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Effect Of Combined Fungicides On The Mycelial Growth Of *Curvularia lunata*

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ABSTRACT
The effects of combine fungicides on mycelial growth of *Curvalaria lunata* was carried out, to determine the effective fungicides and rate of application for control of Fungal pathogen (*Curvalaria lunata*). Five fungicides; Mancozeb, Carbendazim, Mancozeb + Carbendazim, Benomyl and Imidacloprid + metalxyl-m + tebuconazole were evaluated in vitro on *Curvularia lunata* at three levels (manufacturers recommended rate, half the recommended rate and one and half the recommended rate) in a CRD in the laboratory. Mancozeb, Mancozeb+ Carbendazim and Imidacloprid + metalaxyl-m + tebuconazole proved to be the best, showing little or no mycelia growth in 2 – 14 days after inoculation.
Keywords: *Curvalaria lunata*, Fungicides, Mycelia growth, Phytopathogenic fungi

INTRODUCTION

Phytopathogenic fungi are living organisms responsible for nearly half of known diseases in crop plants (Ndikumana, 2013). Among the difficulties with managing foliar disease is timing. Once the symptoms are visible, it is too late for any effective treatment in the current season (Stephen and Pawel, 2015). Fungicides are biocide chemical compounds or biological organisms used to kill or inhibit fungal spores (Margaret, 2004). Chemicals used to control oomycites, which are not fungi, are also referred to as fungicides since oomycites use the same mechanisms as fungi to infect plants (Schnabel and Jones, 2001). 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.

Research on the performance of some fungicides was conducted to measure their effects on the mycelial growth of *Curvularia lunata.*

METHODOLOGY

In vitro Evaluation of Fungicides for the Control of Fungal Leaf Blight of *J. curcas*

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; Sino Agro Chem. Industry Ltd. Guadong, China. Distributed by Jubaili Limited Agrotech), Carbendazim, a systemic fungicide (Forcelet 50% WP; Sino Agro Chem. Industry Ltd. Guadong, China. Distributed by Jubaili Limited Agrotech), Mancozeb (63%) + Carbendazim (12.5%), a combination of contact and systemic fungicide (Fungu force 75.5% WP; Sino Agro Chem. Industry Ltd. Guadong, China Distributed by Jubaili Limited Agrotech), imidacloprid 20% + metalaxyl-m 20% + tebuconazole 2%, a combination of systemics fungicide (Dressforce 42% WS; Sino Agro Chem. Industry Ltd. Guadong, China Distributed by Jubaili Limited Agrotech) and Benomyl, a systemic fungicide (Benomyl 50% WP; Shanghai Shenglian Chemical Co. Limited, China Distributed by Gold Agric Nigeria Limited). 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, for each isolate; 5 fungicides at 3 rates in 4 replications = 5 x 3 x 4 = 60 plates + 4 control = 64 plates.

i.e. For the 4 isolates = 64 x 4 = 256.

The experiment was laid in Completely Randomized Design (CRD) in the laboratory.

Inoculation of Media with Fungal Isolates

A small segment (2mm3) of mycelia mats of each fungal 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.

Preparation of Fungal Inocula

Spore suspensions of the different fungal pathogens used as inocula for *in vivo* bioassay were prepared as follows;

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

***Curvularia lunata*:** The conidiophores are brownish and dark at the middle cells, while the outer cells are lighter. It is 3-5 celled fusiform and typically bend or curved shaped (Plate 1).

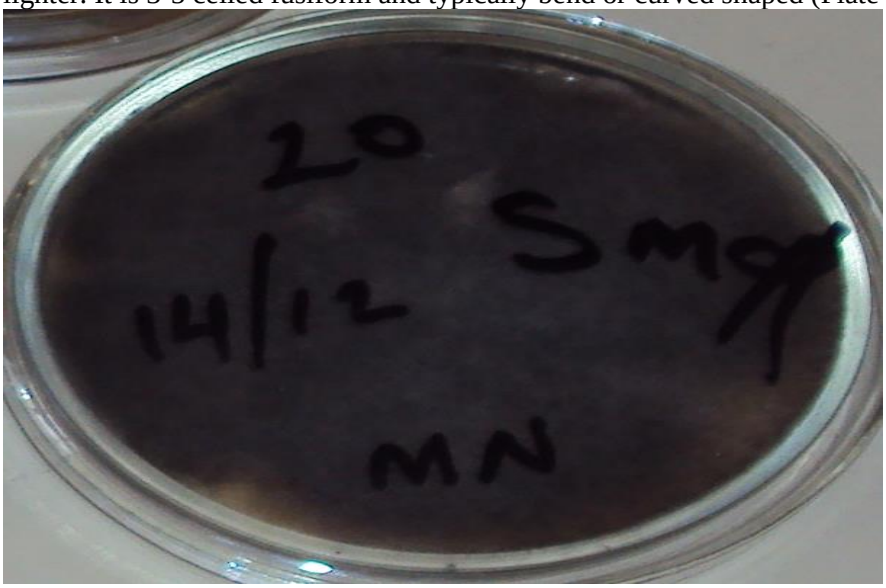


Plate 1: An Isolate of *Curvularia lunata*

Table 1: Fungicides on Mycelia Growth (mm) of *Curvularia lunata*

		Days after inoculation						
Fungicides	**R**ate	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Benomyl	1	2.00b	2.00f	21.38c	29.00c	36.63b	45.00b	50.13b
Benomyl	2	2.00b	2.00f	16.63e	23.25e	29.63d	36.25d	41.63d
Benomyl	3	2.00b	2.00f	13.00f	18.75g	25.25f	31.50f	37.88e
Dress force	1	2.00b	10.00e	13.75f	16.38h	20.25g	22.75h	24.00h
Dress force	2	2.00b	2.00f	9.00g	12.88i	17.13h	18.75i	20.88i
Dress force	3	2.00b	2.00f	8.25g	11.50j	14.25i	16.50j	18.75j
Forcelet	1	2.00b	18.50b	26.50b	32.00b	35.75c	40.25c	43.50c
Forcelet	2	2.00b	14.00c	20.50d	25.50d	28.75e	33.00e	35.25f
Forcelet	3	2.00b	11.50d	16.75e	21.38f	24.50f	27.50g	31.00g
Funguforce	1	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Funguforce	2	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Funguforce	3	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Z-force	1	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Z-force	2	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Z-force	3	2.00b	2.00f	2.00h	2.00k	2.00j	2.00k	2.00k
Control	0	19.13a	37.00a	45.50a	58.75a	72.75a	84.00a	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

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

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

At 4 DAI, control treatment maintained the highest growth of 39.75mm, this was closely followed by 33.50 mm growth values of mycelia when the samples were treated with carbendazim at half recommended rate while, least growth of 2.00 mm were maintained by mancozeb + carbendazim and mancozeb treatments at all application rates. Similar trend of mycelia growth was recorded at 6DAI with control treatment (57.76mm) and carbendazim with (47.75mm) respectively, and no additional growth with mancozeb + carbendazim and mancozeb in all concentrations used. A mycelia growth of 78.75mm was observed in the control treatments at 8DAI, this was followed by a growth of 64.25 mm when carbendazim was applied at half the recommended rate.

At 10 DAI, 85.75 mm mycelia growth was recorded in the control treatment, next was carbendazim applied at half recommended rate with a mycelia growth of 78.25mm (Plate 2). At 12 and 14 DAI, control treatment attained a maximum mycelia growth of 90mm in the Petri-dishes, which is statistically at par with carbendazim at half recommended rate and significantly different from mancozeb + carbendazim and mancozeb at all rates were lowest mycelia growth of 2.00mm was recorded.

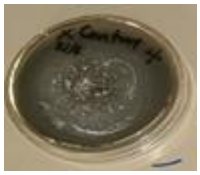
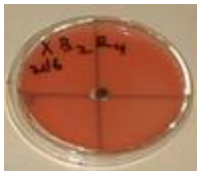
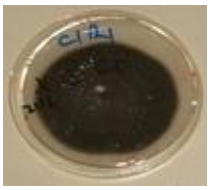
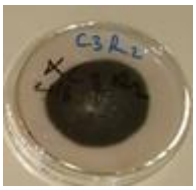
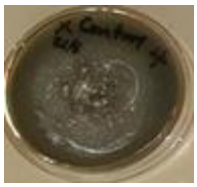
Fungicides	Control	50% Concentration	100% Concentration	150% Concentration
Dress force				
Forcelet				
Benomyl				
Fungu force				
Z-force				

Plate 2: Effects of Different Fungicides and Concentrations on *Curvularia lunata*
Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb+Carbendazim and Z-force-Mancozeb

Effects of fungicides on spores, length and width of micro conidia of *C. lunata* are presented in Table 2. The result shows significant difference among the treatments. Highest number of spores was found in the control treatment with 156250, this was statistically the same with value of carbendazim at rate 1 (148438). No spore was found in mancozeb + carbendazim and mancozeb treatments at all rates in the experiment. With respect to the length of micro conidia of *C. lunata*, highest length of 8.68µm was recorded in the benomyl treatment at rate 1; this was significantly

different from 8.18µm of benomyl treatment at rate 2. A micro conidia width of 4.77µm was recorded in benomyl treatments rate 1 followed by 4.70µm in the control treatment.

Results on the effects of fungicides on macro conidia of *C. lunata* are presented in Table 3 with significant difference among the treatments. High value of spores per millilitre was recorded in control treatment (2347856) followed by carbendazim treatment at rate 1 with 2250000. The length of macro conidia recorded in control treatment was highest (13.78µm), followed by 13.49µm in carbendazim treatment at rate 1, similarly, the width of macro conidia was found to be higher in control treatment with 7.46µm followed by carbendazim at rates 1 and 2 (7.36µm and 7.12µm respectively) which significantly differ from mancozeb + carbendazim and mancozeb treatments at all rates with 0 values.

Table 2: Effect of Fungicides on Micro conidia of *Curvularia lunata*

Fungicides	Rate	Spores/ml	Length (µm)	Width (µm)
Benomyl	1	82031c	8.68a	4.77a
Benomyl	2	54688d	8.18b	4.55bcd
Benomyl	3	117188b	7.86c	4.46cde
Dressforce	1	117188b	7.73cd	4.61bc
Dressforce	2	50781d	7.65cde	4.58bcd
Dressforce	3	23438e	7.38e	4.43de
Forcelet	1	148438a	7.48de	4.59bc
Forcelet	2	121094b	7.42de	4.55bcd
Forcelet	3	89844c	7.38e	4.39e
Funguforce	1	0e	0.00f	0.00f
Funguforce	2	0e	0.00f	0.00f
Funguforce	3	0e	0.00f	0.00f
Z-force	1	0e	0.00f	0.00f
Z-force	2	0e	0.00f	0.00f
Z-force	3	0e	0.00f	0.00f
Control	0	156250a	7.56cde	4.70ab

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

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

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

Table 3: Effects of Fungicides on Macro conidia of *Curvularia lunata*

Fungicides	Rate	Spores/ml	Length(µm)	Width(µm)
Benomyl	1	828125e	12.76c	7.03bc
Benomyl	2	441406g	12.18de	6.59d
Benomyl	3	562500f	11.77e	6.43d
Dressforce	1	617188f	12.72c	6.40d
Dressforce	2	265625h	12.80c	6.64d
Dressforce	3	82031i	12.64cd	6.70cd
Forcelet	1	2250600b	13.49ab	7.36ab
Forcelet	2	1890625c	13.07bc	7.12ab
Forcelet	3	1417969d	12.86c	6.60d
Funguforce	1	0i	0.00f	0.00e
Funguforce	2	0i	0.00f	0.00e
Funguforce	3	0i	0.00f	0.00e
Z-force	1	0i	0.00f	0.00e
Z-force	2	0i	0.00f	0.00e
Z-force	3	0i	0.00f	0.00e
Control	0	2347856a	13.78a	7.46a

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.
Where; Dress force – imidlacloprid + metalaxyl-m + tebuconazole; Forcelet – Carbendazim; Fungu force – Mancozeb + Carbendazim and Z-force - Mancozeb
*Rates 1= half dose, 2= normal dose and 3= one and half dose

DISCUSSION

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. High performance of tebuconazole was also reported by Godfrey (2015) that it had the greatest inhibitory effect in his experiment on *in vitro* inhibitory effect of selected fungicides on mycelial growth of ambrosia fungus causing 100% inhibition irrespective of the fungicide concentration or techniques employed.

Mancozeb a contact fungicide applied alone had a remarkable impact on Colletotrichum and Phomopsis leaf blights at normal rate and on Fusarium and Curvularia leaf blights at one and half recommended concentration. Mostert *et al.* (2000) stated that, Mancozeb was comparable to both Kresoxym- methyl and azoxystrobin regarding its ability to inhibit mycelia growth of *P. viticola.* It inhibits spore germination. Generally, 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).

CONCLUSION

Three most promising fungicides evaluated were; Mancozeb (when used singly), mancozeb + carbendazim and imidlacloprid + metalaxyl-m + tebuconazole proved to be effective in the control of *Curvularia lunata.* -Mancozeb + carbendazim on one hand and imidlacloprid + metalaxyl-m + tebuconazole on the other hand proved to be the most effective on *Curvularia lunata* when applied at manufacturers recommended rate, while mancozeb when used alone was only effective at normal rate.

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