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THE IN VITRO CHEMICAL CONTROL OF *Pythium aphanidermatum*, AN AGENT OF TOMATO ROOT ROTS IN THE NORTH CENTRAL, NIGERIA.

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ABSTRACT

The fungicides benlate (benomyl), ridomil and mancozeb significantly reduced in vitro mycelial growth of Pythium aphanidermatum at all concentrations tested. The research which was carried out between 2005 and 2008 saw ridomil as the most effective compound at low concentrations as complete inhibition of mycelia growth at 50 ppm in vitro was recorded. The in vitro bioassay of fungicides showed that benlate and mancozeb inhibited linear growth of the fungus at 150 ppm and 200 ppm active ingredient (a. i) respectively. The fungicides equally exhibited inhibition of sporangia production and mycelia dry weight of the fungus. All three fungicides tested were effective against P. aphanidermatum and could be used alternatively to reduce the development of fungicide resistance. Furthermore, the fungicides, except mancozeb, could be applied at low concentrations.

Key words: Fungicides, *in vitro* treatment, *P. aphanidermatum*, tomato, root rot.

INTRODUCTION

Tomato is of great nutritional importance because it is an excellent source of vitamins such as vitamins A, C, thiamine, niacin, riboflavin and of minerals like iron and calcium. In view of its uses which include as salad vegetable, puree, paste, ketchup, juice and for cooking soups and stews, it can also be canned or pickled (Chiejina, 2005). The need to increase its production has suffered some set back due to infection of the root and fruit by microorganisms in the field and in storage (Chinoko and Naqvi, 1989; Daradhiyar, 1980 and Chiejina, 2005).

In Nigeria, tomato is mainly cultivated in the region north of 10^{o}N latitude. This covers the savannah belts where its cultivation is supported with irrigation. In the south, the excessive precipitation and associated high relative humidity tend to limit tomato production during the raining season as it favours the multiplication of the pathogen responsible for the root rot. Among the pathogen responsible for root rot of tomato is *Pythium* sp (Agrios, 2005) and *Fusarium oxysporium* (Onyia *et al*, 2005); basal stem rot caused by *Sclerotium rolfsii* (Wokocha and Okereke, 2005). *Pythium aphanidermatum*, one of the causes of root rot disease of tomato, is a destructive soil-borne pathogen which attacks several other plants including monocotyledons and dicotyledons was reported to be responsible for root rot of cowpea (Suleiman, 2010). With hot and humid weather being conducive for growth, the fungus is more devastating in tropical and sub-tropical regions

of the world (Emechebe and Shoyinka, 1985 and Williams, 1975). And in Nigeria, the pathogen is of major concern to vegetative growers in the northern savanna belts which potentially ranked among the world's best vegetable production zones.

Chemical control strategies for the pathogen (*Pythium aphanidermatum*) on tomato root rot have not been described. Systemic fungicides for the control of soil-borne diseases caused by fungi of the order peronosporales were first developed in the early 1970s (Sandler *et al*, 1989). But Wiswesser (1976) and Cremlyn (1980) that systemic fungicide benlate (active ingredient 50% benomyl) was first introduced in 1967. Its wide-spectrum systemic activity has been recognized. Edgington *et al* (1971) described the fungicides as highly toxic to Blastosporae fungi. It is said to be particularly useful as a foliar spray and for seed dressing or soil treatment for the control of grey mold, apple scab, canker, storage and root rots, and leaf spot and for major fungal diseases of soft fruits and vegetables (Cremlyn, 1980).

The objectives of this study were to evaluate the efficacy of three fungicides in inhibiting *P. aphanidermatum* of tomato root rot and to investigate their *in vitro* inhibitory effects on mycelia dry weight and sporangia formation.

MATERIALS AND METHODS

Pathogen and fungicides:

Pythium aphanidermatum isolate, obtained from tomato fields in various parts of Kogi State, was used in this experiment, conducted between 2005 and 2008. The fungicides used were ridomil, mancozeb and benlate (benomyl). The weight of each fungicide was calculated to give definite concentrations in parts per million (ppm) of its active ingredient (i.e.). Stock solutions and suspensions were prepared by adding the desire grammes aseptically to the appropriate ml of sterile distilled water in conical flasks according to Fernando and Linderman (1994). The concentrations used were 50ppm, 100ppm, 150ppm, and 200ppm, and these were used for *in vitro* test as amendments on potato dextrose agar (PDA).

Mycelial growth in fungicide-amended media:

Mycelia growth was measured on PDA amended with different concentrations of the fungicides. The fungicides were filter-sterilized after stock solutions were prepared. The PDA was autoclaved and cooled to 45^{o}C before fungicide solutions were added. A graduated sterile syringe apparatus was used to add 20 ml of molten agar medium to each 100 × 15 mm Petri plate. Then, 7-mm-diameter plugs were cut from actively growing colony margins of *P. aphanidermatum* and placed in the center of fungicide-amended medium in three replicate plates per treatment. Inoculated media contained in the plates were incubated at room temperature (25^{o}C) in darkness for 7 days. Mycelial growth (colony diameter) was measured daily and mean of two colony diameters were taken at right angles to each other, minus the diameter of the inoculum (Wiswesser, 1976 and Cremlyn, 1980). The experiment was a completely randomized design regarding plate placement, with three replicates, and was repeated once. The experiment was concluded after seven day of incubation; the results were collated and analyzed. The average radial measurements of the plates were taken. Percentage inhibitions of each of the fungicide at different concentrations were calculated using the formula by Suleiman and Emua, (2009) as follows:

$$\% \text{ Inhibitions} = \frac{\textit{Diam. control plates} - \textit{Diam. in treated plates}}{\textit{Diam. control plates}} \; x \; \frac{100}{1}$$

Inhibition of sporangia formation:

Evaluation of sporangia production was carried out on both solid and liquid media at 50ppm, 100ppm, 150ppm and 200ppm of the three fungicides. The inoculated plates were observed every other day from the third day for 21 days.

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From the solid medium, the number of mature sporangia produced on PDA impregnated with various fungicide concentrations was counted under the leica microscope; while drops of the isolate on liquid medium were taken from incubated flasks and observed under the microscope (Chinoko and Naqvi, 1989). The mean number of sporangia formation was determined.

Effects of the fungicides on mycelia dry weight of the isolate:

The effects of the fungicides on mycelia dry weight of the isolate was determined on liquid medium at 50ppm, 100ppm, 150ppm and 200ppm ridomil, benlate and mancozeb with the inoculation of 7-day-old culture of the isolate in each flask such that the mycelium matt was uppermost and floated on the medium. The replicated cultures in flasks were incubated at 25^{o}C on the laboratory bench, with occasional gentle hand shaking. Harvesting was carried out five days interval with sterile forceps and oven-dried to constant weight on Whatman's filter paper.

Statistical analysis

All results obtained were analyzed using Simple Descriptive Statistics such as mean and standard error in accordance with Norman, (1995). Means were separated using analysis of variance (ANOVA). ANOVA statistical test used was tested at 5% level of significance. A two-tailed test was used for hypothesis testing. **SPSS 15.0** for windows was used for the statistical analysis. Honestly Significant Difference (HSD) was used for inferential statistical analysis while standard error was used for descriptive statistics.

RESULTS

Mycelial growth in fungicide-amended media

All the three fungicides tested inhibited growth of *P. aphanidermatum*. Benlate (a commercial fungicide) was effective in inhibiting the mycelia extension of the pathogen at all levels of concentrations. The mycelial extension decreased with increase in fungicide concentrations. The total inhibition of mycelia by ridomil and benlate at 50ppm and 150ppm respectively shows their effectiveness in inhibiting mycelia growth. Benlate equally affected the growth habit of the fungus as it produced a fluffy, slightly lobbed, aerial mycelium. The inhibitory effects of the fungicide show level of significance at 0.05% compared with control **(Table 1)**. The result between 50ppm and 100ppm showed no significant difference (P 0.24>0.05). Similarly, between 100ppm, 150ppm and 200ppm was not significant (P 0.31>0.05).

Ridomil fungicide was the most effective in inhibiting the mycelial growth of *P. aphanidermatum*. The fungicide inhibited mycelia growth at all concentrations tested, **(Table 1)**. There was no growth at 50ppm, 100ppm, 150ppm and 200ppm; and were significantly difference compared to the control (untreated plates). The effectiveness of mancozeb increases with increase in concentrations. There was no significant difference between 50ppm, 100ppm and 150ppm (P 0.7 > 0.05). And despite complete inhibition at 200ppm of mancozeb, statistically, there was no significant difference (P 0.84 > 0.05) of 100ppm and 150ppm compared **(Table 1)**.

Inhibition of sporangia formation

Benlate was more effective than mancozeb at inhibiting sporangia formation. With P 0.93 > 0.05, there was no significant difference at 150ppm and 200ppm of benlate (Table 2). In mancozeb, there was a significant difference at all concentrations tested compared with control (P 0.00 < 0.05). The fungicides however delayed sporangia formation compared with the untreated plates (Table 2). The number of sporangia formation in the fungicides decreased with increasing fungicide concentration. There

was however no remarkable difference between the two fungicides with respect to sporangia formation.

Effects on mycelia dry weight

Although benlate tend to be more effective with increasing concentrations on mycelia dry weight of *P. aphanidermatum*, there was essentially no significant difference between the performances at various concentrations. Benlate reduced mycelia dry weight at all the concentrations tested; with no significant difference at 150ppm and 200ppm (P 0.36 > 0.05). Mancozeb reduced mycelia dry weight with increase in concentration of the fungicide. There was however no significant difference between the control and 50ppm; as well as between 100ppm and 150ppm, but there was a significant difference between 200ppm and other concentrations tested where there was 93% inhibition (**Table 3**).

Table 1: Inhibitory effects of fungicides on mycelial growth of *P. aphanidermatum*

Concentration (ppm)	Mean percentage inhibition ± SE (%)benlate	Mean percentage inhibition ± SE (%) ridomil	Mean percentage inhibition ± SE (%) mancozeb
Control (0)	0.00 ± 0.0 [a]	0.00 ± 0.0 [a]	0.00 ± 0.0 [a]
50	98.3 ± 0.6 [b]	100.0 ± 0.0 [b]	98.8 ± 0.4 [b]
100	99.2 ± 0.3 [b c]	100.0 ± 0.0 [b]	99.2 ± 0.3 [b c]
150	100.0 ± 0.0 [c]	100.0 ± 0.0 [b]	99.6 ± 0.2 [b c]
200	100.0 ± 0.0 [c]	100.0 ± 0.0 [b]	100.0 ± 0.0 [c]

For each of the fungicide, means represented by the same letter are not significantly different (P ≤ 0.05)

Table 2: Inhibitory effects of Benlate and mancozeb on Sporangial production of *P. aphanidermatum*

Concentration (ppm)	Mean Sporangia No ± SE ($\times 10^4$) (Benlate)	(Mancozeb)
Control (0)	39.67 ± 0.3 [a]	40.67 ± 0.3 [a]
50	19.33 ± 0.3 [b]	20.33 ± 0.3 [b]
100	14.33 ± 0.3 [c]	15.33 ± 0.3 [c]
150	0.31 ± 0.3 [d]	8.67 ± 0.3 [d]
200	0.00 ± 0.0 [d]	0.00 ± 0.0 [e]

For each of the fungicide, means followed by the same letter are not significantly different (P ≤ 0.05)

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Table 3: The mean mycelial dry weight growth of *P. aphanidermatum* on benlate and mancozeb fungicides

Concentration (ppm)	Mean dry weight ± SE (g) (Benlate)	(Mancozeb)
Control (0)	0.16 ± 0.0 [a]	0.15 ± 0.0 [a]
50	0.12 ± 0.0 [b]	0.14 ± 0.0 [a]
100	0.10 ± 0.0 [c]	0.11 ± 0.0 [b]
150	0.05 ± 0.0 [d]	0.10 ± 0.0 [b]
200	0.04 ± 0.0 [d]	0.03 ± 0.0 [c]

For each of the fungicide, means followed by the same letter are not significantly different ($P \leq 0.05$)

DISCUSSION

Generally, fungicides have for a long time been used against pathogenic micro organisms to help curb their detrimental effects on man, other animals and plants which directly or indirectly affect the well being of human beings. Of the three fungicides tested *in vitro*, ridomil was most effective at low concentrations (50ppm) in inhibiting mycelia growth of *P. aphanidermatum*, where complete inhibition was recorded, a confirmation by Lobna (2006), that root rot of squash can be controlled with ridomil. The study also confirms the effectiveness of benlate and mancozeb with complete inhibition of *P. aphanidermatum* at 150ppm and 200ppm concentrations respectively.

The present investigation has shown that benlate and mancozeb progressively inhibited the radial mycelia growth of *Pythium aphanidermatum* on potato dextrose agar. Benlate inhibited mycelia growth by 99.2% at 100ppm while mancozeb could only achieve 99% inhibition at 150ppm compared to the control treatment. These results seem to differ from those of Wokocha and Ebenebe (1980) who reported that benlate had little or no effect on mycelia growth of soil fungus when applied at 500ppm concentration in an *in vitro* experiment. The effectiveness of ridomil at relatively low concentration suggests that it could be used in combination with other very effective fungicides against very resistance fungi (Fernando and Linderman, 1994).

The effects of these fungicides on mycelia dry weight on *P. aphanidermatum* showed that the highest mean mycelia dry weight of 0.14g was recorded at 50ppm in mancozeb while benlate had 0.12g at the same concentration. The two fungicides continued to show decline in mycelia dry weight with increase concentrations, this observation agreed with the work of Fernando and Linderman, (1994). The number and percentages of sporangia production at various concentrations equally differ. The rate of sporangia production and germination in mancozeb were generally higher than those of benlate at the concentrations tested. Benlate appear to contain some

components that are inhibitory to sporangia production and germination than mancozeb, hence, more sporangia germination in the latter than the former.

The higher sensitivity of the mycelium of *P. aphanidermatum* to these fungicides may be due to any of the following possibilities:

a. Differences in the mode and cites of action and in the degree of solubility of the fungicides in water;
b. Differences in the rates of absorption of the fungicides by the fungus or possible detoxification of the fungicides by the fungus.

CONCLUSION

This study provides information serving as a base line in establishing *Pythium aphanidermatum* as the causal pathogen of severe root rots of tomato and very rampant in Kogi State, Nigeria. The present findings with fungicides agreed with previous findings by other works. It showed that ridomil, mancozeb and benlate are very effective in controlling the disease *in vitro*.

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