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Full Length Research Paper

"Efficacy of mycorrhizal inoculations on seed germination and plant growth of Bambara groundnut, *Vigna subterranea* (TVsu 283)"

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This Research work was conducted to study the effects of mycorhizal fungal inoculation on seed germination and plant growth of Bambara groundnut (*Vigna subterranea*). The impacts of *Glomus mosseae*, *Aspergillus niger*, *Aspergillus fumigatus* and *Malbranchea gypsea* on some plant growth parameters were studied both in their crude and pure forms. Compared to un-inoculated plants, the fungal strains significantly increased plant growth parameters. The highest rate of germination at the 42nd day was observed in the crude fungal inoculation treatments and specifically in *A. fumigatus* crude inoculation as it increased the number of leaves, leaf length, leaf surface area, stem girth, and number of shoots by 54.2, 23.8, 49.1, 20.0 and 40.0%, respectively.

Key words: Mycorrhizal, *Vigna subterranea*, crude and pure forms, plant growth parameters.

INTRODUCTION

In most of the Sub-Sahara African countries, increased population growth leads to hunger, the main cause of malnutrition and food insecurity. This food insecurity relies on low crop productivity, limited by low soil fertility which is accelerated by low soil cation exchange capacity and organic matter, high soil acidity and/or bad cultural practices (Bado, 2002; Yao et al., 2005). All these constraints create disequilibrium between the available quantity of crops produced and the population needs on one hand, and the environment on the other, suggesting that this population is not eating or living safely (Cooke, 1998).

In an effort to enhance crop production, most of our tropical countries rely on chemical fertilizers, which have potentially revealed to be a pollutant to human life and environment (Margni et al., 2002), in addition to their high cost, as the result of lack of own available chemical fertilizer manufacturers.

The lack of food rich in proteins in the world and particularly in developing countries has urged researchers to seek for alternative source of proteins to complete their diet (Ahmed and Abdallah, 2010). Hence, the food insecurity, the environmental pollution and the soil fertility problems need to be solved if promotion of biological and sustainable agriculture is to be accomplished through measures such as improved fallow, intercropping, agroforestry or biofertilizers. Among these strategies, biofertilizers have been reported

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Plate 1. Bambara seeds (TVsu 283).

to increase yield of several crops in Cameroon (Megueni et al., 2006; Ngakou, 2007; Ngakou et al., 2007a, 2008, 2011). Unlike other crop legumes, very little is known about Mycorhiza-*Vigna subterranea* interactions.

Bambara groundnut belongs to the under-utilized grain legumes that possess high crude protein content between 22 and 37% (Adeparusi, 2001; Fasoyiro et al., 2004). It is widely grown in Nigeria and in other African countries like Ghana, Cameroon, Ivory Coast and Togo (Klu et al., 2001). Bambara groundnut is the third most important legume after groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Howell et al., 1994). Hence, there is a need to improve the productivity of this food security crop known to grow on low soil fertility, where it can withstand drought and low rainfall (Berchie et al., 2010).

Therefore, we hypothesize that Mycorhiza symbiosis could alleviate the host plant nutrient requirements, thus resulting to sustainable yield improvement of this valuable crop, so as to substitute chemical fertilizers.

MATERIALS AND METHODS

Site of experiment

The potted experiment was carried out beside the General Biology Laboratory of the Department of Biological Sciences, Osun State University Osogbo, Osun State (Latitude 7.5°N and Longitude 4.5°E, at 246 m elevation) for a period of 6 weeks (July - August, 2014). The culture test was carried out in the Microbiology Laboratory of the Department of Biological sciences, Osun State University Osogbo, Osun State.

Seed collection and treatments

Seeds of Bambara groundnut (*V. subterranea*) with accession number TVsu 283 (Plate 1) were collected from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. The seeds were treated by soaking in 0.02% sodium hypochlorite for 2 min and washed five times with sterilized distilled water (Gholami et al., 2009).

Site of crude inoculum collection

The crude inoculum of *Aspergillus niger* and *Aspergillus fumigatus* was gotten from a sample of native soil obtained from the root of *Araucaria columnaris* while that of *Malbranchea gypsea* was obtained from a sample of soil obtained from the root of *Cassia fistula* at Yidi, Oke-Baale, Osogbo, Osun State (Plate 2). The crude inoculum of *Glomus mosseae* (Plate 3) was obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

Soil preparation and processing

Sandy soils were randomly collected to 6 sacs each of 30 kg from Idi-Iroko Meta Area, Oke-Baale, Osogbo on 9th June, 2014. The soil was sieved to exclude stones and pebbles. The soil was characterized by its dark-brown color and semi-coarse texture. Sterilization of the soil was done by heating in an oven at 100°C. The soil was covered with foil paper to prevent contamination before use.

Seed viability of bambara seeds

Viability of the seeds was determined using a cold water sink-float test (Stuckey and Reese, 1981). Germination tests were then conducted on seeds which were viable.

Pre-germination trials on Bambara seeds

Germination paper sheets (sterile Serviette paper) were arranged in the transparent polyethylene box (10 layers). Rectangular box (170 × 110 × 50 mm) was used for the germination tests. Using a

Plate 2. Site of crude inoculums collection at Yidi, Oke – Baale Area, Osogbo.

Plate 3. Crude Inoculum of *Glomus mosseae* collected from IITA, Ibadan.

measuring cylinder, 20 ml of water was added to the box and excess water was poured off from the germination box before autoclaving. With the use of a forcep, 10 seeds were placed in the autoclaved box in 2 rows/lines. The box was closed tightly and placed near a window where there is natural light. First germination record was taken 6 days after seed setting and then every 48 hour until germination rate reached a plateau (11 days maximum). The contaminated seeds were discarded. After 9 days, the box was opened to allow full seedling development. Seedling vigor was recorded at day 11 (Oyatomi et al., 2010).

Serial dilution

Serial dilution was carried out on the crude inoculums. This was done to reduce the microbial load. One gram of soil was added into the tube containing 9mL of sterile Ringers solution to obtain 1/10 dilution (stock solution) and using a 5 ml sterile syringe, a series of 1/00, 1/1000, 1/10,000, and 1/100,000 dilutions was prepared by adding 1 ml of solution to 9 ml of sterile Ringers solution respectively. Each test tube with the Ringers solution and the crude inoculum was mixed vigorously.

Preparations of the bio-inoculants

Pour plate method was used as culture technique to get pure cultures. Using a 5 ml sterile syringe, 0.5 ml suspension from the dilutions of 10^{-2}, 10^{-4}, and 10^{-6} was transferred onto sterile Petri dishes upon which the already prepared and cool Potato Dextrose Agar (PDA) was poured. Plates were incubated at room temperature for 72 h and observed for growth. Identification of the isolates was done by colonial characterization. The colonies containing the isolates were then sub-cultured in an inoculating chamber on potato dextrose agar to get a pure culture by using the streak plate method. The fungi isolated and identified were: *A. niger, A. fumigatus* and *M. gypsea* designated An, Af and Mg respectively; while *Glomus mosseae* was designated Gm.

Fungal identification

1. With nose mask and hand gloves on, the colonies were first observed visually for the surface color and also on the reverse.
2. Few drops of Lactophenol were put on sterile microscope slide using a pipette.
3. With the use of a forcep, the isolates were placed on the slide, mixed and then covered with sterile cover slip.
4. At low magnification power of microscope (40 objective lens), detailed examinations were done according to Raper and Fennell (1965) and Gams et al. (1985).
5. The microscopic structures (such as conidia, conidiophores, phialides and the mycelia) of each fungus were observed and compared with relevant literatures as reference.

Seedling establishments

Thirty-two buckets of 10 L each were used for planting; all buckets were perforated at the bottom to allow the easy passage of water and to prevent water logging. The buckets were properly labeled for easy identification. Three sterilized seeds of Bambara were sown in each bucket filled with sandy soil; 5-7 cm deep and 10 cm apart and watered regularly. In total, there were eleven groups of thirty-two treatments, with three replications of each bio-inoculant. Three seeds each were sown into each treatment. The control was planted first to avoid contamination. The various groups are listed as follows:

Group A - Sterilized soil + Bambara seeds + crude inoculum of Gm.
Group B - Sterilized soil + Bambara seeds + crude inoculum of An.
Group C - Sterilized soil + Bambara seeds + crude inoculum of Af.
Group D - Sterilized soil + Bambara seeds + crude inoculum of Mg.
Group E - Sterilized soil + Bambara seeds + pure inoculum of Gm.
Group F - Sterilized soil + Bambara seeds + pure inoculum of An.
Group G - Sterilized soil + Bambara seeds + pure inoculum of Af.
Group H - Sterilized soil + Bambara seeds + pure inoculum of Mg.
Group I - Sterilized soil + Bambara seeds + pure inoculum of Gm, An, Af and Mg.
Group J - Sterilized soil + Bambara seeds + crude inoculum of Gm, An, Af and Mg.
Group K - Sterilized soil + Bambara seeds, with no fungi strains (Control).

Harvesting took place 6 weeks after planting. Fresh weight of seedlings were determined immediately after harvesting while dry weight was determined after oven-drying the seedlings at 80°C for 24 h.

Application of the pure and crude inoculums

Ten grams of each of the crude inoculum was mixed with the top soil in each bucket before sowing of Bambara seeds (Pathak et al., 2013). For the pure inoculations, pure cultures of the fungi were suspended in distilled Ringers solution and 10 ml of the solution was inoculated into each bucket using sterile syringe. 10 g and 10 ml each of the crude and pure inoculums respectively, were added to the treatments containing the four fungi acting in concert.

Statistical analysis

Statistical analysis of all tests was carried out using SPSS 15.0 design. Data was analyzed with analysis of variance (ANOVA) at $P<0.05$ level. Tests were conducted in triplicates and values given as mean ± standard error.

RESULTS

Growth parameters

Seed emergency

V. subterranea seeds started emerging from the soil 7 days after planting (7 DAP) in all the 5 treatments. Germination was complete in the field 12 DAP, thus 5 days after the first emergency.

Seed emergence counts

The seed emergence count was done after 7 days of planting and mycorhizal inoculation.

Net assimilation rate / moisture content

Moisture content / net assimilation rate (%) was calculated using the formula:

$$\text{Percentage moisture content} = \frac{(\text{Wet weight} - \text{Dry weight})}{\text{Wet weight}} \times 100$$

Wet and dry weights of roots and shoots

While the wet weights of shoots and roots were measured directly, dry weights were measured after cleaning, rinsing in distilled water and drying for 24 h in an oven at 70°C (Jha and Saraf, 2011). Measurements were taken in gram (g) using weighing balance.

Plant height

The plant was straightened to its fullest length and the length (cm) was measured with a ruler alongside (Harold, 1957).

Number of leaves

Only true leaves were measured and considered as the correct number of leaves (Table 1).

Mycorrhizal dependency

Mycorrhizal dependency (%) which is defined by Gerdemann (1975) as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth on yield at a given level of soil fertility, was measured using the formula (Ajiboye, 2009):

$$\frac{\text{Total dry weights of (Inoculated seedlings- non-inoculated seedlings)}}{\text{Total dry weight of Non-inoculated seedlings}} \times 100$$

DISCUSSION

After sowing, germination occurred 1 week later in all the treatments. This was within the range of 7 to 15 days (Swanevelder, 1998), or 7 to 10 days (Yao et al., 2005) period reported as necessary for germination of Bambara groundnut.

A recent report by Brink et al. (2006) indicated that flowering in Bambara occurs within 30 to 55 days after sowing. This observation and the findings from this research are different from those made by Ocran et al. (1998) that Bambara groundnut flowers within 43 days, while Berchie et al. (2010) revealed date to 50% flowering at between 39 and 41 DAP. The results of this research suggest that dates to 50% flowering for Bambara groundnut significantly vary with the seed variety and the growing area, because after 6 weeks of planting, the Bambara seeds (TVsu283) did not flower.

It was observed at the end of the growing period and from the results of the measured parameters (among many others) that the growth of the Bambara groundnut was more enhanced by the crude mycorrhizal inoculations. Crude *A. fumigatus* increased number of leaves by 54.2% as compared to the non-mycorrhizal treatments (Table 1). This is followed by pure *A. fumigatus* with 37.5% increase, then pure *M. gypsea* with 29.2%, crude *M. gypsea* with 25.0% increases respectively. Next is that of the pure inoculations of the four fungi having 20.8% increase. Following closely are those of the crude inoculations of the four fungi, crude *A. niger* and pure *G. mosseae* all with 12.5% increase. There was no increase in the number of leaves produced by pure *A. niger* as compared to the non-mycorrhizal treatments. Crude *G. mosseae* had a lower number of leaves than the non-mycorrhizal treatments.

A. fumigatus is more effective than the other fungal inoculums because it has been recorded that *A. fumigatus* has an early duplication cycle compared to other filamentous fungi (Michelle and Ian, 2000). Thus more strains of it are available for the Bambara seed to increase its yield and productivity.

The lower yield in the treatments with the four fungal strains is linked to the fungi acting antagonistically. The highest stem length was found in crude *A. niger* with leaf length of 1.9±0.59 cm (Table 2). Crude *A. fumigatus* and pure *A. fumigatus* had the same leaf lengths of 1.7±0.25 cm and 1.7±0.68 cm respectively. Crude *M. gypsea* and pure *A. niger* also had the same leaf lengths of 1.6±0.22 cm and 1.6±0.36 cm respectively, then pure *M. gypsea* having leaf length of 1.4±0.45 cm. The same leaf length was observed in pure *G. mosseae*, pure inoculations of the four fungi, crude inoculations of the four fungi and control with leaf lengths of 1.3±0.18, 1.3±0.15, 1.3±0.25 and 1.3±0.27 cm respectively; the least being crude *G. mosseae* with leaf lengths of 1.2±0.50 cm.

The highest total dry weight was observed in crude *A. fumigatus* with total dry weight of 2.5 ± 0.90 g, followed by pure *M. gypsea* with total dry weight of 2.1 ± 0.50 g (Table 3). Following this were those of crude *A. niger*, pure *A. niger* and pure *A. fumigatus* with total dry weights of 1.5 ± 0.75, 1.5 ± 0.91 and 1.5 ± 0.56 g respectively. Next are those of crude *M. gypsea* and pure *G. mosseae* with total dry weights of 1.4 ± 1.13 and 1.4 ± 1.15 g respectively. Following these are those of the pure and crude inoculations of the four fungi both with 1.1 ± 0.88 g as their total dry weights and crude G. mosseae having 0.7 ± 0.70 g total dry weight. The least total dry weight was found in the non-mycorrhizal inoculations with 0.5 ± 0.17 g total dry weight.

The highest total fresh weight was observed in crude *A. fumigatus* with total fresh weight of 11.1 ± 2.92 g,

Table 1. Number of leaves observed at the end of each specified day.

Treatment	Day			
	14	28	35	42
Crude *G. moseae*	5 ± 1.00	12 ± 1.58	12 ± 1.58	17 ± 1.87
Crude *A. niger*	8 ± 1.14	18 ± 1.90	21 ± 2.76	27± 2.72
Crude *A. fumigatus*	8 ± 1.14	23 ± 1.14	29 ± 2.10	37 ± 1.82
Crude *M. gypsea*	9 ± 0.00	17 ± 1.72	20 ± 1.24	30 ± 2.09
Pure *G. moseae*	5 ± 0.91	17 ± 1.97	21 ± 1.63	27 ± 2.96
Pure *A. niger*	6 ± 0.33	14 ± 1.14	24 ± 0.00	24 ± 1.63
Pure *A. fumigatus*	9 ± 0.33	16 ± 1.14	23 ± 1.14	33 ± 1.90
Pure *M. gypsea*	8 ± 0.80	18 ± 1.15	24 ± 0.00	31 ± 1.14
Pure of the 4	10 ± 1.00	17 ± 0.80	23 ± 1.24	29 ± 0.80
Crude of the 4	8 ± 1.14	16 ± 1.82	23 ± 1.41	27 ± 1.82
Control	9 ± 0.00	15 ± 0.00	18 ± 1.73	24 ± 0.00

Values are mean ± SD of three replicates.

Table 2. Stem Length (cm) observed at the end of each specified day.

Treatment	Day			
	14	28	35	42
Crude *G. moseae*	0.9 ± 0.30	0.9 ± 0.42	1.1 ± 0.44	1.5 ± 0.50
Crude *A. niger*	1.4 ± 0.88	1.8 ± 0.36	1.8 ± 0.34	1.9 ± 0.59
Crude *A. fumigatus*	1.2 ± 0.37	1.5 ± 0.37	1.7 ± 0.40	1.7 ± 0.25
Crude *M. gypsea*	1.1 ± 0.62	1.4 ± 0.51	1.5 ± 0.45	1.6 ± 0.22
Pure *G. moseae*	0.4 ± 0.10	1.0 ± 0.18	1.3 ± 0.33	1.3 ± 0.18
Pure *A. niger*	1.2 ± 0.66	1.4 ± 0.40	1.4 ± 0.15	1.6 ± 0.36
Pure *A. fumigatus*	1.0 ± 0.52	1.2 ± 0.36	1.2 ± 0.33	1.7 ± 0.68
Pure *M. gypsea*	1.1 ± 0.48	1.1 ± 0.33	1.2 ± 0.39	1.4 ± 0.45
Pure of the 4	1.0 ± 0.54	1.2 ± 0.15	1.3 ± 0.55	1.3 ± 0.15
Crude of the 4	0.8 ± 0.33	0.9 ± 0.33	1.2 ± 0.44	1.3 ± 0.25
Control	0.4 ± 0.38	1.1 ± 0.74	1.2 ± 0.45	1.3 ± 0.27

Values are mean ± SD of three replicates.

Table 3. Record of the total dry weight after 42 days of mycorrhizal inoculations.

Treatment	Total dry weight (g)
Crude *G. moseae*	0.7 ± 0.70
Crude *A. niger*	1.5 ± 0.75
Crude *A. fumigatus*	2.5 ± 0.90
Crude *M. gypsea*	1.4 ± 1.13
Pure *G. moseae*	1.4 ± 1.15
Pure *A. niger*	1.5 ± 0.91
Pure *A. fumigatus*	1.5 ± 0.56
Pure *M. gypsea*	2.1 ± 0.50
Pure of the 4	1.1 ± 0.88
Crude of the 4	1.1 ± 0.88
Control	0.5 ± 0.17

Values are mean ± SD of three replicates.

Table 4. Record of the total fresh weight after 42 days of mycorrhizal inoculations.

Treatment	Total fresh weight (g)	Net assimilation rate (%)
Crude *G. moseae*	4.9 ± 2.11	86.0
Crude *A. niger*	8.5 ± 1.83	82.4
Crude *A. fumigatus*	11.1 ± 2.92	77.5
Crude *M. gypsea*	7.8 ± 1.53	82.1
Pure *G. moseae*	6.9 ± 2.25	80.0
Pure *A. niger*	4.8 ± 2.05	69.0
Pure *A. fumigatus*	8.3 ± 1.27	82.0
Pure *M. gypsea*	9.7 ± 1.75	78.4
Pure of the 4	6.4 ± 1.35	83.0
Crude of the 4	5.9 ± 1.70	81.4
Control	4.4 ± 2.02	89.0

Values are mean ± SD of three replicates.

Table 5. Mycorrhizal dependency.

Treatment	Mycorrhizal dependency (%)
Crude *G. moseae*	40
Crude *A. niger*	200
Crude *A. fumigatus*	400
Crude *M. gypsea*	180
Pure *G. moseae*	180
Pure *A. niger*	200
Pure *A. fumigatus*	200
Pure *M. gypsea*	320
Pure of the 4	120
Crude of the 4	120
Control	0

Values are mean ± SD of three replicates.

followed by pure *M. gypsea* with total fresh weight of 9.7 ± 1.75 g (Table 4).

Following this were those of crude *A. niger*, pure *A. fumigatus* and crude *M. gypsea* with total fresh weights of 6.9 ± 2.25, 6.4 ± 1.35 and 5.9 ± 1.70 g respectively. Next are those of crude *G. mosseae* and pure *A. niger* with total fresh weights of 4.9 ± 2.11 and 4.8 ± 2.05 g, respectively. The least total fresh weight was found in the non-mycorrhizal inoculations with 4.4 ± 2.02 g total dry weight.

The highest mycorrhizal dependency was observed in crude *A. fumigatus* with 400% dependency, followed by pure *M. gypsea* with 320% dependency (Table 5). 200% mycorrhizal dependency was found in crude *A. niger*, pure *A. niger* and pure *A. fumigatus*. Crude *M. gypsea* and pure *G. mosseae* both had 180% dependency. Also, both pure and crude inoculations of the four fungi had 120% mycorrhizal dependency. The least mycorrhizal dependency of 40% was found in crude *G. mosseae.*

The Bambara seedlings were uprooted after 6 weeks of Mycorrhizal inoculation for thorough examination (Plate 4).

It can thus be said that the highest growth was found in those inoculated with crude *A. fumigatus.* This is followed by those inoculated with the crude forms of the four fungi, that is, *G. mosseae, A. niger, A. fumigatus* and *M. gypsea* acting in concert. Following this is the *A. niger* inoculated treatment and least being treatment of *G. mosseae*.

Among the pure inoculations, the highest improvement of growth was found in treatment with pure *M. gypsea* and followed closely by those of pure *A. fumigatus.* The third highest improvement was found in treatment of pure *G. mosseae*, followed by those of *A. niger* and the least being treatment of the four fungi acting in concert.

The non - mycorrhizal treatment showed the lowest growth compared to the mycorrhizal treatment, except for the crude *G. mosseae* inoculated treatment which showed lower growth than the non - mycorrhizal treatment.

The dependency of the plant on mycorrhiza ranged widely in the various treatments: the treatment with crude *A. fumigatus* showed the greatest dependency of 400%

Plate 4. Bambara shoots and roots uprooted for thorough examination.

while the least was found in the treatment with crude *G. mosseae* with dependency of 40%. Thus the poor growth and development observed in crude *G. mosseae* – inoculated seedlings can be attributed to the low mycorrhizal dependency. The mycorrhizal dependency of the treatments increased as the root weight increased. It is well known that root production seems to be an important factor in mycorrhizal dependency (Mehraverahn, 1977; Nemec, 1978). Although these results showed a correlation between root dry weight and mycorrhizal dependency, root weight alone cannot be used as a measure of mycorrhizal dependency, and thus agree with Menge et al. (1978) that such relationship ought to be viewed with caution.

The enhanced biomass of inoculated plants has been attributed to increased nutrient uptake in mycorrhizal inoculated plants (Smith and Read, 1997; Ngakou et al., 2007a, 2008).

Conclusion

In conclusion, mycorrhiza improved the plant biomass as compared to un-inoculated treatment. Biofertilizers could thus be recommended to boost the crop productivity and alleviate the long cooking properties of *V. subterranea* seeds. The overall improvement in seedling vigor through a significant increase in various physiological parameters suggests that these fungal strains have a plant-growth promoting ability on Bambara seedlings and hence could be used for seed inoculation for better establishment of seedlings. The plants with enhanced seedling vigour can assist to better plantations. Thus it can be concluded that the use of Bioinoculants or Biofertilizers in agricultural practices may assist to enhance food production.

Improvement of soil fertility and promotion of sustainable agriculture in Osun region is feasible if under-utilized crops such as Bambara groundnut (*Vigna subterranea*) could be inoculated by mycorhizal strains in the field. It is therefore recommended that the results obtained from this research work be forwarded to extension officers in the Ministry of Agriculture and Rural development to tackle problems of seed germination and seedling establishment of this legume with enormous benefits.

Conflict of Interest

The authors have not declared any conflict of interest.

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