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In vitro evaluaion of six fungicides on radial mycelial growth and regrowth of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis)* in Samaru, Nigeria

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ABSTRACT: Six fungicides at three rates (1.5x, 1.0x and 0.5x mg a.i/ml) were evaluated on radial growth and and regrowth of mycelia of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) *in vitro*. It was observed that the fungicides (Benomyl, Benomyl + Thiram, Mancozeb, Metalaxyl-m + Thiomethoxan + Difenconazol, Tricyclazole and Carbendazim + Mancozeb) at all the concentrations tested inhibited mycelial growth and regrowth of the fungus. Benomyl, Benomyl + Thiram and Tricyclazole completely inhibited mycelia growth of fungus at 1.5x, 1.0x and 0.5x mg a.i/ml. Metalaxyl-m +Thiomethoxan + Difenconazole, Carbendazim + Mancozeb partially inhibited radial growth and re-growth of mycelia only at 1.5x mg a.i/ml not at 1.0x and 0.5x mg a.i/ml. Inhibitory effect of all the fungicides on mycelia growth and re-growth was greatest at 1.x5 mg a.i/ml.

Key Words: Fungicides, Castor (*Ricinus communis* , *Fusarium pallidoroseum, in vitro*, mycelia.

Introduction

Castor, *Ricinus communis* L., a monotypic genus belong to the family Euphorbiaceae. It is widely cultivated in the tropics, sub tropics and other warm regions for its seed from which castor oil is extracted (Weiss, 1973; Purseglove, 1968). The important part of the crop is the seeds which contains 50-55% oil (Gobin *et. al.*, 2001; Adefris and Nigussie, 1993; Duke, 1983). Traditionally, castor oil is used in lamps lightening and in medicine. The oil is used in industries as cosmetic base, high grade lubricant and in protecting coatings in paints (Gobin *et al*., 2001; Duke, 1983; Purseglove, 1968). In recent, World production is about 25296 MT per annum with Africa producing 1863MT (FAO, 2003). The three largest importers of castor seeds and oil are the United States, France and United Kingdom with Brazil(60%), India(17%) and China(6%) being the largest growers and exporters (FAO, 2004). Presently in Nigeria, there is high demand for castor oil in industries and the Federal Government is encouraging its production.

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Some reported diseases of castor are Bacterial leaf spot caused by *Xanthomonas ricini* (Anon.1971), Wilt caused by *Fusarium oxysporum f. sp ricini, R*oot rot *(Macrophomina phaseolina*), gray rot caused by *Botrytis ricini* (Anjani *et. al.,* 2004), seedling blight by *Phytphthora palmivora* (Uchida and Aragaki; 1988), Leaf spot (*Cercospora ricinella)* and Alternaria leaf spot (*Alternaria ricini)* (Duke, 1983;Anon. 1971). In India, yield loss of 80-100% has been attributed to fungal diseases (Anjani *et. al.,* 2004) and this affects the income of farmers in terms of foreign exchange. Many *in vitro* studies have demonstrated that some fungicides restrict or prevent the growth of fungal pathogens (Marley and Gbenga 2004; Karaoglanidis *et. al.*; 2004). In the literature there are only few or no reports about the influence of fungicides on mycelial growth of *Fusarium pallidoroseum* from Castor plant. In view of the importance of the crop and the effect of fungal diseases on the yield, there is a need to identify management options for disease(s) associated with this important and relatively new crop.

The aim of the study was to determine *in vitro* effects of selected fungicides on the mycelial growth of *F. pallidoroseum* isolated from castor.

Materials and Methods

Six (6) fungicides were evaluated at three rates (1.5x, 1.0x, and 0.5x, times the field rate recommended by the manufacturer). The fungicides at their manufacturer's recommended rates (x) were Benomyl 30g/20 litres (Benlate 50 WP Dupont), Benomyl + Thiram 1kg/800 ml (Bentex T 40 WP Dupont), Carbendazim + Mancozeb 2.5kg/ha (Team 85 WP African Agro), Tricyclazole 150-200g/ha (Profit agrochemicals), Metalaxy-m +Thiomethoxan + Difenconazol 10g/40kg seeds (Apron Star 44 SD/WP Sygenta) and Mancozeb 6.25g/kg (Dithane M45 80 WP Dupont). The required quantity of each fungicide for each concentration was weighed and dissolved in 5ml of ethanol and made up to 100 ml with freshly prepared PDAS (Potato Dextrose Agar with Streptomycin) and allowed to cool to a pouring temperature of 40-45^{o}C. Twenty milliliters of these PDAS amended with different fungicide rates was poured into 9cm diameter petri-dish. Each plate including the control (without fungicide) on solidification was inoculated in the middle with 14 days old *F. pallidoroseum* culture using 0.5cm cork borer. Labeled petri dishes were placed in an incubator at 28±2 ^{o}C and observed daily for mycelial growth. The experiment was laid in completely randomized design with each rate of fungicide making a treatment replicated 5 times and 1 petri dish representing a replicate.

Radial mycelial growth was taken at seven and fourteen days after inoculation (DAI), by measuring the diameter along two perpendicular lines, from the underside of the petri dishes.

To determine the mycelia re-growth from 14 days old culture was carried out by using a 0.5cm cork borer to cut from actively growing portion of mycelia from fungicide treated Petri-dishes and placed in centre of new plates of fungicide free PDAS. Five plates were inoculated making five replicates for each of the treatments. These Petri dishes were observed for mycelial growth weekly. At 14 days after inoculation (DAI) mycelial radial growth was measured as described earlier to determine the re-growth of mycelia from fungicide free Petri-dishes. Each experiment was repeated once.

Data collected were subjected to statistical analysis using simple ANOVA (SAS 2002) and means separated using Least Significant Difference (LSD).

Results

All the fungicide significantly (P=0.05) inhibited mycelial growth compared to the control at 7 and 14 DAI (Tables 1 and 2). The inhibitory effect of all fungicides generally increased with increase in concentration except Benomyl, Benomyl + Thiram and Tricyclazole which completely inhibited mycelia growth at all the three concentrations. The inhibitory effect of Metalaxy-m + Thiomethoxan + Difenconazol and Carbendazim + Mancozeb at 1.5 and1.0x mg a.i/ml did not differ significantly, but were better than at 0.5x mg a.i/ml. At 14 DAI, however the inhibitory effect of Carbendazim + Mancozeb was significantly decreasing with decrease in concentrations (Table 2). Mancozeb gave the least inhibitory effect on mycelial

growth but there was no significant difference between 1.0x and 0.5 x concentrations, in respect to mycelial growth at 7 and 14 DAI (Table 2).

Table 1: Effect of varying concentrations of fungicides on mycelial growth of *Fusarium pallidoroseum in vitro* at 7 days of inoculation.

Concentration mg ai/ml (x)	Mycelial growth (cm)					
	Benomyl	Mancozeb	Benomyl + Thiram	Metalaxy -m + Thiomethoxan+ Difenconazole	Tricyclazole	Carbendazim + Mancozeb
1.5	0.5b	3.8c	0.5b	0.5c	0.5b	0.5c
1.0	0.5b	6.5b	0.5b	3.1b	0.5b	0.7c
0.5	0.5b	7.0b	0.5b	4.0b	0.5b	3.0b
Control	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a
LSD	0.0	0.8	0.0	0.4	0.0	0.4

Means followed by the same letter in each column are not statistically different (P = 0.05).
x = Manufacturers recommended rate of fungicide
a. i /ml = active ingredient per ml

Table 2: Effects of varying concentrations of fungicides on mycelial growth of *Fusarium pallidoroseum in vitro* at 14 days of inoculation.

Concentration mg ai/ml (x)	Mycelial growth (cm)					
	Benomyl	Mancozeb	Benomyl + Thiram	Metalaxy-m + Thiomethoxan + Difenconazole	Tricyclazole	Carbendazim + Mancozeb
1.5	0.5b	4.2c	0.5b	0.5c	0.5b	0.5d
1.0	0.5b	7.1b	0.5b	3.7b	0.5b	0.9c
0.5	0.5b	7.2b	0.5b	4.7b	0.5b	4.0b
Control	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a
LSD	0.0	0.6	0.0	0.3	0.0	0.2

Means followed by the same letter in each column are not statistically different (P = 0.05).
x = manufacturers recommended rate.
a.i/ ml = active ingredient per ml

Inhibitory effect of all the fungicides on mycelial re-growth also decreased with decrease in the concentrations, except for Benomyl, Benomyl + Thiram and Tricyclazole which completely inhibited mycelial re-growth at 1.5x, 1.0x and 0.5x mg a.i/ml and did not differ statistically (P = 0.05) (Table 3). Metalaxy-m+ Thiomethoxan + Difenconazol and Carbendazim + Mancozeb completely inhibited mycelial regrowth at 1.5x mg a.i/ml. For these two fungicides, 1.0x mg a.i/ml significantly inhibited mycelial re-growth than at 0.5x mg a.i/ml (Table 3). Mancozeb gave the least inhibitory effect on mycelial re-growth; its inhibitory effect at 1.5x mg a.i/ml was significantly more than at 1.0x and 0.5x mg a.i/ml which were statistically the same. All the fungicides at all three concentrations inhibited mycelial re-growth significantly (P=0.05) compared to control.

The fungicides also affected the cultural growth of *F. pallidoroseum* mycelia on solid PDAS. The growth of fungi on mancozeb (DM 45) treated petri dish was flat spreading and not fluffy as in the control at all the three concentrations used (plate1 to 3) while Metalaxy-m + Thiomethoxan + Difenconazol (Apron star) at 1.0 and 0.5 x mg a.i/ml concentrations and Carbendazim + Mancozeb (Team) at 0.5 x mg a.i/ml the growth was similar to control (Plates 1 and 2). None of the fungicides influenced mycelial colour.

Table 3: Effects of varying concentrations of fungicides on mycelial regrowth of *Fusarium pallidoroseum* at 14 days after inoculation.

Concentration mg ai/ml (x)	Mycelial regrowth(cm)					
	Benomyl	Mancozeb	Benomyl + Thiram	Metalaxy-m + Thiomethoxan + Difenconazole	Tricyclazole	Carbendazim + Mancozeb
1.5	0.0b	7.0c	0.0b	0.0d	0.0b	0.0d
1.0	0.0b	8.1b	0.0b	7.3c	0.0b	7.4c
0.5	0.0b	8.2b	0.0b	8.1b	0.0b	8.5b
Control	9.0a	9.0a	9.0a	9.0a	9.0a	9.0a
LSD	0.0	0.3	0.0	0.6	0.0	0.4

Means followed by the same letter in each are column not statistically different at (P = 0.05)
ai/ ml = active ingredient per ml
x = recommended rate of each fungicide

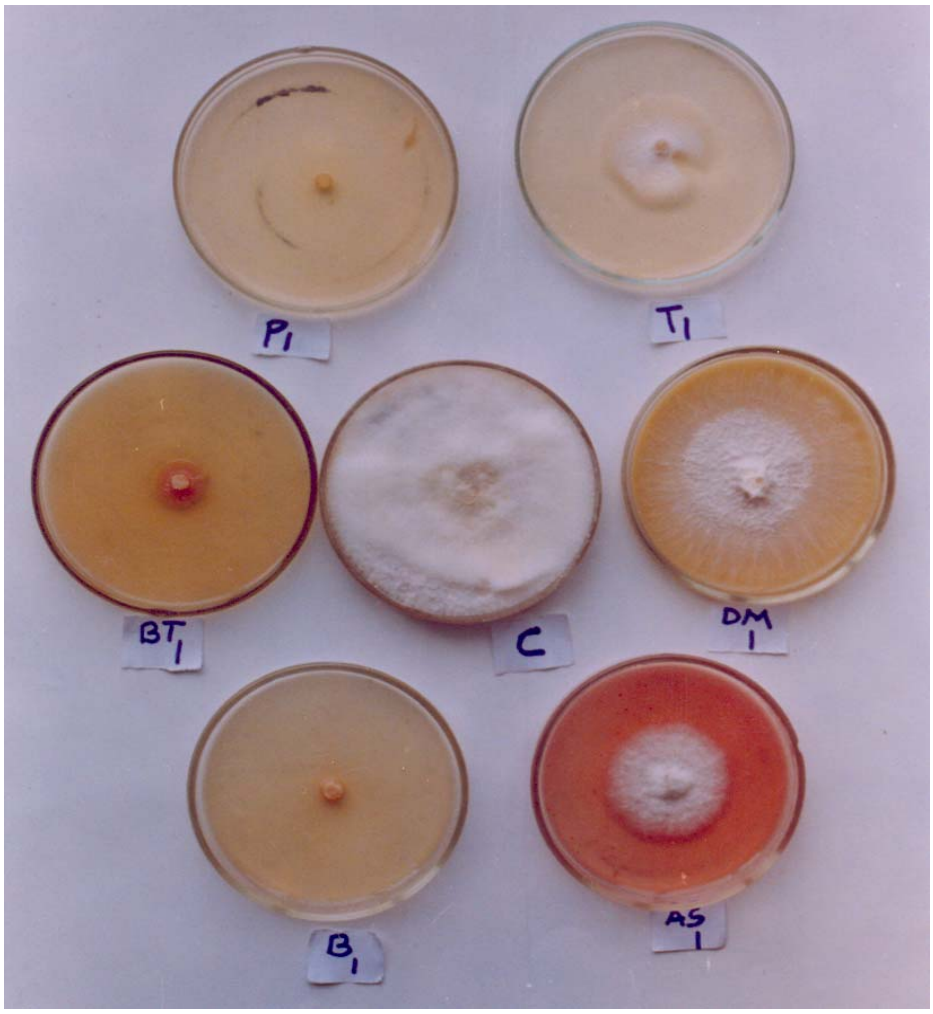

Plate 1: *In vitro* effect of fungicides at 0.5x concentration on the mycelial growth of *F. pallidoroseum* 7 DAI.

Key: P = Tricyclazole; T = Carbendazim + Mancozeb; DM45 = Mancozeb; BT = Benomyl + Thiram; B = Benomyl; AS = Metalaxyl –m + Thiomethozan + Difenconozole; C = Control;

Where x is the manufacturers recommended rate of fungicide

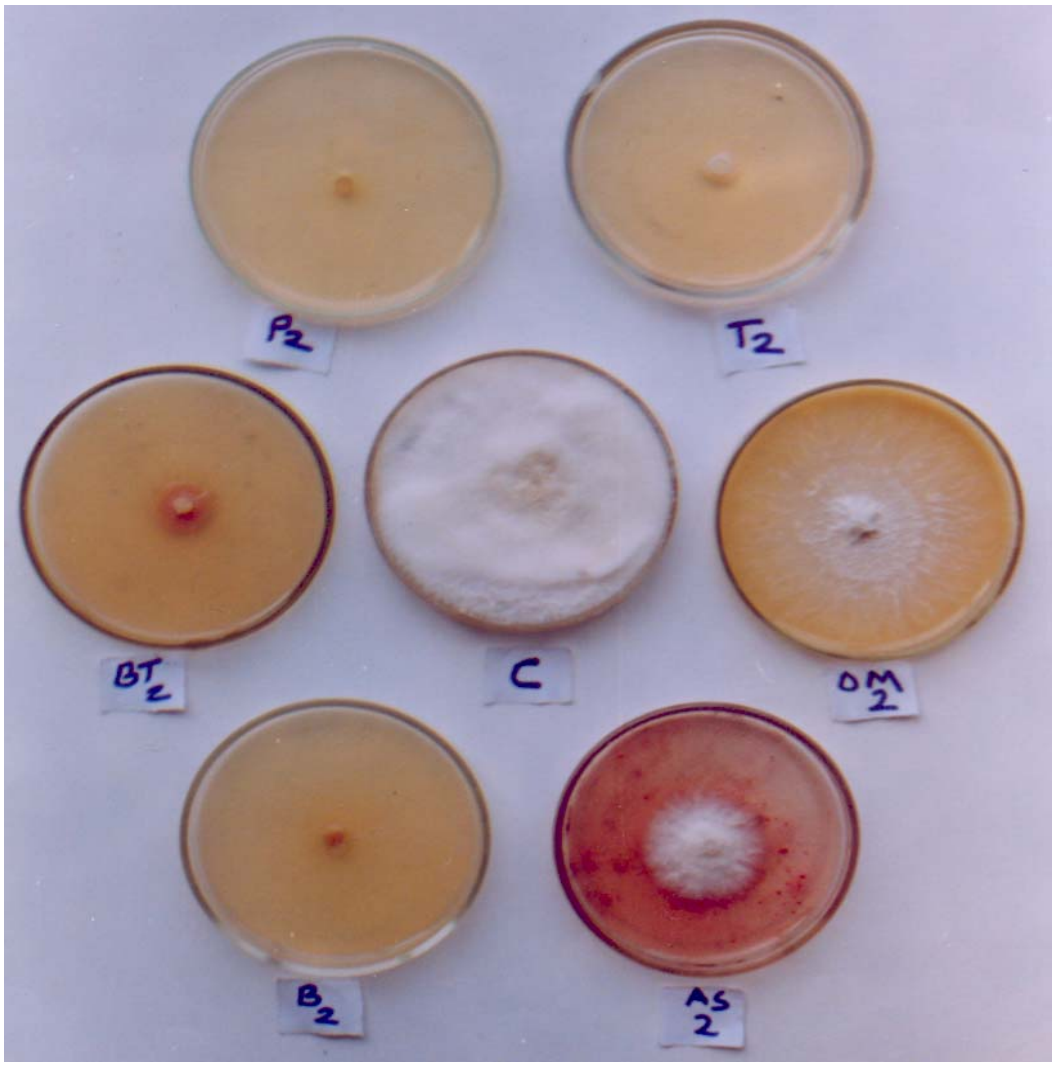

Plate 2: *In vitro* effect of fungicides at 1.0x concentration on the mycelial growth of *F. pallidoroseum*, 7 DAI.

Key

P = Tricyclazole
T = Carbendazim + Mancozeb
DM45 = Mancozeb
B = Benomyl
BT = Benomyl + Thiram
AS = Metalaxyl-m + Thiomethozan + Difenconozole
C = Control
Where x is the manufacturers recommended rate of fungicide

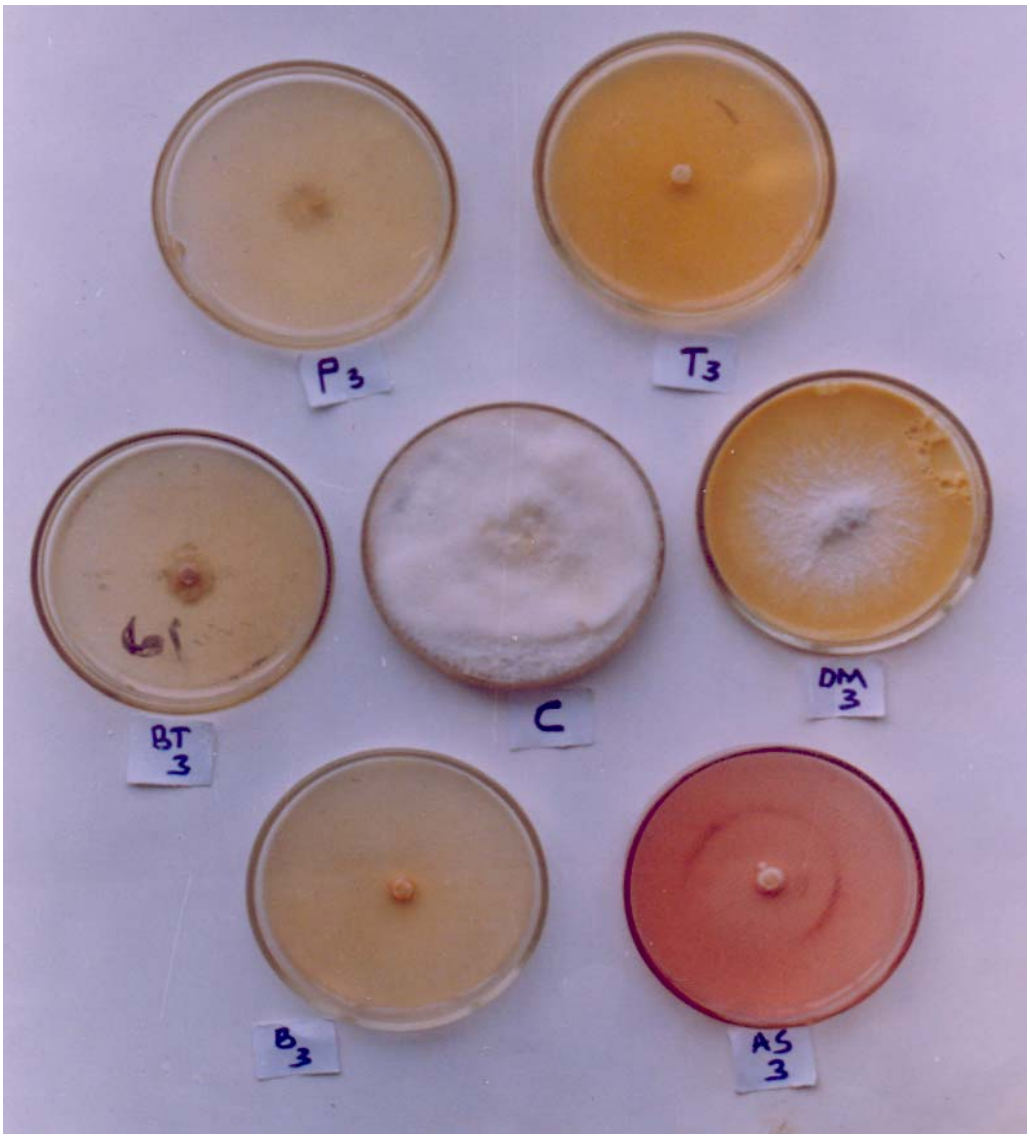

Plate 3: *In vitro* effect of fungicides at 1.5x concentration on the mycelial growth of *F. pallidoroseum*, 7 DAI.

Key

P = Tricyclazole
T = Carbendazim + Mancozeb
DM45 = Mancozeb
B = Benomyl
BT = Benomyl + Thiram
AS = Metalaxyl-m + Thiomethozan + Difenconozole
C = Control
Where x is the manufacturers recommended rate of fungicide

Discussion

All fungicides tested in the laboratory significantly reduced pathogen development when compared with the control. Treatment with Benomyl, Benomyl+Thiram and Tricyclazole had the greatest inhibitory effect on mycelial growth and re-growth at all concentrations (1.5x, 10x and 0.5x mg a.i/ml). Inhibitory effect of Benomyl against many plant pathogenic fungi has been reported by many researchers. Marley and Gbenga (2004) demonstrated the efficacy of Benomyl at 5, 10 and 20 g to control *stenocarpella maydis* on maize *in vitro* and it was found to be effective. Similarly, Karaoglanidis *et. al.;* (2003) reported the effectiveness of Benomyl in controlling leaf-spot caused by *Cercospora beticola* on sugar beet. In this study Mancozeb gave the least inhibitory effect on pathogen. Contrarily, Obagwu (1997) reported Mancozeb to be effective in the *in vitro* control of brown blotch of Bambaranut caused by *Colletotrichum capsici*. The use of Benomyl, Carbendazim and Mancozeb significantly inhibited Physoderma maydis on maize (Brown spot) (Osunlaja, 1999) and there was complete inhibition of sporangia germination at 10,000 ppm a.i of the fungicides. Carbendazim at 50,000 ppm a.i was effective in the control of the disease on field and it result in highest grain yield. Metalaxyl and Benzoid fungicide has been effective in the inhibition of mycelia growth of *Pythium deliense* and *Pythium oligandrum* isolated from damped- off maize (Hani, *et. al.* 2004).

Although this is the first time such a study is been conducted on *F.pallidoroseum* on castor in Samaru, Nigeria. The result shows that the fungicides (Benomyl, Benomyl+Thiram and Tricyclazole, Metalaxyl-m + Thiomethozan + Difenconozole Carbendazim and Mancozeb,) evaluated had inhibitory effect on mycelial growth of *F. pallidoroseum in vitro* at all the three concentrations used especially high concentration.

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