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POTENTIALS OF ORGANIC FERTILIZER APPLICATION AS BIOCONTROL FOR SOME SOIL BORNE FUNGAL PATHOGENS IN OGOJA – NIGERIA.

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ABSTRACT

This study was undertaken to determine the potentials of locally made organic fertilizer in the control of soil borne fungal pathogens. The native mycoflora inherent in the soil samples of the study plot was examined using standard microbiological methods. Assay of the locally sourced organic fertilizer used had *Fusarium* as the only fungus present with a mean spore count of $3.25x10^5$ per gram of compost. Analysis of the composite soil samples before and after application of the organic fertilizer shows high frequency occurrence of six genera of fungi (*Mucor*, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Alternaria*). The mean spore count ranged from $0.25x10^2$/g of compost in the most diluted preparations to 3.2 $x10^5$/g in the least diluted preparations. The 300g/plant and 600g/plant organic fertilizer applications caused drastic reduction in the spore density of *Mucor* and *Alternaria* from 27.72% and 22.83% at Day 7 and 14 respectively to zero (0) in both cases. The spore load of *Aspergillus*, *Rhizopus* and *Penicillium* were not affected as they seemed to proliferate in high percentages of 34.48%, 20.69% and 36.78% respectively for Day 7 and 31.46%, 29.21% and 33.71% respectively for Day 14 in samples treated to 300g/plant organic fertilizer. The decline in the spore load *Mucor* and *Alternaria* is a clear indication that organic fertilizer has potentials as a bio-control substrate for the management of some soil borne fungal pathogens.

Keywords: organic fertilizer, *Mucor*,*Fusarium*, *Alternaria*, biocontrol, soil borne fungal pathogens.

1. INTRODUCTION

The continuous cultivation of crops in same portion of land without rotation has often resulted in the depletion of nutrients. This adversely affects food production in most African countries whose mainstay remains agriculture. Plant pathogens are, in most cases, responsible for many of the acute and chronic diseases of crop plants that often results in severe losses to farmers (Mokhtar & Mougy, 2014). The expanding urbanization, industrialization, rural-urban drift and reduction in arable land have, in most cases, led to a decline in food production in most affected areas (Wokoma *et al.*, 2006). Economic losses to soil borne pathogens are estimated at 50-75% of the attainable yield for many crops (Mokhtar & Mougy, 2014) and about 90% of the 2000 major diseases of principal crops especially vegetables and cereals are caused by soil borne fungal plant pathogens such as *Fusarium*, *Rhizopus*, *Alternaria* (Martinez, 2007). The quest to overcome the rapid depletion of nutrients and to increase soil fertility has led to the augmentation of soil to boost fertility through the addition of manure, compost, chemical fertilizers with lime having timed application to improve and maintain high crop yield.

Considering the recurrent nature of occurrence of many serious diseases of crop plants in the environment year after year, the rapid spread of most of these diseases and the difficulty of managing them following development, it becomes necessary to protect plants rather than to control the disease the plants have been infected (Casey, 2000). Composts made up of various plant materials have been used to control some plant diseases in the field (Pinckard, 1982, Chef *et al.*, 1983, Daughtrey *et al.*, 1990; Hoffman-Hergarten & Sikora, 1993; Wokoma *et al.*, 2006 ; Casey, 2000). This procedure has not produced adequate results.The use of beneficial microbes inherent in organic fertilizers for the control of soil borne fungal pathogens of plants is on the increase because of its efficacy, environmental friendliness and boosting soil fertility. This method also induces system resistance among crop species (Ritika and Utpal, 2013).

Mobambo *et al.*, (1994) reported substantial reduction in the severity of black leaf streak (black sigatoka) disease caused by *Mycosphaerella figiensis* in plantain fields

subjected to regularly application of organic fertilizer in the form of household refuse and animal waste.

This study therefore seeks to explore the potentials and of organic fertilizer as biocontrol agent against some common soil borne fungal pathogens of crops.

2. MATERIALS AND METHODS

2.1 Source of organic fertilizer

The organic fertilizer (OF) used for this study was sourced from a local producer in Ishitamte village, Igoli Ogoja Local Government Area of Cross River State, Nigeria. The fertilizer product was made from locally sourced natural substances and fortified with trace elements and minerals often needed by most plants for better yield. The local manufacturer of the organic fertilizer recommends the application of the product as best in improving the yield of vegetables, cereals and other crops.

2.2 Experimental Plots

Trials of the organic fertilizer were carried out in the month of March, 2013. The location is humid and characterized by an annual rainfall of 1848mm and spread over 10months (February to November). The soil used for the experiment was clayey/loam, with pH 4.8, low effective cation exchange (29mmolkg), low available Mn ($0.2mmolkg^{-1}$), low base saturation and low in nutrients (Ortiz and Vuylsteke, 1995). The plot was first cultivated with cassava and allowed to fallow for a period of 18 months, before being cultivated with cocoa.

2.3 Determination of mycoflora in the organic fertilizer

The mycoflora present in the organic fertilizer was determined using 8 samples randomly selected and thoroughly mixed to obtain a composite sample (Wokoma *et al.*, 2006). One gram (1g) of the organic fertilizer (OF) sample was

dispensed in a 100ml conical containing 10ml of sterile deionized water was added to make 10ml suspension of the sample. The suspension was then shaken thoroughly before making a tenfold serial dilution. Aliquots of 0.1ml from 10^2 and 10^5 were spread-plated onto PDA (Potato Dextrose Agar) and ASDA (Oxoid Acidified Sabouraud Dextrose Agar) in triplicate and incubated in the dark for 48 – 96hours at 28 to 30^OC. After 48 – 96hours incubation, the organisms were subcultured onto ASDA to obtain pure cultures. The cultures were identified after being stained with cotton-blue lactophenol stain (Eja, 2003: Barnett, 1972).

2.4 Determination of Native Soil mycoflora

The population densities of the native soil mycoflora were determined by inoculating 0.1ml aliquots of 10^{-2} and 10^{-3} dilutions of the soil samples obtained from the study area (plot) and spore count was carried out.

2.5 Treatment of plots with organic fertilizer (OF)

Samples of the organic fertilizer intended for the treatment of the plots were applied to the soil at the rates of 300g/plant and 600g/plant (Wokoma *et al.*, 2006). The experiment was set up in a Randomized Complete block design with three replicates. The initial spore load of the mycoflora was determined by sampling the plates before the application of the organic fertilizer (OF) (Loffredo *et al.*, 2008). The fungal populations and types were assayed from samples obtained from the plots after 7 – 14days of the fertilizer treatment to ascertain notable changes in the fungal spore load in the plots where the treatment was applied. This was done by sampling soil in the plots mixed thoroughly and 1g of soil sample used for tenfold serial dilution. Aliquots of 0.1ml were subjected to spore count.

3. RESULTS AND DISCUSSION

The use of organic fertilizers (OF) as bio-control in the field for controlling soil borne fungal pathogens is gradually gaining a widespread application in the agricultural sector owing to its result efficacy in not only improving soil fertility, but in greatly reducing pest and fungal pathogens after soil treatments.

Six fungal organisms in the genera (*Mucor*, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Alternaria)* were isolated from the soil used in the experiment. The percentage of occurrence was 27.72%, 17.93%, 8.69%, 7.61%, 15.22% and 22.83% for the organisms respectively.

Microbial evaluation of the organic fertilizer used for soil treatment revealed the presence of *Fusaruim sp.* Microbiological analysis of the organic fertilizer before use showed that viable *Fusarium* spores ranged from $1.06x10^2/g$ of compost in the most diluted fraction to $1.72x10^5/g$ in the least diluted fraction with little variations (<0.5) in other fractions.

Table 2 shows the mean total spore count of the native mycoflora (fungi) isolated from the study area before and after organic fertilizer application. The counts ranged from $0.86x10^2$/g to $5.03x10^2$/g of organic fertilizer in the control plot. Spore counts from treated soil samples, viable mycloflora ranged from $0.33x10^2$/g to $1.91x10^2$/g in the least diluted preparation and $0.25x10^2$/g to $0.93x10^2$/g in the higher The results of the effects of the organic fertilizer on native soil mycoflora as shown in Table 3 indicates that the organic fertilizer concentrations (300g/plant and 600g/plant) were tolerated by the indigenous mycoflora of the studied plot after 14days of application of the organic fertilizer. This is also evident as there seemed to be no marked changes in the diversity and spore load of the fungal species isolated in the treated composite soil samples after application of the organic fertilizer. Wokoma *et al.,* (2006) in a separate study using similar sample concentrations of 300g/plant and 600g/plant reported that there were no discernable changes in the overall population of the soil fungi before and after organic fertilizer application. Majority of the isolates in this study were also tested by Wokoma *et al.,* (2006), except that *Rhizopus* spp. found associated with the organic fertilizer used in their study. The result shows 28% for the treatment of 300g/plant for day zero (0) had *mucor sp* as the highest occurring soil mycoflora followed by 23% for *Alternaria*, but there appeared to be marked drop to zero percent (0%) for both *Mucor* and *Alternaria* on day 7 and 14 of the 300g/plant application of the organic fertilizer dose (concentration) against the two drastically reduced soil borne fungi and as such potential for the control of these mycoflora in Ogoja soils. Lortio, (2005) advocate a combination of *P. fluoresens* and *Trichoderma harzianum* for the effective control of disease of vanilla crop.

Given the understanding of competition amongst microbial species in quest for carbon source and space, the most competitive mycoflora population will have the highest population and diversity since its growth will out-number the least competitive population (Wokoma *et al.,* dilutions. The result also shows similarity in the fungal species isolated before and after treatment with the organic fertilizer (OF) but with a decline in spore load after treatment, indicating the effectiveness of the organic fertilizer in the control of high populations of the inherent soil borne mycroflora that would have been pathogenic to plants if the plots were cultivated.

2006; Khandelwal *et al.,* 2012; Babu and Pallavi, 2013; Motlagh and Samimi, 2013).

4. CONCLUSION

The locally sourced organic fertilizer (OF) used in this study has proven to be an effective and an eco-friendly biological control agent of fungal soil borne pathogens. The opportunistic fungal pathogens, *Mucor* and *Alternaria* (often found in plant debris) were highly inhibited by the organic fertilizer at 300g/plant and 600g/plant with the populations of other test fungi significantly reduced after 14days of application. With this impressive performance, organic fertilizer application should be encouraged among farmers in Ogoja and possibly, in other farming communities, as an effective and environmental-friendly soil treatment to manage soil-borne fungal pathogens of common staple crops.

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Table 1: spore load of *Fusarium* spp in test fertilizer after 72 hours of incubation

Organic fertilizer	Serial dilution factors of *Fusarium* spp			
Replicates	10^2	10^3	10^4	10^5
A	$1.72x10^5$	$1.63x10^5$	$1.54x10^3$	$1.11x10^2$
B	$1.53x10^5$	$1.60x10^4$	$1.31x10^3$	$1.21x10^2$
C	$1.44x10^5$	$1.72x10^4$	$1.72x10^3$	$1.06x10^2$
Mean	$1.56x10^5$	$1.62x10^4$	$1.35x10^3$	$1.13x10^2$

Table 2 : Population of isolated fungi from native soil samples obtained from the study area before and after organic fertilizer application

Application period (Days)	Fertilizer concentration (g/plant)/ spore count		
	300	600	Control (0)
O	$1.91x10^2$	$0.93x10^2$	$0.86x10^2$
7	$0.72x10^2$	$0.41x10^2$	$5.03x10^2$
14	$0.33x10^2$	$0.25x10^2$	$5.10x10^2$

Table 3: Distribution and frequency of occurrence of fungi species isolated from native soil before and after application of organic fertilizer

Isolated fungi species	Treatment of locations (days)					
	Before OF application			After OF application		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Mucor	57(27.72)	0(0)	0(0)	30(19.35)	0(0)	0(0)
Aspergilus	33(17.93)	32(36.78)	28(31.46)	21(20.00)	45(43.27)	30(42.39)
Fusarium	16(8.69)	7(8.05)	5(5.62)	17(10.97)	11(10.58)	8(8.69)
Penicilium	14(7.61)	18(20.69)	26(29.21)	10(6.45)	19(18.27)	24(25.09)
Rhizopus	28(15.22)	30(34.48)	30(33.71)	23(14.84)	29(27.88)	21(22.83)
Altenaria	42(22.83)	0(0)	0(0)	44(28.39)	0(0)	0(0)
Total	184	87	89	155	104	92

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