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Assessment of the effect of rate and time of application of rice-husk powder as an organic amendment on cowpea (*Vigna unguiculata L.,walp*) inoculated with cowpea mottle virus

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ABSTRACT

A Screen house experiment using potted plants was carried out to assess the effects of time and rate of application of Rice-husk powder as an organic amendment on cowpea (*Vigna unguiculata* L Walp) cv. Ife Bimpe, that were mechanically inoculated with Cowpea Mottle Virus (CM_eV). The results showed that the organic amendment was very effective on plants with soils amended with rice-husk powder, as the plants exhibited better yields and productivity. The amended plants also showed considerable less susceptibility to the virus pathogen compared to the non-amended plants. The results of the experiment further showed that the rate and time of application of the Rice-husk powder was a key factor in the ameliorative effect of this organic amendment in the suppression of the viral inoculum. An application rate of 0.50kg of Rice-husk powder per 10kg of soil two weeks before sowing the cowpea seeds was observed to confer on the plants the highest growth and yield attributes and also the least susceptibility to Cowpea Mottle Virus (CM_eV). On the other hand, cowpea sown on soils amended at a lower rate of 0.125kg of Rice-husk powder per 10kg of soil two weeks after planting were found to have higher susceptibility to Cowpea Mottle Virus (CM_eV). This is indicative that Rice-husk which is cheap, readily available and environmentally friendly offers a promising prospect in Agriculture both as an Organic amendment and in the control of virus disease in Cowpea if applied at recommended rates and time.

Keywords: Rice-husk powder, Rate and Time of Application, Inoculation, Cowpea Mottle virus (CM_eV), Cowpea cv. Ife Bimpe.

INTRODUCTION

Cowpea is important as a food crop throughout West Africa and especially in the Sudan savanna (Singh and Ntare, 1985). This crop provides food, animal feed and cash for the rural populace in addition to benefits to farmlands via *in situ* decay of roots residues and ground cover from cowpea's spreading habits. Cowpea grain provides a cheap and nutritious food for relatively poor urban communities (Quin, 1997). Its cultivation is however hampered by several setbacks such as pests and diseases (Ajibade and Amusa, 2001). Most of these pests and diseases thrive best under high relative humidity, which correlates with high rainfall pattern and atmospheric temperature that are found in humid forest of Southern Nigeria (Adegbite and Amusa, 2008).

Among the numerous pathogens affecting cowpea, viruses are known to infect cowpeas either at one stage or throughout the life of the plant. The effects of virus diseases can be devastating, and they are a major constraint to large-scale production (Singh and Rachie, 1985). Crop losses as a result of diseases caused by plant- infecting viruses, come second only to fungal diseases but plant viruses are much more difficult to control. Nearly 80 virus species occur on arable crops resulting in yield loss worth N700 million per annum (Smart, 2000). Losses due to viral infection of cowpea are between 10% - 100% (Rachie, 1985). Cowpea mottle virus (CM_eV) causes mottling or bright yellow mosaic in cowpea. Leaves are distorted, reduced in size and a witches' broom syndrome is common. Yields may be reduced by more than 75% (Jeyanandarajah & Brunt, 1993). Indeed, Cowpea yield reductions of more than 75% have been reported (Shoyinka *et al*., 1978).

Composted agricultural wastes may be employed in biological control of plant disease (Garrette, 1975). They have since been reported to suppress different types of soil -borne plant diseases (Chen *et al*, 1988; Janisiewicz and Roitman, 1988; Muhammad, 1998; Muhammed *et al.,* 2001). A soil amendment is any material added to a soil to improve its physical properties, such as water retention, permeability,

water infiltration, drainage, aeration and structure (Davis and Wilson, 2005). Schucter (1989), found that various types of agricultural/municipal wastes suppress different types of plant diseases by making plants more vigorous and better able to withstand attack.

Rice husk is the natural sheath or productive cover, which forms the cover of rice grains during their growth. Rice husk represents about 20% by the weight of the rice harvested, about 80% by weight of the raw husk is made of organic components (Anonymous, 1979). During rice refining processes, the husks are removed from grains. It is of little commercial value and because of its high silicon dioxide contents, it is of little or no use to feed either human or cattle. Incorporation of rice husk into soil mixture was found to affect many crops (Sharma *et al.,* 1988). Muhammed *et al*. (2001) observed that rice husks composted soil reduced the incidence of wilting of *Parkia. biglobosa* caused by *Fusarium. solani* in the range of between 31.4% to 70.3%.

Though there is abundant information concerning the use of agricultural wastes for soil amendment to improve crop growth and also in the control of fungal and nematode diseases, little is known about the use of these agricultural wastes to control virus diseases in arable crops. For this reason, this study is aimed at assessing the ameliorative effect of rate and time of application of Rice-husk powder as an organic amendment on Cowpea (*Vigna unguiculata* L., Walp) Cv. Ife Bimpe infected with the Cowpea Mottle Virus.

MATERIALS AND METHODS

Experimental design and plant propagation: Experiments were conducted in the Department of Crop protection screenhouse of the University of Ilorin, situated in the Southern Guinea Savanna of Nigeria, using potted plants to assess the effect of rate and time of application of Rice-husk powder as an organic amendment on Cowpea (*Vigna unguiculata* L.,walp) Cv. Ife Bimpe infected with Cowpea Mottle Virus.

The Cowpea cultivar Ife Bimpe used for the experiment was obtained from the Institute of Agricultural Research and Training, Moor plantation Ibadan, Oyo State, Nigeria. Three seeds were sown per 10-liter (50cm diameter) plastic pot filled with sandy-loam soil. Prior to potting, however, the soil was steam-sterilised at 121^{o}C for 1hour. The plants were thinned down to two per pot after germination. The three rates of application of rice-husk powder to the soil were at 0.125kg/10kg soil, 0.25kg/10kg soil and 0.50kg/10kg soil, while a non-application rate of 0kg/10kg soil served as the control. The three different times of application of the amendment to the soil were at two weeks before planting, at planting and at two weeks after planting. This gave a total of 10 treatment combinations, each was replicated three times.

Sourcing of inoculum and inoculation procedure: The Cowpea mottle virus isolate was extracted from infected leaves sourced from the stock of the Plant Virology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State Nigeria. The infected leaf sample was extracted by homogenization using pre-cooled mortar and pestle in 0.01M Sodium phosphate buffer, at pH 7.2 at the rate of 1g leaf sample to 10ml of buffer. Inoculation was done at 7days after planting by rubbing the inoculum soaked in cotton wool onto the carborundum - dusted 1st and 2nd leaves of the cowpea at the third true leaf stage. The plants and the surroundings were sprayed with cypermethrin at 4 and 6 weeks after planting to further forestall insect infestation.

Data collection and Analysis: Data were collected on weekly basis. Plant height, number of leaves per plant and number of diseased leaves per plant were taken over a period of 8 weeks after inoculation. Yield parameters such as number of pods, dry weight of pods (g) and dry weight of grain (g) were also taken at harvest. The pods were, dried and weighed with the aid of an electronic balance. Threshing was done manually before weighing to get the dry seed weights. The treatment design was a factorial fitted into a randomized complete block design (RCBD). All data were therefore, subjected to analysis of variance (ANOVA) having regards for the factorial nature of the treatment design and the significant differences between them were determined at $P < 0.05$ using the New Duncan Multiple Range Test.

RESULTS

Effect on percentage disease severity: Table 1 shows the results of analysis of variance on the combination effect of the treatments on percentage disease severity over 8 weeks. The result shows that the percentage disease severity, as measured by the number of diseased leaves relative to the total number of leaves on any given plant, was for any given week, lowest in cowpea plants amended at the rate of 0.50kg/10kg soil two weeks before planting and highest in plants amended at the rate of 0.125kg/10kg soil two weeks after planting. For instance, at four weeks after infection, the percentage disease severity was 16.2% in the cowpea plants

amended at planting at the rate of 0.125kg/10kg soil and lowest (8.9%) in plants amended two weeks before planting at the rate of 0.50kg/10kg soil. By the sixth week however there was as a general increase in disease severity for all the treatments. However, plants amended at two weeks before planting at the rate of 0.50kg/10kg soil still had the lowest percentage disease incidence (10.8%),while the plants amended at two weeks after planting at the rate of 0.25kg/10kg soil had the highest percentage disease incidence (25.6%). In all situations however, the unammended plants (control),had the significantly highest levels of disease severity compared with any of the other treatments.

Table1 :The combination of rate and time of application of rice-husk powder as an amendment on Percentage disease Severity on cowpea inoculated with CM_eV

Treatment Combination	2wks	4wks	6wks	8wks
Control (Inoculated, not amended)	0	30.1j	32.1j	38.7g
2APX0.125kg/10kg	0	25.3i	27.6i	29.3g
2BPX0.125kg/10kg	0	11.7c	14.2c	15.1c
AP X 0.125kg/10kg	0	16.2f	19.1fg	21.4e
2APX0.25kg/10kg	0	23.4hi	25.6h	26.1f
2BPX0.25kg/10kg	0	10.6bc	12.4bc	13.1b
AP X 0.25kg/10kg	0	15.8ef	17.9e	19.6de
2APX0.50kg/10kg	0	18.3g	21.1g	23.1f
2BPX0.50kg/10kg	0	8.9a	10.8a	11.1a
AP X 0.50kg/10kg	0	14.3de	16.4de	19.3d

Means within a column followed by the same letter(s) are not significantly different using the New Duncan multiple Range Test at P>0.05

Effect on Plant height: Table 2 shows the effect of the treatments on heights of infected plants. The analysis of variance showed that at the second week, plant heights were not significantly affected by rate of application of the amendment. However, by the third week post inoculation of the virus, there were significant differences in plant heights through to the 7^{th} week. An amendment with rice-husk powder at the rate of 0.50kg / 10kg soil consistently produced the tallest plants from the 2^{nd} , 3^{rd} , 4^{th}, through to the 8^{th} week post infection. It is apparent that amending the soil with rice-husk two weeks before planting generally had the most desirable effect on plant height as such plants consistently had the tallest heights. An amendment with rice-husk either at planting or at two weeks after planting did not produce any significant differences in heights of infected cowpea plants as they were not significantly different from plants infected in non-amended soil.

Evaluation of the different treatment combinations showed that significant differences existed between them. For example, at two weeks post inoculation, a treatment combination of soil amendment at two weeks before planting at the rate of 0.25kg/10kg soil gave the highest heights of 17.7cm, while amendment at planting at the rate of 0.50kg/10kg soil gave the lowest heights (3.8cm), which was not significantly different from amending 2weeks after planting at the rate of 0.125kg/10kg soil (4.8cm). This trend continued through to the fourth week. By the 5^{th} to the 8th week post infection, however, the treatment combination of amendment at two weeks before planting at the rate of 0.50kg/10kg soil produced the tallest plants.

Effect on number of leaves: Table 3 shows the effect of the treatments on number of leaves. Analysis of the result indicates that it is similar to the pattern observed earlier on as with the plant heights. From the second to the third week after inoculation, there was no significant difference in the number of leaves of the cowpea plants. However, from the 4^{th} to the 8^{th} week post inoculation, an amendment rate of 0.50kg/10kg soil had the significantly highest number of leaves. The application of rice-husk as an amendment two weeks before planting also produced the highest number of leaves in plants on such amended soil. By the 8^{th} week, the combination effect of application two weeks before planting at the rate of 0.50kg/10kg soil produced the highest number of leaves (15), while an application of the amendment two weeks after planting at the rate of 0.125kg/10kg soil had the least number of leaves (13.8).

Table 2: Effect of rate and time of application of rice husk on plant height of cowpea Inoculated with Cm_eV

Rate of Application	Week2 (cm)	Week3 (cm)	Week4 (cm)	Week5 (cm)	Week6 (cm)	Week7 (cm)	Week8 (cm)
Control (Inoculated,not amended)	11.5a	15.1ab	15.9c	18.6c	19.1d	21.5d	26.5b
0.125kg/10kg	11.8a	14.2b	15.1c	19.8b	23.4c	24.9b	26.1b
0.250kg/10kg	13.2a	14.3b	16.7b	19.9b	22.2b	23.9c	26.2b
0.50kg/10kg soil	11.3a	15.9a	17.6a	21.1a	23.7a	25.6a	28.3a
S.E	0.8	0.52	0.48	0.94	1.0	1.02	0.74
Time of Application							
2wksAP	10.2b	13.3b	14.9b	18.3b	20.4b	22.1b	24.9b
2wksBP	15.4a	18.9a	20.3a	23.5a	25.5a	28.7a	31.2a
At planting	10.9b	12.4b	13.8b	17.7b	20.3b	21.1b	24.2b
S.E	0.66	0.45	0.42	0.81	0.88	0.88	0.64
Rate X Time of Application							
2wksAP/0.125kg/10kg	4.8d	10.3g	10.7e	14.6ef	20.4bcd	21.5de	8.2c
2wksBP/0.125kg/10kg	16.4a	18.0bc	19.9c	24.9abc	25.7ab	27.4bc	10.4ab
Atplting/0.125kg/10kg	14.0abc	13.7ef	14.6d	19.8cde	24.1abc	25.8bcd	10.9ab
2wksAP /0.25kg/10kg	10.0c	12.4fg	13.6de	17.1cde	20.3bcd	22.0cde	10.3ab
2wksBP / 0.25kg/10kg	17.7a	21.3a	23.3a	25.1ab	27.5a	29.3ab	11.4ab
Atplting/ 0.25kg/10kg	11.8bc	13.6ef	13.2de	17.4def	18.7cd	20.3de	11.3ab
2wksAP/ 0.50kg/10kg	14.6b	17.6bcd	20.7bc	23.6abc	24.3abc	25.7bcd	10.9ab
2wksBP /0.50kg/10kg	15.6ab	19.9ab	21.4ab	26.6a	29.4a	32.9a	11.1ab
Atplting /0.50kg/10kg	3.8d	5.3h	5.9f	13.2f	17.3d	18.0e	5.4c

Means within a column (in each segment) followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P>0.05

Table 3: Effect of rate and time of application of rice husk on number of leaves of cowpea Infected with Cm_eV

Rate of Application	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (Inoculated, not amended)	6.9a	9.2a	11.6b	12.1a	14.6b	15.5bc	16.5d
0.125kg/10kg	5.8a	7.0a	9.7c	11.1a	12.1c	15.6bc	17.8cd
0.250kg/10kg	7.1a	8.5a	11.0b	13.3b	14.2b	16.6b	18.6bc
0.50kg/10kg	5.7a	6.9a	13.2a	15.2a	17.6a	18.1a	19.0a
S.E	0.45	0.80	0.62	0.88	1.31	1.16	1.10
Time of Application							
2wks AP	6.2b	7.2b	6.9b	8.6b	9.6bc	11.6bc	11.8bc
2wks BP	7.6a	9.5a	11.1a	12.3a	13.4a	14.8a	14.9a
At planting	5.4c	6.9b	7.0c	10.1c	10.2bc	10.8bc	10.9bc
S.E	0.39	0.69	0.53	0.76	1.13	1.00	0.90
Rate X Time of Application							
2wksAP/0.125kg/10kg	3.6bc	5.2fg	8.2bc	8.8ef	11.1d	12.3f	13.8g
2wksBP/0.125kg/10kg	6.8a	7.4cd	10.4ab	12.7bc	16.7b	18.9b	20.0ab
At pltng/0.125kg/10kg	7.1a	8.2c	10.9ab	10.9d	11.8d	14.2d	16.3cd
2wksAP 0.25kg/10kg	5.5abc	6.7ed	10.3ab	11.4cd	13.4c	14.5d	14.9ef
2wksBP/0.25kg/10kg	8.0a	10.5a	11.4ab	12.6bc	13.8c	16.1c	17.9c
Atpltng / 0.25kg/10kg	7.7a	8.6c	11.3ab	11.7cd	11.9d	13.1ef	15.6e
2wksAP/ 0.50kg/10kg	5.7abc	8.7c	10.9ab	11.3cd	11.7d	13.7ef	15.2e
2wksBP/0.50kg/10kg	8.1a	9.7b	11.1ab	16.2a	19.9a	21.6a	23.4a
Atpltng/0.50kg/10kg s	3.4c	4.9g	5.4c	7.4f	10.5e	12.3f	14.9ef

Means within a column (in each segment) followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P>0.05

Effect on yield attributes: Table 4 shows the effect on yield of the combination of rate and time of application of rice-husk powder as an amendment on cowpea infected with CM_eV. As with the other parameters, the analysis of the variance shows that the yield attributes in plants amended with rice-husk at the rate of 0.50kg/10kg soil two weeks before planting was the highest compared to the other treatments. Total number of pods per plant was highest (22.6g), in plants amended at the rate of 0.50kg/10kg soil two weeks before planting and lowest (9.0g) in plants amended at planting at the rate of 0.50kg/10kg soil. The grain weights also followed the same trend with plants amended at two weeks before planting at the rate of 0.50kg/10kg soil having the significantly highest weights of 12.8g, and plants amended at planting at the rate of 0.50kg/10kg soil having grain weights of 2.5g.

Table4: The combination of rate and time of application of rice-husk powder as an amendment on Yield attributes of cowpea infected with CM_eV

Treatment Combination	Total no of pods per plant	Wt of pods per (g)	Weight of grain(g)
Control (Inoculated, not amended)	8.2e	8.8e	3.5d
2APX0.125kg/10kg	6.2ef	7.8e	4.0d
2BPX0.125kg/10kg	17.4b	12.8c	9.0c
AP X 0.125kg/10kg	14.0c	8.0e	3.5d
2APX0.25kg/10kg	13.4cd	8.3e	3.3d
2BPX0.25kg/10kg	16.6b	16.8b	11.3b
AP X 0.25kg/10kg	12.0cd	9.5e	3.5d
2APX0.50kg/10kg	5.4g	8.5e	3.0d
2BPX0.50kg/10kg	22.6a	18.0a	12.8a
AP X 0.50kg/10kg	9.4e	10.3de	2.5d

Means within a column followed by the same letter(s) are not significantly different using the New Duncan multiple Range Test at P>0.05

DISCUSSION: The application of organic amendments for the control of plant diseases has been repeatedly explored as an environmentally sensible alternative to the use of synthetic pesticides. The widespread adoption of such an approach has however, been limited by variability in efficacy of such treatments, which in turn has defied resolution due to a general absence of knowledge concerning the mechanisms of disease suppression imparted by these amendments (Cohen *et al*., 2005).

Rice- husk has variously been used both as an amendment to improve crop yield and also in the control of pathogens particularly fungi, nematodes and bacteria. It is apparent from this study that rice-husk, ground into powder could also be used in the control of virus diseases in cowpea. Virus infected cowpea plants that were not amended with rice-husk powder, had growth and yield parameters that were significantly lower than the infected cowpea plants that were amended with rice-husk powder. This is an indication that rice-husk powder could indeed be used as a potent soil amendment to improve the growth and productivity and the suppression of viral diseases in infected cowpea plants.

The mechanism for this control is assumed to be the impartation of competitive advantage to plants growing on the amended soils compared to those on non amended soils. Such plants grew more luxuriantly thereby conferring more photosynthetic advantage. On the other side, plants in non amended soils probably suffered reduced photosynthetic activity occasioned by reduced number of leaves. This reduced photosynthesis is marked during the period of rapid virus increase (Bedrock and Mathews, 1973). Green and Kim (1991) had reported that viruses altered the metabolism of plant cells causing the plants to grow abnormally; a condition that causes both decreased yield and visible symptoms. Reduced photosynthesis due to low chlorophyll levels associated with increased viral symptoms had also been linked to low growth and yield attributes in tomato infected by tomato mosaic virus singly and in combination with Potato virus X (Balogun, 1999).

An amendment with rice-husk powder at the rate of 0.50kg/10kg soil was also found in this study to be the most suitable amongst the other treatments in the control of Cowpea mottle virus in cowpea. Ugwoke and Onyishi (2009), had reported that poultry manure and Mycorrhizae when well managed can be a valuable Agricultural tool in the management of Okra Mosaic Virus, and that an application of up to 40 tons/ha of

poultry manure combined with mycorrhizae increases yield. The early incorporation of the amendment at two weeks before planting, which was found to be the most effective in this study, is attributable to its ability to confer on the plants added nutrients and a longer release period. This finding is in agreement with Mbagwu (1988), who stated that organic manures have a slow and long term release of nutrients and have a tendency for sustainable production.

CONCLUSIONS:

The study shows that rice-husk powder applied to infected cowpea plants as a soil amendment two weeks before planting at the rate of 0.50kg/10kg soil played a significant role in suppressing the pathogenic effects of the virus. It could therefore be used as an effective cultural measure in the management of virus diseases in cowpea. However, further studies on the mechanism by which rice-husk powder achieved the ability to suppress the virus infection is warranted.

Abbreviations : CM_eV: Cowpea mottle virus. AP: After planting. BP: Before planting. AT planting: At planting.

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