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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON PLANT GROWTH PERFORMANCE, PHYSIOLOGICAL CHANGES AND YIELD QUALITY OF SWEET BASIL GROWN IN SOILLESS CULTURE MEDIA

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ABSTRACT

A study was conducted to investigate the growth performance, physiological changes and yield quality of sweet basil (*Ocimum basilicum*) to inoculation by different rate (0, 10, 20 and 30g inoculums) of Arbuscular Mycorrhizal Fungi (AMF) under the soilless culture system. The result showed that plant height, total leaf area, plant fresh and dry weight, root shoot ratio, total chlorophyll content, photosynthetic rate, stomatal conductance, total ethanol soluble carbohydrate and leaf ascorbic acid were significantly influenced by AMF. Oil production increased significantly with increasing the inoculums rate on both fresh and dry weights of basil. Spore counts and percentage of root colonization were not significantly affected by AMF inoculation.

Keywords: Sweet basil, arbuscular mycorrhizal fungi, oil production, photosynthesis rate, root colonization.

INTRODUCTION

Ocimum basilicum or sweet basil is a member of the Lamiaceae family that has a high market demand and economically important uses in food seasoning, pharmaceutical properties (Bais et al., 2002), culinary applications, preservatives, cosmetics and industrial products (Brewer et al., 2011; Makri and Kintzios, 2007). They are also rich in essential oils and have been extensively used in the flavoring of confectionery and baked goods condiments; it has also found wide application in perfumery, as well as in dental and oral products (Simon et al., 1990Quality of sweet basil used in certain fresh and dry products is a function of its secondary metabolite production including essential oils (Hussain et al., 2008; Copetta et al., 2006). According to Gang et al. (2001), essential oils are synthesized and stored in peltate glands especially from leaf structures responsible for oil production. Thus, AMF inoculation is one of the options considered to improve plant growth performance so that the high yield quality of basil can be produced. AMF and plants can share a mutually beneficial relationship (Detloff and Sally, 2010) by forming a mutualistic association with the roots of 90% of land plants (Smith and Read, 1997). Referring to Allen et al. (2003), root surface area greatly increases due to AMF inoculation causing an enhancement in nutrient and water uptake by

the host plant. AMF enhanced water uptake through improved hydraulic conductivity and increasing leaf gas exchange and photosynthetic activity (Ruiz-Lozano et al., 2003; Dell-Amico et al., 2002). Benefit to plant growth, health and survival when plant symbiosis of AMF are established with the relationship of intra-radical and extra-radical root colonization (Robert and Anne Davis, 2004; Rufykiri et al., 2002). Root colonization by AMF can alter plant biomass and carbon use hence may alter the production of secondary metabolites in plants (Toussaint et al., 2007; Strack and Fester, 2006; Copetta et al., 2006; Strack et al., 2003). In addition, the AMF colonize plant roots allowing the plant access to necessary nutrients in exchange for carbohydrates produced by the plant (Graham et al., 2008; Mack and Rudgers, 2008; Smith et al., 2004). However, Smith et al. (2004) studied that there are degrees of mutualism within the plant/fungi relationship mediated by the capabilities of both the plant and fungi. Under certain conditions, Mack and Rudgers (2008) mentioned that mycorrhizal can become parasitic.

Most herbs are grown under glass-house or rain-shelter in order to maximize yield and in order to obtain a regular supply of material all the year round. Coconut coir dust (CCD), a by-product of the coconut industry, has been promoted as an alternative to peat-moss in soilless media. Utilization of this material is considered environmentally sustainable and this has attracted interest among research scientist and glass-house growers. This locally available material has been used as soilless media in this glasshouse study. The aim of the present study was to investigate the potential impact of AMF which have been acknowledged as bio-fertilizer on the herb's quality. Sweet basil was selected as a model plant to examine whether the beneficial effects of AMF on plant development and physiological changes may be extended with some qualitative plant features.

MATERIALS AND METHODS

Media and plant material

The experiment was conducted in the Glasshouse Unit at the Faculty of Agriculture, University Putra Malaysia (UPM), Serdang, Selangor. Seeds of sweet basil were purchased from the local market, germinated for about two weeks in a seed tray containing sandy soil media. Seedlings were maintained in a glasshouse at 28.6±6ºC, with RH is approximately 78±4%. Basil plants were irrigated manually for twice per day. After two weeks, the seedlings had developed one set of true leaves and 2-3 roots, and were individually transplanted into poly bags (40cm long x 25cm wide) containing CCD media.

AMF inoculum preparation

The AMF inoculums were obtained from the Department of Land Management, Faculty of Agriculture, UPM. It consisted of mixed culture of *Scutellospora calospora* and *Glomus mossea*, mass propagated under Setaria grass (*Setaria anceps*

var. *splendida*). Inocula consisted of a mixture of the soil medium, extra-radical hyphae and spores, and colonized root segments (≤2 mm in length) were used for the respective treatments.

Planting and inoculum placement

Two week old basil seedlings, germinated from seeds as mentioned previously, were transferred to the respective polybag. The uniform heights of the grown basils were 30 cm and were placed in each polybag. The respective inoculum treatments were placed into each planting hole before transplanting. Inoculum was placed as a thin layer to a depth of 5 cm to ensure better infection. The un-inoculated seedlings were similarly treated but the inoculum had been steam sterilized at 121ºC for two hours. The observations were recorded for two weeks after transplanting.

Experimental design and plant maintenance

There are four treatments of AMF inoculation; 0g, 10g, 20g and 30g of inoculum. Polybags were arranged in a randomized complete block design (RCBD) with four replicates and 9 plants in each replicate and gave a total of 144 plants. The glasshouse daily temperature was maintained at 28.6 ± 6ºC and the relative humidity (RH) was 78 ± 4%. The plants were watered manually for twice per day with a nutrient solution containing the ion concentrations given by Cooper (1979); with electrical conductivity (EC) maintained between 2.4 – 2.6 ms cm^{-1}. Plants were harvested at 2, 6, and 10 weeks after transplanting (WAT).

Plant growth performance

At harvest, plant height was measured with a measuring tape and total leaf area (TLA) was determined using an Automatic Leaf Area Meter (Model LI-3100, LI-COR, U.S.A.). Roots were washed free of media and blotted gently with paper towels, then later weighted to determine the root fresh weight. Shoot (leaf and stem) and root fresh weight were measured using analytical balance where as plant dry weight (shoot and root) were determined after drying at 80 ºC for 48 hours. The root to shoot ratio (RSR) was determined by using the equation of Hunt (1978) as shown below:

RSR = RDW / SDW

where:

RDW = Total root dry weight

SDW = Total shoot dry weight

Plant physiological changes

Three to four young fully expanded leaves were chosen from each treatment for the determination of total leaf cholorophyll content (Chl), photosynthesis rate (Pn) and stomatal conductance (gs). Leaf discs measuring 10 cm in diameter were

immediately immersed in 20 ml of 80% acetone in an aluminium foil covered glass bottle for approximately 24 hours at 0ºC until all the green color had bleached out. Finally, 3.5 ml of the solution was transferred to measure absorbance at 664 and 647 nm using a light spectrophotometer (UV-3101P, Labomed Inc, USA). The equation for total leaf chlorophyll content is as follows:

Leaf chlorophyll content (mg/mg fresh weight) = $\frac{(7.93*O.D^{1}_{664} + 19.53*O.D_{647}) \times (\text{total volume of 80\% aceton})}{2 \times (\text{total volume of 80\% aceton for O.D reading}) \times LFW}$

where:

^{1} = Optical density

LFW = Leaf fresh weight (mg)

Measurements of the leaf photosynthesis (Pn) rate and stomatal conductance (g_s) for intact leaves were determined using an open path portable open system infrared gas analyser (LI-6400, LICOR, U.S.A.) at 0900 – 1000 hours. The measurement was taken on the abaxial surface with under optimal cuvette conditions (800 µmol m^{-2} s^{-1} PPFD, CO2 flow rate of 400 µmol m^{-2} s^{-1} , 30ºC cuvette temperature, 60% RH and air flow rate set at 500 cm^3 min^{-1}. All the reading were taken within an hour to prevent the leaves adapting to the different conditions of measurement.

Yield quality analysis

Total ethanol soluble carbohydrate (TESC): Fresh samples of basil leaf were collected for TESC determination by Dubois et al. (1956). The phenol–sulfuric acid assay is a broad spectrum method for carbohydrates, measuring both mono- and polysaccharides. One gram of fresh leaf was weighed and digested by hot 80% ethanol (EtOH) two times, each time by 5 ml EtOH and then filtrated by Whatman No. 2 filter paper and the extracts diluted with distilled water to the volume 50 ml. One ml of each sample was placed in the test tube and then 1 ml phenol solution added. The procedure was followed by adding 5 ml of sulfuric acid and shaken vigorously. The yellow-orange color was pipetted off and the wavelength was read at 490 nm by the spectrophotometer machine (Pharmaspec UV-1700 model). The amount of TESC was presented using the glucose standard curve.

Leaf ascorbic acid (LAA) content: LAA was determined using the Direct Calorimetric Determination (Ranggana, 1977). It was carried out using dye, 2-6-dichlorophenol (indophenol method). Five g of fresh leaves were blended with 95 ml of 2% cold metaphosphoric acid (HPO_3). Then, the volume was filtered with filter paper, Whatman No. 2. 1 ml of extract was placed in the cuvette and 4 ml of 2% cold HPO_3 was added. Ten ml of dye was added and shaken. The reading was measured by using a spectrophotometer machine (Pharmaspec UV-1700 model) at 618 nm wavelengths. Unit of leaf ascorbic acid content is ml per 100 g (ml 100 g^{-1}).

Essential oil: Essential oil was obtained from leaves, stems, roots and flowers by a hydro - distillation method with a Clevenger apparatus (British Pharmacopoeia,

1980). Two hundred gram samples were diluted with 2000 ml distilled water (1:10 w/v). Distillation was continued for approximately 4 hour. Oil productions were stored in dark glass bottles at 4 ºC (Marotti et al., 1996).

AMF infection

AMF colonization: At each harvest time, a sample of fresh roots was cut into 1 cm segments and stored in FAA (formalin-acetic acid-50% ethanol) (5:5:90, v/v/v) solution. Roots were then, washed with tap water three times and then acidified with 1% HCl (Hydrochloric acid) for 3 minutes. Roots were stained in 0.05% Trypan blue in water bath at 90 ºC for 15 minutes. Root segment mounted on slides were examined at x100 magnification, using an Olympus BH-2 light microscope containing an ocular crosshair eyepiece. The number of root segments colonized were counted and expressed as percentage of total root segments (Kormanik and Mc Graw, 1982).

$$\text{AMF Colonization (\%)} = \frac{\text{Number of colonized sample}}{\text{Total number of segments examined}} \times 100$$

Spores count: Wet sieving decanting method (Gerdemann and Nicolson, 1963) was used to assess the spore density in the media. Ten gram of soil was collected randomly after harvesting and placed in 200 ml water in a large beaker. Media mixture was stirred for 2 minutes then the mixture was poured into arranging sieves (250, 106 and 42 µm). Media was entrapped to settle at every sieve. The spore suspension was examined under a stereo microscope.

Statistical analysis

Analysis of Variance (ANOVA) on data obtained was performed using Statistical Analysis System (SAS 9.1, SAS Institute, Inc. Cary NC. USA). The treatment means were further separated using Duncan Multiple Range Test (DMRT) at the 5 % level of probability.

RESULTS AND DISCUSSIONS

Effect of AMF inoculation on plant growth performance

The AMF inoculation increased basil plant growth in the glasshouse compared to non-inoculated plants (control). At 6 and 10 WAT, inoculated plants were taller than control plants (Figure 1), but there was no significant effect of inoculum rate on plant height at 2 WAT ($p>0.05$). The highest of AMF inoculation rate (30 g) had significantly increased the plant height at 6 and 10 WAT (c. 6.8 – 22%) compared to non-inoculated plants. Inoculated basil plants produced significantly higher TLA than the control ($p<0.01$) at 6 and 10 WAT. As week increased, TLA also increased until reaching the end of 10 WAT (Figure 2). On 10 WAT, the highest TLA was

observed with 30 g of AMF per plant (c. 15%). Based on Figure 3a, leaf fresh weight on inoculated plant showed a significant increment compared to control plants ($p<0.05$). At 10 WAT, 30 g of AMF gave the highest leaf fresh weight and the result shows a similar trend on stem fresh weight for all treated plants. Maximum value of stem fresh weight at 10 WAT was founded in 30 g of AMF which had increased by 39% compared to those in control (Figure 3b). Similarly, root fresh weight also affected by AMF inoculation although there was no significant difference in plants of 6 weeks old. The highest root fresh weight was recorded with plants of 30 g of AMF at 10 WAT (Figure 3c). According to Figure 4, plant dry weight shown an almost similar pattern with plant fresh weight (Figure 3). By the end of 10 WAT, the highest inoculum rate increased leaf dry weight (c. 27%) compared to control plants (Figure 4a). Stem dry weight was maintained over 10 WAT with a maximum value of 30 g AMF (Figure 4b). Figure 4c indicated that basil root dry weight was influenced by different rate of AMF inoculum at 6 and 10 WAT. At 10 WAT, the highest impact was shown for 30 g of inoculated plant (c. 57%) compared to control plant.

The partitioning between leaf, stem and root growth can be derived from RSR (Figure 5). Inoculated basil plants had significantly higher RSR than the control plants($p<0.01$) at 10 WAT. This could be due to the higher RDW in inoculated plants than the control plants. Contradictly, Kiernan et al. (1984) have found higher RSR in non-inoculated strawberry plants compared to inoculated strawberry plants.

A significant finding of this study was the sweet basil appeared to exhibit a high growth response with increasing of AMF inoculation to the plant root. Plant growth under inoculated of AMF was enhanced during the three months of inoculated period. Plant height, TLA, plant fresh and dry weight and RSR were obtained by 10 WAT. As levels of AMF increased, all parameters were also improved. For all these improvements, it can be said that AMF will increase the root surface area and thereby enhance nutrient uptake potential by arbuscule, hence increase the growth rate of plants (Copetta et al., 2006). Referring to Auge et al. (2001), the formation of hyphae from the AMF penetrated the roots and grows extensively between and within living cortical cells, forming a very large and dynamic interface between symbionts that further enhanced plant growth. At an early stage, there were no significant differences in plant height, leaf and stem dry weight and RSR. This may be due to time factor as mentioned by Ali et al. (2010) of AMF to colonize in root tissue to show desired benefits of the plant growth.

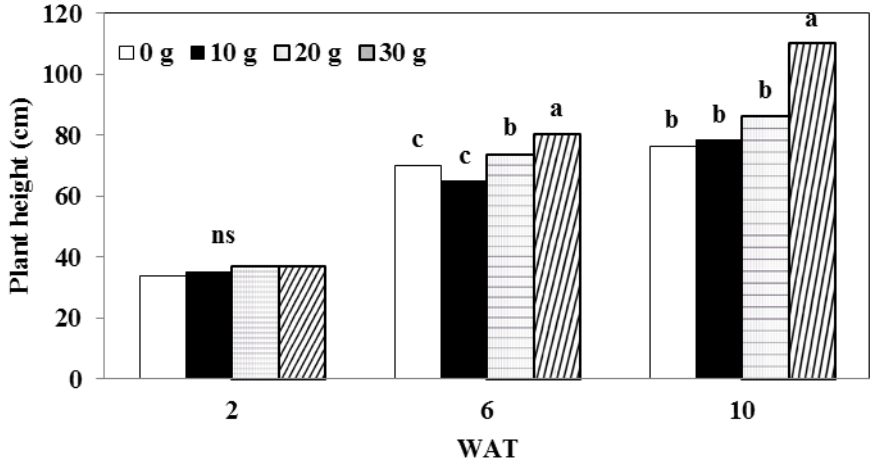

Figure 1. Effect of AMF inoculum rate on the plant height of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

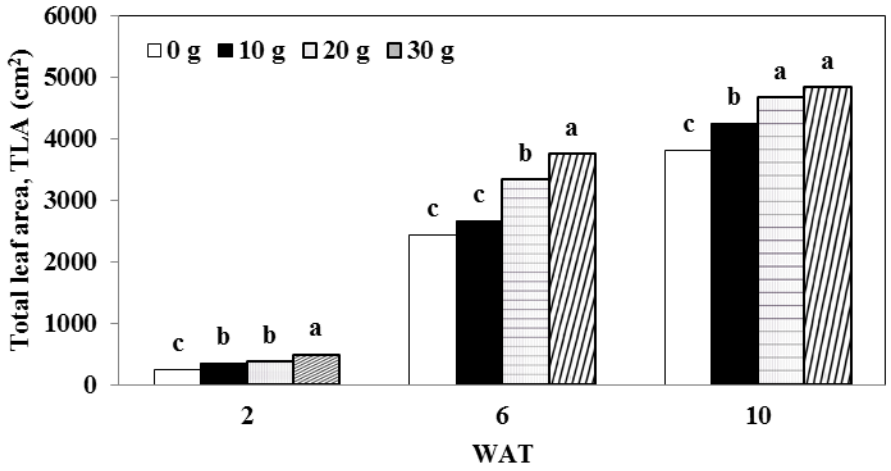

Figure 2. Effect of AMF inoculum rate on the total leaf area of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

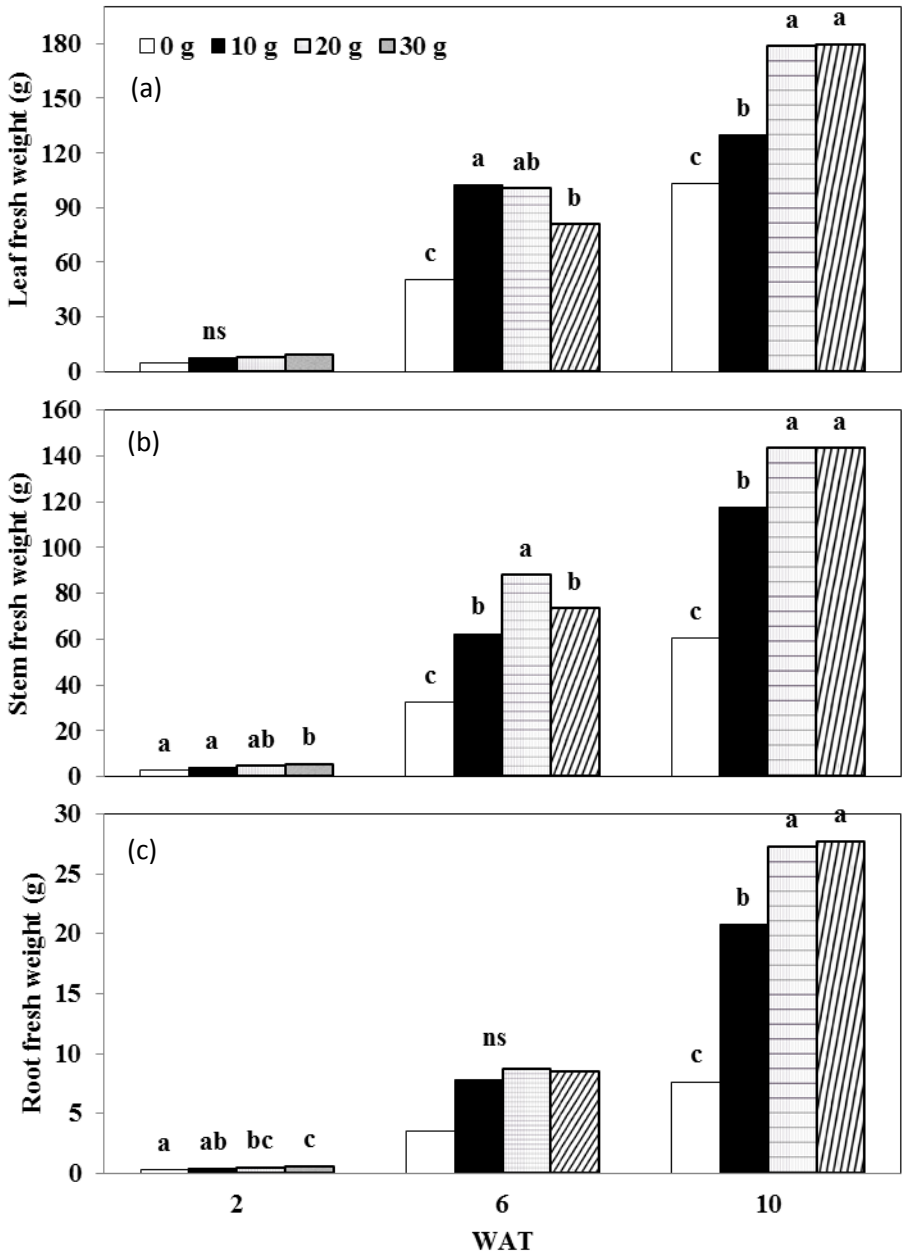

Figure 3. Effect of AMF inoculum rate on (a) leaf, (b) stem and (c) root fresh weight of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

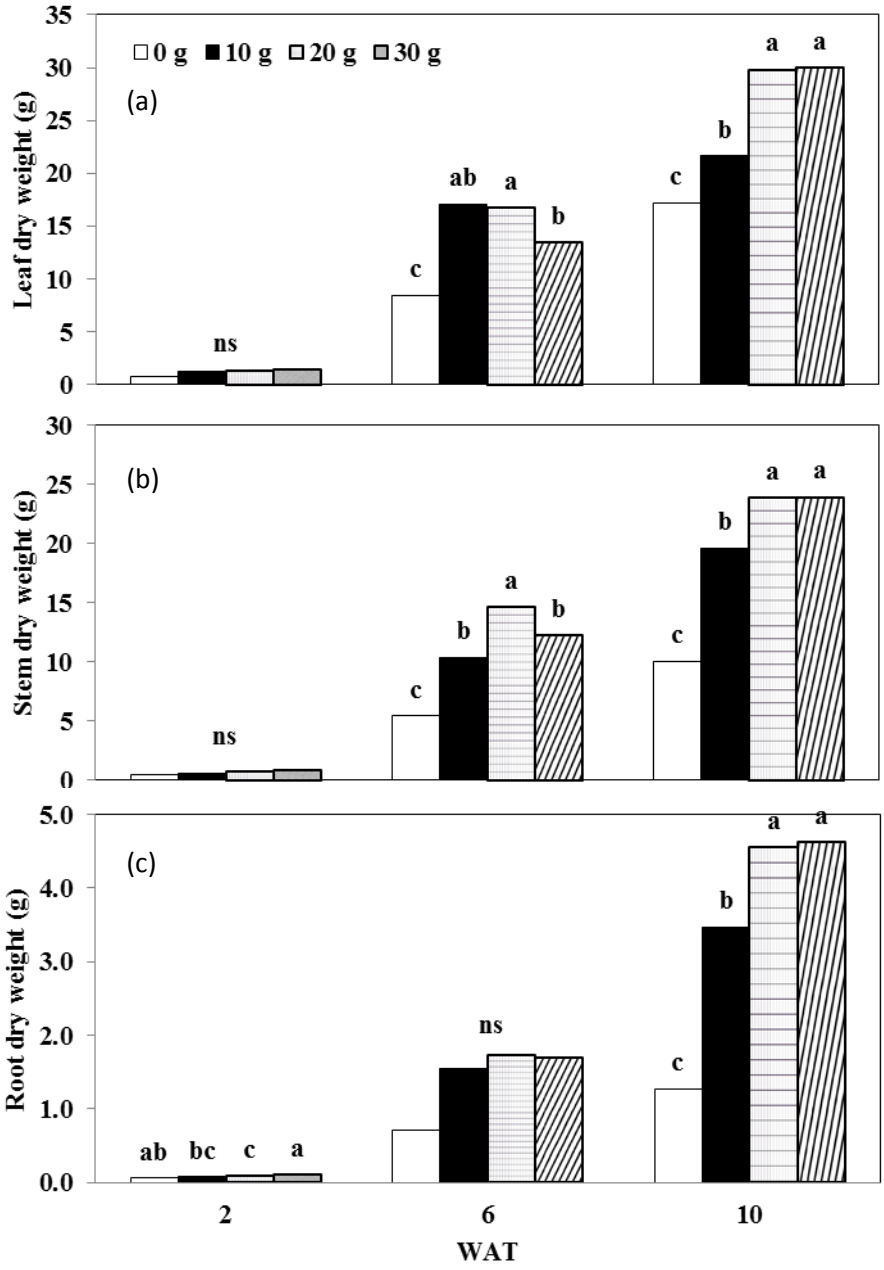

Figure 4. Effect of AMF inoculum rate on (a) leaf , (b) stem and (c) root dry weight of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

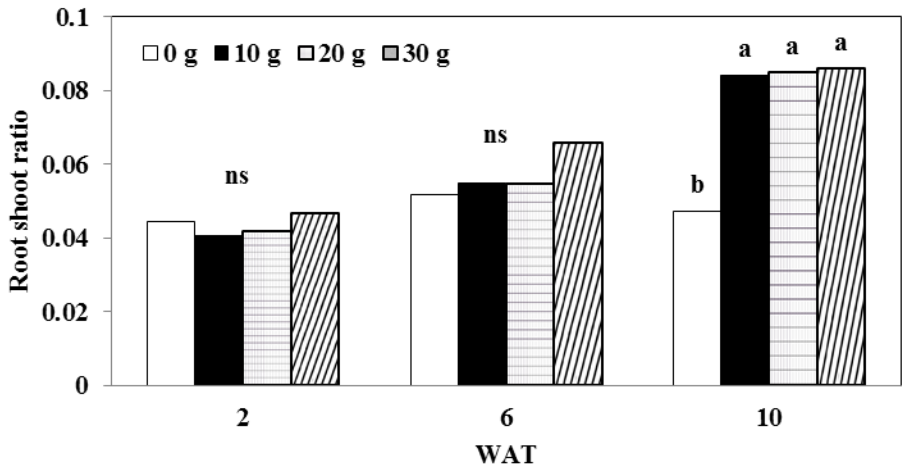

Figure 5. Effect of AMF inoculum rate on root shoot ratio of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

Effect of AMF inoculation on plant physiological changes

Chlorophyll (Chl) is the photoreceptors localized in the chloroplasts that allow plants to absorb energy from light. In this study, it was observed that production of Chl was influenced by AMF inoculums (Figure 6). As the plant grew older (10 WAT), the Chl seemed to increase, but no significant difference until they reached 10 WAT. Chl become gradually increased from 6 to 10 WAT in inoculated plants may be due to the metabolism of the leaf mesophyll (Angela et al., 2011). Increasing of the concentration of total Chl in the leaves of several crops (onion, strawberry, pepper and chickpea) inoculated with AMF species belonging to genus *Glomus* including *G. mosseae* has been found by numerous researchers (Sohrabia et al., 2012; Garmendia et al., 2004; Afek et al., 1990). Similar results were identified in trifoliate orange, a tree whose fruits are widely used in oriental medicine as a remedy for allergic inflammation, when it was inoculated with *G. mosseae* (Wu and Zou, 2012). Referring to Selvaraj et al. (2009), Chl also increased in *Pogostemon patchouli*, an aromatic herb belonging to the mint family and cultivated for its essential oil used in cosmetics, when it was inoculated with isolates of seven indigenous AMF including *G. mosseae*.

The decline in the chlorophyll content was, however, significantly slower than the loss of photosynthetic activity. Photosynthesis (Pn) of basil plants was affected by

AMF rates. Pn was found to be higher for inoculated treatment of 30 g compared to others throughout 10 WAT (Figure 7a). By 10 WAT, 30 g of AMF treatment recorded significantly highest Pn followed by 20 g and 10 g of AMF, and the lowest was recorded at control plant. Plants inoculated with high rates of AMF were found to have higher g_s than control plant in all WAT (Figure 7b). g_s started to ascend from 2 WAT onwards recording the highest g_s was in the 30 g of AMF followed by others treatments. Dell-Amico et al. (2002) reported that AMF enhanced water uptake through improved hydraulic conductivity and increasing photosynthetic activity and g_s. g_s higher in AMF plants would be consistent with higher rates of leaf gas exchange. This physiological adaptation could be necessary to supply carbon needs of fungal symbionts (Auge et al., 2008). As Chl and g_s level were increased, these might be responsible for increased Pn in AMF plants that may be able to assimilate more carbon dioxide and thereby to accumulate more biomass (Krishna et al., 2005; Estrada-Luna and Davies, 2003).

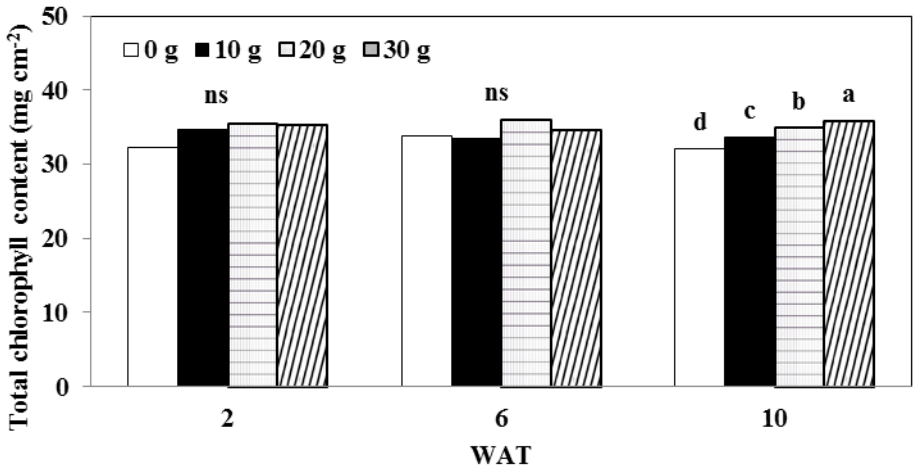

Figure 6. Effect of AMF inoculum rate on total chlorophyll content of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

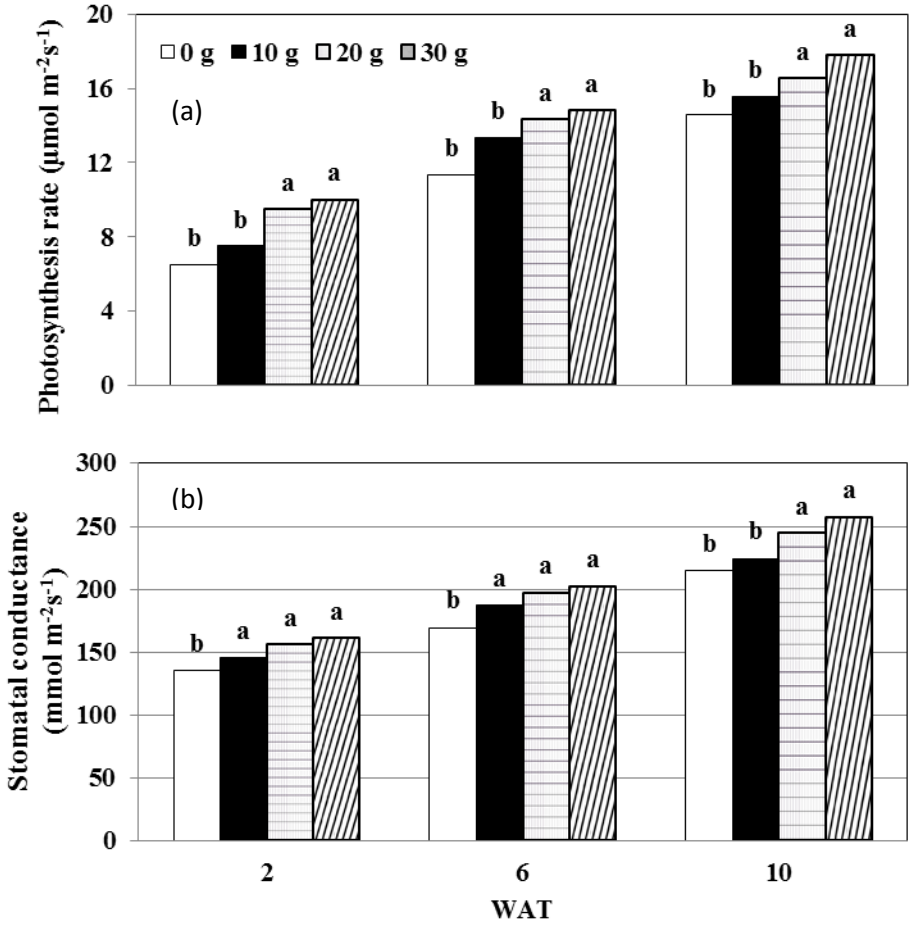

Figure 7. Effect of AMF inoculum rate on (a) photosynthesis rate and (b) stomatal conductance of sweet basil during 10 weeks growing durations. Mean values superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

Effect of AMF inoculation on yield quality

Both TESC and LAA were increased starting at 2 until 10 WAT (Figure 8a and 8b). For TESC, significant differences were found at 10 WAT. The highest value produced on plant treated with 30 g of AMF (48%) at the end of 10 WAT. LAA contents showed a significant difference at 10 WAT. The best value of LAA was obtained on plant inoculated with 30 g of AMF at 10 WAT, 6% compared to control. Increase of AMF enhanced oil production in fresh and dried weight of plant (Figure 9). The graph showed a significantly higher increment for the volume of oil when the plant inoculated with different rate of AMF. Thirty g of inoculums rate had the highest impact on production of plant oil. The dried weight of plant

produced a greater value of oil rather than the fresh weight of plant after infected with AMF inoculation. Total oil production increased significantly to 30 g of AMF for the dried weight of plant (1.645 ml g^{-1}) and fresh weight of plant (1.508 ml g^{-1}). Ascorbic acid is a multi functional compound in plants that is one of the most abundant in green leaves (Marouane et al., 2013). Symbiotic of AMF shown to induce accumulation of secondary metabolites by involving oil production in the medicinal herbs and aromatic crops (Araim et al., 2009; Kapoor et al., 2007). Copetta et al. (2006) had reported that basil inoculated with AMF had longer and more branched roots and their leaves developed more glandular hairs producing more production of oil.

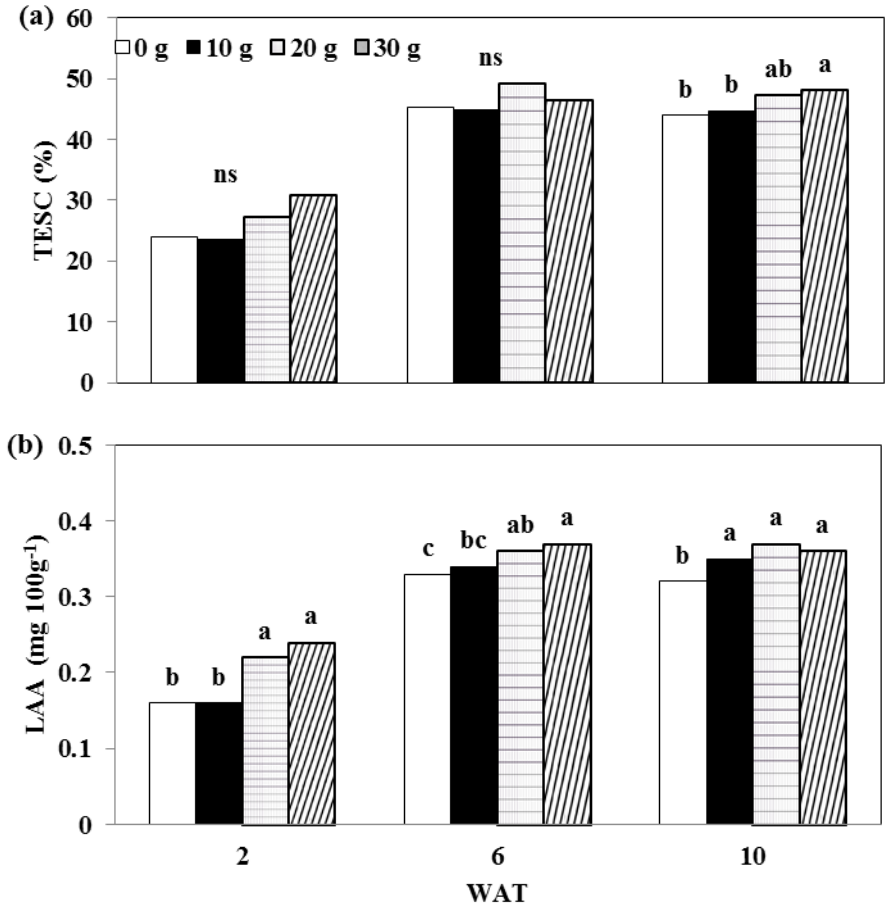

Figure8. Effect of AMF inoculum rate on (a) total ethanol soluble carbohydrate and (b) leaf ascorbic acid of sweet basil during 10 weeks growing duration. Mean values

superscripted by different letters are significantly different by Duncan's Multiple Range Test ($p \leq 0.05$). ns, Non significant.

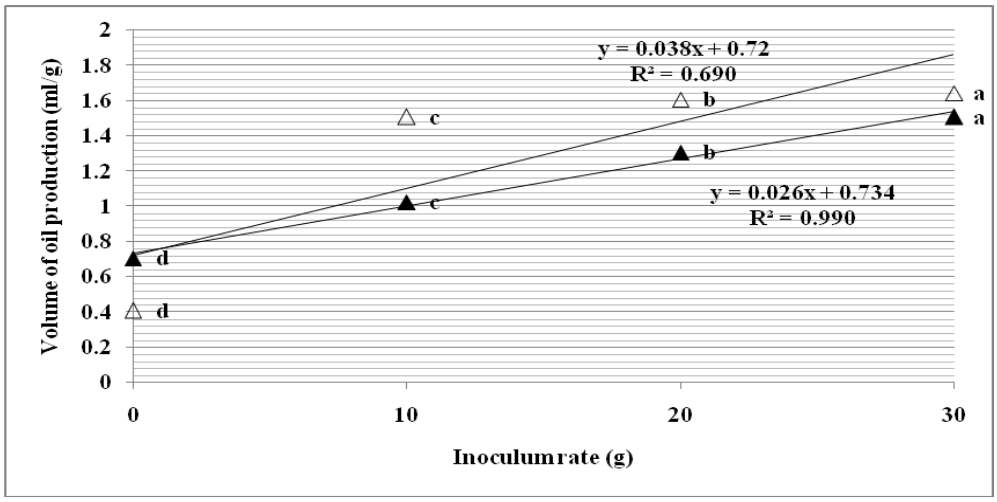

Figure 9. Effect of AMF inoculation on the oil production of fresh and dried weight of sweet basil under different rate of inoculum at 10 WAT. (Symbols represent: fresh weight of basil oil:▲, dried weight of basil oil:△)

AMF infection

Increase of AMF slightly increases AMF colonization and spore count even though there were no significant differences between AMF inoculum. Bever et al. (2001) studied that various species of AMF have different abilities in promoting plant growth by root infection efficiency. Possibly the inoculums used does not contain a fungus beneficial to this species of sweet basil resulting ineffectiveness in root colonization and a number of spores. It is known that the effectiveness of mycorrhization much depends on the specificity of fungus-host plant (species and/or cultivars) (Sensoy et al., 2007). CCD is being used as a peat substitute or amendment to potting mixes with varied results. Spores of the fungi may not germinate greatly in this substrate media where spores become dormant or inactive condition (Sanazzaro et al., 2005; Rabia and Almadini, 2005). Thus, the spores could not produce extensively and unable for root colonization.

CONCLUSION

The result of the study showed the inoculation with 30 g AMF on sweet basil gave potential impact on plant growth performance, physiological changes and yield quality even though AMF inoculation did not show significant effect on AMF root

infection due to spores count and root colonization. The reason could be perfect supply of nutrients in optimal ranges with pH regulated nutrient solution in soilless culture system. This agrees with Koide (1993) where AMF infection might be less or no beneficial under fertilized soil. This also may be related to the spore inoculum is unable to compete with indigenous fungi propogules and other soil microorganisms that probably exist in the substrate which have not steam sterilized in this study. This can explain maybe indigenous fungi and other or soil microorganisms contribute to plant growth for high yield production and quality. Further studies need to be carried out to really maximize the AMF response for plant benefit.

REFERENCES

Afek UR, Menge E, Johnson JA and Pond ELV, 1990. Mycorrhizal species, root age, and position of mycorrhizal inoculum influence colonization of cotton, onion, and pepper Seedlings. *J. Am. Soc. Hort. Sci.* **115:** 938-942.

Ali AA Mustafa, Radziah Othman, Zainal Abidin MA and Ganesan V, 2010. Growth response of Sweet Corn (*Zea mays*) to *Glomus mosseae* Inoculation over Different Plant Ages. *Asian J. of Plant Sci.* **9**(6): 337-343.

Allen MF, Swenson WJI, Querejeta LM, Egerton-Warburton and Treseder KK, 2003. Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. *Annu. Rev. Phytopathol.* **41:** 271-303.

Angela PV, Frederico Lage-Pinto, Leonardo BC da Silva, Maura da Cunha, Jurandi G de Oliveira, Carlos ER., Cristina MM. de Souza and Ricardo AA, 2011. Structural and Ecophysiological Alterations of the Water Hyacinth [*Eichhornia crassipes* (Mart.) Solms] Due to Anthropogenic Stress in Brazilian Rivers. *Brazilian Arch. Biol. Tech.* **54**(5): 1059-1068.

Araim G, Saleem A, Arnason JT and Charest C, 2009. Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. *J. Agr. Food Chem.* **25:** 2255-2258.

Asp N-G, 1993. Nutritional importance and classification of food carbohydrates, In: Plant Polymeric Carbohydrates, Meuser F, Manners DJ, & Seibel, (Ed.), W. Royal Soc. Chem., Cambridge, U.K, pp. 121-126.

Auge RM, Heather DT, Carl ES and Ghazala N, 2008. Hydraulic conductance and water potential gradients in squash leaves showing

mycorrhiza-induced increases in stomatal conductance. *Mycorrhiza* **18:** 115-121.

Auge RM, Stodola, Ann JW, Tims JE and Saxton AM, 2001. Moisture retention properties of a mycorrhizal soil. *Plant Soil* **230:** 87-97.

Bais HP, Walker TS, Schweizer HP and Vivanco JA, 2002. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. *Plant Physiol. Biochem.* **40:** 983-995.

Bever JD, Schultz PA, Pringle A and Morto JB, 2001. Arbuscular mycorrhizal fungi:more diverse than meets the eye, and the ecological tale of why. *Bioscience* **51:** 923-931.

Brewer M, 2011. Natural antioxidants: Sources, Compounds, mechanisms of action, and potential applications. *Compr. Rev. Food Sci. Food Safety* **10:** 221-247.

British Pharmacopoeia, 1980. Vol II.H. M. *Stationary Office, London,* pp: 109.

Coombs J, Hind G and Leegood RC, 1987. Analytical techniques in : *Techniques In Bioproductivity and Photosynthesis.* Coombs J, Hall DO, Long SP, Scurlock JMO. Robert Maxwell, United Kingdom, pp. 20-40.

Cooper AJ, 1979. The ABC of NFT: nutrient film technique: the world's first method of crop production without a solid rooting medium. *Grower Books London*.

Copetta A, Lingua G and Berta G, 2006. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L.var. Genovese. *Mycorrhiza* **16:** 485-494.

Davis JM, 1998. Fresh herb production and marketing. Proceedings of the 1993 New York State vegetable conference. *N.Y. State Veg. Grower's Assn., Ithaca,* pp. 1-2.

Dell-Amico J, Torrecillas A, Rodriguez P, Morte A and Sanchez-Blanco MJ, 2002. Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. *J. Agr. Sci.* **138:** 387-393.

Detloff Sally Jo, 2010. The Effects of Mycorrhizae on the Growth of the Radish, *Raphanus sativus* L. *ESSAI* **8:** 15.

Dubois M, Gilles KA, Hamilton JK, Rebers PA and Smith F, 1956. Calorimetric method for determination of sugars and related substances. *Anal. Chem.* **28:** 350-356.

Estrada-Luna AA and Davies FT, 2003. Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micro propagated Chile Ancho pepper (*Capsicum annuum*) plantlets

during acclimatization and post acclimatization. *J. Plant Physiol.* **160:** 1073-1083.

Gang DR,Wang J, Dudareva N, Nam KH, Simon J, Lewinsohn E and Pichersky E, 2001. An investigation of the storage and biosynthesis of phenylpropenes in sweet basil (*Ocimum basilicum* L.). *Plant Physiol.* **125:** 539–555.

Garmendia I, Goicoechea N and Aguireolea J, 2004. Effectiveness of three *Glomus sp.* in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. *Biol. Contr.* **31:** 296-305.

Gerdemann JW and Nicolson TH, 1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* **46:** 235-244.

Hunt R, 1978. Plant growth analysis. *Studies in Biology Series*. Edward Arnold Ltd., London, U.K, pp. 96.

Hussain AI, Anwar F, Sherazi STH and Przybylski R, 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem.* **108:** 986-995.

Kapoor R, Chaudhary V and Bhatnagar AK, 2007. Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. *Mycorrhiza* **17**(7): 581-587.

Koide RT, 1993. Physiology of the mycorrhizal plant. In: Mycorrhiza synthesis, Tommeruo, IC (Ed.). *Advances in plant pathology*. Vol. 9. Academy Press, New York.

Kormanik PP and McGraw AC, 1982. Quantification of Vesicular-arbuscular Mycorrhizae in Plant Roots. In: Methods and Principles of Mycorrhizal Research, Sheed NC. (Ed.) *American Phytopathological Society, St. Paul* pp. 37-45.

Krishna AK and Govil PK, 2005. Heavy metal distribution and contamination in soils of Thane-Belapur industrial development area, western Indian. *Environ. Geo.* **47:** 1054-1061.

Mack KML and Rudgers JA, 2008. Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *Oikos* **117:** 310-320.

Makri O and Kintzios S, 2007. *Ocimum sp.* (basil): botany, cultivation, pharmaceutical properties, and biotechnology. *J Herbs Spices Med. Plants* **13:** 123-150.

Marotti M, Piccaglia R and Giovanelli EJ, 1996. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. *Agr. Food Chem.* **44:** 3926-3929.

Marouane B, Idoia G and Nieves G, 2013. Enhanced Accumulation of Vitamins, Nutraceuticals and Minerals in Lettuces Associated with Arbuscular Mycorrhizal Fungi (AMF): A Question of Interest for Both Vegetables and Humans. *Agriculture* **3:** 188-209.

Miller JC, Rajzpakse S and Garber RK, 1986. VAM in vegetable crops. *Hort. Sci.* **21:** 974-984.

Rabie GH and Almadini AM, 2005. Role of bioinoculants in development of salt tolerance of *Vicia faba* plants under salinity stress. *Afr. J. Biotech.* **4(**3): 210-222.

Ranggana S, 1977. Manual of Analysis of Fruit and Vegetable Products, 1st edition Tata Mc. Graw Hill, pp. 7-94.

Robert G, Linderman E and Anne Davis, 2004. Varied response of marigold (*Tagetes spp.*) genotypes to inoculation with different arbuscular mycorrhizal fungi. *Sci. Hort.* **99:** 67-78.

Rufykiri G, Thiry Y, Wang L, Delvaux B and Declerck S, 2002. Uranium uptake and translocation by the arbuscular fungus, *Glomus intraradices*, under root-organ culture conditions. *New Phytol.* **156**(2): 275-281.

Ruiz-Lozano JM, Roussel H, Gianinazzi S and Gianinazzi-Perason V, 2003. Defense genes are differentially induced by a mycorrhizal fungus and *Rhizobium sp.* in a wild-type and symbiosis-defective pea genotypes. *Mol. Plant-Microbe Interact.* **12:** 976-984.

Sanazzaro AI, Alberto E, Ruiz OA and Menendez B, 2005. Influence of the arbuscular fungus *Glomus intraradices* on the saline stress physiology of *Lotusglaber*. *Lotus Newsletter* **35:** 29-30.

Selvaraj T, Nisha MC and Rajeshkumar S, 2009. Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of *Pogostemon patchouli* Pellet. Maejo. *Int. J. Sci. Technol.* 3: 222-234.

Sensoy S, Demir S, Turkmen O, Erdinc C and Savur OB, 2007. Responses of some different pepper (*Capsicum Annuum* L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. *Sci. Hort.* **113:** 92-95.

Smith SE and Read DJ, 1997. Mycorrhizal symbiosis. *Academic Press, London,* p 605.

Smith SE, Smith FA and Jakobsen I, 2004. Mycorrhizal fungi cadominate phosphate supply to plants irrespective of grow responses. *Plant Physiol.* **133:** 16-20.

Sohrabia Y, Heidaria G, Weisanya W, Ghasemi-Golezanib K and Mohammadic K, 2012. Some physiological responses of chickpea

cultivars to arbuscular mycorrhiza under drought stress. *Russ. J. Plant Phyisol.* **59:** 708-716.

Strack D and Fester T, 2006. Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. *New Phytol.* **172:** 22-34.

Strack D, Vogt T and Schliemann W, 2003. Recent advances in betalain research. *Phytochemistry* **62:** 247-269.

Toussaint JP, Smith FA and Smith SE, 2007. Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza* **17**(4): 291-297.

Wu QS and Zou YN, 2012. Evaluating effectiveness of four inoculation methods with arbuscular mycorrhizal fungi on trifoliate orange seedlings. *Int. J. Agr. Biol.* **14:** 266-270.