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Research Article

Performance of Fungicides on Mycelial Growth of Colletotrichum Spp

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Research on the efficacy of systemic and contact fungicides on mycelial growth of Colletotrichum was conducted in the Mycology Laboratory, Department of Crop Protection, Faculty of Agriculture, Institute of Agricultural Research, Ahmadu Bello University Zaria, to determine the most effective fungicides in the control of Colletotrichum leaf blight diseases. Each fungicide was amended at three rates viz; Manufacturers recommended rate, 50% less and 50% higher than the recommended rates. Mycelial growth, Macro and Micro conidia were measured. The results show that at 2 days after inoculation (DAI), all the fungicides irrespective of the rates gave statistically similar values of 2.00mm with the exception of control treatment (19.13mm). At 4DAI, the highest mycelia growth was obtained in the control treatment (37.00mm), followed by carbendazim at rate 1 (18.50mm), lowest mycelia growth of 2.00mm was observed with the application of mancozeb and benomyl irrespective of the concentration.. The effects of fungicides on micro conidia of Colletotrichum spp with respect to spores per millilitre, length and width, has significant difference among the treatments. Control had the highest number of spore (53011719) followed by benomyl treatments at rate 1, while no spore was counted in mancozeb + carbendazim and mancozeb treatments at all rates. Result also shows higher spore length in benomyl treatment at rate 1 (4.36µm) followed by rate 2 of the same fungicide (4.22µm). Mancozeb used alone inhibited the mycelial growth of Colletotrichum, others also at combined rates

Key words: Colletotrichum, Mycelia, Fungicides, Conidia, Mancozeb

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INTRODUCTION

Fungicides are important tools for management of plant diseases caused by fungal and oomycete pathogens. However, fungicide usages need to be carefully planned with a good understanding of plant disease epidemics, their components (host, environment and pathogens), fungicide mode of action (biochemical, biological, physical), risk of resistance development and host physiology, among other aspects (Eladi, 1992). Fungicides can either be contact, translaminar or systemic. Contact fungicides are not often taken up into the plant tissue, and protect only the plant where the spray is deposited; translaminar fungicides redistribute the fungicides from the upper, sprayed leaf surface to the lower, unsprayed surface; systemic fungicides are taken up and redistributed through the xylem vessels, by and large, few fungicides move to all parts of a plant (Lopez *et al.,* 2005).

Fungicides kill fungi by damaging their cell membrane, inactivating critical enzymes or proteins, or by interfering with key processes such as energy production or respiration (Hutson and Miyamoto, 1999). Others impact specific metabolic pathways such as the production of sterols or chitin. For example, phenylamide fungicides bind to end inhibit the function of RNA polymerase in oomycetes, while the benzimidazole fungicides inhibit the formation of betatubulin

polymers used by cells during nuclear division (Pattnaik *et al*., 1996). Mode of action determines which fungi will be affected by a fungicide and thus, which diseases can be controlled by using the fungicides. Similarly, fungicides with different modes of action are needed in a disease management program to delay fungicide resistance development (Hewitt, 1998). The study aimed to to determine ascertain the most appropriate fungicide(s) and rate for the control of the diseases.

METHODOLOGY

In vitro Evaluation of Fungicides on the Mycelial Growth of *Colletotrichum* spp

Preparation of Media (Potato Dextrose Ager)

Two hundred grams of peeled slices of Irish potato tubers were boiled in one litre of water. Solution was filtered through doubled layer muslin cloth. Dextrose (20g) and agar-agar powder (15g) were added to the filtrate; volume was made to one litre and reboiled to dissolve agar-agar (homogenized) through stirring. The solution (media) was poured into conical flask and autoclaved at pressure 15psi. Streptomycin sulphate was prepared by diluting 5g with 10ml sterile distilled water and added into the media when it has cooled. The media was allowed to cool in the pouring room.

Preparation of Fungicides (Amendment of Fungicides with Media)

Five fungicides were selected for the experiment viz; Mancozeb, a contact fungicide (Z-Force 80% WP), Carbendazim, a systemic fungicide (Forcelet 50% WP), Mancozeb (63%) + Carbendazim (12.5%), a combination of contact and systemic fungicide (Fungu force 75.5% WP), imidacloprid 20% + metalaxyl-m 20% + tebuconazole 2%, a combination of systemics fungicide (Dressforce 42% WS) and Benomyl, a systemic fungicide (Benomyl 50% WP). The chemicals were prepared at three levels; 0.5x, 1.0x and 1.5x. Where 'x' is the recommended field rate by the manufacturers levelled 1, 2 and 3 respectively. An electric weighing scale was used to measure the chemicals at different rates and mixed up with the media accordingly then dispensed into 9cm Petri-dishes in aseptic condition. However, in each treatment a Petri-dish without any form of fungicide was reserved as control.

Already, lines were drawn at the base of each Petri-dish diagonally to enable the measurement of radial mycelia growth of the fungal pathogens after inoculation. Four repetitions of each treatment were used per isolate. Thus; 5 fungicides at 3 rates in 4 replications = 5 x 3 x 4 = 60 plates + 4 control = 64 plates. The experiment was laid in Completely Randomized Design (CRD) in the laboratory.

Inoculation of Media with Fungal Isolates

A small segment ($2mm^3$) of mycelia mats of the *Colletotrichum* spp isolate was inoculated at the centre of 90.00mm Petri-dishes using a sterile cork borer (2mm). Mycelia growths of the isolates along the perpendicular lines were measured and the average determined (Zafar *et al.,* 2010). This was made at a day interval for fourteen days during which some of the isolates have filled up the Petri-dishes.

At 14 days after inoculation, cultures of the isolates on PDAs from control were harvested through scraping the Mycelia mat using sterile scalpel to determine the spore concentration. The harvested Mycelia was placed in a 250ml beaker, blended in 80ml distilled water, using Binatone 5 speed Turbo Blender (model number; HM - 350S), it was then filtered through double layer muslin cloth. The remaining 20ml of the distilled water was used to rinse the blender and beaker used. A sterile pipette was used to collect 0.1ml of the suspension and placed on the surface of the counting chamber of haemocytometer and covered with a cover slip. The suspension was left for 15 – 20 seconds to allow conidia to settle. Numbers of conidia were counted from square grids in the counting unit of the haemocytometer under electrically powered binocular microscope at x400 magnification. Same procedure was repeated four times per treatment. Spore concentration was calculated using the formula adopted by Marley (2013);

$$C = \frac{n}{256} 4 \times 10^6$$

Where:
C = number of conidia per millilitre
n = number of conidia counted in the chamber
256 = constant volume obtained from 16 x 16 square grids
4×10^6 = constant

The sizes of macro and micro-conidia (width & length) were measured using ocular micrometer mounted on binocular microscope at x400 magnification. Adjusting knobs were used to align conidia with the scale in order to obtain the value. Depending on the number of conidia, up to fifty were measured for each treatment. The whole *in vitro* experiment was run twice.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) procedure using SAS (2012) software. Significant difference among the treatment means were separated using Duncan Multiple Range Test (DMRT).

RESULT

Effects of Fungicides on Mycelia Growth of *Colletotrichum* spp

Results on the effect of different fungicides on mycelia growth of *Colletotrichum spp.*is shown in Table 1. The results show that at 2DAI, all the fungicides irrespective of the rates gave statistically similar values of 2.00mm with the exception of control treatment (19.13mm). At 4DAI, the highest mycelia growth was obtained in the control treatment (37.00mm), followed by carbendazim at rate 1 (18.50mm), lowest mycelia growth of 2.00mm was observed with the application of mancozeb and benomyl irrespective of the concentration. Similar trend of mycelia growth was maintained at 6 and 8 DAI. At 10DAI, the highest growth was recorded with control treatment (72.75mm); this was followed by benomyl at rate 1 with 35.63mm (Plate 7). Also, at 12DAI control treatment had 84.00mm while benomyl recorded 45.00mm. Similar trend was observed at 14DAI in control treatment with a maximum mycelia growth in petridish (90mm) seconded by benomyl applied at rate 1 with 50.13mm. However, no mycelia growth was recorded in mancozeb + carbendazim and mancozeb treatments at all rates used.

Table 1. Effect of Fungicides on Mycelia Growth (mm) of *Colletotrichum* spp.

		Days after inoculation						
Fungicides	**Rate**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Benomyl	1	14.25d	24.63d	39.50c	47.75d	57.00e	64.25e	68.25d
Benomyl	2	10.13f	16.75e	26.38e	35.25e	43.75f	46.38f	49.75e
Benomyl	3	2.00g	11.25f	17.13f	23.00f	27.88g	31.75g	34.50f
Dressforce	1	2.00g	8.13g	14.50g	16.38g	19.50h	22.25h	24.00g
Dressforce	2	2.00g	2.00h	9.38h	11.13h	13.75i	17.50i	18.75h
Dressforce	3	2.00g	2.00h	7.25i	9.50i	11.38j	13.38j	14.63i
Forcelet	1	18.13b	33.50b	47.75b	64.25b	78.25b	88.25b	90.00a
Forcelet	2	15.75c	27.50c	39.50c	52.25c	65.50c	74.75c	85.00c
Forcelet	3	11.50e	23.75d	33.50d	48.25d	61.25d	68.25d	82.25b
Funguforce	1	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Funguforce	2	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Funguforce	3	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Z-force	1	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Z-force	2	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Z-force	3	2.00g	2.00h	2.00j	2.00j	2.00k	2.00k	2.00j
Control	0	24.75a	39.75a	57.76a	78.75a	85.75a	90.00a	90.00a
Significance		*	*	*	*	*	*	*

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance *Rates
1= half dose, 2= normal dose and 3= one and half dose

Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb + Carbendazim and Z-force - Mancozeb

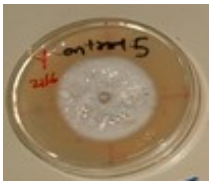
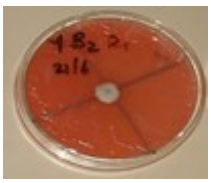
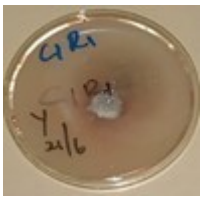
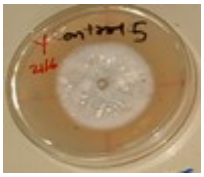
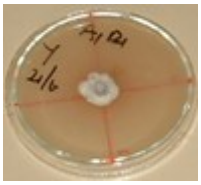
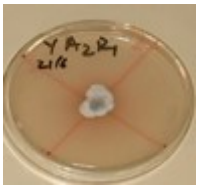
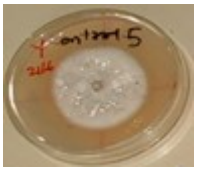
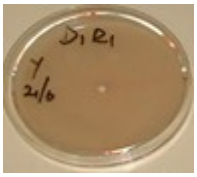
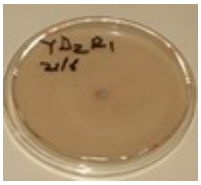
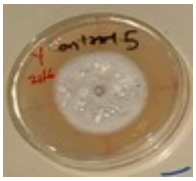
Fungicides	Control	50% Concentration	100% Concentration	150% Concentration
Dress force				
Forcelet				
Benomyl				
Fungu force				
Z-force				

Plate 1. Effects of Different Fungicides Concentration on *Colletotrichum* spp.

Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb + Carbendazim and Z-force - Mancozeb

Table 2 presents the result on the effects of fungicides on micro conidia of *Colletotrichum* spp. with respect to spores per millilitre, length and width with significant difference among the treatments. Control had the highest number of spore (53011719) followed by benomyl treatments at rate 1, while no spore was counted in mancozeb + carbendazim and mancozeb treatments at all rates. Result also shows higher spore length in benomyl treatment at rate 1 (4.36µm) followed by rate 2 of the same fungicide (4.22µm).

Higher micro conidia width of 2.68µ was recorded in imidacloprid + metalaxyl-m + tebuconazole rate 1 while other treatments are statistically the same except for mancozeb + carbendazim and mancozeb that recorded 0 values.

A result on the effects of fungicides on macro conidia of Colletotrichum is presented in Table 3. Number of spores per milliliter showed significant difference among the treatments. Control treatment had the highest number of spores (1324369) followed by benomyl at rate 1 with 722666, while no spore was observed in mancozeb + carbendazim and

mancozeb at all rates. With respect to length of macro conidia of C*olletotrichum*, control maintained the highest value of 15.76µm followed by imidacloprid + metalaxyl-m + tebuconazole at rate 2, Width of macro conidia was more in imidacloprid + metalaxyl-m + tebuconazole rate 1 (3.60µm) and the least was noticed in the benomyl rate 2 with 2.40µm while mancozeb + carbendazim and mancozebs at all rates maintained negative.

Table 2. Effect of Fungicides on Micro Conidia of *Colletotrichum* spp.

Fungicides	Rate	Spores/ml	Length(µm)	Width(µm)
Benomyl	1	11875000b	4.36a	2.55b
Benomyl	2	7488281d	4.22ab	2.50b
Benomyl	3	3242188g	4.05bcd	2.43b
Dressforce	1	8445313c	4.20abc	2.68a
Dressforce	2	6597656e	3.99de	2.53b
Dressforce	3	4332031f	3.62f	2.45b
Forcelet	1	4500000f	4.16bcd	2.53b
Forcelet	2	2273438h	3.97de	2.53b
Forcelet	3	1289063i	3.84e	2.50b
Funguforce	1	0.0j	0.00g	0.00c
Funguforce	2	0.0j	0.00g	0.00c
Funguforce	3	0j	0.00g	0.00c
Z-force	1	0j	0.00g	0.00c
Z-force	2	0j	0.00g	0.00c
Z-force	3	0j	0.00g	0.00c
Control	0	53011719a	4.01cde	2.53b

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

***Rates 1= half dose, 2= normal dose and 3= one and half dose**

Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb + Carbendazim and Z-force - Mancozeb

Table 3. Effect of Fungicides on Macro conidia of *Colletotrichum* spp.

Fungicides	Rate	Spores/ml	Length(µm)	Width(µm)
Benomyl	1	722666b	9.58bc	2.45de
Benomyl	2	484335c	9.40c	2.40e
Benomyl	3	414073d	9.30c	2.45de
Dressforce	1	238251f	9.38c	3.60a
Dressforce	2	152348g	9.82b	2.58bc
Dressforce	3	70413h	9.19c	2.48cde
Forcelet	1	332431e	8.78d	2.55bcd
Forcelet	2	277844ef	8.75d	2.60b
Forcelet	3	148478g	8.50d	2.65b
Funguforce	1	0h	0.00e	0.00f
Funguforce	2	0h	0.00e	0.00f
Funguforce	3	0h	0.00e	0.00f
Z-force	1	0h	0.00e	0.00f
Z-force	2	0h	0.00e	0.00f
Z-force	3	0h	0.00e	0.00f
Control	0	1324369a	15.76a	2.65b

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

*Rates 1= half dose, 2= normal dose and 3= one and half dose

Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb + Carbendazim and Z-force - Mancozeb

DISCUSSION

All fungicides evaluated showed variable response in inhibiting the colony growth of the fungal pathogen according to their nature and specificity when compared with the control treatment. These results are in agreement with a number of earlier *in vitro* studies which have demonstrated that various fungicides may restrict or prevent growth of some fungal pathogens (Tepper *et al*., 1983; Chavan *et al*., 2009; Sultana and Ghaffar, 2010). However, Mancozeb + Carbendazim (Fungu force) and imidacloprid + metalaxyl-m + tebuconazole (Dress force) had the greatest inhibitory effect on mycelia growth in all the leaf blight fungal pathogens identified when applied at the manufacturers recommended rate probably being combined systemic and contact fungicides and combined systemic fungicides respectively.

100% mycelia growth inhibition by the fungicides tested agree with the findings of (Dar *et al.,* 2013; Dubey and Kumar, 2003; Prajapati, *et al.,*2002 andRana and Tripathi (1983), being non-systematic fungicides, they prevent infection largely by inhibition of spore germination and germ tube elongation (Dar, *et al*., 2013). Mancozeb a contact fungicide applied alone had a remarkable impact on Colletotrichum at normal rate.

Fawole *et al.* (2009) stated that, use of higher rate of carbendazim-mancozeb mixture than the recommended does not offer any advantage in the control of target group, rather affects non-target ecologically important groups of microorganisms.Benomyl a systemic fungicide used singly was found to be less effective against any of the fungal pathogens in the *in vitro* trial conducted which is contrary to the findings of many researchers on its inhibitory effects against many plants' pathogenic fungi. This could probably mean that, the fungal pathogens used in the study were resistant to the benomyl fungicide. Tu and Mc Naughton (1980) stated that, many of the fungal pathogens are known to have developed tolerance to benomyl and its related fungicides including several species of *Collectotrichum*.

Fungicide resistance develops when a working mode of action loses its efficacy against target fungal pathogens (Eladi *et al*., 1992). Maymon *et al.* (2006) revealed that, out of 64 isolates of *Collectotrichum gloeosporioides* obtained from infected *Limonium* spp. in Israel, 46 were resistant to benomyl at 10µg/ml and 18 were sensitive to this concentration of fungicides, the sequence analysis of the β-tubutin genes showed that benomyl resistant isolates had an alanine substitute instead of a glutamic acid at position 198 in TUB2.

CONCLUSION

Mancozeb + carbendazim on one hand and imidlacloprid + metalaxyl-m + tebuconazole on the other hand proved to be the most effective, when applied at manufacturers recommended rate, while mancozeb when used alone was also effective on Colletotrichum.

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