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# SPORE PRODUCTION AND INOCULUM FORMULATION OF *CLAROIDEOGLOMUS ETUNICATUM* AND ITS APPLICATION IN MAIZE (*Zea mays*)

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## ABSTRACT

Recently, the demand for AMF (Arbuscular Mycorrhiza Fungi) fertilizer for agriculture and forestry plantations in Indonesia has increased significantly. Meanwhile, the unavailability of an applicable AMF inoculum that's easy to use and inexpensive is one of the constraints on AMF application as a fertilizer in the field. It indicates that the best-formulated inoculum must be found. The study aimed to produce AMF spores using conventional and hydroponic systems, formulate the best AMF inoculum, and measure the growth response in maize (*Zea mays*) after applying the formulated inoculum. At the formulation stage, the research was designed using a Completely Randomized Design (CRD) with three factors: the type of carrier material, temperature, and addition of AMF inoculum. Data were analyzed using SAS software version 9 and further tested using Duncan's Multiple Range Test (DMRT) at 5% alpha (α) level. The results showed that the conventional pot culture technique effectively increases AMF spore density, and the NFT hydroponic technique effectively increases root colonization percentage. The E2 treatment with *Claroideoglomus etunicatum* in pot culture generated the highest spore density of 350 ± 6.97 spores per 10 g of zeolite ($P<0.05$) compared to other treatments and control. Meanwhile, the hydroponic technique had the highest AMF percentage colonization with the E2 treatment at 98% ± 2.66% ($P<0.05$). Analysis of variance in the formulation stage showed the formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of *Zea mays* compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (sodium alginate and 10% *Aloe vera* extract), which makes up the formulated AMF inoculum treated at a temperature of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g. Carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well.

**Keywords:**
Colonization, nutrient film technique, pot culture system, spore density

## INTRODUCTION

Population growth needs sufficient food production to meet food security and combat hunger and poverty. Berners-Lee et al. (2018) stated that crop production is only sufficient to provide food for a projected global population of 9.7 billion in 2050. However, there are some obstacles to increasing food crop production due to the increase in fertilizer costs in recent years. On the other hand, the effects of dependence on chemical fertilizers and pesticides are known to have caused a decline in human health, disruption of ecosystem functions, and environmental degradation (Proia et al., 2013; Rani et al., 2021; Richardson et al., 2014; Rajput et al., 2020;).

Not all plants can grow in extreme weather and adapt to climate change. Therefore, plants need microbes to survive in bad weather and unpredictable climate change. Regarding this problem, a study about exploring and optimizing the beneficial interactions among microbes and plants is needed to support food production without affecting the relationship between humans and their environment.

Arbuscular Mycorrhiza Fungi (AMF) is one of the soil microbes that play an important role in promoting plant growth. AMF functions as a biological fertilizer because of its ability to assist plants in absorbing nutrients through the mechanism of root colonization. The mechanism appears through the formation of intercellular, intracellular, arbuscular, and vesicle hyphae in the roots of host plants. AMF require a host plant in its life cycle through a colonization process in its roots intracellularly, beginning with the pre-symbiotic to the symbiotic phase (Souza, 2015). These functional structures may help plants to grow nicely. The interaction of AMF and host plants is determined primarily by the suitability of the symbiosis between the fungus and host plant in the root system. AMF provide 80% inorganic nutrition for land-living plants (Begum et al., 2019). On the other side, they gain up to 20% monosaccharides from $CO_2$ fixation by plants.

Previous studies have been done by Rosita (2021) and Wulandari et al. (2022), which show that other roles of mycorrhizae improve post-mining soil fertility and provide the essential compounds that plants need. Srivastava et al. (2017) report that AMF as a biological fertilizer increases plant growth by accelerating nutrient uptake from the soil, especially inaccessible nutrients such as Phosphate (P) and Nitrogen (N). In addition to absorbing mineral nutrition, AMF maintains root hydraulic conductivity and enhances plant net photosynthetic capacity and stomatal conductance. Glomus fasciculatum increases photosynthesis, plant productivity, and salt stress tolerance (Ebrahim & Saleem, 2017). Two species of AMF that have optimal effects on plant growth and percentage of mycorrhizal infection are *G. facsiculatum* and *G. etunicatum* (Rahayu, 2014). AMF phylogeny defined *G. etunicatum* as the synonym of *Claroideoglomus etunicatum* (Schüßler & Walker, 2010).

AMF production technology has been developed since the role of AMF for plants is known. The production of AMF inoculum requires a system that allows the production of AMF in large quantities, limited space, and effective management. Aryanto et al. (2018) stated that the interaction of the NFT system and AB Mix nutrition with the host plant Pueraria javanica shows the best biomass and spore production, significantly ($P<0.05$) achieving the highest shoot dry matter, dry root matter, and spores with a percentage of root colonization more than 96%. Rosita et al. (2020) define refers to microscopic observations that have been done on AMF inoculated treatment using Signal grass (*Brachiaria decumbens*) as a host plant, showing AMF colonization reached 55% and spore density amount of 252 % per 10 g media. In contrast, this doesn't occur with non-inoculated treatment.

Nowadays, AMF's inoculum demand for agriculture and forestry plantations in Indonesia is very high. The unavailability of inoculums for application is a major constraint on the use of AMF as a biological fertilizer in the field. Inoculum formulation can be produced in various ways, including tablet formulation, the most inexpensive and effective AMF formulation for field-scale applications. One of the primary standards that must be found in inoculum formulation is inoculum viability, as indicated by the ability of the fungus to colonize host roots and form colonizing structures such as spores, internal hyphae, external hyphae, arbuscles, and vesicles. Using inoculums without a formula usually encounters various obstacles, including the rapid decline in inoculum quality and difficulties in storage, distribution, and application in the field. The objectives of the research were (1) to study AMF spore production techniques using conventional and hydroponic systems and (2) to study the effect of formulated inoculum on the growth of maize (*Zea mays*).

## MATERIALS AND METHODS

**Area study.** The experiments were carried out at the Southeast Asian Ministers of Education Organization Biology Tropical (SEAMEO BIOTROP) greenhouse and Biosystem and Landscape Management (BLM) laboratory, which is located in Bogor, Indonesia, with a latitude of -6.635486261817926 and a longitude of 106.82536873959347.

**Preparation of the planting medium.** Zeolite was used as a planting medium. The first step was to wash the zeolites thoroughly with running water to remove any dust that might be attached to the surface. Once the washing water ran clear, the zeolite was placed in a

holding container to dry in the sun until completely dry. Afterward, the zeolite is carefully weighed and prepared to be placed into a heat-resistant plastic bag. This bag then would be sterilized using an autoclave for 120 minutes at a temperature of 121°C. Two techniques were utilized to produce spores: the conventional pot culture (CVN) and the hydroponic using Nutrient Film Techniques (NFT). Concerning the spore production stage, (1) *Zea mays*, (2) Sorghum bicolour, and (3) *Pueraria javanica* were chosen as host plants. AMF spores of *C. etunicatum* (E) and *G. fasciculatum* (F) were used. Those AMF inocula were obtained from the Biosystem and Landscape Management Laboratory of SEAMEO BIOTROP, with the code numbers BLM_MGL1 and BLM_MGL2. A total of 5 AMF spores were inoculated into each pot in those systems.

Preparation of conventional pot culture (CVN) technique. In establishing the conventional technique using a pot culture system, 5 spores of AMF were inoculated onto the root surfaces and incubated for 4 weeks. The roots not inoculated by AMF were used as the control (K). The inoculation method referred to Rosita et al. (2020). Once the incubation period was over, the seeds were transferred to pots containing 200 g of zeolite as the planting medium. The plants were maintained for 3 months by watering and fertilizing them with water containing 1.42 g (equivalent to 1420 ppm) NPK (nitrogen, phosphorus, and potassium) fertilizer, following the method outlined in Rosita et al. (2020). There were nine tested treatments , including (1) K1= Pot Culture + *Z. mays*; (2) K2= Pot Culture + *S. bicolour*; (3) K3= Pot Culture + *P. javanica*; (4) F1= Pot Culture + *Z. mays* + 5 spores of *G. fasciculatum*; (5) F2= Pot Culture + *S. bicolour* + 5 spores of *G. fasciculatum*; (6) F3= Pot Culture + *P. javanica* + 5 spores *G. fasciculatum*; (7) E1= Pot Culture + *Z. mays* + 5 spores *C. etunicatum*; (8) E2= Pot Culture + *S. bicolor* + 5 spores *C. etunicatum*; (9) E3= Pot Culture + *P. javanica* + 5 spores *C. etunicatum*. Each treatment was repeated in 25 replicates. After the plants were incubated for 3 months, spore density and AMF colonization rate were observed.

Preparation of NFT hydroponic. The plant was previously inoculated with 5 spores of AMF and incubated for 4 weeks. It was then transferred into a pot containing 200 g of zeolite and placed on the NFT hydroponic equipment (Figure 1). The roots not inoculated by AMF were used as the control (K).

Nutrients were delivered to the plant's roots through a stream of water, allowing the deep plant roots to come in contact with a thin layer of flowing nutrients. The water layer was set up to 3 cm in height, and the plants were maintained for 3 months using 1420 ppm AB Mix Hydro J solution (Tripama & Yahya, 2018) to provide nutrients. Considering of Figure 1. there were 9 treatments tested, including (1) K1= NFT + *Z. mays*; (2) K2= NFT + *S. bicolour*; (3) K3= NFT + *P. javanica*; (4) F1= NFT + *Z. mays* + *G. fasciculatum*; (5) F2= NFT + *S. bicolour* + *G. fasciculatum*; (6) F3= NFT + *P. javanica* + *G. fasciculatum*; (7) E1= NFT + *Z. mays* + *C. etunicatum*; (8) E2= NFT + *S. bicolour* + *C. etunicatum*; (9) E3= NFT + *P. javanica* + *C. etunicatum*. Each treatment was repeated in 25 replicates. The number of spore densities and percent of AMF colonization were measured 3 months after treatment.

Spore density and percent colonization of AMF. In determining the density of AMF spores and their colonization percentage, the wet sieving method was used by using graded filters (425 µm, 212 µm, 106 µm, and 63 µm). The host plant's roots were stained, and then ten root pieces, cut to approximately 1-1.5 cm, were observed under a microscope (Tawaraya et al.,1998). The colonization percentage was calculated based on the presence of external hyphae, internal hyphae, vesicles, arbuscular, and AMF spores on the roots (Rosita et al., 2020).

Preparation of formulated inoculum (F1). The inoculant used in this stage had the highest spore density. The selected inoculant weighed as much as 1 kg and was dried in an oven at 60 °C or 40 °C for 3 hours. It was then mixed with 1.75% Natrium Alginate and 10% *Aloe vera* extract. The concentration of Natrium Alginate and *Aloe vera* is determined by Nurlaeli (2012). The mixture that had changed into granules was dried in the oven at 45 °C for 36 hours.

Preparation of formulated inoculum (F2). The inoculant was dried in an oven at 60 °C or 40 °C for 3 hours and then homogenized by adding 1:1 (v/v) gypsum. Furthermore, clay and water were prepared with a ratio of 1: 2.5 (w/v). Clay and water were mixed until homogeneous and then poured over the surface of the inoculant. Afterward, gypsum was added to the mixture. The mixture of materials was printed with a tool measuring 0.8 cm in diameter and 1 cm in height. The printed tablets were dried in an oven at 45 °C for 36 hours.

Application of formulated inoculum in maize (*Z. mays*). Maize seedlings that had been maintained for 2 weeks were prepared. After that, the formulated inoculum F1 and F2 were applied to the seedlings. There were 9 different types of formulations used as treatments, consisting of: (P1) TZ60F1= AMF + 60 $^{o}$C + F1; (P2) TZ60F2= AMF + 60 $^{o}$C + F2; (P3) TZ40F1= AMF + 40 $^{o}$C + F1; (P4) TZ40F2= AMF + 40 $^{o}$C + F2; (P5) TK60F1= 60 $^{o}$C + F1; (P6) TK60F2= 60 $^{o}$C + F2; (P7) TK40F1= 40 $^{o}$C + F1; (P8) TK40F2= 40 $^{o}$C + F2; (P9) TK = no treatment. Each treatment was repeated 10 times. The formulated inoculum was applied by making a hole as deep as ± 3 cm. The inoculum was spread over the rhizosphere area of the maize's roots and then covered with sterilized soil. Maize plants were maintained until

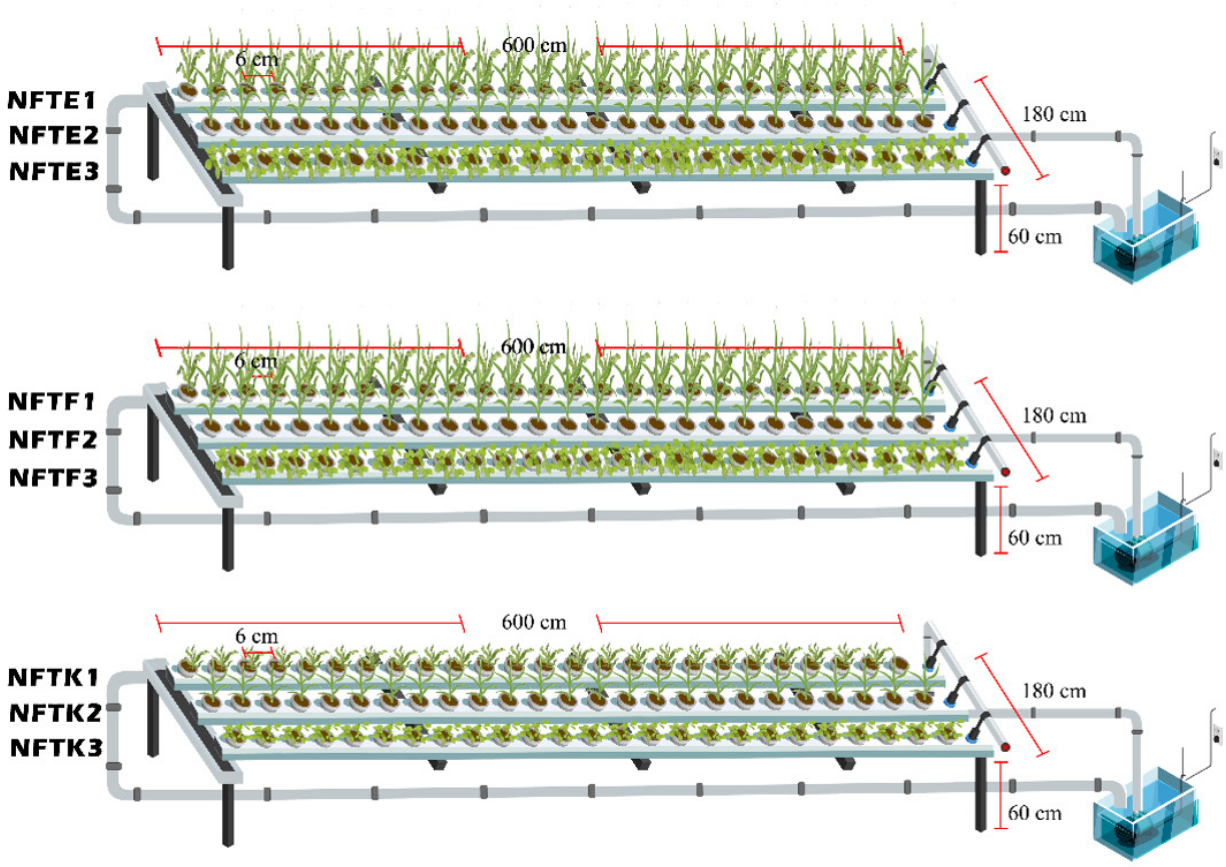


Figure 1. Design of an NFT hydroponic system at the spore production stage

the age of 3 months. Plant height, number of leaves, and dry plant biomass were measured at harvest time. Data were analyzed using SAS software version 9 and further tested using Duncan's Multiple Range Test (DMRT) on a 5% alpha (α) level.

## RESULTS AND DISCUSSION

Results of analysis of variance (Table 1) show that the treatments have significant effects on the parameter of spore density (P<0.05). Spore production using the conventional E2 pot culture technique inoculated with *C. etunicatum* achieved the highest spore density value of 350 ± 6.97 spores per 10 g of zeolite. This means that to produce AMF spores successfully, the most suitable technique is needed, especially in selecting the most suitable host plant and its production technique. Therefore, *Z. mays* and Sorghum were chosen as host plants because of their extensive rooting system. Pot culture is an effective method to produce AMF spores, which is carried out by inoculating effective AMF into certain host plants in a sterilized solid medium (Simanungkalit, 2004). Meanwhile, the determination of Pueraria javanica as the host plant was decided according to Aryanto et al. (2018), who reported that interaction among the NFT technique, AB Mix nutrition, and *P. javanica* as the host plant show the best results in spore production and plant biomass.

The pot culture E2 treatment had the highest spore density of 350 ± 6.97 spores per 10 g of zeolite compared to the other treatments and control. However, the percentage of colonization for this treatment was only 50% ± 6.75%. This occurs because the spores are still in the process of germinating within the media, and the hyphae have not yet penetrated into the root tissue. The result is following Rosita et al. (2020), on microscopic observation of *Brachiaria decumbens*, the number of *C. etunicatum* spores reaches 252 ± 9.82 spores per 10 g of zeolite with a percentage of colonization amount of 55% ± 0.06%, whereas AMF colonization does not occur in non-inoculated plants. On the other hand, Rahayu (2014) stated that *C. etunicatum* has an optimal effect on plant growth.

Table 2 shows that treatment affects significantly to AMF spore production (P<0.05). The results revealed that the NFT hydroponic system inoculated by *C. etunicatum* had the highest percentage of AMF colonization at 98% ± 2.66% (E2) compared to other treatments and controls. This indicates that the NFT hydroponic technique significantly enhanced colonization rates. Rodrigues et al. (2021) and Rahayu (2014) reported that *C. etunicatum* effectively colonizes plant roots, achieving a colonization rate of 84%. *C. etunicatum* is effective in colonizing plant roots because of its high ability to infect. *C. etunicatum* and its spores germinated actively to produce hyphae.

Based on Tables 1 and 2, those show that both spore production and colonization data between conventional (using pot culture) and hydroponics (NFT) generally show contradictory results. This shows that AMF has a mechanism to grow and reach nutritional sources. Mycorrhizae respond to and access nutritional sources through hyphal growth mechanisms. Hyphae are long filamentous structures that form mycorrhizal networks. These hyphae grow and branch in extensions in various directions, exploring the growing medium to find and absorb nutrients. The nutrient concentration influences hypha growth in the media. Hyphae tend to grow faster and branch more in directions with a higher concentration of nutrients. This is the reason why the spore density value in treatments using conventional techniques has higher losses than hydroponic techniques because the nutrients are placed below the surface of the media. Meanwhile, hydroponic treatment produces lower spore density values because the nutrients needed for plant growth are dissolved in the air. The percent value of AMF colonization was the highest in treatments given hydroponic techniques. AMF tends to move to the bottom towards sources of water-soluble nutrients.

The spore germination process can be affected by several factors, such as production technique, host plant, and the amount of organic matter in the planting medium. Organic materials can be obtained through plant maintenance activities, including the application of NPK fertilizer (Susanti et al., 2023), which is known to stimulate spore germination. Referring to Akmal's (2019) findings, the root exudate triggers spore germination, particularly the flavonoid compounds of the flavanol type that promote the growth of AMF hyphae. For plants associated with AMF, receiving adequate sunlight to produce high carbohydrate concentrations is crucial. This result is in line with Sieverding (1991), who reported that AMF species, host plants, growing medium, and environmental conditions affect the time of spore formation. Besides that, mycorrhiza produces organic acids which release fixed P (Ristiyanti et al., 2014). One of the factors that influence the development of mycorrhiza is environmental factors. Two environmental factors that influence AMF development are abiotic and biotic. Abiotic factors include climate, light, temperature, soil fertility, and soil pH (Sastrahidayat et al., 2010), while biotic factors can come from the symbiotic host plants with AMF. AMF inoculation increases P uptake and shoots biomass. The contribution of AMF to P uptake and shoot biomass varied based on the phosphate source. The most excellent P uptake was $CaHPO_4.2H_2O$ (Ca2-P). AMF mobilizes phosphates under stress conditions (low P) and increases contact with P in the soil compared with non-mycorrhizal root systems (Yao et al., 2001). The excessive availability of carbohydrates in plant roots decreases soil fertility, so plants cannot absorb nutrients due to limited root systems and an imbalance of one or more macronutrients (N, P, K). The condition formed an association between roots and AMF. Based on the result, when *C. etunicatum* colonized the maize roots, maize plant roots did not enlarge. The success of AMF colonization of plant roots was proven by the formation of external hyphae, internal hyphae, vesicles, and arbuscles (Figure 2).

When hyphae penetrate the cell wall of plant roots, they create distinctive structures, such as vesicles or arbuscles. Vesicles are thin-walled balloons that form at the ends of hyphae and have round or oval shapes. These structures serve as storage organs for nutrient reserves like lipids. Arbuscles, conversely, have tree-like shapes and are formed from intraradical hyphae branches located between the cell wall and membrane. They are crucial in facilitating nutrient and carbon exchange between AMF and host plants. The high and low levels of AMF colonization are influenced by the type of AMF and the shape of plant roots in the form of fibrous roots, taproots, types of roots, and the environmental conditions of these plants (Akmal, 2019).

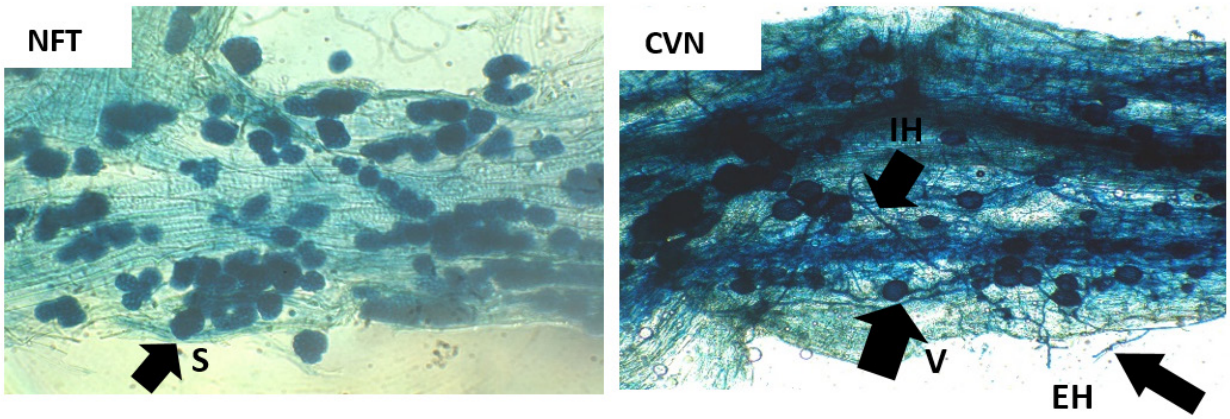


Figure 2. Root tissue microscopic observation of host plant at 3 months after treatment, magnification: 10 x 40 (NFT=hydroponic; CVN= conventional; S= spores; V= vesicles; IH= internal hyphae; EH= external hyphae)

Table 1. Effect of conventional technique in increasing AMF spore production at 3 months after treatment

| Code | Treatment | Spore density/10 g of zeolite | Colonization (%) |
|---|---|---|---|
| K1 | CVN + *Z. mays* | 4 ± 0.58 [g] | 8 ± 0.76 [e] |
| K2 | CVN + *S. bicolor* | 3 ± 0.87 [h] | 8 ± 1.53 [e] |
| K3 | CVN + *P. javanica* | 2 ± 0.96 [i] | 8 ± 1.50 [e] |
| F1 | CVN + *Z. mays* + *G. fasciculatum* | 300 ± 5.16 [b] | 60 ± 4.33 [b] |
| F2 | CVN + *S. bicolor* + *G. fasciculatum* | 190 ± 6.80 [d] | 58 ± 4.65 [c] |
| F3 | CVN + *P. javanica* + *G. fasciculatum* | 198 ± 7.65 [c] | 60 ± 7.94 [b] |
| E1 | CVN + *Z. mays* + *C. etunicatum* | 175 ± 4.38 [e] | 58 ± 5.28 [c] |
| E2 | CVN + *S. bicolor* + *C. etunicatum* | 350 ± 6.97 [a] | 50 ± 6.75 [d] |
| E3 | CVN + *P. javanica* + *C. etunicatum* | 70 ± 4.84 [f] | 70 ± 7.49 [a] |

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

Table 2. Effect of hydroponic technique in increasing AMF spore production at 3 months after treatment

| Code | Treatment | Spore density/10 g of zeolite | Colonization (%) |
|---|---|---|---|
| K1 | NFT + *Z. mays* | 2 ± 0.71 [g] | 3 ± 0.91 [g] |
| K2 | NFT + *S. bicolor* | 5 ± 1.15 [f] | 15 ± 1.91 [f] |
| K3 | NFT + *P. javanica* | 5 ± 1.22 [f] | 15 ± 1.29 [f] |
| F1 | NFT + *Z. mays* + *G. fasciculatum* | 10 ± 1.04 [e] | 80 ± 3.58 [d] |
| F2 | NFT + *S. bicolor* + *G. fasciculatum* | 15 ± 1.08 [d] | 80 ± 4.09 [d] |
| F3 | NFT + *P. javanica* + *G. fasciculatum* | 150 ± 4.16 [a] | 60 ± 2.18 [e] |
| E1 | NFT + *Z. mays* + *C. etunicatum* | 20 ± 1.26 [c] | 88 ± 4.52 [c] |
| E2 | NFT + *S. bicolor* + *C. etunicatum* | 50 ± 3.59 [b] | 98 ± 2.66 [a] |
| E3 | NFT + *P. javanica* + *C. etunicatum* | 15 ± 2.45 [d] | 96 ± 3.62 [b] |

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

Table 3. Effect of temperature and formulated inoculum application on *Zea mays* vegetative growth at 3 months after treatment

| Code | Treatment | Plant height (cm) | Number of leaves | Plant dry biomass (g) |
|---|---|---|---|---|
| TZ60F1 | F1+AMF+60 °C | 120 ± 1.70 [a] | 15 ± 0.84 [a] | 26.3 ± 2.46 [a] |
| TZ60F2 | F2+AMF+60 °C | 110.6 ± 6.47 [ab] | 13 ± 1.26 [b] | 20.7 ± 4.37 [b] |
| TZ40F1 | F1+AMF+40 °C | 104.7 ± 8.78 [b] | 12 ± 0.85 [c] | 20.4 ± 5.14 [b] |
| TZ40F2 | F1+AMF+40 °C | 104.4 ± 4.33 [b] | 12 ± 0.70 [c] | 20.06 ± 0.76 [b] |
| TK60F1 | F1+60 °C | 90 ± 4.35 [c] | 9 ± 0.52 [d] | 15.29 ± 3.18 [c] |
| TK60F2 | F2+60 °C | 89.7 ± 2.38 [c] | 9 ± 0.67 [d] | 15.28 ± 1.62 [c] |
| TK40F1 | F1+40 °C | 86.5 ± 3.78 [cd] | 9 ± 0.42 [d] | 15.27 ± 5.29 [c] |
| TK40F2 | F2+40 °C | 80.9 ± 7.82 [cd] | 9 ± 0.42 [d] | 14.53 ± 3.68 [c] |
| TK | No treatment | 75.4 ± 6.63 [d] | 7 ± 0.42 [e] | 9.03 ± 1.15 [d] |

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

The type of root can affect the presence of an AMF. In fibrous roots, more AMF would be found because the roots spread downward and to the side, making it easier for the symbiosis between mycorrhizae and plants (Hermawan, 2015). Host plants from the Graminae class are more suitable for AMF production because they have a higher percentage of colonization and spore density than those from the Leguminosae (Rini & Rozalinda, 2020).

The best AMF inoculant used in the application stage was the conventional technique code E2 (Pot culture + *S. bicolour* + *C. etunicatum*). The highest spore density was 350 ± 6.97 spores per 10 g zeolite. Furthermore, the inoculum was developed by providing treatment in the form of temperature (60 °C, 40 °C) and adding carrier materials (F1: natrium alginate and 10% *Aloe vera* extract; F2: gypsum).

Table 3 shows that inoculum formulations significantly impact the whole of *Z. mays* growth parameters (height, number of leaves, and plant dry weight) of corn plants 3 months after application in sterile soil media. Based on the result, giving formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of corn plants compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (materials in the form of sodium alginate and 10% *Aloe vera* extract), which make up the formulated AMF inoculum treated at a temperature of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g (Table 3).

The number of AMF spores and the carrier material play an important role in determining the value of plant growth. The number of AMF spores and the type of carrier material greatly determine the success of inoculation and the effectiveness of the symbiotic relationship between AMF and the host plant. Properly managing these two factors can significantly improve plant growth, health, and productivity. Some carriers contain additional nutrients that can support initial spore growth and increase the effectiveness of inoculation. Adding nutrients in the form of alginate is widely used to control the penetration and stability of adhesives made from starch and latex and to regulate the slow release of chemicals in fertilizers and medicines.

Meanwhile, *Aloe vera* has the potential to add high levels of nutrients to plants, as well as growth stimulants (ZPT) in the form of the hormones auxin and gibberellin, which can increase plant growth. In this research, carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well. Previous research reported that *C. etunicatum* has various benefits in increasing growth, nutrient uptake, and plant resistance to abiotic stress (Rosita, 2021). Temperatures above 40 °C are generally too hot for many fungi, including AMF. At very high temperatures, fungal cell proteins and enzymes can denature, disrupting their function and growth. A temperature of 60 °C will damage cellular structures and kill AMF and many other soil microorganisms. AMF can grow well at 30ºC, and the best development of spores to produce mycelia occurs at 28ºC. Temperature can affect the growth and development of AMF. High temperatures affect the growth and formation of mycorrhizal colonies. The development of most mycorrhizal fungi is inhibited if the soil temperature is below 5ºC and the temperature above the soil surface is 35ºC. If the soil temperature reaches 50ºC, it can kill mycorrhizal fungi. From this statement, data was obtained that if the soil temperature reaches 50ºC, it can cause death of AMF.

A good temperature for AMF development is 30ºC, but for mycelial colonization, the best temperature is 28 - 34ºC (Rumiatun, 2024). Inoculum based on G. intraradices spores could remain infectious in moist soil for up to three weeks at temperatures as high as 380C (Haugen & Smith, 1992). The percentage of colonization usually rises in experimental systems between 100 and 300C. However, some plant-fungus combinations can thrive at much lower or higher temperatures (Bowen, 1987). Aryanto et al. (2018) stated that 30°C is the optimal temperature for AMF growth, which can increase spore production in plant feed such as grass. The optimal soil temperature for AMF spore production is usually above the optimal temperature of the host plant. Temperatures below 15°C can inhibit mycorrhizal colonization, while mycorrhizal activity increases with increasing soil temperature. Temperature and nutrition influence the growth and production of spores, which greatly affect the quality and quantity of spore production. In the hydroponic technique, an irrigation system that uses nutrient solutions with the right concentration can increase the production of AMF biomass and spores. The interaction between temperature and nutrition is very significant in AMF spore production. Optimal temperatures and adequate available nutrients can increase spore production. In hydroponic techniques, of using an appropriate irrigation system and balanced nutrition can maximize AMF spore production (Aryanto et al., 2018).

## CONCLUSION

The conventional pot culture technique effectively increases AMF spore density, and the NFT hydroponic technique effectively increases root colonization percentage. The E2 treatment with *C. etunicatum* in pot culture generated the highest spore density of 350 ± 6.97 spores per 10 g of zeolite ($P<0.05$) compared to other treatments and control. Meanwhile, the hydroponic technique had the highest AMF percentage colonization with the E2 treatment at 98% ± 2.66% ($P<0.05$). AMF has a mechanism to grow and reach nutritional sources. Spore density value in treatments using conventional techniques has higher losses than hydroponic techniques because the nutrients are placed below the surface of the media.

The formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of *Zea mays* compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (sodium alginate and 10% *Aloe vera* extract), which makes up the formulated AMF inoculum treated at a temperature

of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g. Carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well.

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