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Research Article

RESPONSE OF TWO SYSTEMIC FUNGICIDES, BEMOMYL AND RIDOMYL FOR *IN VITRO* GROWTH INHIBITION OF *CERCOSPORA ZEAE-MAYDIS*TEHON AND DANIELS, THE CAUSE OF GREY LEAF-SPOT OF MAIZE IN KENYA

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ABSTRACT
The efficacy of two systemic fungicides, benlate and ridomyl at concentrations of 0.5%, 0.25%, 0.125%, 0.063% , 0.32%; and 0.32%, 0.63%, 1.26% 2.52% respectively based on their percentage active ingredients were evaluated for *in vitro* growth inhibition of an isolate of *Cercosporazeae-maydis* obtained from fresh diseased leaves. The variables used for the study of growth inhibition were colony diameter (mm) on green corn leaf decoction agar (GCLDA) and percentage inhibition of spore germination in a moist chamber. There was no significant difference between the two fungicides at different concentrations. Both fungicides completely inhibited growth at all concentrations, even reducing the diameter of the inoculum. Spore germination decreased with increase in concentration for both fungicides with the least in benlate, and it was very low as compared to that in sterile distilled water.

Key Words: *Cercosporazeae-Maydis, Growth Inhibition, Fungicides Benlate, Ridomyl in Vitro*

INTRODUCTION
Maize is the most important food crop in Kenya and the rest of Sub-saharan African region (Pingali and Pandey, 2001; CIMMYT, 2002). It is the staple food crop for 90% of the Kenyan population (Raemakers, 2001) and also plays an important role in the national economy. Currently grey leaf spot of maize caused by *Cercosporazeae-maydis* Tehon and Daniels is a serious disease resulting in a considerable loss in the most maize growing regions of Kenya. The disease was first reported in western Kenya in 1955 (Kung'u and Boa, 1998). Since then it was reported in the maize-growing zones in most of the districts in Kenya (Kinyua, 2004). Yield losses were estimated at 30% - 60% (Murithi and Gathama, 1998). Severe infection develops from teaseling stage of maize growth and this interferes with development due to less photosynthetic production, hence less grains leading to yield losses. A good amount of loss is expected if infection occurs early in the season, and favorable conditions prevail during the infection (Smit and Ward, 1997). Systemic fungicides were found to provide excellent control of this disease in South Africa (Ward, Birch and Nowell, 1994). Although the disease is causing severe yield losses in Kenya no study was undertaken to control it. Therefore, there is need to test the efficacy of fungicides on the growth of the pathogen as a possible management strategy against the disease. The present study was undertaken to determine the effect of two important systemic fungicides, benlate and ridomyl on inhibition of spores and growth of *C. zeae-maydis in vitro*.

MATERIALS ANS METHODS
Spore germination was tested on six concentrations of benlate and ridomyl. The isolate used in this work was obtained from fresh GLS-infected maize leaves from the field. It was stored at 4˚C for subsequent use and sub-cultured on fresh GCLDA.

Spore Germination Method
Spore germination method for evaluating fungicides as suggested by Peterson (1941) was used. Six different concentrations of benlate and ridomil 0.1%, 0.5%, 1%, 2%, 4%, and 6%were prepared in distilled water based on percentage active ingredients of each. One drop of each fungicidal solution and

spore suspension were put on each slide, covered with cover slip and then with moistened filter paper. The slides were incubated in a moist chamber at 28°C. A control was set up with distilled water only. Spore germination was observed over a period of 48 hours. Percentage spore germination at each concentration of the two fungicides and the control was calculated.

Poisoned Food Technique

The poisoned food technique as suggested by Nene and Thapliyal (1979) was used. The growth medium (V-8J Agar) was prepared in a flask and sterilized. To this medium, different quantities of each fungicide were aseptically added to give amended concentrations of 2.52%, 1.26%, 0.63%, and 0.32% for ridomil and 0.5, 0.25, 0.125, 0.063% of benlate based on the percentage of their active ingredient. This medium was poured into petri dishes and left to cool. Five-millimeter discs of the fungal culture were cut using a sterile cork borer and transferred aseptically in the centre of the petriplate. A control was set up with growth medium without the fungicide. The experiment was replicated three times. The plates were incubated at 25°C and the diameter of the fungus measured every week for one month. The mean colony diameters were compared with the control to give a measure of the fungal toxicity.

RESULTS AND DISCUSSION

Results on the effect of benlate and ridomil on percentage spore germination of *Cercosporazeae-maydis* presented in tables 1 show that both fungicides inhibited or greatly reduced spore germination compared to the control (sterile water) in which it was very high. Percentage germination decreased with increase in concentration, and was lower in benlate. No germination was observed at the rates recommended by the manufacturers and above. Inhibition of germination of the spores by the two fungicides suggests that they can be used effectively to manage grey leaf spot of maize since lack of germination will prevent the fungus from penetrating the host tissue.

Table 3 shows the results of the effect of benlate and ridomyl on growth of the fungus. There was significant difference between the control and the different fungicide concentrations whereas there was no significant difference between the various concentrations. Benlate totally inhibited growth of the fungus at all concentrations tested (0.5, 0.25, 0.125, 0.063%) as there was no growth beyond the 5-mm inoculum provided. Even growth on the inoculum was reduced due to fungicide. Results on the mycelia growth inhibition on the inoculum itself are obvious from the table.

Table 1: Percentage conidia germination of *C zeae-maydis* at different concentrations of benlate and ridomyl after 48 hrs of incubation at 25° C in a moist chamber

Benlate		Ridomyl	
% Conc.	**% Spore Germ**	**% Conc.**	**% Spore Germ**
8	0	6.3	0
4	0	3.15	0
2	0	1.16	0.2
1	0.4	0.78	1.3
0.2	1.6	0.16	2.1
Control	96.8	Control	98

Results of the effect of different ridomil concentrations on the growth of *C. zeae-maydis* are also presented in Table 2. Growth of the fungus in the control was significantly different from that of the medium amended with different concentrations of the fungicide, but there was no significant difference between growths at different concentrations. No growth was observed at all fungicide concentrations compared to the control where the rate of growth was very high. However, results presented in Table 2, show growth inhibition even on the 5-mm mycelia discs which were inoculated onto the medium. Growth

was inhibited even at very low concentrations. Doubling the recommended concentration (2.52%) had the least growth compared to the other concentrations.

Table 2: Effect of different concentrations of benlate on the growth of *C zeae-maydis* on V8JA during 4 weeks of incubation at 25° C

Mean Colony Diameter (mm) at One Week Interval									
Benlate					Ridomyl				
Conc.	1st week	2nd week	3rd week	4th week	Conc.	1st week	2nd week	3rd week	4th week
0.5	3.67c*	3.00c*	3.00b*	2.67b*	2.52	3.67c*	3.00c*	2.00d*	2.00c*
0.25	4.00b*	3.67bc*	3.33b	3.33b*	1.26	5.00b	4.00c*	3.33c*	3.00bc*
0..125	5.00b*	4.00bc*	3.67b	3.67b*	0.63	5.00b	4.33b*	4.67b*	3.67b*
0.063	8.33a*	4.67b*	4.00b	3.67b*	0.32	5.00b	4.00bc*	3.33c*	3.00bc*
control	8.33a	13.33a	21.00a	25.00a	control	8.33a	13.33a	21.00a	25.00a
% CV	13.25				% CV	13.25			
SE	0.3967				SE	0.3967			

In a column means followed by the same letter are not significantly (P = 0.05) different from each other according to LSD test.
**No growth but even inhibition on 5 mm inoculation disc used.*

The fungicide was effective even at concentrations lower than the rate suggested by the manufacturers (0.25%), and doubling this rate (0.5%) had a stronger effect since it reduced the rate of growth more than the rest of the other concentrations. The control (V-8JA) had a very high rate of growth compared to the fungicide concentrations.

Shivpuri and Gupta, (2001) used the *in vitro* technique to successfully evaluate the effects of different fungicides against *Sclerotiniasclerrrotiorum*. This can therefore be considered as a quick and effective technique for evaluating a wide range of fungicides. Growth inhibition by both benlate and ridomil imply that the two fungicides can be used effectively in the control of grey leaf spot of maize. Ward *et al.,* (1993, 1997) found that both protectant and systemic fungicides were effectively used to economically manage GLS on maize both in South Africa and United States. Use of fungicides can therefore be considered as one of the effective management strategies against GLS in Kenya and more systemic and contact fungicides should be evaluated for their efficacy against the disease. These two fungicides should also be tested under greenhouse and field conditions.

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