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ARTICLE

The Impact of Bio-Inoculum and Organic Matter on the Sustainability of Micronutrient Levels in Silty Clay Loam Soil Wheat Cultivation

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ABSTRACT

A field experiment was conducted on silty clay loam soil cultivated with wheat to evaluate the effects of bio-inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae* and poultry manure on soil micronutrient concentrations. Treatments included fungal inoculum alone, poultry manure alone, their combination, and a control, arranged in a randomized complete block design with three replications. The combined application of fungal inoculum and poultry manure significantly increased total concentrations of iron, manganese, zinc, molybdenum, copper, and boron in bulk soil to 5890.4, 390.5, 36.5, 1.6, 23.1, and 2.0 mg kg^{-1}, representing increases of 13%, 20.3%, 32.2%, 84%, 23.5%, and 175% over the control. In the rhizosphere, these elements reached 5650.8, 380.7, 33.2, 3.8, 21.4, and 3.5 mg kg^{-1}, with increases of 9.5%, 26.4%, 29.6%, 92%, 38.9%, and 90.1%. Available concentrations in bulk soil rose to 15.3, 10.6, 1.4, 0.4, 0.47, and 1.2 mg kg^{-1}, corresponding to increases of 51.6%, 83%, 42%, 92.5%, 65.9%, and 36.1%, while in the rhizosphere, available values reached 16.2, 11.7, 1.9, 0.7, 0.7, and 1.6 mg kg^{-1}, reflecting increases of 55%, 30%, 35.4%, 90%, 128%, and 93.7%, respectively. These enhancements are attributed to the synergistic effects of microbial activity, organic matter decomposition, and organic acid release, which improved nutrient solubility and accessibility in the root zone. The findings indicate that integrating *Glomus mosseae* inoculation with poultry manure is an effective and sustainable strategy to improve

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soil fertility, increase micronutrient availability, and enhance plant nutrient uptake, providing a practical recommendation for boosting wheat productivity while reducing reliance on chemical fertilizers.

Keywords: *Glomus mosseae* L.; Sustainability; Micronutrients; Organic Matter; Rhizosphere

1. Introduction

The detrimental impact of excessive chemical additions in agricultural systems threatens the sustainability of crop production and the preservation of environmental quality. Therefore, biofertilizers and organic fertilizers are a natural, cost-effective, and ecologically sustainable solution to this issue [1]. Dependence on chemical fertilizers is an imprudent approach for long-term use owing to the elevated production costs, environmental problems arising from factory establishment, and the direct effects on soil and water quality. Hence, biofertilizers and organic fertilizers have been employed as an appropriate substitute for chemical fertilizers [2]. The interplay between organic and biofertilizers is a crucial method that enhances the soil's chemical, physical, and biological characteristics, hence optimizing crop output and quality [3]. Ullah et al. [4] indicated that biofertilization and organic matter can be a valuable strategy for mitigating the adverse effects of drought. It enhances agricultural yield in arid regions during drought by facilitating the interaction between biofertilizers and plants, therefore mitigating and enduring the impacts of drought at both genetic and chemical levels. One adaptation plants employ to cope with drought circumstances caused by diminishing water sources is the development of a robust, extensive root system that facilitates the absorption of water from deep into the soil [5]. The utilization of bio-inoculates promotes root development and the emergence of lateral roots, thereby enhancing the plant's capacity for water absorption [6]. It also enhances plant metabolism to adapt to both stressful and normal situations, facilitating the production of suitable solutes such as proline and calcine, which aid in osmotic control activities. Liu et al. [7] discovered that bio-inoculates alleviated the detrimental impacts of drought and markedly enhanced shoot dry matter, relative water content, leaf water potential, and organic acids by 19.23%, 7.18%, 11.46%, and 33.12%, respectively, in comparison to uninoculated plants. The incorporation of organic matter and bio-inoculates adjusts the soil pH towards neutrality, around 6.7, hence enhancing nutrient availability [8].

Arbuscular mycorrhizal fungi play a significant role in increasing the availability of micronutrients in soil, especially when added in conjunction with poultry manure, which in turn contributes to improving the physical and chemical properties of the soil. This enhances the plant's ability to absorb nutrients from the soil. The fungal outer network provides an excellent mechanism for absorbing nutrients through areas of the soil that are not accessible to plant roots, positively impacting the amount of nutrients absorbed by the plant [9]. *Glomus mosseae* secretes organic acids, phenols, siderophores, and compounds that dissolve insoluble minerals and convert them into soluble forms of micronutrient ions in relatively high pH and low organic matter soils [10]. They also regulate the genes responsible for nutrient transporters within plant roots, enhancing their effective entry into the plant [11]. Many studies have demonstrated the clear effects of *Glomus mosseae* on improving soil hydrological properties and agglomeration, with effects varying depending on the type of fungal isolate and moisture content. Fungi play a clear role in binding soil particles through their mycelium and modifying the agglomeration's absorbency [12]. An improvement in water potential has also been observed, indicating a significant potential for employing *Glomus mosseae* to enhance soil structure under drought conditions, providing a new perspective for soil management under climate change [13]. Integrating environmental and agricultural objectives achieved by soil inoculation with beneficial microorganisms is a tool for enhancing plant productivity and the sustainability of agricultural systems, which rely on soil's natural resources. However, the process of establishing living, vital communities is a challenge for several seasons, and is one of the main goals despite its difficulty. AMFs are soil fungi that have the ability to increase the productivity of a wide range of agricultural crops [14]. The interaction between *Glomus mosseae* and organic matter is an effective and important strategy for enhancing plant growth and productivity in soils poor in organic matter and nutrients, with alkaline pH and high CEC, such as those found in the central and southern regions of Iraq, these additives significantly alter the soil environment by releasing organic acids, which lower soil pH and increase the activity of fungi that prefer acidic environments. This may lead to the establishment of more stable and diverse fungal communities over time, supporting and sustaining ecosystems[15]. *Glomus mosseae* L. is a fungal species (FAM) that engages in a symbiotic association with plant roots. It enhances nutrient accessibility and optimizes plant absorption. These fungal inoculants exude glomalin, a glycoprotein that, when released onto soil particles, aids in the formation and stabilization of soil aggregates, hence enhancing soil structure. This improves the soil's capacity to retain water, particularly under water-stressed circumstances. This protein, including 30–40% carbon molecules, aids in safeguarding the soil against drought[16]. The decomposition of organic waste releases dissolved organic carbon and disintegrates negatively charged organic groups, which prefer to combine with various micronutrients, thus enhancing their long-term availability. It further reduces soil pH and enhances plant resilience to water stress[17,18]. Poultry manure is a significant and efficacious organic fertilizer. It mitigates the detrimental effects of irrigation water salinity and enhances plant resilience to stress. It promotes water holding capacity and aeration, improves soil pore distribution, stimulates root secretions such as organic acids, and lowers the adverse effects of salts in the soil solution. It enhances aeration and oxygen diffusion for soil organisms, hence augmenting biological activity and nutrient accessibility[18]. Poultry dung has several nutrients, such as iron, manganese, copper, and zinc, along with trace quantities of boron and molybdenum[19]. Rich in minerals and physiologically active substances, poultry manure is regarded as one of the most significant sources of organic matter. Its use in combination with the inoculation of soil with arbuscular mycorrhizal fungi (AMF) has been shown in numerous studies to considerably improve inoculation efficiency and increase fungal biomass in the soil. Because poultry manure is a rich source of organic carbon, it creates an environment that is conducive to the growth and multiplication of fungi in the rhizosphere, which increases the generation of spores and the development of symbiotic structures like vesicles and arbuscular[20].

Additionally, through nutrient exchange processes made possible by the fungus, poultry manure increases the availability of vital nutrients, especially phosphorus and micronutrients (including zinc, manganese, and iron), which promote their uptake by plants. By boosting the cation exchange capacity (CEC), encouraging soil aggregation, and raising the organic matter content, it also helps to improve the physical structure of the soil, resulting in a more stable and sustainable environment for the growth of fungal communities[21].

Furthermore, by activating soil microorganisms like mycorrhiza-associated bacteria with humic and fulvic acids and other bioactive substances, poultry manure promotes advantageous interactions between these microbes. Therefore, in sustainable agricultural systems, combining poultry manure with AMF inoculants is a useful tactic to improve crop yield and soil stability[22]. Research has demonstrated that the supplementation of zinc, boron, manganese, iron, copper, and molybdenum enhances wheat grain production and drought stress tolerance, despite these elements not being growth-limiting under adequate water circumstances[23,24]. Arbuscular mycorrhizal fungi (AMF) are a significant class of soil microorganisms that create mutually advantageous symbiotic relationships with the surrounding soil microenvironment and the roots of a variety of plants. Additionally, mycorrhizas contribute to biological processes that increase plant health by protecting plants from biotic and abiotic stresses improving soil structure and quality, and increasing the nutraceutical value of horticultural products[25].

The primary benefit of employing AMF as biostimulants is associated with their function in promoting the uptake of micronutrients and phosphate by plants AMF cannot be mass-produced without plants, even though nearly all plant-beneficial microbes, including certain ectomycorrhizal and mycorrhiza-like ones, may be created by cultivation in fermentation systems Similar to other microbial fertilizers, mycorrhiza mass production requires the invention and selection of biotechniques. The finished product ought to be pathogen-free, viable after storage, have a high colonization potential, and be simple to use[26].

The absence of effective, free-of-host production techniques, as well as formulations that cannot guarantee high-quality end products, sterilized substrates are often used in large-scale pot plant-based cultures to produce AMF. However, in industrial settings, this approach is not financially viable. Another method of producing AMF is soilless hydroponic culture, which yields a greater spore count and a higher-quality inoculum than greenhouse pot cultivation. Because they have stable properties, allow for aeration, and retain enough water for plant growth, inert materials including bark, calcinated clay, expanded clay, and perlite are employed either alone or in combination in the medium composition in both production methods We are using additional biotechnological methods to enhance AMF mass production under soilless culture circumstances [27].

Utilizing root organ culture, aeroponics, and the nutrient film approach, it is worth noting that these methods provide spores at nearly the same cost, with the added benefit of root organ cultivation. It is feasible to continuously collect spores and hyphae from the system by removing and replacing the media in the mycorrhizal side of the plate using the split-plate technique developed by St-Arnaud et al. Alginate and k-carrageenan were used as media ingredients in the cultivation of AMF in 2000–2001 (Vassilev et al., unpublished data). Other natural polysaccharides could be treated similarly. However, because the substrate is costly and the procedure is laborious and time-consuming, it is not commercially viable and is unlikely to be embraced by the industry [28].

To create a low-cost, effective, and clean technology for the production of AMF, industrial microbiologists, plant physiologists, agronomists, and soil scientists must work closely together, as they do in many other biotechnologically based productions of agricultural, plant beneficial products. The formulation phase of mycorrhizal inoculum synthesis, which may involve mycorrhiza-helper bacteria and microorganisms with additional roles like nitrogen-fixing, P-solubilizing, or biocontrol capabilities, may see further advancements in this area. Previous publications of similar approaches have shown encouraging outcomes [29]. One species of arbuscular mycorrhizal fungus (AMF) that is a member of the phylum Glomeromycota is *Glomus mosseae*. It is among the most extensively researched and often found AMF in both natural and agricultural soils.

Important Attributes: *Glomus mosseae* and the roots of the majority of terrestrial plants have a mutualistic, or advantageous, interaction. In the Symbiotic Relationship: After colonizing plant roots, the fungus produces structures like vesicles (for storage) and arbuscules (where nutrition exchange takes place).

The fungus receives its carbohydrates (sugars) from the plant. The fungus enhances the plant's absorption of micronutrients like copper (Cu) and zinc (Zn) as well as minerals that are not easily transportable, like phosphorus (P) [30].

2. Materials and Methods

The experimental site is located in the arid to semi-arid region of southern Iraq. The experiment commenced at the beginning of November and continued until maturity in April, using a sprinkler irrigation system.

Soil analyses were conducted before planting to ascertain certain of the soil's chemical and physical properties. The levels of nutritional components in the field soil and poultry manure were assessed, with the findings presented in **Tables 1** and **2**.

Table 1. Physical and chemical properties of the experimental soil.

Parameters	Value	Unit
particle size distribution		
Sand	59.2	g kg^{-1} soil
Silt	550.5	g kg^{-1} soil
Clay	390.3	g kg^{-1} soil
Soil Texture	Silt Clay Loam	
Electrical Conductivity (EC)	3.2	dS m^{-1}
pH Value	7.1	-
Organic Matter (OM)	7.1	g kg^{-1}
Organic Carbon	13.4	g kg^{-1}
Available Nitrogen	16.0	mg kg^{-1}
Available Phosphorus	7.1	mg kg^{-1}
Available Potassium	113.6	mg kg^{-1}
Fe	5200.3	mg kg^{-1}
Mn	324.6	mg kg^{-1}
Mo	0.25	mg kg^{-1}
B	0.5	mg kg^{-1}

Table 2. Some chemical properties of the organic material used (poultry manure).

Parameters	Value	Unit
Organic Carbon	253.2	g kg^{-1}
Total Nitrogen	28.54	g kg^{-1}
Total Phosphorus	16.6	g kg^{-1}
Total Potassium	18.9	g kg^{-1}
C/N Ratio	8.87	-
pH	7.31	-
EC	4.1	dS m^{-1}
Total Iron (Fe)	4.5	g kg^{-1}
Total Manganese (Mn)	0.5	g kg^{-1}
Total Copper (Cu)	0.38	g kg^{-1}
Total Zinc (Zn)	0.4	g kg^{-1}
Total Boron (B)	0.05	g kg^{-1}
Molybdenum (Mo)	Trace (1–5)	mg kg^{-1}

2.1. Experimental Design

A factorial experiment was performed with a Randomized Complete Block Design (RCBD). The field was divided into three blocks. The experiment was conducted with three replications. There were 18 experimental units, with 6 units in each block, dispersed randomly. The experimental unit has an area of 5 m², with dimensions of 2 m by 2.5 m. In order to examine the impact of the applied treatments on the total accessible concentrations of micronutrients in both the rhizosphere and non-rhizosphere zones, soil samples were only taken at the maturity stage. The LSD test for significant differences was used with the GenStat software.

2.2. Chemical and Physical Analysis

- Soil pH: It was measured in the saturated paste extract of the soil using a pH meter according to the method described in Gao et al. [31].
- Electrical Conductivity (ECe): Electrical conductivity was assessed in the saturated paste extract utilizing an EC meter, following the procedure outlined in Gao et al. [31].
- Organic Matter (OM): The percentage of organic carbon was estimated by wet digestion using potassium dichromate, according to Richards [32], as shown in the following Equation (1):

$$\text{Organic Matter \%} = \text{Organic Carbon \%} \times 1.724 \quad (1)$$

- Bulk Density (g/cm³): The estimation was conducted using the Core Sampler approach, as referenced in Food and Agriculture Organization (FAO) [33].
- Soil Total Nitrogen: Total nitrogen was quantified using the KCl extraction technique, whereas nitrogen content was determined with the Kjeldahl equipment [34].
- Soil Total Phosphorus: Soil phosphorus was extracted using sodium bicarbonate (NaHCO), color-developed using ammonium molybdate and ascorbic acid, then quantified by spectrophotometry, following the Olsen and Sommers technique [33].
- Total Potassium in Soil: Total potassium in the soil was extracted using ammonium acetate (NH4OAC) and estimated using a flame photometer according to Gao et al. [31].
- Soil Texture: Estimated using a hydrometer.
- Determination of iron, molybdenum, and manganese: The estimation is carried out in the soil extract using DTPA extraction solution, and the readings are obtained using an Atomic Absorption Spectrophotometer (AAS).
- Determination of boron: Boron is determined using a spectrophotometer through its reaction with the azomethine-H reagent in a slightly acidic medium to form a yellow-colored complex, which is measured at a wavelength of 240 nm. The extraction is performed using hot water.
- Fungal inoculum: The fungal inoculum of *Glomus mosseae*, an Arbuscular Mycorrhizal Fungi was used. The fungal inoculum was mixed with organic fertilizers before adding it to the soil.

3. Results and Discussion

3.1. Total of Iron, Manganese, Boron, and Molybdenum Concentrations in the Bulk Soil

Figure 1 illustrates the impact of incorporating poultry manure, fungal inoculum, and their interaction on the concentration of trace elements in the bulk soil in comparison to the control treatment. The application of poultry manure to the soil led to increases in the concentrations of B, Mn, Mo, and Fe by 50%, 79%, 10%, and 9%, respectively, in comparison to the control treatment. This increase was particularly pronounced in molybdenum and boron, due to a decrease in pH coupled with an increase in the percentage of organic matter, which binds molybdenum to the complexes, as found in Keeney and Nelson [35]. This rise may be ascribed to the elevated nutritional value of poultry manure, whether these components are incorporated into poultry feed as dietary supplements or are constituents of some pharmaceuticals utilized to address ailments in poultry.

Fe, Mn, Mo, and B concentrations increased by 68.7%, 62.5%, 4.8%, and 4.4%, respectively, when the single fungal inoculum treatment was applied as opposed to the control treatment. The findings show the importance of fungal inoculum in the preparation of nutrients, specifically boron and molybdenum, which improve soil-microorganism interaction and facilitate nutrient absorption by releasing these elements from their compounds in the soil. This aligns with what was mentioned by Marks et al. [36].

The combination of fungal inoculum and poultry manure produced the most significant enhancement in soil micronutrient levels. The percentage increases relative to the control were 175%, 84%, 20.3%, and 13%, respectively. This may result from the fungal inoculum enhancing organic matter breakdown through the secretion of particular enzymes and the release of organic acids that reduce soil pH and solubilize nutrient-containing molecules, thus improving the soil's chemical characteristics. Humic compounds form complexes with micronutrients, preventing their fixation and maintaining their availability to plants. This is considered the optimal option for achieving optimal micronutrient sustainability in the soil and replenishing its natural resources.

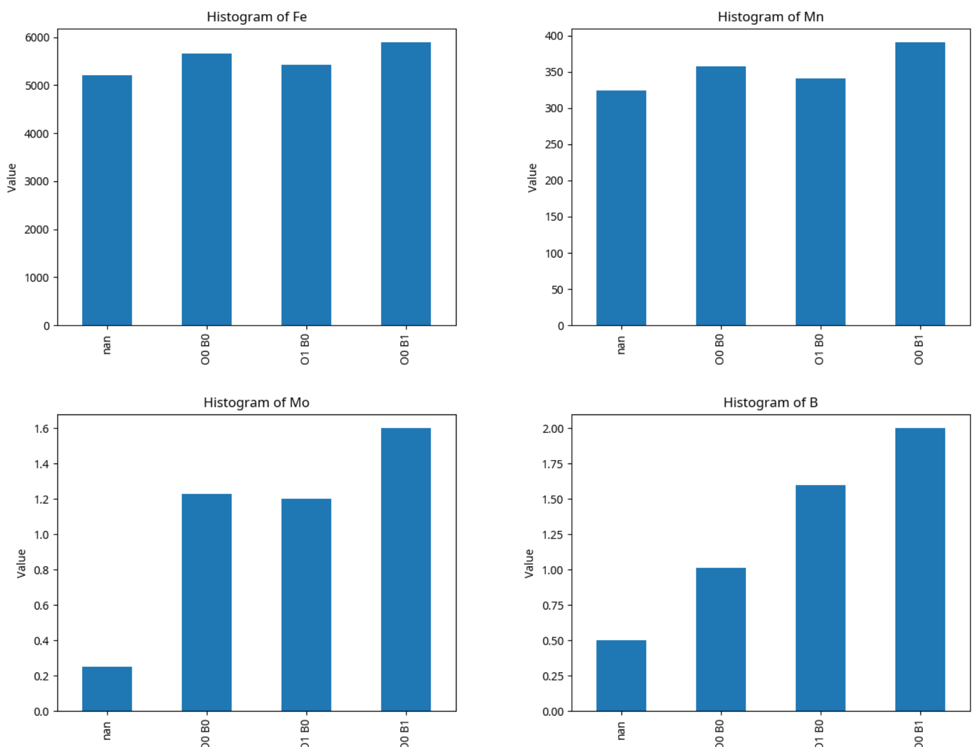

Figure 1. Impact of mycorrhizal inoculation and organic matter on the sustainability of total micronutrient levels in bulk soil.
Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.2. Total of Iron, Manganese, Boron, and Molybdenum Concentrations in the Rhizosphere

Figure 2 illustrates the impact of poultry manure and fungal inoculum, both individually and in combination, on the concentrations of Fe, Mn, Mo, and B in the root zone soil 60 days post-treatment application. Iron concentrations increased under the influence of poultry manure and fungal inoculum alone, and the interaction between them, by percentages of 4.8%, 2.1%, and 9.5%, respectively, compared to the control treatment. The enhancement in the treatment of poultry manure and fungal inoculum individually was minimal in comparison to the interaction treatment. This phenomenon may be ascribed to the interaction between fungal inoculum and poultry manure, which enhances the availability and total concentration of iron in the soil. This occurs due to microbial activity that releases organic acids, facilitating the dissolution of iron bound to the soil, in addition to the iron present in poultry manure, which contains an iron concentration of 4500 mg kg^{-1} as demonstrated by Rana et al.[37].

Manganese concentrations were markedly elevated due to poultry manure and fungal inoculum treatments, with the most substantial rise observed when both were combined. The growth rates were 20% for poultry manure, 15.2% for fungal inoculum, and 26.4% for the combination thereof. This may be ascribed to the influence of manganese on microbial activity, as demonstrated by Molnár et al.[38]. This results from the augmented absorption surface of fungal hyphae and the excretion of organic acids and enzymes that degrade complex substances.

All treatments resulted in substantial increases in soil molybdenum content, with the most pronounced increases observed in the combination of fungal inoculum and poultry manure, at 86%, 76%, and 92%, respectively. Molybdenum is a scarce element in soil, and its concentration markedly rises when soil is amended with poultry manure and fungal inoculum. This enhances fungal activity when organic matter is provided, resulting in the release of elements from their compounds.

Boron concentrations in the soil rose by 75%, 66.6%, and 90.1%, respectively. This may be ascribed to the robust correlation between it and organic matter, since its concentration escalates with its incorporation into the soil. The fungal inoculum increases its accessibility and absorption by the plant, while simultaneously enhancing boron availability through the interaction of organic matter with fungal inoculum, so mitigating the risk of soil toxicity[39].

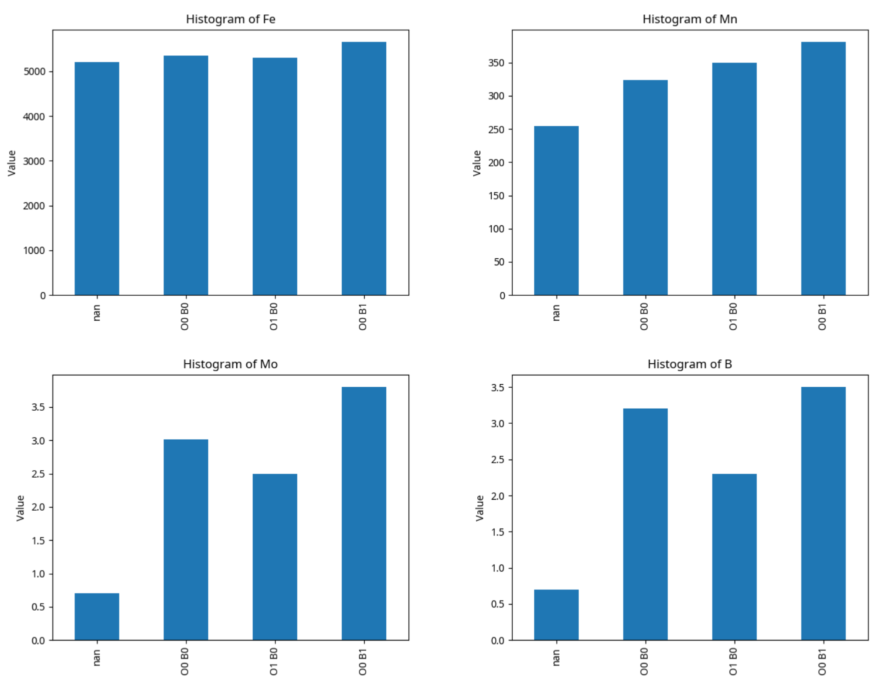

Figure 2. Impact of mycorrhizal inoculation and organic matter on the sustainability of micronutrient levels in rhizosphere soil.

Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.3. Availability of Iron, Manganese, Boron, and Molybdenum Concentrations in Bulk Soil

Figure 3 illustrates the distinct impacts of organic matter addition treatments, fungal inoculum treatments, and their interaction on the availability of Fe, B, Mo, and Mn in the root zone. The presence of these components rose by 36.8%, 86%, 71.4%, and 39.3%, respectively, due to the application of the single poultry manure treatment in comparison to the control treatment. Chicken dung is a key source of nutrients due to its varied quantities, derived from chicken diets as nutritional supplements or the chemical makeup of some therapeutic vaccinations administered to poultry. When added to soil, their concentrations increase, thereby increasing their availability to plants. This is due to its high ionic exchange capacity and its conversion to organic acids such as humic and fulvic acids, which contribute to lowering soil pH and increasing the solubility of mineral elements in the soil solution. This makes them available to plants by forming complexes with organic matter and protecting them from loss. However, we note a decrease in the availability of these elements when adding the fungal inoculum alone: 13.8%, 72%, 50%, and 22.1%, respectively, compared to the control treatment.

Despite the low organic content, fungus inoculation increased the availability of these nutrients. This is because the fungus secretes organic acids and enzymes that help the mineral elements dissolve in the soil. Fungal hyphae extend to areas outside of the roots' reach and increase the absorption surface around the roots.. This improves the uptake of sluggish elements like iron and manganese, and positively influences boron and molybdenum, particularly in soils deficient in these elements. This aligns with the findings published by Vera et al.[40].

The interaction between the fungal inoculum and poultry manure led to the most significant percentage increases in the availability of Fe, B, Mo, and Mn in the rhizosphere soil: 36.1%, 92.5%, 83%, and 51.6%, respectively, in comparison to the control treatment. This may result from the synergistic interaction between the two elements. Organic matter boosts the microbial ecology in the soil, hence augmenting the activity of helpful fungi in solubilizing mineral elements and increasing their availability to plants. The combination of fungal inoculum and poultry manure enhances plant micronutrient absorption by improving soil structure, augmenting microbial activity, and increasing the availability of organic acids and nutrient-dissolving enzymes[41].

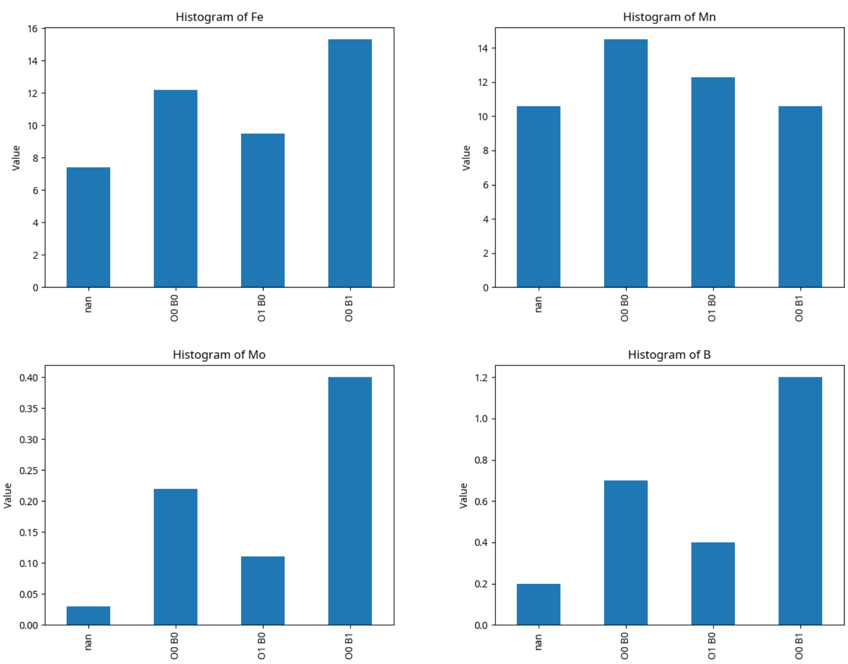

Figure 3. Effect of mycorrhizal inoculation and poultry manure on micronutrient availability in bulk soil.

Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.4. Availability of Iron, Manganese, Boron, and Molybdenum Concentrations in the Rhizosphere

The application of poultry manure alone elevated the concentration of accessible iron in the root zone by 38.85% relative to the control treatment, as shown in **Figure 4**. This may be attributed to the decomposition of organic matter in the soil, resulting in two outcomes: firstly, the generation of organic acids, including humic and fulvic acids. These organic acids can form complexes with trace metals such as iron, zinc, and copper, enhancing their availability and safeguarding them from depletion. Secondly, they decrease the soil pH, which solubilizes iron-containing compounds and releases them into the soil solution [17].

The application of fungal inoculum alone elevated the concentration of accessible iron by 14.8%, which is less than the rise observed with the organic matter treatment. This may result from the soil's low iron and organic matter concentration, as well as the absorption of available iron by microbes, which compete with plants for it. The combination of poultry manure and fungal inoculum resulted in a 55% increase in iron availability in the root soil. This results from the synergistic interaction of the two elements. The nutrient content of poultry manure and its C/N value of 8.8% effectively increased the activity of the fungal inoculum and the accompanying release of elements. Furthermore, the activity of enzymes and organic acids, which adsorb micronutrients to their surfaces due to their high specific surface area, increased the levels of available iron [42].

Treating the soil with poultry manure alone resulted in a 24% increase in the concentration of available manganese in the root soil compared to the control treatment. This is due to the nutrients added by poultry manure to the soil, the role of organic matter in adsorbing micronutrients by forming complexes with them, and the increased acidity of the soil, which facilitates the release of elements from their compounds. The use of fungal inoculum resulted in lower manganese concentrations compared to the treatment with organic matter alone, a 6.8% decrease. This may be due to the soil's poor nutritional status, which reduced fungal inoculum activity due to inadequate nutrient supply [43]. The combination of fungal inoculum and poultry manure produced the highest increase, at 30%. This reflects the synergistic effect of organic matter and microorganisms in increasing manganese availability, as well as other nutrient elements. This positively impacts plant growth and the sustainability of soil and natural resources.

In every treatment, molybdenum concentrations rose noticeably. The concentration of molybdenum increased by 86% just by using organic matter. The concentration of molybdenum increased by 80% when the fungal inoculum was used alone. The soil that was treated with both poultry manure and fungal inoculum (90%) showed the greatest growth. This is because soil acidity has decreased and microbiological conditions have improved [44].

Boron availability increased under the influence of treating the soil with poultry manure and mycorrhizal inoculum alone and in combination with the two, with increases of 90%, 83%, and 93.7%, respectively. This indicates a significant cumulative effect of the interaction treatment, which enhances the availability of this nutrient [45].

3.5. The Total Concentration of Zinc and Copper in the Soil Within the Rhizosphere

The results in **Figure 5** indicate that the only addition of organic matter to the soil elevated the concentrations of copper and zinc in the rhizosphere soil from 15.4 to 19.4 mg/kg and from 25.6 to 30.5 mg/kg, corresponding to increases of 25.9% and 19.1%, respectively. This may result from the elevated nutrient composition of organic matter (poultry dung and bedding), which is included as dietary supplements for poultry or as therapeutic agents for avian diseases (Zhang et al., 2021). The percentage of copper and zinc concentrations was lower in the treatment with the fungal inoculum alone, where the concentrations increased to 17.2 and 27.2 mg kg^{-1}, representing increases of 11.6% and 6.25%, respectively, compared to the control treatment. This demonstrates the function of bioinoculations in augmenting nutrient availability in rhizosphere soil through the decomposition of mineral-containing elements to release nutrients, as well as by excreting organic acids that alter soil pH to acidic levels, thereby enhancing mineral solubility. This is attributable to their function in enhancing the plant's efficacy in nutrient absorption, particularly for slow-moving elements like copper, via the symbiotic relationship between the fungal inoculum and plant roots. The interaction between the fungal inoculum and organic matter was successful, resulting in considerable increases in copper and zinc concentrations of 21.4 and 33.2 mg/kg^{-1}, respectively, which correspond to increases of 38.9% and 29.6% compared to the control treatment. This results from the synergistic interaction between the fungal inoculum and organic matter, which enhances nutrient concentrations in the rhizosphere soil. Organic matter supplies nutrients, whilst fungal inoculants enhance the efficacy of plant absorption. Organic matter contributes to the retention of copper and zinc, safeguarding them from leaching or fixing on clay mineral surfaces by complexing with these metals. The synergistic incorporation of organic matter and fungal inoculants markedly enhances the concentrations of copper and zinc in the root zone. This underscores the critical significance of integrated soil fertility management, both biologically and chemically, which enhances the sustainability of natural resources and reduces dependence on synthetic chemical fertilizers.

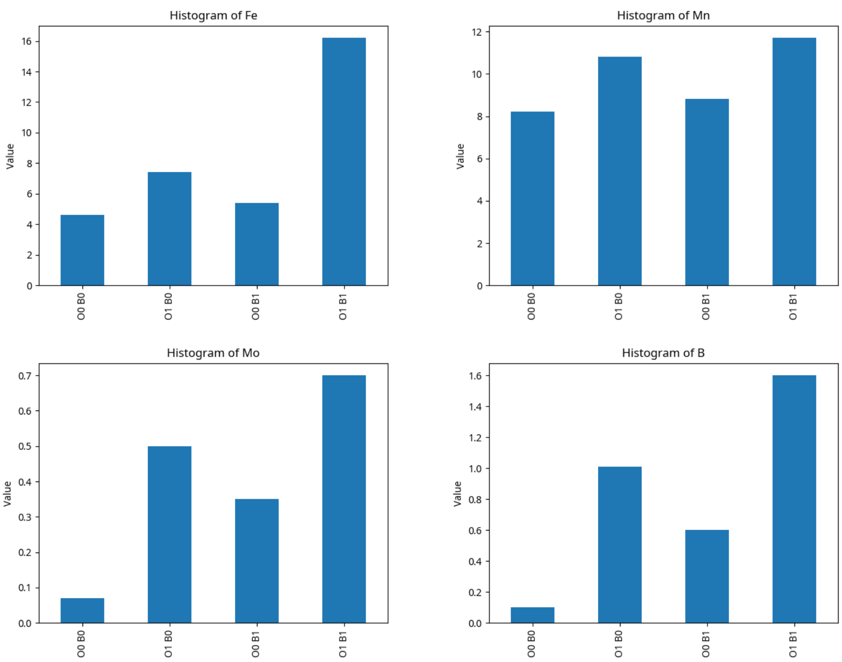

Figure 4. Impact of organic matter and mycorrhizal inoculation on the concentrations of accessible micronutrients in rhizosphere soil.
Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

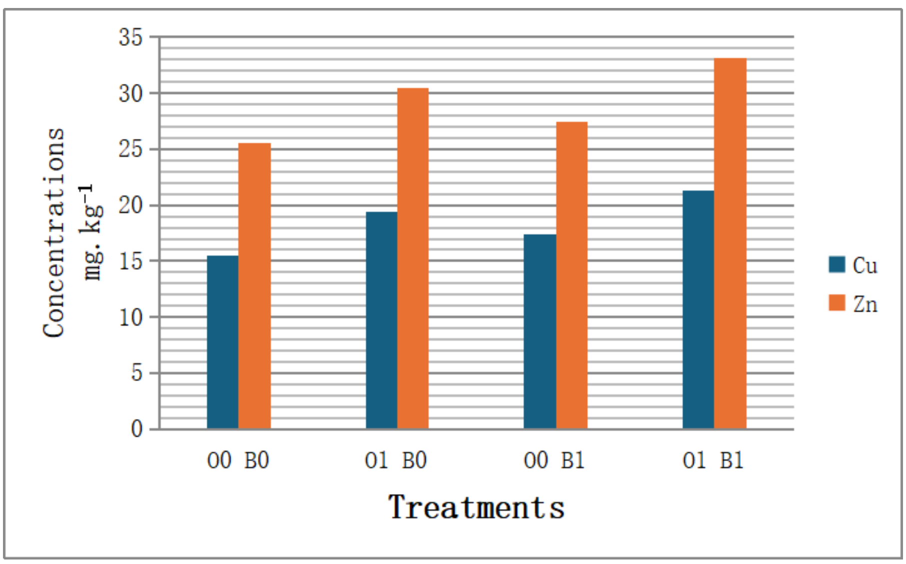

Figure 5. The total concentration of zinc and copper in the soil within the rhizosphere.
Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.6. The Concentration of Available Copper and Zinc in the Soil Within the Rhizosphere

The **Figure 6** findings demonstrate the substantial impact of the organic matter treatment (poultry manure), leading to notable increases in the concentrations of accessible zinc and copper in the rhizosphere. Concentrations rose from 0.16 mg kg in the no-additive condition to 0.34 mg/kg in the poultry manure treatment, reflecting a 52% increase. Zinc concentrations rose from 0.81 mg kg in the control treatment to 1.20 mg/kg in the organic matter treatment, reflecting a 32.5% increase. This results from the elevated levels of micronutrients in organic waste, particularly zinc and copper in degradable organic forms, together with the strong affinity of copper and zinc for organic matter. This facilitated the formation of chelated complexes that increase the availability of these elements. The synergistic link between the fungal inoculum and chicken manure significantly influences the amounts of copper and zinc. The organic matter supplies the fungal inoculum with essential nutrients for development, resulting in enhanced biomass under optimal moisture and aeration conditions. This subsequently enhances the liberation of previously inaccessible nutrients from their compounds into the soil solution. It also promotes root growth and enhances their ability to absorb nutrients. Copper concentration increased to 0.47 mg kg^{-1}, representing a 65.9% increase compared to the control treatment. Zinc concentration increased to 1.4 mg kg^{-1}, a 42% increase. This is consistent with what Al-Juthery et al.[46] reported. Consequently, one may assert that the synergistic interaction between fungal inoculum and organic matter is the most effective strategy for enhancing the use of microelements in soil, improving plant nutrient absorption efficiency, and reducing dependence on chemical fertilizers.

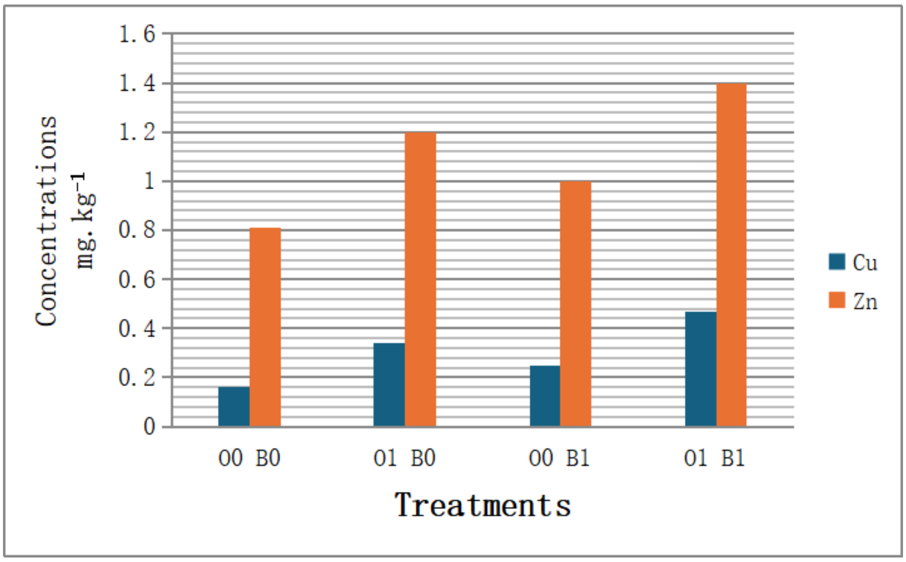

Figure 6. The concentration of available copper and zinc in the soil within the rhizosphere.

Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.7. The Total Concentration of Copper and Zinc in the Soil Outside the Rhizosphere

Figure 7 presents data illustrating the extent of the impact on copper and zinc concentrations outside the rhizosphere following the application of organic matter. The copper content increased to 21.4 mg kg, representing a 14.4% rise compared to the control treatment. The zinc content rose to 33.0 mg kg, reflecting a 19.5% increase compared to the treatment without additional zinc. This is due to the organic waste's provision of micronutrients to the soil, as well as its contribution to improving the physical and chemical properties of the soil. This leads to a reduction in pH and an increase in cation exchange capacity (CEC) due to its elevated specific surface area, while simultaneously decreasing the soil's apparent density, thereby augmenting its porosity. All these parameters enhance the ion exchange mechanism between the roots and the soil solution[47]. In comparing the influence of fungal inoculum on total copper and zinc concentrations outside the rhizosphere with that of organic matter, it is evident that the fungal inoculum exerted a lesser impact on the concentrations of these two elements. The copper concentration rose to 19.5 mg/kg, reflecting a 4.3% increase from the initial treatment. The zinc concentration increased to 30 mg kg^{-1}, representing an 8.7% increase. This indicates that adding the fungal inoculum alone has a lesser effect compared to adding organic matter. It may adversely affect plant growth due to competition between the fungal inoculum and plant roots for nutrients. Microorganisms are recognized for their superior efficiency in nutrient absorption compared to plants. This results in a decline in nutrient supplies, hindering plant growth. Consequently, it is advisable to integrate fungal inoculants into the biofertilization process, incorporating organic waste. This interaction has demonstrated a rise in copper and zinc concentrations to 23.1 and 36.5 mg/kg^{-1}, reflecting increases of 23.5% and 32.2%, respectively. This is due to the synergistic action between the fungal inoculant and organic waste in releasing nutrients present in the organic matter, which constitutes a primary source of food for the fungal inoculant. This enhances its activity and augments biomass, subsequently resulting in a greater nutrient supply and the release of vitamins, organic compounds, and various acids, which serve as food sources. Moreover, these creatures are converted into organic matter after their life cycle, providing sustainable sources of nutrients and natural resources.

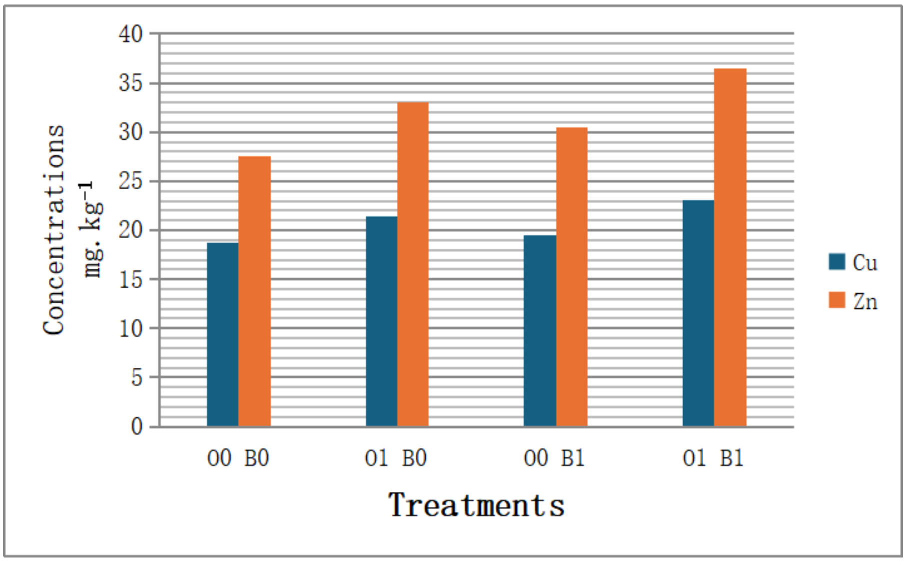

Figure 7. The total concentration of copper and zinc in the soil outside the rhizosphere.

Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

3.8. The Concentration of Available Copper and Zinc in the Soil Outside the Rhizosphere

The data from **Figure 8** demonstrate that the concentration of accessible copper in the soil beyond the root zone was markedly influenced by the incorporation of poultry manure and field bedding, both of which are nutrient-dense, including micronutrients, in comparison to the control treatment. The contents of copper and zinc in the soil beyond the root zone rose to 0.51 and 1.8 mg kg, indicating increases of 64.5% and 26.8%, respectively. This is due to the nutrient content of the manure, which is added as a nutritional supplement to poultry feed and is also used in the formulation of therapeutic drugs. This percentage rise may result in toxicity in the levels of these and other micronutrients. Consequently, chemical investigations of the micronutrient composition in poultry dung should be performed[48].

Copper and zinc concentrations of 0.43 and 1.8 mg/kg, representing increases of 38.7% and 12.7%, respectively, were obtained by applying a fungal inoculum to the soil alone. The previous paragraph covered the circumstances that led to the single fungal inoculum having a negligible effect on nutrient concentrations. The largest concentrations of copper and zinc were simultaneously produced by the interaction between the fungal inoculum and organic matter, with values of 0.7 and 1.9 mg/kg-1, respectively, signifying increases of 128% and 35.4%. This is because the interaction between the fungal inoculum and poultry excrement has a significant effect on the amounts of copper and zinc. These results encourage the shift to integrated fertility management, which combines organic fertilization and biofertilizers to maximize soil sustainability and nutrient recycling. This aligns with the results of Hamid et al.[49] and Al Hasnawi et al.[50].

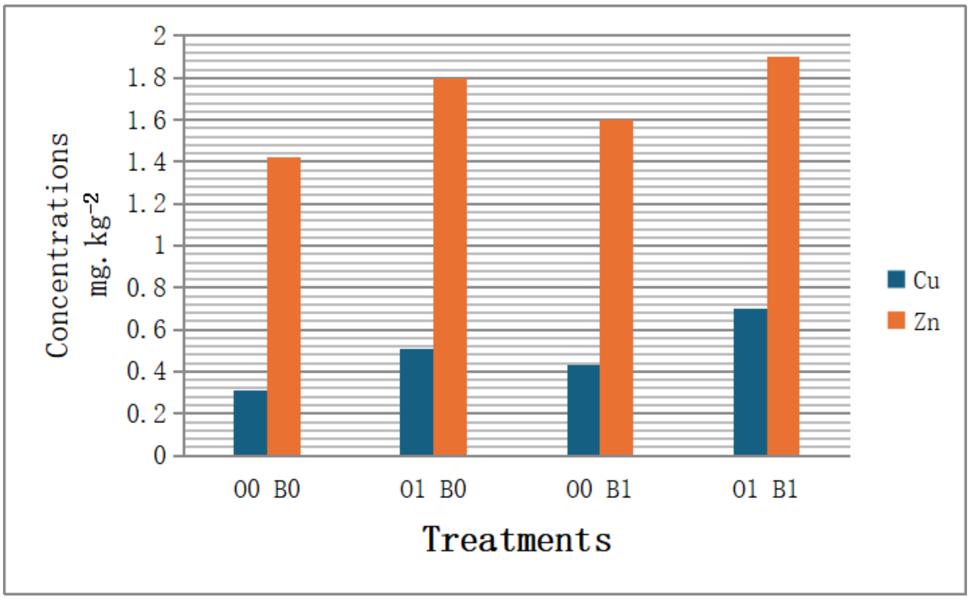

Figure 8. The concentration of available copper and zinc in the soil outside the rhizosphere.

Note: O0B0 without adding, O1B0 adding individual poultry waste, O0B1 adding a single fungal vaccine, O1B1 combined application of poultry manure and fungal inoculum , ≤ 0.05.

4. Conclusion

Organic matter, as a sustainable natural nutrient source, in conjunction with bio-inoculants, especially symbiotic mycorrhizal fungi such as Glomus spp., offers a distinctive method for improving soil fertility, conserving natural resources, and augmenting production. This was accomplished by enhancing the physical and chemical qualities of the soil through the integration of poultry manure and fungal inoculants. The efficiency of micronutrient absorption was improved by expanding the root absorption area, which increased contact surfaces with the soil solution due to heightened microbial activity. The findings indicated that the synergistic interaction between organic matter and fungal inoculants results in a substantial enhancement of plant biomass. This results in enhanced plant development and beneficially affects grain and fruit production. It also decreases agricultural labor expenses by reducing dependence on costly synthetic chemical fertilizers, which have detrimental impacts on soil health, resulting in soil deterioration. These findings endorse contemporary movements advocating for sustainable agriculture and the efficient utilization of natural resources within agricultural systems. The integration of environmental and agricultural objectives achieved through soil inoculation with beneficial microorganisms represents an effective approach to enhancing plant productivity and the sustainability of agricultural systems that rely on the natural resources of the soil.

Author Contributions

Conceptualization, A.M.A., W.S.K., N.S.O., and J.A.K.; methodology, A.M.A., W.S.K., N.S.O., and J.A.K.; formal analysis, A.M.A., W.S.K., N.S.O., and J.A.K.; data curation, A.M.A., W.S.K., N.S.O., and J.A.K.; writing—original draft preparation, A.M.A., W.S.K., N.S.O., and J.A.K.; writing—review and editing, A.M.A., W.S.K., N.S.O., and J.A.K. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data will be available on request from the author.

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Conflict of Interest

The Authors declares that there is no conflict of interest.

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