EFFECTS OF FUNGICIDES ON THE FUNGI ASSOCIATED WITH CASSAVA TUBERS (*Manihot esculenta* Crantz) FROM ILORIN METROPOLIS, NIGERIA

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Abstract
This study investigated in vitro effects of different concentrations of Team fungicide and Z-Force fungicides on the mycelial growth of fungi associated with cassava tuber rot. Team fungicide contained 12% Carbendazim and 63% Mancozeb and only 80% mancozeb was present as Active Ingredient (A.I) of Z-Force fungicide. Aspergillus flavus, Aspergillus niger and Rhizopus stolonifer were isolated from infected tubers after incubation at 27±2°C for one week. Different concentrations of each fungicide (100 mgL^{-1}, 200mgL^{-1}, 400 mgL^{-1} and 500mgL^{-1}) were prepared and their effects on mycelia growths were observed. R. stolonifer was the most pathogenic causing rot symptoms with mean diameter value of 29.10mm while A. niger and A. flavus induced rot with mean diameter of 22.70mm and 17.30mm, respectively. The Team fungicide, at 100mg/L retarded the mycelial growth of A. niger and the inhibitory zone covered 57.57mm which was significantly different from the value recorded for A. flavus (34.00mm) but it had no effect on the mycelial growth of R. stolonifer. The same concentration of Z-Force fungicide was less inhibitory to the growth of the two Aspergillus spp. than Team fungicide. A. niger was observed to have higher inhibitory zone at 200mgL^{-1} out of the two fungicides tested in this study but concentration had no effect on the growth of R. stolonifer. At the highest concentration, the mycelial growth of A. niger and A. flavus was completely arrested. No inhibition was observed in the control experiment. The synergy between carbendazim and mancozeb in Team fungicide was noted. This study aim at comparing the inhibitory effects of Team fungicide and Z-Force fungicide against fungi associated with cassava tubers.

Keywords: Cassava, Mancozeb, Carbendazim, Fungicides, Mycelial growth

Introduction

Cassava (*Manihot esculenta* Crantz) is a woody shrub with an edible root. It grows in tropical and subtropical areas of the world. Cassava is otherwise called "ege" (Yoruba), "akpu" (Igbo), and "rogo" (Hausa). It is a woody shrub of the Euphorbiaceae (spurge) family, native to South America. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy, tuberous root. Cassava is the third-largest source of carbohydrates in the Tropics and is a major staple food in the developing world, providing a basic diet for 502 million people worldwide (Claude and Denis, 1990). Nigeria is the world's largest producer of cassava; a third more than production from Brazil and almost double that of Indonesia and Thailand (FAO, 2013).

A number of different species of bacteria and fungi isolated from cassava tubers showed that post-harvest decay of the crop is a complex matter involving more than a single organism. Soft rot of cassava tubers is caused by a complex fungi; *Lasiodiplodia theobromae*, *Aspergillus niger*, *Aspergillus flavus, Cylindrocarpon candidum*, and *Trichoderma harizianum* (Ekundayo and Daniel, 1973). Other microbial pathogens that can instigate deterioration of the crop include members of the following genera: *Pythium, Mucor, Rhizopus, Penicillium, Fusarium, Cladosporium, Glomerella, Gloeosporium, Rhizoctonia, Bacillus, Xanthomonas, Erwinia, Agrobacterium* and many saprophytic bacteria (Booth, 1976). Diseases caused by these pathogens may cause photosynthetic inefficiency or pre-harvest and post-harvest losses.

Fungicides are biocidal chemical compounds that kill or inhibit the growth of disease-causing fungi. These biocidal agents operate different mode of actions. Contact fungicides act on the surface of the plant and this include mancozeb, a derivative of dithiocarbamic acid but systemic ones are absorbed through the foliage or root and are translocated upward internally by the

plant through the xylem (Agrios, 2005). It has been noted that since fungi differ significantly in morphology and physiology from other forms of life, they may be successfully combated by compounds of low toxicity to other organisms, notably mammals (Edwards *et al.,* 1991).

Team is a systemic and contact fungicide containing 12% Carbendazim and 63% Mancozeb as the active ingredients. Z-force contained 80% Mancozeb as active ingredient and work as contact and broad spectrum fungicide. Mancozeb is a derivative of dithiocarbamic acid. Carbendazim is a carbamate ester-amine. Carbamates are chemically similar to, but more reactive than amides. This research work aimed at detecting the synergy between mancozeb and carbendazim as fungicidal agents.

Materials and Methods

Collection of Samples: Healthy cassava tubers samples were purchased from one of the cassava processing centers in Ilorin metropolis; these were collected in clean polythene-bag and taken to the laboratory. The cassava samples were stored in the laboratory for seven days at room temperature.

Isolation and Identification of Associated Fungi

The cassava tubers were surface sterilized using 70% ethanol and were rinsed with sterile distilled water for three consecutive times. Small portions of the infected areas of the tubers were removed using flamed scalpel and placed into recently cooled agar media in a Petri-dishes. This process was carried out under strict aseptic conditions. The plates were incubated at 28 ± 2^0C for four days after which colonies obtained were further sub-cultured until pure culture of the individual isolates were obtained. The isolates were subjected to microscopic examination. Identification was made with reference to Barnett and Hunter (2010) and Campell and Stewart (1980).

Pathogenicity Test

Healthy cassava tubers were selected for this purpose. The surfaces of the samples were swabbed with 70% ethanol for 1munite and then rinsed with sterile distilled water. The tubers were wounded by dip-cutting each sample with a sterile dissecting loop and inoculated with 5mm disc of pure culture of each isolate. The control tubers were treated with sterile distilled water. All tubers were inoculated at 25 ± 2^{o}C for seven days. All groups were observed for symptoms (Oladiran and Iwu, 1993). The test was carried out in three replicates for each fungal isolate.

Preparation of Fungicidal Solution of Different Concentrations

The different concentrations of fungicidal solutions were prepared in distilled water. Each solution was prepared by weighing 100, 200, 400 and 500mg of the fungicides into 1litre of distilled water.

Effects of Fungicidal Solution on Tested Fungi

The effect of fungicidal solutions on isolates was studied using a method adopted by Ahmed *et. al*. (2012). The Potato Dextrose Agar was poured into sterilized petridishes (9cm diameter) and allowed to solidified. One milliliter of the various concentrations of fungicides was introduced into PDA and allowed to solidify. For each test fungus, 5mm (diameter) of mycelial was cut from advancing margin of each fungal colony. The plug was placed at the centre of each agar medium containing different concentrations of fungicides. Control experiments contained the mycelial plug with sterile distilled water. All the plates were incubated at $25\pm$ ^{o}C. the zone of inhibition was measured and frequency of occurrence was calculated using the method of Pathak and Zaidi (2013).

The frequency of occurrence of fungus was calculated by the following formula:

No. of seeds containing a particular fungus

$$\frac{\text{———————————————————}}{\text{Total seeds used}} \times 100$$

Statistical Analysis
Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS). The means were separated using Duncan's Multiple Range Test (DMRT) at 5 % significant level.

Results and Discussion
After seven days of incubation, three fungi species were isolated from cassava tubers, viz: *Rhizopus stolonifer, Aspergillus flavus* and *A. niger*. From the results of pathogenicity test, the cassava tuber treated with *R. stolonifer* recorded the highest diameter of rot symptoms with mean value of 29.10mm while *A. niger* and *A. flavus* induced rot development with mean diameter of 22.70mm and 17.30mm, respectively (Table 1). For the frequency of occurrence, *R. stolonifer* was responsible for 74% of rot observed, followed by *A. niger* (68%) and *A. flavus* (51%) as shown in Table 2.

The Team fungicide (12% Carbendazim and 63% Mancozeb), at 100mg/L, inhibited the growth of *A. flavus* and the inhibitory zone covered 34.00mm which was significantly different from the value recorded for *A.niger* (57.57mm) but it had no effect on the mycelia growth of *R. stolonifer* (Table 3). The same concentration of Z-Force fungicide inhibited the growth of the two *Aspergiillus spp* lesser than Team fungicide (Table 4). The mycelia growth of *R. stolonifer* resisted against the effects of both fungicides at 100mg/L and 200mg/L but the two fungicides overcame the resistance when the concentrations increased to 400mg/L and 500mg/L respectively. At the highest concentration, the mycelia growth of *A. niger* and *A. flavus* was completely arrested except *A. flavus* treated with Z-Force that recorded 54.00mm (Table 4).

This study revealed that *R. stolonifer* is the most destructive agent associated with cassava tubers. Woolfe (1992) reported that the fungus was responsible for sweet potato rot in Tropics supported this finding. Yusuf and Okusanya (2008) reported that *R. stolonifer* was responsible for 44% rot observed in *Discorea rotundata* against 74% rot reported in this work. Both Team and Z-Fungicide inhibited the mycelia growth of the three fungi. The inhibitory potential of each fungicide increased as the concentraton increased.

Mancozeb is a broad spectrum fungicide used to control a number of fungi diseases such as anthracnose, leaf spot, and rust (USAID, 2012). The toxicity of mancozeb is not restricted to fungi alone but also affects man by causing skin irritation and other chronic skin diseases (Kegley *et al*., 2010). Pozo *et al*.(1994) reported that the Agricultural doze of mancozeb significantly decreased the population of fungi, denitrifying bacteria and aerobic diazotrophs. These results are in conformity with Pathak and Zahid (2013) who had comparatively studied seed dressing fungicides and *Calotropis procera* latex for the control of seed-borne mycoflora of wheat and concluded that mancozeb increased the germination percentage and reduced seed mycoflora. Nghiep and Gaur (2005) confirmed the efficacy of mancozeb against *Aspergillus* and *Rhizopus* species. Carbendazim is systemic in action and its effectiveness had being confirmed by Saleem *et al*, (2012) against *Aspergillus* spp. The application of Team fungicide at rates higher than recommended concentration while not contributing any extra impact on inhibiting the target groups affects the environment negatively by its action on non-target organisms (Fawole *et al.,* 2008).

The results of the work showed the effectiveness of the synergy of mancozeb and carbendazim in Team fungicides.

Table 1: Pathogenicity of fungi isolated from cassava tubers after seven days of inoculation

Fungi Species	**Mean Diameter of Rot (mm)**

Aspergillus flavus	17.30
A. niger	22. 70
Rhizopus stolonifer	29.10
Control	0.00

Table 2: Percentage incidence of isolated fungi from cassava tubers

Fungi	**Frequency of Occurrence**
Aspergillus flavus	51
A. niger	68
Rhizopus stolonifer	74

Table 3: Effects of Team fungicide on the Mycelial growth of isolated fungi.

Fungal Isolates	**Zone of Inhibition (mm)**				
	Control	**100mgL^{-1}**	**200mgL^{-1}**	**400mgL^{-1}**	**500mgL^{-1}**
Aspergillus flavus	0.00±0.00^{a}	34.00±0.58^{b}	57.50±0.61^{b}	63.50±0.06^{b}	85.00±0.00^{a}
A. niger	0.00±0.00^{a}	57.57±0.58^{a}	66.50±0.52^{a}	83.53±0.38^{a}	85.00±0.00^{a}
Rhizopus stolonifer	0.00±0.00^{a}	0.00 ±0.00^{c}	0.00±0.00^{c}	5.03±0.35^{c}	27.50±0.61^{b}

Means with the same superscript letters down the column are not significantly different 0.05 α level

Table 4: Effects of Z-Force fungicide on the mycelial growth of isolated fungi

Fungal Isolates	**Zone of Inhibition (mm)**				
	Control	**100mgL^{-1}**	**200mgL^{-1}**	**400mgL^{-1}**	**500mgL^{-1}**
Aspergillus flavus	0.00±0.00^{a}	4.00±0.06^{b}	23.50±0.03^{b}	72.50±0.29^{a}	85.00±0.00^{a}
A. niger	0.00±0.00^{a}	37.57±0.38^{a}	37.67±0.88^{a}	44.50±0.58^{b}	54.00±0.58^{b}
Rhizopus stolonifer	0.00±0.00^{a}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	3.00±0.29^{c}	6.00±0.25^{c}

Means with the same superscript letters down the column are not significantly different 0.05 α level

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