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Application of Organic Amendments and Botanical Foliar Sprays against Bacterial Diseases of Mungbean (*Vigna radiata* L.) in South Eastern Nigeria

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ABSTRACT

A Field trial was conducted at the Eastern farm of Michael Okpara University of Agriculture, Umudike, during the 2012 farming season to assess the potentials of some organic soil amendments and some botanicals as foliar sprays in the control of some common bacterial diseases associated with mungbean (*Vigna radiata* L.) which include bacterial blight, halo blight, leaf spot and bacterial wilt. The soil organic amendments employed as control measure included leaves of *Azadirachta indica*, *Delonix regia*, poultry droppings and kitchen ash while the plant extracts that served as foliar sprays in control of these bacterial diseases were: neem seed (*A. indica*), ginger stem (*Zingiber officinale*) and bitter kola seed (*Garcinia kola*), water was used as a control while streptomycin sulphate served as a standard check. *A. indica* and *D. regia* leaves were first allowed to decompose before application as organic amendments. All the experiment was in a randomized complete block design (RCBD) and replicated three times. Results obtained showed that organic amendments sourced from kitchen ash improved growth of mungbean, sustained total pod yield and suppressed disease incidence and severity. Foliar spray trials results showed that *A. indica* was the best in enhancing growth, yield (seed weight) and most effective in reducing disease incidence and disease severity more than other plant extracts.

INTRODUCTION

Mungbean (*Vigna radiata*) is a food legume that is very rich in protein and essential amino acids with the exception of the sulphur amino acids, methionine and cysteine which may be nutritionally limited. It is a good source of soluble carbohydrate, and contains very high amount of crude fiber (Duke, 1983; Onimawo and Egbekun, 1998). However, this all important food crop is beset by a number of bacterial diseases namely: bacterial blight, halo blight, bacterial wilt and bacterial spot which result in yield loss.

Bacterial blight caused by *Pseudomonas syringae* is an important disease of Mungbean. The presence of *P. syringae* reduces the profitability of Mungbean due to yield losses. *P. syringae* is primarily a seed-borne pathogen. Similarly halo blight caused by *P. savastanoi* pv. *Phaseolicola* is another important bacterial disease of mungbean that is spread mainly by rain splash and symptom takes 7-10 days to appear after infection, causing an extensive yellowing and halo surrounding a small (1-2mm) dark, water-soaked (shiny) spot. On infected pods, a shiny circular lesion develops and a cream-coloured drop containing millions of bacterial cells may ooze out from the lesion (Varma *et al.*, 1992).

Bacterial spot is caused by *Curtobacterium flaccumfaciens* and is often present at low level in most crops. Symptoms consist of large irregular, dry, papery lesions on leaves that coalesce to form large brown dead tissues. These areas dry out to a tan colored patch which may tear and fall out, giving the leaf a ragged appearance. The disease is a seed borne and may occur as early as the seedlings cotyledonary stage (Shih, 2005).

Bacterial wilt caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*). The key symptom is wilt, being more rapid in younger plants. Adventitious roots form on the lower stem to compensate for blockages caused by the extensive colonization of the bacterium in the vascular tissue. Plants may be stunted and chlorotic. The pith of wilted plants can be water soaked, brown or hollow. Plants may wilt entirely over days and the rapid wilting also distinguishes this from fungal wilt. The disease can also be distinguished from other wilt disease by placing infected tissue, freshly sectioned into water to observe masses of bacterial ooze streaming out. The pathogen infects root through wounds and colonizes the vascular tissues causing blocking of the vascular bundles which are the xylem and phloem (Varma *et al.*, 1992).

These bacterial diseases have become major constraints in production of Mungbean especially in the South-eastern Nigeria (Thakur *et al.*, 1997; Basandrai *et al.*, 1999). Though bacterial incidence can be reduced using an integrated pest management (IPM) strategy by incorporating crop rotation, pathogen-free seeds, crop hygiene, chemical seed treatment and application of

foliar bactericides yet these have been found to be of limited use in control of this disease (Mayeux, 1992). This paper therefore aims at using some available soil organic amendments and botanicals as foliar sprays in reducing the incidence and severity bacterial diseases and improving yield of Mungbean in South Eastern ecological zone of Nigerian.

MATERIALS AND METHODS

Land Preparation

An experimental trial was conducted during the months of July-September 2012 in the Eastern farm of Michael Okpara University of Agriculture, Umudike, which is located within the lowland Rainforest and between latitude 05°29N, longitude 07°32E and mean elevation of 122m above sea level.

Before the land preparation analysis characterization of the soil was carried out by collecting some soil samples randomly from the experimental field in a diagonal pattern and the samples taken to the Soil Science laboratory of the university to determine the soil composition and characteristics. After which the land was cleared then ploughed and harrowed into ridges.

Seed Planting and Agronomic Practices

Each bed measured 4m x 6m with a population of 900-950 seedlings per treatment. Seeds were planted three per hole with spacing of 20cm x 20cm between seeds and 1m between beds, seed were placed in the soil at the depth of about 1.5cm. Seedlings were later thinned down to two per stand two weeks after seed emergence i.e. at three- leaf stage (Cheng, 1990). Weeding of the experimental plots was done two weeks after planting (WAP) and repeated two week thereafter. The weeding was done manually by hoeing and rouging of the plots. Mungbean variety used for all the trials was NM-94. Other routine agronomic practices were also observed (Opeke, 2006; Agugo and Opara, 2008).

Preparation of Organic Amendments: Soil organic amendments materials used were as follows; *A. indica* leaves, *D. regia* leaves, Poultry droppings, Kitchen ash, and Streptomycin sulphate (synthetic antibiotic) and a control (without any treatment) Before the application of these materials (except streptomycin). For effective results the organic manure (animal dung and plant materials) were first left for two week before planting for proper decomposition and assimilation of nutrients into the soil. After decomposition 3.50kg of each of the product was weighed out (except Streptomycin sulphate which weighed 3.0g of product) so that each treatment received 3.3kg in three- split application of the product at two weeks intervals.

The products (the organic amendments) were applied three weeks after planting using side dressing method of application due to increased number of stands and the close spacing (Walter, 1990).

Plant botanicals: The plant extracts employed were; Neem (*Azadirachta indica*), Ginger (*Zingiber officinale*), Bitter Kola (*Garcinia kola*) and the control (streptomycin sulphate and water). 50g of the plant parts (*A. indica*, *Z. officinale* and *G. kola*) were weighed washed and air-dried which was later ground into powder using a mechanical hand grinder. After grinding, each plant parts powder was dissolved in 100ml of sterile water and left for 48hrs. Suspension of each plant extract was filtered using cheesecloth and the filtrate diluted to 50% w/v before being used as foliar sprays.

Application of foliar sprays was by means of spraying the aqueous plant extracts with a hand spray (atomizer) on the leaves of the crops until there was a runoff, the crop stems and branches were also sprayed thoroughly three weeks after planting.

Thus the formula:

$$\text{Percentage (\%)} \text{ Disease Incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants sampled}} \times \frac{100}{1}$$

All data collected were analyzed using analysis of variance (ANOVA) of SAS 2009 model while the treatment means were separated using Fishers Least Significant Difference (LSD) at probability of 5% ($P \geq 0.05$) according to Steel and Torre (1981).

RESULTS

The data on Soil analysis and Characterization showed that the soil was a Loamy sand poor in organic carbon

Data Collection and Analysis

Data were collected at 2 weeks after application of treatment, based on the following parameters; length of vine(cm) number of branches, number of pods, weight of the pods per plant (g), weight of the seeds per hectare (kg). Also disease severity ratings were recorded on a 1-6 scale as stated below;

- 0= No disease symptoms
- 1= Two or few spots scattered on the leaf
- 2= about $\frac{1}{4}$ (25%) of the leaf affected
- 3= about $\frac{1}{2}$ (50%) of the leaf affected
- 4= about $\frac{3}{4}$ (75%) of the leaf affected
- 5= Entire leaf surface affected or leaf almost dead

Collection of all data were made two weeks after application of treatments at two weeks interval and data was collected by obtaining the number of plants that were affected by the disease over the total number of the plants that were not affected, multiply 100:

and nitrogen and so affected crop performance significantly (Table 1). The result on organic amendments had no significant effect on number of branches two weeks after application (Table 2). However, as time progressed, there were significant differences when compared with the control experiments from the data regarding number of branches. For instance kitchen ash significantly produced plants with more number of pods (15.67cm) which were as good as those of standard chemical, streptomycin (18.02cm) which differed ($P \geq 0.05$) from the control plants (Table 3).

Table 1: Analysis and characterization of the soil sample of Umudike farm

Soil	Characteristics
Texture	Loamy sand
Sand	61.33
Silt (%)	6.00
Clay (%)	32.66
Organic carbon (%)	1.35
organic matter(mg/kg)	15.0
Nitrogen (%)	0.11
Ca (cmo/kg ⁻¹)	2.93
Ma (cmo/kg ⁻¹)	1.26
K (cmo/kg ⁻¹)	0.16
Na (cmo/kg ⁻¹)	0.23
P (cmo/kg ⁻¹)	5.20
Ex. acidity (cmo/kg ⁻¹)	1.10
ECEC (cmo/kg ⁻¹)	5.69
pH (H2O)	4.70

On the vine length (cm) from two weeks up to four weeks after treatments (WAT) the data showed significant ($P \geq 0.05$) differences in treatments. At two WAT all the treatments had significant increase in vine lengths with the exception of *A. indica* + *D. regia* that had shorter length (44.30cm) and these differed statistically ($P \geq 0.05$) from others. Regarding number of pods there was significant ($P \geq 0.05$) improvement with all treatments when compared with the control. However, the highest mean pod number per plant was recorded with plants treated with kitchen (20.00) ash which

showed superiority ($P \geq 0.05$) over all other treatment. While in terms of seed weight (Table 4) it was noted that despite the low performance of *A. indica* + *D. regia* initially at two, four and six WAT it still produced highest seed weights of 1040.93kg per plot at the end of total harvest followed by kitchen ash treated plants 1030.65kg. It was also observed that the total seed weights produced by poultry dropping and antibiotics treated plants did better than the control which consistently produced crops with the lowest seed weights (Table 4).

Table 2: Effect of Soil Organic Amendments on Growth Parameters of Mungbean (4 WAT)

Treatments	No. branches /plant	Vine length /plant	No. Pod /plant
<i>A. indica</i> + <i>D. regia</i>	8.27	27.40	3.40
Poultry dropping	8.90	36.40	4.98
Kitchen ash	7.90	35.50	1.57
Streptomycine Sulphate	9.40	33.15	0.40
Control	8.70	34.75	1.17
LSD ($P \geq 0.05$)	NS	3.32**	0.87**

**= Highly Significant at 5% Probability; NS= Not significant at 5% Probability

WAT = Weeks after Treatments

Table 3: Effect of Soil Organic Amendments on Growth Parameters of Mungbean (8 WAT)

Treatments	No. branches /plant	Vine Length (cm)/plant	No. Pod /plant
<i>A. indica</i> + <i>D. regia</i>	14.83	58.25	12.49
Poultry dropping	13.60	55.55	13.65
Kitchen ash	15.20	54.55	20.16
Streptomycine Sulphate	18.02	49.15	18.20
Control	11.67	44.30	8.10
LSD ($P \geq 0.05$)	2.01**	3.63**	1.20**

**= Highly Significant at 5% Probability; NS= Not significant at 5% Probability

WAT = Weeks after Treatments

The effect of different organic treatments on the disease incidence and disease severity of mungbean is presented in Tables 5 and 6. Significant differences were observed on disease incidence; kitchen ash treated plants consistently produced the best results with the least percentage disease incidence from the fourth to

eight weeks after treatments and a mean disease incidence of 17.94% when compared with the control (40.45%). In terms of disease severity however, there was no differences, however, at the eight week after treatment, significant ($P \geq 0.05$) differences were recorded between all treatments and the control (Table 6).

Table 4: Effect of Soil Organic Amendments on Seed weight of Mungbean at harvest (4-10 WAT)
Seed weight

Treatments	4wks (g)	6wks (g)	8wks (g)	10wks (g)	Tot pod wt/Plt (g)	Seed wt /ha (kg)
<i>A. indica</i> + <i>D. regia</i> leaves	57.7	82.2	128.7	151.1	419.7	1040.93
Poultry dropping	39.4	47.7	79.6	127.2	293.9	730.48
Kitchen ash	48.3	51.2	137.3	177.8	414.6	1030.65
Streptomycin sulphate	26.8	48.3	97.7	138.9	311.7	770.96
Control	33.0	41.4	61.0	88.9	224.3	560.10
LSD(P≥0.05)	8.22**	7.79	36.56**	43.31**	69.29**	170.32**

** = Highly Significant at 5% Probability; NS = Not significant at 5% Probability

WAT = Weeks after Treatments

Table 5: Effect of Soil Organic Amendments on Disease incidence of Mungbean (2-8 WAT)
Disease Incidence

Treatments	2Wks (%)	4Wks (%)	6Wks (%)	8Wks (%)	Mean% Dis. Inc
<i>A. indica</i> + <i>D. regia</i> leaves	11.67	20.00	23.37	46.37	25.35
Poultry dropping	8.37	15.00	18.37	42.67	21.10
Kitchen ash	5.00	8.37	18.37	40.00	17.94
Streptomycin sulphate	6.70	15.00	18.37	35.00	18.77
Control	8.37	16.70	20.00	61.67	40.45
LSD(P≥0.05)	2.52**	2.01**	NS	9.0*	4.51*

* = significant at 5%; ** = highly significant; NS = Not significant at 5% alpha level.

Table 6: Effect of Soil Organic Amendments on Disease severity of Mungbean (2-8 WAT)
Disease severity

Treatments	2Wks	4Wks	6Wks	8Wks	Mean Dis. Sev.
<i>A. indica</i> + <i>D. regia</i> leaves	3.17	3.40	3.60	3.37	3.32
Poultry dropping	3.17	3.57	3.67	3.90	3.37
Kitchen ash	3.00	3.37	3.53	3.67	3.37
Streptomycin sulphate	3.07	3.30	3.37	3.50	3.39
Control	3.17	3.77	3.87	4.83	3.92
LSD(P≥0.05)	NS	NS	NS	0.42*	0.40*

* = significant at 5%; ** = highly significant; NS = Not significant at 5% alpha level.

Table 7 shows that *A. indica* had the best effect on all parameters tested with regard to the foliar sprays on disease of mungbean: *A. indica* aqueous extract achieved the best reduction of the disease severity and disease incidence (1.63 and 28.22% respectively), which

differed significantly from the control $P < 0.05$. Also *A. indica* had the highest score on number of pods (23.20g), and seed weight (41.68g) per plant which was significantly better than control (8.6g and 2206g respectively).

Table 7: Effect of Foliar Sprays on Disease, Growth and Yield of Mungbean

Treatments	Disease severity	Disease Inci. (%)	No. of branches	Vine length (cm)	No. of pod/plt	Seed wt /plt (g)	Seed wt /ha (kg)
Neem	1.63	18.22	17.40	53.00	23.20	41.68	1660.72
Ginger	1.86	20.99	16.60	49.00	22.00	33.64	1340.56
G. Kola	1.98	25.78	15.40	49.80	18.20	39.80	1590.20
Streptomycin	1.82	25.17	16.00	51.60	16.60	28.96	1150.84
Control	3.82	56.16	1380	45.40	8.60	22.06	880.24
LSD $P \leq 0.05$	0.72*	9.23**	NS	NS	3.74**	10.74**	26.85**

*, ** = significant at 5% and highly significant at 5% alpha levels respectively. NS = Not significant at 5%.

DISCUSSION

From the results available Kitchen ash treatments were better as it was able to sustain the crops resulting to promotion of the yield and growth of mungbean more than any other treatment applied during the period of this study. Messiaen (1992), observed that kitchen ash was be able to suppressed the disease; and according to the Bailey and Lazarovits (2002) kitchen ash is rich in nitrogen and may have effect on soil borne disease by releasing allelo-chemicals generated during product storage. Kitchen ash may also have reduced the acidity in the soil thereby making nutrients in the soil more available to the plant resulting in high seed yield. It was also observed from the results obtained that Kitchen ash increased growth parameters in addition to suppressing disease incidence and severity of mungbean. The implication of this result may be that the treatment selectively induced partitioning of plant nutrients to the pods in preference to vegetative growth of the plant.

The results further indicated that bactericidal potential of the active ingredient (azadiractin) of *A. indica* which inhibits the spread of bacterial pathogen which was demonstrated when Williams (2005) used compost manure to control bacterial wilt of tomato caused by *Ralstonia solanacearum* as the survival of the pathogen was much higher in the control experiment. This is in conformity with similar work done by Koki and Tajul (2003) where extracts from *A. indica* was used to prevent the growth of *Aspergillus niger* in grapes. *A. indica* is one of the most promising species for consideration as potential grain protectant and the oil spray can help prevent bacterial disease of crops (Williams, 2005).

From the result, it appeared that all the plant extracts used as foliar sprays had the potential to reduce disease severity of bacterial diseases of mungbean (*V. radiata*) when compared with the control. However, *A. indica* leaves proved to be the most superior because it possesses the ability to inhibit the development of bacterial leaf spot disease of *V. radiata*, which is similar to the work done by Balm (2003) where he used *A. indica* extracts to control bacterial blight disease of cucumber.

According to the work done by Williams (2005) plant extracts are systemic in function like some known bactericides and has significant effect on the pathogen resulting in most cases to the bactericidal activity which in turn lead to significantly increased growth parameters and crop yield. Inhibition of bacteria disease and control of the pathogen in potted *V. radiata* has been demonstrated by other workers in previous work (Balm, 2003; Koki and Tajul, 2003).

Various workers have also emphasized the advantage and potential of botanicals wood ash in controlling plant disease (Gould, 2005; Pfleger, 2008). Although chemical bactericides may be effective in control of bacterial diseases but of the hazards which

they pose to environment tend to discourage farmers (Pfleger, 2008). Therefore, the need for pollution free and cheaper alternative control measures like the use of kitchen ash and plant extracts for resource poor farmers is a welcome development.

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