Effect of salinity on spore germination, hyphal length and root colonization of the Arbuscular Mycorrhizal Fungi

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Abstract. Various environmental factors influence the growth and development of Arbuscular Mycorrhizal Fungi (AMF), one of which is salinity. Salinity can affect several stages of AMF growth. The research aims to study the influence of salinity towards germination, growth, and performance of AMF, namely *Gi. margarita* and *G. etunicatum*. The level of salinity given ranges from 0 - 10,000 ppm with an interval of 2,000 ppm. Spore germination experiments were carried out in petri dish culture while evaluation was carried out in open pot culture with sorghum as host. The results showed that increasing salinity decreased the initial day of germination, the percentage of germination and hyphal growth. The effect is greater on *Gi. margarita* than *G. etunicatum* on all parameters measured. An increase of salinity up to 10,000 ppm decreased the percentage of *Gi. margarita* root colonization by 69.07% and *G. etunicatum* by 37.78%. Nevertheless, the effect of salinity observed in this study towards germination and growth of AMF was more categorized as delaying rather than inhibiting or stopping.

1. Introduction
Arbuscular Mycorrhizal Fungi (AMF) can increase the resistance of host plants in various environmental stress conditions by increasing nutrient uptake, mitigating drought stress, promoting disease resistance, and improving soil structure. Many studies have been conducted to study the role of AMF in enhancing plant growth and productivity, including under salinity stress.

Several studies have shown the positive impact of AMF inoculation to increase growth and tolerance to salinity. Various mechanisms, such as (a) producing antioxidant enzymes [1], (b) increasing photosynthetic activity and water use efficiency [2], [c] improving rhizosphere and soil conditions, (d) producing growth hormones [3], (e) increasing nutrient absorption capacity [4] and (f) accumulating dissolved ingredients in plant tissues [5]. However, there are still very limited studies conducted to study how salinity may affect the growth and development of AMF.

Soil salinity can affect the growth and activity of AMF through several mechanisms, both separately and interactively with host plants, ranging from spore germination, hyphal growth, root colonization, and spore production. Spores are very important propagules of AMF, and the role of AMF for the growth of host plants depends on the ability to germinate rapidly and form root colonization [6].

The obligate nature AMF is a technical obstacle to observe the effect of salinity towards their growth and performance. The most probable observable phase in the AMF life cycle from the complex
interactions to host plants is spore germination because it is apart from the presence of the host plant. The life cycle begins with the germination of isolated spores or sporocarps formed in the soil or sometimes in the roots of plants.

Germination of AMF spores is influenced by various factors, such as periods of spore dormancy that may differ between species [7], host plant root exudates, soil moisture, temperature, pH, light and carbon dioxide [8,9], and salinity [10]. But all of that depends on the presence of suitable host plants. Based on the description, the purpose of this study was to study the effect of salinity levels on spore germination and hyphal growth and root colonization of AMF.

2. Materials and Methods

2.1 Experimental design

The experimental design to test the effect of salinity on AMF was performed using Factorial Completely Randomized Design with two factors. The first factor is the species of AMF namely *Gigaspora margarita* [M1] and *Glomus etunicatum* [M3]. The second factor is the level of salinity consisted of 0 [S0]; 2,000 [S1]; 4,000 [S2]; 6,000 [S3]; 8,000 [S4]; and 10,000 ppm [S5].

2.2 Spore germination test

The spore germination test was carried out using the petri dish culture method. Petri dishes are filled with river sand that has been washed with its top coated by filter paper. The media is moistened with NaCl solution according to the treatment until the filter paper is moist. For each dishes, approximately 15 AMF spores of *Gi. Margarita* and *G. etunicatum* were inoculated. The test was conducted in three replications. The cultures were incubated in a dark room at a temperature of 25°C. Observation of germination begins on the second day after treatment and is carried out daily until the 15th-day post-incubation. After 15 days of incubation, hyphal length was measured using the gridline intersect method [11]. Parameters observed in this test were the initiation day of germination, the percentage of germination and hyphal growth.

2.3 Arbuscular Mycorrhizal Fungi colonization test

The colonization test of mycorrhizal fungi was conducted by using an open pot culture filled with river sand as a growing medium and *Sorghum bicolor* as a host plant. Culture pots are filled with ± 200 g of river sand per pot. The media is moistened with NaCl solution according to the treatment. Germinated seeds of *S. bicolor* are planted into culture pots, and mycorrhizal fungi are inoculated before planting the host. After the sixth week, roots were harvested to observe the percentage of colonization. Root samples [± 2 g fresh weight] were washed and cut in 1 cm segment. The segment was dipped into 10% KOH solution and heated for 15 minutes. After cooling, the root segments are bleached with an alkaline hydrogen peroxide solution [3 mL of 25% NH₃ + 30 mL 6% H₂O₂ v/v] for 20–30 minutes. Furthermore, the root segments were soaked in 1N HCl for 10 minutes followed by immersion in 0.05% Trypan blue for one night. The root samples were then dipped into a solution of the lacto glycerol detaining solution. Root preparation for observing root colonization was performed according to Giovannetti and Mosse procedures [12]. Root colonization was calculated using the colonized root length method as described by Kormanik and Mc Graw [13]. Observations were made with a microscope at 40× magnification.

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\% \text{ Root colonization} = \frac{\text{Number of AMF positives segments}}{\text{Total number of segments scored}} \times 100
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2.4 Statistical Analysis

Data obtained were subjected to Analysis of Variance (ANOVA). Data obtained were arcsine-transformed before analysis. The means were compared by Duncan’s Multiple Range Tests at 5% of confidence level.
3. Results and Discussion

3.1 Effect of Salinity on Spore Germination

Effect of salinity level on spore germination of *Gi. margaritas* and *G. etunicatum* are shown in Table 1. In general, the increase in salinity has a negative impact on the germination of both mycorrhizal spores, both on the day the spores start to germinate and the percentage of spore germination. This result is in accordance with the Hajiboland’s result [14] which showed a decrease in AMF spore germination under increasing salinity levels. However, the salinity treatment of 2,000 ppm produced no significant differences with control.

Based on the results, it can be seen that each species of mycorrhizal spores gave different responses to the level of salinity. Spores of *G. etunicatum* was able to germinate earlier than *Gi. margaritas* at all levels of salinity treatments. Furthermore, *Gi. margaritas* were not able to germinate until the end of observation (15th day’s post-incubation) at the salinity of 10,000 ppm. The different results may be explained due to the size of the spore, where as *Gi. Margaritas* produced a larger spore size than *G. etunicatum*. Smaller spores were able to germinate earlier [15] due to faster hydration process in order to initiate germination. Delayed hydration process will also lead to the delay of AMF spore germination [16].

| NaCl levels (ppm) | Initiation day of germination1) | Percentage of germinated spores2) |
|-------------------|-------------------------------|----------------------------------|
|                   | *Gi. margarita* | *G. etunicatum* | *Gi. margarita* | *G. etunicatum* |
| 0,000             | 3 [a]             | 2 [a]             | 73.33 [cd]      | 80.00 [d]       |
| 2,000             | 4 [a]             | 3 [a]             | 60.00 [c]       | 66.67 [c]       |
| 4,000             | 8 [b]             | 8 [b]             | 40.00 [b]       | 60.00 [c]       |
| 6,000             | 12 [c]            | 10 [b]            | 26.67 [ab]      | 53.33 [b]       |
| 8,000             | 13 [c]            | 10 [b]            | 20.00 [a]       | 40.00 [b]       |
| 10,000            | 0 [a]             | 14 [c]            | 0.00 [a]        | 13.33 [a]       |

1) And 2) Values followed by the same letter are not significant as Duncan tests \(P = 0.05\)

The hydration process is one of the important stages in AMF spore germination in addition to other stages, namely activation, tube germination, and hyphae formation. It was further stated that the mechanism of spore germination begins with the entry of water into the spore, followed by the hydration of the components like organelles and macromolecules in the spores. Then the enzyme becomes active to start the cellular metabolic cycle. Two to ten days after spores are activated; germination tubes appear and are followed by hyphae growth [8, 10, 14, 17, and 18]. In this study spore germinated on the 2nd day for *G. etunicatum* and day 3rd day for *Gi. margarita*. The results further showed that the spores could germinate quickly in normal conditions.

In addition to the negative effect on initiation day of germination, the percentage of germinating spores (Table 1) and the length of the growing hyphae (Table 2) were also strongly influenced by the level of salinity. The results showed that the effect of salinity on the percentage of germinated spores has the same pattern as the initiation day of germination from the two species tested. In normal conditions (without any influence of salinity) both spores were able to germinate well indicated by a high percentage of germination 77.33–80.00%. However, salinity level up to 10,000 ppm decreased the percentage of germinated spores of *Gi. Margaritas* and *G. etunicatum* were 100% and 83.34% respectively on the 15th-day post-incubation.

In Table 2, it showed that increasing level of salinity negatively impacts on the length of the growing hyphae on each AMF species. It was further suspected that the results were in correlation with the initiation day of germination. Spores that germinated earlier will certainly have a longer hyphae growth until the last observation day. The length of hyphae produced by both types of AMF is
also different, where the length of *Gi. margaritas* hyphae were shorter than *G. etunicatum*. The difference in the response of the two types of AMF used is allegedly also influenced by the intrinsic nature of each type of AMF [17].

**Table 2. Salinity effect on the hyphal length of *Gigaspora margarita* and *Glomus etunicatum***

| NaCl levels (ppm) | *Gi. margarita* | *G. etunicatum* |
|-------------------|-----------------|-----------------|
| 0,000             | 65.28 (c)       | 74.28 (c)       |
| 2,000             | 40.37 (b)       | 42.95 (b)       |
| 4,000             | 12.48 (a)       | 21.66 (b)       |
| 6,000             | 6.92 (a)        | 8.55 (a)        |
| 8,000             | 0.00 (a)        | 3.20 (a)        |
| 10,000            | 0.00 (a)        | 0.00 (a)        |

Column values followed by the same letter are not significant as Duncan tests (*P* = 0.05)

### 3.2 Effect of Salinity on Percentage of Root Colonization

The results of testing the effect of salinity on root colonization in pot culture with *S. bicolour* plants are presented in Table 3. The results showed that both AMF species were able to colonize host roots at all levels of salinity treated. However, increasing salinity causes a reduced percentage of root colonization by AMF. The decrease in root colonization differed between the two AMF species whereas an increase in salinity up to 10,000 ppm caused a decrease in root colonization by *Gi. margaritas* and *G. etunicatum* by 69.07% and 37.78% respectively.

**Table 3. Salinity effect on the percentage of root colonization of *Gigaspora margarita* and *Glomus etunicatum***

| NaCl levels (ppm) | Percentage of root colonization (%) |
|-------------------|------------------------------------|
|                   | *Gi. margarita* | *G. etunicatum* |
| 0,000             | 64.82 (c)       | 76.58 (c)       |
| 2,000             | 56.78 (b)       | 70.65 (c)       |
| 4,000             | 32.85 (a)       | 58.86 (b)       |
| 6,000             | 28.69 (a)       | 52.64 (b)       |
| 8,000             | 20.76 (a)       | 48.75 (b)       |
| 10,000            | 20.05 (a)       | 47.65 (b)       |

Column values followed by the same letter are not significant as Duncan tests (*P* = 0.05)

Effect of salinity on the percentage of root colonization by *Gi. margaritas* and *G. etunicatum* gave different results if compared with the results of spore germination testing without using host plants (Table 1 and 2). Both AMF species were able to form root colonization at all levels of salinity even though the colonization decreased with increasing levels of salinity. It was further suspected that the presence of host plants and rhizosphere gave benefits to AMF spores so that they could germinate and colonize host plant roots [7, 8, 9, and 16]. Hence, the Negative effects of salinity towards spore germination may be suppressed in the presence of host plants. The presence of host plants was able to create conditions that can stimulate the germination of AMF spores.

The role of host plants to stimulate spore germination and colonization in roots is related to the presence of phytochemical signalling from the roots [19]. Germination of mycorrhizal spores is highly dependent on the availability of abundant simple carbohydrates in the roots of the host plant. It is stated that the roots of host plants can excrete one or more metabolites to stimulate mycorrhizal
growth. Characteristic of mycorrhizal hyphae that directly contacted with the root of the host plant was described as fan-shaped with lateral branching [20]. While in conditions without host plants, the fan-shaped structure is not formed. The phenomenon showed that specific mycorrhizal morphogenesis occurs in conditions or compatible with the host plants.

Some researchers have studied the growth and branching of mycorrhizal fungal hyphae which are controlled by signals from the roots of host plants [21]. Special signals from host plants can affect the pre-infection stage of mycorrhizal hyphae even though the mechanism cannot be fully explained. Other researchers stated that germination and colonization of mycorrhizal fungi besides being influenced by the presence of host plants also depend on environmental factors such as soil, climate, and strains of mycorrhizal fungi [8]. Table 3 shows that although the two carbuncular mycorrhizal fungi spores were able to germinate and form a symbiosis with host plants, colonization performance from Gi. Margaritas are lower than G. etunicatum.

4. Conclusions
The results showed that salinity could have a detrimental effect on spore germination, hyphal growth and root colonization of AMF. Adverse effects of salinity on spore germination AMF is more of a delay in germination than preventing or damaging the process. Furthermore, the effect of salinity is greater on spores of Gi. Margarita than to G. etunicatum.

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