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DRY BIOMASS ACCUMULATION OF *TALINUM TRIANGULARE (JACQ. WILLD)* INOCULATED WITH ARBUSCULAR MYCORRIHIZAL FUNGI (AMF) UNDER *FUSARIUM* -INFESTED SEEDLINGS IN CALABAR, NIGERIA.

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Abstract

With *Talinum triangulare* being one of the most important leafy vegetable in the world, it is important to improve its growth and biomass yield. Soil-borne plant pathogens are difficult to control, and application of individual biocontrol agent is often limited. To this end, arbuscular mycorrhizal fungus (AMF) application is needed to improve the biomass accumulation and reduced the level of *fusarium root rot* colonization in *Talinum triangulare* seedlings in calabar was investigated in pot experiment to determine the influence of AMF on the above plant. The *Talinum triangulare* seedlings inoculated with two AMF species *(Glomus clarum* and *Glomus gigaspora*) were infested with 10ML of *Fusarium* spores suspension under screen house experiment. The results indicated that inoculation with *G. clarum* in combination with f*usarium (Gc*$^{+}$*F)* recorded the highest percentage *Fusarium root rot* colonization while the lowest significant ($P \leq 0.05$) mean value of 8.33% was found in *G. gigaspora* inoculated plants in combination with *Fusarium (Gg*$^{+}$*F).* Inoculation with AMF consistently improve the root, stem, and total dry matter of *Talinum triangulare* seedlings over (control) plants. The root, stem, and total plant biomass of the inoculated seedlings with *G. clarum* (*Gc*) were significantly ($P \leq 0.05$) highest than the control treatment and lowest dry root, stem and total dry weight was recorded on control 0.12, 0.44 and 1.33 g plant $^{-1}$. However, the leaf dry weight and root: shoot ratio (R/S) did not show any significant ($P \geq 0.05$) difference between treatments. The enhancement of dry biomass of root, stem and total dry weight is an important mechanism of disease tolerance in mycorrhizal plants. In conclusion, the results show that inoculation with AMF, *G. gigaspora* inoculated plants in combination with *Fusarium (Gg*$^{+}$*f)* have the potential to reduce the negative effect of *Fusarium* from colonizing *Talinum triangulare* plants. However, *G. clarum* was the best in promoting seedlings biomass.

Keyword: Arbuscular Mycorrhizal fungi. *Fusarium oxysporum*, Dry biomass accumulation, seedlings inoculated, *Talinum triangulare.*

1.0 Introduction

As growers attempt to manipulate more of the interrelated factors in plant production, disease management is becoming increasingly complex. Individual methods of disease control will be blended with each other and with methods of production. Soil-borne pathogens impose particular challenges on the field of plant protection. Usually their propagules remain infectious in the soil for several seasons and the soil itself, as their environment with its numerous contributing variables still remains to be a "blackbox" to some extent. Furthermore, the ban on chemical soil desinfectants like methyl bromide and in general, the growing awareness on health and environmental issues and the increase of organic production make it necessary to search for alternatives in the control of soil-borne pathogens.

As for many other cases, nature itself offers promising mechanisms to deal with these particular challenges. Arbuscular mycorrhizal fungi (AMF) have enormous potential for use as bioprotectant (Xavier & Boyetchko 2004). AMF-mediated bioprotection has been exploited and accepted as a key practice for disease control (Garcia-Garrido 2009). According to Sikes (2010), the bioprotective effect of AMF depends upon various factors such as host genotype, AMF species involved and the efficiency of root colonisation. Furthermore, several researchers have suggested that establishment of AMF in plant roots reduces the damage caused by pathogens, and improves plant resistance to the biotic stresses (Garmendia *et al*. 2004; Vierheilig and Bago, 2005), but the underlying mechanisms of bioprotection remain unknown (Pozo and AzcónAguilar 2007). *Talinum triangulare* is well consume as a leafy vegetable in Calabar. However, despite complain by the local farmers on yield reduction cause by soil borne infestation, these farmers still do their best to enable them meet the targeted growing population who consumed these vegetable daily, Therefore, the present study was conducted based on the hypothesis that AMF could promote the growth, biomass and reduce *fusarium* colonization of *Talinum triangulare* seedlings. A pot culture experiment was performed in the screen house to determine the influence of (*Glomus clarum* and *Glomus gigaspora*) on dry biomass of root, stem and leaf of *Talinum triangulare* infested with *fusarium root rot*.

2. Materials and methods

The field work was carried out from March 2025 to June 2025 and lasted for 12weeks in the Screen House, Botanic Garden and Research Centre of the Department of Plant Science and Biotechnology, University of Cross River State, Calabar. Calabar South is a Local Government Area in Cross River State. The state is one of the South- South States of Nigeria. Calabar lies in the tropical high rainforest agro-ecology of the Equatorial climatic belt of Nigeria (Latitude $5^0$00' and $5^0$40'N, longitude $8^0$04' and $8^0$62'E) and about 700m above sea level (Iwena, 2008). It has a bimodal annual rainfall distribution that ranges from 2500 – 3500mm with a mean annual temperature range of 22.2^0c to 38.2^0c and a relative humidity that ranges from 75 – 90%. The

top soil (0-4cm depth) was collected from the experimental sites and sieved soil was sterilized by heating using the hot air oven at 180^0C for 2 hours and used as the growth medium. The seeds of *Talinum triangulare* were obtained from the Crop Science Department, College of Agriculture, Ovonum-Adun, Obubra Local Government Area of Cross River State, in February, 2025 and were subjected to pre-treatment according to methods adopted by Annih *et al.,* (2020). Two AM Fungi species (*G. clarum* and *G. gigaspora*) of inocula consisting of spores, mycelium and infected root fragments were purchase from the International Institute for Tropical Agriculture (IITA), Ibadan. The experiment was a 7 x 3 completely randomized design (CRD) which comprises of seven treatments with three replicates, totally 21 pots.

The treatments consisted of Uninoculated (control), Inoculated with *Glomus clarum* (*Gc*), Inoculated with *Glomus gigaspora* (*Gg*), Inoculated with *Fusarium oxysporum* (*Fo*), Inoculated with *Glomus clarum* in combination with *Fusarium oxysporum* (Gc^+Fo), Inoculated with *Glomus gigaspora* in combination with *Fusarium oxysporium* (Gg^+Fo), Inoculated with *Glomus clarum* in combination with *Glomus gigaspora and Fusarium oxysporum* (Gc^+Gg^+Fo). The pots (39 cm diameter and 49 cm deep) were each filled with 9kg of the sterilized soil and were arranged on the concrete floor in the Screen House, Botanic Garden and Research Centre of the Department of Plant Sciences and Biotechnology of the University of Cross River State. These were watered to field capacity and left to drain overnight. 40g of crude inocula was placed 3cm below the surface of the soil in AMF designated pots before sowing to produce mycorrhizal plants (Rabic and Almadini, 2005). Seedlings with same height were choosing after thinning at one week after emergence (WAE), 10ml of spore's suspension of *Fusarium* was applied by pipettes just below the collar region around the hypocotyls of the *Fusarium* designated plants (Sohriabi *et al.,* 2015). To confirm *F. Oxysporium* colonization at harvest (12WAE), a number of *Talinum triangulare* plants roots with symptoms of longitudinal cracks, red streaks, dark spot and necrosis on the roots were collected and transferred to the laboratory to re-isolate *Fusarium* on PDA plates to ensure the *Fusarium* infection Al-hmoud and Al-momany (2015). The plates were incubated at room temperature (28 ± 1^0c) for 7 days.

After the incubation period, root colonization was scored positive, when a typical colony was developed. They were determined by counting the number of colonized roots segment out of twenty roots segment tested Alam *et al.,* (2011). The percentage of *Fusarium* roots rots colonization was calculated using the following equation:

$$\text{FRR Colonization} = \frac{Number\ of\ segments\ coloniz.\ by\ fus.\ spp}{Total\ number\ of\ segments} \text{ x } 100$$

Harvested *Talinum triangulare* seedlings were separated into roots, stems and leaves. Their biomass was then determined using an electronic scale balance (Model: XS4001S),

after oven drying them at 80^0c until a constant dry weight (Sohrabi *et al.,* 2015). The total dry weight was computed as the sum of the dry weights of root, stem and leaf. The dry weight data obtained were then used to calculate the root: shoot ratio. The root: shoot ratio (R:S) of each plant was calculated as a ratio of root dry weight to shoot dry weight: weight to shoot dry weight:

$$\frac{dry\ weight\ of\ root}{dry\ weight\ of\ shoot}\ gg$$

2.2 Statistical Analysis

Data analysis involved calculating standard errors from replicate readings. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0, with two-way ANOVA and Duncan's Multiple Range Test ($P \leq 0.05$) used to determine significant differences between mean values.

3. Results

3.1 Evaluation of *Fusarium Root Rot* Colonization of *Talinum triangulare*

Evaluation of *Fusarium* root rot colonization percentage of *Talinum triangulare* plants is presented in Table 1. Plants inoculated with *G. clarum* in combination with *Fusarium* (Gc^+F) recorded the highest percentage *Fusarium* root rot colonization of 30.00%. While, the lowest significant ($P \leq 0.05$) mean value of 8.33% was found in *G. gigaspora* inoculated plants in combination with *Fusarium* (Gg^+F).

3.2 Plant Biomass Accumulation

The effect of *Fusarium* and AM Fungi inoculation on the biomass accumulation of *Talinum triangulare* plants as revealed by the dry weight measurements is presented in Table 2. Generally, the dry weight measurements (root, stem, leaf, total dry weight) of inoculated *Talinum triangulare* plants showed higher mean values than the un-inoculated control plants. There was no significant ($P \geq 0.05$) effect on the leaves dry weight of *Talinum triangulare* plants, no significant ($P \geq 0.05$) difference between the treatments. The highest leaf dry weight of 2.31g/plant^{-1} was recorded in plants inoculated with *G. clarum*. Meanwhile, the highest significant ($P \leq 0.05$) root, stem and total dry weight mean values of 1.13, 2.24 and 5.69 g/plant^{-1} respectively were obtained in *Talinum triangulare* plants inoculated with *G. clarum* (Table 2).

3.3 Root: Shoot Ratio

There was no significant ($P \geq 0.05$) effect of *Fusarium,* AM Fungi inoculation on the root: shoot ratio of *Talinum triangulare* plants as shown in Table 2. The mean values did not showed any significant ($P \geq 0.05$) difference between the treatments. Plants inoculated with *G. clarum* had the highest non-significant ($P \geq 0.05$) root: shoot ratio mean value of 0.55g/g and the lowest of 0.27g/g was found in un-inoculated control plants.

Table 1. Effect of *Fusarium oxysporium* Colonization on *Talinum triangulare* Seedling at Harvest (%)

Treatment	% *Fusarium* col. at harvest 12 WAE
Control	0.00±0.00[a]
Gc	0.00±0.00[a]
Gg	0.00±0.00[a]
F	23.33±6.00 [c]
$Gc^{+}F$	30.00±15.27 [c]
$Gg^{+}F$	8.33±6.00[b]
$Gc^{+}Gg^{+}F$	25.00±7.63 [c]

*Means of three replicates ± S.E.M, Means within each column followed by different letters are significantly different at ***P≤0.05*** according to Duncan's Multiple Range Test.

WAE: Weeks After Emergence; ***Gc*****:** *Glomus clarum*; ***Gg***: *Glomus gigaspora*; ***F***: *Fusarium*; ***$Gc^{+}F$***: *Glomus clarum + Fusarium*; ***$Gg^{+}F$***: *Glomus gigaspora + Fusarium;* ***$Gc^{+}Gg^{+}F$***: *Glomus clarum + Glomus gigaspora + Fusarium*

Table 2. Effect of *Fusarium oxysporium* and Mycorrhizal Inoculation on the Dry Biomass of *Talinum Triangulare* Seedlings at Harvest 12WAE.

Treatment	Root dry weight (g plant^{-1})	Stem dry weight (g plant^{-1})	Leaf dry weight (g plant^{-1})	Total dry weight (g plant^{-1})	Root: Shoot ratio (g g^{-1})
Control	0.12±0.05^{a}	0.44±0.15^{a}	0.76±0.15^{a}	1.33±036^{a}	0.27±0.03^{a}
Gc	1.13±0.15^{c}	2.24±0.59^{b}	2.31±0.65^{a}	5.69±1.38^{b}	0.53±0.06^{a}
Gg	0.69±0.27^{b}	1.79±0.67^{a}	2.27±0.22^{a}	4.76±1.18^{ab}	0.37±0.01^{a}
F	0.79±0.17^{b}	1.71±0.31^{a}	1.68±0.33^{a}	4.33±0.42^{ab}	0.51 ±0.18^{a}
Gc^{+}F	0.68±0.14^{b}	1.88±0.77^{a}	2.00±0.66^{a}	4.56±1.59^{ab}	0.45±0.12^{a}
Gg^{+}F	0.48±0.21^{a}	1.40±0.53^{a}	1.43±0.62^{a}	3.32±1.35^{ab}	0.33±0.10^{a}
Gc^{+}Gg^{+}F	0.46±0.16^{a}	1.28±0.29^{a}	1.59±0.28^{a}	3.33±0.72^{ab}	0.33±0.05^{a}

*Means of three replicates ± S.E.M, Means within each column followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

WAE: Weeks After Emergence; ***Gc*****:** *Glomus clarum*; ***Gg***: *Glomus gigaspora*; ***F***: *Fusarium*; ***Gc^{+}F***: *Glomus clarum + Fusarium*; ***Gg^{+}F***: *Glomus gigaspora + Fusarium;* ***Gc^{+}Gg^{+}F***: *Glomus clarum + Glomus gigaspora + Fusarium*

4.0 Discussion

Mycorrhizal fungi are known to affect growth of most plant species through various ways. The results of the present study clearly showed the beneficial effects of two AM fungi inoculation (*G. clarum* and *G. gigaspora*) on *Fusarium* colonization and the dry biomass accumulation of *Talinum triangulare***.** Based on the results, there was no *fusarium* root rot colonization in control and AMF treated *Talinum triangulare* seedlings due to non- *fusarium* inoculation (Table 1). Mainwhile, significant ($P \leq 0.05$) lower percentage *fusarium root rot* colonization were observed in seedlings treated with *G. gigaspora* inoculated plant in combination with *fusarium* ($Gg^{+}F$). This could be due to the ability of the AMF to colonize the root before pathogen establishment, therefore, reducing the negative effect of *fusarium* root rot colonization. This present work is in harmony with report of Sohrabi *et al.*, (2015). Who reported that mycorrhizal colonization reduced the percentage of *fusarium* colonization in infected chickpea plants; but just Gm+Fus treatment led to significant increase in level of *fusarium* colonization compared with +Fus treatment. In recent study, the highest significant ($P \leq 0.05$) *fusarium root rot* colonization in plant inoculated with *G. clarum* in combination with *fusarium* ($Gc^{+}F$) (Table 1). This could be that direct (via interference competition) or indirect (via exploitation competition) interactions have been suggested as mechanisms by which AM fungi could not reduce the abundance of pathogenic fungi in roots let to increase colonization by the pathogen. Thus, in the absence of competition from other microorganisms, the population density at the plateau is related to the specific capacity of a strain to colonize the plants. This result is in relation with that of Caron *et al.*, (1986). Who documented that tomato plants inoculated with *G. intraradices* and *fusarium oxysporum f. sp. radices-lycopersici* had lower pathogen population levels than plants inoculated with the pathogen alone. Our findings are connected with the findings of Thygesen *et al.*, (2004). Who noted that root colonization by AM Fungi can decrease the development of fungal root pathogen in their host plants. From the present study, all growth parameter under consideration like; root, stem, total dry weight of plants were recorded to be significantly ($P \leq 0.05$) higher (Table 2) in AMF treated plants than the control set. This might be due to the fact that AMF increases nutrient solubility present in soil and enhance nutrient uptake which facilitated the increment in growth parameters. The present findings are in agreement with the findings of Momraz *et al.*, (2018); who documented on four selected vegetables crop; Rouphael *et al*., (2010). Who documented that AMF can also enhance Phosphorus availability under nutrient deficiency/availability typical of organic farming systems? However, the significant ($P \leq 0.05$) reduction in dry matter production (Table 2) in AMF in combination with *fusarium* inoculated plants in this present work could be due to the fact that AM Fungi contributes to the multipartite interaction by reducing the root and shoot dry weight and lowering the phosphorus status and photosynthetic activity, especially in plants that were inoculated with *fusarium* 2WAE. Or it is noteworthy that in the presence of a biotic stress caused by *fusarium*, AM Fungus could not influence

the root and stem dry weight of *Talinum triangulare* plants. These findings are consistent with previous reports by Momarz *et al.,* (2018). The non-significant ($P \geq 0.05$) leaf dry weight and root:shoot ratio in this experiment (Table 2). This could be due to the direct effect of f*usaruim* on the plants, maybe, AMF may have competed with the roots for photosynthates, thereby having negative leaf and root: shoot ratio. This present research is in harmony with the results of Diagne *et al.,* (2020). Who documented a higher root: shoot ratio in tomato? Several studies reported that endophytic fungi such as AMF can promote plant rooting by stimulation of auxin production in mycorrhizaed roots or release of auxin-lik compounds from hyphae (Ruzzi and Aroca, 2015; colla *et al*., 2015). Giri *et al*., (2003). Who reported that root and shoot dry weight were higher in mycorrhizal than non-mycorrhizal plants. Our findings is in agreement with their results. The significant ($P \leq 0.05$) variation of total dry weight (Table) values within the treatments in this present research is similar to findings of Kim *et al.,* (2017). Who reported difference in responses exist between fungal and plant species and environmental conditions. This could be due to species diversity. The varying responses to the different fungi species suggest that symbiotic efficiency is determined not only by the species of plant and races of the fungus, but also by the host species and environmental condition (Diagne *et al.,* 2020; Eifediyi *et al.,* 2010; Oyeyemi *et al.,* 2017).

5. Conclusions

The findings further enhance our understanding of the potential of arbuscular mycorrhizal fungi as biocontrol agents for the management of wilt diseases in *Talinum triangulare* plants. Their utilization revealed distinct defense mechanisms. AMF demonstrated a protective effect by increasing the mycorrhizal surfaces, thereby improving plant growth and promoting robust biomass accumulations and ultimately strengthening their resistance against pathogens. On the other hand, AMF acted directly on the pathogens. While treatments, including the application of *fusarium,* and their combined synergistic treatment exhibited a lower *fusarium root rot* colonization percentage, the distinct synergistic effect was not prominently observed. This study highlighted the specificity of biocontrol agents in their interaction with pathogens. Although *G. gigaspora* exhibited effectiveness in combating *Fusarium root rot,* which demonstrated a lower colonization rate. Additionally, *G. clarum* demonstrated superior biomass accumulation in these case. The results taken together emphasize the potential of AMF as sustainable alternatives in agriculture, offering a viable solution to reduce reliance on fungicides and promote environmentally and health-conscious practices (i.e., the current findings align with the hypothesis outlined in the introduction).

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Competing interests

The authors declare that they have no competing interest.

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