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Combined Effects of Indigenous Arbuscular Mycorrhizal Fungi (AMF) and NPK Fertilizer on Growth and Yields of Maize and Soil Nutrient Availability

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Abstract: The excessive application of mineral fertilizers in maize cultivation leads to progressive soil contamination in the long term and increases the cost of production. An alternative to reduce over-fertilization is to perform a partial replacement with microbes that promote nutrition and growth, such as Arbuscular Mycorrhizal Fungi (AMF). A pot experiment which was followed by two field experiments was performed with and without the application of indigenous AMF in combination with five nitrogen–phosphorus–potassium (NPK) fertilization rates (100% NPK = N120P60K60; 75% NPK = N90P45K45; 50% NPK = N60P30K30; 25% NPK = N30P15K15; control = N0P0K0). The objective was to investigate whether the soil application of indigenous mycorrhizal fungi inoculum combined with NPK fertilization can provide higher maize yields and soil-available N, P, and K than chemical fertilization can alone. The greenhouse results showed that the application of AMF with a 50% NPK treatment significantly increased the plant's growth, root colonization, leaf chlorophyll content, and N, P, and K tissue content. The results from the field conditions showed that there was a highly significant yield after the treatment with AMF + 50% NPK. The study also revealed that mycorrhizal fungi inoculation increased the available soil N and P concentrations when it was combined with a 50% NPK dose. This suggests that the inoculation of fields with AM fungi can reduce the chemical fertilizer application by half, while improving soil chemistry. The results suggested that AMF inoculation can be used in integrated soil fertility management strategies.

Keywords: biofertilizer; inorganic fertilizer; mycorrhizal inoculant; plant nutrition; soil health; soil microorganisms; soil nutrients; sustainable agriculture

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1. Introduction

Maize (*Zea mays*) is one of the most commonly grown crops in the world. It is a rich source of carbohydrates and proteins, and it is consumed as a staple food by millions of people on every continent. Maize is also used in a variety of industrial products, from animal feed to biofuels [1]. It is a popular crop in Africa and its production is of great importance in terms of the calorie intake that is provides in Uganda. The maize yields in Uganda are relatively low (2 tons/ha) when it is compared to countries like South Africa with average yields of 4–6 tons/ha [2–4]. The main reasons for the low maize yields in Uganda are due to the effects of climate change factors such as poor management, pests, and diseases, the low rate of the utilization of improved seeds, drought, heat, and erratic rainfall [5,6]. Furthermore, two of the main constraints to increasing the maize productivity among Uganda's smallholder farmers are soil infertility (phosphorus-deficient soils) and economic constraints on farmers who have limited incentives to purchase mineral fertilizers [7,8]. The current nutrient use estimates are low, with Uganda applying less than 10 kg/ha of NPK fertilizers (0.8; 0.3; 0.3 kg/ha of N, P, and K, respectively) [9,10]. The inappropriate use of mineral fertilizers has resulted in reduced yields and reduced soil fertility. In contrast, the overuse of inorganic fertilizers is associated with major environmental and health problems such as water pollution and reduced soil microbial diversity [11,12]. Improving productivity through novel cropping system approaches that integrate in situ soil fertility improvement techniques is, therefore, a top priority. Soil microbes that improve the nutrient availability and uptake efficiency are, therefore, valuable technologies. Attempts are now being made to increase the yields to increase productivity and reduce the use of chemical fertilizers [13]. One strategy to improve the plants' growth and yield is their inoculation with Arbuscular Mycorrhizal Fungi (AMF) [14].

AMF is a group of soil microbes that live symbiotically with most crop species and trees. These microorganisms play important roles in soil processes such as nutrient cycling and uptake, maintaining soil health, and affecting the plant's growth by altering their root structure [14,15]. These fungi have been widely shown to play an important role in promoting plant water and nutrient uptake, mainly phosphorus (P), and also they influence the plant's growth by maintaining the soil health [16]. This is due to the extensive mycelial network of the symbiont in the soil and its association with phosphorus-solubilizing microorganisms (PSMs) to release organic acids and dissolve the less-soluble phosphates or other minerals that are immobilized by soil constituents [17]. Improved AMF-mediated growth is a result of an improved soil structure and nutrient status by the mycelium, which acts as a soil binder, reducing the nutrient and organic matter loss, increasing the water and mineral retention and increasing the plant productivity [18]. AMF are considered to be a bioremediation agent of stress as they can stimulate growth under extreme conditions [19,20]. They play an important role in overcoming the depletion of non-renewable phosphorus resources, so this technology reduces the quantitative use of chemical fertilizers [21,22]. Studies have shown that mycorrhizal fungi inoculation significantly increases the biomass accumulation and yield [23–25]. Several studies that have been conducted in Senegal and Kenya have reported significant increases in the yield with long-term mycorrhizal inoculation [26–28]. Selecting efficient microorganisms to produce qualitative and quantitative biofertilizers is a key point to consider when one is applying AMF technology to African agriculture. However, most commercial mycorrhizal inoculants either contain species from another region or do not contain viable reproductive organs, and thus, do not result in mycorrhizal colonization [29]. Therefore, the efficacy of AMF inoculation declines due to the environmental changes the occur. Moreover, inoculation with exotic commercial mycorrhizal inoculants is not successful in the presence of high levels of native arbuscular fungi [30,31]. An alternative to this problem is to use indigenous mycorrhiza from the soil. This approach has many advantages in restoration programs such as a high adaptability to the physicochemical conditions and a low environmental risk. Moreover, several examples have highlighted the benefits of the double inoculation of AMF and NPK for promoting plant growth [32–34]. However, to our knowledge, a double inoculation with AMF and NPK in maize has not been practiced in Uganda. A previous study selected AMF strains (*Gigaspora*, *Scutellospora*, *Glomus*, *Acaulospora*, *Archaospora*, *Enttrophosporaa*, and *Paraglomus*) that had been isolated from the rhizosphere of maize which was grown in eastern Uganda [35]. This study aimed to evaluate the use of these indigenous mycorrhizal fungi as inoculants in combination with different NPK doses to improve the maize yields. The objective of this study was to investigate the minimum NPK fertilizer application rate that would be required to improve the maize productivity. With this in mind, it was hypothesized that the indigenous AMF inoculation performance would increase or decrease depending on the N, P, and K ratios. It was also hypothesized that a double fertilization with AMF and NPK would improve the plant tissue's N, P, and K status, biomass, leaf chlorophyll content, grain yield, and soil chemical properties.

2. Materials and Methods

2.1. Pot Experiment

The pot experiments were conducted from March to June 2021 at the Faculty of Agriculture, Uganda Martyrs University, Nkozi (6°52′00″ N and 3°43′00″ E), Central Uganda. The soil that was used in this experiment is characterized as being clay-loam, and it was collected from a maize farm in Serere, eastern Uganda (1°32′0″ N and 33°27′3″ E). The soils were collected from the top layer at 0–30 cm and had a pH of 5.0 and total N, P, and K concentrations of 930.00, 4.62, and 73.7 mg/kg soil, respectively. The soil was sieved (2 mm sieve), sterilized at 60 °C for 48 h, and mixed homogeneously. About 6 kg of dry soil (plastic bag) was used to fill the pot. The pot size was 4021.24 cm^3 (diameter = 16 cm; height = 20 cm).

A factorial complete randomized design included the NPK fertilizer and AMF inoculum treatments with 4 replicates (5 × 2). The treatments were: (1) 100% NPK = N120P60K60, consisting of 120 kg/ha N as urea, 60 kg/ha P as triphosphate, and 60 kg/ha K as potash; (2) 75% NPK = N90P45K45; (3) 50% NPK = N60P30K30; (4) 20% NPK = N30P15K15; (5) control = N0P0K0. These five levels of fertilizer were combined or not with AMF. The 100% NPK dose corresponded to the recommended rate that is adequate to reach the greatest economic yield in Uganda [36]. The fertilizers used were triple phosphate (TSP 46% P_2O_2, Falcon fertilizer), urea (Bora Bora Fertilizer 46% N), and potash (MOP 60% K_2O, Falcon fertilizer).

The inoculum was prepared using traditional pot culture techniques [37]. The AMF genera from the morphological identification were propagated in maize roots [35]. This is because maize is the most commonly used host plant for the mass propagation of AM fungi [38]. The maize was grown in plastic pots (13 cm × 9 cm) supporting 2.5 kg of a 3:2 soil–sand mixture. The soils were preheated to destroy the native mycorrhizal spores and their corresponding mycoparasites. The maize plants were allowed to grow to field capacity for five weeks with regular irrigation. We stopped watering them for 4 weeks to ensure that the harsh conditions promoted the AMF sporulation [39]. The indigenous AMF inoculum contained 50 infectious AMF propagules/g representing 7 genera (*Gigaspora*, *Carpospore*, *Glomus*, *Acrospora*, *Archaeospora*, *Enttrophospora*, and *Paraglomus*) [35].

Longe 5 maize cultivars that were published on by the Namulonge Research Institute (Kampala, Uganda) were used in this study. This maize variety was chosen because it has been described as being widely adapted and preferred by smallholder farmers in Uganda [40]. Before the planting stage, the seeds were surface sterilized using 70% ethanol for 5 min and washed 5 times with sterile distilled water. The seeds were sown in the center of each pot. The average minimum and maximum temperatures inside the greenhouse during plant growth were 20 °C and 29 °C, respectively, and the average relative humidity was 75% and in a 11:13 h light: dark photo-period. The plant shoot height was measured using a tape measure which was placed between the collar and the sheath of the last fully emerged leaf during the 8-week growth period, after which the plants were harvested. The shoot samples were oven dried at 60 °C for 72 h, and the dry biomass was recorded. Next, the dried shoots were ground, and the N, P, and K tissue contents were quantified. The pot contents were washed using a 2 mm sieve to isolate the roots which were kept in ethanol (70%) at 4 °C for a further evaluation of the mycorrhizal root colonization. The roots were stained with 0.05% trypan blue [41], and the percentage of the colonization was determined using the method that was described by Trouvelot et al. [42]. The stained root tissue was examined using a compound microscope (Motic, MIPLUS20, Speed Fair Co., Ltd., Causeway Bay, Hong Kong) at 400× magnification. The chlorophyll index of maize leaves was measured using a soil plant analysis development meter (SPAD meter) (SPAD 502 plus, Konica Minolta, Osaka, Japan).

2.2. Field Experiments

The best-performing AMF–NPK combination (AMF + 50% NPK) was selected from the pot trial and it was compared with the recommended dose of NPK (120:60:60 kg/ha) and the control (no treatment). The field tests were conducted at two locations (Kumi and Nkozi) in central and eastern Uganda during the 2 rainy seasons from June to August 2021 and from March to May 2022. The experimental area was divided into 6 m × 6 m plots which were separated by a 1 m wide alley and laid out in a Randomized Complete Block Design (RCBD). The NPK fertilizers were applied manually and incorporated into the soil. Inoculum (50 g/hole) was placed at the time of seeding. The maize plants (variety Longe 5) were sown at a plant density of 4 plants m^{-2} with there being a row spacing of approximately 50 cm and 75 cm between the rows. The weeds were manually controlled 4 weeks after sowing. The effects of the treatments on the mycorrhizal root colonization were evaluated 5 weeks after planting on four plants which were randomly selected along a 1 m transect within each plot. The mycorrhizal root colonization was measured using the same method as described for the pot experiments. The grain yield was assessed at the harvest stage after reaching physiological maturity. The maize cobs from each plot were shelled, labeled, and stored in a solar dryer for 5 days. After sun drying, the grains were weighed, and this is expressed in tons/ha.

The soils were sampled from each plot after two growing seasons. The soils were then placed in a zip-lock freezer bag and stored at 4 °C until they were used. Soil chemical properties such as pH, organic matter (O.M), total N, total P, and total K were determined using the methods that were described by Okalebo et al. [43]. The soil pH was measured in a 2.5:1 soil–water suspension. The content of the total N and P was measured in a digest which was obtained by treating the soil samples with hydrogen peroxide, sulpuric acid, selenium, and salicylic acid. The total K contents of the soils were measured by the complete oxidation of the samples using Kjeldahl procedures, which was followed by a spectrometric analysis.

An analysis of variance (ANOVA) was performed using Minitab 21 software to determine the effect of the AMF–NPK combinations on the root colonization, maize yield, and soil chemical properties. A Tukey's comparison test was used to compare means at a $p \leq 0.05$ significance level. A Pearson correlation coefficient was used to determine the effect of the root colonization on the shoot nutrient content, shoot dry weight, leaf chlorophyll content, and maize yield.

3. Results

3.1. Pot Experiment

3.1.1. Effects of the Application of AMF–NPK Fertilizer Combinations on the Maize Shoot Height and Shoot Dry Weight

Significant variations in the final plant height and shoot dry weight of the maize plants are recorded in Table 1. The indigenous AMF inoculation which was combined with the NPK fertilizers resulted in significant improvement in the growth in terms of height and dry weight when it is compared to the controls. Based on Table 1, it is observed that each treatment had a significant effect ($p < 0.05$) on the mean maize height. The highest plant height was found with the treatment with AMF + 50% NPK (97 cm), while the lowest plant height was found with the control treatment (no mycorrhizae and no NPK fertilizer), with is reaching 75 cm. The treatment with AMF + 50% NPK was shown to be significantly different from the controls and the other treatments ($p < 0.05$). The average height of the AMF + 50% NPK-treated maize was 28% higher than it was for the control.

Table 1. Effect of AMF–NPK fertilizer combinations on shoot height (cm), dry weight (g), root AMF colonization, and leaves chlorophyll content in maize.

Treatment	Final Plant Height (cm)	Shoot Dry Weight (g)	Root Colonization %	Leaves Chlorophyll Content (SPAD Units)
Control	75.75 e	75.00 d	0.0 f	31 ± 5 c
100%NPK	85.00 d	84.25 c	0.0 f	41 ± 8 b
AMF	87.50 d	86.00 c	45.6 c	39 ± 4 b
AMF + 25% NPK	89.25 d	88.75 c	34.5 e	40± 5 b
AMF + 50% NPK	97.00 a	99.00 a	63.0 a	47 ± 5 a
AMF + 75% NPK	92.25 c	90.00 b	39.7 d	46 ± 4 a
AMF + 100% NPK	94.00 b	91.50 b	58.6 b	46 ± 4 a

SPAD units: soil plant analysis development. The means that share the same letters within a group in each column are not significantly different by ANOVA and Tukey's multiple comparison test ($p < 0.05$).

In terms of the biomass production of the maize, there were also significant effects of the different treatments, in which the AMF + 50% NPK treatment presented the highest values of the dry weight of the shoots (99 g), while the lowest shoot dry weight was found in the control (75 g).

3.1.2. Effects of the Application of AMF–NPK Fertilizer Combinations on the Maize Root Colonization and Leaf Chlorophyll Content

The colonization of the plant roots by indigenous mycorrhizae was affected by the inoculum ($p < 0.001$) (Table 1). The AMF + 50% NPK treatment (63%), which was followed by the AMF + 100% NPK treatment (58.6%), the AMF treatment (45.6%), the AMF + 75% NPK treatment (39.7%), and the AMF + 25% NPK treatment (34.5%), had significant high root colonization rates when they were compared to that of the control (Table 1).

The effects of different treatments on leaf chlorophyll content are shown in Table 1. The chlorophyll concentrations were highest (47 ± 5 SPAD units) with the AMF + 50% NPK treatment and they were the lowest (31 ± 5 SPAD units) with the control (no AMF and no NPK fertilizer). The treatments with AMF + 75% NPK and AMF + 100% NPK had similar effects on the leaf chlorophyll content with concentrations of 46 ± 4 SPAD units.

3.1.3. Effects of the Application of Indigenous AMF and NPK Fertilizer on the N, P, and K Uptake in the Maize Shoots

Based on the analysis of variance, the application of AMF and different doses of NPK fertilizers had a significant ($p < 0.05$) effect on the content of phosphorus, nitrogen, and potassium in the shoots of maize plants when they were compared with the control (Table 2). The results show that the accumulation of N, P, and K in plants that were treated with AMF + 50% NPK was the highest.

Table 2. Effect of AMF–NPK combinations on mean N, P, and K content (mg/g plant dry shoot weight).

Treatment	P Uptake in the Shoots	N Uptake in the Shoots	K Uptake in the Shoots
Control	1.15 e	11.3 f	9.41 e
100%NPK	1.71 d	18.62 e	10.74 d
AMF	2.41 c	19.25 e	11.90 c
AMF + 25% NPK	1.85 d	20.44 d	11.72 c
AMF + 50% NPK	3.17 a	28.00 a	16.55 a
AMF + 75% NPK	2.43 c	23.10 c	12.23 c
AMF + 100% NPK	2.53 b	27.58 b	14.11 b

Values that are not followed by the same letters in the same column are significantly different according to Tukey test ($p < 0.05$).

3.2. Field Experiment

After the pot experiment, the AMF + 50% NPK treatment performed well and was therefore selected for a comparison in the field trials with the recommended dose of NPK (120:60:60 kg/ha) and the control (no AMF and no NPK fertilizer).

3.2.1. Effect of AMF+ 50% NPK and 100% NPK on Maize Root AMF Colonization and Density of AMF Spores in the Soil

The effect of the inoculation and the NPK application on the maize was significant in terms of the mycorrhizal colonization percentage. AMF with 50% of the recommended dose of NPK significantly ($p < 0.05$) increased the mycorrhizal colonization percentages of the maize that was grown on farms in Kumi and Nkozi (Table 3). The root colonization was more extensive in the plants that were treated with AMF + 50% NPK than in the plants that were treated with 100% NPK and in the control in both of the fields. The treatment of 100% of the recommended dose of NPK also increased the mycorrhizal colonization percentage in Kumi and Nkozi.

Table 3. Effect of treatments on mean spores and root colonization.

	Spore per 100 g Soil		% of Root Colonization	
Treatments	**Kumi**	**Nkozi**	**Kumi**	**Nkozi**
Control	3464 c	2343 c	40 ± 15 c	30 ± 20 b
AMF + 50% NPK	4327 a	4175 a	75 ± 09 a	68 ± 13 a
100% NPK	3894 b	2956 b	65 ± 11 b	63 ± 08 a

Values that are not followed by the same letters in the same column are significantly different according to Tukey test ($p < 0.05$).

In both of the fields, the number of spores in the soil was significantly different according to the treatment that was applied (Table 3). The maize plots that received the AMF + 50% NPK treatment showed the highest number of AMF spores in the soil, which was followed by the one that received 100% NPK, and the control had the lower number of spores.

3.2.2. Effect of AMF + 50% NPK and 100% NPK on Maize Yield

A significant difference was observed among the treatments ($p < 0.05$) (Table 4). In both fields, the highest yield was observed for the maize that was treated with the combination of AMF and 50% NPK, and the lowest yield was observed for the control. Similarly, the weight of 1000 grains varied depending on the treatment that was used. The average weight of 1000 grains which was obtained was higher in the 'AMF + 50% NPK' treatment than it was the control and 100% NPK treatments.

Table 4. Effect of treatments on mean dry grain yield (tons/ha) and weight of 1000 dry grains (kg).

	Grain Yield (tons/ha)		Weight of 1000 Dry Grains (kg)	
Treatments	**Kumi**	**Nkozi**	**Kumi**	**Nkozi**
Control	2.7 ± 2.0 c	2.6 ± 2.0 c	2.2 ± 0.4 c	0.8 ± 0.3 a
AMF + 50% NPK	6.5 ± 0.9 a	6.2 ± 1.0 a	2.8 ± 0.1 a	1.1 ± 0.2 a
100% NPK	2.9 ± 1.1 b	3.7 ± 1.1 b	2.4± 0.2 b	0.5 ± 0.2 b

Mean comparisons were performed by the Tukey multiple comparison test. Values that are not followed by the same letters in the same column are significantly different according to Tukey test ($p < 0.05$).

3.2.3. Effects of AMF + 50% NPK and 100% NPK on the Soil Chemical Properties

There were significant effects of the different treatments on the soil nutrient status (N and P) contents of the soil of the rhizosphere in both of the maize fields ($p < 0.05$) (Table 5). In general, there is a tendency that the highest values of the soil nutrient status, which were measured after two rainy seasons, were the highest under the AMF + 50%

NPK treatment. The values of the soil nutrient status in this treatment were higher than those in the control and the 100% NPK treatment (Table 5). In contrast, the different treatments had no significant effects on the soil pH ($p > 0.05$), O.M ($p > 0.05$), and K ($p > 0.05$) (Tables 5 and 6).

Table 5. Effect of treatments on soil chemical properties.

	Kumi			Nkozi		
	Treatments			Treatments		
	Control	AMF + 50% NPK	100% NPK	Control	AMF + 50% NPK	100% NPK
pH	6.15 a	6.10 a	6.13 a	5.80 a	5.67 a	5.73 a
% TN	0.47 c	0.64 a	0.60 b	0.31 c	0.46 a	0.44 b
% O.M	2.09 a	2.11 a	2.13 a	0.93 a	0.93 a	0.93 a
P (mg/kg)	9.52 c	32.6 a	13.3 b	6.21 c	13.7 a	10.4 b
K (mg/kg)	108.3 a	111.1 a	113.2 a	167.7 a	161.8 a	170.9 a
CEC	8.6	-	-	4.32	-	-

TN—total nitrogen; OM—organic matter. Data within a row that are not followed by the same letter are statistically different according to Tukey test ($p < 0.05$).

Table 6. Pearson correlation between root colonization on shoot nutrient content, shoot dry weight, and leaf chlorophyll content.

	P Uptake	N Uptake	K Uptake	Final Plant Height (cm)	Shoot Dry Weight (g)	Leaf Chlorophyll
% root colonization	0.912	0.866	0.877	0.881	0.854	0.742

4. Discussion

In both of the field experiments, the best root mycorrhizal colonization was achieved with the AMF + 50% NPK treatment. These results confirmed those from the pot experiments. The low mycorrhizal fungi colonization at the recommended dose of 100% NPK indicates that the over-application of mineral fertilizers in agroecosystems adversely affects the AMF symbionts, thus reducing the AMF abundance and benefits that are provided by the symbionts [44]. Excessive fertilization significantly reduced the AMF colonization due to the higher nutrient availability in the soil after the chemical addition [45]. According to [46–49], after applying the chemical fertilizers, the plant can directly absorb enough nutrients to reduce its dependency on AMF symbiosis. The plant reduces the allocation of photosynthates and reduces the AMF colonization after the chemical fertilizers [22]. All of these studies that are cited have previously shown that the excessive application of chemical fertilizers significantly reduced the AMF colonization.

In this study, the performance of indigenous AMF inoculants in combination with different doses of NPK fertilization led to significant changes in the plant growth parameters. Previous studies also showed the significant response of AMF + NPK fertilization to the plant's growth and yield [50,51], suggesting that the AMF activity is sensitive to the number of nutrients in the soil.

Plants that are grown under P-limited conditions are better prepared for colonization by mycorrhizal fungi [52]. In this study, the amount of P in the soil was lower than that which is required for optimal growth and maize production, resulting in a significant increase in the colonization rate when it is compared to the application of high concentrations of NPK. Moreover, the number of AMF spores that were found in field soils indicated that the native AMF population was not negligible (~3464 and 2343 spores per 100 g of dry soil for Kumi and Nkozi, respectively). The uninoculated control plants in both of the field studies showed the presence of native AMF propagules and relatively little natural mycorrhizal colonization. In this study, it was found that inoculated indigenous fungal colonization was not site-specific, but it depended on the infectivity of the species in the inoculum. Moreover, native AMF in both of the fields might not be better adapted to the local conditions than the indigenous inoculated fungi are. This is consistent with a study by

Bender et al. [53], showing that an exotic AMF inoculum could be established in the field. The establishment of exotic AMF may be due to the low density of native AMF in soil or a moderate P content in the soil. Thus, native AMF communities can be stimulated when the exotic species are also adapted to local niche requirements and work in synergy with native species. The mycorrhizal colonization of plant roots is also determined by several factors, including the presence and quality of native populations. Although exotic AMF strains often do not form mycorrhizal associations [54], this was not seen in this study, and all of the treatments with AMF significantly increased the maize root mycorrhizal colonization when they were compared to the control. The same results from different field sites may also be related to a lack of trophic competition with native strains, as reported in another study [55].

Regarding the effect of the treatments on the leaf chlorophyll content, the total chlorophyll was, on average, 47 SPAD units in the AMF + 50% treatment. This value is above the minimum (25.44 SPAD units) that is required by maize [56]. Furthermore, nutrient uptake by the plants depends on the root system growth and nutrient availability in the rhizosphere. An appropriate strategy for the plants to adapt to nutrient deficiencies is to establish symbiosis with the mycorrhizal fungi which can extend the nutrient uptake beyond the level of root system development and expansion [19]. Chu et al. [57] reported that the phosphorus concentrations in plants with mycorrhizae with a low phosphorus supply were significantly higher than they were in the treatments without mycorrhizae. The results showed that improved N, P, and K tissue contents are positively correlated with mycorrhizal colonization (Pearson correlation = 0.91). In other words, the tissue contents of N, P, and K increased with an increasing mycorrhizal colonization. This strong positive correlation between the mycorrhizal root colonization and the shoot NPK content was also reported by Abdel-Fattah et al. [58]. The results of this study can be seen as positive for researchers, and they also demonstrate the importance of using AMF in maize production. However, this study did not elucidate the effects of the AMF + NPK combination on the intake of other essential nutrients such as Zn, Ca, Cu, and S.

Reducing the use of artificial fertilizers and adding AMF resulted in higher grain yields. The best results were obtained with a 50% reduction in the fertilizer in combination with the AMF inoculant, thereby increasing the grain yields by more than 140% in both of the fields in comparison to that of the control. This result is relevant when one is considering that the yield increase was obtained by reducing the fertilizer by 50%. The results that are presented in this study demonstrate the environmental benefits of using AMF to improve maize productivity. AMF can mobilize and deliver nutrients to the maize to achieve the best results in terms of growth and grain yield with half of the full NPK fertilizer recommended dose. Similar results were reported for pineapple, with the AMF inoculation and application of half of the fertilizer dose promoting the highest levels of fruit mass and organoleptic variables. An increase in the sorghum yield was reported by Ramadhani et al. [59], who also showed that soil degradation can be reduced by significantly reducing the fertilizer in combination with AMF. Using this indigenous inoculant, the results appear to indicate that maize responds better to the inoculant when it is combined with the N60P30K30 fertilizer.

Furthermore, based on the results from the fields, the highest N and P soil contents were found with the treatment with AMF + 50% NPK, whereas the lowest N and P soil contents were found with the control treatment. The application of a double inoculation (AMF + NPK) is effective in increasing the soil N and P contents ($p < 0.05$), but not in increasing the soil pH and the K content ($p > 0.05$). The soil nutrient content (N and P) was significantly correlated with the root colonization, and it was significantly higher when the chemical fertilizer quantity was moderate, suggesting that different responses of AMF may be mediated by the amount of fertilizer that is applied.

5. Conclusions

The results support the hypothesis that plants inoculated with indigenous AMF inoculum that is combined with 60, 30, and 30 Kg/ha of N, P, and K, respectively, will achieve higher productivity and exhibited growth and yield values when they are compared to the plants that were inoculated with inorganic fertilizer alone or without fertilizer. The combination of AMF and 50% NPK also improved the soil's chemical properties. Therefore, AMF appears to be a good alternative to NPK fertilization, or at least, it can cut the need for large amounts of synthetic fertilizer (NPK) by half. Agricultural management practices based on AMF treatment can provide an economical, environmentally friendly, and sustainable ways to increase the soil fertility and yields.

However, further studies using biomolecular tools are needed to determine whether the plant roots were simultaneously colonized by multiple AMF strains in the field. Further studies are needed to determine whether the introduced AMF species persist in the field.

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