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Effect of Mycorrhiza and Nitrogen Fixing Bacteria on Growth Characteristics and Nutrient Uptake of Maize (*Zea mays* L.) at Different NP Levels.

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ABSTRACT
A pot experiment was conducted from July to October 2024 in the plastic house belonging to the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, located at (35 32 18.4 N and 45 21 55.3E) to examine the effect of mycorrhiza, nitrogen-fixing bacteria, and the interaction between them on maize (Zea mays L.) growth and nutrient uptake at different levels of NP fertilizers (0%, 25 %, 50 %, 75 %, 100 %) of dose, using urea and triple superphosphate as the source of NP. A Factorial experiment was conducted using a complete randomized design (CRD) with three replicates in silty clay soil collected in Kani Panka at a depth of 15–30 cm. Before harvesting, the plant height and chlorophyll intensity were measured, and then the plants were harvested to measure root and shoot dry weight, root colonization, and N, P, Fe, Mn, Zn, and Cu concentration in dry shoots. The results show that co-inoculation led to significantly greater plant height 85.67 cm, chlorophyll intensity (61.00 SPAD), root dry weight (3.46 g) and shoot dry weight (8.18 g) at 75% NP, while root colonization (77.67%) was highest at 50% NP fertilizer. Maize shoot N, P, Fe, Mn, Zn, and Cu content were respectively observed at (18.80 g Kg^{-1}, 1.37 g Kg^{-1}, 94.37 µg g^{-1}) at 50 % NP, (69.00 µg g^{-1}, 94.83 µg g^{-1}, and 44.00 µg g^{-1}) at 75 % NP fertilizer. Additionally,inoculation by Azotobacter *chroococcum* alone significantly increased plant growth and nutrient uptake, but mycorrhiza resulted in less effective for increases in plant growth and nutrient uptake compared to control.

***Keywords*:** Azotobacter *Chrococcum,* Biofertilizers, Chemical fertilizers, Maize, Mycorrhiza.

INTRODUCTION

Maize (*Zea mays* L.), one of the most significant food crops after wheat, and is evolving into a strategic commodity with significant economic value that supports both farmers' incomes and the national economy. Due to its many uses like food for animal feed and other industrial products, it is a significant source of carbohydrates, proteins, fiber, oils, vitamins, and nutrients. The second most grown crop after wheat, maize contributes significantly to the world's food supplies with an estimated global production of 1137 million tonnes. [1 and 2].

Chemical fertilizers are vital as they supply plants with nutrients that Their concentrations in the soil below the critical level. Current agricultural structures rely heavily on the non-stop application of mineral inputs, which include mineral fertilizers, usually nitrogen (N), phosphorus (P), and potassium (K), that contribute to overall growing yields. However, additionally, they lead to the decline of the soil quality, soil degradation, soil health, human health, and climate change around the world [3]. and increase the environmental pollution and increase costs [4]. As a result, there is a need for innovative and sustainable agricultural practices to tackle these challenges.

Using microbial biofertilizers has been considered a valuble alternative way for enhancing soil fertility and soil health and increasing plant growth and crop yield in sustainable agriculture practices. The application of these environmentally friendly biofertilizers enhances nutrient uptake and water absorption, promotes plant growth, and decreases environmental pollution. These prospective biological fertilizers are crucial for soil productivity, sustainability, and environmental preservation, as they serve as eco-friendly and cost-effective resources for farmers [5 and 6].

Co-inoculation of maize seeds with mycorrhiza and Azotobacter *chroococcum is* based on competitive and efficient Azotobacter fixing atmospheric nitrogen, which supplies nitrogen, which is the most crucial element for plant development and metabolic activities. Mycorrhizal fungi Boost plant water and nutrient absorption, improving fertility, health, and structure of soil and providing various advantageous applications in agriculture [7 and 8].This study aims to investigate the possibilities of sustainable agriculture methods, namely the use of nitrogen-fixing bacteria and mycorrhiza as biofertilizers to improve maize development, nutrient absorption, soil health, and environmental resilience and so lower the use of chemical fertilizers. This work aims to find environmentally appropriate substitutes for conventional mineral fertilizers by looking at the cooperative action of these biofertilizers in enhancing nutrient absorption and yield.

Materials and Methods

Study location

The experiment was carried out in the plastic house of the Department of Horticulture in the College of Agricultural Engineering Sciences, University of Sulaimani, With GPS reading of (35 32 18.4 N and 45 21 55.3 E), and in the laboratory of soil microbiology within the Natural Resource Department during 20th Jun to 5th Oct 2024. The soil used in this study was silty clay and belongs to the Vertisol order based on the USA soil taxonomy. The soil sample was collected from the research field station in Kani Panka (35 22 31.6 N and 45 43 13.5 E), which is 44 km east of Sulaimani (Table,1). We collected the soil at a depth of between 15-25 cm. The soil samples were air-dried, ground, and sieved through a 4 mm sieve and stored in the trashcan in the soil microbiology laboratory.

Table 1: some physical, chemical and microbiological properties of the soil used in the experiments.

Soil properties	Values
Sand (g kg^{-1})	46
Silt (g kg^{-1})	510
Clay (g kg^{-1})	444
Texture	Silty clay
pH	7.80
ECe $dS.m^{-1}$ at 25C	0.23
Organic matter (g kg^{-1})	12.20
Calcium Carbonate (g kg^{-1})	231.6
Available Nitrogen (mg kg^{-1})	0.11
Available phosphorus (mg kg^{-1})	5.89
Available potassium $mmol^{-1}$	35.50
Total Bacteria	1.6×10^7 g/ml
Total Fungi	1.96×10^5 g/ml

(6 kg) of sieved soil that was collected was added to each pot (20 cm in diameter and 30 cm) depth. The urea fertilizer 46% N was used as the nitrogen source at the rate of (100 kg ha^{-1}) applied in two doses: the first at planting and the second one month after planting. Phosphorus was added as triple superphosphate, which contains 20% phosphorus, at a rate of (80 kg ha-1), and it was applied all at once when planting following fertilizer guidelines [29].

The Agricultural Research Directorate in the Sulaimani Ministry of Agriculture and Water Resources provided us sterilised maize seeds. All pots both inoculated and non-inoculated will have five levels of NP fertilizers (0% NP, 25%NP, 50%NP, 75%NP and 100%NP) of the recommendation dose added to them. Arbuscular mycorrhizal fungus were obtained by cultivating a host plant in sterile soil under carefully monitored conditions, mycorrhizal fungi were able to colonies it and produce mycorrhizal inoculum. Following adequate colonisation, the substrate containing the infected root fragments, hyphae, and spores was removed and utilized as the inoculum then it was added to some of the potted soil. To apply the microbial inoculants, 120 grams of mycorrhizal inoculum—containing spores, hyphae, root fragments, and colonized root pieces—was thoroughly mixed into the potting soil. In addition, 3 milliliters of a pure Azotobacter chroococcum culture were applied directly into each planting hole. Some pots received only the mycorrhizal inoculum, others only Azotobacter chroococcum, and a third group received a combination of both inoculants.Then the maize seed were planted in each pot treatment. With three replicates per treatment, the pots were set in plastic house benches in a total randomized configuration CRD. Following germination, the seedlings were thinned to four maize plant per pot and grown in the plastic house under natural light. To ensure consistent soil moisture, pots were routinely weighed and watered to maintain approximately 70% of the field capacity throughout the duration of the experiment. he plant height and chlorophyll intensity were measured following development prior to harvest; subsequently, the plants were taken to assess root colonization. Following shoot and root biomass was sampled at 10 weeks after planting and put in oven drying at 65 C° for 72 hours. The dried shoot and root were weighted to find the shoot and root dry weight (g), and the shoot ground was sieved with 0.5 mm and we determined N using the Kjeldahl method , P Measured colorimetrically using the molybdenum blue method, Fe, Mn, Zn, and Cu Quantified using Atomic Absorption Spectrophotometry (AAS) in the dry shoot was ascertained. We used XLSTAT version 12 program for statistical analysis. Least Significant Difference LSD at a significant level of at $p \leq 0.05$ was used to all possible comparisons among the mean values after they demonstrated their significance in the general test.

Results and Discussion

Plant Height (cm)

Azotobacter chroococcum and mycorrhiza co-inoculation greatly increased plant height in maize at all NP fertiliser levels ($p < 0.05$), as shown in Table 2. Compared to the plants that didn't receive any inoculation (48.08 cm), the tallest plants (85.67 cm) were seen when both Azotobacter chroococcum and mycorrhiza were used together at the 75% NP application level. The height of the plants increased significantly with Azotobacter chroococcum inoculation alone, reaching 80.33 cm at 75% NP. With a peak value of 75.67 cm at the same NP level, mycorrhiza by itself also markedly increased plant height. These findings imply that moderate NP fertilisation and microbial biofertilisers work in concert, possibly as a result of increased nutrient availability and uptake efficiency.

While some treatment differences were not statistically significant, the statistical analysis (LSD at $p \leq 0.05$) confirmed that several treatments differed significantly from the control. This suggests that the relationship between the type of biofertilizer and nutrient availability influences how tall plants react to microbial inoculation and NP levels.

The rising of maize plant height in co-inoculated plants of *Azotobacter chroococcum* and Mycorrhiza may be due to the importance of bacteria in increasing plant growth by nitrogen fixation and supplying available nitrogen to the soil, which is taken up by the plant. Nitrogen is also important for photosynthetic rate and vegetative growth, and as a result, for increasing plant height [9], and might result from mycorrhiza's ability to increase a plant's access to nutrients through hyphae that can reach through soil that plant roots cannot, allowing plants to obtain nutrients through mycorrhizal pathways [9].

Chlorophyll Intensity (SPAD)

The data in Table 2 shows that when maize was grown with both mycorrhiza and *Azotobacter chroococcum*, it had a much higher chlorophyll intensity than the control group, and this intensity increased with higher NP levels. The highest chlorophyll intensity reached was 61.00 SPAD at 75% NP, while the control group had the lowest value of 24.75 SPAD. When the plants were only treated with mycorrhiza, the chlorophyll intensity also increased, with the best result being 53.83 SPAD at 50% NP, compared to the control's 24.75 SPAD at 0% NP. Table 2 shows that the maize plant treated with *Azotobacter chroococcum* had a notable increase in chlorophyll intensity, reaching a high of 56.33 SPAD with 50% Application, while the control group at 0% NP had a lower value 24.75 SPAD.

The heightened chlorophyll concentration in maize plants co-inoculated with mycorrhiza and Azotobacter chroococcum may result from the conversion of atmospheric nitrogen into soil-accessible forms, hence facilitating nitrogen absorption by the plants. which enhance plant growth, which leads to a rise in the total chlorophyll content of maize plant leaves, which increases the green color of the plant and contains more chlorophyll, which gives the important value of chlorophyll intensity on maize plants since N is the contributes in forming chlorophyll or it is a part of its structure. [10].

Table 2: Effect of mycorrhiza, *Azotobacter chroococcum* and their interaction with NP on maize plant height and chlorophyll intensity*.

Treatments	Plant height (cm)	Chlorophyll intensity (SPAD)
NP 0	48.08 c	24.75 d
NP 25	62.17 b	40.67 c
NP 50	70.83 a	48.92 a
NP 75	71.33 a	47.75 a
NP 100	62.00 b	42.50 b
NP 0 + Azo	51.17 f	27.50 e
NP 25 + Azo	67.33 c	47.00 b
NP 50 + Azo	76.67 b	56.33 a
NP 75 + Azo	80.33 a	55.50 a
NP 100 + Azo	66.33 c	49.00 b
NP 0 + My	51.00 d	26.50 g
NP 25 + My	65.33 b	45.33 bc
NP 50 + My	75.67 a	53.83 a
NP 75 +My	75.67 a	53.00 a
NP 100+ My	65.50 b	46.50 b
NP 0 + Azo + My	55.33 ij	29.00 hi

NP 25 + Azo + My	68.67 de	50.00 d
NP 50 + Azo + My	78.67 b	56.67 b
NP 75 + Azo + My	85.67 a	61.00 a
NP 100 + Azo + My	67.00 ef	53.00 cd

*: Similar letter or letters in each column separately means non- Signifiant difference between them and via versa
P0: control, NP: Nitrogen and Phosphorus, Azo: Azotobacter, My: Mycorrhiza, Azo + My: Azotobacter with Mycorrhiza mean the same letters are not significant at ($p \leq 0.05$).

Shoot dry weight (g)

Table 3 demonstrates that co-inoculating maize plants with microbial biofertilizers, mycorrhiza, and Azotobacter *chroococcum* of various levels of NP application significantly improves shoot dry weight compared to the control heights. Shoot, dry weight was observed in 75 % NP (8.18g), while the lowest value was (4.06 g). When the soil inoculated with mycorrhiza significantly affected increasing shoot dry weight at different NP applications compared to the control, the maximum shoot dry weight observed at 75% NP was (7.45 g) compared to the control (4.06g). However, inoculated plants with only Azotobacter *chroococcum* shoot dry weight of maize plants significantly increased by (8.60 g) at 50% NP application compared to the control (4.06g).

The increase of maize shoot dry weight in co-inoculated plants may be due to nutrient and water uptake from the soil rhizosphere, which increases Plant growth, resulting in shoot dry weight increase [11], or maybe due to the role of Azotobacter *chroococcum* to fix atmospheric nitrogen, Available nitrogen in soil has not been estimated, which is taken up by plants and increases growth and biomass, resulting in increased shoot dry weight, and due to the production of phytohormones by bacteria, which increase more plant growth and shoot dry weight [12].

Root dry weight (g)

Root dry weight of maize plants are significantly different with the application on co-inoculantion of mycorrhiza with Azotobacter *chroococcum*. Inoculation treatment produced a higher root dry weight (3.46g) observed in 75% NP compared to the control (1.32g). Inoculation of the maize plant with *Azotobacter chroococcum*, shown in Table 3, significantly increased root dry weight under different NP percentage applications, producing the highest value, which was (2.86 g), compared to control produced a mean of (1.32 g). The result showed that inoculating the maize plant with mycorrhiza significantly increased root dry weight at 75% NP, which produced the maximum value (2.67g).

The significant role and capacity of mycorrhiza to Root zone area and increase root surface, extend diameter, density, and branching—all of which improve the plant's ability to absorb more water and nutrients—may be the cause of the co-inoculated maize shoots' increased dry weight. Causing the plant to have more ability to growth with more root biomass rise root dry weight. Also may be due to the role of bacteria *Azotobacter chroococcum* by fixing atmospheric nitrogen which uptake by maize plant and increase plant growth and biomass resulting in increasing root dry weight [9].

Root colonization (%)

Table 3 demonstrates that inoculation with microbial biofertilizers significantly increased root colonization percentages in maize plants across all NP fertilizer levels. The highest root colonization (77.67%) occurred at the 50% NP application with co-inoculation of *Azotobacter chroococcum* and mycorrhiza, while the lowest root colonization (24.83%) was observed at the control 0% NP level. Interestingly, mycorrhiza alone also showed a substantial improvement in root colonization, reaching its maximum at 50% NP (74.00%). Similarly, compared to the control at a value of (24.83%) at 0% NP level, *Azotobacter chroococcum* alone enhanced colonization at each, with a peak value of (65.67%) at 50% NP level compared to the control (24.83%) at 0% NP level. The highest degree of colonization, which was 50% NP fertiliser, implies that moderate nutrient supply creates the best circumstances for microbial colonisation. At this stage, the plant needs more nutrients, which makes the roots exude more, which attracts and supports microbial communities. On the other hand, low NP levels (0%) might stop plants from growing and exuding, which would make it harder for microbes to settle in. High NP levels (above 50%), which we will not go into here, often make plants less reliant on microbial symbionts, which leads to less colonization. The microbes' complementary roles in co-inoculated treatments are responsible for the increased root colonization. While Azotobacter produces plant hormones like auxins and gibberellins that aid in root growth, mycorrhizae collaborate with plant roots by sending out tiny threads into the soil to find water and nutrients, particularly P. Low phosphorus greatly increases root infection, and mycorrhizae are not sensitive to phosphorus. Better resource uptake is made possible by enhanced microbial colonization and root architecture. These microbes' cooperative relationship probably creates an environment that is conducive to colonization and nutrient uptake in rhizospheres [13]. These findings support previous studies on microbial consortia improving plant-microbe symbiosis [14].

Table 3: Effect of mycorrhiza, *Azotobacter chrococcum* and their interaction with NP on maize shoot dry weight, root dry weight and root colonization.

Treatments	Shoot dry weight (g)	Root dry weight (g)	Root Colonization %
NP 0	4.06 c	1.32 d	24.83 e
NP 25	5.71 b	2.01 b	35.67 d
NP 50	7.20 a	2.48 a	63.42 a
NP 75	7.05 a	2.34 a	57.42 b
NP 100	6.01 b	1.75 c	51.08 c
NP 0 + Azo	4.22 f	1.40 de	27.33 f
NP 25 + Azo	6.40 c	2.19 b	36.50 e
NP 50 + Azo	8.06 a	2.83 a	65.67 a
NP 75 + Azo	7.56 b	2.86 a	59.67 b
NP 100 + Azo	6.37 c	2.01 bc	52.67 cd
NP 0 + My	4.74 g	1.48 f	29.83 g
NP 25 + My	6.02 de	2.31 c	41.67 f
NP 50 + My	7.42 a	2.41 bc	74.00 a
NP 75 +My	7.45 a	2.67 a	67.67 b
NP 100+ My	6.33 cd	1.85 de	59.67 c
NP 0 + Azo + My	4.92 gh	1.54 ij	33.00 jk
NP 25 + Azo + My	6.59 bcd	2.53 cd	46.00 gh
NP 50 + Azo + My	8.10 a	2.61 c	77.67 a
NP 75 + Azo + My	8.18 a	3.46 a	71.00 b
NP 100 + Azo + My	6.66 bcd	2.14 ef	62.00 c

P0: control, NP: Nitrogen and Phosphorus, Azo: Azotobacter, My: Mycorrhiza, Azo + My: Azotobacter with Mycorrhiza mean the same letters are not significant at ($p \leq 0.05$).

Nitrogen uptake (g Kg^{-1})

The use of biofertilizer had a significant ($p \leq 0.05$) impact on the nitrogen uptake in plant shoots. Data in Figure 1 show co-inoculation of maize plants with microbial biofertilizers, mycorrhiza with *Azotobacter chroococcum* significantly enhances shoot nitrogen content across varying NP fertilizer levels compared to non-inoculated control. The highest N content (18.80 g kg^{-1}) was recorded at 50% NP application under co-inoculation, while the lowest value (12.05 g kg^{-1}) occurred in the non-inoculated control at 0% NP level. Then, followed by inoculation with *Azotobacter chroococcum* alone, its value achieved peak N content at 50% NP (17.98 g kg^{-1}), surpassing the control (12.05 g kg^{-1}) compared with the control. In the same way, maize plants treated only with mycorrhiza had the highest nitrogen uptake at 50% NP (17.50 g kg^{-1}), which was much better than the control (12.05 g kg^{-1}). Co-inoculation consistently outperformed single treatments, indicating synergistic microbial interactions.

The enhanced N uptake and subsequent improvement in shoot dry weight can be attributed to the following mechanisms: *Azotobacter chroococcum* takes nitrogen from the air and turns it into ammonium (NH_4^+) that plants can use, which directly increases nitrogen levels and helps plants grow more. Azotobacter synthesizes auxins and gibberellins, stimulating root elongation and shoot development and thereby increasing biomass [15]. Co-inoculation brings together mycorrhiza's ability to make phosphorus available and Azotobacter's ability to fix nitrogen, providing a balanced supply of nutrients that helps plants grow. The best possible synergy between moderate fertilisation and microbial inoculants, which improves nutrient uptake, is reflected in the increase in shoot nitrogen concentration up to 50% NP application. After this, too much NP input reduces the benefits of microbes, may dilute nutrients, and can cause microbial or physiological stress, which lowers the amount of N in plant shoots. [16,17 and 18].

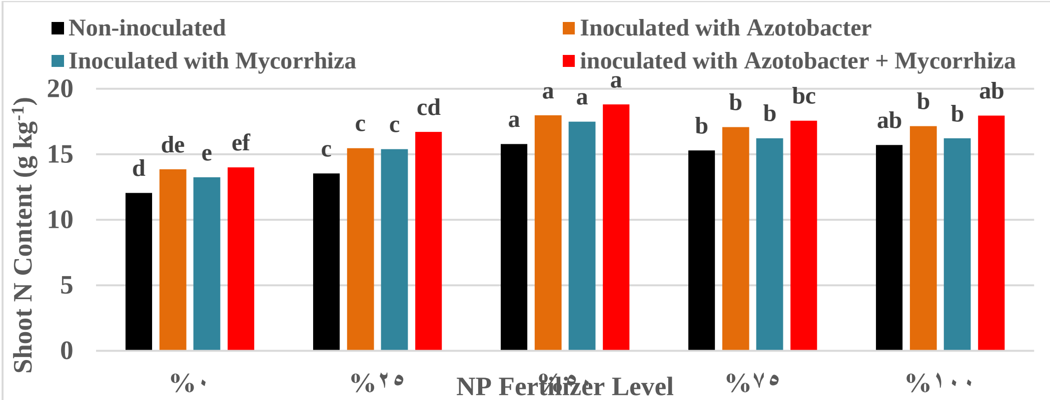

Figure 1: Effect of mycorrhiza and *Azotobacter chrococcum* inoculated and non – inoculated and their interaction on maize nitrogen uptake at different NP levels.

Figure 2 displays the coefficient of determination (R^2) for shoot nitrogen uptake at various NP levels. Inoculating with *Azotobacter chroococcum* produced the greatest value 0.9019, while co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza came in second at 0.9009 and *mycorrhiza treatment had the lowest value 0.8919.*

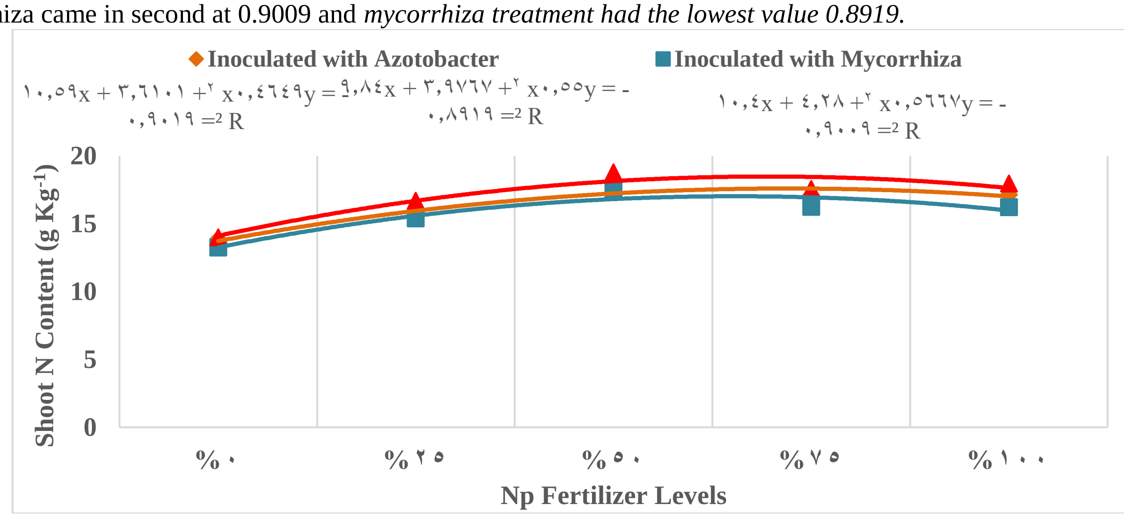

Figure 2: The relation between nitrogen uptake and NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chrococcum* and their interaction on maize plant

Phosphorus uptake (g Kg^{-1})

Figure 3 Phosphorus (P) content in shoots was also significantly ($p \leq 0.05$) affected by increasing biofertilizer treatments at different NP levels. The highest P content (1.37 g kg^{-1}) was recorded in the co-inoculation of *Azotobacter chroococcum* with mycorrhiza treatment at 50% NP level, reflecting an increase of P content compared with non-inoculated control treatment (0.49 gkg^{-1}), followed closely by mycorrhiza alone (1.30 gkg^{-1}) at 50 % NP level. Azotobacter had a significant effect on P content, peaking at 75% NP (1.14 gkg^{-1}) compared to the control (0.49 gkg^{-1}) at NP levels. Significant increase in P content: these values far exceeded those of the control treatment, particularly under lower NP percentage levels, where phosphorus availability is typically constrained. Mycorrhiza plays a crucial role in phosphate solubilization and uptake, especially in soils with limited available phosphorus. The extra radical hyphae of mycorrhizal fungi extend beyond the depletion zone, accessing less available P pools [19]. This is particularly valuable in phosphorus-fixing soils, where a large proportion of applied phosphorus becomes unavailable to plants due to chemical immobilization. Therefore, the use of biofertilizers such as mycorrhiza can reduce dependence on chemical phosphorus fertilizers, thereby minimizing nutrient runoff and associated environmental risks. [20]. The nitrogen in NP fertilizer has a role in promoting root growth and architecture, boosts plant metabolism, mycorrhizal colonization under balanced conditions and Nitrogen improves phosphate transporter activity, Therefore, nitrogen supports phosphorus uptake but does not replace the need for biofertilizers that target P availability directly [23].

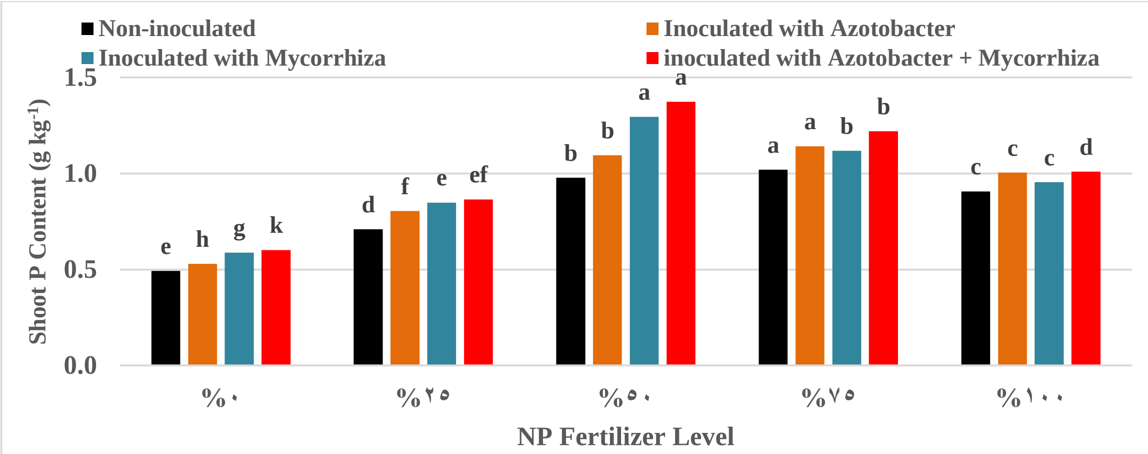

Figure 3: Effect of mycorrhiza and Azotobacter *chrococcum* inoculated and non – inoculated and their interaction on maize Phosphorus uptake at different NP levels.

Figure 4 displays the coefficient of determination (R^2) for shoot phosphorus content at various NP levels. Inoculating with mycorrhiza produced the greatest value 0.8864, while co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza came in second at 0.885 and *Azotobacter chroococcum treatment had the lowest value 0.6565.*

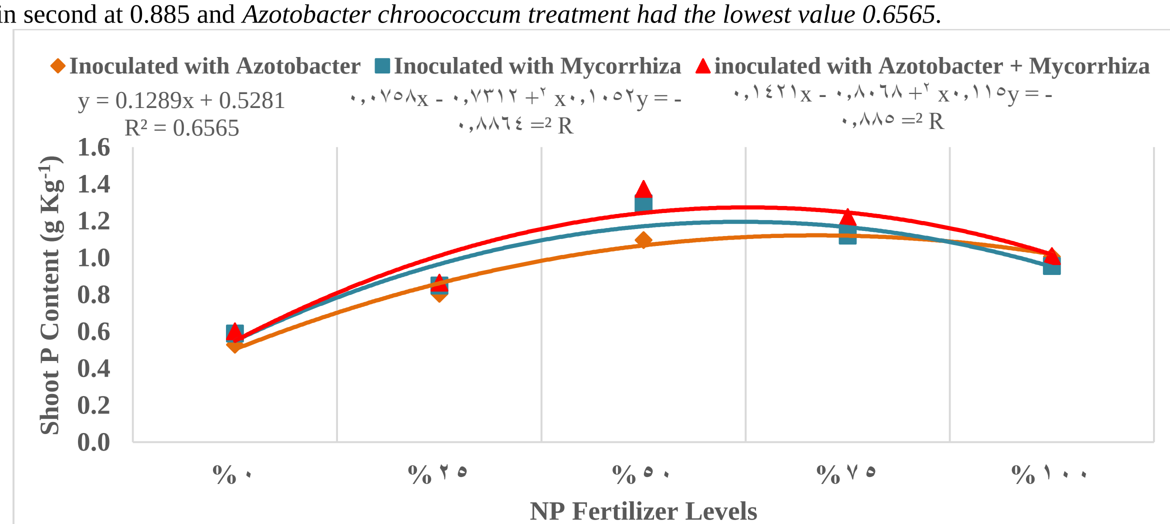

Figure 4: The relation between Phosphorus uptake (g Kg^{-1}) and NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chrococcum* and their interaction on maize plant.

Iron uptake (µg g^{-1})

Under different NP levels, the use of microbial biofertilizers significantly ($p \leq 0.05$) raised the iron uptake in maize plants. Figure 5. Co-inoculation with mycorrhiza and *Azotobacter chrococcum* markedly increased Fe content compared to non-inoculated controls. The highest Fe content (94.37 µg g^{-1}) was observed at 50% NP under co-inoculation of Azotobacter and mycorrhiza, while the lowest value (19.43 µg g^{-1}) occurred at 0% NP at control without inoculation. Across all NP levels, the trend showed enhanced Fe uptake under microbial treatments. Similarly, at 50% NP, both the concentration of Fe by mycorrhiza (85.40 µg g^{-1}) and by *Azotobacter (*90.93 µg g^{-1}) treatments demonstrated higher Fe content compared to the control (19.43 µg g^{-1}). It means co-inoculation of Azotobacter and mycorrhiza, caused 4.86 times increase in Fe concentration. There are several reasons why biofertilizer treatments result in better iron acquisition. Mycorrhiza improves the uptake of iron by expanding the absorptive area of the roots and releasing it through acidification and enzymatic activity. However, it is known that Azotobacter produces siderophores, which are low-molecular-weight substances that bind and solubilize iron, increasing its availability to plants [21]. Additionally, the changes caused by microbes in the soil around plant roots can Ready iron in soil is not appreciated and is essential particularly in alkaline soils where iron is often lacking. This finding highlights the potential of biofertilizer strategies in addressing micronutrient deficiencies, which are a widespread problem in intensive cropping systems [22 and 23].

Because there is less Fe available in the soil at high NP levels (100 units), the concentration of iron (Fe) drops dramatically. Increased soil pH, potential root stress, and nutrient antagonistic interactions—particularly phosphorus binding with iron—are the main causes of this. In the absence of helpful microorganisms like Azospirillum (Azo) and mycorrhiza (My), which aid in enhancing iron availability, these conditions restrict iron uptake [30].

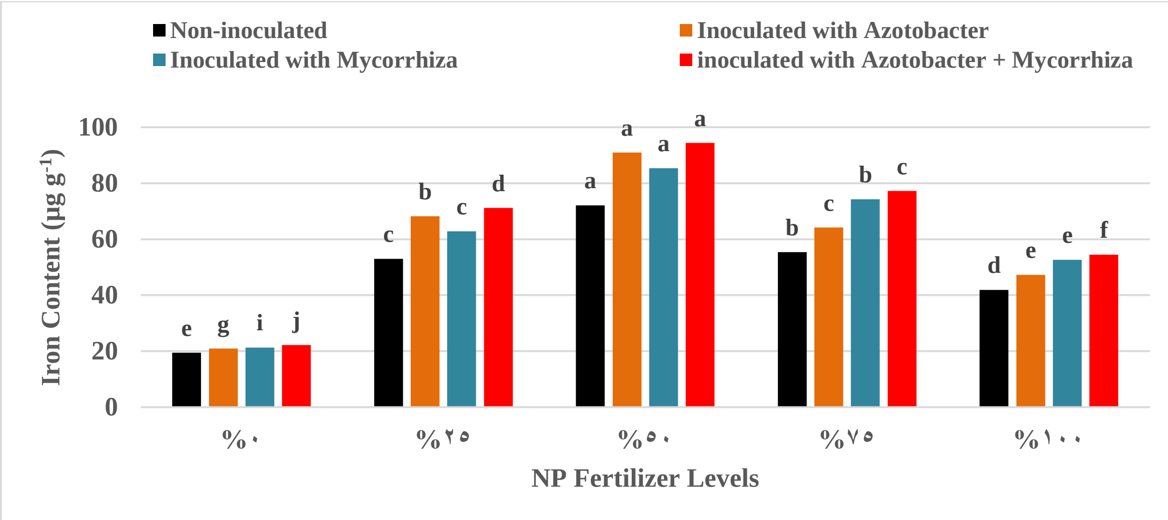

Figure 5: Effect of mycorrhiza and Azotobacter *chrococcum* inoculated and non – inoculated and their interaction on maize iron uptake at different NP levels.

Figure 6 displays the coefficient of determination (R^2) for iron uptake at various NP levels. Inoculating with mycorrhiza produced the greatest value 0.9883, while co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza came in second at 0.9747 and *Azotobacter chroococcum* treatment had the lowest value 0.9191.

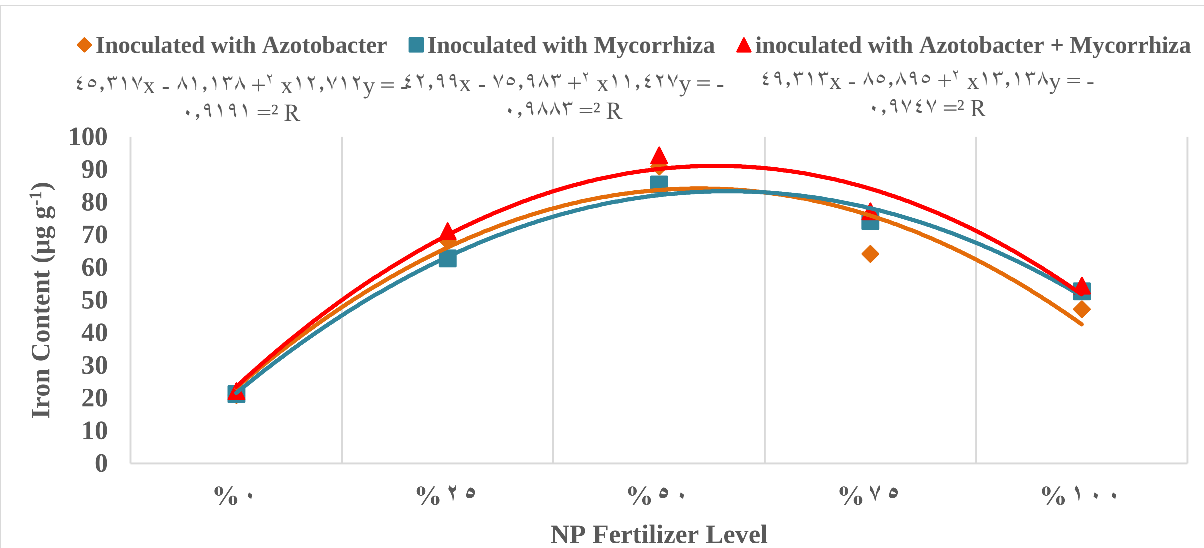

Figure 6: The relation between iton uptake NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chrococcum* and their interaction on maize plant.

Manganese uptake (µg g^{-1})

The data in Figure 7 demonstrates that co-inoculation of maize plants with microbial biofertilizers, *mycorrhiza*, and *Azotobacter chroococcum* at various levels of NP application significantly improved manganese uptake of plant tissues relative to the control. The highest Mn concentration was observed in the 75% NP application with co-inoculation *Azotobacter chroococcum* and mtcorrhiza, reaching (69.00 µg g^{-1}), while the lowest value was recorded at the control 0% NP, which was (21.38 µg g^{-1}). When the soil was inoculated with only mycorrhiza, it significantly increased Mn uptake in maize plants compared to the control across all fertilizer levels, with the maximum Mn content observed at 75% NP being (49.32 µg g^{-1}) versus (21.38 µg g^{-1}) in the control. Similarly, inoculation with only *Azotobacter chroococcum* significantly increased Mn uptake, particularly at 75% NP, where Mn concentration reached (47.30 µg g^{-1}) compared to the control.

Also, Azotobacter chroococcum might help by making organic acids and siderophores that move Mn in the area around plant roots. The combined action of these microorganisms could enhance overall nutrient availability, leading to improved Mn uptake in plant tissues. Moreover, *Azotobacter chroococcum* is known to fix atmospheric nitrogen and produce phytohormones such as auxins and gibberellins, which may stimulate root development and micronutrient uptake [24].

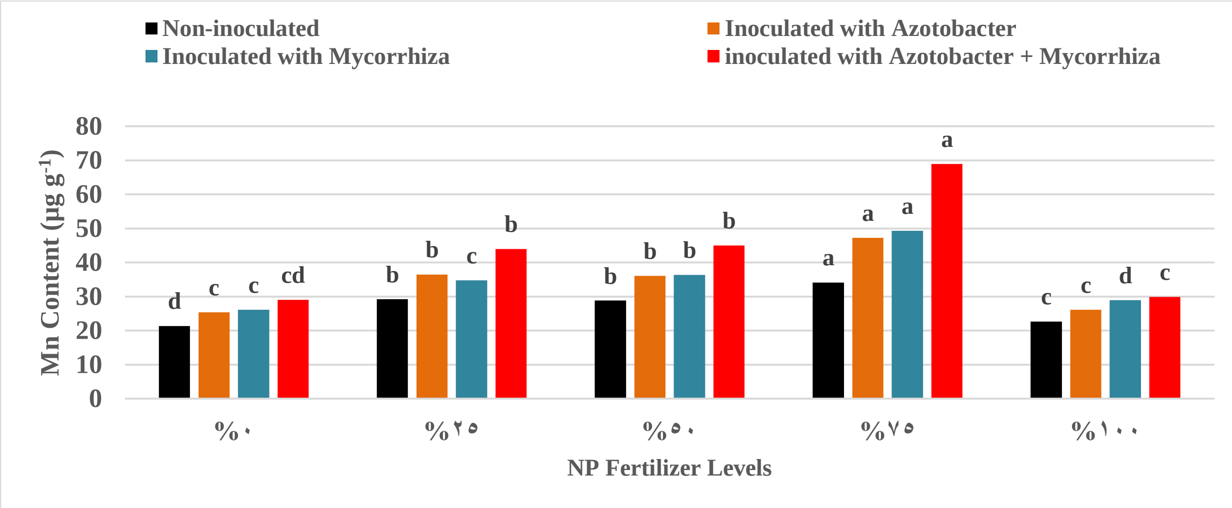

Figure 7: Effect of mycorrhiza and *Azotobacter chrococcum* inoculated and non – inoculated and their interaction on maize manganese uptake at different NP levels.

Figure 8 displays the coefficient of determination (R^2) for shoot manganese content at various NP levels. Inoculating with *Azotobacter chroococcum* produced the greatest value 0.6658, while inoculation with only mycorrhiza came in second at 0.6067 and co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza treatment had the lowest value 0.5628.

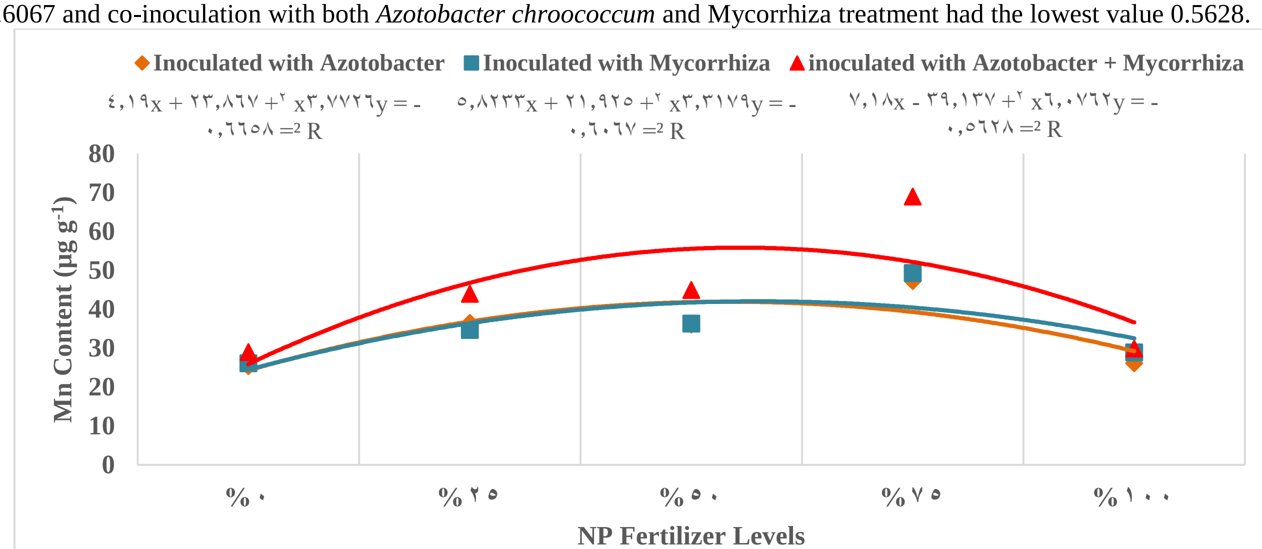

Figure 8: The relation between manganese uptake and NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chroococcum* and their interaction on maize plant.

Zinc uptake (µg g^{-1})

The study reveals that co-inoculating maize plants with mycorrhiza and Azotobacter chroococcum significantly enhanced zinc (Zn) uptake across varying NP fertilizer levels (Figure 9). The highest Zn concentration (94.83 µg g^{-1}) occurred at 75% NP with co-inoculation of Azotobacter chroococcum with mycorrhiza while the lowest value was observed in the non-inoculated control at 0% NP (18.28 µg g^{-1}). Individual inoculations also improved Zn accumulation. Mycorrhiza alone achieved peak Zn content at 75% NP (82.58 µg g^{-1}), surpassing the non-inoculated control (18.28 µg g^{-1}) at the same NP level. Similarly, Azotobacter alone showed maximum Zn uptake at 50% NP (85.33 µg g^{-1}), significantly higher than the control (18.28 µg g^{-1}). Co-inoculation consistently outperformed single treatments, suggesting synergistic microbial interactions.

The enhanced Zn uptake likely stems from multiple interconnected mechanisms, Mycorrhizal hyphae extend the root system, accessing Zn from a larger soil volume and secreting phosphatases or carboxylates that solubilize Zn bound to soil particle [25]. *Azotobacter* produces organic acids (e.g., gluconic acid), lowering rhizosphere pH and increasing Zn solubility, making it more plant-available. *Azotobacter*'s nitrogen fixation improves overall plant vigor, indirectly supporting Zn uptake, while mycorrhiza enhances phosphorus acquisition, which may stabilize Zn transport within. Azotobacter-derived auxins stimulate root branching, increasing surface area for Zn absorption, while mycorrhizal colonization amplifies this effect by improving root longevity and function. Co-inoculation balances soil nutrient availability, preventing toxicity at high NP levels (e.g., 100% NP) while ensuring sufficient nutrients at moderate levels (75% NP) to sustain microbial activity [26 and 27].

Compared to iron (Fe) and manganese (Mn), zinc uptake responded more strongly to fertiliser levels and microbial treatments (Azotobacter and Mycorrhiza). This advantage is probably due to the fact that microbial activity mobilises zinc more efficiently through organic acids and enhanced root absorption. However, because of their restricted mobility, sensitivity to soil pH, and possible antagonistic effects from high fertiliser phosphorus levels, Fe and Mn are less responsive. Therefore, the combined effects of biofertilizers and moderate fertiliser use have a greater positive impact on zinc availability and uptake [31].

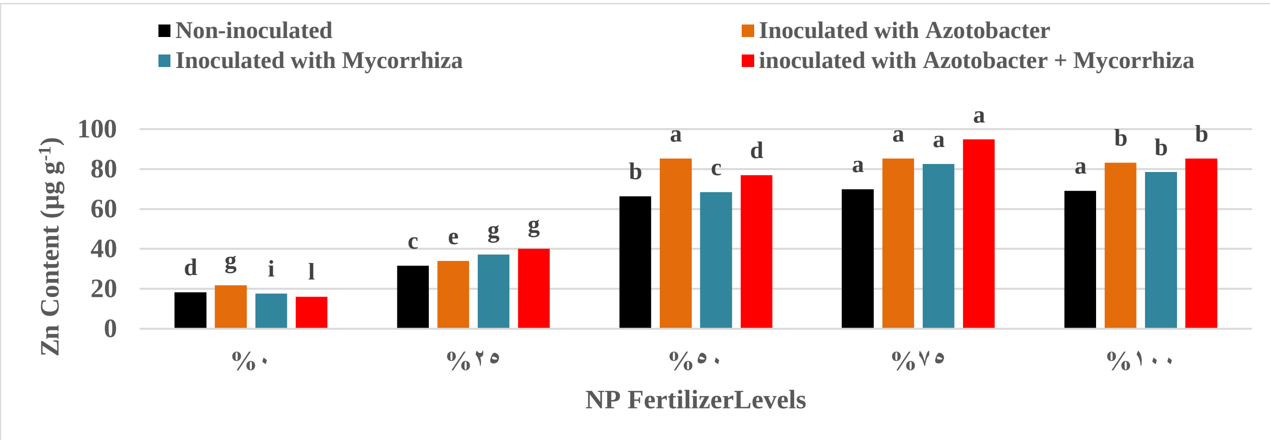

Figure 9: Effect of mycorrhiza and *Azotobacter chrococcum* inoculated and non – inoculated and their interaction on zinc uptake at different NP levels.

Figure 10 displays the coefficient of determination (R^2) for shoot zinc uptake at various NP levels. Inoculating with mycorrhiza produced the greatest value 0.9687, while co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza came in second at 0.9614 and *Azotobacter chroococcum* treatment had the lowest value 0.8863.

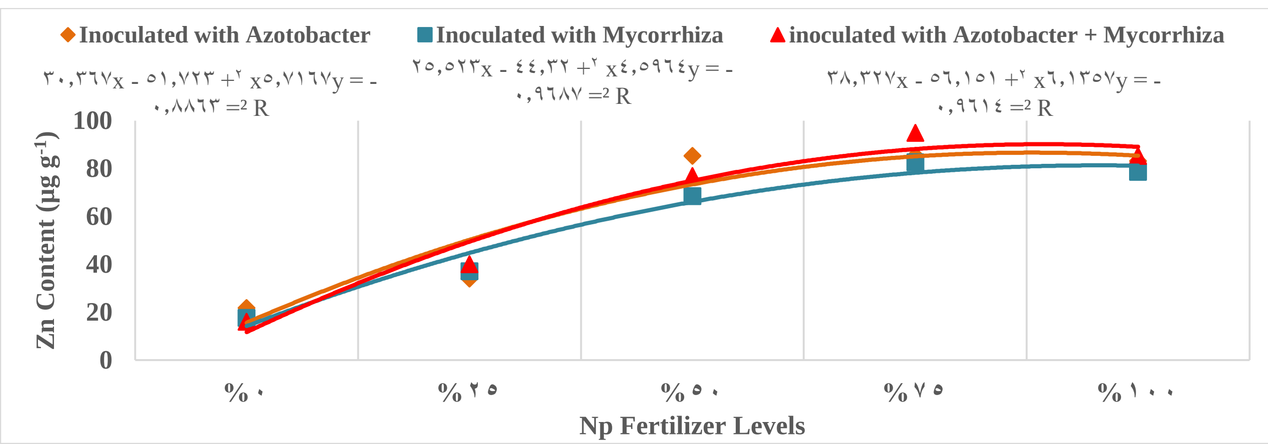

Figure 10: The relation between zinc uptake and NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chrococcum* and their interaction on maize plant.

Cupper uptake (µg g-1)

Co-inoculation of maize plants with microbial biofertilizers, *mycorrhiza*, and *Azotobacter chroococcum* significantly enhanced cupper uptake in plant tissues across different NP fertilizer levels compared to the control Figure 11. The highest Cu content was recorded at 75% NP under co-inoculation of Azotobacter *chroococcum* with mycorrhiza, reaching (44.00 µg g^{-1}), while the lowest value (35.13 µg g^{-1}) was observed in the NP control at 0% NP. Inoculation with *Azotobacter chroococcum* alone significantly improved Cu uptake, particularly at 75% NP (41.53 µg g^{-1}), followed by mycorrhiza (41.40 µg g^{-1}). Both microbial treatments showed higher Cu levels than the controls at all NP levels. Even at lower NP levels (25% and 50%), co-inoculated and singly inoculated treatments outperformed the non-inoculated control, indicating the role of microbial biofertilizers in enhancing micronutrient uptake.

Mycorrhizal fungi are well-known for forming symbiotic associations with plant roots, increasing the effective root surface area through extraradical hyphae. This extension into the soil matrix enhances the plant's ability to access immobile nutrients like Cu, as well as trace elements On the other hand, *Azotobacter chroococcum* promotes root colonization through multiple mechanisms: it produces phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which stimulate root branching and elongation, thereby creating more colonization sites for beneficial microbes [28].

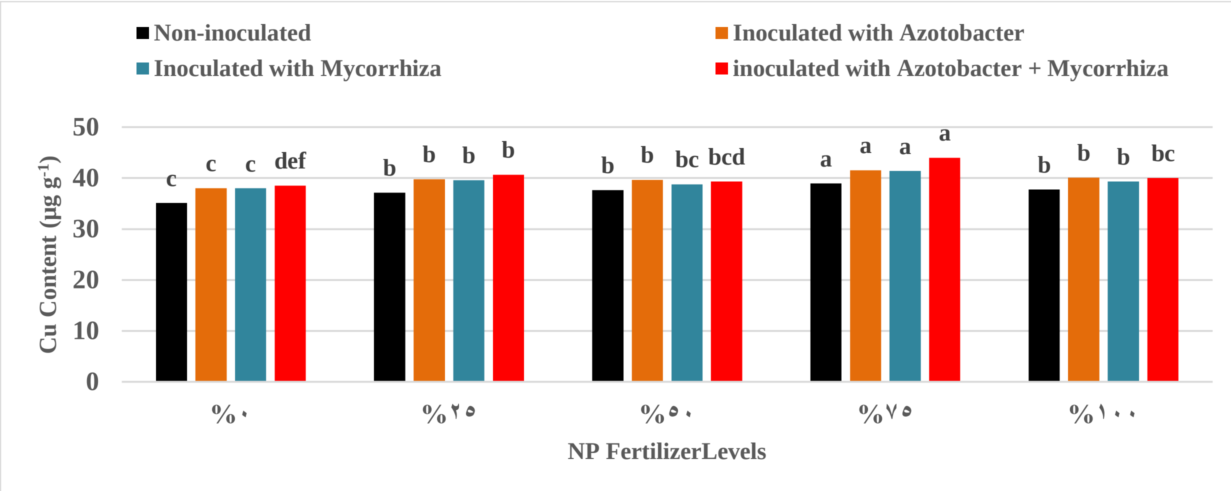

Figure 11: Effect of mycorrhiza and *Azotobacter chrococcum* inoculated and non – inoculated and their interaction on maize cupper uptake at different NP levels.

Figure 12 Figure 10 displays the coefficient of determination (R^2) for shoot cupper uptake at various NP levels. Inoculating with *Azotobacter chroococcum* produced the greatest value 0.7765, inoculation with mycorrhiza came in second at 0.4836 and co-inoculation with both *Azotobacter chroococcum* and Mycorrhiza treatment had the lowest value 0.3865.

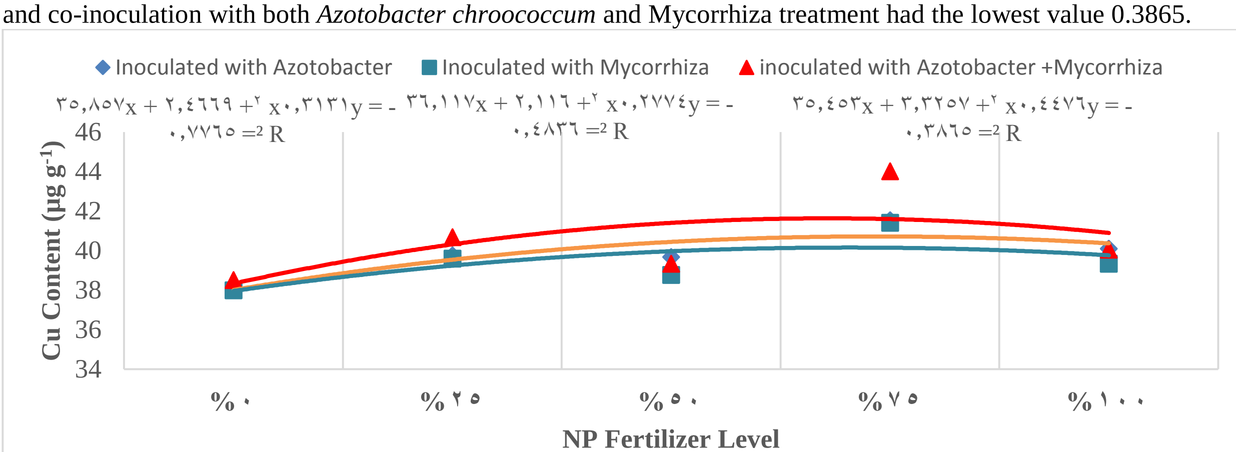

Figure 12: The relation between cupper uptake and NP levels for maize plant inoculated with mycorrhiza, *Azotobacter chrococcum* and their interaction on maize plant.

Conclusion

The application of co-inoculation of *Azotobacter chroococcum* with mycorrhiza *Glomus mosseae*, particularly in combination, significantly improved maize growth parameters such as plant height, root dry weight, shoot dry weight, chlorophyll intensity, and root colonization and nutrient concentration of N, P, Fe, Mn, Zn, and Cu in maize plants At addition of NP to a certain level in addition Inoculating maize plants only with mycorrhiza significantly increases their growth parameters and nutrient concentration under different NP-level fertilizers. When the maize plant inoculated with *Azotobacter chroococcum* significantly increases growth parameters and nutrient concentration under different NP fertilizers. The most pronounced improvements were observed at 50% and 75% of the recommended NP chemical fertilizer rates, indicating that using microbial biofertilizer inoculants can compensate for reduced chemical inputs without compromising plant performance.

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دور السماد الحيوي (المايكورايزا) والازوتوبكتر في خصائص النمو وامتصاص بعض المغذيات للذرة الصفراء تحت مستويات مختلفة من سماد (NP).

ريكار رسول إسماعيل **عمر على فتاح**

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الخلاصة

نفذت تجربة سنادين خلال شهر تموز ولغاية ايلول 2024 في قسم البستنة كلية علوم الهندسة الزراعية/جامعة السليمانية (*35 32 18.4 N/45 21 55.3E*) لدراسة تأثير المايكورايزا والبكتريا المثبة للنايتروجين و التداخل بينهما في نمو وامتصاص العناصر الغذائية لنبات الذرة الصفراء (*Zea mays L.*) و تحت مستويات مختلفة من السماد الكيمياوي*NP* (*100%, 75%, 50%, 25%, 0%*)الموصي به، استعمل سماد اليوريا وسوبرفوسفات الثلاثي كمصدر لـ*NP*. أجريت التجربة وفق التصميم العشوائي الكامل (*CRD*) بثلاث مكررات لكل معاملة في تربة طينية غرينية. اذ جمعت من منطقة كاني بانكه على عمق *15–30* سم. قبل حصاد النباتات تم قياس إرتفاع النباتات و شدة الكلوروفيل ثم حصدت النباتات وتم قياس الوزن الجاف للمجموع الجذري و الخضري، والنسبة المئوية للإصابة بألمايكورايزا وتركيز العناصر الغذائية (*Cu, Zn, Mn, Fe, P, N*) في الوزن الجاف للمجموع الخضري. اظهرت النتائج أن التلقيح الثنائي اعطي زيادة معنوية في إرتفاع النبات 85.67 سم، شدة الكلوروفيل (*SPAD 61.00*)، الوزن الجاف للجذور 3.46 غم والوزن الجاف للمجموع الخضري 8.18 غرام تحت مستوي *75 NP ٪*. بينما أعلي قيمة لنسبة الإصابة بألمايكورايزا كانت (*%77.67*) سجلت في مستوي *50 NP ٪*. أعلى قيمة للعناصر الغذائية علي التوالي (*Cu, Zn, Mn, Fe, P, N*) كانت (*18.80*غرام كغمم$^{-1}$)(*1.37* غم كغم$^{-1}$)(*94.37* غم كغم$^{-1}$) سجلت في*NP 50* ٪ و (*69.00* مايكروغرام غم$^{-1}$)، (*94.83* مايكروغرام غم$^{-1}$) و (*44.00* مايكروغرام غم$^{-1}$) في *75 NP ٪*، أضافة إلي ذلك تلقيح النباتات ب *Azotobacter chroococcum* أدي ألي زيادة معنوية في نمو النباتات و إمتصاص العناصر الغذائية ولكن تأثيرها أقل من التلقيح الثنائي، وان التلقيح بالمايكورايزا لها تأثير علي زيادة نمو وإمتصاص العناصر الغذائية مقارنة بمعاملة المقارنة (*Control*).

الكلمات المفتاحية: مايكورايزا، *Azotobacter chroococcum*، سماد الكيمياوي، نبات الذرة، سماد الحيوي.