IN-VITRO INHIBITION OF GROWTH OF SOME SEEDLING BLIGHTINDUCING PATHOGENS IN *Amaranthus hybridus* BY COMPOST-INHABITING MICROBES

MUHAMMED, H. M.
Department of Biological Sciences,
Ibrahim Badamasi Babangida University,
Lapai, Niger State, Nigeria
E-mail: habibamaliyu@gmail.com.
Phone No: +234-806-564-7965

Abstract

The study was carried out to isolate and identify the microbes associated with seedling blight diseases of Amaranthus hybridus with a view of finding a possible control methods. The fungi and bacteria species were isolated using baiting and serial dilution methods, respectively, and identified based on their morphological/biochemical characteritics. The fungi species isolated were Aspergilus niger, A. flavus, Fusarium oxysporum and Rhizopus nigricans. While the compost inhabiting microbes that were used for the antagonistic study were A. niger, A. flavus, F.avenaceum, R. airhizus, Debaryozyma hansenii, Bacillus subtilis, Staphylococcus aureus and Strephtococcus feacalis. The zone of growth inhibition of B. subtilis and R. nigricans was the highest with (33 mm), while there was no growth inhibition in A. niger and A. flavus and F. oxysporum. The suppressveness of some compost microbes such as B. subtilis could be used to inhibits the growth of inducing- pathogens that causes seedling blight diseases in A.hybridus.

Keywords: Seedling blight diseases, *Amaranthus hybridus*, Compost inhabiting microbes.

Introduction

Amaranthus hybridus L., popularly called “Smooth amaranth”, belongs to the family of Amaranthaceae. It is an annual herbaceous plant of 1- 6 feet high. The leaves are alternate petioled. About 3 – 6 inches long, dull green, and rough, hairy, ovate or rhombic with wavy margins. The flowers are small, with greenish or red terminal panicles. Taproot is long, fleshy red or pink. The seeds are small and lenticellular in shape; with each seed averaging 1 – 1.5 mm in diameter and 1000 seeds weighing 0.6 – 1.2 g. It is rather a common species in waste places, cultivated fields and barnyards. In Nigeria, *A. hybridus* leaves combined with condiments are used to prepare soup (Oke, 1983; Mepha *et al*., 2007 and Akubugwo *et al*., 2007).

Vegetables are of great nutritional value and they are important sources of vitamins and minerals, thus, essential components of human diet. Vegetables are important components of most food consumed in Nigeria. Consumption of vegetables as part of the daily diets intake, provides consumers with some vital minerals such as Calcium, Phosphorus, and vitamins such as vitamins A, C, B, D, and K, for healthy growth, development and proper functioning of the human body (Muhammed, 2011).

Vegetable seedlings often do not grow well under humid conditions, particularly if the soil is cold and wet. The farmer then has the choice of keeping weak, sickly plants or undertaking the costly operation of replanting. This kind of damage is usually caused by one or more soil-borne fungi that attack under conditions unfavourable for rapid seed germination and growth (Muhammad, 1998). Seedling diseases are caused by a variety of different fungi attacking plants in the early growth stage. Seedling diseases are often a complex of two or more different fungi infecting a plant. Composts are very effective as an organic manure because it fosters a more diverse soil environment in which a myriad of soil organisms exists. Composts act as a food source and shelter for the antagonists that compete with plant pathogens. Some micro organisms prey on and parasitize plants, and there are those that are beneficial which produce antibiotics as bio control such as *A. niger, Trichoderma harzianum* and *B. cereus.* Studies in the pasts have shown that successful disease suppression by compost has been less frequent in soils than in potting mixes (Hoitink *et al*., 1991). This is probably why there has been much research concerning compost-amended potting mixes and growing media for green house plant production than research on compost-amended soils for field crop production. Since composted agricultural wastes has been reported to suppress different types of soil- borne plant diseases (Chen *et al.*, 1998). This study was aimed at investigating the inhibition of mycelia growth of some seedling blight- inducing pathogens in *Amaranthus hybridus* by composts (soil amendment with cow dung, goat dung and chicken droppings.

Materials and Methods

Isolation of Pathogenic Fungi from the Infected Material

The infected seedlings of *Amaranthus hybridus* were obtained from the Botanical garden of the Ibrahim Badamasi Babangida University Lapai, North Central Nigeria and brought to the Laboratory for isolation and identification of the samples.

The infected samples of *Amaranthus hybridus* were rinsed in tap water and the necrotic portions were excised and cut into 1mm pieces and surface sterilized with 10% Sodium hypochlorite for 30 seconds and rinsed in four successive changes of sterile distilled water, after which the sections were then blotted dry on clean, sterile paper towels. They were then plated on PDA in 3 replicates and incubated for 36 hours at 32 ± 2^0C under 12-h photoperiod (Muhammad *et al.*, 2001).

Purification of Isolates

For this activity, as soon as growth colonies were recognized in the mixed-culture, they were sub-cultured aseptically by transferring them on to fresh culture plates of PDA and NA three times, before a single pathogen was obtained in a plate. The sub-culturing was done by using sterilized loop and needle to obtain pieces of growing mycelium of fungi from parts and edges of the mixed cultures.

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 seconds in warring blender at the rate of 5g of mycelium per mililitre of sterile deionized water. The resulting suspensions were used as inoculum. The inoculum was freshly prepared before the applications. Three weeks old vegetable seedlings growing in oven-sterilized topsoil (0.5cm) contained in 15cm 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 disease. The pathogens were later re-isolated from the inoculated plants and compared with the initial isolates.

Preparation of Composts

The composts were prepared from cow dung, goat dung, chicken droppings, as described by Abo, S and Badr, E (2001). Separate polythene bags were weighed with 30kg of cow dung, goat dung, and chicken droppings each. The polythene bags were sealed and a small hole was made in each bag to facilitate decomposition and microbial activities. Each of the composts was watered daily and allowed to decompose for 21, 24 and 27 days respectively.

The Soil Amendments

Thirty (30), four litre plastic bowls filled with 1.2kg sterilized soil, were arranged on a bench in a Green house maintained at temperatures between 30^{o}C – 40^{0}C during the months of October and December 2010. And inoculated as described with the pathogenic fungi identified from *Amaranthus hybridus*. The soil in each replicates of 3 plastic bowls was amended with 2.6g cow dung, 1.5g goat dung, 1.8g chicken droppings and watered daily. Each pathogen was replicated three times. Another set up was made inoculated but not amended a control treatment. The replicates were allowed to stand for 0,5,10 and 20 days respectively (Muhammad, 1998).

Isolation and Identification of Microorganisms developed from the composts

Ten gramm of soil composts samples were taken from the green house studies. The samples were serially diluted to 10^{-10} to isolate the secondary microorganisms. One ml of the dilution at 10^{-2} was plated on PDA in triplicates, using the pour plate method. After 48 hours of incubation at 32^{0}C the Petri dishes were examined for the pattern of growth of microorganisms present. Those colonies that had clear zones of inhibition of growth of other microorganisms around them were then sub-cultured on PDA until a pure culture was established. The secondary microorganisms isolated were identified as described by (Barnett, H and Hunter, B 1996).

Inhibitory effects and measurement of the inhibition zones from the antagonists on the pathogens

The target pathogen was inoculated on PDA at four equidistant peripherals points while the antagonists were inoculated at the centre of 90 mm diameter Petri plate's. The inoculated plates were incubated upside down at 32^{0}C and were observed every 12 hours for inhibition of growth. Zones of inhibition of growth of the pathogen were measured along two pre-determine perpendicular lines drawn at the centre of each Petri dish. The mean of the three measurements was recorded for each pathogen (Muhammad, *et al.,* 2003).

Statistical analysis (ANOVA) are performed following a completely randomised design to test the significant effects and means compared using the LSD test($p \leq 0.05$) as outlined by Duncan(1955).

Results

Seedling blight pathogens isolated from *Amaranthus hybridus*

Isolation from the blighted seedlings revealed the association of the following fungal species, *Aspergilus niger*, *Aspergillus flavus Fusarium oxysporum, Rhizopus nigricans and* upon testing the pathogenicity of these isolated fungi, the severity of the disease was 25-50% from day 0-5, the infection was high on day 10 with 73% (Table 1). It then decreased from day 15-20 with 67%.

Table 1: Soil Ammendments of *Amaranthus hybridus* from Day 0-20

Days	Mean value	Disease incidence (%)
0	10.703 b	25
5	16.814 e	50
10	19.813 a	73
15	17.816 c	67
20	16.629 d	67

Showing the means and disease incidence (%) of effects of soil amendment on *A. hybridus* each value represents the mean of 5 values. Different letter(s) within row represent values that are significantly different at p= 0.05 based on ANOVA Duncan test.

Table 2: Microbes isolated from the composted wastes

	Microbes isolated from each of the composted wastes and their occurrence							
Composted wastes	*A.niger*	*A.flavus*	*Debaryozema hansenii*	*Fusarium avenaceum*	*Rhizopus airhizus*	*Staphylococcus aureus*	*Strephtococcus feacalis*	*Bacillus subtilis*
Cow dung	√	√	nil	√	√	√	nil	√
Goat dung	√	√	√	√	nil	nil	nil	nil
Chickens droppings	√	√	nil	nil	√	nil	√	nil

The five major fungi and three bacteria species found associated with the composted wastes were *Aspergilus niger*, *Aspergilus flavus*, *Debaryozema hansenii*, *Fusarium avenaceum, Rhizopus airhizus*, *Staphylococcus aureus, Strephtococcus feacalis* and *Bacillus subtilis* as shown in Table 2. *A. niger* and *A. flavus* were all isolated from the three composted wastes, *D. hansenii* was isolated from goat dung while *F.avenaceum* was isolated from cow and goat dungs respectively; *R.airhizus* was isolated from both cow dung and chicken droppings respectively; *S.aureus* and *B.subtilis* were isolated from cow dung while *S.feacalis* was isolated from chicken droppings respectively (Table 2).

Table 3: Zones of growth inhibition (mm) induced by seedling blight- inducing pathogens on compost inhabiting microbes

Compost inhabiting microbes: (mm)	Seedling blight- inducing pathogens (mm)			
	A.niger	*A.flavus*	*F.oxysporum*	*R.nigricans*
A.niger	30	_	_	30
A.flavus	_	_	20	10
F.avenacum	_	_	_	_
R.airhizus	_		30	_
D.hansenii	_	_ _	30	20
B.subtilis	_	_	20	33

S.aureus	31	30	18	20
S.feacalis	_	_	17	30

Effects of growth competetion of *Aspergillus niger*
The inhibitory effects of *A. niger,* on *A.niger* and *R.nigricans* inducing pathogens inhibits their growth by 30mm zone of inhibition. However, no growth was recorded on *A. flavus* and *F.oxysporum* (Table 3).

Effects of growth competetion of *Aspergilus flavus*
A. flavus inhibited the growth of *F. oxysporum* and *R. nigricans* to 10 and 20 mm, respectively; but had no growth inhibition effect on *A. niger*.

Effects of growth inhibition of *Fusarium avenacum*
F. avenaceum had no growth inhibition on all the inducing pathogens as shown in Table 3.

Effects of growth inhibition of *Rhizopus airhizus*
R. airhizus inhibited the growth of *F.oxsporum* at 30mm zone of inhibition however, no growth inhibition effects on *A. niger*, *A. flavus* and *R. nigricans.*

Effects of growth inhibtion of *Debaryozyma hansenii*
D. hansenii inhibited the growth of *R. nigricans* and *F. oxysporum* to 20 and 30 mm respectively; but had no growth inhibition effects on *A. niger* and *A. flavus* (Table 3).

Effects of growth inhibition of *Bacilus subtilis*
B.subtilis had no growth inhibition effects on *A. niger* and *A. flavus* (Table 3), but there is inhibition of growth on *F. oxysporum* and *R. nigricans* to 20 and 33 mm.

Effects of growth inhibition of *Staphylococcus aureus*
The zones of inhibition of *A. niger, A. flavus* and *R. nigricans* inhibited the growth of *S. aureus* with an average of 31, 30, 20 mm respectively; the mycelial growth of fungi covers the plate after five days incubation. However, for *F.oxysporum* it was 18mm.

Effects of growth inhibition of *Strephtococcus feacalis*
From the table (3), *S. feacalis* had no growth inhibition effects on *A. niger* and *A.flavus* as it covers the whole of the plate after five days incubation but it inhibited the mycelial growth of *F. oxysporum* and *R. nigricans* to 17 and 30 mm.

Discussion
The results of the experiment revealed that *A. niger, A. flavus,* and *F. oxysporum* and *R. nigricans* are pathogenic microorganisms which affect vegetables seedlings the most common symptoms are damping-off of seedlings which are physiological signs of seedling blight disease as revealed by (Nunez, 2001). *B. subtilis* was responsible for bacterial blight of seedling as reported by (Koch *et al.*, 1977). Richard (2000), reported that *F. oxysporum* and *R. nigricans* are highly effective in inducing the seedling blight diseases of most vegetables in moist environments. Seedling blight infections generally arise from seed-borne *Fusarium* species that can damage seedlings in warm, arid soils (Colhoun 1970,Glynn *et al.*, 2006).

The role of compost-inhabiting microbes in inhibiting the growth of the inducing- pathogens has been reported by Schueter, (1989). Young *et al*. (1974), reported that two mechanisms of action might be into play for *B. subtilis* to induce the mycelia growth inhibition on inducing- pathogens. 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 by Young *et al*. (1974), to be located in walls of strains of *B. subtilis*, suggesting that this might have diffused out into the surrounding medium, causing the zones of inhibition observed.

These results revealed that *Aspergilus niger* isolates obtained from composted wastes exerted typical mycoparasitism against *Fusarium oxysporum*, results that also agree with those of Venkatasubbaiah and Safeeulla (1984), who found that *Aspergilus niger* isolated from the rhizosphere of coffee seedlings was antagonistic to the collar rot pathogen.

In this study, *A.niger,* had no growth inhibition effects on *A. flavus* and *F. oxysporum* there was competition of growth between the inhabiting microbes and the seedling blight inducing- pathogens this was revealed earlier by Muhammad and Amusa (2003), that there was no zone of inhibition between *A.niger* and *F.oxysporum*, as *A.niger* did not induce inhibitory zones on the fungus.
Currently, biocontrol of the seed-borne plant pathogenic fungi has been studied using bacterial and fungal antagonists. Strains of *Pseudomonas* species, *Bacillus* species, *Trichoderma* species and isolates of *Fusariun oxysporum* isolated from the rhizospheres of crop plants and composts, have shown efficacy not only against plant pathogens, but also in helping the plants to mobilize and acquire nutrients (Glynn *et al*., 2006).

Compost-inhabiting microbes as revealed by the present study had some growth inhibitory effects on some of the seedling blight-inducing pathogens isolated from *Amaranthus hybridus.* The inhibitory activities of the compost-inhabiting microbes can play a significant role in suppressing seedling blight pathogens of some vegetables. The inhabiting microbes and their antagonistic properties may serve as a potential bio control for seedling blight diseases of vegetables in the future.

References

Abo, S. & Badr, E. (2001). Antagonistic effects of some compost. *Phytopathology, 68, 183 –210.*

Akubugwo, I. E., Obasi, N. A., Chinyere, G. C. & Ugbogu, A. E. (2007). Nutritional and chemical value of *Amaranthus hybridus* L.leaves from Afikpo, Nigeria. *African Journal of Biotechnology*, *6(24), 2833-2839.*

Barnett, H. L. & Hunter, B. B. (1996). *Ilustrated genera of imperfect fungi.* Minnesota: Burgess Publishing Company.

Chen, Y., Inbar, Y. & Hadar, Y. (1998). Composted agricultural wastes as potting media for ornamental plants. *Soil Science*. *145-298.*

Colhoun, J. (1970). Epidemiology of seed-borne *Fusarium* diseases of cereals II. Infection of seedlings by *Fusarium culmorum* and *Fusarium avenaceum* in relation to environmental factors. *Trans.Br. Mycology Society, 51: 397-404.*

Glynn, N. C., Ray, R., Edwards, S. G., Hare, M. C., Parry, D. W., Barnett, C. J. & Beck, J. J. (2006). Quantitative of *Fusarium* species and *Microdochium* spp. PCR assays to evaluate seed treatments for the control of *Fusarium* seedling blight of wheat. *Journal of Applied Microbiology*, *102, 1645-1653.*

Hoitink, K., Daniel, L., & Dindal, L. (1991). The Ecology of Compost. *Agricutural Review, 25, 110-220.*

Koch, T., Daniel, K., & Steve, J. (1977). *Rhizoctonia solani: Seedling blights of vegetables and Field crops. Florida Cooperative Extension Service, Institutes of Food and Agricultural Sciences.* University of Florida. http://plantpath.ifas.ufl.edu/takextpub/factsheets Pp. 1-10.

Muhammad, S., Suberu, H. A., Amusa, N. A. & Magaji, M. D. (2001). The Effect of soil Amendment with saw dusts and Rice husks on the growth and incidence of seedling disease in *Tamarridus indica* Linn caused by *Macrophomina phaseolina* and *Rhizoctonia solanii*. *International Agriculture Research, 2, 40-46.*

Muhammad, S. (1998). Effects of soil amendments with rice husks on *Fusarium* wilt of pepper *capsicum annuum*. *The Beam: Arts Sciences, 4, 108-112.*

Muhammad. S. & Amusa, N. A. (2003). In-vitro inhibition of growth of some seedling blight inducing-pathogens by compost inhabiting microbes. *African journal of Biotechnology*, *2, 161-164.*

Muhammed, H. M. (2011). Invitro inhibition of growth of seedlingblight inducing pathogens in some vegetables by compost inhabiting microbes in Bida Local Government area of Niger State Nigeria. A thesis submitted to the Department of Biological Sciences Usmanu Danfodiyo University Sokoto, Nigeria.

Mepha, H.D., Eboh, L and Banigbo, D.E.B (2007). Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *African Journal of Food Agriculture Development*, *7 (1), 1-18.*

Nunez, J. (2001). Fungi combinations often found in vegetables Disease. *Western farm Press for Western Agriculture, 24, 456-510.*

Oke, O. L. (1983). Amaranth. In: handbook of tropical foods. Chan HT (ed). New York: Marcel-dekker, Inc, Pp 1-14.

Richard, I. (2000). *Fundamentals of horticulture.* New Delhi: Tata McGraw-Hill Publishing Company Ltd, Pp 348-369.

Schueter, C. (1989). Antiphytopathogenic properties of biogenic wastes compost. *Agriculture Ecosystem and Environment, 27, 477-482.*

Venkatasubbaiah, P. & Safeeulla, K. M. (1984). *Aspergilus niger* for biological control of *Rhizoctonia solani* on coffee seedlings. *Tropical pest Management, 30, 401-406.*

Young, F. E., Tupper, J. & Strominger, J. L. (1974). Autolysis of cell walls of *Bacillus subtilis*: Mechanism and Possible Relationship to Competence. *Biological Chemistry, 249, 3600-3602.*