

Effect of six fungicides on sporulation of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) in Samaru, Nigeria

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Abstract: Six fungicides at three rates i.e. one and half, one and half of recommended rates (1.5x, 1.0x and 0.5x mg a.i/ml) were evaluated on the spores of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) in vitro. It was observed that the fungicides (Benomyl, Benomyl + Thiram, Mancozeb, Metalaxyl-M + Thiomethoxan + Difenconazole, Tricyclazole and Carbendazim + Mancozeb) at all the concentrations tested it inhibited sporulation of the fungus. Benomyl, Benomyl + Thiram and Tricyclazole completely inhibited sporulation at 1.5x, 1.0x and 0.5x mg a.i/ml. Metalaxyl-M + Thiomethoxan + Difenconazole, Carbendazim + Mancozeb partially inhibited sporulation only at 1.5x mg a.i/ml, not at 1.0x and 0.5x mg a.i/ml. The inhibitory effect of all the fungicides on sporulation was greatest at 1.5x mg a.i/ml.

Keywords: fungicides; castor; *Ricinus communis*; *Fusarium pallidoroseum*; in vitro, Mycelia, Potato dextrose agar (PDA)

I. Introduction

Castor, *Ricinus communis* L., a monotypic genus belong to the family Euphorbiaceae. It is widely cultivated in the tropics, subtropics and other warm regions for its seed from which castor oil is extracted (Purseglove, 1968; Weiss, 1973). The important part of the crop is the seeds which contain 50–55% oil (Duke, 1983; Adefris and Nigussie, 1993; Gobin *et al.*, 2001). Traditionally, castor oil is used for Lightening of lamp and in medicine as a purgative in Egypt and the Mediterraneans (Adefris and Nigussie, 1993). The oil is used in industries as a cosmetic base, high-grade lubricant and as protective coating in paints (Purseglove, 1968; Duke 1983; Gobin *et al.* 2001). In recent years, world production is about 25 296 MT per annum with Africa producing 1863 MT (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, due to the great potentials found in castor seeds and oil there is high demand for castor oil in industries and the Federal Government is looking for ways to encourage its production so as to diversify the economy. Some reported diseases of castor are bacterial leaf spot caused by *Xanthomonas ricini* (Anon, 1971), wilt caused by *Fusarium oxysporum* f. sp *ricini*, root rot (*Macrophomina phaseolina*), gray rot caused by *Botrytis ricini* (Anjani *et al.* 2004) seedling blight by *Phytophthora palmivora* (Uchida and Aragaki, 1988), leaf spot (*Cercospora ricinella*) and Alternaria leaf spot (*Alternaria ricini*) (Anon, 1971; Duke, 1983). In India, yield loss of 80–100% has been attributed to fungal diseases (Anjani *et al.* 2004) and this has affected 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 (Karaoglanidis *et al.* 2003; Marley and Gbenga 2004). In the literature there are only a few reports about the influence of fungicides on mycelia 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 the *in vitro* effects of selected fungicides on the sporulation of *F. pallidoroseum* isolated from castor.

II. Materials And Methods

Six fungicides were evaluated at three rates (1.5x, 1.0x, and 0.5x, the field rate as recommended by the manufacturer). The fungicides at their manufacturer's recommended rates (x) were Benomyl 30 g/20 liters (Benlate 50 WP Dupont), Benomyl + Thiram 1 kg/800 ml (Bentex T 40WP Dupont), Carbendazim + Mancozeb 2.5 kg/ha (Team 85 WP African Agro), Tricyclazole 150–200 g/ha (Profit agrochemicals), Metalaxyl-M + Thiomethoxan + Difenconazol 10 g/40 kg seeds (Apron Star 44 SD/ WP Sygenta) and Mancozeb 6.25 g/kg (Dithane M45 80 WP Dupont). The required quantity of each fungicide for each concentration was weighed and dissolved in 5 ml 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°C. Twenty milliliters of these PDAS amended with different fungicide rates was poured into 9 cm diameter Petri dishes. Each plate including the control (without fungicide) on solidification was inoculated in the middle with 14 day old *F. pallidoroseum*

culture using a 0.5 cm cork borer. Labeled Petri dishes were placed in an incubator at 28 + 28°C and observed daily for mycelial growth. The experiment was laid in completely randomized design with each rate of fungicide making a treatment replicated five times and five Petri dishes representing a replicate. At 14 days after inoculation, mycelia were harvested from the culture using a sterilized scapel. Numbers of spores were observed using a haemocytometer. For each fungicide and their various rates, conidial suspension was prepared from 5 petri dishes. To obtain 10% spore concentration, 10 ml of the initial inoculum was added to 90 ml of sterile distilled water. Using a pipette, 1 ml of the suspension was mounted on the surface of the counting chamber of the haemocytometer, and the cover-slip was slid back and forth against the chamber until spores were hermatically attached together as a result of which Newton's rings appear and observed under a light microscope and the number of spores counted per chamber. Spores were counted four times to represent four

replications for each fungicide rate and their means recorded and using the equation; $\frac{n}{256} \times 4 \times 10^6$ to give the

total number of conidia in 1 ml of the conidia suspension (Booth, 1971). Where: n=number of conidia counted in the chambers, 256= constant volume obtained from 16x16, 4×10^6 = constant. . Data collected were subjected to statistical analysis using simple ANOVA (SAS 2002) and means separated using Least Significant Difference (LSD). Means of different rates per fungicide were compared.

III. Results

The effects of various fungicides on sporulation of *Fusarium pallidoroeseum in vitro* are summarized in Table 1. The differences among the different concentrations in relation to sporulation were statistically significant (P=0.05). All the concentration of fungicides evaluated significantly inhibited sporulation when compared with the control. Benomyl, Benomyl +Thiram and Tricyclazole completely inhibited sporulation. The inhibitory effect of this fungicides did differ statistically (P = 0.05), all of them had similar trend at 1.5x, 1.0x and 0.5x mg a.i/ml. On the other hand Mancozeb and Carbendazim + Mancozeb could not inhibit sporulation at all the 3 concentrations used even though there was decrease in sporulation as concentration increases. However, metalaxyl-M + Thiomethoxan + Difenconazole completely inhibited sporulation at 1.5x mg ai/ml ,while at 1.0x and 0.5x mg a.i/ml there was partial inhibition when compared with the control. However, Benomyl, Benomyl + Thiram and Tricyclazole completely inhibited mycelial growth therefore no spores were formed. Meanwhile, Mancozeb and Carbendazim +Mancozeb did not have any significant differences with the control. All the concentrations of the fungicides used significantly inhibited sporulation when compared with the control. Benomyl, Benomyl + Thiram, Tricyclazole had the greatest inhibitory effect on sporulation of fungi. Mancozeb gave the least inhibitory effect on *Fusarium pallidoroeseum*, while Carbendazim + Mancozeb and Thiomethoxan + Metalaxyl-M + Difenconazole gave partial inhibition especially with increase in concentration to 1.0x and 1.5x mg a.i/ml.

IV. Discussion

In vitro evaluation of Benomyl by other researchers showed that the fungicide inhibited sporulation of *Verticillium* sp (Sivaprakasan and Rajagopalan (1974), *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* (Shimfe, 1984), *Curvularia* sp (Bawa, 1992). On the contrary, Yar'adua (1986) in his *in vitro* study of fungicides found that at 500ppm concentration benomyl did not inhibit the sporulation of *Alternaria porri* completely. Mancozeb at all the rates of 1.5x, 1.0x and 0.5x mg a.i/ml partially inhibited sporulation of *Fusarium* sp *in vitro*. Although report shows that it has been effective on other fungal pathogens. Obagwu (1997) reported Mancozeb to be effective *in vitro* in the control of brown blotch of Bambaranut caused by *Colletotrichum capsici*. Singh *et al.* (1974) reported that mancozeb was effective against *Curvularia lunata*. However, Mancozeb has been reported to enhance sporulation of *F. oxysporum* Agrawal *et al.* (1974). Although this is the first time these fungicide formulations are been evaluated, Carbendazim + Mancozeb effectively controlled mycelia growth at 1.5x and 1.0x mg a.i/ml while Tricyclazole was able to completely inhibit sporulation at all the concentrations used in this study, Apron star (Thiomethoxan + Metalaxyl -M + Difenconazole) had high inhibitory effect on sporulation of *Fusarium pallidoroeseum* at 1.5x mg a.i/ml than at 1.0 and 0.5x mg ai/ml which is in agreement with previous research where it has been effective for the control of other pathogenic fungi. Singh (1983) reported that application of Metalaxyl on Pearl millet plant infected with *Sclerospora graminicola* controls it. Bawa (1992) report the efficacy of Metalaxyl plus Carboxin (Apron plus) on mycelial growth and sporulation of *Curvularia* sp *in vitro*. The report shows that efficacy increased with increase in concentration. In this study Tricyclazole and Carbendazim + Mancozeb had complete and partial inhibitory effect respectively on sporulation of *Fusarium pallidoroeseum in vitro*

Table 1: Effects of varying concentrations of fungicides on sporulation of *Fusarium pallidoroseum* in vitro at 14 days after inoculation.

Concentration mg ai/ml (x)	Conidia ml(4x10 ⁶)					
	Benomyl	Mancozeb	Benomyl + Thiram	Thiomethoxan + Metalaxy-M + Difenoconazole	Tricyclazol	Carbendazim + Mancozeb
1.5	0.00b	36.44b	0.00b	0.00d	0.00b	26.8d
1.0	0.00b	40.50b	0.00b	98.85c	0.00b	34.6c
0.5	0.00b	51.87b	0.00b	105.72b	0.00b	54.8b
Control	168.0a	168.0a	168.0a	168.0a	168.0a	168.0a
LSD	0.0	0.004	0.0	0.0015	0.0	0.0048

Means followed by the same letter in each column are not statistically different at (P =0.05)

a.i/ ml = active ingredient per ml

x = recommended rate of each fungicide

V. Conclusion

These inorganic fungicides can be used to protect castor plant from *F.pallidoroseum* since some of the fungicide was able to inhibit mycelial growth and eventually inhibiting spore formation. Also with increased rate the efficacy was better. However, there is the need to evaluate these fungicides on the field.

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