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EFFECT OF MYCORRHIZAL INOCULATION ON THE GROWTH OF *Capsicum annuum L.* IN NEMATODE INFESTED SOIL.

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ABSTRACT

Pepper (*Capsicum annuum* L.) is spicy shrub in the family of Solanaceae highly susceptible to nematodes. This vulnerability has limited its production in the nematode infested soils of the Cross River basin of Nigeria. The use of chemical nematicides is considered as environmental and ecological risk as some useful soil organisms are usually adversely affected. Arbuscular mycorrhizal fungi (AMF) application has been considered an eco-friendly option of controlling soil borne pathogens including nematodes. This study investigated the interaction effect of inoculation with *Glomus mosseae* (AMF) and *Pratylenchus coffeae* (nematode) on the growth of pepper seedlings. Inoculation with *G. mosseae* increased plant height by 13.37% in nematode inoculated soil and 33.7% in soil without nematode. Seedlings' total dry weight was increased by 4.93% in nematode inoculated soil and 6.06% in soil without nematode. Mycorrhizal colonization was reduced by 12.5% in nematode infested seedlings while nematode count was reduced by 75% in mycorrhizal pepper seedlings. Foliar N, P and K yield were in all cases significantly (P = 0.05) higher in *G. mosseae* inoculated seedlings than in uninoculated ones. The results obtained in this work consistently show that arbuscular mycorrhizal fungi can enhance the growth of *Capsicum annuum* in nematode infested soil.

Keywords: Abuscular mycorrhiza, *Capsicum annuum* growth, nematode infested soil.

1. INTRODUCTION

Pepper (*Capsicum annuum* L.) is a herb which grows up to 30-40cm in height. It is widely cultivated in some parts of Nigeria for its spicy flavoured and mild pungent fruits. As important as pepper is, it is not flourishing in all parts of Nigeria due to its high vulnerability to nematode attack. This is the case with the soil of Cross River basin in Nigeria (Okon and Imuk, 2011). Nematode infestation and feeding on the roots of plants usually interfere with the roots' water and mineral absorption (Marro *et al.,* 2014) and subsequent conduction to the shoot leading to poor growth and reduced yield. Management of nematodes in agricultural systems is mostly by the use of chemical nematicides (Noling, 2016). These chemicals are not only expensive but eco-unfriendly and most of them do not meet the environmental safety regulations (Harrier and Watson, 2004).

Pepper is known to be mycorrhizal and its growth and yield has been shown to be increased by arbuscular mycorrhizal fungi (AMF) inoculation (Olsen *et al.,* 1999; Okon and Solomon, 2014). One of the most widely acknowledged benefits of AMF is its ability to enhance stress tolerance in plants such as occur during nematode infestations (Serfoji *et al.,* 2010; Okon and Imuk, 2011; Schouteden *et al.,* 2015) thereby reducing its harmful effects (de la Peña, 2006). However, for this to be achieved, more than 38% root colonization by the AMF must be attained (Saleh and Sikora, 1984). Colonization by AMF increases tolerance to nematode infection mainly by: (i) improving or maintaining better host water and nutrient status thus offsetting the deleterious effect of the pathogen (Augé, 2001; Smith and Smith, 2011; Baum *et al.,* 2015), (ii) altering the host's root morphology through increased growth and proliferation (Gutjahr and Paszkowski, 2013); (iii) compensating for injuries inflicted by the nematode through increased nutrition or competition for photosynthates (Maia *et al.,* 2006).

Information is lacking on the interaction of AMF and nematode on the growth of *Capsicum annuum* in the Cross River basin soil of Nigeria. This study was undertaken to investigate the effect of mycorrhizal inoculation on *C. annuum* grown in nematode infested soil with a view to proffering solution to this problem so as to encourage cultivation of this spice by gardeners

who may wish to plant it in their home gardens or even go into large scale production.

2. MATERIALS AND METHODS

2.1 Soil sterilization and nursery preparation.

Top soil (0-20cm) was sieved to remove pebbles, root fragments and other debris. The soil was then moistened and steam sterilized in an oven at 160°C for 72 hours. Seedlings of *Capsicum annuum* L. var. *annuum*. were broadcast on the moistened sterile soil and raised for four weeks before being transplanted to their respective treatments.

2.2 Isolation and identification of nematodes

Nematodes for this work were extracted from the roots and rhizosphere soil of an established capsicum plant in Calabar using the Cobb's sieving and decanting technique followed by the Baerman funnel technique (Southey, 1986). The most dominant isolate, *Pratylenchus cofeae* was used for this investigation.

2.3 Source of AMF

The arbuscular mycorrhizal fungus *Glomus mosseae* used was obtained from USDA-ARS, Beltsville, USA and maintained by propagating in sorghum pot cultures (Ferguson and Woodhead, 1982).

2.4 Experimental design and layout

Twelve black perforated nursery bags (24×33cm) were each filled with 8kg of sterile top (0-20cm) sandy loam soil. Twenty-four uniformly sized capsicum seedlings were selected and transplanted two to a nursery bag. Mycorrhizal inoculation was done at the time of transplanting by placing the seedlings on 100g of crude inoculum of *Glomus mosseae* consisting of about 600-650 spores in the planting hole. Nematode treatment designated plants were inoculated with 100ml suspension containing 95-100 eggs of *Pratylenchus coffeae*.

The experimental treatments consisted of nematode and mycorrhiza (N^+M^+); nematode without mycorrhiza (N^+M^-); mycorrhiza without nematode (N^-M^+) and No nematode, no mycorrhiza (N^-M^-) as control laid out in a completely randomized design (CRD) with three replications each.

2.5 Growth parameters measurement

Twelve weeks after transplanting, seedlings' heights were measured with a metre rule. The plants were harvested with care after thoroughly watering the soil to loosen it from the roots. Each plant was separated into roots, stem and leaves and oven dried to a constant dry weight at 70°C in their respective appropriately labeled envelopes.

Foliar nutrient contents were determined for N, P and K using the method of Udo *et al.* (2009).

Mycorrhizal fungus colonization of roots was determined by collecting feeder roots at the time of harvesting, fixing in 50% ethanol, clearing and staining using the method of Koske and Gemma (1989). Colonization was estimated using the modified grid transect method of Giovanetti and Mosse (1980). Quantification of nematode infection of capsicum roots was done by assessing the number of nematodes per gram of root following the method of Hussey and Barker (1973) and Jaizme-Vega (2006).

All the data obtained were subjected to analysis of variance using SPSS for windows version 19. Means were separated at (P=0.05) using Duncan's multiple range test.

4. RESULTS

Inoculation with *Glomus mosseae* increased plant growth as expressed in height and biomass yield values while inoculation with *Pratylenchus coffeae* reduced plant growth (Table 1). Colonization with *G. mosseae* significantly (P = 0.05) reduced the infestation by *P. coffeae* and its negative effect on *C. annuum* seedlings. Percentage root mycorrhizal colonization was significantly (P = 0.05) reduced by inoculation with *P. coffeae* (Table 1) while nematode infestation of capsicum roots was significantly reduced by *G. mosseae* inoculation.

The effect of *G. mosseae* and *P. coffeae* inoculation on leaf foliar nutrient yield is as presented in Table 2. Foliar N and P yield was significantly (P = 0.5) increased by AMF inoculation while K uptake was not significantly affected in nematode infested soil.

4. DISCUSSION

The results of this work have demonstrated that the growth of *Capsicum annuum* var. *annuum* is reduced by nematode attack and inoculation with *G. mosseae* can reduce the deleterious effect of this nematode. This result is in agreement with

the findings of other workers on other plant species (Vaast *et al*., 1998). In mycorrhizal plants, the damage caused by the parasitism could have been compensated for by increased plant nutrition, competition for infection site or photosynthates, changes in root morphology, histopathological, biochemical and physiological alterations, and promotion of defense mechanisms to react against the pathogen, all caused by the *G. mosseae*. It has also been suggested that these factors can as well act in synergy Azcón-Aguillar and Barea, 1996; Maia *et al*., 2006).The reduction of growth of the host plants by the nematode could have been caused by the depressed nutrition status resulting from the attack by the parasite as indicated by the reduced foliar phosphorus and nitrogen yield in nematode infested plants (Table 2) resulting from the attack by the parasite without mycorrhizal inoculation which had the lowest nitrogen and phosphorus yield. On the other hand, better nutrient status has been shown to increase tolerance to plant parasitic nematode infection (Pettigrew *et al*., 2005).

Inoculation with AMF has been shown to ameliorate the negative effects of nematode infestation in pants. Several studies have demonstrated that AMF hyphae usually extend out from their host roots to explore and extract nutrients from beyond their depletion zone (Bücking and Kafle, 2015). Increased phosphorus uptake could have enhanced better root growth and proliferation (Parmar and Sharma, 1996), thereby increasing the absorption surface area for the increased absorption of other nutrients. AMF has been reported instrumental to as much as 90% phosphorus uptake by mycorrhizal plants (Vander Heijden *et al*., 2015). The reduction in nematode infestation by AMF inoculation could have possibly resulted from direct competition for nutrient and space (Vos *et al*., 2014), induced systemic resistance (Pieterse *et al*., 2014), altered rhizosphere interactions due to mycorrhizal root exudates discharged to the soil (Vos *et al*., 2012) or a combination of all these. AMF have been shown to produce phytoalexins in the roots (Morandi, 1996) which could have possibly reduced the level of infestation by the nematodes.
Reduction in nutrient uptake by nematode infestation has been reported by some earlier researchers (Okon and Imuk, 2011). One possible mechanism for this could be through the interference with nutrient flow from the AMF to the host plant caused by the nematode (Cofcewicz *et al*., 2001). This reduction in nutrient uptake would then have led to the reduced root growth and reduced AMF colonization in mycorrhizal plants. It has been shown that P transfer between host and fungus occurs in arbuscule-containing cells (Harrison *et al*., 2002; Isayenkov *et al*., 2004). The reduction in the proportion of arbuscules containing cells in nematode infested plants might have then resulted in the interference with P transfer between the host and the AMF thereby suppressing its uptake. The reduction in the uptake of other nutrients: nitrogen and potassium is likely an indirect effect resulting from phosphorus deficiency.

5. CONCLUSION

The findings from this work have shown that with suitable AMF inoculation, *Capsicum annuum* can be cultivated in Calabar with success its high nematode rich soils not-withstanding and that the deleterious effect of plant parasitic nematodes on this plant can be checked using arbuscular mycorrhizal fungus such as *G. mosseae*.

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Table 1.: Effect of *Glomus mosseae* and *Pratylenchus coffeae* inoculation on height and biomass yield of *Capsicum annuum*.

Treatment	Plant Height (cm)	Plant Total Dry weight (g plant^{-1})	%AMF Colonization	Nematode count per 100g of roots
N^+M^+	*22.50^b	11.36^b	69.64^a	67.00^b
N^-M^+	16.70^c	10.79^c	0.00^e	140.00^a
N^-M^+	26.40^a	12.26^a	75.00^b	0.00^e
N^-M^-	15.80^{cd}	11.56a^b	0.00^e	0.00^e

*Mean of three replicates. Means within each column followed by different letters are significantly different at P=0.05 according to Duncan's multiple range test. N^+M^+: Inoculated with nematode and mycorrhiza; N^+M^-: Inoculated with nematode without mycorrhiza; N^-M^+: Inoculated with mycorrhiza without nematode; N^-M^-: Control (no nematode, no mycorrhiza).

Table 2.: Effect of *Glomus mosseae* and *Pratylenchus coffeae* inoculation on.

Treatment	Foliar nutrient yield (mg plant^{-1})		
	Nitrogen(N)	Phosphorus(P)	Potassium(K)
N^+M^+	*46.84^a	8.87^c	12.45^b
N^+M^-	21.41^d	2.25^d	13.40^a
N^-M^+	42.26^{ab}	12.43^a	12.06^b
N^-M^-	31.17^c	10.78^b	9.17^c

*Mean of three replicates. Means within each column with different letters are significantly different at P=0.05 according to Duncan's multiple range test. N^+M^+: Inoculated with nematode and mycorrhiza; N^+M^-: Inoculated with nematode without mycorrhiza; N^-M^+: Inoculated with mycorrhiza without nematode; N^-M^-: Control (no nematode, no mycorrhiza).

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