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Full Length Research paper

In-vitro inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes

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Compost-inhabiting bacteria were studied for their effect on seedling blight inducing pathogens. *Aspergillus niger*, *Trichoderma harzianum*, *Bacillus cereus* and *Bacillus subtilis* were the microbes found associated with cow dung, sawdust and rice husk composted soils. *Sclerotium rolfsii*, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Macrophomina phaseolina* were isolated from blighted seedlings of Cowpea, while *S. rolfsii*, *P. aphanidermatum, Helminthosporium maydis* and *Rhizoctonia solani* were isolated from blighted maize seedlings. When these compost - inhabiting microbes were paired with the seedling blight inducing pathogens, *T. harzianum* grew on the mycelia of all the test fungal pathogens. *B. cereus* reduced the mycelia growth of *Sclerotium rolfsii*, *F. oxysporum* , *P. aphanidermatum*, *H. maydis* and *R. solani,* with inhibitory zones ranging from 35.5% to 53.3%. *B. subtilis* in culture also inhibited the mycelia growth of all tested pathogenic fungi with inhibitory zones of between 40.0% to 57.8%. The inhibitory activities of the compost-inhabiting microbes might partly be responsible for the efficacy of compost in reducing seedling blight diseases of crops.

Key words: seedling blight, growth inhibition, sawdust, cow dung, rice husk, compost soil.

INTRODUCTION

Composted organic material such as plant debris and animal manure add nutrient to the soil thereby increasing the soil fertility. This improves plant growth and makes the plant less prone to infection by pathogens (Muhammad et al., 2001). Organic substrates had been reported to have lower bulk density, hence better root-substrate relation (Ayodele, 1997). It has also been reported to provide adequate nutrients to the seedlings and reduces their predisposal to soil borne pests and diseases (Muhammad et al., 2001). Schueter (1989) found that various types of agricultural/municipal wastes suppress different types of soil-borne plant diseases by making plants more vigorous and better able to withstand attack**.** Muhammad et al. (2001) also observed that sawdust composted soil reduced incidence of seedling blight of *Parkia biglobosa* caused by *Fusarium solani* ranged from 30% to 74.2%. While rice-husks composted soil reduced in the incidence of wilting of *P. biglobosa* caused by *F. solani* ranged from 31.4% to 70.3%.

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Seedling blight diseases of crops have remained very serious constraints to cowpea and maize production in the drier savannas and Sahel, the principal zones of their production. Apart from having a wide host range, most of the fungal pathogens produces scleroria that remain viable in soils for many years and it has been very difficult to find a suitable sources of resistance genes, especially in the cowpea genotypes. Consequently, most of the effort has been directed towards development suitable control options.

Since composted agricultural wastes as 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). They could be employed as biological control of plant disease (Garrette, 1975). Morever, the prohibitive cost of pesticidal application as well as its effects on the environment makes it less acceptable to the resource poor farmers, in the drier savannas and Sahel agroecologies of Nigeria. The present study was aimed, therefore, at investigating the inhibition of mycelia growth of some seedling blight inducing pathogens of cowpea and maize by compost (soil composted with Cow dung, sawdust and Rice husks) inhabiting microbes.

MATERIAL AND METHOD

Isolation of pathogenic fungi

Cowpea and Maize seedlings with symptoms of infections were obtained from the research plots in the permanent site of the Usman Dan fodiyo University, Sokoto, in northwestern Nigeria and brought to the plant pathology laboratory. The infected samples were rinsed in tap water, and the necrotic portions were excised and cut into 2-mm pieces, surface sterilized with 10% sodium hypochlorite for 30 s and rinsed in 4 successive changes of sterile distilled water. These were then plated on Potato Dextrose Agar (PDA) and incubated for 6 days at 32^0C under 12- h photoperiod. The pathogens were identified using cultural and morphological features with reference to Barnett and Hunter (1982) while the confirmatory identification was made by the International Mycological Institute, CABI Bioscience, UK Plant Clinic.

Pathogenicity of the isolates

The mycelia suspension of the isolates was produced in V8 broth medium in 250 ml conical flasks for 6 days. The mycelium of each isolates was filtered through the cheesecloth, gently pressed to remove excess liquid and blended for 3 s in Warring blender at the rate of 5 g of mycelium per milliliter of sterile deionized water. The resulting suspensions were used as inoculum. The inoculum was freshly prepared before the applications. Three-weeks- old seedlings growing in oven-sterilized topsoil (0.5 cm) contained in 15 cm diameter pots were inoculated with the mycelia suspension of the fungal isolates. The plants were then placed on benches in greenhouse and observed for symptoms of the diseases. The pathogens were later re-isolates from the inoculated plants and compared with the initial isolates.

Composting

Pot experiment was employed in this study. Fifty-five litre plastic buckets (Perforated) were filled each with 5 kg of soil obtained from the biological garden Usmanu Danfodiyo University Sokoto, and arranged on a bench in the green house at Biological Garden. Each bucket was amended with 50 g rice-husk, saw-dust or cow dung mixed thoroughly and watered every other day. Five buckets were used for each of the treatment. The treatments were allowed to decompose for six weeks.

Isolation of the antagonistic microorganisms

One gram of the composted soil samples was placed in 9ml of sterile distilled water in McCartney bottles shaken vigorously on a vortex mixer for 10mins and then serially diluted from which 1 ml of 10^{-4} to 10^{-6} dilutions were plated on PDA. The plates were incubated for 6 days at 26^0C and the pathogens were identified as described above. Another set of inoculated plates were incubated at 32^0C for 48 h and then examined for the growth of microorganisms present which were then sub-cultured on nutrient agar until pure cultures were established. Plate counts (cfu) was used to determine the inoculum load or population of the isolates. The rate of occurrence of each of the isolates was also determined.

Inhibitory effects of the antagonistic microorganisms

The target pathogen was inoculated on PDA at four equidistant peripheral points while the composts inhabiting microorganisms were inoculated at the centre of 90mm diameter petri plates. Each inoculation was replicated three times. In another set, the target pathogens were inoculated at the centre of 90 mm diameter petri plates and composts inhabiting microorganisms at four equidistant peripheral points. Each inoculation was also replicated three times. The inoculated plates were incubated upside down at 28^o C and were observed every 12 h for inhibition or otherwise of their growth. Zones of growth inhibition of the pathogen were measured along two pre-determined perpendicular lines drawn at the centre of each Petri dish. The mean of the three measurements were recorded for each pathogen.

RESULTS

The result of the experiment revealed that *Sclerotium rolfsii*, *Fusarium oxysporum, Pythium aphanidermatum* and *Macrophomina phaseolina* were isolated from blighted seedlings of Cowpea, while *S. rolfsii, P. aphanidermatum, Helminthosporium maydis*, and *Rhizoctonia solani* were found associated with the blighted seedlings of maize. All the isolated fungi from the blighted cowpea and maize seedlings were found to be pathogenic on the host crops. The four major fungi found in the compost were *Aspergillus niger*, *Trichoderma harzianum*, *Bacillus cereus* and *Bacillus subtilis* (Figure 1).

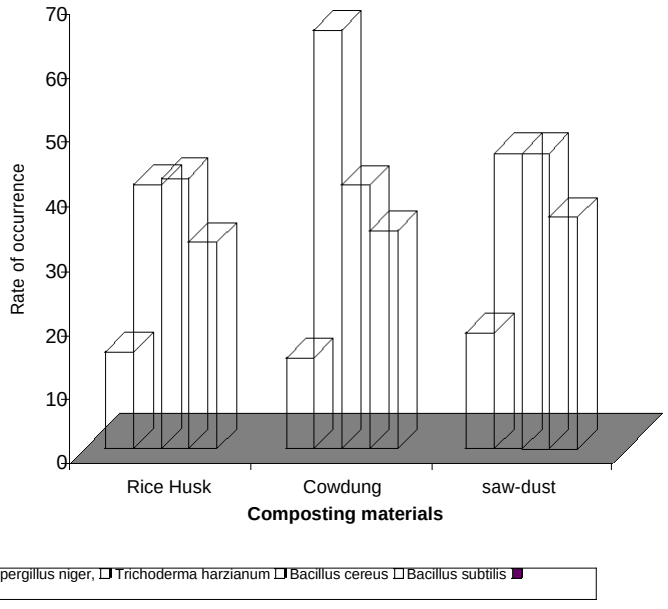

Figure 1. Rate of occurrence of compost-inhabiting microbes.

The zones of inhibition of mycelia growth *S. rolfsii*, *F. oxysporum, P. aphanidermatum, M. phaseolina, H. maydis* and *R. solani* induced by *B. cereus* ranges between 45.5 to 72.2%. However it was observed that the zone of inhibition of growth of *R. solani* induced by *B. cereus* was gradually reduced from 45.5% (3 days of incubation) diameter to 11.11% (5 days of incubation) because *R. solani* continued to grow into inhibitory zone.

Table 1. Zones of growth inhibition (%) induced by compost-inhabiting microbes on seedling blight inducing pathogens.

Seedling blight inducing pathogens	Compost-inhabiting microbes: zones of growth inhibition (%)		
	A. niger	*B. cereus*	*B. subtilis*
F. oxysporum	-	57.7	62.2
S. rolfsii,	-	68.8	75.6
P. aphanidermatum	-	72.2	74.4
H. maydis	-	57.7	58.8
M. phaseolina	-	64.4	57.7
R. solani	-	45.5	44.4

Bacillus subtilis on the other hand also induced inhibition growth of between 44.4 -75.5% on most the test pathogens.

The estimated population of *A. niger* in the compost was least compared with the other microbes, it ranges between 2.4 - 2.6x10^6cfu/g while *T. harzanium* has the highest inoculum density which ranges from 6.2 x 10^6cfu/g in cow dung composted soil to 4.6 x 10^6cfu/g in rice composted soil. The level of cfu/g compost of *B. cereus* range from 4.6 in sawdust to 6.1 in cow dung composted soil. While *B. subtilis* had 3.5 to 3.6 levels of cfu/g compost in the composted soil (Figure 2).

It was also observed that *T. harzianum* grew so fast that it covered the whole plate thereby growing on the mycelia of the pathogens. There was no zone of inhibition of the pathogen found under this set up. *A. niger* did not to induce inhibitory zones on any of the tested fungus.
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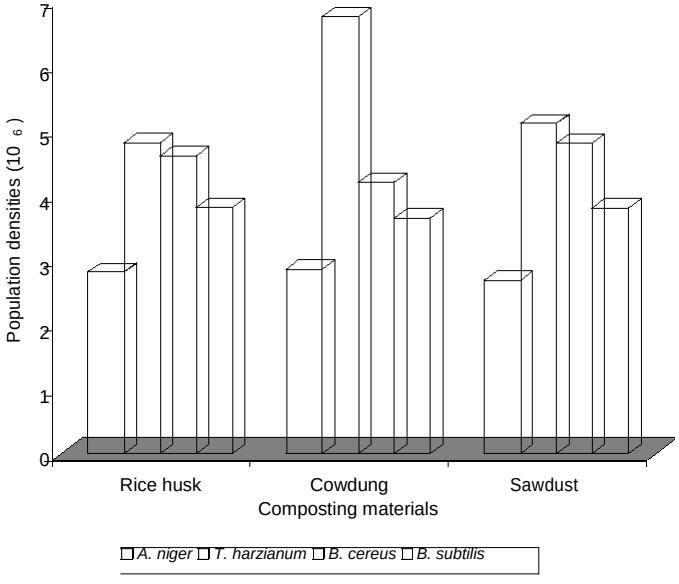

Figure 2. Mean population densities of compost-inhabiting microbes in composted soil.

DISCUSSIONS

The role of the compost-inhabiting microbes in inhibiting the growth of the pathogens has been earlier reported (Schueter, 1989). *B. cereus* and *B. subtilis* inhibited the mycelia growth of all the tested fungal pathogens in this investigation. Bankole and Adebanjo (1998) reported that soils inoculated with *B. subtilis, B. cereus and Trichoderma spp* reduced seedling infection and that the efficacy of antagonists increased with increase in dose Lytic enzymes are known to be produced by *B. cereus* (Csuzi, 1978), these enzymes and other antibiotics produced by *B. cereus* have been reported to have antagonistic effects on some microorganisms (Dorherty and Preece, 1988) . Both substances, produced by *B. cereus* , may have been responsible for inhibiting the growth of the pathogens observed in the study and may probably playing an important role in the prevention of seedling blight diseases under study.

B. subtilis was found to induce the mycelia growth inhibition on all the tested pathogens. Two mechanisms of action might be into play. One might be the production of biologically active metabolites, which inhibited the growth of the pathogens. The other might be its rapid growth and spread on moist surfaced agar plates, which prevented the establishment of the pathogens. A lytic factor has been reported to be located in walls of strains of *B. subtilis* (Young et al., 1974), suggesting that this might have diffused out into the surrounding medium, causing the zones of inhibition observed. Also *B. subtilis* has been reported of having the ability to produces at least five antibiotics namely subtilin, bacitracin, bacillin, subtenolin and Bacilomycin (Abo-El-Dahab and El-Goorani, 1974). These substances might have acted in concert to inhibit the growth of the pathogens used in this study. Afouda (1999) reported that plants infected with *M. phaseolina* and treated with *B. subtilis* showed the lowest blight incidence of 13% and the highest percentage germination of 72%, compared to the control which had blight incidence of 70% and germination percent of 40%. Noronha et al. (1995) also reported that cowpea seeds treated with *B. subtilis* significantly reduced seedling mortality and was superior to seed treatment with quitozene, a fungicide.

T. harzianum was found to grow on the mycelium of the all the tested pathogens in this study. *Trichoderma* species are filamentous fungi which have been found to show potential as biological agents against seed and root rotting pathogens and for managing post harvest diseases (Okigbo and Ikediugwu, 2000) . *T. harzianum* is known to produce extracellular cell wall degrading enzymes such as chitinases, β-1,3-glucanases and cellulases which are important features of mycoparasites for the colonization of their host fungi (Lorito et al., 1994; Di Pietro, 1995).

Chet and Baker (1980) observed that soils that are naturally suppressible to *R. solani* contained a high

natural population of *T. harzianum*, while Strashnov et al. (1985) reported that application of *T. harzianum* by coating tomato fruits reduced *R. solani* fruit rot by up to 85% under laboratory condition. *T. harzianum* has also been used in Northern Nigeria in control of basal stem rot diseases of tomato caused by Sclerotium (*Corticum rolfsii*) (Wokocha et al., 1986) . Similarly, *T. harzianum* from New York soils have been used for biological control of cowpea seed rot caused by *Pythium* sp. (Hadar, *et al*., 1984). Bankole and Adebanjo (1998) also reported the hyper- parasitism of the *Pythium aphanidermatum* by *T. viride.*

Compost-inhabiting microbes as revealed by the present study had inhibitory effects on the seedling blight inducing pathogens of maize and cowpea in northwestern Nigeria. Therefore, inhibitory activities of the compost-inhabiting microbes play a significant role in suppressing seedling diseases induced by soil-borne pathogens.

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