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Efficiency of *Glomus aggregatum*, Azotobacter, Azospirillum and Chemical Fertilizer on Growth and Yield of Single Cross Hybrid 4452 Maize

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ABSTRACT

The efficiency of arbuscular-mycorrhizal fungi (*Glomus aggregatum*), azotobacter (*Azotobacter chroococcum*), azospirillum (*Azospirillum lipoferum*) and chemical fertilizer on the growth and yield of single cross hybrid 4452 maize was evaluated at the laboratory of the Soil Science Department, Faculty of Agriculture, Kasetsart University at Kamphaeng Saen campus and in a field experiment at the National Corn and Sorghum Research Center in Nakhon Ratchasima province. Pellets of arbuscular-mycorrhizal inoculum containing 30-50 spore/g and inoculum of azotobacter and azospirillum in granular form were produced using fine compost as a carrier having 10^7-10^8 cfu/g in the population. The results revealed that corn growth in terms of plant height was greater for the microbially inoculated and fertilizer treatments than the control. The quantity of mycorrhizal spores in the mycorrhizal treatment was higher than in the other treatments. The rhizosphere of the soil used was suitable for azotobacter, with more than 10^6 cfu/g found. The nitrogen, phosphorus and potassium contents of microbial-inoculated plants that had been subjected to chemical fertilizer treatment were greater than in the control. The difference among treatments of the residual soil nitrogen after harvest was not significant.

The grain yield from the greenhouse experiment produced interesting results. The minimum yield found in the control treatment was 49.80 g/plant, while those of the microbial-inoculated treatments were higher. The yield of the azotobacter+azospirillum, azotobacter+arbuscular-mycorrhizal-fungi+chemical-fertilizer and the azotobacter+chemical-fertilizer showed the greater yields, with 216.72, 211.32 and 198.42 g/plant, respectively, compared with 174.72 g/plant from the maize treated with chemical fertilizer. The grain yield from field experiment using azotobacter+chemical-fertilizer was the greatest (706.25 kg/ha). The sole application of arbuscular-mycorrhhizal inoculation gave a grain yield as high as that of the chemical fertilizer treatment (5,993.75.0 and 5,825.00 kg/ha, respectively). The yield of azotobacter+azospirillum+arbuscular-mychorrhizal-fungi+chemical fertilizer was 5,987.50 kg/ha and the lowest yield was found in the control (3,543.25.0 kg/ha).

Keywords: arbuscular-mycorrhizal fungi, azotobacter, azospirillum, *Glomus aggregatum*, maize

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INTRODUCTION

The soil rhizosphere contains several kinds of microorganisms, which vary greatly depending on environmental conditions. These microorganisms are either beneficial and stimulate plant growth or deleterious causing plant disease. Mycorrhizal fungi exist around plant roots in a form of symbiosis, such as Azotobacter and Azospirillum, which can freely hold nitrogen and release some kinds of hormones that are useful for field crops and vegetables (Mala *et al*., 1997). Generally, the amount, activities and efficiency of Azotobacter and Azospirillum in nitrogen fixation are low, due to limits in the amount of organic carbon and nitrogenase enzyme that are inhibited by oxygen around the plant roots (Sylvia *et al*., 1998). The important role of arbuscular-mycorrhizal fungi has been widely documented. They exist symbiotically around the roots of various agronomical and horticultural plants, such as flower plants, ornamental plants, pot plants, fruit plants and perennial plants (Bolan, 1991). These fungi can enhance the efficiency of the uptake of many mineral elements by the plant, particularly phosphorus, which is difficult to dissolve in soil. Long-term fertilization of nitrogen in field plots revealed decreasing amounts of some mycorrhizal fungi, whereas other species increased (Porras-Alfaro *et al*., 2007). Sannazzaro *et al*. (2006) found that *Glomus intraradices* could accelerate *Lotus glaber* to recover satisfactorily from salt-affected conditions. Plants with mycorrhizal fungi could resist flooding conditions better than those without the bacterial association (Neto *et al*., 2006). If the advantages of mycorrhizal fungi are considered and some of their disadvantages neutralized, they could provide an alternative to existing agricultural cropping improvement practices

Therefore, it of interest to apply the proper species and strain of these microbial inocula into the soil rhizosphere to enhance plant growth. Moreover, a study of the efficiency of arbuscular-mycorrhizal fungi, azotobacter and azospirillum together with the different forms of these three microbial inocula to increase maize yield production, as well as to sustain soil fertility, may result in reducing the cost of maize production and sustaining soil productivity.

MATERIALS AND METHODS

The experiment used single cross hybrid 4452 maize from the Suwan Farm, Kasetsart University. This hybrid is undergoing study of all its cultivation practices before its distribution to farms.

Preparation of Azotobacter culture and inoculum

A pure culture of *Azotobacter chroococcum* obtained from the Soil Science Department, Faculty of Agriculture, Kasetsart University at KamphaengSaen Campus was placed in 100 mL of modified Ashby's medium (20 g Mannitol, 0.2 g K_2HPO_4, 1.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.1 g K_2SO_4, 5.0 g $CaCO_3$, 0.002 g $Na_2MoO_4.2H_2O$ and 1 L distilled water) for 7 d in a 250 mL flask and aerated by shaker at 150 rpm. Inoculation of the azotobacter was carried out in 100 mL of medium in a carrier of 250 g mashed and sterilized compost and then the mixture was incubated at room temperature for 1 week. Later, another 250 g of mashed and sterilized compost was added and then incubated further for 1 week. The population of azotobacter in each pellet was determined by a spread plating method on modified Ashby's medium. The inoculum was placed in a plastic bag and kept below 4°C in a refrigerator until used. The inoculation of this biofertilizer was carried out by the seed inoculation method using gum acacia as the sticker. Every seed contained about 0.1 g of inoculum.

Preparation of Azospirillum culture and inoculum

A pure culture of *Azospirillum lipoferum* was obtained from the Soil Science Department, Faculty of Agriculture, Kasetsart University at Kamphaeng Saen Campus and cultured in the medium as described by Okon *et al*. (1977), which consisted of solution A, containing 6 g K_2HPO_4, 4 g KH_2PO_4 and 500 mL distilled water, and solution B, containing 0.2 g $MgSO_4$. $7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2$, 1.0 g NH_4Cl, 5.0 g malic acid, 3.0 g NaOH, 0.05 g yeast extract, 0.002 g Na_2MoO_4. $2H_2O$, 0.001 g $MnSO_4.2H_2O$, 0.0014 g H_3BO_3, 0.0004 g $Cu(NO_3)_2$, 0.0021 g $ZnSO_4$, 0.002 g $FeCl_3$, 2 mL Bromothymol blue (0.5% alcoholic solution) and 500 mL distilled water. Both solutions A and B were sterilized separately and mixed at a hot temperature.

Azospirillum lipoferum was cultured in a 250 mL flask containing 100 mL of medium for 7 d and aerated by shaker at 150 rpm. The granulated inoculum was prepared by spraying onto dry, mashed and sterilized compost at the ratio of *Azospirillum lipoferum* in 100 mL per 250 g compost. Then, the mixture was incubated at room temperature for 1 week. Later, an additional 250 g of dry, mashed and sterilized compost was added and incubated further for 1 week. The inocula were kept in a black plastic bag at 4°C in a refrigerator. Maize seed was inoculated using gum acacia as the sticker and every seed contained about 0.1 g of inoculum.

Preparation of arbuscular-mycorrhizal fungi

A pure culture of *Glomus aggregatum,* an arbuscular-mycorrhizal fungi, obtained from the Soil Science Department was used. Mycorrhizal pellets were produced by quantitatively propagating the fungi spores using pot inculum with sorghum as the host plant, as described by Mala (1995). The pellets formed a cylindrical pill of size 6×6 mm (diameter × length) by mixing the inoculum with mashed dry compost and rock phosphate in the ratio of 80:15:5, respectively, and then adding 1% gum acacia and slightly sprinkling with water, before putting in a mold to produce the pellets. The pellets were dried for 24 h at room temperature. The average dry weight of each pellet was 0.6 g and contained 30-40 viable mycorrhizal spores. Inoculation using the soil inoculation method involved one seed being inoculated with one pellet of inoculum both in the greenhouse and the field experiment.

The greenhouse experiment

This study aimed to determine the efficiency of the inocula of pelleted mycorrhizal fungi, azotobacter, azospirillum and chemical fertilizer on single cross hybrid 4452 maize using a randomized complete block (RCB) design comprising 16 treatments (Table 1) and four replications. In this study, each pot was filled with 8 kg of Kamphaengsaen soil series, sown with four maize seeds and after 1 week of germination, one seedling was kept in the pot. The chemical fertilizer treatment involved 5g/plant of an N:P:K fertilizer with the ratio of 15:15:15. As for the treatments of azotobacter and azospirillum, seed inoculation after Mala (2007) was used. Apart from the arbuscular-mycorrhizal fungi treatment, each growing hole for maize was filled with four mycorrhizal pellets together with four maize seeds.

The field experiment

The field experiment was carried out at the National Corn and Sorghum Research Center in Nakhonratchasima Province using the RCB design, comprising 16 treatments with four replications. Treatments and seed inoculation were similar to the greenhouse study. The plot size was 4 m × 3 m, with spacing of 0.75 m between rows and 0.25 m within rows. Three maize seeds were planted in each growing hole and two germinants were removed after 1 week of germination. Fertilizer was provided after thinning at 40 kg/rai (250 kg/ha) using an N:P:K formula with a ratio of 15:15:15.

Table 1 Various codes and treatment details for greenhouse and field experiments.

Treatment code	Treatment detail			
	Azotobacter (seed inoculation;0.2g/seed)	Azospirillum(seed inoculation; 0.2g/seed)	Arbuscular mycorrhizal fungi (soil inoculation;1 pellet/seed)	Chemical fertilizer
Control	-	-	-	-
Azotobacter(T)	+	-	-	-
Azospirillum(S)	-	+	-	-
Glomus aggregatum(M)	-	-	+	-
Chemical fertilizer(F)	-	-	-	+
T+S	+	+	-	-
T+M	+	-	+	-
T+F	+	-	-	+
S+M	-	+	+	-
S+F		+	-	+
M+F	-	-	+	+
T+S+M	+	+	+	-
T+S+F	+	+	-	+
T+M+F	+	-	+	+
S+M+F	-	+	+	+
T+S+M+F	+	+	+	+

Data collecting procedure

Plant growth in terms of plant height was checked at 4, 6, 8 and 10 week after growing. The grain yield was measured after harvest. The N content in the soil and N, P and K levels in the plants after harvesting were determined according to Attanand *et al*. (1989). The changes in the azotobacter population in the root zone during the growth period of the maize at 4 and 8 week and at harvest were studied using the spread plating method in modified Ashby's medium (Mala, 2007). The mycorrhizal spores in the soil were evaluated at 4, 6, 8 and 10 week and at harvest by the wet sieving method (Brundrett *et al*. 1994). The data were analyzed and the average mean differences compared using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Analysis of the inocula indicated that arbuscular-mycorrhizal fungi were present with 30-50 spores/g, whereas azotobacter and azospirillum were found in quantities of 10^7-10^8 cfu/g. The effects of these microorganisms are discussed below.

Growth of single cross hybrid maize

The growth of maize, in terms of plant height both in the greenhouse and field experiments are shown in Tables 2 and 3, respectively. The maize grew well and the plant heights for different periods after planting were significantly different. In the greenhouse treatment the plant heights that appeared to be clearly outstanding were the azotobacter+chemical-fertilizer treatment (T+F), the azotobacter+azospirillum+chemical-fertilizer treatment (T+S+F) and the azospirillum+arbuscular-mycorrhizal-fungi+chemical-fertilizer treatment (S+M+F). For the in-field experiment, the outstanding treatments were the azotobacter+chemical-fertilizer treatment (T+F), the arbuscular-mycorrhizal-fungi+chemical-fertilizer treatment (M+F) and the azotobacter+azospirillum+mycorrhizal-fungi+chemical-

Table 2 Plant height of single cross hybrid 4452 maize at week 4, 6, 8 and 10 after planting in the greenhouse.

Treatment	Plant height (cm)			
	4 week	6 week	8 week	10 week
Control	98.75d-e	145.75d-f	159.00d-f	166.75b-c
Azotobacter(T)	103.13c-e	148.75c-f	156.50d-f	162.00b-c
Azospirillum(S)	114.75a-d	161.50a-e	168.50a-f	175.25a-c
Glomus aggregatum(M)	97.25e	138.00f	152.00e-f	163.00b-c
Chemical fertilizer (F)	108.50a-e	154.25c-f	161.00d-f	168.50b-c
T+S	101.00d-e	155.50c-f	162.50c-f	167.50b-c
T+M	106.13b-e	140.75e-f	149.00f	159.25c
T+F	124.50a	181.00a	185.50a-b	189.75a
S+M	114.75a-d	162.50a-e	168.00a-f	177.00a-c
S+F	113.00a-e	169.00a-c	176.25a-d	181.25a-b
M+F	120.25a-b	156.00b-f	165.25b-f	172.75a-c
T+S+M	106.50b-e	153.75c-f	158.50d-f	165.50b-c
T+S+F	121.00a-b	178.00a-b	183.50a-c	189.50a
T+M+F	120.10a-b	168.00a-c	184.80a-b	189.60a
S+M+F	118.75a-c	180.75a	187.50a	191.00a
T+S+M+F	121.50a-b	164.50a-d	172.75a-e	178.75a-c
F-test	**	**	**	**
CV(%)	9.17	8.58	7.88	6.89

Table 3 Plant height of single cross hybrid 4452 maize at week 4, 6, 8 and 10 after planting in the field experiment.

Treatment	Plant height (cm)			
	4 week	6 week	8 week	10 week
Control	94.08b-c	160.04d-g	194.21e	196.87c
Azotobacter(T)	88.50c	145.08g	197.96d-e	200.50c
Azospirillum(S)	94.07b-c	156.30f-g	203.13b-e	202.58c
Glomus aggregatum(M)	100.25b	166.29c-g	204.50a-e	205.71b-c
Chemical fertilizer (F)	114.42a	182.42a-e	217.71a-b	223.08a-b
T+S	95.45b-c	159.25d-g	200.58c-e	203.29c
T+M	96.62b-c	173.67b-f	195.78d-e	199.09c
T+F	116.42a	192.79a-b	215.50a-c	225.08a
S+M	90.96b-c	158.75e-g	197.00d-e	195.63c
S+F	98.79b-c	169.67b-f	195.92d-e	208.54a-c
M+F	120.46a	199.96a	219.09a-b	225.17a
T+S+M	100.17b	168.46c-g	205.38ab-e	213.00a-c
T+S+F	111.46a	182.79a-d	211.33ab-d	213.79a-c
T+M+F	110.50a	173.50b-d	210.50a-d	213.10a-c
S+M+F	115.36a	181.38a-e	217.13a-b	209.08a-c
T+S+M+F	120.78a	187.37a-c	220.99a	225.21a
F-test	**	**	**	**
CV(%)	6.73	8.32	4.84	5.41

fertilizer treatment (T+S+M+F). The increase in plant growth with azotobacter and azospirillum in this case were similar and were slower than the effect from chemical fertilizer. In the initial stage, growth of both microorganisms in soil was slow, since there was a lack of organic material around the root zone. However, after the root exudation of maize was expressed, the population and activity of those microorganisms increased.

Quantity of arbuscular mycorrhizal fungi in the root zone

The quantities of mycorrhizal spores in the root zone of the field experiment are shown in Table 4. Generally, arbuscular-mycorrhizal fungi spores were found in the soil in all treatments, because some were already present in the soil prior to the experiment. However, in the treatments that included mycorrhizal fungi, the mycorrhizal spores were more abundant than in the treatments without. Azotobacter and azospirillum not only promoted the growth of plants, but also promoted the growth of arbuscular-mycorrhizal fungi. This resulted in the great quantity of arbuscular mycorrhizal fungi than in the control treatment. The mycorrhizal spores in the treatment inoculated azotobacter and azospirillum, which then tended to be in greater numbers than in non-inoculated treatments, while those treatments applied with chemical fertilizer caused a reduction of mycorrhizal spores.

Quantity of azotobacter in the root zone

The quantities of azotobacter in the root zone over different growth periods are shown in Table 5, based on the data collected at week 4, 8 and 12 after planting. The quantities of all azotobacter, both in the greenhouse and field experiments, were significantly different at the 95% confidence level. The quantity of azotobacter in the control treatment was the lowest among all treatments. In all treatments with azotobacter, azotobacter survived optimally in all periods of

Table 4 Quantity of mycorrhizal spore spore/10 g soil in the root zone of single cross hybrid 4452 maize in the field experiment at different periods.

Treatment	week 4	week 6	week 8	week 10	at harvest
Control	29g	59c-d	56e	58f	20f
Azotobacter(T)	63fg	65c-d	79d-e	69ef	60d-f
Azospirillum(S)	70fg	34d	93c-e	67e-f	67d-f
Glomus aggregatum(M)	106b-f	83b-d	136b-d	117b-e	100c-d
Chemical fertilizer (F)	83e-g	82b-d	90c-e	66e-f	143a-c
T+S	87d-g	86b-c	101c-e	74d-f	64d-f
T+M	161a-b	147a	165a-b	126a-d	101c-d
T+F	99c-f	91bc	96c-e	87c-f	40e-f
S+M	140a-e	148a	134b-d	133a-c	163a-b
S+F	77fg	71b-d	107b-e	77d-f	90c-e
M+F	146a-c	150a	123b-d	153a-b	136a-c
T+S+M	143a-d	143a	136b-d	132a-c	126b-c
T+S+F	86d-g	79b-d	103b-e	80d-f	110c-d
T+M+F	96c-f	144a	149b-c	154a-b	136a-c
S+M+F	178a	141a	152b-c	175a	122b-c
T+S+M+F	96c-f	119a-b	212a	114b-e	178a
F-test	*	*	*	*	*
CV(%)	48.82	45.60	42.60	44.66	52.08

Table 5 Quantity of Azotobacter propagule (10^5 cfu/g) in the rhizosphere of single cross hybrid 4452 maize after different periods of growth.

Treatment	Green house experiment			Field experiment		
	week 4	week 8	week12	week4	week 8	week12
Control	13.79e	21.27b	20.14b	22.52c	41.18f	39.49f-g
Azotobacter(T)	32.94a	24.49b	29.42b	63.45a	101.33a	78.21a
Azospirillum(S)	27.17a-c	28.31a-b	28.62b	59.55a-b	69.61c-e	56.90b-f
Glomus aggregatum(M)	30.11a-b	30.51a-b	25.39b	42.79b	44.46f	46.38d-g
Chemical fertilizer (F)	28.18a-c	28.24a-b	27.89b	43.77b	47.47f	43.49f-g
T+S	30.35a-b	27.57a-b	21.76b	48.79b	97.85a-b	68.35a-c
T+M	22.30b-e	23.92b	22.88b	48.20b	81.49b-c	73.70a
T+F	14.33e	25.54b	25.31b	43.05b	71.87c-d	62.62a-d
S+M	21.12c-e	20.07b	27.47b	35.24b	77.02c	66.75a-c
S+F	23.01b-d	26.16b	24.11b	54.34b	52.75e-f	43.07f-g
M+F	17.41d-e	23.33b	24.31b	64.16a	47.54f	46.60d-g
T+S+M	15.97d-e	27.41a-b	33.07a	68.35a	55.37d-f	45.75d-g
T+S+F	16.56d-e	27.51a-b	24.33b	58.51a-b	72.46c-d	51.21c-g
T+M+F	17.42d-e	26.46b	27.79b	63.62a	66.23c-e	50.48c-g
S+M+F	26.57a-c	29.37a-b	28.14b	47.04b	71.35c-d	55.15b-g
T+S+M+F	15.18d-e	38.00a	25.06b	49.21b	77.07c	70.51a-b
F-test	*	*	*	*	*	*
CV(%)	34.82	27.57	55.80	57.30	31.15	29.69

the maize growth. This microorganism can co-exist with azospirillum and arbuscular-mycorrhizal fungi and it was present in greater quantities than in the control treatment.

Quantity of nutrients in maize

The analysis of the greenhouse experiments indicated that the levels of all three elements (nitrogen, phosphorus and potassium) in the stems of single cross hybrid 4452 maize in all treatments were significantly different at the 95% confidence level (Table 6).

The greatest quantity of nitrogen (0.85%) was found in the treatments that included azotobacter+azospirillum+arbuscular-mycorrhizal-fungi and the chemical fertilizer treatment (T+S+M+F), whereas the nitrogen content was the lowest (0.53%) in the control treatment. In the other treatments, the nitrogen content ranged between these two extreme values and was not significantly different among treatments. However, all treatments showed a likelihood of promoting the uptake and accumulation of nitrogen in the maize compared with the control treatment. All treatments with chemical fertilizer had a nitrogen concentration that tended to be greater than in the maize treated with azotobacter+azospirillum+arbuscular-mycorrhizal-fungi, but, the concentrations were not statistically significant.

The concentration of phosphorus in the stems of the maize in the greenhouse experiment are also shown in Table 6. The greatest quantity of phosphorus (0.17%) was found in the arbuscular-mycorrhizal fungi treatment (M). However, further inspection showed that all treatments affected the uptake of phosphorus in the maize, with an average phosphorus accumulation of 0.10%. The azotobacter+ azospirillum treatment had greater phosphorus

Table 6 Nitrogen, phosphorus and potassium contents in the shoot of single cross hybrid 4452 maize at harvest period.

Treatment	Green house experiment			Field experiment		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	0.53b	0.12a-c	2.60d	0.39	0.07b	1.78
Azotobacter(T)	0.69a-b	0.13a-b	2.80b-d	0.56	0.10a-b	2.40
Azospirillum(S)	0.56a-b	0.14a-b	2.65d	0.51	0.08b	2.92
Glomus aggregatum(M)	0.63a-b	0.17a	2.81a-d	0.51	0.08b	3.12
Chemical fertilizer (F)	0.79a-b	0.08b-d	3.40a	0.53	0.07b	2.66
T+S	0.70a-b	0.12a-c	2.74b-d	0.52	0.10a-b	2.72
T+M	0.59a-b	0.12a-c	2.71c-d	0.59	0.13a	2.54
T+F	0.66a-b	0.06d	3.21a-c	0.51	0.08b	2.66
S+M	0.58a-b	0.09a-d	2.71c-d	0.48	0.09a-b	2.80
S+F	0.66a-b	0.07b-d	3.07a-d	0.48	0.10a-b	2.56
M+F	0.77a-b	0.07b-d	3.32a-b	0.48	0.08b	2.32
T+S+M	0.64a-b	0.09b-d	2.80b-d	0.57	0.11a-b	2.46
T+S+F	0.61a-b	0.06d	2.89a-d	0.52	0.08b	2.60
T+M+F	0.62a-b	0.07c-d	2.90a-d	0.52	0.09a-b	2.66
S+M+F	0.72a-b	0.06c-d	3.00a-d	0.51	0.08b	2.94
T+S+M+F	0.85a	0.10a-d	2.90a-d	0.47	0.07b	2.78
F-test	*	*	*	ns	*	ns
CV(%)	29.19	60.90	14.36	20.79	33.07	22.30

than the treatment of chemical fertilizer which produced the lowest phosphorus content (0.07%). The sole application of azotobacter, azospirillum and arbuscular mycorrhizal fungi encouraged higher P content, but those inocula applied with chemical fertilizer tended to reduce the shoot P content.

The shoot concentrations of potassium in the maize harvested in each greenhouse treatment were significantly different (Table 6). The greatest content (3.40%) was found in the chemical fertilizer treatment (F), whereas the lowest (2.60%) was found in the control treatment. The average contents of the other treatments are shown in Table 6.

The high average concentration of potassium (3.09%) based on all treatments that included chemical fertilizer, showed that treatment with chemical fertilizer promoted greater potassium accumulation than the treatment with arbuscular-mycorrhizal fungi, azotobacter and azospirillum that had average concentrations of potassium of 2.89, 2.87 and 2.84%, respectively.

Nutrient accumulation in the stem of the maize after harvesting in the field experiment at the National Corn and Sorghum Research Center in Nakhonratchasima Province was slightly different from that observed in the greenhouse experiment (Table 6). Thus, the quantities of nitrogen and potassium in the plants in each treatment in the field experiment were not significantly different, whereas the quantities of phosphorus were significantly different at the 95% confidence level.

Although the quantities of nitrogen accumulated in the maize in the field experiment were not significantly different, there was some likelihood that there was an effect, as in the control treatment, the quantity of nitrogen was found to be the lowest (0.39%). For the azotobacter, azospirillum, arbuscular-mycorrhizal fungi and chemical fertilizer treatments, the average quantities of nitrogen were not significantly different.

The maize in the azotobacter+arbuscular-mycorrhizal-fungi treatment (T+M) had the greatest quantity of phosphorus (0.13%), while that in the control treatment, the chemical fertilizer treatment (F) and the azotobacter+azospirillum+arbuscular-mycorrhizal-fungi+chemical-fertilizer treatment (T+S+M+F) all had the same lowest value (0.07%). The inoculation of arbuscular mycorrhizal fungi enhanced the P content. This is the major beneficial effect of mycorrhizal fungi to a host plant, namely that it can take up more P from the soil (Brundrett, 2009).

In addition, it was found that all treatments with chemical fertilizer yielded rather low quantities of phosphorus with an average of 0.08%, whereas those treatments with azospirillum, azotobacter and arbuscular-mycorrhizal fungi yielded an average quantity of phosphorus of 0.10, 0.09 and 0.09%, respectively.

The quantities of potassium in the maize (Table 6) were not significantly different. However, it appeared that the control treatment contained the lowest quantity of potassium; in other treatments, the quantities of potassium were greater. In particular, the greatest quantity of potassium was found in the treatment with arbuscular-mycorrhizal fungi; however, it was not sufficient to be significantly different. Meanwhile, the average quantities of potassium in all treatments with azotobacter, azospirillum, arbuscular-mycorrhizal fungi and chemical fertilizer were nearly similar (2.60, 2.72, 2.70 and 2.64%, respectively).

Quantity of nitrogen in the soil after harvesting

The quantity of nitrogen in the soil after harvesting is shown in Table 7. The quantity of soil nitrogen in each greenhouse treatment was significantly different at the 95% confidence level, whereas that in the field experiment was not.

In the greenhouse experiment, the soil nitrogen content in the control treatment was the lowest (0.07%) whereas in other treatments, it ranged between 0.10 and 0.11% and in the field experiment the range was about the same (0.17-0.18%).

Maize yield

The maize yields for both the greenhouse and field experiments are shown in Table 7 and were consistent with each other. The yields were significantly different at the 95% confidence level.

The outstanding yields in the greenhouse experiment resulted from the treatment of azotobacter+azospirillum+chemical-fertilizer (T+S+F), followed by the treatment of azotobacter+arbuscular-mycorrhizal-fungi+chemical-fertilizer(T+M+F), arbuscular-mycorrhizal fungi+chemical-fertilizer(M+F) and azotobacter+chemical-fertilizer(T+F), which yielded 216.72, 211.32, 203.28 and 198.42 g/plant, respectively. Rather low yields were recorded from the control treatment and the only azospirellum treatment (49.80 and 53.58 g/plant, respectively).

In the field experiment, the outstanding yields were from the treatments of azotobacter+chemical-fertilizer (T+F), arbuscular-mycorrhizal-fungi+chemical-fertilizer (M+F), arbuscular-mycorrhizal-fungi (M) and azotobacter+azospirillum+arbuscular-mycorrhizal-fungi+chemical fertilizer (T+S+M+F), with rates of 1,073.0, 998.0 and 958.0 kg/rai, respectively, followed by the yield from only chemical fertilizer treatment (932.0 kg/rai). The yield from other treatments was lower, with the lowest from the control treatment (567.0 kg/rai).

Considering the average yields from all treatment, yields from both the greenhouse and field experiments are consistent with each other. Thus, from the greenhouse experiments, the treatments with chemical fertilizer, arbuscular-

Table 7 Residual nitrogen content in the soil after harvest and the yield of single cross hybrid 4452 maize in green house and field experiments.

Treatment	Green house experiment		Field experiment	
	N (%)	Yield (g/plant)	N (%)	Yield (kg/rai)
Control	0.07b	49.80d	0.18	567.0e
Azotobacter(T)	0.10a	66.54d	0.18	680.0d-e
Azospirillum(S)	0.10a	53.58d	0.18	815.0b-d
Glomus aggregatum(M)	0.11a	68.52d	0.18	959.0a-c
Chemical fertilizer (F)	0.10a	147.42a-c	0.17	932.0a-d
T+S	0.11a	64.56d	0.18	697.0c-e
T+M	0.10a	101.28c-d	0.18	750.0b-e
T+F	0.11a	198.42a	0.18	1073.0a
S+M	0.10a	113.34b-d	0.17	756.0b-e
S+F	0.10a	172.32a-c	0.17	757.0b-e
M+F	0.11a	203.28a	0.18	998.0a-b
T+S+M	0.10a	63.84d	0.18	710.0c-e
T+S+F	0.10a	216.72a	0.18	841.0a-d
T+M+F	0.10a	211.32a	0.18	826.0b-d
S+M+F	0.10a	181.62a-b	0.18	849.0a-d
T+S+M+F	0.10a	174.72a-b	0.18	958.0a-c
F-test	*	*	ns	*
CV(%)	14.23	59.04	7.45	23.00

mycorrhizal fungi, azotobacter and azospirillum, had average yields of 166.69, 139.74, 137.18 and 130.09 g/plant, respectively, whereas from the field experiments, they were 904.2, 850.7, 816.9 and 797.9 kg/rai, respectively.

CONCLUSION

Azotobacter, azospirillum and arbuscular-mycorrhizal fungi are microorganisms that can be involved in planting single cross hybrid 4452 maize. An application of arbuscular-mycorrhizal fungi can enhance maize growth as much as that from chemical fertilizer. The alternative application of azotobacter, azospirillum and arbuscular-mycorrhizal fungi, together with a reduced quantity of nitrogen and phosphorus chemical fertilizer is possible. However, the yields may vary depending on the diverse conditions of the soil. In other words, the efficiency of each type of microorganisms may vary in accordance with the nature of the soil. In soil of low fertility, azotobacter and azospirillum will hold nitrogen and transfer it to the plant to help in its growth and yield, while arbuscular-mycorrhizal fungi will make phosphate dissolve and transfer to the plant. If the soil is fairly fertile, the plant growth and yield resulting from microorganisms may be observable, even if not clearly. However when microorganisms together with chemical fertilizer were used, maize growth and yield were markedly increased. The long-term application of these biofertilizers may enhance the improvement of the physical and biological conditions of the soil, making it more suitable to plant growth. This may provide a suitable alternative practice to reduce the costs in corn production.

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