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Effect of Compost Quality and Microbial Population Density of Composts on the Suppressiveness of *Pythium myriotylum*, Causal Agent of Cocoyam (*Xanthosoma sagittifolium*) Root Rot Disease in Cameroon

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Abstract

The effect of eight compost types made from four different grass species mixed with poultry and pig manure and microbial population in compost were evaluated on the suppressiveness of the root rot disease on cocoyam. The experimental layout was a complete randomized design with five replicates conducted in a screen house in the Faculty of Agronomy and Agricultural Sciences, University of Dschang. Compost was used in the ratio of 1:1 by weight (2kg compost: 2kg soil/plant). It was inoculated 48 hours before planting to enable compost-pathogen interaction. Compost microbial populations were estimated using dilution plating with appropriate media (tryptic soil agar, TSA) for Heterotrophic bacteria and Actinomycetes isolation agar for Actinomycetes respectively.

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The physicochemical properties (pH-H_2O, CEC, N, K, Na, Mg and Ca) of compost were determined and analysed. Disease incidence and severity was significantly ($P \leq 0.05$) reduced in all compost amended pots than the control at 12 weeks after inoculation. The density the microbial groups (Heterotrophic and Actinomycetes) reported to have contributed positively in the suppression of the disease. Microbial populations of Heterotrophic bacteria and Actinomycetes in the different compost types significantly influenced cocoyam root rot severity as the correlations were highly positively significant for Heterotrophic bacteria ($R^2 = 0.911$) and for Actinomycetes ($R^2 = 0.862$) respectively resulting to low root rot disease incidence and severity. The most suppressive compost was compost made of *Tithonia diversifolia* with the highest microbial populations irrespective of animal dung (34 x 10^{11}/ml for poultry and 30 x 10^{11}/ml for pig) which registered the least disease incidence and severity (24.5% and 8% respectively). Correlation analysis of physicochemical properties of compost revealed that disease suppression is associated with the activities of microbial populations and physicochemical qualities of compost.

***Key words*:** Cocoyam root rot disease; Compost types; Microbial populations; Physiochemical properties

1. Introduction

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) commonly called «white macabo» is one of the most important tuber crops in the Araceae family that is cultivated worldwide and serves as a staple food for more than 400-500 million people in the tropics and subtropics. In Cameroon, this tuber crop ranks second after cassava [1]. It is cultivated in all the agro-ecological zones of Cameroon, with the main producing areas being the western highland and the humid forest zones. Cultivated for its tubers, all the plant parts (corms, cormels, flowers and leaves) are consumed [2, 3]. It serves as an important source of income for farmers especially the women in Cameroon [3, 4]. However, its production in Africa represents only about 24% of the world production due to the root rot disease caused by a soil borne plant pathogen, *Pythium myriotylum* Dreschl which causes stunted growth, leaf yellowing and tuber yield reduction [5, 6]. It has been reported to cause yield losses of about 90% in Cameroon [7]. The use of cultural practices does not provide sustainable control of the disease [8]. Farmers try to establish cocoyam plantations on virgin soils but to no avail as this is factorised with the use of infected planting materials and land limitation. Due to the difference in genetic base and rareness in flowering, research to develop high yielding cocoyam resistant cultivars to the root rot disease were not successful [6, 7, 9, 10]. Research has shown that, the use of compost as alternative to chemical control reduces disease severity and losses caused by soil borne plant pathogens [11, 12, 13] as they are colonised by antagonistic microorganisms which compete with the pathogen for available food sources. The objective of this study was therefore to evaluate the effect of compost quality and microbial population on the suppressiveness of *Pythium myriotylum,* causal agent of cocoyam root rot disease.

2. Materials and methods

2.1. Experimental site

The study was conducted in the screen house of the Department of Plant Protection, Faculty of Agronomy and Agricultural Sciences (FAAS), University of Dschang with the following geographical characteristics (altitude:1400m, latitude: between 5°10' and 5°30'N and longitude: between 9°50'and 10°20'E). The soils are mostly highly evolved ferralsols characterized by a typical sudano-guinnean climate with two seasons: a long rainy season (from mid-March to mid-November) and a short dry season (from mid-November to mid-March). The average rainfall of the locality ranges between 1800-2000 mm annually while temperatures range from 21-24^{o}C. The relative humidity is generally above 60 % even during dry season.

2.2. Compost composition and preparation

Eight compost types were prepared using four different grass species based on their availability and previous research works. The grass species used were, *Tithonia diversifolia, Chromolaena odorata, Pennisetum purpureum* and *Ageratum conyzoides*. These plant species were chopped into pieces of 5 to 10 cm to facilitate decomposition. Two animal dung (poultry and pig manure) were added at a rate of 11 and 10 kg respectively to ameliorate the efficiency and quality of the compost. The composts were built in heaps with a chimney at the centre of each compost heap to facilitate ventilation and increase microbial activities. They were watered weekly and turned at two weeks interval to facilitate homogenous decomposition and maintain equilibrium between microbial activities. Compost maturity was determined by taking periodic temperature readings using a soil thermometer until a constant temperature of about 30.5^{o}C obtained.

2.3. Determination of physicochemical properties of compost and soil samples

Soil and each compost samples were carried to the Laboratory of Soil Analysis and Environmental Chemistry, FASA, University of Dschang and analysed for physicochemical properties (pH, CEC, N, Ca, Mg, Na, and K). Compost samples were dried at room temperature for 48 h and clods ground. Soil samples were sieved in a 2 mm sieve and fine fragments (< 2 mm) used for laboratory analysis.

2.4. Determination of microbial population in compost

Microbial population per compost type was determined using dilution plating in two replicates. 10 g of dry weight of each compost type was suspended in a 90 ml flask containing the sterilised NaCl solution [3, 14]. From this suspension, a 10 fold serial dilution was prepared and from the 10 th fold, 0.1 ml of the diluted solution was pipette into 90 mm diameter Petri dishes containing different media corresponding to the type of microbial population to be determined. Tryptic Soy Agar (TSA) for heterotrophic bacteria and Actinomycetes Isolation Agar for Actinomycetes. All plates were incubated at 26°C (3 days for Heterotrophic bacteria and 4 days for Actinomycetes). The plate counts were used to determine mean population density of each targeted microbial group.

2.5. Pathogen culture and inoculum preparation

The pathogen was sub-cultured on a potato dextrose agar (PDA) medium amended with 500mg of Chloramphenicol for purification that has been autoclaved at 121°C for 15 min. The pathogen was then incubated at 27°C for 5 days. *P. myriotylum* culture was blended in sterilised distilled water for 2 min to produce a solution of mycelia strand (inoculum) [14].

2.6. *Plant material, experimental design and compost application*

Suckers of average weight 75g were soaked in 15L of water mixed with 50g of Ridomil Plus 66 WP (contact and systemic fungicide) for 12 hours and dried for 24 h at room temperature before planting [3, 14]. In the screen house, the pots were arranged in a completely randomised design with five replicates. Experimental factors consisted of compost and pathogen inoculation. Polyethylene bags were filled with compost and soil in a 1:1 ratio (2 kg compost: 2 kg of soil/bag/plant) and then inoculated with 18.5 ml inoculum and kept for 48 h to allow pathogen interaction with the compost before planting. Five sample plants were selected from each treatment for data collection. Data were collected from the 6th week at two weeks intervals to 12 weeks after planting (WAP).

2.7. *Statistical analysis*

Data collected were analysed using the Statistical Analyses System (SAS) program. ANOVA was used to determine the homogeneity between variables. Student-Newman-Keuls test was used to compare differences between means of the variables at P = 0.05. Correlations were performed between physicochemical properties and microbial population densities to determine the influence of these variables on disease suppression. Correlations that were significant at 5% were considered influential on disease suppression.

3. Results

3.1. *Physicochemical analysis of compost*

Chemical analysis revealed that compost mixtures with poultry manure gave higher values of chemical elements than those with pig manure. Soil that was used as control had the least physicochemical properties compared to all compost types. The pH was high in all compost types with values ranging from 6.6 to 6.7 for compost mixtures with poultry manure and from 5.8 to 6.9 for compost mixtures with pig manure indicating that pH were all within base range and thus, could favor bacteria than fungi growth. Compost mixtures of *A. conyzoides* had the least pH value (5.8) with pig manure compared to other compost types with the same animal dung (Table 1). Compost mixtures with *T. diversifolia* had the lowest organic carbon irrespective of animal dung (5.3% for poultry manure and 6.6% for pig manure), the highest nitrogen content (2.8 g/kg for poultry and 2.6 g/kg for pig dung) and the lowest C/N ratio (19.1 for poultry and 25.7 for pig). Similar organic matter (OM) contents were observed in all compost types irrespective of the animal dung indicating that they were all at the same level of decomposition after composting. The soil had the least OM (2.4%) (Table 1).

3.2. *Relationship between physicochemical properties of compost and disease suppression*

The correlation analysis revealed that physicochemical properties in the different compost types significantly influenced cocoyam root rot severity. The correlations were highly negatively significant for OM ($R^2 = 0.815$), for Mg ($R^2 = 0.719$), for Na ($R^2 = 0.756$) and for CEC-E ($R^2 = 0.777$) respectively. However, a very low negative correlation was observed between Organic C ($R^2 = 0.183$), C/N ($R^2 = 0.115$), K ($R^2 = 0.161$) and CEC-pH7 ($R^2 = 0.289$) respectively and disease severity (Table 2). Consequently, low root disease severities were associated with high physicochemical properties in the different compost types.

Table 1: Physicochemical properties of compost and soil used in the study

Compost types	pH-H_2O	Org. C (%)	Total N(g/kg)	C/N	Ca^z	K^z	Mg^z	Na^z	OM (%)	CEC-pH7^z	CEC-E
Mixture with poultry manure											
T. diversifolia	6.9	5.3	2.8	19.1	19.6	4.8	7.6	1.1	9.1	30.7	27.8
C. odorata	6.9	5.8	2.6	21.8	17.1	3.5	7.3	0.7	9.9	29.4	27.6
P. purpureum	6.7	5.9	2.5	23.2	15.8	2.0	6.7	0.6	10.2	28.3	25.7
A. conyzoides	6.6	5.7	2.7	21.1	14.9	1.9	4.9	0.4	9.8	26.9	22.1
Mixture with pig manure											
T. diversifolia	6.8	6.6	2.6	25.7	15.3	2.4	6.8	0.8	11.4	26.7	26.6
C. odorata	6.5	6.8	2.5	26.7	14.8	1.9	6.5	0.6	11.7	24.3	25.3
P. purpureum	6.2	6.9	2.4	28.6	12.8	1.9	5.0	0.6	11.9	23.5	21.8
A. conyzoides	5.8	6.6	2.4	26.8	12.3	1.8	4.9	0.4	11.4	21.6	19.8
Soil	6.4	1.4	1.1	12.9	5.2	0.8	1.8	0.1	2.4	8.9	7.8

$^{z\,=}$ (meq/100g of compost)

Table 2: Correlation coefficients between physicochemical properties of compost and disease severity on roots

Compound	Slope	Intercept	R^2	Probability
pH-H_2O,	-1.986 ± 1.911	14.741 ± 14.199	0.697	0.037*
Organic matter	-0.402 ± 0.366	5.885 ± 4.113	0.815	0.008**
Total Nitrogen	-1.263 ± 1.401	4.579 ± 3.567	0.668	0.049*
C/N	-0.061 ± 0.034	2.832 ± 3.182	0.115	0.373NS
Ca	-0.177 ± 0.161	3.355 ± 2.547	0.582	0.003**
K	-0.452 ± 0.252	2.072 ± 1.350	0.161	0.084*
Mg	-0.885± 0.3649	3.774 ± 1.106	0.719	0.029*
Na	-2.128 ± 0.288	2.979 ± 1.068	0.756	0.018*
CEC-pH7	-0.161 ± 0.152	4.383 ± 4.145	0.289	0.135NS
CEC-E	-0.432 ± 0.173	5.847 ± 1.821	0.777	0.014*

**: significant at 1%; *: significant at 5% and NS: not significant ($p > 0.05$)

3.3. Microbial population density of composts

Heterotrophic bacteria population was high in all compost types compared to Actinomycetes. Microbial populations were highest in compost made of *T. diversifolia* compared to the other compost types irrespective of animal dung. The least microbial populations were observed in compost mixtures of *A. conyzoides* irrespective of the animal dung (20.0 x 10^{11}/ml for poultry and 15.5 x 10^{11}/ ml for pig) in the case of Heterotrophic bacteria and (12.5 x 10^{11}/ ml for poultry and 9.5 x 10^{11}/ml for pig) in the case of Actinomycetes (Table 3).

Table 3: Microbial population densities in different composts used in the study

Compost type	**Heterotrophic bacteria** (x 10^{11}/ ml compost)	**Actinomycetes** (x 10^{11}/ ml compost)
T. diversifolia + poultry	34.0 a*	28.5 a
T. diversifolia + pig	30.0 b	25.5 b
C. odorata + poultry	29.5 bc	21.5 c
C. odorata + pig	27.5 c	18.5 d
P. purpureum + poultry	26.5 d	16.5 d
P. purpureum + pig	23.5 e	14.5 e
A. conyzoides + poultry	20.0 f	12.5 ef
A. conyzoides + pig	15.5 g	9.5 f
Soil	0 h	0 g

*Means within a column followed by the same letter are not significantly different ($p = 0.05$)

The correlations were highly negatively significant for Heterotrophic bacteria ($R^2 = 0.911$) and for Actinomycetes ($R^2 = 0.862$) respectively. Consequently, low root disease severities were associated with high microbial population densities of both microorganism types (Table 4).

Table 4: Correlation coefficients between microbial population in compost and disease severity on roots

Microbial groups	**Slope**	**Intercept**	**R^2**	**Probability**
Heterotrophic bacteria	-0.101 ± 0.092	4.270 ± 2.667	0.911	0.008**
Actinomycetes	-0.114 ± 0.091	3.904 ± 2.067	0.862	0.000**

**: significant at $p \leq 0.01$

3.4. Disease incidence and severity on cocoyam root rot

Data analysis indicated a high significant difference ($P < 0.05$) between the different compost types and disease incidence and severity. Compost mixtures with poultry manure showed a slight increase in disease suppression than compost mixtures with pig manure. The least root rot disease severity was observed in plants which received compost mixtures of *T. diversifolia* followed by *C. odorata* (Table 5).

Table 5: Disease incidence and severity (%) on cocoyam roots with respect to compost types, 12 WAP

Treatment(compost types)	Disease incidence (%)	Disease severity (%)
Soil (control)	89.6 a*	38 a
A. conyzoides + pig manure	47.6 b	28 b
A. conyzoides + poultry manure	45.2 b	26 b
P. purpureum + Pig manure	42.6 bc	23 bc
P. purpureum + poultry manure	40.8 d	22 c
C. odorata + Pig manure	32.4 e	18 d
C. odorata + poultry manure	30.9 e	16 d
T. diversifolia + pig manure	26.4 f	11 e
T. diversifolia + poultry manure	24.5 f	8 e

*Means within a column followed by the same letter are not significantly different (p = 0.05).

4. Discussion

Several research works have shown that compost made using similar methods but different feedstock may perform differently in soil borne disease suppression [15, 16]. Suppression of *Pythium* spp by compost might depend mainly on a number of factors such as the nature of composted material, the level of organic matter content and fertility, the pH values of the soil, the combine activities of compost resident microbial groups and physicochemical properties of the compost [16]. The pH values of the compost types were high than the optimal pH values for growth and pathogenicity of *P. myriotylum* (6.1 – 6.2) as reported by [6, 16, 17]. These could justify why the different compost types used in this study were suppressive to the cocoyam root rot disease compare to the control (soil). Compost has been shown to stimulate soil microbial activities as well as microbial populations such as *Pseudomonas* (a bacterium) which are potentially antagonistic to *Pythium* spp in the field [16, 18] thus, reducing plant disease severity. The fact that soil had no microbial populations to compete with the pathogen probably because it was sterilized and coupled with its very low content in nutritive elements, gave it a very low disease suppressive potential thus making the plants very susceptible to pathogen infection and hence the highest disease incidence and severity observed. Compost mixtures of *T. diversifolia* and *C. odorata* had the highest microbial population densities and mineral nutrients as the correlations were high and positively significant for Heterotrophic bacteria and Actinomycetes thus accounting for their highest disease suppressive potentials. Compost mixtures of *A. conyzoides* had a very low disease suppressive potential. In fact, it was established that low root disease severities are usually associated with high microbial population densities in compost. This is in accordance with [18, 19] works which reported that the suppression of *Pythium* spp by compost mainly depends on the combine activities of compost resident microbial groups and compost quality. Also, the highest biocontrol agents must have compete continuously with the pathogen for available food sources necessary for spores germination, propagules and mycelia growth hence reduction in root infection. This is in relation to [20] work which showed that the suppression of damping-off of cotton caused was related to the reduced *P. ultimum* sporangium germination following compost amendments. The fact that poultry manure is very high in nutritive elements coupled with the fact that antibiotics are a common additives to poultry diets, might have induced plant resistance to the pathogen thus making plants which received compost mixtures of poultry manure to show low disease severity than pig manure. In addition, low nutrient wastes may be more valuable for crops if composted with high nutrient wastes like poultry manure [19, 20]. Also, [20] examined three types of manure and found that, the suppression of damping-off due to *P. ultimum* on cotton seedlings increased from poultry to steer manure and to dairy manure compost.

5. Conclusion

The suppressive effect of compost on cocoyam root rot disease varies according to compost feedstock due to the combine effect of increased levels of plant nutrients and high microbial population present in compost. Cocoyam plants that received compost showed high disease suppression than the control. The most suppressive composts were those made from *T. diversifolia* follow by *C. odorata. A. conyzoides* compost showed the highest disease incidence and severity. There was a high negative correlation between high microbial populations (Heterotrophic bacteria and Actinomycetes) and physicochemical properties of compost and low disease severity and incidence. Compost mixtures with poultry manure gave higher disease suppression with least disease severity than those with pig manure irrespective of the grass species used.

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