



Can arbuscular mycorrhiza fungi and NPK fertilizer suppress nematodes and improve tuber yield of yam (*Dioscorea rotundata* 'cv' ewuru)?

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Abstract

Poor soil fertility and nematodes limit yam tuber yield and quality. Arbuscular mycorrhizal fungi (AMF) and fertilizers may suppress nematodes and improve yam productivity. We evaluated the extent AMF and fertilizer suppressed nematodes and improved yam performance. Tuber weight, mycorrhizal colonization of roots and nematode populations were evaluated with eight treatments; Control (No amendments), 90-50-75, kg N- P₂O₅-K₂O ha⁻¹ (NPK), (AMF) (2g/kg soil), nematodes (5000 juvenile/pot), and their combinations. Tuber weight was higher in NPK+AMF and NPK+nematode treatments than AMF+nematode. NPK+AMF improved tuber weight by 17.5% and 32% compared with sole NPK or AMF respectively. Compared with control, nematodes did not reduce tuber weight but, AMF+nematode reduced it by 49.4%. NPK reduced AMF colonization of roots and reduced nematode population on tuber, in roots and soil by 34%, 42.6% and 41% respectively. NPK+AMF treatment was superior to either NPK or AMF in improving tuber yield while NPK was superior to AMF in suppressing nematodes in roots, soil, and tuber.

Keywords: Arbuscular mycorrhizal fungi, nematodes, Nigeria, mineral fertilizer, root colonization, yam tuber weight.

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Introduction

Yams (*Dioscorea* spp) are tropical plants with large reserve of food in their underground tubers (Okoli and Onwueme, 1986). They are important staple food crops in many parts of West Africa, and are prominent during socio-cultural festivals. Yam represents stored wealth, which can be sold all-year-round by the farmer or marketer because they can be stored relatively for longer duration in comparison with other tropical fresh produce (Aidoo, 2009). White yam, *Dioscorea rotundata* is indigenous to West Africa and it is an important economic crop in the yam belt zone of West Africa. Nigeria accounted for more than 70% of the world production of yam (Ekanayake and Asiedu, 2003). Many farm-families depend on the tubers for food, cash, local food security and other traditional uses. In spite of the importance and increased demand for yam tubers, its production and productivity has steadily declined (APMEU, 2001). The steadily declining tuber yields of yam per unit area is caused among other factors by decreasing soil fertility, high labour requirement, problems of pests and diseases. Amongst the various constraints to production of yam, nematode pests are of significant importance (Bridge et al., 2005). Application of fertilizer has been reported to increase yam productivity (Kolawole, 2013). Hence, mineral fertilizer application is considered a quick way of meeting the nutrient requirement of yam.

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Plant parasitic nematodes have been observed to reduce yield, food quality, market value and sprouting of yam. Reduction of 20-30% in tuber weight at harvest has been reported (Kwoseh, 2000). Although the use of pesticides to control nematode is feasible and effective, but they are costly, scarce or highly toxic for both the user and the environment, constituting serious health hazards (FAO, 2014). Concerns of the environment, crop production and food safety have made researchers to spur many measures to manage nematode pests on crops. For the control of nematodes in yam production, alternative management practices such as hot water treatment of tubers (Coyne et al., 2007), use of cover crops and chemical fertilizers (Kolawole, 2013) have been explored.

Importance of association of arbuscular mycorrhizal fungi (AMF) with yam for natural soil fertility has been demonstrated (Dare et al., 2012). AMFs are important elements of the soil microflora in agroecosystems, which form a mutualistic symbiosis with most plant species. AMFs have been implicated in increasing the availability and uptake of soil phosphorus and trace elements, thereby enhancing host plant growth (Ceballos et al., 2013). Root colonization by AMF, in general, favors plant development by increasing nutrient uptake, hormonal activity, growth rate and consequently yield (Hart et al., 2014), but is also associated with pathogen suppression (Hol and Cook, 2005). Plant parasitic nematodes may enhance or depress colonization of roots and sporulation of mycorrhizal fungus. Likewise, mycorrhizae may decrease or increase nematode penetration, development and reproduction. Previous studies showed the efficiency of indigenous AMF on yam growth (Tchabi et al., 2016).

The present study aimed at determining the effects of NPK mineral fertilizer, AMF inoculation on the performance of yam planted in plant parasitic nematodes infested soil.

Material and Methods

The experiment was conducted at the Teaching and Research Farm, Ladoké Akintola University of Technology, Ogbomoso (Lat. 8° 10' N: Long. 4° 10' E) Oyo State, Nigeria.

Top soil (0-30 cm depth) was collected randomly with soil auger at the Teaching and Research Farm, Ladoké Akintola University of Technology, Ogbomoso. The soil was bulked, mixed thoroughly, air dried, and sieved through 2 mm mesh size sieve. Thereafter, the soil was sterilized by autoclaving at 120°C and 15psi for 30 minutes and later air dried for 72 h. Sub sample of the autoclaved soil was collected and taken to the laboratory for plant parasitic nematode bioassay and chemical analyses. The initial chemical properties of the soil (sandy loam in texture; sand 810 g kg⁻¹, silt 90 g kg⁻¹, and clay 100 g kg⁻¹) are as follows; pH-H₂O) 6.5, total organic carbon 5.3 g kg⁻¹, total nitrogen 0.22 g kg⁻¹, Bray P 6.9 mg kg⁻¹, Exchangeable cations: K 0.22, Ca 1.48 and Mg 0.96 in cmol kg⁻¹.

Thirty kg soil each was weighed into thirty two plastic pots (30-liter volume) perforated at the bottom to prevent water logging and allow good water drainage. The pots were properly labeled.

Eggs of *Meloidogyne incognita* were extracted from galled roots of *Celosia argentea* using the method described by Hussey and Barber (1973).

There were eight treatments namely: 90 - 50 - 75 kg N - P₂O₅ - K₂O ha⁻¹ (NPK) which is the recommended rate for yam in the zone; Arbuscular Mycorrhizal Fungi (AMF) at the rate of 2 g/kg soil; Nematodes (5000 eggs and juveniles per pot); AMF + NPK; AMF + Nematode; NPK + Nematode; NPK + Nematode + AMF; and Control (no fertilizer, AMF and nematode). The experiment was arranged in completely randomized design with four replicates.

Healthy white ware yams (cultivar 'ewuru', popular among farmers) were obtained from the local market, steam sterilized at 50-55°C and cut into yam setts under sterile condition. Planting was done by opening holes in the autoclave sterilized soil inside the pots and one 200-300 g yam sett was placed in each hole with the cut surface turned upward at an angle of about 45° and covered with soil and capped 2 weeks after planting (WAP). Staking began 5 months after planting with 4 m long bamboo stakes. The pots were manually weeded by hand pulling as at when necessary.

Arbuscular Mycorrhizal Fungi (AMF) was placed in the planting hole in designated pots at the rate of 60 g/pot. This inoculum contained soil spores, roots of plants used in propagating the inoculum and hyphae. Two weeks after sprouting of yam, each appropriate pot was inoculated with approximately 5000 eggs and juveniles of *Meloidogyne incognita*. Inoculation was done by slowly dispensing 5 ml of the previously prepared nematode suspension into holes around the yam plant. NPK compound fertilizer (15 - 15 -15) (5 g/pot) and the excess N (1.33 g/pot) and K (0.63 g/pot) were met using urea and muriate of potash, respectively. The fertilizers were applied in a ring form and lightly incorporated into the soil about 30 cm

away from the growing yam at five months after planting.

At 48 WAP, the pots were upturned over polyethylene sheet. The roots and tubers were gently separated and the adhering soils washed off. The tubers were weighed on weighing balance and weight per treatment was recorded. Post harvest soil samples were collected from individual pots for nematode bioassay.

Pin-pie extraction method ([Whitehead and Hemming, 1965](#)) was used for root and soil nematode extractions within 3 hours after harvesting of the yam tubers. Nematodes were extracted within 48 hours after tubers were collected from the pots. Modified Baermann technique was used for extraction. Nematodes species were identified by means of morphology and morphometrics coupled with guide by the identification key for agricultural important plant-parasitic nematodes ([Mekete et al., 2012](#)).

At final harvest, galls on the yam root and tuber were assessed following the method of [Sasser et al. \(1984\)](#) on 0 – 5 rating scale where 0 = no gall; 1 = 1 – 2% gall; 2 = 3 – 30% gall; 3 = 31 – 50% gall; 4 = 51 – 70% gall and 5 = 71 – 100% gall.

At twelve weeks after sprouting, root samples were collected from designated plants by digging 30 cm away from the main yam plant to get fine roots. The roots were washed with water and then cut into about 10 cm long pieces, put inside bottles containing ethanol alcohol, and later transferred to the laboratory for mycorrhizal count. Percentage mycorrhizal colonization of roots was determined by gridline-intersect method ([Giovannetti and Mosse, 1980](#)), after clearing the root with KOH and staining with chlorazol black E ([Brundrett et al., 1984](#)).

Data were subjected to analysis of variance (ANOVA) using SAS software ([SAS, 2009](#)) and treatment means compared using least significant difference (LSD) at 5% probability level.

Results

Plant parasitic nematode bioassay of soil after inoculation

There was no nematode found in the soil that was examined immediately after sterilization before yam planting.

Yam tuber weight

Tuber weight was significantly higher in NPK+AMF and NPK+nematode treatments compared with AMF+nematode treatment which had significantly lowest tuber weight (Table 1). The combined application of NPK and AMF improved yam tuber weight by 17.5% and 32% compared with the sole application of NPK or AMF respectively. Inoculation of juvenile nematodes into the soil compared with the control (no nematodes, no fertilizer) had no significant effect on yam tuber weight. However, AMF+nematode treatment reduced tuber weight by 49.4% compared with the control. Compared with the nematode alone treatment, application of NPK fertilizer in nematode infested soil improved tuber weight by 28.4% and combined application of NPK fertilizer and AMF only improved tuber weight by 12%. That is, the effect of combined application of NPK fertilizer and AMF on yam tuber weight in nematode infested soil was neither additive nor synergistic.

Table 1. Effects of NPK fertilizer and arbuscular mycorrhizal fungi (AMF) on tuber weight and AMF colonization of yam roots grown in nematode infested soil

Treatments	Tuber weight (kg/pot)	AMF colonization (%)
Control		Nil
NPK alone	0.85	Nil
AMF alone	0.70	72.5
Nematode (NEM) alone	0.73	Nil
NPK+AMF	1.03	48.5
AMF+NEM	0.40	65.0
NPK+NEM	1.02	Nil
NPK+AMF+NEM	0.83	49.3
LSD _(0.05)	0.46	4.2

AMF colonization of yam roots

Significantly highest AMF colonization of yam roots was observed in the AMF treatment followed by AMF+nematode treatment, while NPK+AMF and NPK+AMF+nematode treatments had significantly lowest AMF root colonization values (Table 1).

Nematode infestation

Root gall was significantly higher in the AMF+nematode treatment than for the sole nematode treatment (Table 2). Other treatments were not significantly different from each other. All the treatments had no significant effects on yam tuber gall formation (Table 2).

Table 2. Effects of NPK fertilizer and arbuscular mycorrhizal fungi (AMF) on gall index on roots and tuber and nematode population in soil, root and tuber of yam grown in nematode infested soil

Treatments	Root gall index	Tuber gall index	Nematode population (number L ⁻¹)		
			Root	Tuber	Soil
Nematode alone	1.3	3.7	1677	831	1585
AMF+Nematode	2.8	2.3	1185	727	1421
NPK+Nematode	1.8	3.0	962	548	933
NPK+AMF+Nematode	2.0	3.0	930	664	1139
LSD(0.05)	1.25	ns	380	200	412

Nematode population recorded on yam tuber grown in sole nematode infested soil was significantly more than that recorded for NPK+nematode treatment. That is, application of NPK fertilizer reduced nematode population on yam tuber by 34% (Table 2). Other treatments were not significantly different from each other.

Yam roots harvested from sole nematode infested soil had significantly highest nematode population than the other treatments which were not significantly different from each other (Table 2). Post harvest soil nematode count was the highest in sole nematode infested soil. Inoculation of AMF in nematode infested soil only significantly reduced post harvest nematode population in yam roots, whereas application of NPK fertilizer significantly reduced post harvest nematode populations in the roots, tubers and soil. Combined application of NPK fertilizer and AMF only significantly reduced nematode population in the roots (Table 2).

Discussion

Generally, plants with mycorrhizae and nematode parasites yield less than mycorrhizal plants without nematodes and more than non-mycorrhizal plants with nematodes. This was also true in the present study except that the non-mycorrhizal plants with nematodes out yielded plants with mycorrhizae and nematode parasites. The observation in the present study that AMF did not improve yam tuber weight compared with the control is in contrast with previous reports (Odoh and Oluwasemire, 2015; Tchabi et al., 2016). The observation is however similar to the report of Sidibe et al. (2015) for yam. Banuelos et al. (2014) observed that in fertilized plants single inoculation with AMF and nematodes caused plant growth depressions, which were counteracted when AMF and nematode inoculation were combined in *Impatiens balsamina* plants. This is contrary to what was observed in this study where yam tuber yield of fertilized pots with either AMF or nematode inoculation had the highest weight.

Although AMF generally suppressed nematode population on yam roots, tuber and soil similar to the report of Tchabi et al. (2016), but the effect was not as pronounced as that of the application of NPK mineral fertilizer.

In the present study also, arbuscular mycorrhizal colonization of yam roots was reduced with NPK fertilizer application. This is similar to the observation of Kolawole et al. (2015). High P-supply reduced AMF colonization of maize root (Liu et al., 2016). Isolates of AMF differ in their sensitivity to soil and plant P levels and therefore fertilizer application may alter the activity of the symbiosis (Sylvia and Schenck, 1983). There are contradictory reports on the role of fertilizers in influencing AMF in soil. Many reports indicated a negative influence (Vivekanandan and Fixen, 1991) while a few reported a positive influence of fertilizers on AMF (Kolawole, 2013). Previous studies have however found increased levels of AM fungal inoculum and root colonization rates in low-input as compared with intensive farming systems in the field (Douds et al., 1995).

Conclusion

The effect of combined application of NPK fertilizer and AMF on yam tuber weight in nematode infested soil was neither additive nor synergistic. AMF was less effective in suppressing nematodes compared with NPK fertilizer. NPK fertilizer reduced the negative effect of nematodes on yam tuber weight; significantly reduced nematode populations in the roots, soil, and on the tuber. Nematodes did not reduce yam tuber weight but reduced tuber quality.

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