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Characteristics and antifungal effect of composts on *Fusarium oxysporum* F. SP. *lycopersici* incitant of *Fusarium* wilt of tomato (*Solanum lycopersicum* L.)

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Key words: Mycelial growth, organic amendments, composting, physical, chemical properties.

Abstract

A laboratory study was carried out to evaluate some physical and chemical properties of three composts namely; composted poultry manure (CPM), composted cow dung (CCD) and composted neem-leaf (CNL) used as soil organic amendment and their efficacies was tested against Fusarium wilt of tomato. The properties of the composts showed that they were matured and therefore ideal to be use as soil amendments. Chemical analyses revealed that the composts had pH as 6.8 (CNL), 7.8 (CPM) and 8.0 (CCD). Electrical conductivity (EC) was rated low with values as 1.3µScm^{-1}, 1.2µScm^{-1} and 1.1µScm^{-1} for CNL, CPM and CCD respectively. Neem leaf-based compost exhibited higher total C (39.0 g kg^{-1}), NO_3^{-1}-N (135.7 mg kg^{-1}) and K (64.9 cmol (+) kg^{-1}), where as CPM had higher total N (2.9 g kg^{-1}), NH_4^+-N (24.6 mg kg^{-1}), Ca (14.0 cmol (+) kg^{-1}) and Ma (7.2 cmol (+) kg^{-1}). The *in vitro* experiment consisted of four treatments – extract of CPM, CCD and CNL, and distilled water as control were arranged in a complete randomized design (CRD) replicated five times. The result showed that extract of CPM significantly reduced radial growth of the pathogen to 2.2 cm and inhibited growth by 51%. The effectiveness of CPM is attributed to the attainment of suitable physical and chemical properties that favours the survival and activity of the antagonistic micro-organisms that suppress Fusarium wilt of tomato. Further research to identify the biocontrol agents in composts and to test their efficacies as potential alternative to synthetic fungicides is recommended.

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Introduction

Composting has been defined as intense microbial activity leading to decomposition of most biodegradable materials usually mixture of organic materials which results to organic residue stability (Boulter *et al*., 2000: Weltzien 1991). The process is considered to be the most efficient treatment in producing an environmentally safe and agronomically advantageous soil organic amendment of acceptable operational costs. The microbial community in compost converts degradable organic matter in to more stable, humified forms and inorganic products and releases heat as metabolic waste product (Ciavatta *et al*., 1993).

Composts have been widely accepted as soil amendments or as amendments to growth media for production of agronomic and horticultural crops (Craft and Nelson, 1996). One of the beneficial properties of compost-amended plant growth media is the microbial induced suppression of soil-borne plant pathogens and diseases (Postma *et al*., 2003). Abbasi and co-workers (2002) reported increased activities of biocontrol agents in the rhizosphere of tomato due to application of animal manure composts and reduced severity of soil-borne diseases of tomato. The disease suppressive characteristics of composts are attributed to a combination of Physico-chemical properties and biologic attributes (Lozano *et al*., 2009). Hointink and Fahy, (1986) reported that nitrogen content, cellulose and lignin content, pH, EC and moisture content are implicated in the control of plant pathogen. The main mechanisms of action for disease suppression in compost is said to be based on stimulation of microbial competition which involves a substantial part of the total soil microbiota or a specific group of antagonistic micro organisms (Pascual *et al*., 2002).

Fusarium oxysporum f. sp. *lycopersici* is a soil borne fungus that affects tomato plants which is a crop of great economic importance (Suarez-Estrella *et al*., 2007). Tomato production is significantly reduced by this pathogen because it can destroy roots of tomato at growth stages (Kim and Kim 2008). Many types of compost were reported to have suppressed Fusarium wilts in different crops. Suppression of *F. oxysporum* f.sp. *radicis-lycopersici* with the use composted poultry manure was reported by Cheuk *et al*. (2005). Control of Fusarium seedlings blight was also reported by Boyd-Wilson and Walter (2002) using composted radiate pine bark. Similarly composted hard wood bark of coffee was successfully used in the control of *F. oxysporum* f. sp. *chrysanthemi* (Trillas-Gay *et* al., 1986). Application of chemical fungicide to control this disease induced reduction of useful soil microorganisms and environmental hazards. Various organic amendments have a fungicidal and fungitoxic effect on soil-borne fungi which will reduce our dependency on synthetic pesticide for the control of Fusarium wilt of tomato. This study was therefore conducted to investigate the chemical properties of the composts and to test their efficacy on *Fusarium oxysporum* f. sp. *lycopersici* under *in vitro* conditions.

Materials and methods

Preparation of composts

Composts were prepared using cow dung, poultry manure, and neem leaves to give three compost types tagged as CCD (composted cow dung), CPM (composted poultry manure) and CNL (composted neemleaves). Two pits of 120cm x 90cm x 120cm (lbh) were dug for each compost types for subsequent turning to ensure good aeration and proper decomposition. Cow dung and poultry manure were collected from Gombe main cattle market and Gombe State poultry production unit, respectively, while fresh neem-leaves were collected from the premises of Federal College of Horticulture, Dadin Kowa, Gombe, State.

Each compost source was put in its respective pit up to a thickness of 20cm and followed by a 10cm layer of sawdust. Small quantity of soil was spread to cover the sawdust to aid decomposition. This procedure was sequentially repeated until the pit was filled up completely to a height of 120cm. The compost pits were watered to moist condition and covered with polythene sack to raise the temperature to speed up the decomposition process. Pits were watered for continuous microbial activities every week or twice a week depending on the moisture condition of the pits. After four weeks, the contents of each pit were turned and emptied into a new pit of same dimension which was reserved for this purpose and were subsequently turned into the new pit using shovel, every three weeks to ensure uniform decomposition and proper aeration. The composting period lasted for three months after which the different composts were individually packed into 50kg polythene bags as composted poultry manure (CPM), composted neem-leaf (CNL) and composted cow dung (CCD) respectively. Data on p^H, temperature (^oC) and C: N were taken on 1, 7, 14, 28, 49, 70 and 91^{th} days of composting.

Physical and chemical properties of the composts

Composite samples from each of the compost (CCD, CPM and CNL) were separately collected air-dried, ground and sieved through 1mm sieve and subjected to physical and chemical analyses in the laboratory. The pH was measured using pH Electrode. Total N was determined using Kjeldahl method (Bremner and Mulvaney, 1982), while organic matter was estimated by Walkley and Black method (Nelson and Sommers, 1982). Exchangeable cations (Ca, Mg, and K were measured with the atomic absorption spectrophotometer (Wright and Stuczynski, 1996). EC was determined from a 2:1 (vol/vol) mixture of distilled water and compost with a portable EC Metre. For determination of ammonium nitrogen and nitrate nitrogen, 5g of air-dried compost was mixed with 50ml of KCl and water respectively and vigorously shaken for 45 minutes and then filtered. The concentration of ammonium-nitrogen and nitrate-nitrogen in the filtrates was measured by the indol-phenol method as previously described by Mulvancy (1996).

Effect of composts on mycelial growth of Fusarium oxysporum f. sp. lycopersici

The experiment was laid in a completely randomized block design (CRD), consisting of four treatments; Poultry manure compost extract (PMCE), Cow dung compost extract (CDCE), Neem leaf compost extract (NLCE) and distilled water as control (DW). The treatments were replicated five times. The three composts were prepared to compost extracts according to the procedures of Weltzien (1991) at 1kg compost dissolved in 5 litres of distilled water, fermented for even days before use. Seven-day-old culture of *Fusarium oxysporum* f sp. *lycopersici* was used, where a 6mm plug of the culture of this pathogen was removed and plated on autoclaved PDA contained in 20 Petri dishes. Three drops (0.1ml) from each of the compost extracts were separately added to the respective Petri dishes after the inoculation. Three drops of distilled water were equally applied to serve as the control. The dishes were covered and sealed with masking tape to avoid contamination. These were incubated under fluorescent light at 27 ± 2°C for one week. The radial growth of the pathogen was measured and the average of the measurements were used to calculate the percent inhibition rate according to the formula used by Rini and Sulochana, (2007) as follows: $PI = [(C- T) /C] X 100$, where PI= Percent inhibition rate, C= Radial growth of the pathogen in control plates (cm), T= Radial growth of the pathogen in treated plates (cm).

Statistical analysis

The data on the radial growth and percent inhibition were statistically analysed using GenStat Release 7.2 DE (PC/Windows XP) Copyright 2007, Lawes Agricultural Trust (Rothamsted Experimental Station) and treatment means were separated using LSD at 5% level of significance.

Results and discussion

Physical and chemical Properties of the Composts

Data on pH, temperatures and C: N of the composts during three months composting were depicted on

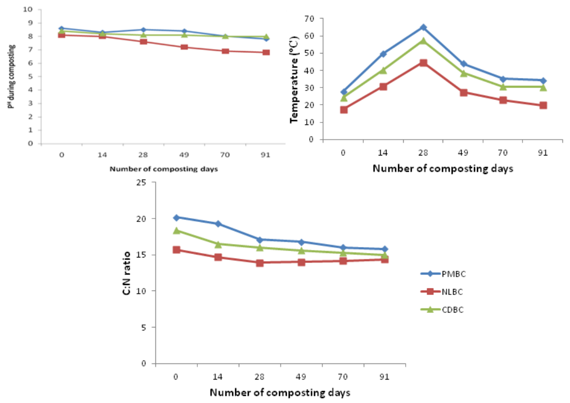

Fig. 1. Evolution of pH (A) temperature in °C (B) and C: N (C) during composting

Fig. 1(C) shows that the temperatures of all compost piles were higher than ambient temperatures during composting process. The temperature of CPM (65.1 °C) and CCD (57.4 °C) were higher than CNL (44.5 °C) at 28th days of composting. This was due to activities of thermophilic microorganisms (Litterick and Hamler, 2004), which indicated that CPM and CCD have a good biological activity. The starting pH of CPM, CCD, and CNL were 8.6, 8.4, and 8.1 respectively (Figure 1B). The pH drop during the rmophilic due to accumulation of organic acids reflects high rate of organic matter degradation and these acids are used later on as substrate by other microorganisms. During the cooling down and maturation stages the pH drops to a neutral value (Chefez *et al.*, 1998). The pH value settles to between 8.1-8.6 as the compost stabilizes. These pH favours the development of actinomycetes and alkaline bacteria (Boulter *et al.*, 2000). However, pH of all the compost pile decreased to 7.8, 8.0 and 6.8 for CPM, CCD and CNL respectively. This is perhaps due to the release of organic acids following the decomposition of organic substances. The pH value of compost is looked as an indicator of process of decomposition and stabilization. Ideally, the pH value of compost should be neutral to slightly acid (6.0~7.5) and efforts should be made to control if it exceeds about 8.5.

In Fig. 1(C) result indicated that the initial C: N of CPM (20.2) and CCD (18.4) were higher than CNL (18.4). The final C: N varies from 14.4, 15.0 and 15.3 for CNL, CCD and CPM respectively, and these are the ideal ratios for mature compost as organic amendment (Boulter *et al.*, 2000). C:N ratio is a traditional parameter, which has been used to evaluate the compost maturity and stability as it defines the agronomic quality. The composting process results in the fall of C/N ratio because the microorganism activities: the conservation of nitrogen and transformation of carbon to CO_2 and humic substances. It has been stated that when C/N ratio is less than 20, the compost is mature and can be used without any restrictions (Garcia *et al.*, 1992). The chemical properties of the composts used in all the experiment was presented in Table 1. The result shows that pH values ranged between neutral (6.8), slightly alkaline (7.8) to moderately alkaline (8.0) for CNL, CPM and CCD respectively. Similarly all the composts have higher EC values. The highest total C is recorded on CNL followed by CCD. Composted poultry manure has the lowest total C. Total N content is highest in CPM then CCD while CNL has the lowest total N. Higher C:N was recorded in all the composts with CNL having the highest value followed by CCD and CPM respectively. Also, higher NH_4^+-N content was observed in CPM then CCD, whereas, CNL had the highest NO_3^- -N. The Ca and Mg contents were also rated high with higher values obtained in CPM, where as K is significantly higher in the CNL.

The result of the analyses of the composts at maturity showed that all composts have high pH and EC values, resulting in high microbial activities (Noble and Coventry, 2005). This result is in compormity with the findings of Al-Dahmani *et al.* (2003) who reported effective suppression of *Xanthomonas vesicatoria* which compost extracts having pH values that ranged from 7.0 to 8.3 and EC values of 0.8 to 4.0 μScm^{-1} . The total organic carbon and nitrogen in all the compost were rated high in accordance with the classification of Esu (1991). This is perhaps one of the reasons why all compost extracts were effective in suppressing Fusarium wilt of tomato. Organic carbon serves as source of energy for high proliferation of beneficial micro organisms (Bailey and Lazarovits, 2003). Lazarovits (2001) and Islam and Tayota (2004) observed that addition of organic amendments containing high nitrogen significantly reduced the population of the pathogen causing Verticillium wilt, common scab of potato and plant parasitic nematodes. The C: N ratio of all the composts was high and this is the ideal C: N ratio often found in compost that suppresses Fusarium wilt. However, if the C/N ratio was lower, *Fusarium* wilt which has preference to excess of N becomes more severe (Hoitink *et al.* 1997).

Table 1. Physicochemical properties of the composts used in the experiments.

Parameters	CNL*	CPM	CDD
P^H (H_20)	6.8	7.8	8.0
EC (µScm^{-1})	1.3	1.2	1.1
Total C (g kg^{-1})	39.0	27.0	30.0
Total N (g kg^{-1})	2.7	2.9	2.8
C:N	14.4	9.3	10.7
NH_4-N (mg kg^{-1})	10.2	24.6	20.9
NO_3-N (mg kg^{-1})	135.7	98.4	77.2
Ca (cmol (+) kg^{-1})	6.5	14.0	11.8
Ma (cmol (+) kg^{-1})	4.3	7.2	6.7
K (cmol (+) kg^{-1})	64.9	57.0	49.5

*CNL: Composted Neem- leaf, CPM: Composted poultry manure, CCD: Composted cow dung.

The three compost extracts were also rich in NH_4-N and NO_3-N. Brady (1974) reported that nitrate-nitrogen can be readily assimilated by plants and resident soil microorganisms, but where carbon-based organic residue are available, micro organisms tend to utilize NH_4+ more quickly than plants. However, many fungi are capable of utilizing both NH_4+ and NO_3- forms while others may use one or the other. Considerable evidence has support the view that ammonia liberated following application of high nitrogen amendments is responsible for killing pathogens (Shian *et al.*, 1999). This may explain why amendments with a low C: N ratio is most often found to suppress plant diseases (Bailey and Lazarovits, 2003). Generally, high NO_3- decreases Fusarium wilt incidence and severity (Hoffland *et al.*, 2000). However, excessive N-fertility may result in increase Fusarium wilt severity. The opposite effect may occur when composts are produced from organic residue with high C: N ratio. High C: N ratio compost immobilize nitrogen, thus making plants deficient in nitrogen resulting in lack of growth and increase susceptibility to pathogens (DeCauster and Hointink, 1999). This is why saw dust was incorporated in the composts so as to reduce nitrogen immobilization and to improve the suppressive ability of the antagonistic microbes in the composts. High calcium content in the composts enhanced disease suppression as plants deficient in calcium favours Fusarium wilt development

Table 2. *In vitro* effect of three compost extracts on the mycelial growth of *Fusarium oxysporum* f. sp *lycopersici* at seven days after inoculation.

Compost extracts	Mean radial growth (cm)	Inhibition (%)
PMC*	2.2d	51a
NLC	3.1b	31c
CDC	2.4c	46b
CNL	4.5a	00d
SE±	0.11	2.69

*PMC: Poultry manure-based compost, CDC: Cow dung based-compost, NLC: Neem-leaf based compost.

The results in the Table 2 showed the *in vitro* effect of three different compost extracts on mycelial growth of *Fusarium oxysporum* f. sp. l*ycopersici*. All the tested compost extracts significantly (*P*=0.05) exhibited lower radial growth of the pathogen, compared with the control (4.5cm). Poultry manure compost extracts (CPM) was more effective in reducing the radial growth of the pathogen (2.2cm), followed by Cow dung and Neem-leaf compost extracts with 2.4cm and 3.1cm respectively. Higher percent inhibition was recorded with the used CPM which inhibited growth of the fungus by 51%. This is followed by CCD (47%) and CNL (31%) respectively.

The result of the evaluation of compost extracts for the control of *Fusarium oxysporum* f. sp. *lycopersici* in the laboratory shows that CPM, CCD and CNL individually suppressed the growth of the pathogen. The compost extracts contain antagonistic micro organisms and active antifungal compounds (El-Masry *et al*., 2002) which cause significant reduction in mycelial growth of the pathogen on PDA. The higher reduction of the mycelial growth was recorded on plates where CPM was applied reducing pathogen growth by 51%. Application of CCD also reduced mycelial growth of the pathogen 47%. This is perhaps due to high number of antagonistic bacteria such as *Bacillus subtilis, B. thurigiensis*, *Achromobacter xylosoxydance, Pseudomonas pseudoalkaligenes*, and *P. fluorensens* in the CPM as reported by Ben-Jenana *et al*. (2009). The least reduction of the fungal growth was achieved by the use CNL which reduced the growth by 31%, this may be attributed to low organic carbon and nitrogen compared to the other composts. The CPM is the most effective in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici* compared to the other composts. This finding is in agreement with the findings of Kerkeni *et al*. (2007) who reported significant reduction of mycelial growth of *Fusarium oxysporum* f. sp. *radisis-lycopersici* using animal manure compost on tomato. He asserted that compost extracts contain biocontrol agents that are effective in controlling the soil borne pathogen. In a previous work conducted *invitro*, Kerkeni *et al*. (2008) also showed that compost extracts applied *in-vitro* inhibited the growth of *Fusarium oxysporum* f. sp. *radisis-lycopersici* by 42.6%. Similar finding was also discovered by BenJenana *et al*. (2009) who equally disclosed the inhibition of the mycelial growth of *Pythium aphanidermatum* on tomato by animal manure based-compost extracts. Weltzien (1990) obtained an inhibitory effect on *Botrytis cinera* by using compost extracts made from cattle and poultry manure.

Reduction of the mycelia growth of *Fusarium oxysporum* f. sp. *radisis-lycopersici lycopersici* by CPM and CCD was probably due to the presence of suppressive agents that produced antifungal compounds in the extracts. Quarles (2001) stated that compost extracts contain millions of bacteria, fungi, and other micro-organisms that inhibit the growth of soil borne pathogens. The least reduction of mycelia growth was recorded based on the application of CNL, which is relatively lower than what was recorded on CPM and CCD. The inferiority in activity of CNL compared to the other two compost extracts may be attributed to the presence of fewer or less antagonistic agents in the extract. The suppression effect exhibited by NLCE was mainly due to the presence of antifungal compounds such as Limonoids contained in the extract (Sivakumar, 2009).

Conclusion

Based on the findings of this study it could be concluded that the use of CPM effectively suppresses *Fusarium oxysporum* f. sp. *lycopersici*. This is attributable to favourable environmental condition created by the compost which favours the proliferation and antagonistic activity of the bio-control agents present in the compost. Further research to identify the biocontrol agents in composts and to test their efficacies in field as potential alternative to synthetic fungicides is recommended.

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