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The effect of the combination of arbuscular mycorrhiza and rhizobacteria and doses of NPK fertilizer on the growth of *Sorghum bicolor* (L.) Moench

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Abstract. *Sorghum bicolor* (L.) Moench is one of the agricultural commodities that has important economic value. The use of fertilizer is done to increase productivity in order to follow the demand for sorghum which is quite high. Farmers widely use chemical fertilizers because they are cheap and easy to apply, but it has a negative impact on the environment. Sorghum growth naturally depends on microbes in the root area such as arbuscular mycorrhiza and rhizobacteria which have been known to increase sorghum growth. Arbuscular mycorrhiza and rhizobacteria as biological fertilizers can reduce the use of chemical fertilizers. This research aimed to study the effect of a combination of arbuscular mycorrhiza and rhizobacteria and doses of NPK fertilizer on sorghum growth. This study used two treatment factors, namely combination of microbes (arbuscular mycorrhiza, *Azospirillum* sp. and *Klebsiella* sp.) and doses of NPK (without NPK, 25% NPK, 50% NPK, 75% NPK, 100% NPK). Sorghum seeds of each treatment were individually grown on zeolite medium and were maintained in the greenhouse for two months. Plant growth parameters, colonization percentage of arbuscular mycorrhiza, rhizobacterial population, and root phosphate content were measured. Combination of *Azospirillum* sp. and *Klebsiella* sp. significantly increased shoot height and number of leaves. Combination of arbuscular mycorrhiza, *Azospirillum* sp. and *Klebsiella* sp. produced the highest dry weight. The dose of 25% NPK significantly increased all plant growth parameters. Combination of two rhizobacteria produced the highest root P content.

1. Introduction

The interaction between microbes in the rhizosphere has many good effects on plants. Microbes that have synergetic effects on plants include arbuscular mycorrhiza (AM) and plant growth-promoting rhizobacteria (PGPR). Several studies have shown that AM and PGPR can improve plant growth through increased absorption of nutrients from the soil [1]. Interactions between AM and rhizobacteria can influence plant physiology [2], nutrient acquisition [3], inhibition of plant pathogenic fungi [4], and enhancement of root branching [5]. Some bacteria are only able to associate with certain AM fungi. This shows that rhizobacteria have a high specificity of AM [6]. This can be caused by the presence of certain bacteria depending on the exudates released by some AM fungal species. Besides,



germination and growth rate of AM can also be affected by the presence of several types of rhizobacteria [7].

PGPR grows in plant roots and functions to increase plant yield through mechanisms such as improved mineral nutrition, disease suppression, or phytohormone production [8]. PGPR can interact with AM fungi [9]. Meanwhile, AM is mostly found in agricultural land [10]. AM plays an important role in the nutrition cycle [11]. AM through extramatrical hyphae can help the absorption and translocation of more nutrients than without symbiosis with AM [12] especially phosphate ions [13]. AM provides nearly 80% of plant phosphate requirements [14]. In addition, AM is able to provide macro and micronutrients such as N, K, Mg, Cu and Zn which are available little in the soil [15]. The AM association in host plants has an impact not only on increasing plant growth but helping plants to withstand environmental stress [16]. The combination of AM and PGPR can be inoculated simultaneously to improve plant growth.

Excessive use of chemical fertilizers can adversely affect the environment. The uncontrolled use of chemical fertilizers is one of the causes of the decline in the quality of the biological, physical, and chemical fertility of the soil. This has resulted in a decrease in the quality of agricultural land, resulting in lower land productivity. Its ability to reduce the impact of environmental pollution is proven to be in line with its ability to reduce the dose of chemical fertilizer use. One way to overcome environmental problems is by using biological fertilizers. AM and PGPR inoculation can improve plant growth. The use of microbes can increase the availability of nutrients for plants. While the use of chemical fertilizers has a low efficiency on the availability of nutrients that can be absorbed by plants. This is because these microbes produce root growth compared to plants that are not colonized by AM or PGPR. Mills and Jones [17] reported that good growth roots can increase the absorption of nutrients in the soil.

Sorghum bicolor (L.) is an agricultural commodity that has important economic value. This plant is able to live on marginal land especially in the tropics because it is able to adapt to low fertility soils [18]. A symbiosis between *S. bicolor* and AM can increase tolerance to *S. bicolor* drought stress and increase yield [19]. In addition, symbiosis with PGPR can increase the productivity of *S. bicolor* [20]. The use of a combination of AM and rhizobacteria as biological fertilizers can reduce the use of chemical fertilizers. The combination composition of AM and rhizobacteria will produce maximum plant growth. The combination of both as biofertilizers is also able to reduce the use of chemical fertilizers. Therefore, this research aimed to study the combination of arbuscular mycorrhiza and rhizobacteria and doses of NPK fertilizer on sorghum growth.

2. Materials and Methods

2.1. Inoculants, NPK, and experimental designs

This study uses 2 types of microbes namely arbuscular mycorrhiza and rhizobacteria (*Klebsiella* sp. and *Azospirillum* sp.). Factorial randomized complete design with two treatment factors, namely microbial combination and NPK fertilizer dosage. The first factor is the combination of microbes with 8 levels consisting of K (control), M (mycorrhizal), A (*Azospirillum* sp.), P (*Klebsiella* sp.), MA (mycorrhizal+*Azospirillum* sp.), MP (mycorrhizal+*Klebsiella* sp.), AP (*Azospirillum* sp.+*Klebsiella* sp.), and mix (mycorrhizal+*Azospirillum* sp.+*Klebsiella* sp.). While the second factor is the variation in the dose of NPK fertilizer with 5 levels consisting of N0 (without fertilizer), N1 (25% NPK), N2 (50% NPK), N3 (75% NPK), and N4 (100% NPK). Each combination treatment consisted of 3 replications so that 120 experimental units were obtained. The planting medium used in this study is zeolite.

2.2. Host plant preparation and planting

The seeds used as host plants are *S. bicolor*. Before planting, *S. bicolor* seeds were sterilized by the surface. Sorghum seeds were soaked in 70% alcohol for 1 minute then rinsed with sterile distilled water 3 times. Sorghum seeds are germinated on filter paper which has been moistened with sterile

aquadest to grow roots for five days. The seeds are ready to be planted on zeolite media until they are two weeks old.

The planting stage was done using 200-g pot (15 cm depth and 10 cm diameter). A total of 3 of 2-weeks old sorghum plant were planted at 150 g of zeolite. A total of 10 g AM inoculum was inoculated around the roots of the host plant. The organic material was added to the pot until approximately 1 cm from the top of the pot. Plants were maintained for five weeks in a greenhouse. *Azospirillum* sp. and *Klebsiella* sp. were given at 1 week after planting (WAP) and 3 WAP with 5 mL pot⁻¹. Plants are watered every day. NPK fertilizer is given only once a week at 2 WAP with the appropriate dose of each treatment.

2.3. Harvesting, percentage of AM colonization, rhizobacterial population, and root phosphate content

Sorghum plants were harvested after 5 weeks of day of plantation. Before harvesting, the shoot height and number of leaves were determined. The roots were cleaned from zeolite and washed with water. The shoots and roots were separated. The fresh weight of shoots and roots were weighed, then the shoots were dried in the oven at 80 °C for 24 hours until the weight was constant. Some roots were dried in the oven at 80 °C for 24 hours until the weight is constant and the other part was used to observe root colonization, percentage of AM colonization, and phosphate root content. The fresh weight and dry weight of the plants obtained were a combination of fresh weight and shoot and root dry weight. Analysis of root colonization was carried out based on the root coloring method [21]. The roots were washed with distilled water until the roots were clean from the planting media. The roots were then heated in 10% KOH (w/v) for 30 minutes at 60 °C, then rinsed up to three times. The roots were soaked in 2% HCl (v/v) for 1 minute, then rinsed with distilled water. The roots were colored by soaking in trypan blue 0.05% (w/v), then soaked in 50% (v/v) glycerol. The AM colonization structure was observed using a compound microscope (Olympus BX53 system, Japan). Calculation of the percentage of root colonization using the slide method [22].

Calculation of the rhizobacterial population uses the plate count method [23]. A total of 5 g of zeolite was taken from the root area of the composite 3 replications of each treatment. A total of 1 g of zeolite was put in a tube containing 9 mL of sterile aquadest and homogenized using vortex for 1 minute at 1000 rpm. As much as 1 mL is transferred to the next tube, doing the same until 10⁻³ dilution. A total of 0.1 mL of 10⁻¹ and 10⁻³ dilution results were put into sterile Petri-dish and selective media was poured (Pikovskaya agar for *Klebsiella* sp. and Caceres agar for *Azospirillum* sp.), then incubated for 3–7 days at 28 °C. The population of rhizobacteria is calculated using Stuart™ Scientific colony counter (Bibby Sterlin Ltd, US).

The P content of roots was measured using the ascorbic acid method [24]. The roots were washed using running water and placed in 10 mL of distilled water in 16 x 100 mm screw-cap tubes. The tubes were heated for 60 minutes. A total of 0.5 mL mixture of reagents containing ammonium molybdate, ascorbic acid, and antimony potassium tartrate in a single solution was reacted with 3 mL of extract root extract from the root of the Erlenmeyer flask. The standard P solution and sample solutions were added 2 mL of P color reagent and homogenized, then incubated for 30 minutes. The absorbance standard P solution, then blanks and sample solutions. P content in the solution was measured using a spectrophotometer at 800 nm. Roots content is expressed in content (in mg P L⁻¹).

2.4. Data analysis

The variables observed consisted of shoot height, number of leaves, plant fresh weight, plant dry weight, percentage of root colonization by AM, and phosphate content of roots. The data obtained were analyzed by a statistical variance of ANOVA followed by DMRT (Duncan Multiple Range) tests at the level of 5% using SPSS version 23.

3. Results and Discussion

3.1. Growth of host plants

Most AM and rhizobacterial inoculation treatments increased *S. bicolor* growth although not always significant. However, the results of the observations indicated that the dry weight of plants did not significantly affect all treatments. The inoculation of *Azospirillum* sp.+*Klebsiella* sp. treatment significantly increased shoot height and number of leaves compared to the control and other treatments. AM+*Azospirillum* sp.+*Klebsiella* sp. treatment also significantly increased shoot height, but did not increase the number of leaves significantly. The inoculation treatments of *Klebsiella* sp., AM+*Azospirillum* sp., and AM+*Klebsiella* sp., tended to be better in increasing shoot height and number of leaves than controls, AM, and *Azospirillum* sp. in AM treatment inoculations increased shoot height but not significantly compared to the control. However, the number of leaves in the AM treatment was better than the control, *Azospirillum* sp., and *Klebsiella* sp. The inoculation treatment of *Azospirillum* sp. and *Klebsiella* sp. increase shoot height but not significantly compared to controls. However, shoot height in the inoculation treatment of *Azospirillum* sp. did not significantly affect the control and other treatments (Table 1).

Table 1. *Sorghum bicolor* growth after 5 weeks grown in the nursery.

Treatments	Shoot height (cm)	Number of leaves	Plant dry weight (g)
Control	19.29 ^{ab}	1.29 ^{ab}	0.14 ^a
AM	23.73 ^{abc}	1.80 ^{bc}	0.21 ^a
<i>Azospirillum</i> sp.	16.97 ^a	1.09 ^{abc}	0.11 ^a
<i>Klebsiella</i> sp.	25.16 ^{bc}	1.67 ^{abc}	0.18 ^a
AM+ <i>Azospirillum</i> sp.	26.52 ^{bc}	1.87 ^{bc}	0.18 ^a
AM+ <i>Klebsiella</i> sp.	27.02 ^{bc}	1.78 ^{bc}	0.24 ^a
<i>Azospirillum</i> sp.+ <i>Klebsiella</i> sp.	30.03 ^c	2.00 ^d	0.24 ^a
AM+ <i>Azospirillum</i> sp.+ <i>Klebsiella</i> sp.	28.43 ^c	1.87 ^{bc}	0.43 ^a

Each value represents the mean of three replicates. Values (along each column) sharing the same letter are not significantly different at the 5% ($p \geq 0.05$) level as determined by DMRT.

The decrease of NPK fertilizer concentration was able to increase the percentage of AM colonization in *S. bicolor* roots. The highest percentage of AM colonization was found in the treatment without fertilizer. The percentage of AM colonization decreased along with the increase in NPK fertilizer concentration. The lowest percentage of colonization was found in the 100% NPK treatment. In general, AM colonization could be increased the growth of host plants. The observations showed that the treatment of 25% NPK was able to increase shoot height, number of leaves, plant fresh weight, and plant dry weight significantly compared to other treatments. The shoot height and the number of leaves without NPK fertilizer treatment tended to be better than the 50% NPK, 75% NPK, and 100% NPK treatments. However, without fertilizer treatment did not significantly influence the fresh weight and dry weight of plants. The fresh weight and dry weight of the 50% NPK treatment tended to be better than without fertilizer, 75% NPK, and 100% NPK treatments. However, shoot height and the number of leaves in the 50% NPK treatment did not increase significantly compared to 75% NPK and

100% NPK treatments. The treatment of 75% NPK and 100% NPK was not able to significantly increase shoot height, number of leaves, fresh weight, and dry weight (Table 2).

Table 2. *Sorghum bicolor* growth and root colonization after 5 weeks grown in the nursery.

Treatments	Shoot height (cm)	Number of leaves	Plant fresh weight (g)	Plant dry weight (g)	Colonization percentage (%)
Without fertilizer	43.28 ^c	2.90 ^c	1.07 ^{bc}	0.26 ^b	68.33 ^c
25% NPK	56.26 ^d	3.57 ^d	2.62 ^d	0.43 ^c	55.83 ^{bc}
50% NPK	19.71 ^b	1.51 ^b	1.74 ^c	0.32 ^{bc}	47.50 ^b
75% NPK	3.97 ^a	0.36 ^a	0.63 ^{ab}	0.07 ^a	10.83 ^a
100% NPK	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a

Each value represents the mean of three replicates. Values (along each column) sharing the same letter are not significantly different at the 5% ($p \geq 0.05$) level as determined by DMRT.

3.2. *Sorghum bicolor* root phosphate content

Most of the root phosphate content increased significantly at the concentrations of 75% NPK and 100% NPK in AM, AM+*Klebsiella* sp., and AM+*Azospirillum* sp treatments. The root phosphate content in AM and AM+*Azospirillum* sp. increased significantly at 100% NPK concentration. The phosphate content of AM treatment at 25% NPK concentration and 50% NPK tends to be better than without fertilizer treatment. Meanwhile, the phosphate content of the root in the treatment of AM+*Azospirillum* sp. not significantly different from the treatments of without fertilizer, 25% NPK and 50% NPK.

Meanwhile, the root phosphate content in AM+*Klebsiella* sp. significantly different from the concentration of 75% NPK. The root phosphate content of AM+*Klebsiella* sp. at concentrations of 25% NPK, 50% NPK, and 100% NPK tend to be better than without fertilizer treatment. Meanwhile, the phosphate content of roots in the AM+*Klebsiella* sp.+*Azospirillum* sp. Treatment significantly increased at a concentration of 25% NPK. Meanwhile, the phosphate content of the root of the AM+*Azospirillum* sp. treatment no significant effect on the without fertilizer, 25% NPK, 75% NPK, and 50% NPK treatments (Table 3).

Table 3. *Sorghum bicolor* root phosphate content after 5 weeks grown in the nursery.

Treatments	Without fertilizer	25% NPK	50% NPK	75% NPK	100% NPK
AM	3.47 ^{ab}	4.68 ^{bc}	4.07 ^{abcd}	4.99 ^{cd}	5.08 ^d
AM+ <i>Klebsiella</i> sp.	2.96 ^a	4.07 ^{abcd}	4.93 ^{cd}	5.05 ^d	4.55 ^{bcd}
AM+ <i>Azospirillum</i> sp.	3.80 ^{abcd}	3.78 ^{abcd}	4.19 ^{abcd}	4.99 ^{cd}	5.18 ^d
AM+ <i>Klebsiella</i> sp.+ <i>Azospirillum</i> sp.	4.55 ^{bcd}	5.33 ^d	3.91 ^{abcd}	3.31 ^{ab}	4.01 ^{abcd}

Each value represents the mean of three replicates. Values (along each column) sharing the same letter are not significantly different at the 5% ($p \geq 0.05$) level as determined by DMRT.

3.3. Rhizobacterial population

In general, the population of *Klebsiella* sp. the highest was in the concentration of 25% NPK in each treatment. The concentration of 25% NPK increased the population of *Klebsiella* sp. in the control, AM treatment, *Azospirillum* sp., AM+*Azospirillum* sp., AM+*Klebsiella* sp., and AM+*Azospirillum* sp.+*Klebsiella* sp., but did not occur in the *Klebsiella* sp. and *Azospirillum* sp.+*Klebsiella* sp. treatment. The treatment of AM+*Klebsiella* sp. produced the highest population of *Klebsiella* sp. compared to other treatments. Meanwhile, *Klebsiella* sp. could not grow on the 75% NPK and 100% NPK. The population of *Klebsiella* sp. in the AM treatment did not occur at a concentration of 50% NPK either. However, the population growth of *Klebsiella* sp. still happened in the treatment of AM and *Azospirillum* sp.+*Klebsiella* sp. on the giving of 75% NPK (Figure 1).

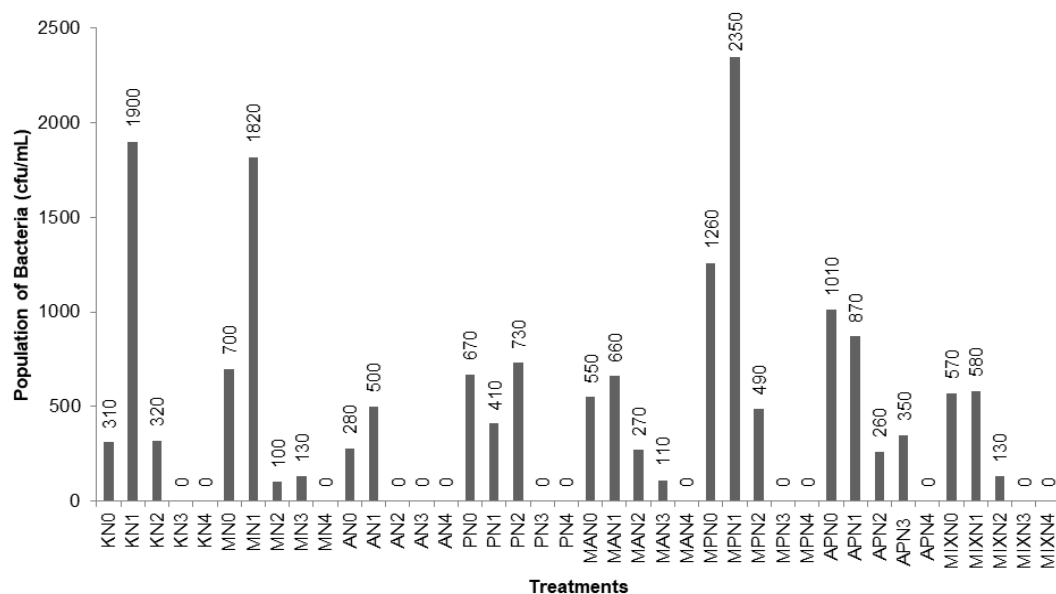


Figure 1. A Number of *Klebsiella* sp. population on *S. bicolor* of planting medium.

In general, the highest population of *Azospirillum* sp. was on the 25% NPK in each treatment. The concentration of 25% NPK increased the population of *Klebsiella* sp. in the control, AM, *Azospirillum* sp., AM+*Azospirillum* sp., AM+*Klebsiella* sp., and AM+*Azospirillum* sp.+*Klebsiella* sp. treatments, but did not occur in the *Klebsiella* sp. and *Azospirillum* sp.+*Klebsiella* sp. treatments which produced the highest population of *Klebsiella* sp. in the without fertilizer treatment. The *Azospirillum* sp.+*Klebsiella* sp. treatment produced the highest population of *Azospirillum* sp. compared to other treatments. Whereas, *Azospirillum* sp. could not grow on the 75% NPK and 100% NPK. The population of *Klebsiella* sp. of the AM treatment did not occur at the concentration of 50% NPK either. However, the population growth of *Klebsiella* sp. still happened in AM, AM+*Azospirillum* sp., and *Azospirillum* sp.+*Klebsiella* sp. on giving of 75% NPK (Figure 2).

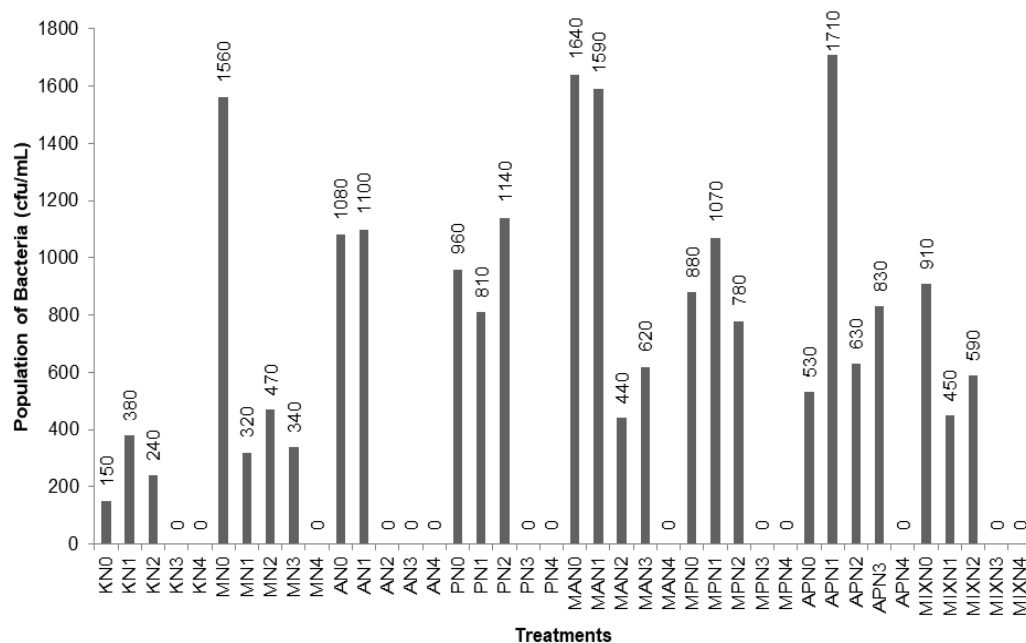


Figure 2. A Number of *Azospirillum* sp. population on *S. bicolor* of planting medium.

3.4. Arbuscular mycorrhizal growth in *S. bicolor* root

Arbuscular mycorrhiza colonized *S. bicolor* root in treatment without administration of NPK, 25% NPK, 50% NPK, and 75% NPK. Arbuscular mycorrhiza is able to form a new colonization structure at the root of the host plant. The structures of arbuscular mycorrhizal colonization observed include external hyphae, internal hyphae, and vesicles (Figure 3).

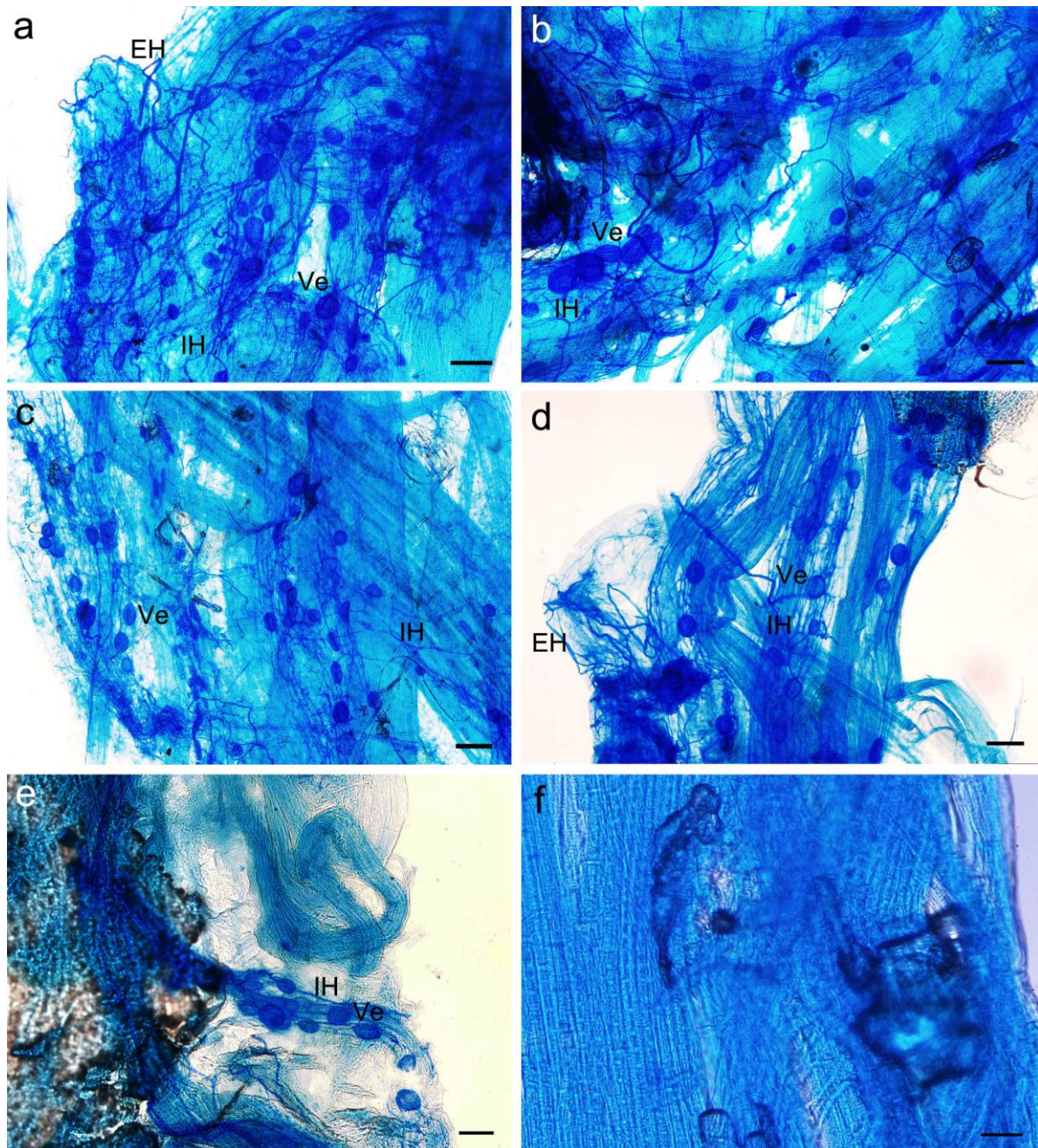


Figure 3. AM colonization of *S. bicolor* roots. **a, b** without fertilizer. **c** 25% NPK. **d** 50% NPK. **e** 75% NPK. **f** 100% NPK. *Ve* vesicle, *IH* internal hyphae, *EH* external hyphae. Scale bar 100 µm (**a, b, c, d, e**), 50 µm (**f**).

3.5. Discussion

The growth of *S. bicolor* can be affected by the combination of AM and rhizobacteria. In general, the combination treatment of AM and rhizobacteria was able to improve several growth parameters including shoot height and number of leaves compared to controls although not always significant. Combination treatment of *Azospirillum* sp.+*Klebsiella* sp. significantly increasing shoot height and number of leaves compared to controls and other treatments (Table 1). This result is also following Afifi *et al* [20] that the combined use of *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* was able to significantly increase nitrogenase, dehydrogenase and phosphatase activities,

plant height, dry weight plant and number of branches. Kapulnik *et al* [25] also showed that total shoot and root weights, total N content, plant height and leaf length were significantly increased by rhizobacteria inoculation. The dry weight of plants in each treatment was not significantly different from the controls (Table 1). The result shows that *S. bicolor* plant tissue has a high water content. The results of the Sarig *et al* [26] showed that *Azospirillum* inoculation was able to increase 19% in *S. bicolor* total stover dry-matter yield which had been planted for 60 days. In addition, rhizobacterial inoculation is able to increase dry weight plants [27]. This cannot be assumed to be different because the planting period used is not the same as this study.

AM colonization of *S. bicolor* root is influenced by the amount of nutrients present in the growing media. The provision of NPK fertilizer with various concentrations (without fertilizer, 25% NPK, 50% NPK, 75% NPK, and 100% NPK) resulted in a different percentage of AM colonization. The highest percentage of colonization was found in the treatment without fertilizer which reached 68.33%. The higher the concentration of fertilizer given the lower AM colonization is formed. At concentration of 100% NPK AM colonization is not formed (Table 2). The higher the amount of phosphate present in the soil, causes a decrease in AM colonization at the root of the host plant. Increased soil phosphate causes the influence of host plant growth because AM colonization will decrease. It can be said that AM associations in host plants do not have a beneficial effect [28].

The dose of 25% NPK significantly improved the shoot height, number of leaves, fresh weight, and dry weight compared to other treatments (Table 2). The biomass of the plants (dry weight basis) increased at NPK concentrations of 26%, 29%, and 35% compared to those without NPK under optimal irrigation. Water use efficiency also increased based on fresh weight yield at NPK concentrations including 37%, 42% and 55% [29].

In general, the majority of treatments are able to increase the phosphate content in the roots even though it is not always significant. The concentration of 75% NPK and 100% NPK in AM, AM+*Klebsiella* sp., and AM+*Azospirillum* sp. which is able to increase the phosphate content of the roots significantly. Conversely, treatment of AM+*Klebsiella* sp.+*Azospirillum* sp. significantly increases the root phosphate content at a concentration of 25% NPK. Thus, an increase in NPK fertilizer concentration was not positively correlated with an increase in phosphate content in roots. This shows that the high dose of NPK in the growing medium is not absorbed by the root of the host plant. According to Holevas [30], phosphate available in the soil is phosphate which can be absorbed by plants.

Most of the population of *Klebsiella* sp. significantly increased at a concentration of 25% NPK in each treatment. While most of the growth of *Klebsiella* sp. did not occur at a concentration of 75% NPK and 100% NPK (Figure 1). The presence of *A. bradense* in the rhizosphere increased VAM colonization and biomass. While the N input due to *Azospirillum* is decreased, it may be a competition for carbohydrates [31]. Populations of *Azospirillum brasilense* reached 2.5×10^6 to 1.3×10^6 cfu g soil⁻¹ at 30 days after inoculation [32]. Most of the population of *Azospirillum* sp. also increased significantly at a concentration of 25% NPK in each treatment. However, the average growth of *Klebsiella* sp. did not occur at a concentration of 75% NPK and 100% NPK (Figure 2). Population of rhizobacteria increases through the use of biofertilizers, organic manure and chemical fertilizer systems. The use of biofertilizers can reduce the amount of chemical fertilizer use. The use of 50% in combination with 50% inorganic biofertilizer and through the N and PK through chemical fertilizers is able to produce maximum yield for onion cultivation [33].

AM colonization of *S. bicolor* roots can be characterized by the formation of new structures formed. New structures formed in the roots of *S. bicolor* include external hyphae, internal hyphae, and vesicles (Figure 3). According to Sieverding [34] reported that new structures were formed at the root of host plants such as hyphae, vesicles, arbuscules, and spores due to AM colonization. In addition to nutrients present in the soil, the formation of new structures resulting from AM colonization is also strongly influenced by temperature and soil pH [14, 35, 36].

4. Conclusion

Combination of arbuscular mycorrhiza, *Azospirillum* sp. and *Klebsiella* sp. produced the highest dry weight of *Sorghum bicolor*. The dose of 25% NPK significantly increased all plant growth parameters. Combination of two rhizobacteria produced the highest root P content.

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