EFFECT OF ARBOSCULAR MYCORRHIZAL FUNGI BIOFERTILIZER ON THE GROWTH OF CASHEW SEEDLING

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ABSTRACT

The effects of several arbuscular mycorrhizal fungi (AMF) on the growth, nutrient uptake (nitrogen, phosphorus, and potassium), and acid phosphatase activity of two promising numbers of Anacardium occidentale seedling were evaluated. The experiment was conducted in the green house of Indonesian Spices and Medicinal Crops Research Institute (BALITRRO) in 2002 for six months on a randomized design with two factors and four replicates. First factor was isolate (six isolates of AMF and one control) consisting of: 1) control; 2) Glomus aggregatum; 3) Glomus etunicatum; 4) Mycofer; 5) Glomus sp.; 6) a mixture of Glomus sp1, Glomus sp2, Glomus sp3, Glomus sp4, Glomus etunicatum, Gigaspora margarita, Gigaspora sp., and Entrophospora sp., and 7) Gigaspora sp. The second factor was two cashew promising numbers: Asembagus and Wonogiri. The results showed that AMF inoculation significantly affected the growth of cashew. Mycofer and mixed AMF were more effective to Wonogiri promising number, while for Asembagus promising number inoculation of mycofer was more effective. Inoculation with mycofer to Asembagus promising number increased the uptake of P and K nutrients by 65 and 53% while inoculation with mycofer and mixed AMF to Wonogiri promising number increased the uptake of N, P, and K nutrients by 55, 38, and 17%, and by 18, 31, and 17%. Moreover, the AMF inoculation resulted in higher phosphatase activity. In mycorrhizal Asembagus promising number infected by mixed AMF, the increment of phosphatase activity was 136.5%, whether in Wonogiri promising number infected by mycofer, the increment of phosphatase activity was 80% than control.

Key words: Anacardium occidentale, promising number, growth, phosphatase activity

INTRODUCTION

Cashew (Anacardium occidentale) is a horticultural plant and mostly developed in Eastern Region of Indonesia (dry land and climatic condition). The plant has economic value which produced 157,675 ton of cashew nut with export value of US$77,755,000 in 2008 (SECRETARIAT OF DIRECTORATE GENERAL OF ESTATE CROPS, 2009). Since 1977 until 2010, the cultivation area of cashew had increased sharply, from 58,391 to 578,761 ha, and produced 9,123 to 157,675 tons of cashew nut.

Generally, the land of cashew cultivation area was classified as marginal soil with low fertility status and organic matter content. Utilization of bio-fertilizer in situ to support the growth of seedling before transplanting into the degraded land is needed.

Arbuscular mycorrhizal fungi (AMF) benefit plants by allowing them to grow and produce in relatively harsh mineral stress environments. The main role of mycorrhizal associations is to acquire nutrients by exploring the soil volume with hyphae that is both more responsive and extensive than the roots themselves (BRUNDRETT, 2004). A plant associated with mycorrhiza (mycorrhizal plant) is more efficient in absorbing nutrients from soil solution, more rapidly in assimilating the phosphate element and enhancing the absorption of N, S, Zn, and other elements (MOSSE, 1981). Moreover the nutrients (P, N, Zn, and Cu) enhance most in host plants grown in many soils, however, K, Ca, and Mg are enhanced when plants are grown in acidic soils (CLARK and ZETO, 2000).
Soils contain organic and inorganic phosphorus compounds. The enzymes involved in the cleavage of phosphate from organic substance are collectively called phosphatases (Raddersma and Gierson, 2004). Soils normally contain a wide range of microorganisms capable of releasing inorganic orthophosphate from organic phosphate of plant and microbial origin (Tate, 2000). Microorganisms produce variety of phosphatases that mineralize organic phosphate (Tisdale, 1985 in TAO et al., 2008) found that the importance of roots associated with particular microorganism known as antitropic mycorrhizae has been shown to increase phosphate uptake by a number of crops.

The aim of the study was to investigate the influence of several AMF inoculum types on the growth of two promising numbers of cashew, NPK nutrient uptake, and acid phosphatase activity in cashew root.

MATERIAL AND METHOD

The experiment was conducted in the green house and Eco-physiology Laboratory of Indonesian Spices and Medicinal Crops Research Institute (BALITTO), Bogor for six months in 2002. The cashew seedlings used were Asembagus and Wonogiri promising numbers. The inoculums were AMF types isolated and cultured from the rhizosphere of cashew, and AMF types from mineral soil. Growing media used (2 kg soil/pot) was Podzolic soil with fertility status as follows: acidic soil pH, medium C organic content and N total, very low available P, low exchangeable Ca and Mg, medium exchangeable K and Na, high cation exchange capacity, and clay loam soil texture.

The experiment was arranged using a completely randomized design with two factors and four replicates. First factor was isolate (six isolates of AMF and one control) consisting of : 1). control; 2). Glomus aggregatum; 3). Glomus etunicatum; 4). Mycofer; 5). Glomus sp.; 6). a mixed of Glomus sp1, Glomus sp2, Glomus sp3, Glomus sp4, Glomus etunicatum, Gigaspora margarita, Gigaspora sp., Enthopospora sp., and 7). Gigaspora sp. The second factor was two cashew promising numbers: 1). Asembagus and 2). Wonogiri. AMF (250-300 spores/plant) was inoculated along with planting the cashew seeds. Every experiment unit was applied with 50 ml of 1% Hyponex/pot every 3 days.

Plant growth parameters (plant height, number of leaf, and stem diameter) were measured every month until 6 months after planting (MAP). The weights of leaf, stem, and root; leaf area index (LAI); percentage of AMF infection in the root; total uptake of N, P, and K nutrients; and acid phosphate activity were measured by method of acid phosphatase activity (Dodd et al., 1987) at the end of the experiment. The percentage of AMF infection in the root was measured by the equation:

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\% \text{ infection} = \frac{\Sigma \text{root infected by AMF}}{\Sigma \text{root observed}} \times 100\%
\]

RESULT AND DISCUSSION

Plant Growth

The effect of cashew promising number was significant to the number of leaf at 5 and 6 MAP, and was significant to very significant to the seedling height at 4 until 6 MAP. However, it was not significant to influence stem diameter (Figure 1). Generally, Wonogiri promising number had performed better plant growth than Asembagus one.

The effect of AMF inoculation was significant to the number of seedling leaf and height, and affected significantly to very significantly to stem diameter at 2 until 6 MAP (Table 1). Inoculation of mycorhiza resulted in the best seedling growth compared to other AMF treatments and control.

Interaction between cashew promising number and AMF inoculation was significant to very significant on the number of seedling leaf, height, and stem diameter at 2- 6, 4-6, and 2-6 MAP, respectively. Growth response of cashew seedling to the AMF inoculation was different between the two promising numbers of cashew. On Asembagus promising number, infection of mycorhiza followed by Glomus sp. resulted in better seedling growth, while on Wonogiri promising number infected by mycorhiza and mixed AMF resulted in better seedling growth than other types of AMF (Table 2, 3, and 4). Increasing number of cashew leaf on Asembagus promising number due to mycorhiza inoculation was 16.1% of control, while it was 7.7% of control on Wonogiri promising number infected by mixed AMF.

![Figure 1. Effect of different promising number to the leaf number and height of cashew](image)
### Table 1. Effect of AMF inoculation to the leaf number, height, and stem diameter of cashew

| Treatment          | 2 MAP | 3 MAP | 4 MAP | 5 MAP | 6 MAP |
|--------------------|-------|-------|-------|-------|-------|
| **Leaf number Jumlah daun** |       |       |       |       |       |
| Control            | 11.1 ab | 13.7 bc | 19.8 ab | 21.9 c | 20.3 b |
| Glomus aggregatum  | 10.7 b | 13.8 bc | 20.2 ab | 23.4 bc | 21.2 b |
| Glomus etunicatum  | 10.3 c | 12.9 c | 19.1 b | 21.8 c | 21.4 b |
| Mycor              | 11.7 a | 14.7 ab | 22.1 a | 25.8 a | 24.5 a |
| Glomus sp.         | 11.8 a | 14.4 ab | 21.6 a | 24.7 ab | 22.3 ab |
| Mixed AMF          | 9.6 c  | 14.0 bc | 18.4 b | 21.7 c | 21.2 b |
| Gigaspora sp.      | 11.2 ab| 15.7 a  | 20.1 ab | 22.4 a | 21.7 b |

**Plant height Tinggi tanaman (cm)**

| Treatment          | 55 ab | 56 a  | 57 a  | 58 abc | 59 abcd |
|--------------------|-------|-------|-------|--------|---------|
| Control            | 25.3 a | 27.1 a | 32.9 b | 35.2 b | 37.1 ab |
| Glomus aggregatum  | 25.0 a | 27.2 a | 35.1 a | 37.0 ab | 38.7 ab |
| Glomus etunicatum  | 23.3 a | 25.5 a | 32.5 c | 34.9 b | 36.3 b |
| Mycor              | 24.8 a | 27.3 a | 36.3 a | 38.5 a | 39.8 a |
| Glomus sp.         | 24.9 a | 26.9 a | 36.1 a | 36.7 ab | 38.0 a |
| Mixed AMF          | 24.8 a | 27.4 a | 32.2 a | 34.8 b | 37.6 b |
| Gigaspora sp.      | 20.4 b | 22.6 b | 26.2 a | 25.8 c | 29.7 c |

**Stem diameter Diameter batang (mm)**

| Treatment          | 27.8 bc | 30.2 ab | 36.6 bc | 35.2 bc | 34.5 d |
|--------------------|---------|---------|---------|---------|--------|
| Control            | 6.09 ab | 6.42 ab | 6.91 bc | 7.38 bc | 7.74 bc |
| Glomus aggregatum  | 6.04 a  | 6.67 a  | 7.03 ab | 7.70 ab | 8.09 ab |
| Glomus etunicatum  | 6.09 a  | 6.61 a  | 7.16 ab | 7.67 ab | 8.14 a |
| Mycor              | 6.23 a  | 6.87 a  | 7.36 a  | 8.2 a  | 8.18 a |
| Glomus sp.         | 6.21 a  | 6.70 a  | 7.31 a  | 7.75 a  | 8.19 a |
| Mixed AMF          | 5.53 b  | 6.53 a  | 6.83 bc | 7.13 cd | 7.36 ed |
| Gigaspora sp.      | 5.01 c  | 5.60 bc | 6.63 a  | 6.91 c  | 7.12 ed |

**Note:** Numbers followed by the same letters in the same column are not significantly different at 5% DMRT

**Keterangan:** Angka yang diikati huruf yang sama pada setiap kolom tidak berbeda nyata pada uji Duncan taraf 5%

### Table 2. Interaction effect between promising number and AMF to the leaf number of cashew

| AMF (Main Plot/Sub Plot) | 4 MAP | 5 MAP | 6 MAP |
|-------------------------|-------|-------|-------|
| **Asembagus**            |       |       |       |
| Control                 | 33.31 abc | 32.61 de | 35.19 cde |
| Glomus aggregatum       | 34.42 abc | 36.30 bcd | 38.38 abc |
| Glomus etunicatum       | 31.79 c | 34.54 cd | 35.91 cd |
| Mycor                   | 35.59 abc | 37.65 abc | 39.17 abc |
| Glomus sp.              | 36.64 a  | 37.23 abc | 38.40 abc |
| Mixed AMF               | 27.9 d   | 29.16 ef | 33.43 de |
| Gigaspora sp.           | 24.93 d  | 26.89 f  | 27.98 f |
| **Wonogiri**            |       |       |       |
| Control                 | 32.53 abc | 37.76 abc | 39.00 abc |
| Glomus aggregatum       | 35.84 abc | 37.64 abc | 39.02 abc |
| Glomus etunicatum       | 33.14 abc | 35.35 bcd | 36.70 bcd |
| Mycor                   | 36.96 a  | 39.29 ab | 40.56 ab |
| Glomus sp.              | 35.49 abc | 36.15 bcd | 37.60 abc |
| mixed AMF               | 36.56 a  | 40.45 a | 41.68 a |
| Gigaspora sp.           | 27.50 d  | 30.16 ef | 31.43 ef |

**Note:** Numbers followed by the same letters on the same column are not significantly different at 5% DMRT

**Keterangan:** Angka-angka yang diikati oleh huruf yang sama pada masing-masing kolom tidak berbeda nyata pada uji Duncan taraf 5%

Effect of AMF inoculation was significant to the weights of leaf, stem, and root of cashew seedling (Figure 2). Increments of leaf, stem, and root weights of cashew due to AMF inoculation were 8-38, 7-31, and 2%. 

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Inoculation of mycofer resulted in the highest weights of cashew leaf, stem, and root, and significantly higher than control and Gigaspora sp. inoculation treatments. Interactions between cashew promising number and AMF inoculation were very significant to the weight of leaf, and significant to the weight of root (Figure 3). The two promising number seedlings of cashew showed rather different response to the AMF inoculation. Asembagus promising number seedlings infected by mycofer showed the best leaf and root weights, then followed by Glomus sp. and Glomus etunicatum. In addition, these three AMF showed better leaf and root weights compared to other AMF treatments. Besides, Wonogiri promising number seedling infected by mycofer and mixed AMF showed better leaf and root weights compared to other AMF treatments. Leaf weight of Asembagus promising number due to mycofer inoculation was 60.34% higher than control, while on Wonogiri promising number the leaf weights due to mycofer and mixed AMF inoculations were 18.55 and 15.04% higher than control, respectively. It was considered that mycofer and mixed AMF were more effective to Wonogiri promising number, while on Asembagus promising number inoculation of mycofer and single type of AMF (Glomus etunicatum and Glomus sp.) were more effective. As proposed by Sieverding (1991) that the effectiveness of certain types of Arbuscular mycorrhiza to the host plant may vary and are determined by environmental conditions and host plant species.

Promising number of cashew, AMF inoculation, and their interaction significantly affected leaf area index of cashew seedling at 6 MAP (Figure 4). Cashew inoculated with mycofer and mixed AMF showed higher leaf area compared to other AMF treatments. The increasing leaf area of those mycorrhizal plants were each 37.42 and 17.18% compared to control. Asembagus promising number significantly showed better leaf area than Wonogiri promising number. Furthermore, the highest leaf area values were performed by promising numbers of Asembagus, infected by mycofer, and Wonogiri, infected by mycofer and mixed AMF. Better mycorrhizal plant growth has been attributed extensively to ability of AMF to expand the volume of soil for which mineral nutrients are made available to plants compared to what roots themselves would contact (Clark and Zeto, 2000). Therefore, suitable symbiosis between Mycorrhiza and plant roots may increase the absorption and transition of nutrients from the soil to the roots and improve plant growth (Turk et al., 2006).

**Nutrient Uptake**

Instead of improving growth performance of cashew, AMF application might increase the uptake of N, P, and K nutrients by plant. The two promising numbers showed different response, in case of plant nutrient uptake, to the AMF inoculation (Figure 5). The increasing N, P, and K uptakes of Asembagus promising number inoculated with mycofer were 2, 65, and 53%. When it was inoculated

![Figure 2. Effect of AMF inoculation to the weight of leaf, stem and root of cashew](image)

![Figure 3. Effect of promising number and AMF to the weight of leaf and root of cashew](image)
with Glomus etunicatum, the N, P, and K uptakes were 15, 36, and 28%, and when inoculated with Glomus sp. they were 2%, 39% and 26% higher than control. Mycofer and Glomus sp influenced more in enhancing the uptake of P and K, whether Glomus etunicatum might influence in the uptake of N, P, and K nutrients. Although Glomus aggregatum colonized >80% of Asembagus promising number root, the nutrient uptake was lower than those colonized by Glomus etunicatum and mycofer.

SIEVERDING et al. (1986) in SIEVERDING (1991) detected that P uptake of cassava infected by G. fasciculatum (70.5% of root colonization) was 3.6 mg P/plant, lower than by Acaulospora mellea (32.5% of root colonization) 5.2 mg P/plant. The increments in the uptake of N, P, and K of Wonogiri promising number infected by mycofer were 55, 38, and 17%, followed by mixed AMF (18, 31, and 17%), and Glomus aggregatum (22, 15, and 31%). DA SILVEIRA et al. (2002) found that responses of the avocado plants to the different arbuscular mycorrhiza species after 7 months of grafting were distinctly different. Scutellospora heterogama, A. scrobiculata, G. etunicatum, and G. clarum more thoroughly colonized the root system of the avocado plants, resulting in enhanced nutrient uptake and higher accumulation of reserve substances, which favored vegetative growth. Mycorrhiza through the external mycelium, supported the plant by increasing rhizosphere soil volume of about 12-15 cm² per cm infected root. The internal hyphae enhanced the contact of the root with the medium, or increased the soil volume explored for nutrient uptake (SIEVERDING, 1991).

**Acid Phosphatase Activity of Cashew Root**

Soil organic P was mineralized enzymatically. Mineralization of organic P may release considerable amounts of inorganic P in favorable conditions of moisture, pH and root activity (TISDALE, 1985 In TURNER et al., 2005). BOLAN (1991) reported that solubilization of soil P is achieved by the release of organic acids and phosphatase enzymes. The phosphatase enzyme is produced by the root of higher plants as well as by numerous microorganisms. Phosphatases are phosphate esterases with hydrolyze phosphoesters, as a rule, phosphoric monoesters such as phosphatidic esters of higher plants as well as by numerous microorganisms. Phosphatases are phosphate esterases with hydrolyze phosphoesters, as a rule, phosphoric monoesters such as phosphatidic esters (MENGEL and KIRBY, 2001). The chief attribute of VAM fungi is their ability to absorb soluble phosphate (and other nutrient) beyond the phosphate depletion zone in soils of low phosphorus status. Symbiosis of AMF was alternative mechanisms increasing phosphate level which occurred directly through external hyphae and indirectly through its effect on acid phosphatase activity.
In mycorrhizal root of Asembagus promising number, increasing acid phosphatase activity was 11.5 up to 136.5% over to non mycorrhizal plant, mixed AMF registered the highest value followed by Glomus aggregatum and mycofer. In mycorrhizal root of Wonogiri promising number, increasing acid phosphatase activity was 5.5 up to 80% over to non mycorrhizal plant, mycofer had the highest value followed by Glomus aggregatum, mixed AMF and G. etunicatum (Figure 6). Higher phosphatase activity in mycorrhizal plant has been reported by GOICOECHEA et al. (1996). The increasing acid phosphatase activity in mycorrhizal alfalfa seedling roots even in control or during drought period when compared to non mycorrhizal were 41.2 and 50%.

CONCLUSION

The current study had shown that inoculating Anacardium occidentale seedling with AMF enhanced growth performance, N, P, K nutrient uptakes, and acid phosphatase activity. Mycofer and mixed AMF were more effective to Wonogiri promising number, while for Asembagus promising number inoculation by mycofer was more effective. Inoculation with mycofer to Asembagus promising number increased the uptake of P and K by 65 and 53%, whether inoculation with mycofer and mixed AMF to Wonogiri promising number increased the uptake of N, P, and K by 55, 38, and 17%. Moreover, the AMF inoculation resulted in higher phosphatase activity. In mycorrhizal Asembagus promising number infected by mixed AMF, the increment of phosphatase activity was 136.5%, while in Wonogiri promising number infected by mycofer the increment of phosphatase activity was 80% higher than control.

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