

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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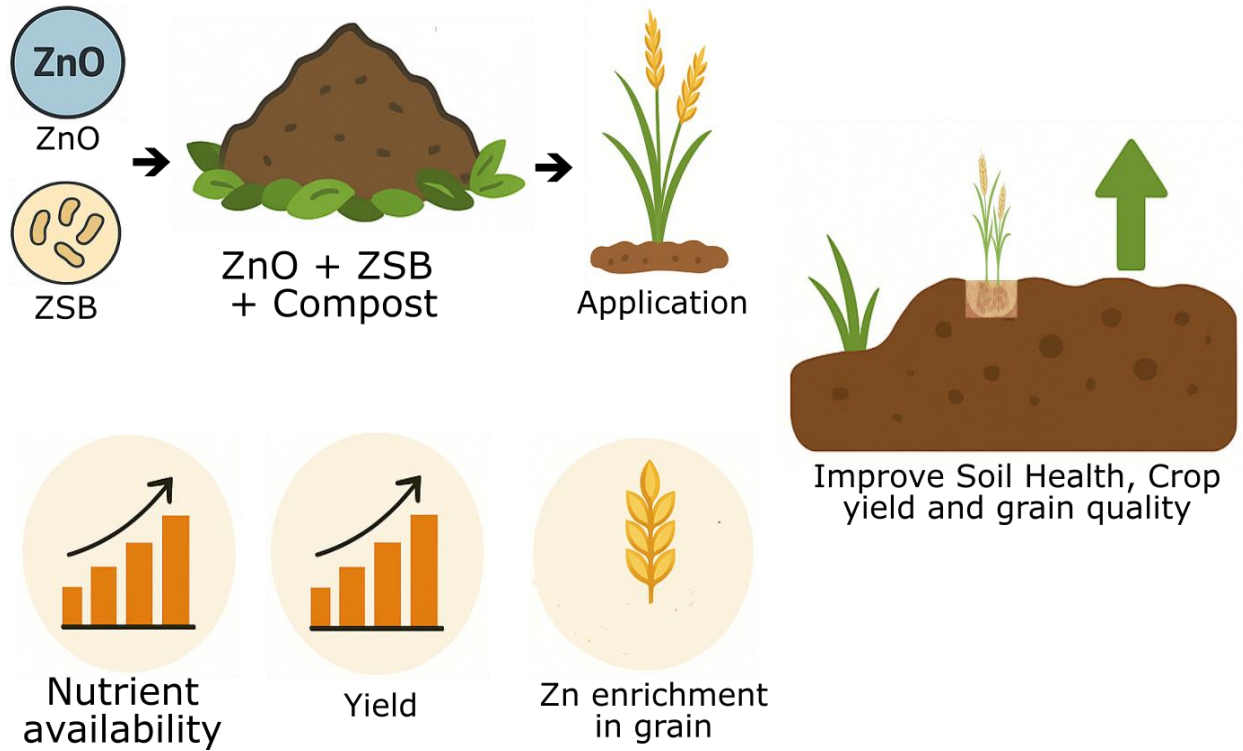
12 **Abstract**

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 increasing interest in Zn-enriched organic amendments, most available studies have  
79 focused on humid or semi-controlled environments (Waterlot et al., 2024), while evidence from  
80 arid and semi-arid regions remains limited (Feliziani et al., 2025). High soil pH, low organic  
81 matter, and rapid micronutrient fixation pose unique challenges in these systems, often reducing  
82 the effectiveness of conventional Zn fertilizers (Sethi et al., 2025). Consequently, field-based  
83 evaluations of bioactivated Zn-enriched compost under arid conditions are still scarce, highlighting  
84 the need for location-specific validation (Sreenivasa, 2012; Prasath et al., 2018). 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, these experiments were conducted under closely  
91 monitored research-managed conditions, which may not fully reflect the variability and constraints  
92 of farmer-managed arid agroecosystems. The present study advances this earlier work by  
93 validating the performance of microbial-assisted Zn-enriched compost across two distinct farmer-  
94 field locations under real agronomic practices. This multi-location, field-scale assessment  
95 integrates soil biochemical indicators, crop productivity, grain Zn biofortification, and economic  
96 returns, thereby providing new insights into the scalability, adaptability, and on-farm feasibility of  
97 bio-organic Zn management strategies in nutrient-deficient arid soils.

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

106 From an environmental perspective, the utilization of compost, particularly when derived from  
107 agricultural, municipal, or agro-industrial residues, contributes to sustainable waste management  
108 by diverting organic matter from landfills and mitigating greenhouse gas emissions. Composting

109 stabilizes organic residues, reducing methane generation and leachate formation, while also  
110 sequestering carbon in soils—an important consideration in climate change mitigation.  
111 Furthermore, the inclusion of beneficial microbes and micronutrient enrichment in compost  
112 contributes to soil biodiversity and resilience, supporting the ecological functions that underpin  
113 sustainable

## 114 **2. Materials and Methods**

### 115 **2.1. Compost Preparation**

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

138 The bacterial strains used in the present study had already been characterized for zinc and  
139 phosphorus solubilization, catalase and urease activity, exopolysaccharide, siderophore and indole

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

## 152 **2.2. Field trial**

153 Two field experiments were conducted at separate arid locations, Bahawalpur (29.3544° N,  
154 71.6911° E) and Bahawalnagar (29.1903° N, 72.6343° E), to evaluate the effects of ten (10)  
155 different treatments on crop performance. The weather data, including monthly average  
156 temperature and rainfall (mm) during the cropping season (2021-22), were presented in fig. 1. The  
157 treatments included the sole and combined application of a market source of zinc (ZnSO<sub>4</sub>), a cheap  
158 source of zinc (ZnO), compost, zinc solubilizing rhizobacteria, and microbial-assisted zinc-  
159 enriched compost. The experiment consists of ten treatments; T0: Control; T1: ZnSO<sub>4</sub>; T2: ZnO;  
160 T3: Compost; T4: ZSB; T5: compost + 2% ZnO; T6: ZnSO<sub>4</sub> + ZSB; T7: ZnO + ZSB; T8: compost  
161 + ZSB; T9: compost + 2% ZnO + ZSB (microbial-assisted Zn-enriched compost). A randomized  
162 complete block design (RCBD) was used with three replications per treatment at each site to ensure  
163 statistical reliability. The control treatment (T0) represents the prevailing farmers' field practice in  
164 the study area, where recommended doses of N, P, and K fertilizers are applied without  
165 supplemental zinc or organic amendments. This treatment, therefore, reflects the actual baseline  
166 production system commonly adopted by farmers in arid regions.

167 Before sowing, composite soil samples were collected from the 0–15 cm depth across each site  
168 and analyzed for baseline fertility status. The soils at both locations were characterized as deficient  
169 arid soils with the average properties: pH (7.69 and 8.1), electrical conductivity (EC) (1.27 and  
170 1.42 dS m<sup>-1</sup>), organic matter (0.43 and 0.51%), total nitrogen (0.032 and 0.035%), available

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

### 183 **2.3. Soil and plant samples collection and analysis**

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

### 191 **2.4. Plant analysis**

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

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

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

## 217 **2.5. Soil chemical analysis**

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

## 226 **2.6. Soil biochemical analysis**

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

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

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

## 246 **2.7. Economic analysis**

247 The economic analysis was estimated, and subsequently, the costs and returns of various items  
248 used in this study for wheat cultivation, as normally practiced by farmers (control), and the  
249 application of a market source of zinc and microbial-assisted zinc-enriched compost (Khan et al.,  
250 2012) were apportioned. Gross margin was calculated by the following formula to make  
251 comparisons.

$$252 \quad \text{Gross Margin (GM)} = \text{Total Revenue (TR)} - \text{Total Variable Cost (TVC)}$$

253 Economic comparisons were made using the control treatment as a benchmark representing  
254 farmers' conventional fertilization practices. This approach ensures that profitability assessments  
255 realistically reflect on-farm decision-making conditions rather than idealized experimental  
256 scenarios.

## 257 **2.8. Statistical Analysis**

258 The statistical analysis in this study was conducted using a combination of multivariate techniques.  
259 Analysis of Variance (ANOVA) was employed to test for significant differences between group  
260 means. Principal Component Analysis (PCA) was done to reduce dimensionality and identify the  
261 gradients of variation in the dataset, facilitating visualization of patterns and clustering. Pearson  
262 correlation coefficients were calculated to quantify the strength and direction of linear associations

263 between pairs of continuous variables. All analyses were performed using OriginPro 2021b and  
264 Statistix 8.1 software, with significance levels set at  $\alpha = 0.05$ .

### 265 **3. Results**

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

#### 272 **3.1. Comparative effect of bio-organic and organic fertilizers on soil biochemical properties**

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

286 In Trial I, the treatment (compost + 2% ZnO + ZSB) led to a maximum CFU count ( $34.1 \times 10^6$   
287 CFU g<sup>-1</sup> soil), showing an increase of 50.3% over the control. Microbial biomass carbon (MBC)  
288 and microbial biomass nitrogen (MBN) were significantly elevated, with increases of 21% and  
289 26%, respectively, compared to the untreated control. Additionally, ammonium-N and nitrate-N  
290 concentrations increased by 25% and 23%, respectively. Similarly, in Trial II, the Compost + 2%  
291 ZnO + ZSB treatment again resulted in the highest values, with CFU increasing by 39%, MBC by  
292 27%, MBN by 32%, ammonium-N by 28.2%, and nitrate-N by 27.7% relative to the control.

293 Among individual and dual component treatments, Compost + ZSB and compost + 2% ZnO  
294 showed superior performance compared to sole applications. Compost + ZSB increased MBC by  
295 16% in Trial I and 22% in Trial II, whereas compost + 2% ZnO enhanced nitrate-N by 12% in  
296 both trials. The integration of compost, zinc (in the form of ZnO), and zinc-solubilizing bacteria  
297 (ZSB) synergistically improved soil biochemical properties, underscoring the potential of  
298 microbial-assisted zinc-enriched compost as a sustainable soil amendment in enhancing soil  
299 fertility and microbial function.

### 300 **3.2. Multivariate analysis of the studied parameters compares the effectiveness of bio-** 301 **organic and inorganic fertilizers to improve soil health**

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

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

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

In both Trial I (Bahawalpur) and Trial II (Bahawalnagar), the application of microbial-assisted zinc-enriched compost demonstrated a significant improvement in wheat growth, particularly in terms of plant height, shoot dry biomass, 1000-grain weight and grain yield (Fig. 3). Among the treatments, the combined application of Compost + 2% ZnO + ZSB (T9) yielded the most pronounced effects. In Trial I, the Compost + 2% ZnO + ZSB treatment increased plant height by 16.7% and shoot dry biomass by 17.6% compared to the control. Similarly, in Trial II, this treatment resulted in a 19.4% increase in plant height and a 19.8% increase in shoot dry biomass over the control. The next most effective treatments were Compost + ZSB (T8) and Compost + ZnO (T5). Compost + ZSB enhanced plant height by 12.5% and 12.1% and shoot biomass by 15.1% and 12.8% in Trials I and II, respectively. Compost + ZnO led to increases of 9.7% and 13.6% in plant height, and 4.7% and 10.5% in biomass for Trials I and II, respectively.

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

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

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

Based on the results obtained from both Trial I (Bahawalpur) and Trial II (Bahawalnagar), the application of microbial-assisted zinc-enriched compost significantly improved the concentration

355 of macronutrients (N, P, K) and micronutrients (Fe and Zn) in wheat grains compared to the control  
356 and individual application of inorganic fertilizers (Fig. 4). In Trial I, the combined treatment of  
357 Compost + 2% ZnO + ZSB (T9) demonstrated the highest nutrient enhancement across all  
358 measured parameters. This treatment led to an increase of 16.5% in nitrogen (N), 13.9% in  
359 phosphorus (P), and 11.3% in potassium (K) contents compared to the control. In terms of  
360 micronutrients, grain iron (Fe) content ( $30.4 \text{ mg kg}^{-1}$ ) and zinc (Zn) content ( $38.5 \text{ mg kg}^{-1}$ ) were  
361 recorded, which were increased by 27.6% and 37.3%, respectively, relative to the control.  
362 Similarly, in Trial II, the Compost + 2% ZnO + ZSB treatment consistently outperformed all other  
363 treatments. Compared to the control, it increased grain nitrogen by 15.9%, phosphorus by 15.0%,  
364 potassium by 13.7%, iron by 27.9%, and zinc by 39.4%. The treatments ZnO + ZSB and  $\text{ZnSO}_4$  +  
365 ZSB also showed considerable improvements over the sole applications of ZnO or  $\text{ZnSO}_4$ ,  
366 suggesting the synergistic effect of zinc-solubilizing bacteria (ZSB) in mobilizing native and  
367 supplemented zinc sources.

368 The Compost + ZSB treatment also enhanced nutrient accumulation in grains, albeit to a lesser  
369 extent than the Compost + 2% ZnO + ZSB. Sole compost and ZSB treatments moderately  
370 increased nutrient content compared to the control, while the sole application of  $\text{ZnSO}_4$  and ZnO  
371 resulted in lower improvements than when these sources were bioactivated. Overall, the combined  
372 application of compost, ZnO, and ZSB (Compost + 2% ZnO + ZSB) was the most effective  
373 strategy for promoting the biofortification of wheat grains with essential nutrients in both trials.

### 374 **3.5. Correlation analysis demonstrates the interactive effect of improved soil fertility** 375 **parameters on yield**

376 Correlation analysis revealed significant positive relationships among soil biochemical  
377 parameters, bacterial population in term of colony forming units ( $\text{CFU g}^{-1}$  soil), microbial biomass  
378 carbon (MBC), and microbial biomass nitrogen (MBN) and key soil nutrients (N, P, K, Fe, and  
379 Zn) with crop yield (Fig. 5). Specifically, CFU exhibited a strong positive correlation with MBC  
380 ( $r = 0.82$ ,  $p < 0.01$ ) and MBN ( $r = 0.76$ ,  $p < 0.01$ ), indicating that enhanced microbial abundance  
381 supports higher microbial biomass. MBC and MBN were significantly correlated with available  
382 nitrogen ( $r = 0.79$  and  $0.74$ , respectively;  $p < 0.01$ ), suggesting active microbial involvement in  
383 nutrient mineralization and cycling. Available phosphorus and potassium were also positively  
384 correlated with microbial indicators (MBC–P:  $r = 0.68$ ; MBN–K:  $r = 0.63$ ;  $p < 0.05$ ), underscoring  
385 the role of microbial processes in improving nutrient availability. Moreover, micronutrients such

386 as Fe and Zn showed moderate but significant correlations with MBC ( $r = 0.59$  and  $0.57$ ,  
387 respectively;  $p < 0.05$ ), suggesting microbial-mediated enhancement of micronutrient solubility.  
388 Importantly, crop yield demonstrated strong positive correlations with MBC ( $r = 0.85$ ), MBN ( $r =$   
389  $0.80$ ), and CFU ( $r = 0.78$ ), as well as with macronutrients N ( $r = 0.83$ ), P ( $r = 0.77$ ), and K ( $r =$   
390  $0.75$ ). These findings collectively indicate that microbial activity and biomass are closely linked  
391 to nutrient availability and are reliable predictors of soil fertility and crop productivity.

### 392 **3.6. Comparison of bio-organic and inorganic ( $ZnSO_4$ ) fertilizers for cost of production and** 393 **net returns for one hectare of wheat**

394 In the study area, the total cost of cultivating one hectare of control (untreated),  $ZnSO_4$  (market  
395 source of zinc) and microbial-assisted zinc-enriched compost (bio-organic fertilizer) of wheat  
396 comprised several components, including land preparation, sowing, seed, fertilizer application,  
397 plant protection (weedicide and pesticide), manual labor, irrigation, and harvesting and threshing  
398 operations (Table 2). The production cost per hectare was calculated as Rs. 230,371 for control  
399 wheat, Rs. 235,353 for  $ZnSO_4$ -treated wheat, and Rs. 239,438 for the microbial-assisted zinc-  
400 enriched compost-treated wheat. The treated wheat incurred higher costs than the control  
401 (untreated) wheat, mainly due to the application of zinc fertilizers. For wheat cultivation, the total  
402 production cost also accounted for opportunity costs, calculated as a 12% markup from land  
403 preparation to fertilizer application. These amounted to Rs. 14,529 for control, Rs. 15,063 for  
404  $ZnSO_4$ , and Rs. 15,446 for the microbial-assisted zinc-enriched compost. Manual labor costs were  
405 Rs. 2965, Rs. 3,033, and Rs. 3,478, respectively. As detailed in Table 3, the total cost (TC) was  
406 broken down into Total Variable Cost (TVC) and Total Fixed Cost (TFC). The TVC included  
407 expenses related to land preparation, seed, fertilizers, labor, markup, and harvesting and threshing.  
408 The estimated TVC per hectare was Rs. 167,484 for control, Rs. 172,534 for  $ZnSO_4$ , and Rs.  
409 176,551 for the microbial-assisted zinc-enriched compost. TFC, on the other hand, included fixed  
410 expenses such as land rent and *abiana* (government water charges), which collectively amounted  
411 to Rs. 62,887 per hectare. Land rent was treated as an opportunity cost, as most farmers in the  
412 region owned their land. The total cost of production (TC) was derived by summing the TVC and  
413 TFC.

414 The gross margin, net return, and revenue-to-cost (R/C) ratio were calculated for the cultivation of  
415 one hectare of wheat treated as control,  $ZnSO_4$ , and microbial-assisted zinc-enriched compost  
416 (Table 3). Based on Equation 1, the gross margins were estimated at Rs. 111,997 for control, Rs.

417 112,304 for ZnSO<sub>4</sub> and Rs. 130,578 for the microbial-assisted zinc-enriched compost. Net returns  
418 were determined by deducting the total costs (TC) from total revenue (TR). When land rent (LR)  
419 was included, the net returns amounted to Rs. 49,110 for control, Rs. 49,417 for ZnSO<sub>4</sub>, and Rs.  
420 67,691 for the microbial-assisted zinc-enriched compost. Excluding land rent, the net returns  
421 increased to Rs. 110,885, Rs. 111,192, and Rs. 129,466, respectively. Likewise, the revenue-to-  
422 cost ratios, including land rent, were 1.21 for control and ZnSO<sub>4</sub>, and 1.28 for the microbial-  
423 assisted zin-enriched compost. Without considering land rent, these ratios improved to 1.67, 1.65,  
424 and 1.74, respectively. The similarity in revenue-to-cost ratios between the ZnSO<sub>4</sub> treatment and  
425 the control reflects the limited economic benefit of conventional zinc fertilization under calcareous  
426 arid soils, where zinc fixation reduces plant availability. This outcome underscores the necessity  
427 of biologically assisted Zn delivery systems, which demonstrated superior profitability in the  
428 present study.

#### 429 **4. Discussion**

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

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

447 Biological indicators of soil fertility, including colony-forming units (CFU), microbial biomass  
448 carbon (MBC), and microbial biomass nitrogen (MBN), were improved by the Compost + 2%  
449 ZnO + ZSB treatment. This demonstrates not only the proliferative effect of compost as a microbial  
450 substrate but also the synergistic interactions between microbial inoculants and native soil  
451 microbiota, which enhance microbial colonization and community stability (Dincă et al., 2022; Lu  
452 et al., 2025). ZSB plays a critical mechanistic role by releasing organic acids (Sethi et al., 2025),  
453 siderophores (Zhu et al., 2025), and hydrolytic enzymes that mobilize nutrients (Mujumdar et al.,  
454 2024), improve rhizosphere activity, and stimulate beneficial plant–microbe interactions (Jalal et  
455 al., 2024; Feng et al., 2024). Furthermore, the observed increases in nitrate-N and ammonium-N  
456 concentrations indicate enhanced nitrogen transformation pathways, including ammonification,  
457 nitrification, and mineralization processes, actively mediated by microbial communities (Duan et  
458 al., 2023; Chen et al., 2025). These transformations improve nitrogen turnover and ensure steady  
459 nutrient availability to crops, thereby supporting higher productivity and nutrient use efficiency.  
460 The pronounced improvement in soil biochemical properties under the integrated application of  
461 compost + ZnO + ZSB can be attributed to synergistic interactions between organic matter inputs,  
462 microbial activity, and zinc chemistry in alkaline arid soils. Compost provides a stable carbon  
463 source that stimulates microbial proliferation and enzymatic activity, thereby accelerating organic  
464 matter decomposition and nutrient mineralization (Gao et al., 2024; Meng et al., 2025). The  
465 concurrent increase in microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)  
466 observed in this study reflects enhanced microbial turnover and nutrient immobilization-  
467 mineralization dynamics, which are critical indicators of soil functional fertility (Edrisi et al.,  
468 2019).

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

478 Long-term studies have demonstrated that repeated compost and microbial inputs progressively  
479 enhance soil organic carbon sequestration, microbial functional diversity, and nutrient buffering  
480 capacity, leading to sustained yield improvements over time (Wang et al., 2022; Wang et al., 2025;  
481 Shu et al., 2022). Continuous organic inputs promote stable soil aggregates, improved water-  
482 holding capacity, and resilient microbial communities, which are particularly critical in arid  
483 agroecosystems (Liang et al., 2025). Although the present study was conducted over a single  
484 cropping season, the observed improvements in microbial biomass and soil organic matter suggest  
485 strong potential for cumulative long-term benefits, as reported in extended compost-based  
486 fertilization trials.

487 Plant growth, measured in terms of plant height and shoot dry biomass, was significantly improved  
488 by the Compost + 2% ZnO + ZSB treatment. These improvements are attributable to enhanced  
489 nutrient availability, particularly zinc and nitrogen, resulting from the synergistic effect of the  
490 organic matrix, micronutrient enrichment, and microbial solubilization. Zinc plays a critical role  
491 in auxin synthesis and enzyme activation (Wang et al., 2023b), and its increased availability in a  
492 bioavailable form likely stimulated vegetative growth. Moreover, the compost component  
493 improved soil physical structure and provided a steady nutrient supply (Kelbesa, 2021), while ZSB  
494 enhanced micronutrient solubility through the production of organic acids and siderophores  
495 (Asghar et al., 2024).

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

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

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

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

523 Additionally, compost-mediated improvements in soil structure and water-holding capacity likely  
524 alleviated abiotic stress typical of arid soils, indirectly supporting plant growth and nutrient uptake  
525 (Rehman et al., 2023; Wang et al., 2023a). The observed enhancement in 1000-grain weight and  
526 grain yield under ZSB-based treatments aligns with earlier studies demonstrating that microbial  
527 inoculants improve root architecture, nutrient acquisition efficiency, and assimilate partitioning  
528 during grain filling (Galindo-Castañeda et al., 2022).

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

536 The economic analysis of wheat cultivation under different zinc fertilization treatments  
537 demonstrated that although input costs were higher for treated plots, especially for microbial-  
538 assisted zinc-enriched compost, the overall profitability was enhanced, particularly under bio-  
539 organic fertilization. This aligns with research, which reported that the addition of external nutrient

540 sources in wheat production generally increases variable costs, especially when micronutrient  
541 fortification is involved (Ali and Tsou, 1997). Despite the increased costs, the economic benefits  
542 in terms of gross margins and net returns were significantly higher for the microbial-assisted zinc-  
543 compost treatment. This improvement may be attributed to enhanced nutrient use efficiency and  
544 improved plant growth parameters due to the synergistic effects of compost and beneficial  
545 microbes. It is evident from the literature that microbial inoculants can promote nutrient  
546 solubilization and uptake, translating into higher yields (Sammauria et al., 2020) and better  
547 financial returns. The higher net income in the microbial-assisted zinc-enriched compost treatment  
548 relative to the control suggests that bio-organic-assisted biofortification not only improves soil  
549 health and crop yield but also enhances farm-level economic resilience. This strategy offers a  
550 sustainable solution for nutrient-deficient soils, supporting the transition towards low-input, high-  
551 efficiency farming systems (Akbar et al., 2020).

552 Although initial input costs were higher with microbial-assisted zinc-enriched compost, the  
553 significant gains in yield and net return justify its adoption. The data strongly support integrating  
554 microbial-assisted composts into conventional fertilization regimes to improve agronomic  
555 performance and economic returns under resource-constrained conditions. The present study was  
556 conducted under specific soil and climatic conditions and over limited cropping seasons, which  
557 may restrict the generalization of results across diverse agroecological zones. Long-term effects  
558 on soil microbial diversity, nutrient cycling, and micronutrient buildup were not assessed, and the  
559 findings were restricted to wheat without testing in other cropping systems. Future research should  
560 therefore focus on multi-location and multi-season trials, long-term soil health monitoring, and  
561 extending the approach to different crops. Additionally, developing cost-effective microbial  
562 inoculant formulations and delivery mechanisms will be crucial to enhance adoption, particularly  
563 by resource-constrained farmers.

## 564 **5. Conclusion**

565 The integrated application of microbial-assisted zinc-enriched compost (Compost + 2% ZnO +  
566 ZSB) enhances wheat growth, yield, grain nutrient biofortification, and soil fertility compared to  
567 sole amendments. This combined treatment consistently outperformed others across both trials,  
568 markedly increasing plant height, biomass, grain weight, and yield, while significantly elevating  
569 macronutrient (N, P, K) and micronutrient (Fe, Zn) concentrations in grains. Soil chemical and  
570 biological parameters also showed pronounced improvements, with enhanced organic matter,

571 microbial biomass, and nutrient availability, underscoring the synergistic effects of organic,  
572 microbial, and zinc amendments. These findings highlight the efficacy of integrated nutrient  
573 management strategies involving microbial-assisted zinc-enriched compost for sustainable wheat  
574 production and soil health restoration, offering promising avenues for addressing micronutrient  
575 deficiencies in cereal crops.

#### 576 **Data Availability Statement**

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

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#### 590 **Competing interest**

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

592

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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

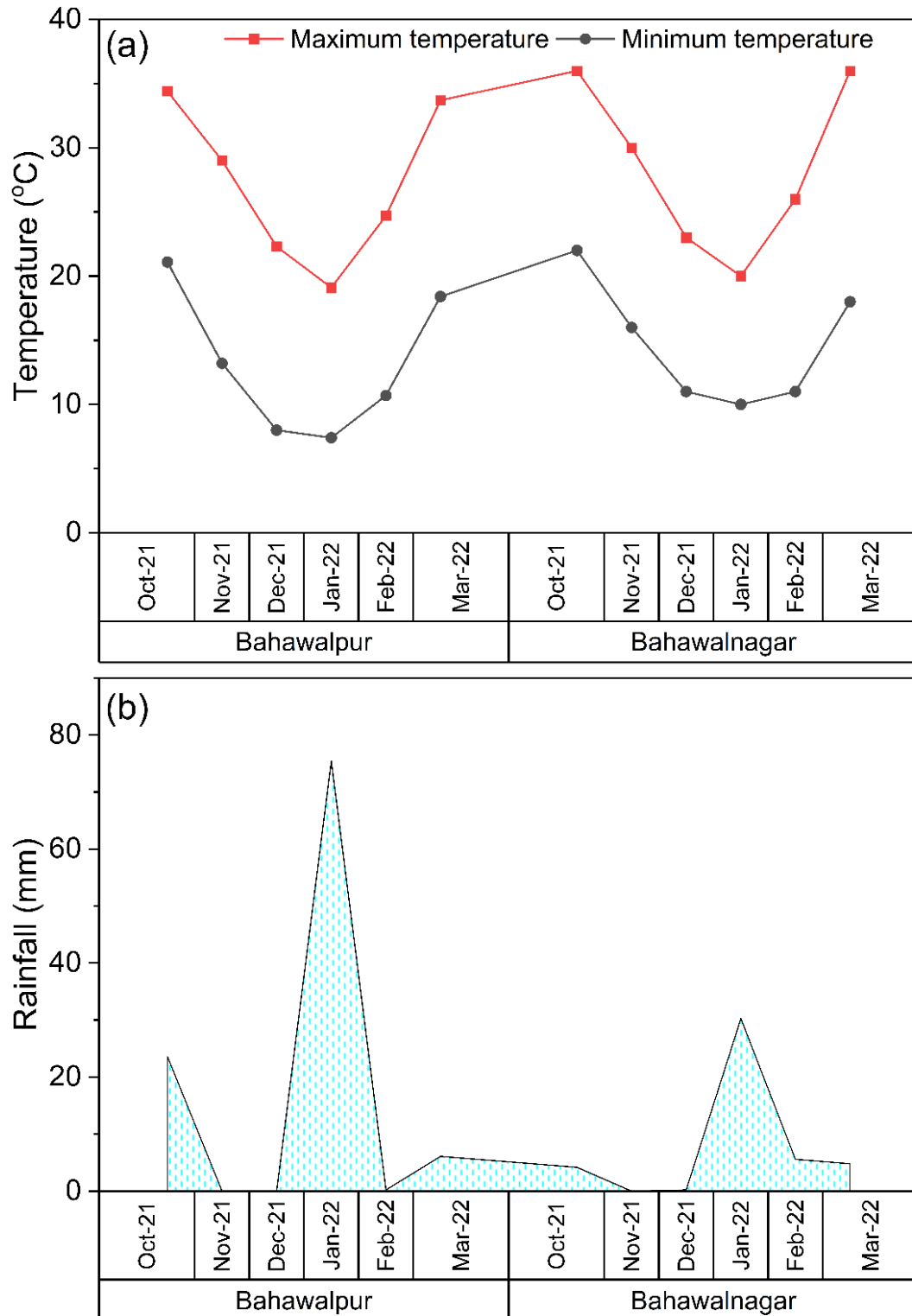
Activity	Costs of production per hectare (Rs.)		
	<b>Control (farmer's conventional practice)</b>	<b>Market Source of Zinc (ZnSO<sub>4</sub>)</b>	<b>Microbial-assisted zinc-enriched compost</b>
<b>Land preparation</b>	17,297	17,297	17,297
<b>Seed</b>	14,826	14,826	14,826
<b>Fertilizers</b>	88,956	88,956	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

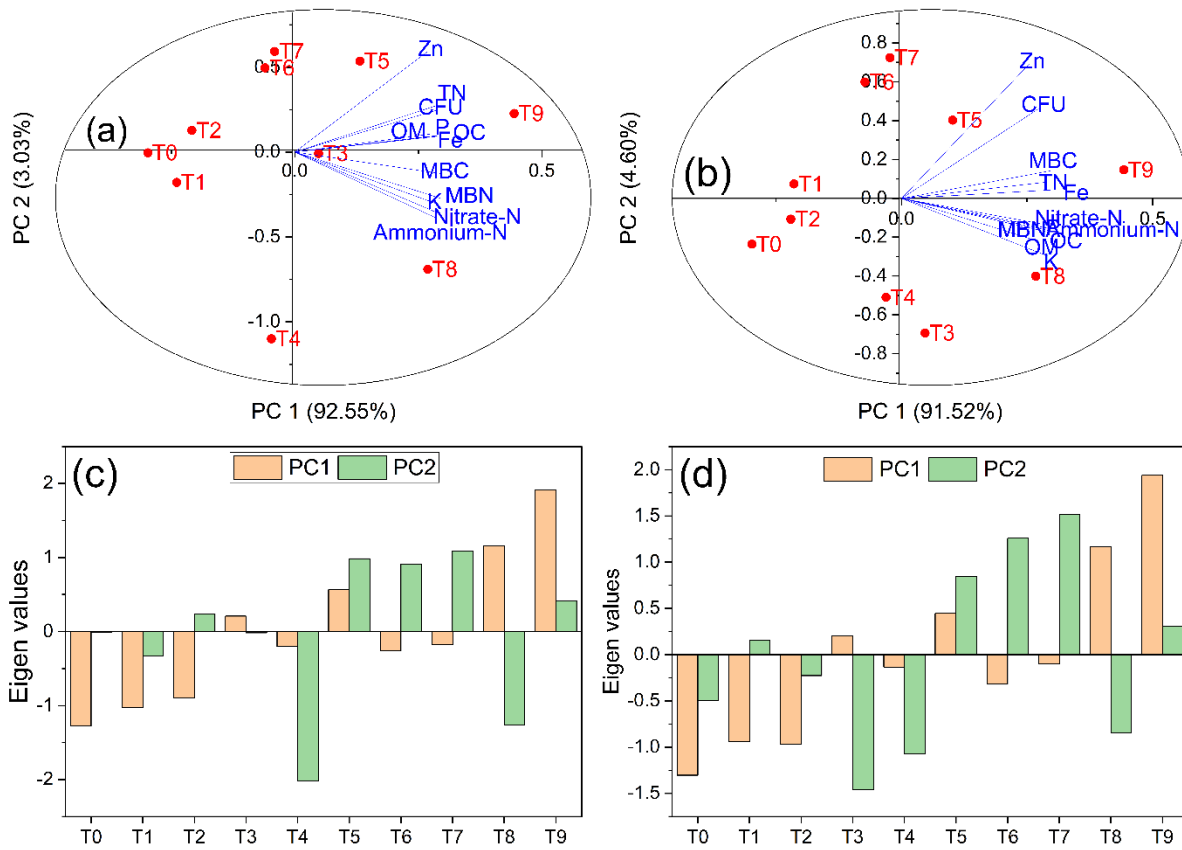
**Table 3:** Gross margin, net income and revenue to cost (R/C) ratio

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

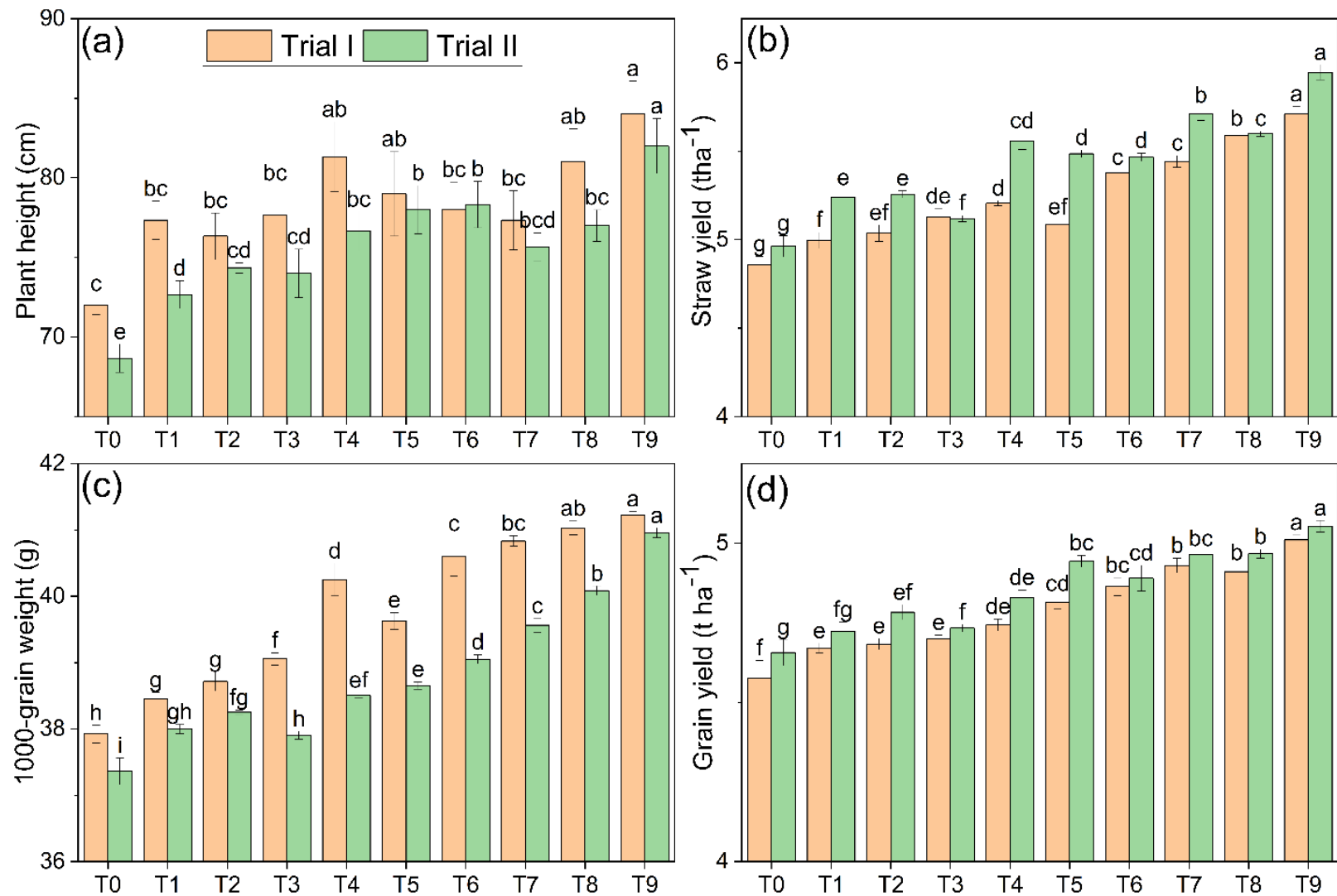
Yield is presented as average of both trials conducted in Bahawalpur and Bahawalnagar. \*: significant increase in net income by the application of microbial-assisted zin-enriched compost



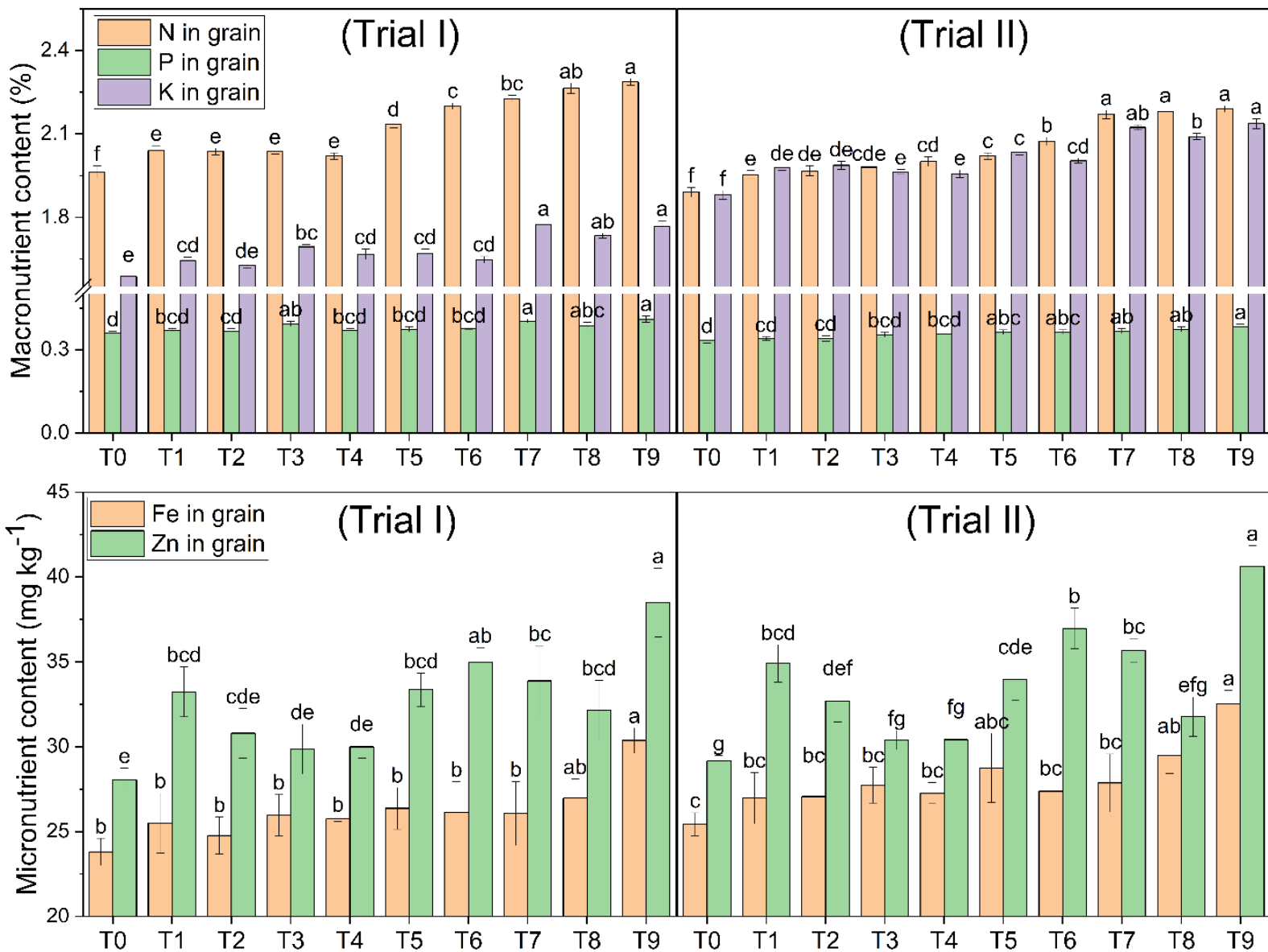
**Figure 1.** Climate data from experimental sites during the 2021-22 cropping season. Data is presented as the monthly average temperature (a) and total rainfall (mm) in a month (b)



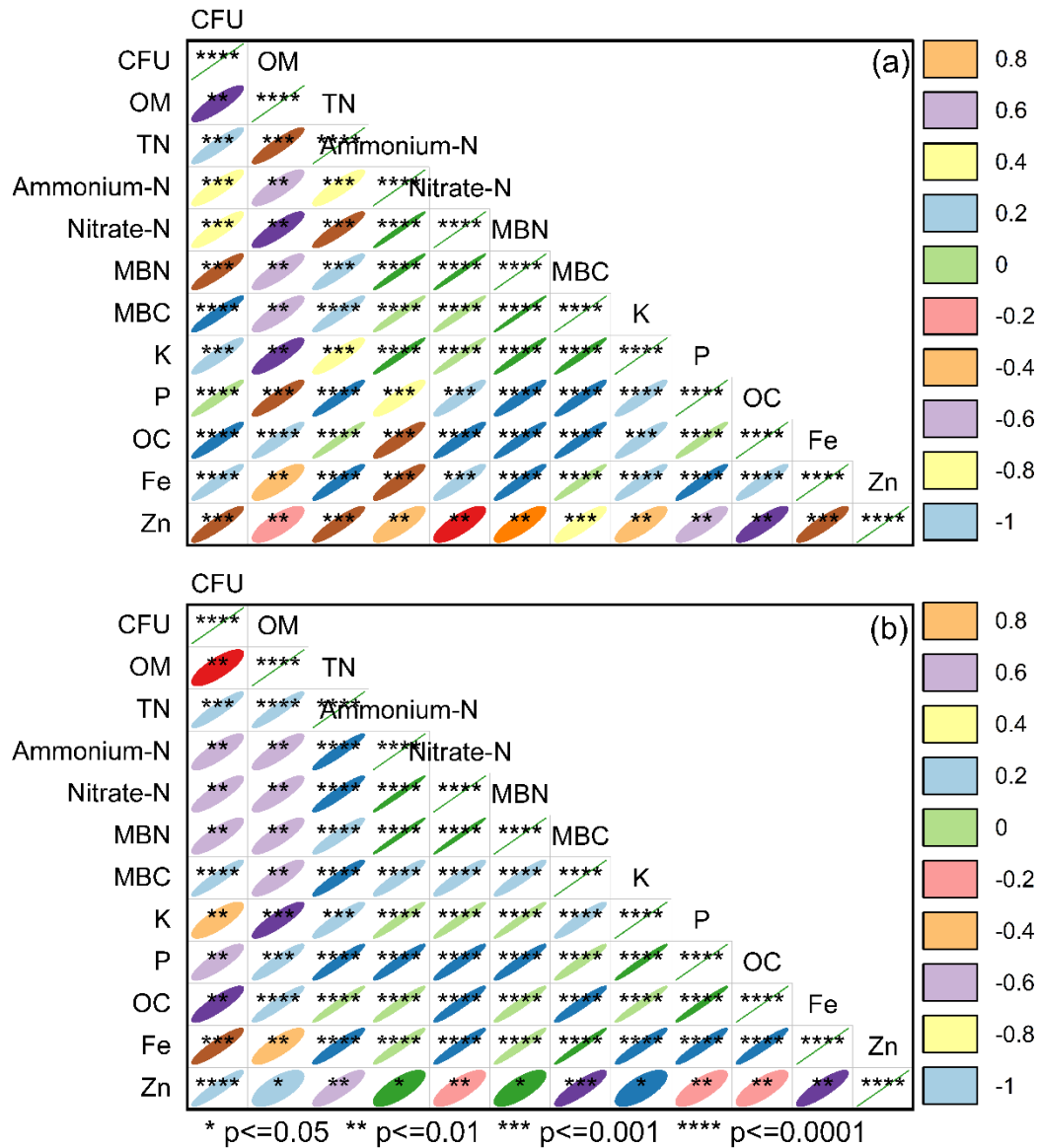
**Figure 2.** Principal component analysis (PCA) demonstrates the effectiveness of microbial-assisted zinc-enriched compost to improve soil biochemical properties. (a): PCA - trial I (Bahawalpur); (b): PCA - trial II (Bahawalnagar); (c): eigenvalues – trial I; (d): eigenvalues – trial II; Zn: DTPA-extracted 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)