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RESEARCH ARTICLE

Effects of Bio-Amendment of Coconut Dust with Empty Fruit Bunch Compost on the Efficacy of Mycorrhizae Under Deficit Fertigation

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Abstract It has been known that the application of beneficial fungi and compost, has a favourable effect on easing water deficiency stress in plants, hence helping to boost agricultural activities in times of climate uncertainty. In this study, the influence of arbuscular mycorrhizal fungi (AMF) in combination with oil palm empty fruit bunch compost (EFB) on the growth, yield, and physiology of chilli under deficit fertigation was investigated. Throughout the study, five-week-old chilli seedlings were fertigated daily with 100% and 60% of daily evapotranspiration (ET) readings. Three days after transplanting, 10g of sandy soil containing roughly 120-150 mycorrhizal spores was applied to the root zone. Physiological data such as real-time photosynthesis and stomatal conductance were measured at vegetative, early flowering, fruit setting, and maturity or harvesting stages. Meanwhile, yield and morphological measurements were recorded at the end of the study. It was discovered that the addition of EFB to the coconut coir dust media enhanced the beneficial effects of AMF on all parameters including total biomass, chlorophyll fluorescence Fv/Fm, total chlorophylls, photosynthesis rate and stomatal conductance regardless of fertigation levels. The study also revealed that AMF inoculation alone was less effective than non-inoculation + EFB. In conclusion, it is suggested that incorporation of AMF and EFB compost positively affect the yield, growth and physiology of chilli under deficit fertigation.

Keywords: Fertigation, water deficit, PSII, evapotranspiration, compost.

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Introduction

Chilli is one of the most commonly consumed vegetables and spices in Malaysia. According to the Agriculture Department, the nation produced roughly 24,428 tonnes per year, which was less than its domestic demand of 55,420 tonnes in 2018. Chilli usually consumed in the form of fresh fruit, dried or fine powder. In addition to being used mostly for cooking, chilli, which contain the active ingredient capsaicin, are also used in traditional and clinical therapeutics to relieve pain and inflammatory conditions (Fattori *et al*., 2016). The predominant method of cultivating chilli peppers in Malaysia is a fertigation system, which is heavily reliant on the application of water and fertilisers. However, the global supply and demand of fertilisers have been subject to uncertainties, leading to a rise in prices that has imposed a burden on farmers, which may eventually result in a decline in national output.

Deficit irrigation (DI), also known as continuous deficit irrigation (CDI), is a water-saving technique that has been extensively studied as a more efficient way to use water in dry regions (Valcárcel *et al*., 2020;

Agbna *et al*., 2017; Costa *et al*., 2007). This technique has the potential to significantly increase water productivity by maximising per unit of water taken up and used by plant, as opposed to per unit of land area. When irrigation efficiency is increased, water use efficiency (WUE) is greater compared to full irrigation (Agbna *et al*., 2017). In some situations, the addition of water is a waste, resulting in a decrease in WUE (Moriana *et al*., 2003). Moreover, less irrigation will prevent excessive or unnecessary nutrient loss through leaching, thereby improving groundwater quality (Ünlü *et al*., 2006).

The study conducted by Ruiz-Lau *et al*. (2011) investigated the impact of water deficit on chilli revealed that a deficit in water only resulted in a reduction in the fresh mass of fruit per plant. However, there was no statistically significant difference observed in the dry weight of fruit when compared to plants that received full irrigation. Yang *et al*. (2018) reported that the increase in water deficiency led to an increase in the build-up of total soluble solids concentration (TSSC). Meanwhile, the fruit quality was found to be superior in regions with water deficit and increased storage length, owing to a higher concentration of reducing sugars (Bai *et al*., 2023; Zhu *et al*., 2016; Nadler & Heuer, 1995). The accumulation of sugars is thought of as a natural means of defence against water deficit, as it aids in cellular osmotic adjustment (Ahmad *et al*., 2019).

The symbiotic colonisation of plant roots by AMF is related with a variety of physical, nutritional, physiological, and cellular effects (Ruiz-Lozano, 2003). The AM-plant association provides carbon energy in the form of photosynthates to fungus, while the plant benefits from increased nutrient uptake and tolerance to biotic and abiotic challenges (Ruiz-Lozano *et al*., 2006). Furthermore, Khalvati *et al*., (2005) and Auge' *et al*. (2001) found that the external mycelium of mycorrhizae is capable of enhancing soil aggregates, which in turn increases water absorption, phosphorus uptake, and other nutrient uptake in roots. Numerous studies have demonstrated the efficacy of AM in reducing the detrimental effects of abiotic and biotic stress including heavy metal, salt, drought and disease stress (Pozo de la Hoz *et al*., 2021; Dastogeer *et al*., 2020; Li *et al*., 2019; Ferrol *et al*., 2016;). Through the regulation of stomatal behaviour, osmotic adjustment and the enhancement of antioxidant enzymes activities to protect cell from oxidative damages, plants growing in the presence of AMF typically have an improved series of defence mechanisms in response to stress especially water deficit (Wu *et al*., 2007; Wu *et al*., 2006; Caravaca *et al*., 2005).

Despite the considerable amount of research conducted on AMF, there remains a dearth of comprehensive studies on the efficacy of AMF in coconut coir dust, a substrate characterised by low water retention capacity and high salinity levels, particularly in an elevated temperatures of tropical climate. The present study investigates the potential beneficial effect of AMF inoculation and empty fruit bunch compost amendment on morpho- physiology and yield of chilli grown in coconut coir dust-based media under deficit fertigation.

Materials and Methods

Mycorrhizal Fungi Preparation

Fine sandy soil was used to grow maize as medium to multiply the spores' density of *Glomus* sp. After 3 months, plants were removed and the soil media was kept as *Glomus* sp. inoculum. Ten gram of harvested soil sample was used to check for number of spore present using the wet sieving technique (Daniels & Skipper, 1982). Approximately 120-150 spores were used as inoculum source to inoculate each plant.

Experimental Design and Treatments

The experiment was carried out under rain shelter, Taman Pertanian Universiti, Universiti Putra Malaysia. Five week old chilli seedlings were transplanted into 20 x 20 cm white polybags in a randomized complete block design (RCBD) arrangement of treatments. Two different media used were coconut dust amended with EFB compost (3:1 v/v) and coconut dust (100%). The EFB compost consist of nitrogen (N) 1.5-2.0%, phosphate (P) 0.2-1.0%, potassium (K), 1-3%, Magnesium (MgO) 0.4-1.0%, humic acid 5.0-7.0% and organic matter (OM) plus trace elements 25-50%. A total of eight treatments with five replicates each were designed that are Control (100% evapotranspiration value (ET)), 60%ET, 100%ET+EFB, 60%ET+EFB, 100%ET+AM, 60%ET+AM, 100%ET+AM+EFB, 60%ET+AM+EFB. For mychorrhizal fungi treatments, 30g of sand containing approximately 120-150 spores were put around the rooting area. Fertigation (fertilizer+irrigation) was given manually using Cooper formulation according to daily evapotranspiration reading as follow:

$ET_C = K_c \times ET_0$ where ET_C = sample evapotranspiration or crop water use
Kc = crop coefficient for chilli
ET_0 = pan coefficient

The values of crop coefficient (Kc) of chilli were taken from FAO tabulated Kc values specific for tropical conditions. Those Kc values were 0.3 (Kc_{ini}), 0.6 (Kc_{dev}), 0.75 (Kc_{mid}) and 0.95 (Kc_{end}). Where Eto is the reference evaporation calculated using pan evapotranspiration method class A (Doorenbos & Pruitt, 1977).

Estimation of Root Colonization

The percentage of roots colonized by AMF was performed following the method by Phillips & Hayman (1970) and calculated using formula by Giovannetti & Mosse (1980). 1 cm long root were cleared and heated for 1 hours at 90^0 C in 10% KOH before staining for 5 minutes in 0.05% trypan blue in lactophenol.

$$\text{Root colonization (\%)} = \frac{\text{No. of colonized segments}}{\text{Total No. of segments examined}} \times 100$$

Fruit Fresh Weight, Fruit Dry Weight and Plant Biomass

Plants were grown for 120 days after transplanting. Fruits were harvested at matured stage when the colour of fruits changed from green to intermediate reddish colour and weighed to determine the total fresh weight. Then, 10 uniform samples were subjected to oven-drying at a temperature of 72°C until a constant mass was achieved. The fruit dry weight was then measured by subtracting the mass of the dried fruit from the mass of the fresh fruit (Dorji *et al.*, 2005). The oven drying procedure was also done for shoots, stems and roots for their dry weight determination.

Photosynthesis, Stomatal Conductance, Relative Chlorophyll and Maximum Quantum of Yield PSII

Photosynthesis and stomatal conductance readings were taken at vegetative, early flowering, fruit setting and harvesting stage using an open-path portable photosynthesis system (Li-600XT, LI-COR, Lincoln, Nebraska-USA) with ambient atmospheric settings. Three uppermost fully expanded leaves were chosen from different angles to represent photosynthesis and stomatal conductance values for each plant. Relative chlorophyll content was measured using a SPAD-502 meter (Minolta chlorophyll meter SPAD-502, Spectrum Technologies, Inc., Plainfield, IL). Meanwhile, the maximum quantum of yield PSII (Fv/Fm) was recorded using a portable photosynthetic efficiency analyzer (PEA) (model Handy-PEA, Hansatech Instrument Ltd., Norfolk, UK).

Statistical Analysis

Data were analysed using GraphPad Prism software package (http://www.graphpad.com). Significance of different data was assessed using one-way analyses of variance followed by LSD post-hoc tests. Significantly different values are represented on graphs using different letters.

Results and Discussion

Root Colonization

In this study, fertigation was applied using daily evapotranspiration values (Doorenbos & Pruitt, 1977). Figure 1 shows that 100%ET+AMF+EFB had the highest root mychorhizal inoculation followed by 60%ET+AMF+EFB. The needs in adding EFB compost into coconut dust media in term of root colonization can been seen in 100%ET+AMF+EFB and 100%ET+AMF, where the later recorded significantly lower root infection than the previous. The AMF colonization of the root is essential for determining the efficacy of this fungi on plant growth. According to Benaffari *et al*. (2022), Soka & Ritchie (2016), Gryndler *et al*. (2009) and Ravnskov *et al*. (2006), the interaction of AMF with organic matter greatly increases root colonisation and yield. This could be the explanation to the positive finding in this study that the media must be supplemented with organic matter as opposed to being coconut coir dust 100%.

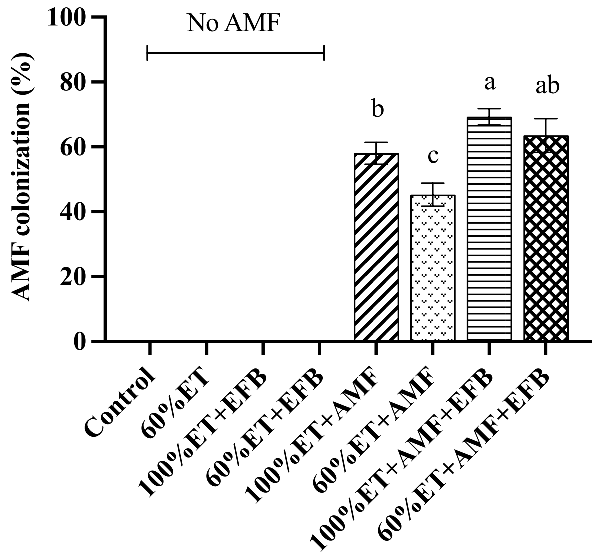

Figure 1 Root colonisation of chilli under deficit fertigation. Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means ($p \leq 0.05$). Values represent the mean ±SE of n = 4.

Fruit Yield and Plant Biomass

Fresh fruits are the most common form in which chillies are traded and thus, information pertaining to fresh fruits is important. Compared to control plants, the AMF and EFB-enhanced media fertigated with 100%ET yielded the highest fresh produce (1.61 fold), whereas 60%ET-treated plants produced the lowest (-1.84 fold) (Figure 2(A)). Under deficit fertigation, the addition of EFB alone or in combination with AMF exhibited a significant difference compared to 40%ET. Since water is recognised to be the most limiting abiotic factor on plant development and production, the loss in fresh yield seen under deficiency fertigation was expected (McElrone *et al*., 2021). Intriguingly, the AMF effect into coconut coir dust media was seen to be less effective in the absence of EFB, despite receiving full fertigation, with no significant difference between 60%ET+EFB and control.

Deficit fertigation plants treated with either or both AMF and EFB significantly produced higher fruit dry weight (Figure 2(B)) and plant biomass against the control (Figure 3). It is recognised that severe water stress inhibits biomass production in many plants. However, moderate water deficiency has variable effects on plant growth. Reports from previous studies (Li *et al*., 2019; Lee & Oh, 2017), exposure of plants to mild water stress did have a significant effect on growth and biomass, with significant accumulation of protective secondary metabolites and antioxidant enzymes likely being the factors responsible for the tolerance traits in the plants.

Coconut coir dust, although has been known to have good water absorption, proved as the least effective material in providing prolonged evaporation restriction (Wickramaarachchi *et al*., 2020; Kalaivani & Jawaharlal, 2019). Furthermore, in addition to having a higher evaporation resistance than coconut coir dust, compost was also shown to be rich in non-pathogenic beneficial microbes and fungi as plant growth promoter (Martínez-Cano *et al*., 2022; Stella & Sashikala, 2016).

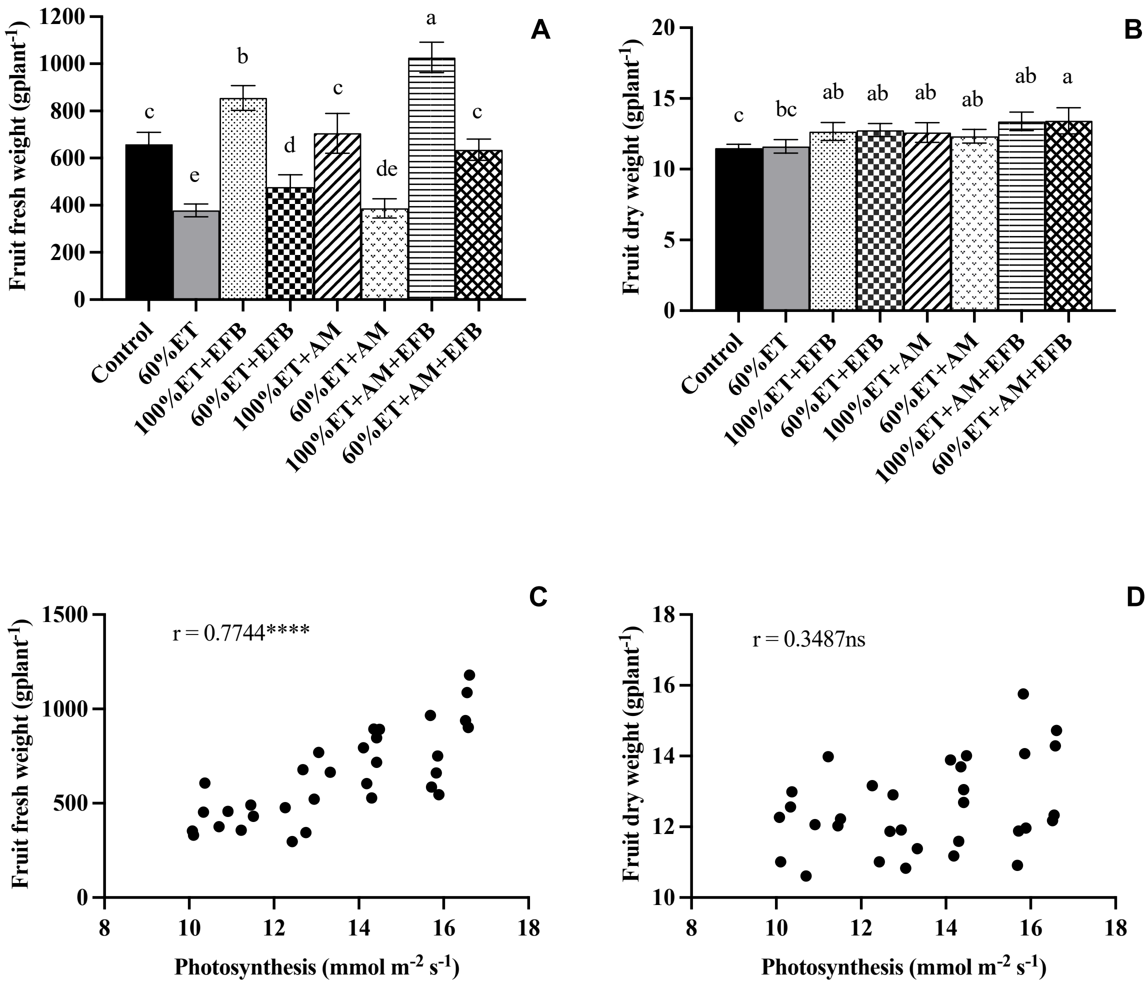

Figure 2 Fruit fresh weight (A), fruit dry weight (B) of chilli and correlation of each parameter toward photosynthetic activity (C,D). Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means ($p \leq 0.05$). Values represent the mean ±SE of n = 4. The correlation analysis was done based on Pearson correlation coefficient

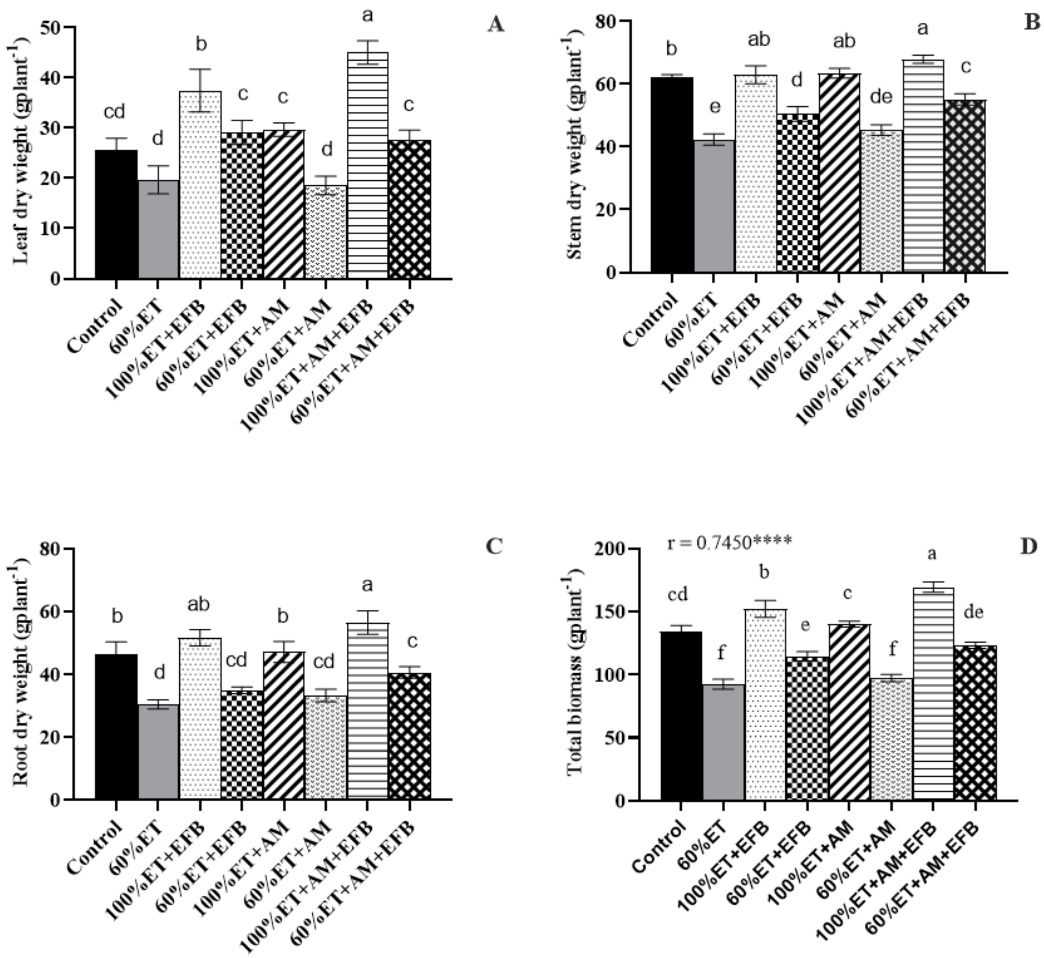

Figure 3 Leaf dry weight (A), stem dry weight (B) , root dry weight (C) and total biomass (D) of chilli under deficit fertigation. Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means (p≤0.05). Values represent the mean ±SE of n = 4. The r value indicates the correlation of total biomass vs photosynthesis

Maximum Quantum of the PSII and Relative Chlorophyll Content

Figure 4(A) demonstrates that deficit fertigation significantly decreased Fv/Fm in 60%ET and 60%ET+EFB compared to control, but not in 60%ET+AM+EFB plants. The viability of chlorophylls and their constituents, especially the PSII, are significant indications of a plant's health, with the two having a substantial effect on the effectiveness of photosynthetic activity. While total chlorophylls indicates the general health of a leaf, Fv/Fm reveals the photoinhibition state in PSII, which affects the electron transport chain process of photosynthesis (Aro *et al*., 1993, Murata *et al*., 2007). Photoinhibition is a process of photooxidation of D1 protein in PSII core during stress when the chlorophyll antenna absorbs more light than it can utilise, resulting in oxidative degradation of the proteins (Aro *et al*., 1993). The damaging reactions are reported to be mediated by singlet oxygen produced during charge recombination reaction between $P680^{+}$ and the reduced secondary electron acceptor (QA^{-}) (Vass, 2011). Therefore, in other words, the higher the Fv/Fm ratio, the greater the photosynthetic activity (Karim & Johnson, 2021). The present study also supports the idea where photosynthetic activity and Fv/Fm was found to be positively correlated (Figure 4 (C)).

In addition, Figure 4(B) demonstrates that, all treatments had significantly increased the level of chlorophylls in comparison to control, with the exception of the 60%ET treatment, which showed no difference. No significant difference between control and 60%ET indicates that moderate deficit fertigation in this study would still maintain the chlorophyll levels in the leaves. This suggest that the higher chlorophylls in plants treated with AMF and/or EFB under 60% evapotranspiration value may be the result of better acclimation in facilitating more energy absorption by the photosynthetic apparatus, which could result in improved photosynthesis activity, without compromising PSII efficiency (Figure 4 (A)). The correlation analysis of photosynthesis and total chlorophylls revealed a positive and significant (Figure 4 (D)). Numerous studies indicate that AMF symbiosis in the root system maintains or increases total chlorophylls under water deprivation (Ye *et al*., 2022; Zhang *et al*., 2018; Zuccarini, 2007). Meanwhile, according to Hashem *et al*. (2018), AMF inoculation may downregulates the chlorophyll degradation enzyme chlorophyllase while preserving genes and enzymes favourable for chlorophyll synthesis under water deficit stress.

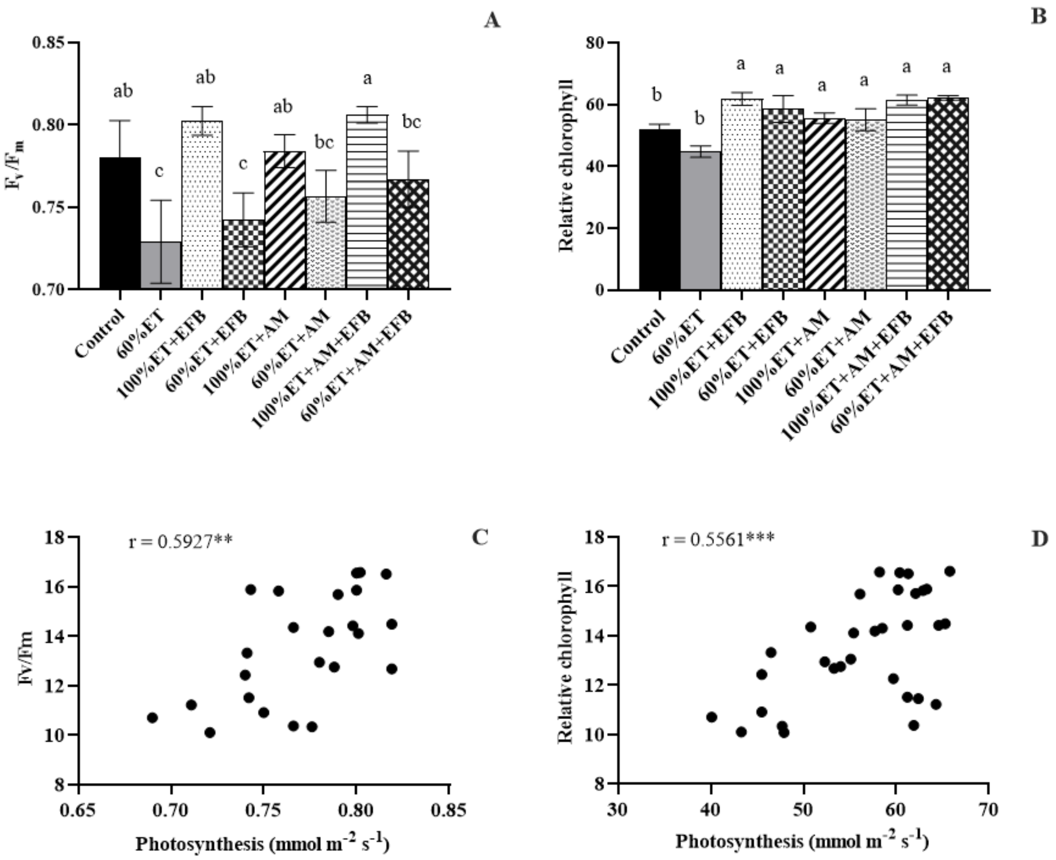

Figure 4 Maximum quantum of yield PSII (A), Relative chlorophyll content (B), of chilli and correlation of each parameter toward photosynthetic activity (C,D). Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means (p≤0.05). Values represent the mean ±SE of n = 4

Photosynthetic Activity and Stomatal Conductance

Water is required as the electron donor in Photosystem II, facilitating the electrons during light-dependent reactions and the opening of stomata for gas exchange. This study monitored the activity of photosynthesis to examine how plants respond physiologically to deficit fertigation at four stages (Figure 5). Incorporating AMF and EFB with full and moderate fertigation, particularly 100%ET+AMF+EFB and 60%ET+AMF+EFB, respectively, yielded consistently better photosynthetic activity compared to the other treatments across all developmental phases. On the other hand, it was observed that the 60%ET treatment exhibited a significantly lower value during the fruiting and maturity stages. However, no significant difference was found when compared to the control and 60%ET+AMF treatments during the vegetative and early flowering stages, respectively. However, the deficit fertigation had less of an impact on the photosynthetic activity of 60%ET+AMF+EFB plants. In fact, it was significantly higher than all other deficit fertigation treatments (with or without AMF) and 100%ET-fertigated plants. The introduction of AMF has not only been shown to effectively mitigate the detrimental effects of water deficit and drought in greenhouse-grown plants, but also in field conditions (Li *et al*., 2019; Barzana *et al*., 2014). As demonstrated in this study, AMF inoculation and EFB addition improved photosynthetic activity during deficit fertigation. In addition, our correlation analysis demonstrates that increased carbon fixation and carbohydrate synthesis through photosynthesis contributed to enhanced growth and development (Figure 2 (D)). Previous studies have reported the positive effect of AMF on photosynthetic activity and other relevant physiological parameters under abiotic stress (Chandrasekaran *et al*., 2019; He *et al*., 2017; Mena-Violante, *et al*., 2006), thus supporting our findings.

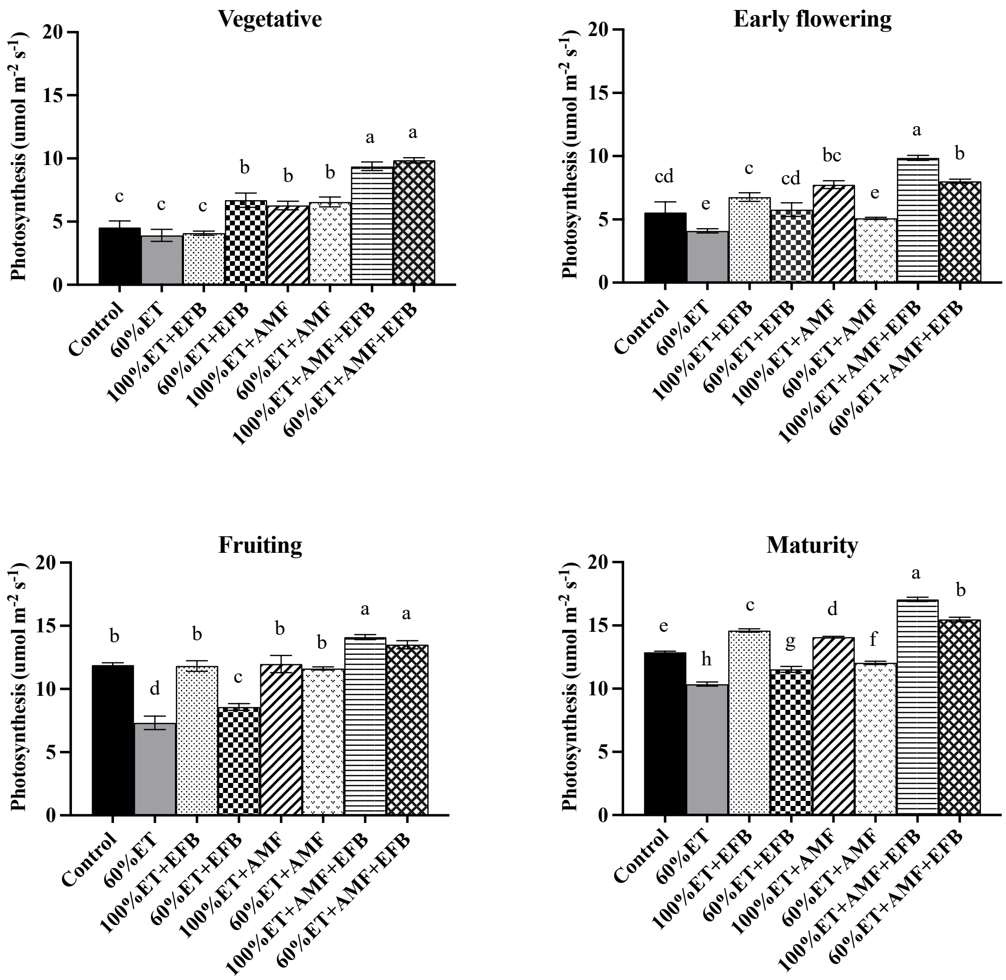

Figure 5 Photosynthesis activity at vegetative, early flowering, fruiting and maturity stage. Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means ($p \leq 0.05$). Values represent the mean ±SE of n = 5

Stomatal conductance is a measure of stomatal opening and one of the most often studied physiological parameters alongside photosynthetic activity (McAusland *et al*., 2016; Lawson & Blatt, 2014). This study assessed stomatal conductance at different development stages (Figure 6). In general, the stomatal conductance has increased during the growing period, peaking at maturity. The stomatal conductance of plants treated with full fertigation 100%ET+AMF+EFB consistently differed significantly from all other treatments. In contrast, 100%ET+AM did not differ significantly from the control at any stage except maturity, validating our prior hypothesis that AMF inoculation in coconut coir dust without EFB addition was less effective. The favorable effect of AMF and EFB under deficit fertigation was observed in 60%ET+AMF+EFB, which had considerably better stomatal conductance in the vegetative, early flowering, and maturity stages than 60%ET, 60%ET+EFB, and 60%ET+AM. In fact, it was significantly greater than plants treated with complete fertigation at maturity. Mycorrhizal symbiosis and compost were reported to have adjusted stomatal aperture in water-stressed plants to prevent excessive water loss, while still permitting carbon uptake into the leaves (Shakib *et al*., 2019; Pedranzani *et al*., 2016; Porcel *et al*., 2015). Inoculation of AMF into the root system was also discovered to induce the abscisic acid response, which regulates stomatal conductance in response to abiotic stress, hence assisting plants in adapting to environmental changes (Ludwig-Müller, 2010).

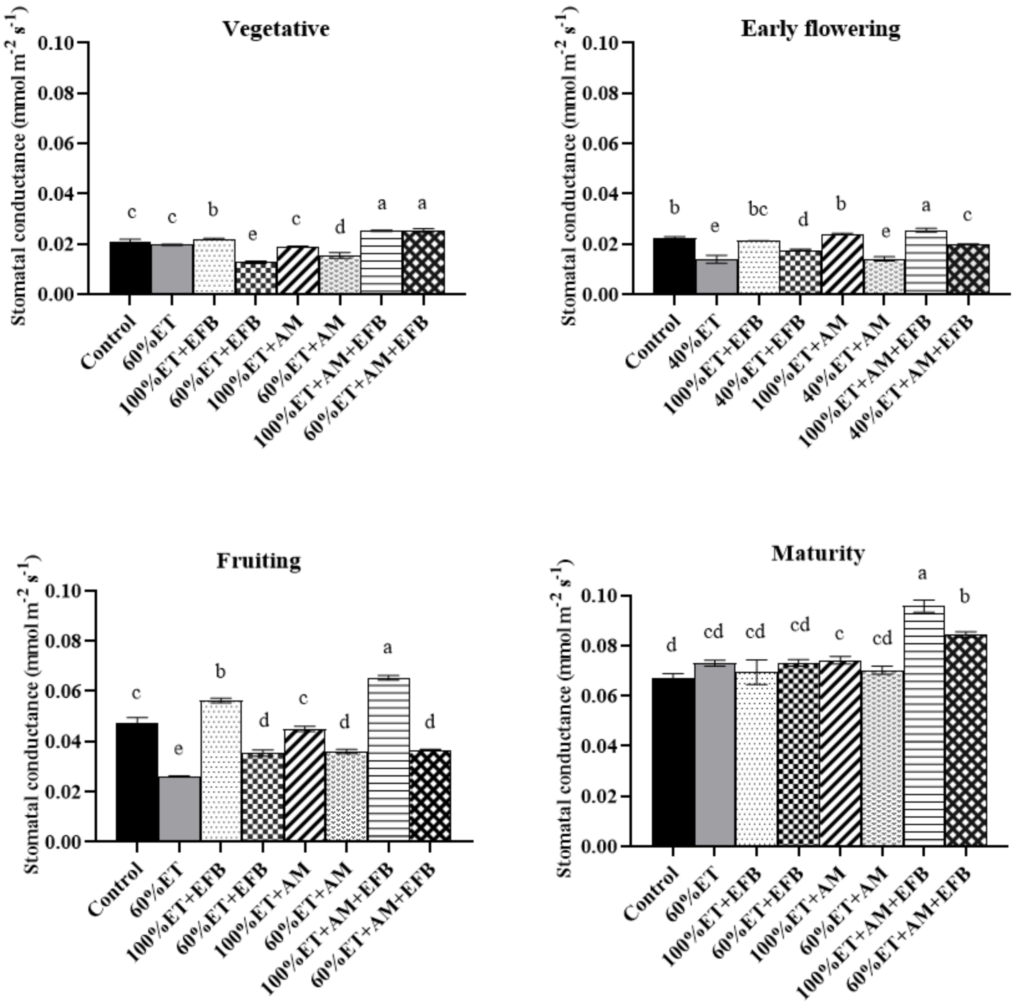

Figure 6 Stomatal conductance at vegetative, early flowering, fruiting and maturity stage. Data was analysed using a one-way ANOVA followed by LSD's post hoc test to assess the significance between means ($p \leq 0.05$). Values represent the mean ±SE of n = 5

Conclusions

This study demonstrates that the addition of AMF+EFB into coconut coir dust resulted in enhanced economic fruit fresh weight and improved the morpho-physiology of chilli plants. Inoculation of AMF, however, was less effective without EFB into the media in many parameters tested. This biological approach is an environmentally safe alternative to the conventional methods involving heavy use of fertilizers and pesticides and has the potential to be widely implemented in organic vegetable production systems. Furthermore, the utilization of AMF and EFB is recommended as an essential approach towards achieving sustainable agriculture, considering the rising production costs and the uncertain impacts of climate change.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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