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EFFECT OF KAEMPFEROL ON THE ESTABLISHMENT OF ARBUSCULAR MYCORRHIZAL FUNGI ON OIL PALM SEEDLING ROOTS

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) could associate beneficially with more than 80% of terrestrial plants roots including oil palm roots and as an alternative to reduce chemical fertilizer applications. Oil palm root exudate such as flavonoid is important in the interaction between the crop and AMF. Flavonoids exhibit a strong stimulatory effect on AMF hyphal growth, hyphal differentiation and root colonization, plus improving plant-microbe interactions. The present studies were conducted with the following objective to determine the flavonoid efficiency on AMF establishment in oil palm roots. Exogenous kaempferol as one of the flavonoid types was applied at four concentrations (0, 2.5, 5.0 and 10.0 ppm) with three AMF sources (*Glomus mosseae*, mixed AMF and non-inoculated) with complete fertilizer. Each pot contained sand mixture with one-month-old oil palm seedling. The planting time is twelve weeks in the net shelter house. The oil palm growth and root development significantly affected by the treatment (*G. mosseae**10 ppm kaempferol) since plant height, root length and phosphorus uptake showed significant interactions between the treatments with 20.91%, 27.24%, and 102.33% difference, compared to non-inoculated plant at the same concentration, respectively. The results for shoot biomass, root biomass, root volume, root diameter, phosphorus in soil and root infection showed the highest value compared to AM mixed species and non-inoculated plants.

Keywords: flavonoid, kaempferol, mycorrhiza, oil palm, root exudates

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

INTRODUCTION

Plant synthesizes a wide range of flavonoids in shoot and root tissues during vegetative growth and development. The synthesis and accumulation of flavonoid were observed on various root parts, such as on root hair surfaces, in root cap cells and at the root tips. Flavonoids can also be released from border cells and decomposing root cap (Hassan *et al*., 2012). They are one of the largest groups of polyphenolic compounds and plant secondary metabolites (Eva *et al*., 2006). Flavonoids are currently considered to play an important role inside the roots (Amalesh *et al*, 2011). The exudate played an important role in the establishment of AMF symbiosis (Steinkellner *et al*., 2007). The AMF is known widely to improve plant nutritional status through enhancement of essential nutrients and water uptake by increasing root surface area (Mechri *et al*., 2015). The fungi have the ability to form mutualistic symbiosis with various important agricultural plants. The formation and function of AMF induced considerable morphological, physiological, biochemical, and molecular changes for both the host and the symbion (Widiastuti *et al*, 2007).

Nutrient cycle in ecosystem works well in the presence of AMF. The extramatrical hyphae extends the zone that can be reach by the root thus facilitate the absorption and translocation of nutrients. In the other hands, AMF also helps in increasing the availability and supply of slowly released element like phosphate to the plant (Sajid *et al.,* 2014). Phosphorus is often found in limited quantity in soils, because of its poor solubility and low diffusion rates. Besides, a large amount of the P present in the soil is immobilized in organic compounds, which must be hydrolyzed by plant or microbial phosphatases before plants can take it up. Therefore, in 40% of arable land, limited P availability causes P deficiency, which usually leads to a stimulation of the biosynthesis of phenolic compounds, including flavonoids (Cesco *et al.,* 2010).

Flavonoid is able to influence AMF by increasing the level of AMF colonization (Scervino *et al*., 2005; Steinkellner *et al*., 2007; Amalesh *et al*., 2011). Flavonoids can be important in the establishment of AMF in oil palm since plants able to get several benefits from AM fungi such as better survival, growth, nutrient uptake, and reproduction rate. The AM fungi are also essential in improving the resistance of plants to pathogens (Mechri *et al.,* 2015).

This present study was undertaken with the objective to determine the effect of kaempferol and AMF sources on development of oil palm seedlings.

MATERIALS AND METHODS

The mixture of soil: sand (1:1, v/v) was used as growth medium .Serdang series soil was taken at Ladang Dua, UPM. The sand was washed to remove any chemical and dried under sunlight. The soil mixture was sterilized twice using autoclave at 121 °C for 60 minutes. The soil mixture was

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

allowed to cool for 24 hours before filling 500 g per pot (125 mm diameter x 101 mm height).The inoculum was initially produced in pot culture using maize (*Zea mays*) as a host. The spore inoculum density per 10 g of dry soil was determined by wet sieving and decanting technique using different sieve sizes of 400, 250, 106 and 45 µm (Gupta and Mukerji, 2002). The spores were collected in petri dish with parallel line underneath and counted under stereo microscope (Gerdeman and Nicolson, 1963) to estimate the number of spore inside the inoculum. Ten gram (about 300 spores) of spore inoculum was added into the soil. Spores inoculum of *Glomus mosseae* and mixed AMF (*Scutellospora sp* and *Glomus mossea)*) were obtained from Laboratory of Soil Microbiology, Land Management Department, Faculty of Agriculture, Universiti Putra Malaysia. The study was carried out in shelter house at Ladang 15, Universiti Putra Malaysia, Malaysia. Four rates of kaempferol (0, 2.5. 5 and 10 ppm) were applied to different types of AM fungi (*Glomus mosseae*, mixed AMF and non-inoculated) (Scervino *et al*., 2005). Each pot contained sand mixture with one-month-old oil palm seedling. The planting time is twelve weeks in the glasshouse. Ten mL of fertilizer was applied in a form of Hoagland solution every alternate day by drenching technique (Hoagland and Arnon, 1950). Ten mL of kaempferol was applied to each plant every three weeks by drenching technique. The treatment was arranged using completely randomized design (CRD) with three replications. The oil palm seedlings were harvested at twelve weeks. Stock solution was prepared in 100 ppm. 10 mg kaempferol was weighed and put in 100 ml 70 % methanol. From the stock solution, different concentrations were prepared. Stock solution was stored in 4 °C while the flavonoid was freshly prepared every application time. Upon harvest at week 12, the plant samples were washed properly to remove all soil. The plant height was measured by standard measuring tapes from the soil surface to the tip of the main stem. The plant height was expressed as centimeter (cm). The fresh roots were used to analyze root morphology before they were oven dried. Some fresh roots were stored in 95% alcohol for root infection analysis. The plant samples were dried in oven at 70 °C temperature until constant weight achieved and The dried weight of shoots and roots were weighed by using the digital balance (QC 35EDES-Sortorius- Germany) before grinding process for chemical analysis. Soil was also sampled for analysis of chemical properties. The morphology of oil palm roots was determined after harvest by using root scanner Model WinRhizo Pro 2008 - Epson Perfection V700 Photo (Regent Instruments Inc., Quebec, Canada). The adhering soil was rinsed off from root samples with distilled water and placed in the root scanner. Total root length (cm), total surface area (cm^2), total volume (cm^3) and root diameter (mm) were determined. Available phosphorus in soil was determined using Bray II method (Bray and Kurtz, 1945). Two grams of air dried soil (2.00 mm sieved) was weighed into 50 ml falcon tube and react with 14 ml of extracting solution (0.03 NH_4F and 0.1 HCl). The soil suspension was shaken for 45 s by using the wrist inversion technique. All the extract was filtered through Whatman No. 42 filter paper into the plastic vial. The final product has been

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

analyzed by Auto Analyzer (Lachat instruments, Quik Chem® FIA+ 8000 series). Plant nutrient (phosphorus) was determined according to Kjeldahl method (Bremner and Mulvaney, 1982). For the root infection studies, fresh roots were rinsed and approximately 1 g biomass of fine tertiary root hairs was cut to one cm lengths (segments) and stained using the method of Koske and Gemma (1989).

$$Mycorrhizal\ root\ colonization\ (\%) = \frac{number\ of\ mycorrhizal\ segments\ (stained)}{total\ number\ of\ sampled\ segment}$$

The data were recorded and analyzed using analysis of variance (ANOVA) by Statistical Analysis Software (SAS) version 9.3 for Windows. The significant difference of treatment means was determined using the Least Significance Different's test at ($P \leq 0.05$).

RESULTS AND DISCUSSION

There was an interaction between kaempferol concentrations and the different AMF types on the oil palm's growth (Table 1). The shoot biomass of oil palm seedlings was not significant. However, *G. mosseae* treated plant showed higher shoot biomass than AM mixed species treated plant with 4.04% difference. Among the kaempferol concentration, 10 ppm was the best concentration for the oil palm seedlings to have higher shoot biomass while 5 ppm showed the lowest shoot biomass. The difference between 10 ppm and 5 ppm was 6.24%. The best combination of treatment was AM mixed species treated plant at 10 ppm, which was 2.22 g/ plant.

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Table 1: Mean square for plant growth of oil palm seedlings

Source of variation	df	Shoot biomass (g/ plant)	Root biomass (g/ plant)	Plant height (cm)	Root length (cm)	Root volume (cm^3)	Root diameter (mm)	Phosphorus in soil (mg kg^{-1}/ plant)	Phosphorus in tissue (%)	phosphorus uptake (mg/ plant)
Kaempferol concentration (KC)	3	0.14^{ns}	17.55**	5.96**	37.95**	4.08*	1.76^{ns}	0.62^{ns}	1.15^{ns}	1.92^{ns}
AMF types (M)	2	0.66^{ns}	1.29^{ns}	0^{ns}	14.1**	2.33^{ns}	1.38^{ns}	2.33^{ns}	0.02^{ns}	1.39^{ns}
KC*M	6	1.19^{ns}	2.44^{ns}	8.01**	9.04**	0.87^{ns}	0.99^{ns}	0.44^{ns}	0.29^{ns}	1.2^{ns}
Error	12									
Total	13									
CV		16.91432	8.781598	5.176159	6.694762	22.96251	11.94842	41.82539	23.29207	31.10739

*significant at 5% level; **significant at 1% level; ns - not significant

The root biomass of the oil palm seedlings was not significantly different since there was no interaction between kaempferol concentrations and different types of AMF. However, the kaempferol concentrations had an effect towards the plant heights. Figure 1 showed root biomass was significant at 10 ppm kaempferol. There was significant effect on plant height of oil palm seedlings. *G. mosseae* treated plant showed the highest plant height at 10 ppm kaempferol with 42.2 cm/ plant with 20.91% difference, compared to non-inoculated plant at the same concentration (Figure 2).

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Figure 1: The effects of kaempferol concentration on root biomass of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.

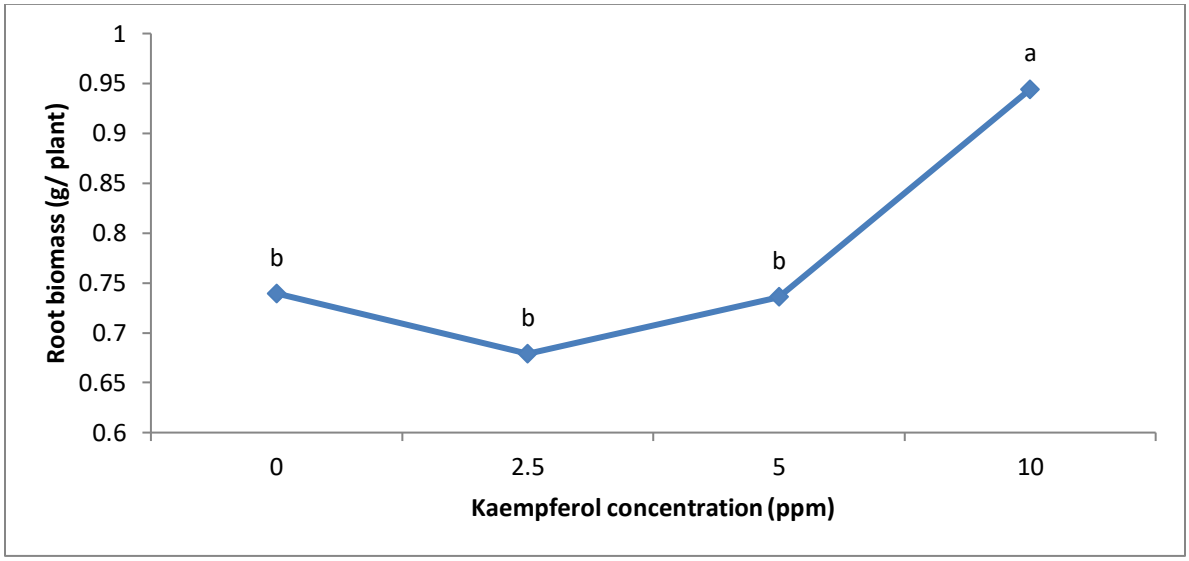
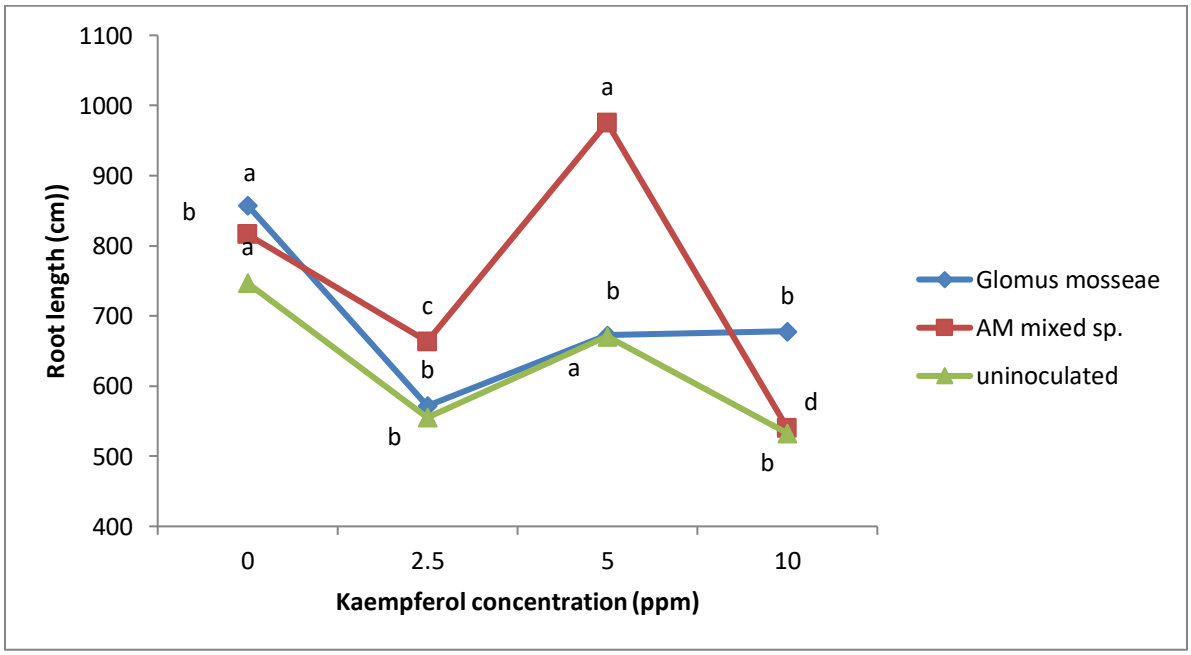


Figure 2: The effects of kaempferol concentration and mycorrhizal types on plant height of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.

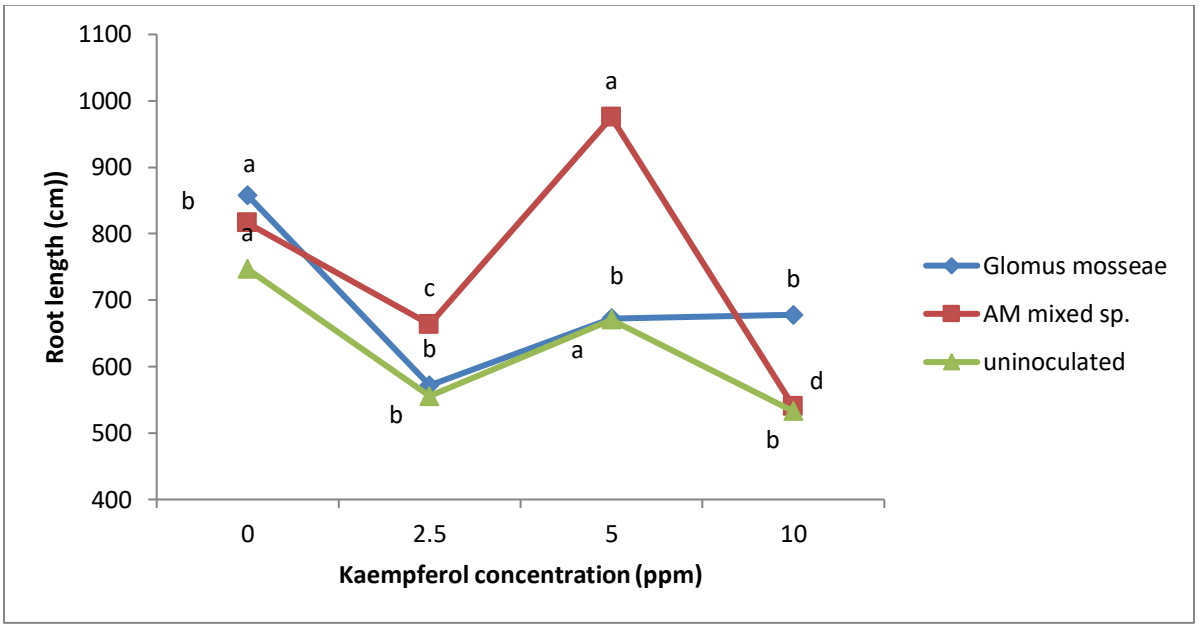


ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

The root length of oil palm seedling also showed significant effect towards the treatments. *G. mosseae* treated plant at 10 ppm showed the best among other treatment which was 677.85 cm/ plant compared to non-inoculated plant with 27.24% difference. However, AM mixed species showed the highest root length at 5 ppm with 975.3 cm/ plant (Figure 3).

Figure 3: The effects of kaempferol concentration and mycorrhizal types on root length of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.

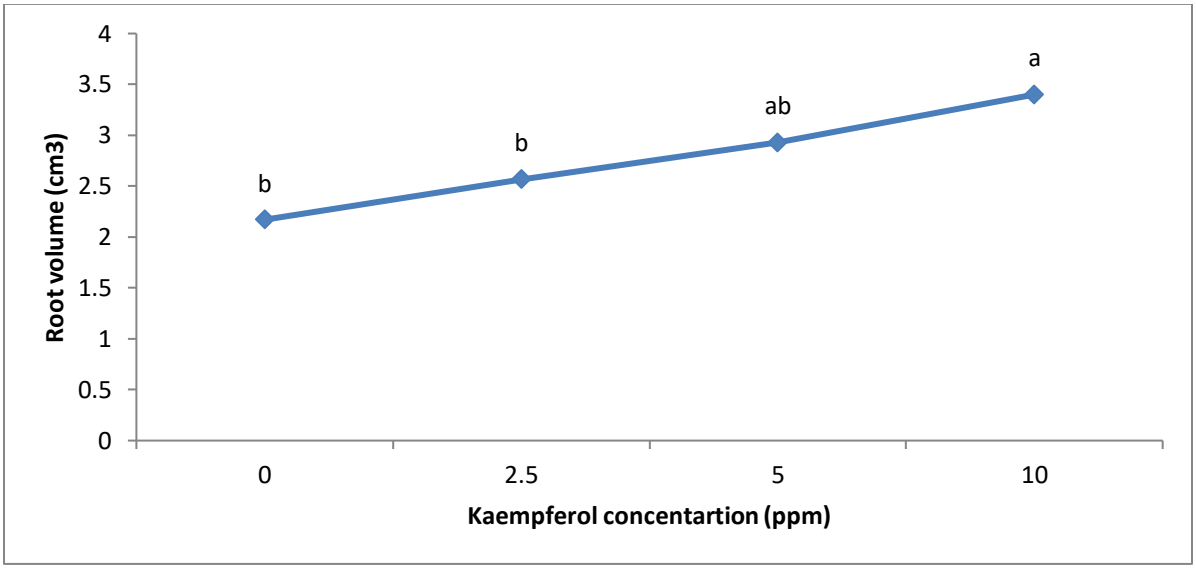


The root volume of oil palm seedlings showed no significant interaction between kaempferol concentration and AMF types. However, the kaempferol concentration itself showed significant effect of root volume. Ten ppm kaempferol showed the highest root volume which was 3.40 cm3, compared to 0 ppm kaempferol, with 56.65% difference (Figure 4).

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Figure 4: The effects of kaempferol concentration on root volume of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.



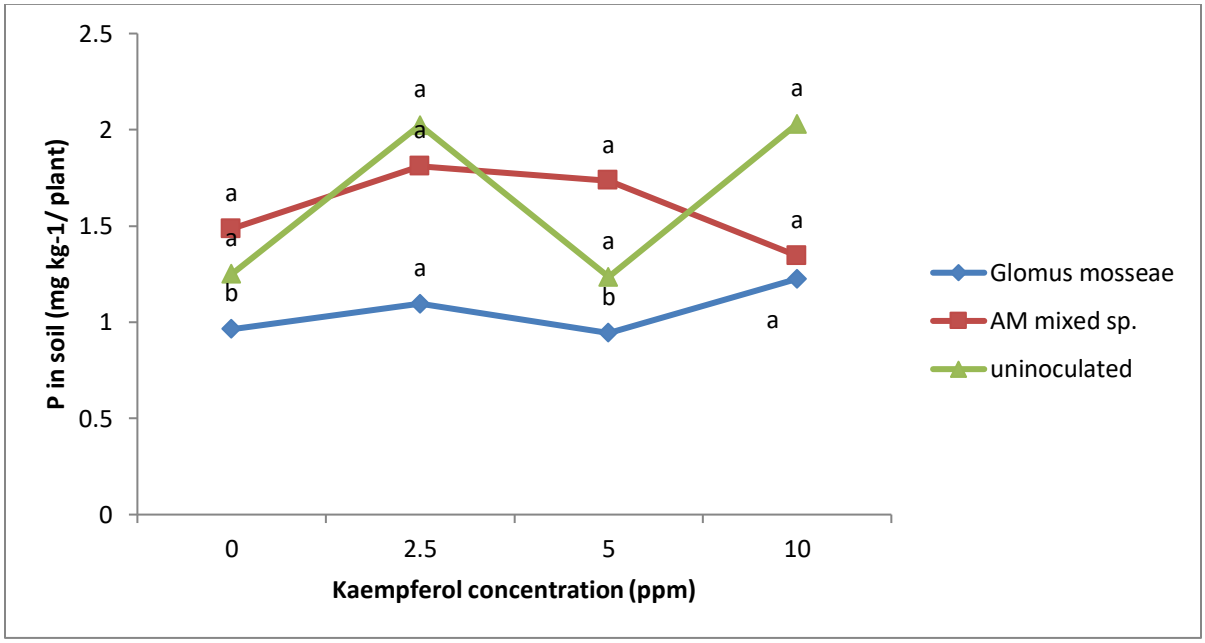
The root diameter of oil palm seedling was no significant since there was no interaction between AMF types and kaempferol concentration. However, *G. mosseae* treated plant showed the highest root diameter than AM mixed species treated plant and non-inoculated plant with 0.14% and 9.19%, respectively. Among the kaempferol concentration, 10 ppm was the best concentration for the oil palm root in order to have higher root volume. The difference between 10 ppm and 0 ppm was 12.36%.

The analysis of phosphorus in soil showed there was no significant interaction between AMF types and kaempferol concentration. Yet, *G. mosseae* treated plant showed the least amount of P in soil compared to AM mixed species and non- inoculated plant. Non- inoculated plant showed unstable trend along the concentrations while AM mixed species showed the decreasing amount of P in soil along the concentration (Figure 5).

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Figure 5: The effects of kaempferol concentration and mycorrhizal types on phosphorus in soil of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.

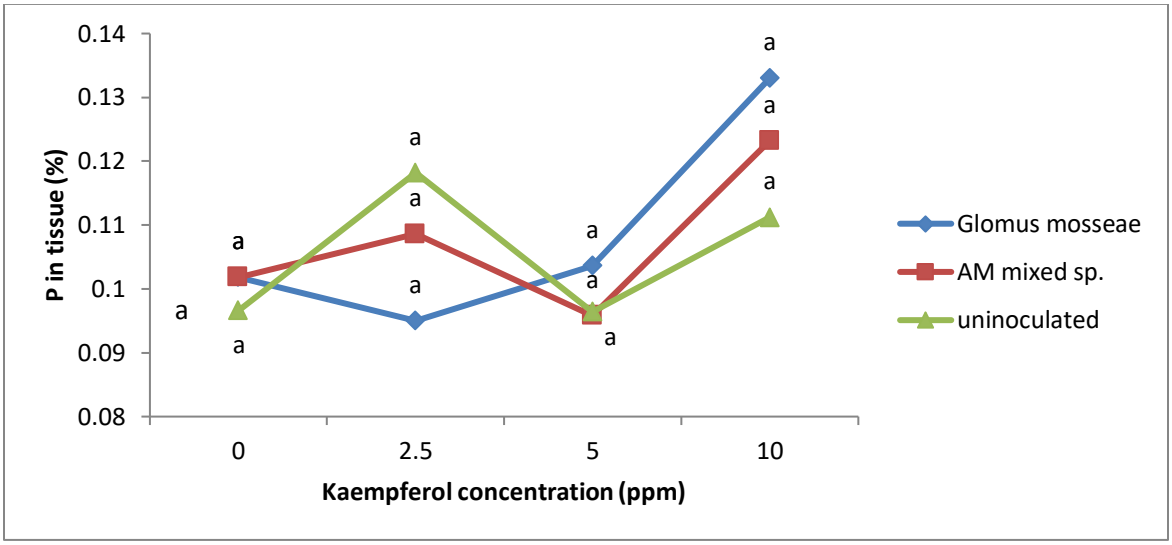


The determination of phosphorus in oil palm seedling's tissue showed no significant interaction between AMF types and kaempferol concentration. Yet, *G. mosseae* treated plant still showed the best AMF among the types with 2.60% higher than non-inoculated plant. Ten ppm kaempferol showed highest percentage of phosphorus in tissue with 22.38% compared to 0 ppm kaempferol. The best combination of the treatment was Glomus mosseae at 10 ppm kaempferol (Figure 6).

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Figure 6: The effects of kaempferol concentration and mycorrhizal types on phosphorus in tissue of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.

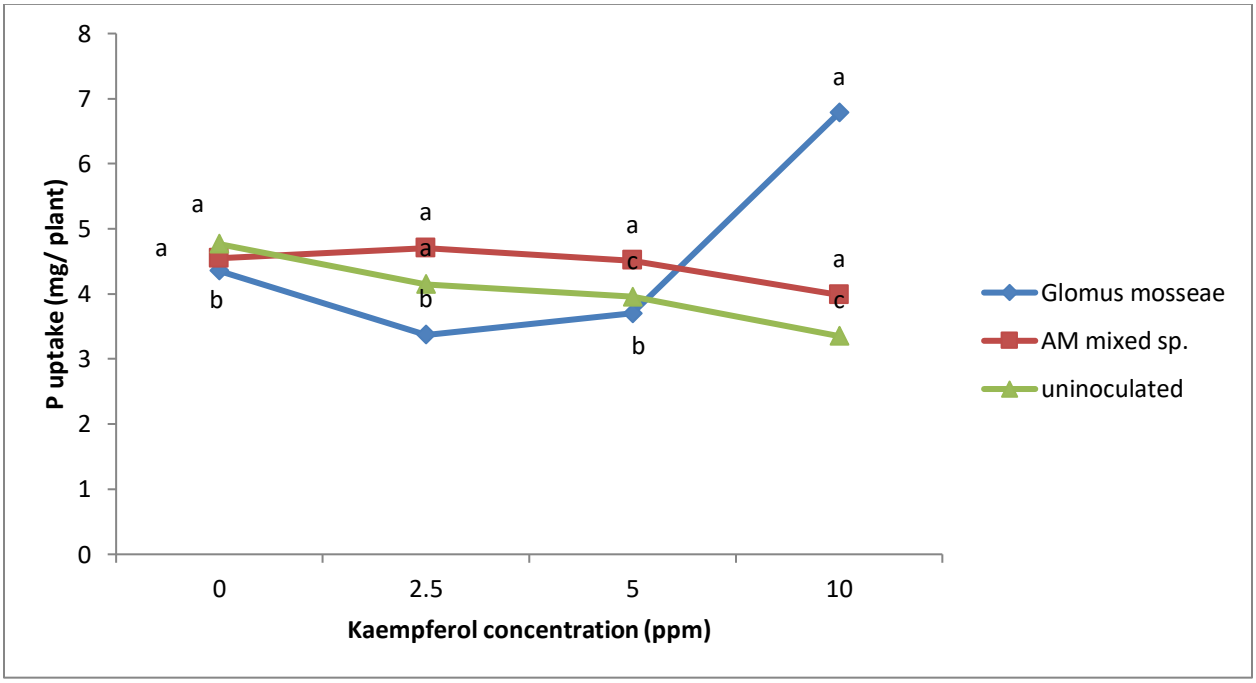


The P uptake of oil palm seedling also showed significant effect towards the treatments. *G. mosseae* treated plant at 10 ppm showed the best among other treatment which was 6.781 mg/ plant compared to non-inoculated plant with 102.33% difference (Figure 7). The root infection of oil palm seedlings root was not significant because no interaction between AMF types and kaempferol concentration. However, *G. mosseae* showed higher root infection than AM mixed species with 12.37% difference. Among the kaempferol concentration, 10 ppm was the best concentration for the oil palm root infection. The difference between 10 ppm and 0 ppm was 15.47%. The best combination of the treatment was G. mosseae at 10 ppm, which was 94.17%.

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

Figure 7: The effects of kaempferol concentration and mycorrhizal types on phosphorus uptake of oil palm seedlings. Means with the same letter between kaempferol concentrations are not significantly difference.



The study showed that individual *G. mosseae* was able to significantly increase biomass of oil palm seedlings when the application kaempferol was at 10 ppm. The result is consistent with studies by Naher *et al.*, (2013) that showed growth and yield improvement of *Abelmoschus esculentus* with *G. mosseae* inoculation. Plants improved the growth with the presence of AMF. The root infection by the AMF escalates active absorptive surface area and encourages nutrient and water uptake even tough in a condition of water stress. AMF colonization expands disease suppression capability of the host plant. AMF are vast in nature and the essential element of tropical soil system (Naher *et al.*, 2013).

The flavonoid kaempferol positively affected *G. mosseae* development in oil palm roots but negatively affected development of the mixed AMF which contained both *G. mosseae* and *Scutelospora sp*. The increased plant growth could be due to stimulatory effect of the flavonoids on root colonization as suggested by Scervino *et al.* (2007) that flavonoid stimulate the presymbiotic fungal growth resulting in a greater number of entry points, which can lead to higher root colonization. As spore germinates, the AMF grows towards the host plant, finally penetrating the root and forming its intraradical structures. Scervino *et al.* (2007) observed that a

ISSN: 2455-6939

Volume:04, Issue:02 "March-April 2018"

higher number of entry points in the presence of the flavonoids crysin, luteolin, morin, and rutin always resulted in an enhanced colonization rate by *Gigaspora* or *Glomus* species.

The establishment of the symbiosis is the outcome of complex exchange of signals between the AMF and the host. However, certain flavonoids in roots are proven to be involved in enhancing the level of root colonization and regulation of AM symbiosis such as in clover plants (Scervino *et al*., 2007). Flavonoids are one of the largest groups of polyphenolic compounds and plant secondary metabolites, and they play an important role in plants as defence and signalling compounds in reproduction, pathogenesis and symbiosis (Eva *et al*., 2006).

Growth of oil palm roots inoculated with *G. mosseae* was significantly enhanced compared to plants inoculated with mixed AMF applied with 10 ppm kaempferol. This could be due to competitive situation at root colonization. Consistent with the fast rate of root colonization and early peak in colonization vitality, *G. mosseae* was the most effective competitor (Jan *et al*. 2008).

Oil palm has a poorly developed root system with very little root hairs, hence low ability to take up enough nutrients including phosphorus. Establishment of AMF inside the oil palm root enhanced the ability of the roots to uptake more P from soil. Phosphate is less mobile in soil and rapid uptake by the growing plants result in a depleting zone around the roots. The external hyphae of AMF can help to absorb phosphate beyond the depletion zones around the root hairs and transport it to the root tissues (Abdel-Fattah *et al.*, 2014).

CONCLUSIONS

The study proved that kaempferol influenced the establishment of AMF in oil palm roots. Addition of kaempferol positively affected infection of *G. mosseae*, but not influencing the mixed AMF that contain mixture of *G. mosseae* and *Scutelospora sp*. Oil palm biomass and root development significantly increased with inoculation of *G. mosseae* compared to the mixed AMF. Concentrations of kaempferol also influence plant growth where 10 ppm kaempferol significantly affect the oil palm growth. Oil palm seedlings inoculated with *G. mosseae* in the presence of kaempferol improved growth compared to plants inoculated with mixed AMF.

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