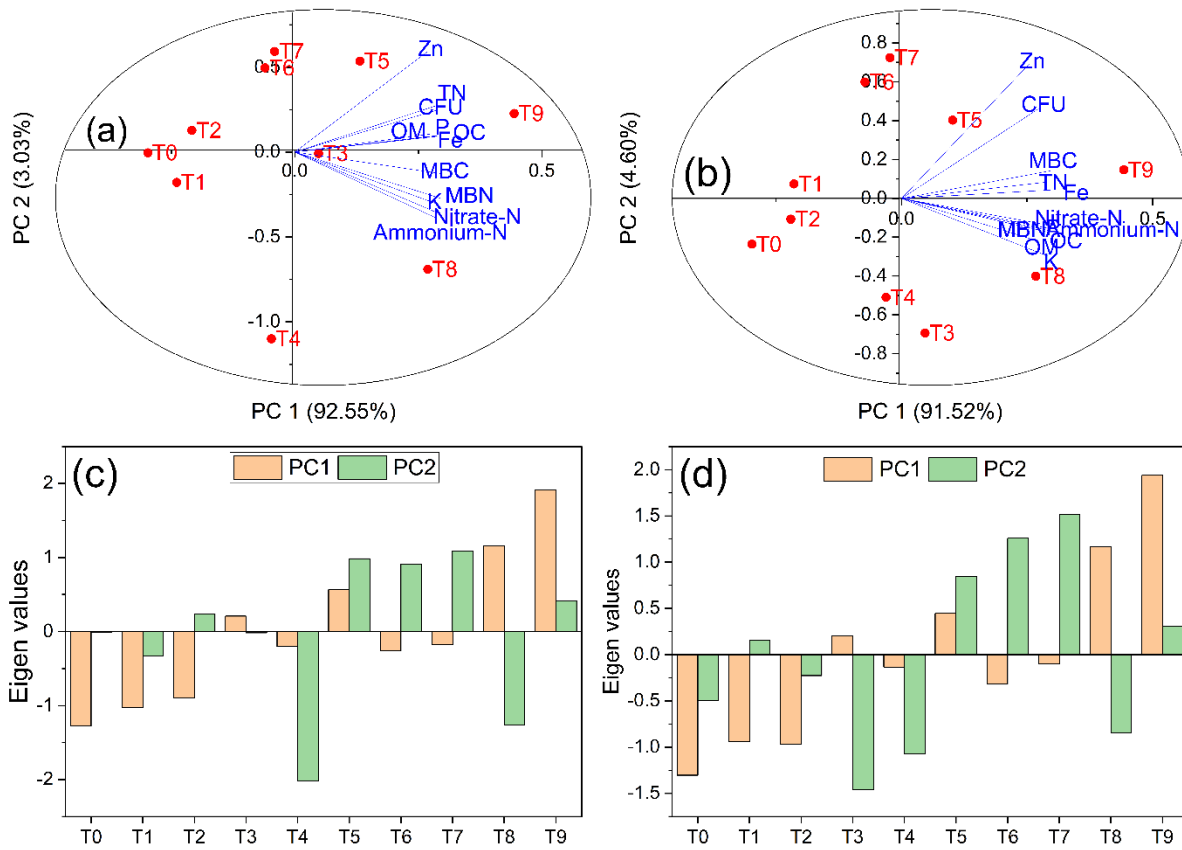
**Table 3:** Gross margin, net income and revenue to cost (R/C) ratio

<b>Parameters</b>	<b>Control</b>	<b>Market Source of Zinc (ZnSO<sub>4</sub>)</b>	<b>Microbial-assisted zinc-enriched compost</b>
<b>Grain yield (mounds/ha)</b>	115.5	117.4	125.75
<b>Price (Rs./mound)</b>	2,170	2,170	2,170
<b>Revenue A (grain yield × price) (Rs.)</b>	250,635	254,758	272,878
<b>Biomass yield (mounds/ha)</b>	122.75	128	145.75
<b>Price (Rs./mound)</b>	235	235	235
<b>Revenue B (biomass yield × price) (Rs.)</b>	28,846.25	30,080	34,251
<b>Total revenue</b>	279,481.25	284,838	307,129
<b>Gross margin (Rs.) = TR-TVC</b>	111,997	112,304	130,578
<b>NR with LR (Rs.) = TR-TC</b>	49,110	49,417	67,691 *
<b>NR without LR (Rs.) = TR-TVC</b>	110,885	111,192	129,466
<b>Revenue/cost ratio with LR = TR/TC</b>	1.21	1.21	1.28
<b>Revenue/cost ratio without LR = TR/TVC</b>	1.67	1.65	1.74

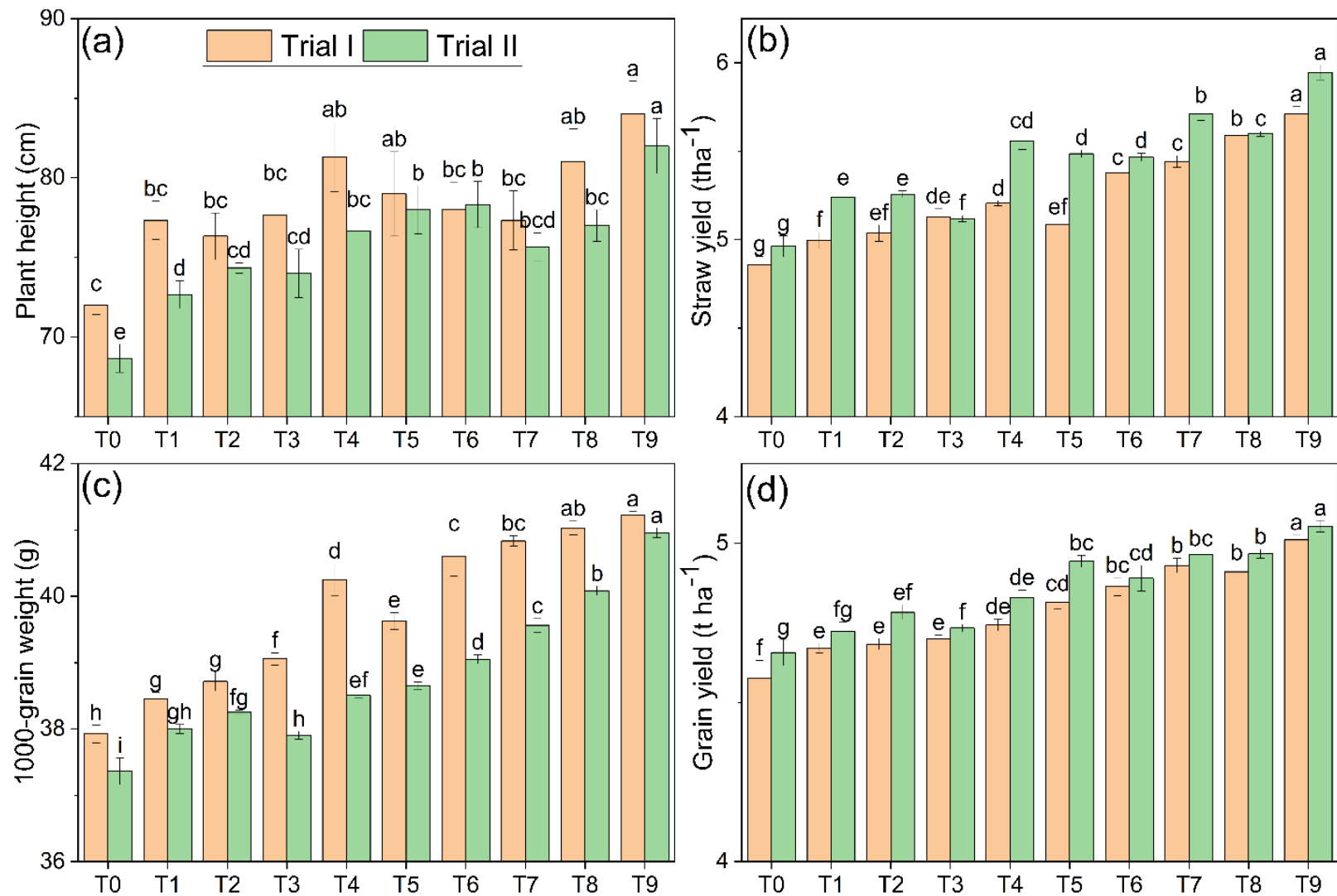
Yield is presented as average of both trials conducted in Bahawalpur and Bahawalnagar. \*: significant increase in net income by the application of microbial-assisted zin-enriched compost



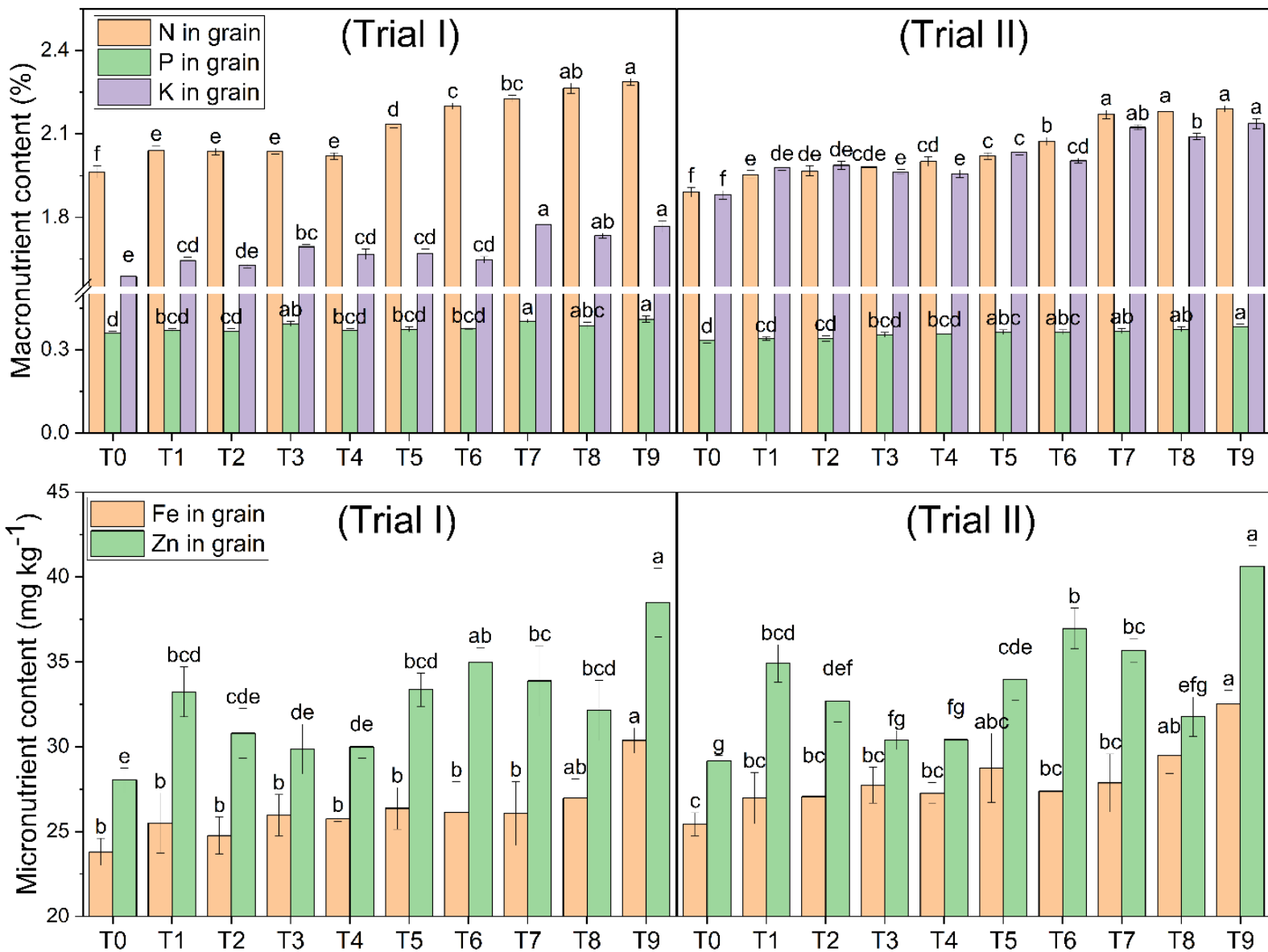
**Figure 1.** Climate data from experimental sites during the 2021-22 cropping season. Data is presented as the monthly average temperature (a) and total rainfall (mm) in a month (b)



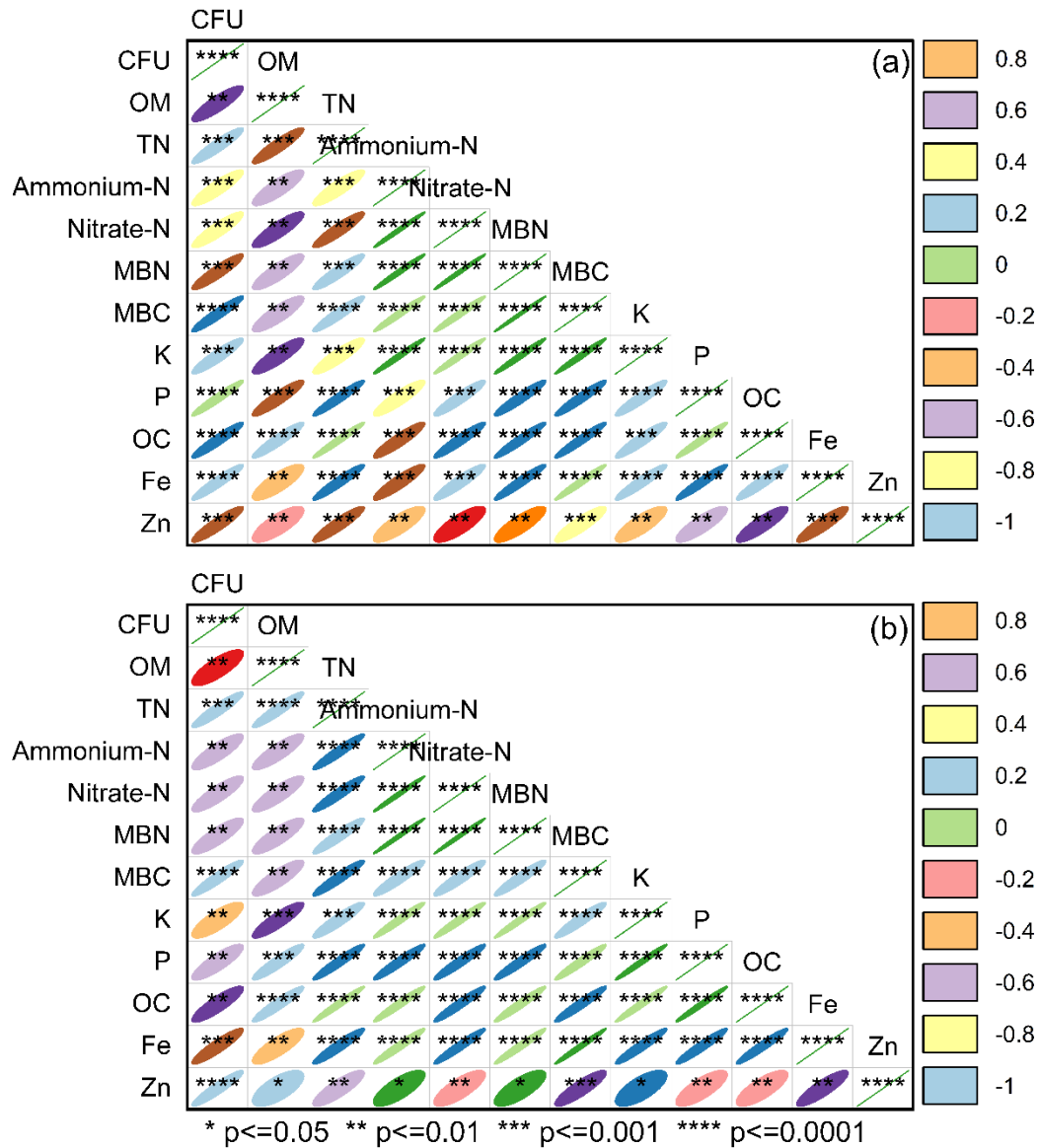
**Figure 2.** Principal component analysis (PCA) demonstrates the effectiveness of microbial-assisted zinc-enriched compost to improve soil biochemical properties. (a): PCA - trial I (Bahawalpur); (b): PCA - trial II (Bahawalnagar); (c): eigenvalues – trial I; (d): eigenvalues – trial II; Zn: DTPA-extracted zinc; TN: total nitrogen; CFU: colony forming units; OM: organic matter; TOC: total organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; Fe: DTPA extracted iron; P: Olsen phosphorous; K: extractable potassium



**Figure 3.** Effect of microbial-assisted zinc-enriched compost on the growth and yield of wheat. The same letter(s) on the bars represent statistically non-significant variation at 5% probability ( $n = 3$ ,  $p \leq 0.05$ ). Trial I: Bahawalpur; Trial II: Bahawalnagr; (a): plant height; (b): straw yield; (c): 1000-grain weight; (d): grain yield



**Figure 4.** Effect of microbial-assisted zinc-enriched compost on the macro- and micronutrient concentration in wheat grain. The same letter(s) on the bars represent statistically non-significant variation at 5% probability ( $n = 3, p \leq 0.05$ ). Trial I: Bahawalpur; Trial II: Bahawalnagar



**Figure 5.** Pearson's correlation analysis was used to describe the relationship between the studied soil parameters. The thickness of the ellipses represents the correlation coefficient (R) and the asterisks represent the significance based on *p-value*. a: Trial I (Bahawalpur); b: Trial II (Bahawalnagar)