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ARTICLE

Evaluating the Interaction of Mycorrhizal Fungi, Azotobacter, and Biochar in Enhancing Cucumber Productivity and Soil Health

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ABSTRACT

This experiment evaluated the effects of the mycorrhizal fungus *Glomus mosseae*, *Azotobacter chroococcum* bacteria, and Biochar on the characteristics of the root system, and yield of the cucumber plant, *Cucumis sativus L.*; for this purpose, experiment designed: the first factor is a combination of Mycorrhizae (M) at 35 g plant^{-1}, Azotobacter (A) 15 ml plant^{-1} with a microbial density of 2.2, and three concentrations (0, 5, 10%) of Biochar sprayed on the plant. The results of the research demonstrated that using mycorrhizae, Azotobacter bacteria, and phosphate rock with half the mineral recommendation (MAR) and spraying Biochar at a concentration of 10% gave the highest rate of infection of the roots with mycorrhizae, amounting to 80%, and the highest dry weight of the root system reached 84.53 g. The highest number of total bacteria was 8.74 log Cfu g m^{-1} of soil, the highest plant height reached 375.0 cm, the highest dry weight of the shoot reached 101.66 g plant^{-1}, and the highest yield for the greenhouse was 4.501 ton greenhouse^{-1}, followed by the treatment of adding Mycorrhiza with phosphate rock and half the mineral recommendation (MR) with Biochar at a concentration of 10%, then treatment with the addition of mycorrhizae with Azotobacter bacteria with half the mineral recommendation (AR) with 10% of Biochar. It is possible to eliminate half of the mineral recommendation by using these fertilizers, reduce the harmful impact of pollution on the environment and enhance sustainability in agriculture.

Keywords: Biochar; Phosphate Rock; Azotobacter; Mycorrhizal; Mineral Fertilizer

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1. Introduction

Microbial biofertilizers work to improve plant growth by enhancing the absorption and availability of plant nutrients. Biofertilizers stabilize atmospheric nitrogen and dissolve phosphate and potassium compounds, as well as compounds of many other elements, while also producing plant growth regulators, enzymes that promote plant growth, organic acids, and siderophores. Many types of bacteria, including nitrogen-fixing bacteria, such as the free-living bacteria Azotobacter, facilitate phosphorus uptake, the most important of which are mycorrhizae. Mycorrhizae encourage and increase the avaliablity of many nutrients through several mechanisms, with phosphorus being the most significant[1]. Mycorrhizae are considered important biological fertilizers; they are the most crucial fungi present in the soil environment, providing a symbiotic relationship between the fungal mycelium and the roots of various plants. Mycorrhizal hyphae can extend over long distances, reaching tens of meters, forming a biomass that enhances plant growth by terms of providing water and nutrients, as well as supporting growth under stress conditions, such as drought and salinity[2]. Mycorrhizae play a major role in converting phosphorus compounds and other elements into available forms through a series of microbial transformations. To increase the benefit of phosphate rock as a source of phosphorus, phosphate-dissolving bacterial and fungal techniques, especially mycorrhizal fungi, were employed. These fungi have a great ability to secrete many organic compounds that dissolve complex phosphorus compounds in the area surrounding the roots, as well as increase the movement of phosphorus element toward the root in the presence of hyphae, which can reduce the distance of spread by up to 90% and increase the surface area for absorption[3].

The use of organic fertilizers in conjunction with biofertilizers, such as mycorrhizal fungi and Azotobacter bacteria, has a significant impact and is considered one of the most effective ways to improve the physical, chemical, and biological properties of the soil. Among these environmentally friendly organic fertilizers is vermicompost, which studies have proven to enhance productivity. It improves soil propertie and creates a safe and healthy ecosystem for food production. It can also serve as a bio-resistance, replacing the need for chemical pesticides that negatively affect public health and the environment, thus providing desirable qualitative characteristics to plant products and the desired original taste, free of health and environmental pollutants. The colonization of plant roots by nonpathogenic microbial biofilms enhances nutrient mobilization, integration, and biogeochemical cycling, concentrating nutrients essential for plant growth, disease resistance, and productivity[4]. Microbial biofilms significantly contribute to the decomposition of organic matter, nutrient cycling, and polysaccharide production, making them crucial for soil structure, health, nutrient availability, water holding capacity, porosity, soil density, and erodibility[5]. Research highlights the critical role of the fungus Glomus intraradices in mobilizing organic nitrogen[6]. A review identified several phosphorus-solubilizing microbes, including *Pseudomonas sp*., *Azotobacter*, *Agrobacterium sp*., *Paenibacillus*, and *Bacillus sp*., discussing their importance in plant growth and crop yield through the mineralization and solubilization of insoluble phosphorus[7]. *Bacillus pumilus* and *P. pseudoalcaligenes*, two biofilm-forming potassium-mobilizing bacteria, improved carbohydrate accumulation and membrane permeability in rice under salinity stress by enhancing the solubility, mobility, and uptake of potassium[8].

The research aims to determine the efficiency of using some biofertilizers, such as phosphate rock and Biochar sprayed on the plant and their effect on the growth and yield of the cucumber plant *Cucumis sativus L*.

The aim of this study is also to find answers to these questions: (1) How to increase the proportion of Biochar in soil? (2) How the presence and absence or different abundance of some biofertilizers, with phosphate rock and Biochar in the soil, affects the weight of roots and leaves and other properties for cucumber plant *Cucumis sativus*.

2. Materials and Methods

A field experiment was carried out in the fall season (2022) on the cucumber crop inside a plastic house at the research station of the College of Agriculture/Anbar University to study the effect of the mycorrhizal fungus *Glomus mosseae*, the bacteria *Azotobacter chroococcum*, and phosphate rock in interaction with Biochar, which was sprayed on the plant in growth and yield of cucumbers under protected cultivation. **Table 1** shows some of the physical and chemical properties of field soil.

Table 1. Some chemical and physical properties of greenhouse soil before planting.

Characteristic	Unit	Measurement
EC	dSm^{-1}	3.1
pH	-	7.6
CEC	$Cmol.kg^{-1}$ Soil	19.2
O.M	%	0.7
Soil texture	-	Sandy loam
N	mg kg $soil^{-1}$	73.2
P		7.2
K		106.3
Sand	%	607
Silt		249
Clay		144
Total microbes	1.5×10^{-3}	Cfu g^{-1}

2.1. Multiplication of Mycorrhizal Vaccine

A previously identified mycorrhizal fungus vaccine, *Glomus mosseae*, was used from the microbiology laboratories at the College of Agriculture/University of Anbar. It consists of (spores + infected roots + dry soil). This vaccine was propagated by planting yellow corn seeds in plastic pots, each containing 10 kg of soil sterilized with an autoclave at a temperature of 121 °C for 20 minutes. I added 25 grams of inoculum under the surface layer of the potting soil at a depth of about 5 cm. I planted 5 yellow corn seeds in one pot, then the seedlings were thinned out after a week of germination to three seedlings in each pot. The shoots were removed two months after germination, and the soil mixture and the roots cut into small pieces were placed in sterile plastic bags and kept in a cool, dry place until used as a vaccine. Samples of them were also examined under a microscope to confirm whether the roots were infected with mycorrhizae after staining[9].

2.2. Isolation, Purification, and Diagnosis of Azotobacter Bacteria

Soil dilutions were prepared dilutions by adding 10 grams of soil sample to 90 milliliters of sterile water in 250-milliliter beakers, thoroughly mixing to achieve the first dilution at 1:10. Subsequent serial decimal dilutions were carried out up to the seventh dilution (10^{-7}) by transferring 1 milliliter of the soil suspension from each preceding dilution into tubes containing 9 milliliters of physiological solution. For each soil sample, I inoculated the liquid medium (sucrose mineral salts) as described by[10] Specifically, 1 milliliter from the 10^{-5}, 10^{-6}, and 10^{-7} soil dilutions was used to inoculate test tubes containing 9 milliliters of the sterilized liquid medium, which had been prepared and autoclaved for 20 minutes at 121 °C and 15 psi, with three replicates for each dilution. The test tubes were incubated at 28 °C for 5 days. Observations for the presence of a brown membrane (brown ring) indicated the positive initial growth of *Azotobacter spp*.[11]. The Azotobacter bacteria were then purified and diagnosed, and microscopic and biochemical tests were subsequently conducted according to[12], which showed that they were of the *A. chroococcum* type. The Azotobacter biofertilizer was prepared by packing a quantity of peat moss into thermal bags, each bag containing 2 kg of peat moss. It was sterilized in an autoclave at a temperature of 121 °C. It was then placed in sterile beakers, and the bacterial inoculum was added to the holder. It was added to the beaker containing 2 kg of peat moss. Sterilized 500 ml of liquid nutrient broth containing *A. chroococcum* bacteria. The inoculum was added gradually and left for 24 hours in the laboratory to ensure the spread and distribution of the bacterial cells on the peat moss carrier. Then, it was used in the field experiment[13].

2.3. Preparing Biochar

Biochar was prepared according to the method[14] by placing 15 liters of chlorine-free water in a special plastic container with a capacity of 30 liters; then an air pump model RS-702 was submerged into the container under the surface water, and 100 ml of molasses was added to the water to feed the microorganisms. Then, 1.5 kg of solid vermicompost was placed inside a piece of light cloth (similar to gauze), made into a bundle, and suspended in the water. The cover was put on to prevent the entry of insects and dust, and it was left for 72 hours with continuous ventilation[15]. After that, the cloth was taken out and the resulting extract, **Table 2** show the Characteristics of the Biochar used in the study. which is called Biochar, was diluted with 100 ml L^{-1} and then sprayed on the plants.

Table 2. Characteristics of the Biochar used in the study.

Characteristic	Value	Unit of Measurement
pH	6.05	-
EC	1.3	dsm^{-1}
N	2.8	%
P	1.31	%
K	2.14	%
Total bacteria	1.9×10^{-7}	Cfu g^{-1} dry soil
Total fungi	2.4×10^{-3}	Cfu g^{-1} dry soil

2.4. Experiment Transactions

A factorial experiment was conducted with two factors:

The first factor is a combination of biofertilizer (Mycorrhiza, azotobacter with rock phosphate, plus half the fertilizer recommendation) and a comparison treatment of 100% mineral fertilizer, as follows:

M1: Only mycorrhizal (M), to which half the fertilizer recommendation was added.

M2: Azotobacter (A) bacteria, to which half the fertilizer recommendation was added.

M3: Mycorrhizal (M) + Azotobacter (A), to which half the fertilizer recommendation was added.

M4: Mycorrhizal (M) + phosphate rock (R), to which half the fertilizer recommendation was added.

M5: Azotobacter (A) + phosphate rock (R), to which half the fertilizer recommendation was added.

M6: Mycorrhiza (M) + Azotobacter (A) + Phosphate rock (R), to which half the fertilizer recommendation was added.

M7: 100% complete fertilizer recommendation (S) for cucumber plants.

The second factor is Biochar

Three concentrations of Biochar prepared for addition have been approved:

Without addition, and its symbol is V0.

(2) Add 5% of Biochar, symbol V1.

(3) Add 10% of Biochar, code V2.

A randomized complete block design (RCBD) was adopted in implementing the field experiment with two factors (7 combinations randomly distributed among sectors representing replicates).

2.5. Fertilization

(1) Biofertilization: 50 grams of mycorrhizal inoculum were placed in each hole at a depth of 5 cm from the soil surface. As for the bacterial isolates, they were prepared in the liquid culture medium, Nutrient Broth 15 ml per plant, then placed on peat moss and added to each hole.

(2) Phosphate rock: Phosphate rock, which contains 21.47% P_2O_5, was added at a rate of 300 kg P_2O_5 $hectare^{-1}$ per hole in one batch before planting. **Table 3** shows the chemical properties of the phosphate rock used in the experiment.

Table 3. Some chemical characteristics of the phosphate rock used in the study.

Characteristic	Value	Unit of Measurement
Total P	8.63	%
$CaCo_3$	28.5	%
F	0.58	%
S	1.48	%
Si	2.29	%
Fe	1490.00	mg L^{-1}
Mg	777	mg L^{-1}
Zn	393	mg L^{-1}

Source: Phosphate Fertilizer Manufacturing Plant\Akashat\Ministry of Industry \Iraq.

(3) Add Biochar at a concentration of (0, 5 and 10%) and spray it on the plant using a 20-litre sprayer. The process of adding Biochar was carried out in three stages (20 days after planting, the beginning of flowering, and after setting). The time interval between one addition and another was 15 days, and the spraying process was carried out early in the morning.

(4) Mineral fertilization: Mineral fertilization was added according to the fertilizer recommendation for cucumber plants under protected conditions. Nitrogen fertilizer was added at a rate of 500 kg N ha^{-1} (urea fertilizer), divided into three batches: the first before planting, the second batch one month after planting, and the third two months after planting with irrigation water. During fertilization, phosphate fertilizer was added at a rate of 300 kg P_2O_5 ha^{-1} (triple superphosphate) in one batch before planting, mixing with the soil, while potassium fertilizer was added at a rate of 300 kg K_2O ha^{-1} (potassium sulfate), added in one batch before planting, mixing with the soil. This is for the full fertilizer recommendation treatment, while half of the recommendation was added to the biofertilizer combination.

2.6. Estimating the Percentage of Roots Infected with Mycorrhizae

The percentage of infection of roots with the mycorrhizal fungus *Glomus mosseae* was estimated by estimating

the percentage of infection in root pieces that were stained according to the method described in[9]. Ten root pieces, 1 cm long, representative of each sample, were randomly selected, placed on a glass slide, and examined with an optical microscope (X40). The infection rate was calculated according to the equation:

$$\begin{aligned}\text{Percentage of roots infected with mycorrhizal fungi} &= (\text{Number of infected root pieces})/ \\ &(\text{The total sum of the root pieces}) * 100\end{aligned} \quad (1)$$

2.7. Estimation of Mycorrhizal Dependence

The following equation estimated the relative mycorrhizal dependence:

$$\begin{aligned}\text{RMD} &= \text{dry weight of mycorrhizal plants} - \\ &\text{dry weight of non} - \text{mycorrhizal plants}/ \\ &\text{dry weight of mycorrhizal plants} \times 100. \\ &\text{Its value ranges from } (0{-}100\%) \\ &(\text{Al} - \text{Samarrai and Suhail, 2018}).\end{aligned} \quad (2)$$

2.8. Studied Attributes

The characteristics of the root system, shoots, and yield characteristics of cucumber plants were measured, which are:

Percentage of roots infected with mycorrhizal fungi (%), dry weight of the root system (g of plant^{-1}), total bacteria in the soil (Cfu g^{-1}), plant height (cm), dry weight of the shoot (g of plant^{-1}), house yield Plastic (1 ton of plastic house), mycorrhizal dependence.

2.9. Statistical Analysis

Data were collected and the results were analyzed statistically using SAS software using analysis of variance. The averages were compared using the least significant difference (L.S.D) at a 5% significance level.

3. Results

3.1. Effect of G. Mosseae Inoculum and Plant Host Type on the Percentage of Root Mycorrhizal Infection (%)

Table 4 shows the effect of the type of plant host and inoculation with mycorrhizal fungi on the rate of infection of the roots. There was a significant effect on the rate of infection achieved with the use of mycorrhizal inoculum, amounting to 81.21%, while it reached 5.5% without adding the inoculum. A non-significant superiority of infection was observed in the roots of the cucumber plant. The highest infection rate was 43%, compared to the infection rate of 42% in the roots of yellow corn. It was also found that the interaction of cucumber plants with the use of the vaccine achieved the highest infection rate of 84.33%, while the lowest infection rate was 4.00% in soil not inoculated with mycorrhizae.

The reason for the superiority of inoculation treatments with the mycorrhizal fungus *G. mosseae* in the rate of infection of the roots with mycorrhizal fungi is attributed to the efficiency of the inoculum used and the response of the plant host. There was no significant superiority between the cucumber and yellow corn plants in terms of the infection rate, and the two plants responded to the infection greatly due to their need for phosphorus and the susceptibility of their roots. The results agree with Damodaran et al. (2012) in a study conducted in India on cotton plants, where the percentage of roots infected with mycorrhizal fungi ranged between 37% and 73%, depending on the type of inoculum, soil type, and environmental conditions.

3.2. The Logarithm of the Total Number of Bacteria after Harvesting

Figure 1 reveals the impact of combining biofertilizers with phosphate rock and Biochar on the logarithm of the total number of bacteria after harvesting. The triple combination MAR produced the highest bacterial count, with 8.7 log colony-forming units (CFU) per gram of soil, significantly surpassing all other combinations. Following MAR, the combinations MA and A recorded bacterial counts of 8.67 and 8.59 log CFU per gram of soil, respectively. The AR and MR combinations showed counts of 8.51 and 8.19 log CFU per gram of soil, while the mycorrhizal treatment M yielded 7.55 log CFU per gram of soil. The mineral fertilization treatment S had the lowest bacterial count at 5.55 log CFU per gram of soil. The triple combination MAR with half the fertilizer recommendation outperformed the S treatment by 57.47%. The concentrations of Biochar did not significantly affect the bacterial count. Spraying with 10% Biochar resulted in the highest bacterial count of 8.04 log CFU per gram of soil,

Table 4. Effect of *G. mosseae* inoculum and plant host type on the percentage of root mycorrhizal infection (%).

Plant Host / Pollination	Yellow Corn	Cucumber	Average Pollination	L.S.D (0.05) For Vaccination
Inoculated	77.3	85.13	81.21	2.63
Not vaccinated	7	4	5.5	
Average plant host	42	43	L.S.D (0.05)	
L.S.D (0.05) For the plant host	2.63	To interfere between the plant host and pollination		3.71

compared to 7.99 and 7.89 log CFU per gram of soil for the 5% and 0% concentrations, respectively.

Significant differences were observed in most interaction treatments between biological combinations with phosphate rock and Biochar concentrations. The interaction of the MAR combination with 10% Biochar spray achieved the highest bacterial count, 8.83 log CFU per gram of soil, significantly exceeding other interactions. This treatment showed a 64.73% increase compared to the lowest interaction treatment, which was the mineral recommendation without spray (S), recording the lowest bacterial count of 5.36 log CFU per gram of soil.

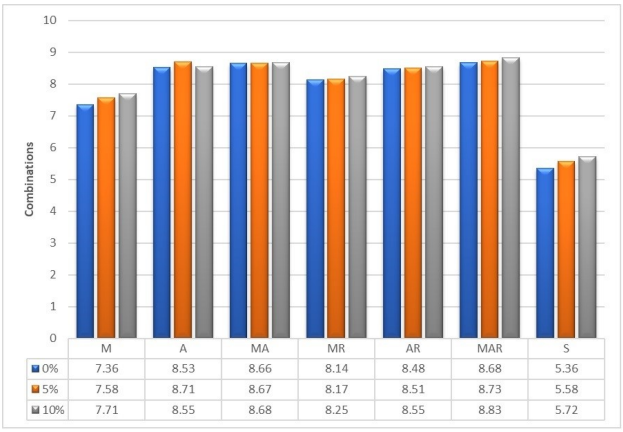


Figure 1. Effect of the biological combination with phosphate rock and Biochar spray on the logarithm of the total number of bacteria after planting.

3.3. Dry Weight of Shoots (g Plant^{-1})

Figure 2 shows the effect of combining biofertilizers with phosphate rock and Biochar spray on the dry weight of cucumber plant shoots. The results indicate significant differences among the treatments. The MAR combination yielded the highest average shoot dry weight at 122.66 g per plant, significantly higher than all other combinations. Following MAR, the MR and MA combinations recorded shoot dry weights of 106.21 g and 98.22 g per plant, representing increases of 15.48% and 24.88%, respectively. Additionally, the Mycorrhizal (M) treatment resulted in a significantly higher dry weight compared to the Azotobacter (A) treatment, with dry weights of 97.66 g and 86.22 g per plant, respectively. The mineral fertilization (S) treatment recorded the lowest average shoot dry weight at 83.22 g, while the triple combination MAR showed a 47.39% increase over this treatment. Biochar concentrations also had a significant impact on dry weight, with a 10% spray yielding the highest rate of 105.28 g per plant, compared to 5% and 0% concentrations, which recorded 97.90 g and 90.99 g per plant, respectively. Significant differences were observed for most interaction treatments between the biological combinations with phosphate rock and Biochar concentrations. The interaction between the MAR combination and a 10% Biochar spray produced the highest average shoot dry weight at 130.66 g per plant, significantly outperforming all other interactions and showing a 66.80% increase compared to the lowest interaction treatment, the mineral recommendation (S) without Biochar, which recorded 78.33 g per plant[17–19].

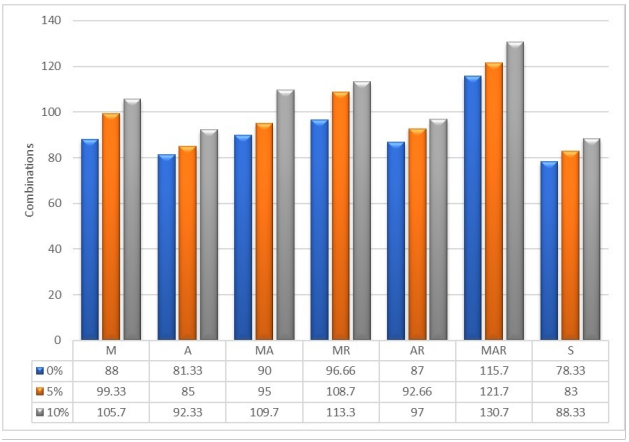
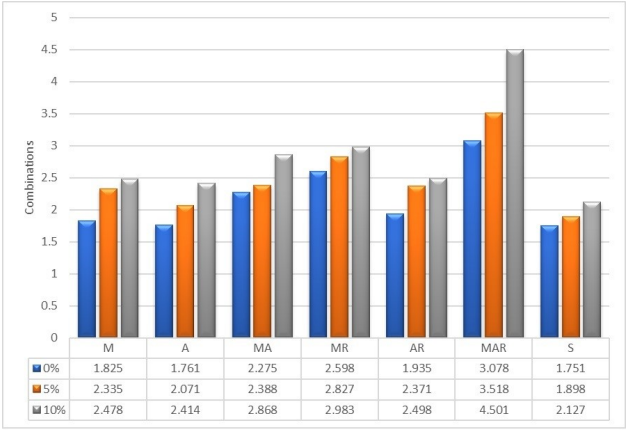


Figure 2. Effect of the biological combination with phosphate rock and Biochar spray on the dry weight of the shoot (g plant^{-1}).

3.4. Yield of Plastic House (Ton of Plastic House $^{-1}$)

Figure 3 illustrates the impact of combining biofertilizers with phosphate rock and Biochar spray on cucumber plant yield in the greenhouse. The MAR combination produced the highest yield, with 3.699 tons per greenhouse, significantly outperforming all other combinations. The MR combination followed with a yield of 2.803 tons per greenhouse, then the MA and AR combinations with yields of 2.510 and 2.368 tons per greenhouse, respectively. The table also indicates that the Mycorrhizal (M) treatment resulted in a significantly higher yield than the Azotobacter (A) treatment, with yields of 2.212 and 2.082 tons per greenhouse, respectively. The full mineral recommendation (S) treatment had the lowest yield, at 1.925 ton per greenhouse. A 10% Biochar spray achieved the highest yield of 2.881 tons per greenhouse, with significant increases of 15.8% and 32.4% compared to the 0% and 5% concentrations, which yielded 2.175 and 2.487 tons per greenhouse, respectively. The interaction between the MAR combination and 10% Biochar spray resulted in the highest greenhouse yield, recording 4.501 tons per greenhouse, significantly surpassing all other interaction treatments and showing a 157% increase compared to the lowest yield from the full mineral recommendation (S) without Biochar, which was 1.751 tons per greenhouse.



	M	A	MA	MR	AR	MAR	S
0%	1.825	1.761	2.275	2.598	1.935	3.078	1.751
5%	2.335	2.071	2.388	2.827	2.371	3.518	1.898
10%	2.478	2.414	2.868	2.983	2.498	4.501	2.127

Figure 3. The effect of the biological combination with phosphate rock and Biochar spray on the greenhouse yield (ton of plastic greenhouse^{-1}) for cucumbers under protected cultivation.

3.5. Mycorrhizal Dependency %

Table 5 shows the mycorrhizal dependence % of the dry weight of the root system, the dry weight of the shoots, and the yield of the greenhouse. The dry weight of the root system of the cucumber plant inoculated with mycorrhizae was only 39.6 grams compared to 22.3 grams for the non-inoculated plant (comparison treatment without any addition). Thus, the mycorrhizal dependence of the dry weight of the plant is 39.6 grams. The root weight was 52.3%, and the dry weight of the shoot was 75.4%, 31.8% and 57.8% for both the pollinated and non-pollinated plants and the mycorrhizal dependence, respectively. The data for the cucumber yield in the greenhouse was 1,662 kg Plastic House^{-1} for the pollinated plant and 908 kg for the non-pollinated plant, while the plant was 45.3% dependent on mycorrhizae in the case of the greenhouse crop compared to the non-pollinated plant. The results agree with[20, 21], who stated that mycorrhizal reliability varies depending on the type of plant, soil type, genus, and type of mycorrhiza, as well as the surrounding environmental conditions, which in their study ranged between 15 and 80% depending on the factors mentioned.[22] also stated that the reliability of mycorrhiza of mulberry plants depends on the genus and type of mycorrhizae, and the type *F. mosseae*, followed by *A. scrobiculata*, then R. intraradices in terms of physiological characteristics, plant growth, and qualitative characteristics of mulberry yield, which agreed with what was reported that *F. mosseae* was better than *D. tortuosum* in the mycorrhizal dependence of some canopy trees in terms of increasing the shoot and root system and the chlorophyll content of the leaves.

4. Discussion

Tables 4 and **5** and **Figures 1, 2** and **3** reveal that incorporating biofertilizers, specifically the mycorrhizal fungus (M) and Azotobacter (A) along with phosphate rock (R) and using Biochar (V) as a spray, while applying half the recommended fertilizer dosage for cucumber plants, significantly enhances various growth characteristics. These characteristics include the percentage of roots infected with mycorrhizal fungi, the dry weight of the root system, the total bacterial count in the soil, plant height, the dry weight of the shoot, and the yield per plastic house. The triple combination of MAR demonstrated superior performance in both vegetative growth and yield compared to the binary combinations, which were also exceptional to single treatments. The 10% concentration of Biochar yielded the best results across all

Table 5. Mycorrhizal dependence % of dry weight of root and shoot total and greenhouse yield.

Plant Treatment	Dry Weight of Root (g)	Dry Weight of Shoots (g)	Plastic House Yield (kg)
Pollinated plant	42.6	75.4	1662
Unpollinated plant	20.3	31.8	908
Mycorrhizal dependence	52.30%	57.80%	45.30%

measured characteristics compared to the 5% and 0% concentrations.

The mycorrhizal fungus boosts plant growth and nutrient availability, resulting in higher relative growth rates. This enhancement is due to the fungus's capacity to extend beyond the root system, thereby increasing soil exploration and nutrient absorption, especially phosphorus. Additionally, mycorrhizae trigger physiological responses in plants, such as root branching and enzyme secretion, which further enhance nutrient uptake. This mechanism clarifies why using mycorrhizae leads to greater phosphorus availability from phosphate rock and subsequent improvements in plant growth and productivity.

The addition of biofertilizers also increased bacterial and fungal densities in the soil, likely due to the soil being home to billions of microorganisms per cubic centimeter. Adding specific bacteria and mycorrhizal fungi enhances these microbial communities, leading to improved plant growth and yield, it has improved the characteristics of plant growth and the formation of a dense root system, and this means providing a suitable rhizosphere area for the growth, activity, and reproduction of microorganisms and increasing their numbers [23–25]. These microorganisms usually decrease in number in soils poor in nutrients or infertile, as they are necessary for building their bodies and carrying out vital processes. Providing all the requirements that the microbial mass in the soil needs for its activity in terms of adding biofertilizers with organic vermicompost in the form of interactions, whether double or triple, has had a significant impact and led to a significant increase in the numbers of bacteria in the soil, especially with the triple interaction. When adding fertilizers of all three types and at the highest levels that give the highest number, it has an important effect in increasing its activity in decomposing organic matter, consuming what it needs, and providing the main nutrients to the plant [26–28]. The addition of biofertilizer, whether in the case of a combination consisting of mycorrhizal fungi and nitrogen-fixing bacteria or a complete combination consisting of mycorrhizal fungi, nitrogen-fixing bacteria, and phosphate rock with any level of Biochar, did not differ significantly between them, but in all cases it was superior to the no-addition treatment (recommendation). Adding biofertilizer also increases the logarithm of the total number of bacteria and the colonization of these organisms in the inoculated soil. This is due to the positive effect of growth-regulating substances [30–32]. Spraying Biochar on plants enhances stomatal permeability, leading to improved absorption of macro- and micro-nutrients. This is because Biochar promotes cell growth and elongation, resulting in an expanded root system, increased nutrient uptake, and enhanced shoot growth due to the presence of plant growth regulators such as auxins, cytokinins, abscisic acid, and gibberellins [29, 33, 34]. Biochar application also boosts chlorophyll content, total protein, and sugar levels in plants while stimulating auxin formation to soften cell walls and improve water absorption [35, 36]. These benefits collectively lead to better plant growth and higher yields, particularly when using a higher concentration (10%) of Biochar mixed with biofertilizers, as demonstrated in this research [37, 38].

5. Conclusions

Adding an integrated combination of bacterial and fungal biofertilizers together gave better results in increasing root and vegetative growth indicators, yield, and microbial density in the soil and obtaining high growth and productivity standards for cucumber plants compared to single biofertilization. The use of Biochar sprayed on the plant also gave a clear significant increase in growth characteristics and yield. This effect increased by increasing the concentration from 5% to 10%, as well as in cases of interaction with combinations of biofertilizers and phosphate rock. Using a combination of biofertilizers with phosphate rock and Biochar sprayed on the plant with 50% chemical fertilization gave better results than the full mineral recommendation. Therefore, it is possible to dispense with half of the mineral recommendation by using these fertilizers, reduce the economic cost, reduce the

harmful impact of pollution on the environment and human health, and enhance sustainability in agriculture.

Author Contributions

Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision N.A.J.K.A.-S.; Conceptualization, methodology, investigation, resources, data curation, writing—original draft preparation, N.F.Y.; investigation, resources, data curation, writing—original draft preparation, writing—review, editing, visualization and supervision Z.K.K.A.-S.; Conceptualization, methodology, investigation, resources, data curation, writing—original draft preparation, A.A.-D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Tajalee, G., Verma, A., Ayoub, L., et al., 2022. Increasing nutrient use efficiency in crops through biofertilizers. The Pharma Innovation Journal. 11(6), 2003–2010.

[2] Aljawasim, B.M., Khaeim, H., Manshood, M., 2020. Assessment of arbuscular mycorrhizal fungi (*Glomus spp.*) as potential biocontrol agents against damping-off disease Rhizoctonia solani on cucumber. Journal of Crop Protection. 9(1), 141–147.

[3] Alkobaisy, J.S., Lafi, A.S.A., Abdel Ghani, E.T., 2020. Influence of using Mycorrhizae (MH) with Vermicompost (VRF) on soil properties, soybean (Glycinemax) growth and yield. Sys Rev Pharm. 11(6), 347–351.

[4] Bargaz, A., Lyamlouli, K., Chtouki, M., et al., 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front Microbiol. 31(9), 1606. DOI: https://doi.org/10.3389/fmicb.2018.01606

[5] Wang, J., Zhong, H., Zhu, L., et al., 2019. Arbuscular mycorrhizal fungi effectively enhances the growth of Gleditsia sinensis Lam. seedlings under greenhouse conditions. Forests. 10(567), 2–10.

[6] Thirkell, T.J., Cameron, D.D., Hodge, A., 2016. Resolving the nitrogen paradox of arbuscular mycorrhizas: Fertilization with organic matter bring considerable benefits for plant nutrition and growth. Plant, Cell and Environment. 39, 1683–1690.

[7] Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology. 8, 00971. DOI: https://doi.org/10.3389/fmicb.2017.00971

[8] Jha, Y., 2017. Potassium mobilizing bacteria: Enhance potassium intake in paddy to regulates membrane permeability and accumulate carbohydrates under salinity stress. Brazilian Journal of Biological Sciences. 4, 333–344.

[9] Gerdmann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decating. Transactions of the British Mycological Society. 46, 234–244.

[10] Becking, J.H., 1981. The family Azotobacteraceae. In: Starr, M.P. (Ed.). The Prokaryotes. Springer-Verlag: Berlin/Heidelbery, Germany; New York, NY, USA. pp. 795–817.

[11] Baron, E.J., Feingold, S.M., 1990. Diagnostic microbiology, 8th ed. C. V. Mosby Company: St. Louis, MO, USA. pp. 11–20.

[12] Holt, J., Krieg, N.R., Sneath, P.K.A., et al., 2004. Bergey's manual determinative bacteriology, 9th ed. Williams and Wilkins: Baltimore, MD, USA. pp. 120–129.

[13] Brenner, D.J., Krieg, N.R., Staley, J.T., 2004. Bergey's manual of bacteriology. Williams and Wilking: Baltiomore, MA, USA; London, UK. pp. 90–110.

[14] Wang, K.H., Radovich, T., Pant, A., et al., 2014. Integration of cover crop and Biochar for soil and plant health management in a short-term vegetable cropping system. Applied Soil Ecology. 82, 26–37.

[15] Chaichi, W., Djazouli, Z., Zebib, B., et al., 2018. Effect of biochar on fababean growth and yield. Compost Science and Utilization. 26(4), 279–285.

[16] Damodaran, P.N., Udaiyan, K., Roh, K.S., 2012. Mycorrhizal dependency in certain Indian cotton cultivars. Research in Plant Biology. 2, 55–66.

[17] Hassan, D., Thamer, T., Mohammed, R., et al., 2023. Calibration and Evaluation of AquaCrop Model Under Different Irrigation Methods for Maize (*Zea mays* L.) in Central Region of Iraq. In: Kallel, A., Barbieri, M., Rodrigo-Comino, J., et al. (Eds.). Selected Studies in Environmental Geosciences and Hydrogeosciences. CAJG 2020. Advances in Science, Technology & Innovation. Springer: Cham. DOI: https://doi.org/10.1007/978-3-031-43803-5_10

[18] Jaafer, A.A., Mohammed, R.J., Hassan, D.F., 2020. Studying the thermodynamic parameters for the evaluation of potassium availability by adding organic matter.

Biochemical & Cellular Archives. 20(1), 785–789.

[19] Jafaar, A.A., Mohammed, R.J., Hassan, D.F., 2022. Effect of phosphorus fertilizer and irrigation level on desert soil management and potato yield. International Journal of Agricultural & Statistical Sciences. 18(2), 689–696.

[20] Arty, B., Budi, S.W., 2021. Mycorrhizal dependency and growth response of *A. chinensis* (Osbeck) Merrill and *Pongamia pinnata* (L.) pierre in soil media with low pH and high aluminium. IOP Conference Series: Earth and Environmental Science. 870, 1–9.

[21] Akol, A.M., Nassif, N., Jaddoa, K.A., et al., 2021. Effect of irrigation methods, tillage systems and seeding rate on water consumption, biological yield and harvest index of wheat (*Triticum aestivum* L.). International Journal of Agricultural & Statistical Sciences, 17, 1467–1477.

[22] Mei Shi, S., Chen, K., Gao, Y., et al., 2016. Arbuscular mycorrhizal fungus species dependency governs better plant physiological characteristics and leaf quality of mulberry (*Morusalba* L.) seedlings. Frontiers in Microbiology. 7, 1–12.

[23] Jafaar, A.A., Mohammed, R.J., Hassan, D.F., et al., 2023. Effect of foliar seaweed and different irrigation levels on water consumption, growth and yield of wheat. IOP Conference Series: Earth and Environmental Science. 1252(1), 012057.

[24] Giovannini, L., Palla, M., Agnolucci, M., et al., 2020. Arbuscular Mycorrhizael fungi and associated microbiota as plant biostimulants: Research strategies for the selection of the best performing inocula. Agronomy. 10(1), 1–14.

[25] Mohammed, I.Q., Alkobaisy, J.S., 2024. Effect of bio, vermicompost and mineral fertilizers on some soil properties and soybean (*Glycine Max* L.) growth and productivity. Proceedings of the Fourth Al-Noor International Conference for Science and Technology (4NICST2022); Istanbul, Turkey; 17–18 August 2022. pp. 15–28.

[26] Khalifa, K.M., 2016. The effect of pollination with Mycorrhizae and Azotobacter in increasing the efficiency of chemical fertilizer application of wheat crop (*Triticum aestivum* L.) growing in gypsum soil. Al-Furat Journal of Agricultural Sciences/Third Agricultural Conference. 12(4), 30–37.

[27] Meng, L.P., Wua, Y., Zha, Q., et al., 2023. Secondary vegetation succession on the Loess Plateau altered the interaction between arbuscular mycorrhizael fungi and nitrogen-fixing bacteria. Forest Ecology and Management. 530, 120744.

[28] AlJanaby, Z.A.A., Obeed, Z.O., Hamza, L.M., et al., 2020. The effect of compost NP and salicylic acid spray for quantity and quality of luxury variety grapes (*Vitis vinifera* L.). Plant Archives. 20(1), 2691–2694.

[29] Alkobaisy, J.S., Abdel Ghani, E.T., Mutlag, N.A., et al., 2021. Effect of vermicompost and biochar on the growth and yield of broccoli and some soil properties. IOP Conference Series: Earth and Environmental Science. 761, 1–6.

[30] Mohammed, S.J., Alkobaisy, J.S., Saleh, J.M., 2019. Effect of earthworm on lettuce production through the recycling of organic and bio- compost production. Asian Soil Research Journal. 2(1), 10.

[31] Mohammed, R.J., Suliman, A.A., 2023. Land suitability assessment for wheat production using analytical hierarchy process and parametric method in Babylon Province. Journal of Ecological Engineering. 24(7), 75–87.

[32] Mohammed, R.J., Hameed, I.A., Thamer, T.Y., 2022. Effect of using different types of well water in Karbala Governorate on soil and plant. Ecological Engineering & Environmental Technology. 23(3), 202–207.

[33] Levinsh, G., Vikmane, M., Kirse, A., et al., 2017. Effect of vermicompost extract and vermicompost-derived humic acids on seed germination and seedling growth of hemp. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. 71(4), 286–292.

[34] Ali, Z.A., Hassan, D.F., Mohammed, R.J., 2021. Effect of irrigation level and nitrogen fertilizer on water consumption and faba bean growth. IOP Conference Series: Earth and Environmental Science. 722(1), 012043.

[35] AlJanabi, A.Z., Ameer, A.J., Israa, H., et al., 2019. Effect of adding different levels of organic manure and potassium fertilizer in the yield growth of wheat (*Triticum aestivum* L.). Plant Archives. 19, 791–795.

[36] Alkobaisy, J.S., 2023. Factors affecting mycorrhizael activity. In: Nogueira de Sousa, R. (Ed.). Arbuscular Mycorrhizal Fungi in Agriculture - New Insights, 1st ed. IntechOpen: Zagreb, Croatia pp. 1–12.

[37] Wang, M., Liu, S., Zhang, S., et al., 2016. QTL mapping of seedling traits in cucumber using recombinant inbred lines. Plant Breeding. 135(1), 124–129.

[38] Wani, F., Ahmad, D., Ali, T., et al., 2015. Role of microorganisms in nutrient mobilization and soil health—A review. Journal of Pure and Applied Microbiology. 9, 1401–1410.