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Effects of Two Types of Fertilizer and *Arbuscular Mycorrhiza* on Damping off Disease (*Macrophomina phaseolina*) of Cowpea (*Vigna uniguiculata* L. Walp)

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Abstract: A screen house experiment was carried out to determine the effect of organic fertilizer, compost, inorganic fertilizer, and single super phosphate and *Arbuscular mycorrhiza* on damping-off disease of cowpea. The experimental design used was completely randomized design (CRD). There were fifteen treatments which were replicated three times. 2g of *Macrophomina phaseolina* (damping off disease) was infested on 10kg of soil sample in each pot with (30 by 75) cm spacing. *Arbuscular mycorrhiza*, compost (poultry based) and single super phosphate (SSP) were applied at the rate of 2250kg/ha, 1670kg/ha and 676kg/ha respectively. Three seeds of cowpea were sown into each pot, but later thinned into two plants per pot. Data on germination percentage at 7, 14, 21 and 28 days, number of leaves of cowpea at 2, 4, 6 and 8 weeks after planting, weight of pods per plant, incidence and severity at which pathogens affected the cowpea plant per treatment and growth yield of each treatment were collected and analyzed using analysis of variance (ANOVA) and means separated using the Duncan multiple range test. The plants that were treated with 676kg/ha single super phosphate produced higher cumulative weight of harvested fruits per plant followed by *Arbuscular mycorrhiza*, which did not have any negative effect on environment. The values of phosphorus in the post harvest soil analysis revealed that lower values of phosphorus were recorded in Single Super Phosphate treated pots, while plants treated with *Arbuscular mycorrhiza* at 500kgha^{-1} produced higher P value than the pre-planting soil analysis indicating the absorption of large amount of the elements. It was therefore recommended that *Arbuscular mycorrhiza* could be used to optimize seed yield in cowpea.

Keywords: Legume, Waste, Organic and Inorganic Manure, Poultry Waste

1. Introduction

Cowpea (*Vigna unguiculata*) belongs to the family of *Fabaceae*. It is one of the most important food legumes originally cultivated in the tropical regions of the world, but now it's grown in the hot in semi-arid to sub-humid tropics covering Asia, Africa, Southern Europe, Central and South America [7, 8]. It is compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton because it is shade tolerant, which makes it an important component of traditional intercropping systems, especially in complex and elegant subsistence farming system of the dry savannas in sub-Saharan Africa[5].

Cowpea is a common food item in the southern United States where it is often called field peas. It fixes atmospheric nitrogen through its root nodules and grows well in poor soil with more than 85 percent sand and less than 0.2 percent organic matter. It is an annual crop that can be planted as cover crop to check erosion and can also be used as forage crop [4].

Some cowpea varieties have creeping stems, while others are upright in growth. The seed colour may be white, mottled-brown, red, black or purple. Ife brown, FARV-13, Farin juda, Alabama, Kwara, Kudi as well as New Era

Bombay C70, C725 and C1346 are common varieties of cowpea grown in West African countries. Other varieties include black-eyed, pink-eyed, purple hull, brown-eyed pea, Crowder pea, cream [7, 8].

Vigna unguiculata is one of the major sources of protein in the diets of both human and animals. It is mainly grown for food. Its leaf and the green pod are used as salad and vegetable. The harvested seeds can either be boiled or roasted in many ways for human consumption. The seeds are usually ground and cooked in form of doughnut or fried in oil in form of balls like bean cake. It is also used in preparing soups as well as in livestock feed [2].

Cowpea performs better on well drained sandy loam soils, but can also do well on sandy or clay soils. The seeds are planted on mounds, ridges or on flat seed beds. They are manually sown at three seeds per hole of 3-4 cm deep. Cowpea is planted between August and September in southern Nigeria and between July and August in the North where it can be grown two times in a year [3].

A number of disease and insect pests are reported as major problems contributing to the low yield of the crop. Insects like helisthus, aphids, green stick or vegetable bugs, lasser constalk borer, myriads, Mexican bean bettle, bean leaf beetles, cowpea curculios, flower thrips and weevils (when in storage) are possible insect pests. Diseases which attack and cause low yield include Fusarium wilt, bacteria canker, southern stem blight, cowpea mosaic virus, *Cercospora* leaf spot, rust and powdery mildew and damping off disease, root knot disease, which also is a severe root disease induced by root knot disease, which also is a severe root disease induced by root knot nematodes (*Meloidygyne spp*.), which cause damage to cowpea [10].

Macrophomina phaseolina is a soil-borne pathogen which causes damping-off disease (charcoal rot) of many young plants. Infestation of this organism results into the destruction of young plants; it affects the leaves, roots, stems and pods .Researchers have adopted different control measures against this infestation and crop breeders have developed improved varieties, which show high level of tolerance to the pathogen by treating the seeds with fungicide, practicing crop rotation, shifting cultivation and complete destruction of infected plants [3].

Fertilizers are used to boost plant vigour and act as resistance mechanism to diseases, but they are rather expensive and not within the reach of the farmers. Hence, an alternative measure had to be taken – a measure that will be environmentally friendly and leaves no residual effect on plants, environment and the consumers.

Arbuscular mycorrhiza fungi (AMF) are used to control diseases and improve crop yield. They are also known to increase P uptake, enhance the uptake of other nutrients by the root system and thus are beneficial in biological nitrogen fixation of rhizobium, biological control of root pathogen and drought [6]. AMF are one of the more important groups of soil organisms that play critical role in protection against pathogen. The evidence suggests that the organic farming system of AMF with greater crop resulted in enhanced nutrient uptake and therefore, is used as a substitute to reduce the use of fertilizer [9].

Compost (poultry based manure) is organic manure in the soil that enhances symbiotic relationship between microorganisms in the soil and is effective in amending the soil. It also improves organic carbon content as well as phosphorus and potassium levels of the soil [5].

The reduction in the occurrence of diseases in mycorrhiza field is a factor that needs consideration in integrated systems for pest management in agricultural production. To overcome damping off disease in cowpea, the use of *Arbuscular mycorrhiza (Glomus deserticola*) single super phosphate and poultry manure was tried on this crop. Therefore the objective of this study was to compare the effect of poultry-based compost, single super phosphate and *Arbuscular mycorrhiza* on damping-off disease of cowpea by *Macrophomina phaseolina*.

2. Materials and Methods

The experiment was conducted in the screen house of the Institute of Agricultural Research and Training (IAR & T), Nigeria. *Arbuscular mycorrhiza*, single super phosphate (SSP) and compost were used at 500kg ha^{-1}, 2250 kg ha^{-1} and 150kg ha $^{-1}$ respectively. For control pots, there were *Macrophomina phaseolina* infested and non-infested pots. This resulted into five (5) treatments. The treatments were:

i. Control + Macrophomina phaseolina
ii. Control – Macrophomina phaseolina
iii. Arbuscular mycorrhiza at 500 kg ha^{-1}
iv. Compost (poultry base) at 2250 kg ha^{-1}
v. Single super phosphate (SSP) at 150 kg ha^{-1}

The experiment was laid out in a Completely Randomized Design (CRD) with three replicates. The soil used for this experiment was prepared by sterilizing at 270°C using hot air method, and was allowed to cool before filling into 10kg plastic pots. The plastic pots were arranged at 30 x 70 cm spacing. Soil samples were randomly taken from the sterilized soil for pre-planting analysis.

Variety of cowpea seed planted were Ife Brown, which were obtained from the seed store of the Institute of Agricultural Research and Training (I. A. R. & T.), Ibadan and planted at 3 seeds per pot. They were later thinned to 2 seedlings per pot at one week after germination. *Arbuscular mycorrhiza*, single super phosphate (SSP) and compost were randomly applied into pots prior to planting. Management practices such as weeding, staking, thinning were duly carried out.

Data collected were on growth parameters including germination percentage at 7, 14, 21, 28 days, number of leaves at 2, 4, 6, 8 weeks after planting, number of pods per plot and weight of pods. Soil samples were taken for post-harvest analysis to ascertain its nutrient contents. All data were subjected to statistical analysis using SAS 2000 (version 8), while means were separated using Duncan Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Physical and Chemical Properties of Pre-Planting Soil Analysis

The physical properties of the soil analyzed that the soil was 775.2g kg^{-1} sand, 155.6 g kg^{-1} clay and 69.2 g kg^{-1} silt in texture. The soil pH value was 5.9, which was slightly acidic. The available phosphorus level was 7.4 mg kg^{-1}, which was low for the planting of cowpea according to [11] who reported that below 10 mg kg^{-1} is low for planting cowpea. Organic carbon, organic matter and nitrogen in the soil were 124 g kg^{-1}, 215 g kg^{-1}, and 15 g kg^{-1} respectively (Table 1).

Table 1. *Physical and chemical properties of pre-planting soil sample.*

Parameter	Value
pH	5.9
N (g kg^{-1})	15
Na (cmol kg^{-1})	0.57
Av. P (mg kg^{-1})	74
K (cmol kg^{-1})	0.36
Organic carbon (g kg^{-1})	12.4
Ca (cmol kg^{-1})	0.95
Mg (%)	0.77
Organic matter (g kg^{-1})	215
Sand (g kg^{-1})	775.2
Silt (g kg^{-1})	69.2
Clay (g kg^{-1})	155.6

3.2. Effects of Poultry- Based Compost, Single Super Phosphate and Arbuscular mycorrhiza on the Growth of Cowpea

Table 2. *Comparative effect of compost (poultry based), single super phosphate and Arbuscular mycorrhiza on the germination percentage of cowpea at 7, 14, 21, and 18 days.*

Treatments	7 days	14day	21 days	28days
Compost (poultry based)	100.0a	50.0a	100.0a	100.0a
Single super phosphate	75.0ab	50.0a	100.0a	100.0a
Arbuscular mycorrhiza	75.0ab	50.0a	83.3a	83.3a
Control + M. phaseolina	50.0ab	25.0b	50.0b	50.0b
Control – M. phaseolina	50.0ab	50.0a	75.0a	75.0a

Means with the same letters on the same column are not significantly different from each other at $P < 0.05$.

The germination percentage of cowpea at 7, 14, 21, 28 days after planting (DAP) as shown in Table 2 were significantly different from each other. Compost at 7 DAP had the highest germination percentage, while SSP and AM had the same value. There was no significant difference in the germination percentages of the control, plants with *M. phaseolina* only, the compost and Single Super Phosphate treated pots at 7 DAP. At 14 DAP germination percentage of compost treated plants became low compared to germination percentage at 7 DAP this is probably due to the fact the compost was releasing its minerals into the soil and this at first had shocking effect on the seeds and seedlings. Also at 14 DAP, there was no significant difference in all the treatments except for the *M. phaseolina* treated only plants which reduced from 50% germination to 25% due to the effect of the pathogen on the seedlings. The 21 and 28 DAP; compost and SSP had the highest germination percentage, while the control with *M. phaseolina* had the least germination percentage. However, Control without *M. phaseolina* had better germination percentage than that of *M. phaseolina* starting from 14, 21, and 28 DAP. This revealed that the *M. phaseolina* had negative effect on the germination of cowpea but, can be control with the use of either Compost (poultry based) or Single Super Phosphate for better germination.

3.3. Effect of Compost (Poultry Based), Single Super Phosphate and Arbuscular mycorrhiza on the Number of Leaves of Cowpea at 2, 4, 6, and 8 Weeks After Planting (WAP)

The numbers of leaves of plants treated with the 5 treatments were significantly different ($P < 0.05$) different at 2, 4, 6 and 8 WAP. Plants treated with poultry-based compost had the highest number of leaves at 2WAP. At 4WAP, the leaves treated with SSP increased and had the same number of leaves with compost followed by AM at 4 WAP. The control with *M. phaseolina* had low number of leaves when compared with the control without *M. phaseolina*. At 4, 6 and 8 WAP, there was no significant difference in the number of leaves of plants treated with Compost, Single Super Phosphate and AM. This means that any of the three can be used against *M. phaseolina*, better growth and yield. Due to high cost and unavailability of inorganic fertilizer (Single Super Phosphate) to farmers, the use of AM or compost is recommended due to their ecofriendly potentials and effectiveness in the control of *M. phaseolina*. In all, compost had the highest number of leaves (Table 3). The higher number of leaves as observed gave room to the plants for photosynthesis to take place which in helped in the manufacture of food for the plants to enhance good nutrient and high vigor for better yield.

Table 3. *Comparative effect of compost (poultry base), single super phosphate and Arbuscular mycorrhiza on the number of leaves of cowpea at 2, 4, 6 and 8 weeks after planting (WAP).*

Treatment	2WAP	4WAP	6WAP	8WAP
Compost (poultry base)	6.00	11.33a	21.00a	31.67a
Single super phosphate	5.00	11.00a	19.00ab	28.67a
Arbuscular mycorrhiza	5.00	10.00a	18.00ab	27.67a
Control + M. phaseolina	4.00	7.00b	10.67c	14.00c
Control – M. phaseolina	4.60	8.00ab	12.33bc	20.00ab
	ns			

Means with the same letters on the same column are not significantly different from each other at $P < 0.05$.

3.4. Incidence and Severity of M. phaseolina that Affected Cowpea Plants Per Treatment

The effect of these treatments on the incidence and severity of damping off disease revealed that control with *Macrophomina phaseolina* had the highest incidence (33.3%) and severity (3.3) at which the pathogen affected cowpea plants (Table 4). The table indicated 50% of the plant was affected. However, *Arbuscular mycorrhiza* (AM) had the highest effect on pathogen, the incidence was 18.0% and severity was 1.0. This showed that AM control the occurrence of the disease greatly, which is in agreement with [9] who reported that *A. mycorrhiza* is one of the most important groups of soil organism apart from it playing critical role in the protection against pathogen. At 6 and 8 WAP (though not reflecting on the table because there was no in value of the incidence and severity from what was observed for 4 WAP), the soil-borne disease was very severe on the control with *M. phaseolina* and least severe on the control with *M. phaseolina* followed by control without *M. phaseolina* and less severe with *A. mycorrhiza* and SSP at 8WAP. This shows that there were no significant differences in the mean numbers of incidences and severity. To control *M. phaseolina* on cowpea plant AM is recommended for use.

Table 4. *Incidence and severity of M. phaseolina on cowpea plants in response to the treatments applied.*

	Incidence		Severity	
Treatment	**2WAP**	**4 WAP**	**2 WAP**	**4 WAP**
Compost (poultry base)	50.00a	33.67a	3.67a	1.66ab
Single super phosphate	38.00ab	18.00ab	2.00ab	1.00ab
Arbuscular mycorrhiza	36.00ab	18.00ab	1.67ab	1.00ab
Control + M. phaseolina	66.67a	33.33a	3.67a	3.33a
Control – M. phaseolina	50.00a	33.33a	3.00a	2.33a

Means with the same letters on the same column are not significantly different from each other at $P < 0.05$.

3.5. Effect of Compost (Poultry Based), Single Super Phosphate and Arbuscular mycorrhiza on Growth Yield of Cowpea

The mean number of pods produced that was highest among all treatments was the application of rate of 455kgha^{-1} of SSP. The number of pods of 11 was observed for SSP, this was followed by mean of 10.33 for AM treated plants, this was not significantly different from plants treated with *A. mycorrhiza*, but significantly different from all other treatments (Table 5). The least was the control + *M. phaseolina* which had an average of 4.33 pods. From all indications, the lower the number of pods the lower the yield, and the higher the number of pods the higher the yield, all things been equal. With respect to the weight of harvested pods of cowpea per plant, plants treated with SSP produced significantly highest yield per plant than the control and other treatments in this experiment (11.0g per plant and 455 kg ha^{-1} (Table 5).

Table 5. *Yield parameters of cowpea in compost (poultry based), single super phosphate and Arbuscular mycorrhiza treated soils.*

Treatment	No. of pods /treatment	Weight of pod	No. of pods per ha (kg)
Compost (poultry base)	6.67^{b}	6.27^{bc}	278.67^{ab}
Single super phosphate	11.00^{a}	10.26^{a}	455.00^{a}
Arbuscular mycorrhiza	10.33^{a}	8.01^{ab}	356.00^{a}
Control + *M. phaseolina*	4.33^{c}	2.57^{c}	115.11^{c}
Control – *M. phaseolina*	5.67^{bc}	4.82^{c}	214.22^{b}

Means with the same letters on the same column are not significantly different from each other at $P < 0.05$.

It was observed by [1] that P is essential for flowering and pod filling formation that sometimes large quantity of P may be present in the soil, but not available to the growing plant because it is insoluble in such situation therefore the use of AM is recommended for plant to use the soil available P that is essential for cowpea growth, which AM is good at. Also, since there was no significant difference in compost, SSP and AM treated plants, it is better to adopt the use ecofriendly AM to overcome the stress of procurement of the other two.

3.6. Results of Post-Harvest Soil Analysis on Some Elements

The values of Ca content of post-harvest soil samples of *A. mycorrhiza* recorded highest significantly value of 1.02%, but Mg values were not significantly different among the treatments. Na value was significantly lower in control than with *M. phaseolina* (3.4 g kg^{-1}); K (g kg^{-1}) and P (100.5 g kg^{-1}) were significantly higher in *A. mycorrhiza* than other treatments. The low P value (0.08g kg^{-1}) recorded in control with *M. phaseolina* proved the ability of AM to encourage the absorption of P on soil as reported by [9] that AM is very effective in helping plants absorb P from soil, which invariably prevents P runoff that leads to eutrophication (undesired biological growth and productivity) because agriculture is a source of phosphate pollution in the environment. Increased P uptake by mycorrhiza can help to reduce the quality of this nutrient added to soil and decrease accumulated P soil and water (Table 6).

Table 6. *Result of post – harvest analysis of the soil with the different treatments.*

Treatment	Ca	Mg	Na	K	P	pH
Before planting soil	0.95^{a}	0.77^{ab}	0.57^{b}	0.36^{a}	7.40^{c}	5.90^{ab}
Arbuscular mycorrhiza	1.02^{a}	0.84^{a}	0.63^{a}	0.46^{a}	10.05^{d}	6.20^{ab}
Compost (Poultry based)	0.95^{a}	0.77^{ab}	0.57^{a}	0.36^{a}	6.85^{c}	5.96^{ab}
Single super phosphate	0.93^{a}	0.73^{ab}	0.55^{a}	0.31^{a}	4.25^{c}	6.08^{ab}
Control + *M. phaseolina*	0.84^{ab}	0.63^{b}	0.34^{ab}	0.14^{ab}	0.80^{c}	6.01^{ab}
Control – *M. phaseolina*	0.84^{ab}	0.69^{b}	0.48^{ab}	0.29^{ab}	6.85^{ab}	6.56^{a}

Mean of the same letters on the same column are not significantly different from each other at $P < 0.05$.

For the first 7 days of planting, germination percentage of cowpea in various treatments were encouraging, even up to the 28 days after planting, except for plants treated with *M. phaseolina* that had stunted growth. At 21 days after planting, the effect of pathogen became visible. At 4 WAP, plants with SSP fertilizer had the highest number of leaves and were greener than other plant treatments. At 6WAP, there were different symptoms on all the treated plants, but less in A. mycorrhiza and SSP. Other treatments affected by pathogen were severe: some of the leaves developed brown spots and later dropped off. The dropping or wilting of leaves was mostly severe on controls with and without *M. phaseolina*, which showed that planting of cowpea without treatments such as these would lead to great reduction of yield through wilting of leaves and undeveloped flowers (Tables 3 and 4).

The cumulative growth, yield and weight of pods results revealed that SSP fertilizer gave the highest yield of cowpea. However, SSP fertilizer are rather expensive and may not be within the reach of the farmers, hence alternative measure that will be environmental friendly and leave no residual effect on plants, the environment and consumers. *A. mycorrhiza* can give such result.

4. Conclusion

The experiment was carried out to evaluate the effect of *A. mycorrhiza*, single super phosphate (SSP) and compost on the damping off disease of cowpea. From the results obtained, the following observations were made: plants treated with SSP at 150kgha^{-1} produced higher cumulative harvested pods per treatment, but was not significantly different from other treatments. However, among other treatments, plants treated with *A. mycorrhiza* were next to SSP, which had higher cumulative harvested pods of 356kgha^{-1}. This confirmed the report of [1], that P is essential for flowering and pod filling formation and that even though large quantity of P may be present in the soil, they may not be available to the growing plant because it is insoluble in such situation hence *A. mycorrhiza* can be used. The use of 150kgha^{-1} of SSP is therefore recommended for farmers since most agricultural crops perform best at this level of application of fertilizer.

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