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# Synergistic Interaction of Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Bacteria on *Sorghum bicolor* (L.) Moench Growth under Saline Condition

INDRIATI RAMADHANI[1*] AND SRI WIDAWATI[1*]

[1]*Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences-LIPI, Jalan Raya Jakarta-Bogor Km 46, Cibinong 16911, Indonesia*

*I Ramadhani and S Widawati contributed equally to this work.

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a food source and a raw material in biofuel-ethanol production. Arbuscular mycorrhizal fungi (AMF) and phosphate-solubilizing bacteria (PSB) are called rhizosphere microorganisms, which are useful microorganisms that enhance plant growth. Rhizosphere microorganisms also increase plant's resistance to environmental stress, while NPK fertilizer used on agricultural land are found to increase crop yields only. However, its continuous application has a negative impact on the environment. Therefore, this research aimed to study the synergy between AMF, and PSB with NPK fertilizer in influencing the sweet sorghum growth in saline condition. Two treatment factors were used, which are microbes combination (arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria), and NPK doses without NPK, 25% NPK, 50% NPK, and 100% NPK. A complete randomized design was used as an experimental design with 3 replications for each treatment. Furthermore, zeolite was used to grow sweet sorghum seeds individually and were maintained for one month in a greenhouse. Pots were watered with 50% seawater (freshwater: seawater = 1:1) every day to keep the moisture. Plant growth parameters were also measured, which includes AMF colonization in the roots, number of AMF spores, and PSB population in the planting medium. The combination of AMF, PSB, and NPK in sweet sorghum increased the plant height, number of leaves, plant fresh weight, plant dry weight, and total of plant P, although not always significant. AMF+PSB+25% NPK produced the highest number in all parameters. Therefore, the synergy between AMF, and PSB with NPK fertilizer is able to increase the plant growth in saline condition.

Key words: arbuscular mycorrhizal fungi, NPK, phosphate-solubilizing bacteria, saline, sweet sorghum

Sorgum manis (*Sorghum bicolor* (L.) Moench) merupakan sumber makanan dan bahan baku dalam produksi bioetanol. Jamur mikoriza arbuskular (JMA) dan bakteri pelarut fosfat (BPF) disebut sebagai mikroorganisme rizosfer, yang merupakan mikroorganisme yang berguna untuk meningkatkan pertumbuhan tanaman. Mikroorganisme rizosfer juga meningkatkan ketahanan tanaman terhadap cekaman lingkungan, sedangkan pupuk NPK yang digunakan pada lahan pertanian ternyata hanya meningkatkan hasil panen saja. Namun, penerapannya yang berkelanjutan memiliki dampak negatif terhadap lingkungan. Oleh karena itu, penelitian ini bertujuan untuk mempelajari sinergi antara JMA, dan BPF dengan pupuk NPK dalam mempengaruhi pertumbuhan sorgum manis dalam kondisi salin. Dua faktor perlakuan yang digunakan, yaitu kombinasi mikroba (jamur mikoriza arbuskular dan bakteri pelarut fosfat), dan dosis NPK tanpa NPK, 25% NPK, 50% NPK, dan 100% NPK. Rancangan acak lengkap digunakan sebagai desain penelitian dengan 3 ulangan untuk tiap perlakuan. Selanjutnya, zeolit digunakan untuk menumbuhkan biji sorgum manis secara individu dan dipelihara selama satu bulan di rumah kaca. Pot disiram dengan 50% air laut (air tawar: air laut = 1: 1) setiap hari untuk menjaga kelembaban. Parameter pertumbuhan tanaman juga diukur, yang meliputi kolonisasi JMA di akar, jumlah spora JMA, dan populasi BPF dalam media tanam. Kombinasi JMA, BPF, dan NPK dalam sorgum manis meningkatkan tinggi tanaman, jumlah daun, berat basah tanaman, berat kering tanaman, dan P total tanaman, meskipun tidak selalu signifikan. JMA + BPF + 25% NPK menghasilkan nilai tertinggi di semua parameter pertumbuhan. Oleh karena itu, sinergi antara JMA dan BPF dengan pupuk NPK mampu meningkatkan pertumbuhan tanaman dalam kondisi salin.

Kata kunci: bakteri pelarut fosfat, jamur mikoriza arbuskula, NPK, salin, sorgum manis

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a plant which is widely cultivated due to its usefulness as a food source and a raw material in biofuel-ethanol production (Dar *et al*. 2018). Its sufficient carbohydrate content consists of approximately 62% carbohydrates, being a potential sweet sorghum component (Barcelos *et al*. 2016). It is a suitable bioethanol feedstock in Eastern Indonesia (Hasibuan and Nazir 2017). Previous studies showed that sweet sorghum has a higher energy output than sugarcane, sugar beet, corn, and wheat (Dar *et al*. 2018).

*Corresponding author: Phone: +62-8567364656; Email: indriatiramadhani@gmail.com

Soil salinity is an environmental condition which is toxic to plants (Liang *et al*. 2018). The high $Na^+$ and $Cl^-$ ion contents in saline soils reduce the nutrient absorption, nutritional balance, and disrupt plant metabolic processes (Arzani and Ashraf 2016). In addition, high soil salinity reduces soil porosity and aeration (Khataar *et al*. 2018). Also, it reduces water absorption and causes physiological dryness (Saxena *et al*. 2017).

Production of alternative food substitutes for rice is needed for coastal communities in Indonesia. Sweet sorghum can be used as one of the potential food to be developed in coastal areas (Marles *et al*. 2018). Sweet sorghum has quite high carbohydrates, but people prefer to use it for biofuel production and feedstock (Dar *et al*. 2018). Several studies have also been conducted to find formulas that can help sweet sorghum grow in saline stress condition. One of them is the use of rhizosphere microbes such as arbuscular mycorrhizal fungi (AMF), which have been shown to help sweet sorghum to grow in saline conditions (Wang *et al*. 2019).

Rhizosphere microorganisms are useful microorganisms, known to increase plant growth and enhance resistance to environmental stress (de Zelicourt *et al*. 2013). A typical AMF is a symbiotic mutualism relationship between soil fungi and plant roots, which is able to assist plants in nutrient absorption and increase its tolerance to environmental stress (Smith and Read 2008). While phosphate-solubilizing bacteria (PSB) are rhizosphere microorganisms, which assist plants in nutrient absorption, such as phosphate (Awasthi *et al*. 2011). In addition, PSB are discovered to increase the bioavailability of phosphate in mycorrhizal plants (Toro *et al*. 1997).

NPK fertilizer used on agricultural land are found to increase crop yields, change the crop quality, and the soil productivity (Zhong and Cai 2007). However, mineral fertilizer application on soil causes shift in microbial community (Chu *et al*. 2007). While microbial application enhances plants' tolerance to environmental stress (Bhardwaj *et al*. 2014). Thus, NPK fertilizer combined with potential microbes reduces its impact on the agricultural land. Previous studies showed that the combination of PSB and AMF was well studied in several types of plants such as *Helianthus tuberosus*, *Triticum aestivum*, and *Sorghum bicolor* (Rupaedah *et al*. 2014; Minaxi *et al*. 2013; Nacoon *et al*. 2020). However, little known whether the combination of PSB and AMF is able to increase plant growth in saline condition. Therefore, this research aimed to study the synergy between AMF and PSB with the NPK fertilizer in influencing sweet sorghum growth in saline condition.

## MATERIALS AND METHODS

**Preparation of Planting Media**. Two hundred and sixty grams of zeolite grade 1 (0.7–1.4 mm in diameter) was placed in a pot measuring 13 cm high and 10 cm in diameter, covered and sterilized in an autoclave at 121°C for 15 minutes. NPK fertilizer used was blue in color and granular, easily soluble in water, and has the main content of N (16%), P (16%) and K (16%). Before planting sweet sorghum, NPK were first added to the media with several concentrations, namely 0% NPK (0 g NPK), 25% NPK (0.625 g NPK), 50% NPK (1.250 g NPK), and 100% NPK (2.500 g NPK). This preparation was carried out in a sterilized room.

**Biostimulant Preparation, Plant Root Inoculation, and Planting**. Microbes used were arbuscular mycorrhizal fungi (AMF) and phosphate-solubilizing bacteria (PSB). The arbuscular mycorrhizal fungi used were obtained from the soil (pH = 5.6) of the Tasikmadu sugar cane plantation, Karanganyar, central Java, Indonesia. The phosphate-solubilizing bacteria used were *Pseudomonas fluorescens* isolate ILPL1 which isolated from the rhizosphere *Amaranthus hybrydus* and *Ipomoea aquatica* on the coast of Laki Beach, Seribu Islands (Widawati 2011). Two treatment factors were used, which are microbes combination (AMF and PSB), and NPK doses without NPK, 25% NPK, 50% NPK, and 100% NPK. A complete randomized design was used as an experimental design with 3 replications for each treatment. A biostimulant in the form of liquid inoculant, isolated from *Pseudomonas fluorescens,* was made by culturing 1 ose needle into 50 mL Pikovskaya media (5 g $Ca_3(PO_4)_2$. 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl; 0.1 g $MgSO_4$. $7H_2O$; 0.2 g KCl; 10 g glucose; 0.5 g yeast extract; 20 g agar; $MnSO_4$ and a little $FeSO_4$, 1000 $l^{-1}$ aquadest) (Gaur 1981), placed in a shaker for 5 days then transferred into 500 mL liquid Pikovskaya and placed in a shaker again. The inoculant was homogeneous and contained bacterial $10^8$ cfu/mL. Then, the sorghum seeds were washed with 70% alcohol rinsed 3 times with sterilized distilled water and left to germinate in a Petri-dish containing a sterilized filter paper. After growing 2 leaves, sweet sorghum root was inoculated with arbuscular mycorrhizal spores and a slightly wet tissue containing

20 AMF spores,was attached to sweet sorghum roots. Then, sweet sorghum root was placed into the planting hole with 5 mL of liquid PSB inoculant and covered with zeolite. Plants were maintained for one month in a greenhouse, while they were watered with 50% seawater (freshwater: seawater = 1:1) every day to maintain the moisture of the zeolite. Fifty mL of 50% seawater is used to water the sweet sorghum seeds to grow sprouts every day. After the seedlings have grown sprouts, they were watered with 100 mL of seawater every day. Thus, after 2 months, sweet sorghum plants were harvested to determine the AMF colonization in the root, AMF and PSB population in the planting medium. The parameters measured include plant height, number of leaves, fresh weight, dry weight of plants, and the absorption of P in leaves (total P content of plants). The bacterial population in the planting mediumwere calculated by the number plate method (Marschner and Dell 1994) with a dilution method of $10^{-1}$ to $10^{-7}$. A total of 5 g of zeolite was taken from the composite where plant roots were harvested and used for 3 repeated tests. A total of 1 g of zeolite was taken and placed into a test tube containing 9 mL of sterilized aquadest ($10^{-1}$ dilution) and homogenized using vortex for 1 minute at 1000 rpm. One mL sample was taken from the $10^{-1}$ dilution and transferred to a $10^{-2}$ dilution tube: this procedure was repeated until the $10^{-7}$ dilution. Also 0.1 mL sample was taken from $10^{-3}$, $10^{-5}$, and $10^{-7}$ dilutions into a sterilized Petri-dish, while Pikovskaya media was added to it. Bacterial population calculations were carried out after incubation for 3–7 days at 28 °C. The calculation of PSB population on Pikovskaya media was carried out only on *P. fluorescens* colonies that formed the clear zone (halo zone).

**Analysis of Root Colonization**. Root colonization analysis by AMF was carried out following the root staining method (Brundrett *et al*. 1996). These test's procedures are as follows: The roots were washed clean 3 times with distilled water, the roots were soaked in 10% KOH (w/v) heat at 60 °C for 30 minutes, the solution was removed and the roots were washed 3 times with distilled water, the roots were soaked again in 2% HCl (v/v) for 2−3 minutes, then the solution was removed and the roots were soaked the 3rd time in 0.05% (w/v) hot trypan blue dye solution at 90 °C or put in an autoclave at a pressure of 15 psi for 10 minutes, the solution was removed and the roots were soaked the 4th time in a destaining solution that was 50% (v/v) glycerol, then the roots were cut into ± 1 cm. Ten pieces of roots were taken randomly and arranged in a row on the glass object. Root colonization by AMF was observed and calculated under a compound microscope by the slide method (Giovannetti and Mosse 1980). The colonization percentage of AMF was calculated from the following equation: Percentage of AMF colonization = (Root length infected/Root length observed) × 100%.

**Calculation of AMF Spore Density in Planting Media**. Calculation of AMF spore density after harvest was conducted using 10 g of growth media by the wet-sieving sucrose centrifugation method (Brundrett *et al*. 1996). The filtrate (spores) from the filtration (water mixed with spores) were added into a Petri-dish with gridline attached underneath (size 0.5–1 cm).

**Analysis of N and P Content in Plants**. Measurement of N and P contents in plants follows the method from the soil research book compiled by Sulaeman *et al*. (2005). Leaves were dried at 50 °C until moisture content was lost. A total of 0.2 g of leaves were dissolved in 2 mL of acidic solution, then were extracted using a destruction block at a temperature of 170 °C or until the solution was clear. The solution was filtered and diluted with distilled water to a volume of 10 mL. A total of 1 mL of solution was dissolved in 1 mL of P dye and added 3 mL of distilled water. Furthermore, the absorbance was measured with a spectrophotometer with a wavelength of 450 nm.

**Data Analysis**. The data obtained were analyzed with ANOVA statistical variants followed by DMRT (Duncan Multiple Range Test) at 5% using SPSS version 23.

## RESULTS

**Growth of Sweet Sorghum.** In general, sweet sorghum seedlings inoculated with AMF, PSB, different NPK concentrations, and harvested after 1-month have significantly increased of plant growth, though the gradual increase of NPK did not always correlate with an increase in plant growth at the $p<0.05$ level (Table 1, Figure 1a). All treatments significantly increased plant height compared to the control treatments. The 25% NPK treatment improved plant height more than 50% NPK and 100% NPK. The PSB+25% NPK treatment significantly increased plant height compared to PSB only, PSB+50% NPK, and PSB+100% NPK. The AMF+25% NPK treatment significantly increased plant height compared to PSB only, AMF+50% NPK, and AMF+100% NPK (Table 2). The AMF+PSB+25% NPK treatment significantly increased plant height compared to AM+PSB only,

AMF+PSB+50% NPK, and AMF+PSB+100% NPK. The AMF+PSB+25% NPK treatment significantly increased plant height compared to the control and other treatments (Table 1, Figure 1b). All treatments did not significantly increase the number of leaves compared to the control treatments. The 50% NPK treatment significantly increased the number of leaves compared to control, 25% NPK, and 100% NPK. The PSB+100% NPK treatment did not significantly increase the number of leaves compared to control, PSB only, PSB+25% NPK, and PSB+50% NPK. A similar result was also observed for AMF+NPK treatments. The AMF+100% NPK treatment also did not significantly increase the number of leaves compared to control treatments, AMF only, AMF+25% NPK, and AMF+50% NPK. The AMF+PSB+25% NPK and AMF+PSB+50% NPK treatments significantly increased the number of leaves compared to the control and other treatments (Table 1, Figure 1).

All treatments did not significantly increase plant fresh weight compared to the control treatments. The 50% NPK treatment significantly increased plant fresh weight compared to control, 25% NPK, and 100% NPK. The PSB, PSB+25% NPK, and PSB+50% NPK treatments significantly increased plant fresh weight compared to the control treatments, and PSB+100% NPK. A similar result was also observed for AMF+NPK treatments. The AMF+100% NPK treatment also did not significantly increase plant fresh weight compared to the control treatments, AMF, AMF+25% NPK, and AMF+50% NPK. The AMF+PSB+25% NPK treatment significantly increased plant fresh weight compared to AMF+PSB only, AMF+PSB+50% NPK, and AMF+PSB+100% NPK. The AMF+PSB+25% NPK treatment significantly increased plant fresh weight compared to the control and other treatments (Table 1). All treatments did not significantly increase plant dry weight compared to the control treatments. The 50% NPK treatment significantly increased plant dry weight compared to control, 25% NPK, and 100% NPK. The PSB, PSB+25% NPK, and PSB+50% NPK treatments significantly increased plant dry weight compared to control and PSB+100% NPK. A similar result was also observed for AMF+NPK treatments. The AMF+100% NPK treatment also did not significantly increase plant dry weight compared with control, AM only, AMF+25% NPK, and AMF+50% NPK. The AMF+PSB+25% NPK treatment significantly increased plant dry weight compared to AMF+PSB only, AMF+PSB+50% NPK, and AMF+PSB+100% NPK. The AMF+PSB+25% NPK treatment significantly increased plant dry weight compared to the control and other treatments (Table 1).

All treatments did not significantly increase a total of plant P compared to the control treatments. The 25% NPK treatment significantly increased the total of plant P compared to control, 50% NPK, and 100% NPK. The 100% NPK treatment did not significantly increase the number of leaves compared to control treatments. The PSB+25% NPK treatment significantly increased the total of plant P compared to control, PSB+50% NPK, and PSB+100% NPK. The PSB+100% NPK treatment did not significantly increase a total of plant P compared to control. The AMF+25% NPK and AMF+50% NPK treatments significantly increased the total of plant P compared to control treatments, AMF, and AMF+100% NPK. The AMF+PSB+25% NPK treatment significantly increased the total of plant P compared to the control and other treatments (Table 1).

**Microbial Populations on Sweet Sorghum of Planting Medium.** In general, the highest population of PSB found on planting media after 1-month, was in PSB inoculation without NPK addition. The addition of 100% NPK concentration significantly decreased the PSB population. Subsequently, the PSB population also increased on AMF and NPK addition. The increased PSB population started from AMF addition without NPK. The PSB population increased gradually until the addition of AMF with 50% NPK. However, the PSB population decreased significantly with AMF and 100% NPK (Table 2).

The decreased NPK concentration increased the colonization percentage of AMF in *S. bicolor* roots. Therefore, the highest colonization percentage of AMF was produced in AMF inoculation without NPK but with 25% NPK addition. The percentage of AMF colonization decreased significantly until the addition of 100% NPK. Although, PSB addition also increased the colonization percentage of AMF in sweet sorghum roots. The highest colonization percentage of AMF was produced in the PSB inoculation without NPK but with 25% NPK addition. The colonization percentage of AMF decreased along with the increase in NPK concentration. Thus, the lowest colonization percentage was found in the PSB inoculation with 100% NPK (Table 2).

The highest number of AMF spores in the sweet sorghum planting media was in the AMF inoculation with 25% NPK addition but without NPK. The highest number of AMF spores in *S. bicolor* roots planting

Table 1 Effect of AMF, PSB and NPK concentrations on various growth parameters of sweet sorghum growth after 4 weeks

| Treatments | Plant height (cm) | Number of leaves | Plant fresh weight (g) | Plant dry weight (g) | Total of plant P (%) |
|---|---|---|---|---|---|
| Control | 16.00$^{a}$ | 4.00$^{a}$ | 0.50$^{a}$ | 0.15$^{a}$ | 0.03$^{a}$ |
| 25% NPK | 39.60$^{de}$ | 4.00$^{a}$ | 1.50$^{bc}$ | 0.45$^{bc}$ | 17.02$^{d}$ |
| 50% NPK | 37.33$^{cd}$ | 5.00$^{b}$ | 1.50$^{bc}$ | 0.46$^{bc}$ | 5.60$^{b}$ |
| 100% NPK | 29.00$^{b}$ | 4.00$^{a}$ | 0.93$^{ab}$ | 0.28$^{ab}$ | 1.11$^{a}$ |
| PSB | 42.00$^{ef}$ | 5.00$^{b}$ | 1.83$^{bc}$ | 0.56$^{bc}$ | 11.68$^{c}$ |
| PSB+25% NPK | 46.67$^{gh}$ | 5.00$^{b}$ | 1.83$^{bc}$ | 0.55$^{bc}$ | 20.23$^{de}$ |
| PSB+50% NPK | 46.33$^{gh}$ | 5.00$^{b}$ | 1.50$^{bc}$ | 0.44$^{bc}$ | 16.98$^{d}$ |
| PSB+100% NPK | 35.00$^{c}$ | 4.00$^{a}$ | 1.17$^{ab}$ | 0.35$^{ab}$ | 1.12$^{a}$ |
| AMF | 42.00$^{ef}$ | 5.00$^{b}$ | 1.83$^{bc}$ | 0.54$^{bc}$ | 11.38$^{c}$ |
| AMF+25% NPK | 47.00$^{gh}$ | 5.00$^{b}$ | 1.87$^{bc}$ | 0.56$^{bc}$ | 17.41$^{d}$ |
| AMF+50% NPK | 44.67$^{fg}$ | 5.00$^{b}$ | 1.50$^{bc}$ | 0.46$^{bc}$ | 17.00$^{d}$ |
| AMF+100% NPK | 35.00$^{c}$ | 4.00$^{a}$ | 1.07$^{ab}$ | 0.32$^{ab}$ | 0.79$^{a}$ |
| AMF+PSB | 49.00$^{hi}$ | 5.00$^{b}$ | 2.33$^{cd}$ | 0.71$^{cd}$ | 23.02$^{e}$ |
| AMF+PSB+25% NPK | 58.33$^{j}$ | 6.00$^{c}$ | 3.67$^{e}$ | 1.11$^{e}$ | 29.96$^{f}$ |
| AMF+PSB+50% NPK | 50.00$^{i}$ | 6.00$^{c}$ | 3.00$^{de}$ | 0.90$^{de}$ | 17.33$^{d}$ |
| AMF+PSB+100% NPK | 37.67$^{cd}$ | 4.00$^{a}$ | 1.17$^{ab}$ | 0.35$^{ab}$ | 1.17$^{a}$ |

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.

media was in the AMF inoculation with PSB and 50% NPK addition. However, the number of AMF spores did not always correlate with the increase in NPK concentration. The highest number of AMF spores in the PSB and NPK was in the AMF+PSB+50% NPK. Therefore, the addition of PSB and NPK did not significantly affect the number of AMF spores (Table 2).

**Arbuscular Mycorrhizal Fungi Colonization on Sweet Sorghum.** Arbuscular mycorrhiza colonized *S. bicolor* root in treatment with AMF+PSB+25% NPK, AMF+25% NPK, AMF, PSB+AMF, AMF+PSB+50% NPK, AMF+50% NPK, and AMF+PSB+100% NPK. However, the control treatment was not found in the AM colonization structure. AM is able to form a new colonization structure at the root of the sweet sorghum. The structure of arbuscular mycorrhizal colonization observed is vesicular (Figure 2a−h).

## DISCUSSION

In general, the synergy between AMF, PSB, and NPK fertilizers increase the sweet sorghum growth in saline condition.Previous study has shown that the combination of AMF, PSB, and NPK could increase the growth of sweet sorghum (Ramadhani *et al*. 2019). Salt in growth medium affect plant growth and development because salt reduces nutrient acquisition in plants (Ruiz-Lozano *et al*. 2012). Also, the high level of salt in the soil causes very important ecological and agronomic problems (Yaish and Kumar 2015). Hence, AMF increases the host plant growth under salt pressure (Zuccarini and Okurowska 2008). The soil microorganisms in rhizosphere also increase the nutrient availability and enhance the IAA production (Armada *et al*. 2015; Marulanda *et al*. 2009). In other treatments, the combination of selected microbes,

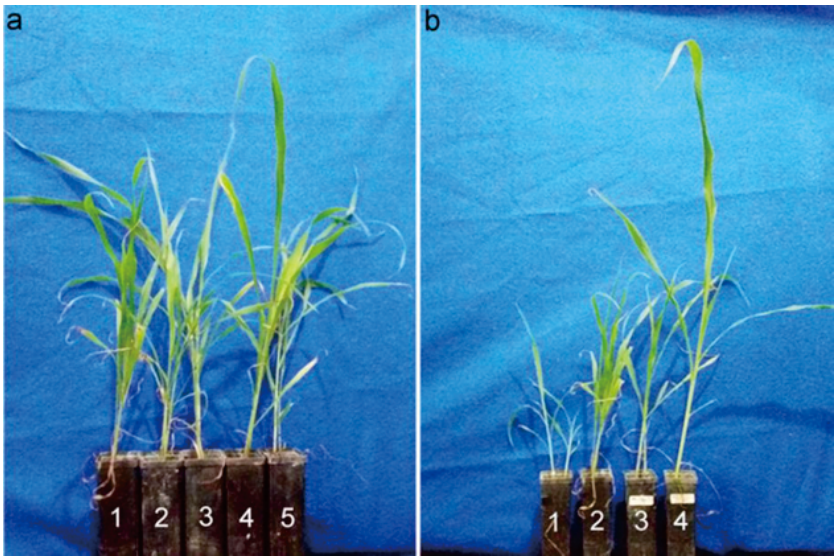


Fig 1 Growth of *Sorghum bicolor* 1 months after planting. **a** (1) 25% NPK; (2) AMF; (3) PSB+AMF; (4) PSB; (5) AMF+PSB+25% NPK. **b** (1) control; (2) PSB+100% NPK; (3) AMF+100% NPK; (4) AMF+PSB+25% NPK.

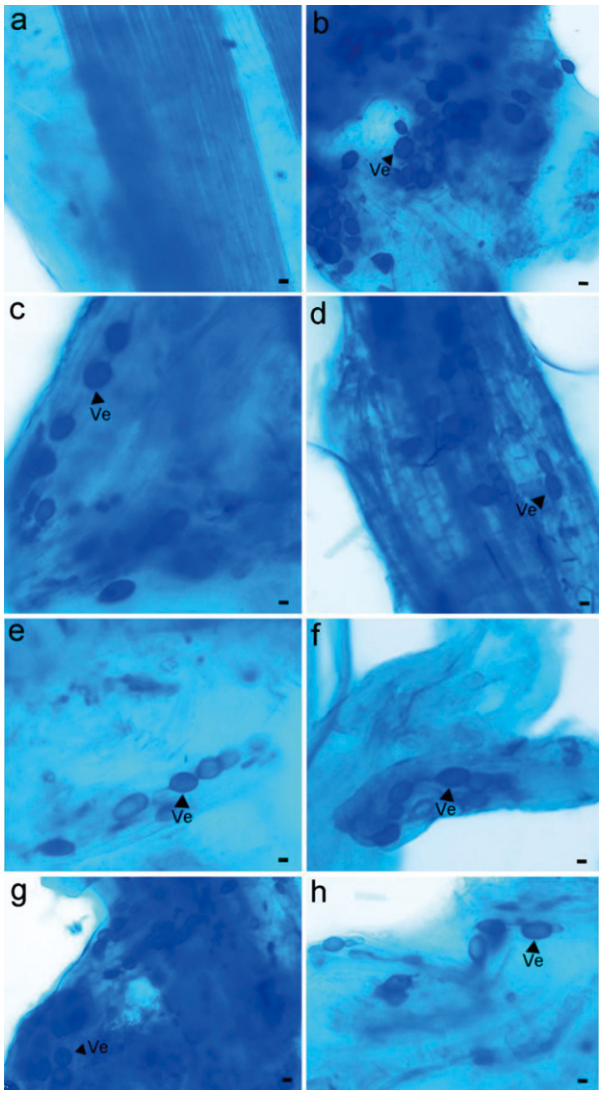


Fig 2 AMF colonization on sweet sorghum roots. **a** control. **b** AMF+PSB+25% NPK.**c** AMF+25% NPK. **d** AMF. **e** PSB+AMF. **f** AMF+PSB+50% NPK. **g** AMF+50% NPK. **h** AMF+PSB+100% NPK. *Ve* vesicle. Scale bars 20 µm (**a**–**h**).

Table 2 Effect of AMF, PSB, and NPK concentrations on microbial populations on sweet sorghum of planting medium

| Treatments | Number of viable PSB cells (cell/g zeolite) | Colonization percentage of AM (%) | Number of AM spores (spores/10 g zeolite) |
|---|---|---|---|
| Control | $0.0^{a}$ | $0.0^{a}$ | $0.0^{a}$ |
| 25% NPK | $0.0^{a}$ | $0.0^{a}$ | $0.0^{a}$ |
| 50% NPK | $0.0^{a}$ | $0.0^{a}$ | $0.0^{a}$ |
| 100% NP K | $0.0^{a}$ | $0.0^{a}$ | $0.0^{a}$ |
| PSB | $58.7 \times 10^{6\,c}$ | $0.0^{a}$ | $0.0^{a}$ |
| PSB+25% NPK | $36.7\times 10^{6\,b}$ | $0.0^{a}$ | $0.0^{a}$ |
| PSB+50% NPK | $33.0 \times 10^{6\,b}$ | $0.0^{a}$ | $0.0^{a}$ |
| PSB+100% NPK | $0.4 \times 10^{6\,a}$ | $0.0^{a}$ | $0.0^{a}$ |
| AMF | $0.0^{a}$ | $82.0^{gh}$ | $21.0^{f}$ |
| AMF+25% NPK | $0.0^{a}$ | $83.3^{gh}$ | $25.0^{g}$ |
| AMF+50% NPK | $0.0^{a}$ | $50.0^{d}$ | $16.0^{d}$ |
| AMF+100% NPK | $0.0^{a}$ | $10.0^{b}$ | $2.0^{b}$ |
| AMF+PSB | $18.3 \times 10^{6\,ab}$ | $80.0^{f}$ | $3.0^{b}$ |
| AMF+PSB+25% NPK | $22.7 \times 10^{6\,ab}$ | $90.0^{i}$ | $3.0^{b}$ |
| AMF+PSB+50% NPK | $31.0 \times 10^{6\,b}$ | $60.0^{e}$ | $18.0^{e}$ |
| AMF+PSB+100% NPK | $0.5 \times 10^{6\,a}$ | $20.0^{c}$ | $6.0^{c}$ |

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.

*Pseudomonas,* and AMF, increase their host plant growth in saline conditions (Hidri *et al*. 2016).

Our result shows that AMF, PSB, and NPK inoculation in sweet sorghum increase plant height, the number of leaves, plant fresh weight, plant dry weight, and total P compared to control treatments, though it is not always significant. In other treatments, the salinity reduces plant height, size, and yield on the crop of Brassica (Zamani *et al*. 2010). Moreover, the salinity may reduce the crop yield by interfering with the water and nutritional balance of the host plant (Islam *et al*. 2001). Sweet sorghum plant in saline condition with the AMF+PSB+25% NPK treatment showed significant increase in height, number of leaves, plant fresh weight, plant dry weight, and total P. In other treatments, 25% NPK increased all growth parameters of sweet sorghum plant. However, this study was not conducted under saline condition (Ramadhani *et al*. 2019).

Our result showed that the AMF+PSB+25% NPK treatment significantly increased plant fresh and dry weight compared to the control and other treatments. g. Based on the host plant growth data, the higher the NPK concentration, the more inhibiting thehost plant growth. The host plantgrowth at the highest concentration of treatment, namely 100% NPK alone, was more inhibited than the combination of PSB, AMF, and 100% NPK. This is because high salt concentrations can inhibit the host plant growth by reducing chlorophyll content and disrupting the photosynthesis process. However, the presence of AMF can help the absorption of mineral elements and increase the efficiency of photosynthesis, then improving the growth of the host plant (Porcel *et al*. 2012). Similarly, Hajiboland *et al*. (2010) had reported that high salinity reduced tomatoes' production. The estimation of potential yield losses due to salinity is about 20% of total yield (Ashraf and Harris 2005). Our result also showed that the AMF+PSB+25% NPK treatment significantly increased the total plant P compared to the control and other treatments. Similarly, the colonization of AMF is well known to increase host nutrient acquisition, particularly P (Smith and Read 2008). Moreover, the combined inoculation of AMF and phosphate-solubilizing fungi gave better uptake of P (Goenadi and Sugiarto 2000; Cabello *et al*. 2005).

The highest population of PSB was obtained in the inoculation of PSB without NPK addition. According to previous studies, *Pseudomonas* is known for high salinity tolerance (Vyas *et al*. 2009). In other treatments

*Pseudomonas fluorescens* has been shown to have excellent plant colonization and potential plant growth promotion abilities (Oteino *et al*. 2013). The highest colonization percentage of AM was produced in the AM inoculation without NPK but with 25% NPK. The lowest percentage of colonization was found in the inoculation of PSB with 100% NPK. The highest number of AM spores in sweet sorghum roots planting media was in the inoculation of AM with 25% NPK addition but without NPK. Similarly, in other treatments, AMF colonization was lowest in plants with the highest fertilizer dosage (Omorusi and Ayanru 2011).

Our result showed that arbuscular mycorrhizal fungi colonized sweet sorghum root in the treatment with AMF+PSB+25% NPK, AMF+25% NPK, AMF, PSB+AMF, AMF+PSB+50% NPK, AMF+50% NPK, and AMF+PSB+100% NPK. The structure of arbuscular mycorrhizal colonization observed was vesicular. Similarly, new structures of AMF colonization were formed at the root of host's plant such as hyphae, vesicles, arbuscules, and spores (Sieverding 1991). In contrast, colonization of plants' roots by other AMF species were reduced in the presence of NaCl indicating that salt suppresses the formation of AMF colonization structures (Giri *et al*. 2007; Sheng *et al*. 2008). Therefore, this indicated that AMF's ability to live in saline condition depends on the concentration of NaCl in the soil and the AMF species.

Synergistic interaction of AMF, PSB, and NPK improve the sweet sorghum growth in saline condition. The AM+PSB+25% NPK treatment in saline condition produced the highest plant height, leaves number, plant fresh weight, plant dry weight, and total P of sweet sorghum.

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## AUTHOR CONTRIBUTIONS

All authors have reviewed the final version of the manuscript and approved it for publication. SW designed the study; SW conducted the research and collected the data; IR and SW analysed the data; IR and SW wrote and reviewed the paper. Therefore, IR and SW are the main contributors to this manuscript.

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