IN VITRO ASSESSMENT OF FUNGICIDES FOR THE CONTROL OF SORGHUM
ANTHRACNOSE INDUCED BY COLLETOTRICHUM GRAMINICOLA (CESATI) WILSON.

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ABSTRACT
Foliar spraying of fungicides is not widely practiced in sorghum production for a number of reasons, namely, high cost of chemicals, most cumbersome and not economically profitable, therefore, there is need to evaluate the efficacies of some selected fungicides in the laboratory with a view to recommending them as seed dressers for the control of anthracnose on the crop. In view of this, three fungicides, benomyl at 1.5g a.i./l, metalaxyl at 1.0g a.i./l and mancozeb at 6.25g a.i./l were evaluated under laboratory conditions to assess their effects on the mycelia growth of Colletotrichum graminicola, arranged in a completely randomized design. The three fungicides inhibited mycelia growth of Colletotrichum graminicola. Compared to the control check (sterile distilled water) which covered the plate (6.7cm), benomyl, metalaxyl and mancozeb caused a reduction in the mycelia growth to 34.3%, 20.9% and 58.2%, respectively. The colony diameter of the fungus was significantly reduced to 2.3cm, 1.4cm and 3.9cm, respectively (p=0.05) by these fungicides at lower concentrations of benomyl (0.19g a.i./l), metalaxyl (0.13g a.i./l) and mancozeb (0.78g a.i./l). Thus, metalaxyl gave the most effective control of the fungus at P=0.05.

INTRODUCTION
Anthracnose of sorghum caused by Colletotrichum graminicola (Ces) Wilson (Colletotrichum sublineolum (Ces.)) is an important disease of sorghum in West Africa and other production area (Marley et al., 2004). It is highly destructive and has been a major limiting factor to stable sorghum production in Nigeria (Anas et al., 2001). Although, the disease is found in all sorghum growing areas of Nigeria, it is of particular importance in Southern Guinea savanna zone, which is comparatively more humid than the sudan, sahelian considerable (Akpa et al., 1996). Anthracnose is a serious disease affecting both local and improved
varieties of sorghum, causing 30-50% yield loss in susceptible cultivars (Thomas et al., 1996; Marley et al., 1997 and Marley et al., 2001).

Plant diseases can be controlled by the use of chemicals (Asala and Gwary, 2002) and host resistance (Marley et al., 2001), but in West Africa, many local land races and introduced varieties lack satisfactory resistance to anthracnose (Marley et al., 1997). The use of chemical fungicides (Benlate, Apron star and Dithane M-45) is a quick and effective means of controlling fungal diseases in plants. The potent activities of fungicides had been reported previously by other workers. Lim (1980) reported that some mango isolates of Glomella cingulata (C. gloesporoides) were extremely sensitive to benzimidazole compounds (a group to which benomyl and carbendazim belong). Foliar spraying of fungicides is not widely practiced in sorghum production for a number of reasons, namely, high cost of chemicals, most cumbersome and not economically profitable. Since the Colletotrichum graminicola is mainly soil/seed-borne (Adegboye and Adeoti, 2005), there is need to evaluate the efficacies of some selected fungicides in the laboratory with a view to recommending them as seed dressers for the control of anthracnose on the crop.

The specific objective of the study is therefore, to evaluate the efficacies of benomyl (Benlate 50% W.P., Dupont), Mancozeb (Dithane M-45, 80% W.P. Dupont) and Thiamethoxam + Metalaxyl-M + difenoconazole (Apron Star 42 W.S; Syngenta) fungicides on the mycelia growth of Colletotrichum graminicola in vitro.

**MATERIALS AND METHODS**

**Preparation of agar medium.**

Potato dextrose agar (PDA) (oxoid) was prepared according to the Manufacturer’s specifications and autoclaved at 121° C and 1.1kg/cm³ for 15 minutes. The sterilized medium was subsequently allowed to cool to 45°C following which 10 ml of the medium to which streptomycin at the rate of 25µg/ml (PDAs) had been added was poured aseptically into 9cm diameter Petri-dishes, covered and allowed to solidify.

**In-vitro assessment of the sensitivity of C. graminicola to fungicides.**

Diseased specimens were collected from the experimental plots at different stages of crop growth. In the laboratory, samples were surfaced sterilized and washed in sterile distilled water, plated on PDA and incubated for 7 days at 24-30°C. Thereafter, subculturing was done on PDAs to get pure culture of the organism from the specimens.

The pure cultures were maintained on PDAs and sub-cultured after every three weeks.

Observations by researchers in Nigeria over many years indicate that the loss due to the disease may
be Three fungicides — benomyl (Benlate 50% W.P., Dupont), Mancozeb (Dithane M-45, 80% W.P. Dupont) and Thiamethoxam + Metalaxyl-M+ difenoconazole (Apron Star 42 W.S; Syngenta) were tested against the fungal isolate at the recommended dosage concentration of 0.6, 0.4 and 2.5 kg of commercial products per hectare respectively (Table 1). To assess the fungicides, different proportions of the above dosages were incorporated into 250 ml of potato dextrose agar (PDA). The tested rates for each fungicide were designated as 2x, x, 0.5x, 0.25x, 0.125x and 0.063x (i.e. 6.00, 3.00, 1.50, 0.75, 0.38 and 0.19 gm a.i / litre for Benlate; 4.00, 2.00, 1.00, 0.50, 0.25 and 0.13 gm a.i / litre for Apron star; 25.00, 12.50, 6.25, 3.13, 1.56 and 0.78 gm a.i/litre of PDA for Dithane M-45); where x is the manufacturer’s recommended dose of the fungicide to be dissolved in 1.0 litre of water assuming that the rate per hectare is to be dissolved in 200 litres of water.

To prepare PDA-fungicide mixture, the following method was adopted. Active ingredients in gramme per 250ml (1.50, 0.75, 0.38, 0.19, 0.95 and 0.05 for Benlate; 1.0, 0.5, 0.25, 0.13, 0.07 and 0.03 for Apron star; 6.25, 3.13, 1.56, 0.39, 0.1 and 0.025 for Dithane M-45) of the fungicide were added separately into 500ml conical flask containing 250ml of molten Potato dextrose agar amended with streptomycin (PDAs). The flasks were shaken carefully to ensure even distribution of the fungicides (Adegboye and Adeoti, 2005)

The following methods were adopted to ensure that equal quantity of the medium was poured into each plate. McCartney bottles were sterilized along with the media in the autoclave. Using three McCartney bottles for each concentration of a fungicide, 24ml of the concentration (filling the bottle up to a marked point on the neck) was poured into each Petri-dish. The whole process was carried out in an aseptic condition. Three Petri-dishes were poured for each concentration. The plates were allowed to set for 24 h. after which an 0.6cm (diameter) cork borer was used to transfer the fungus from 7-day old culture on to the centre of each plate. According to Adegboye and Adeoti (2005), control experiment consisting PDAs plates alone were similarly inoculated for each fungicide concentration in the experiment. The plates for different concentrations of the fungicides were arranged in completely randomized design on the laboratory bench at 24-30°C. Fungitoxicity was assessed by measurement of linear (diameter) growth of the fungus after 7 days.
Table 1: Chemical nature, trade and common names of fungicides evaluated in the laboratory for their efficacies against *Colletotrichum graminicola*.

<table>
<thead>
<tr>
<th>Trade name, mode of action of the fungicide (systemic or contact)</th>
<th>Common name of active ingredient</th>
<th>Chemical nature of active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benlate – systemic</td>
<td>Benomyl</td>
<td>50% Methyl 1-(butylcabinayl)benzimidazole</td>
</tr>
<tr>
<td>Apron star- systemic /broad spectrum</td>
<td>Apron star</td>
<td>20% thiamethoxam, 2% w/w metalaxyl-M, 2% w/w difenoconazole</td>
</tr>
<tr>
<td>Dithame M-45- contact</td>
<td>Mancozeb</td>
<td>2.5% Zn ion + 2% Mn as in maneb.</td>
</tr>
</tbody>
</table>

**RESULTS**

Table 2, showed the radial growth of *C. graminicola* on plates incubated for 7 days after inoculation at different concentrations on PDA fungicide amended medium. It was observed that uniform olive green colony of *C. graminicola* produced in the control incubated under continuous darkness. The concentrations of the fungicides used significantly inhibited mycelial growth up to a quarter of the manufacturer’s recommendations after which results obtained differed among fungicides tested compared with the control. The inhibitory effects significantly \((P < 0.05)\) varied among different concentrations of the fungicides (Table 2). Some of the concentrations of the three fungicides completely inhibited mycelial spread throughout the experimental period. When the fungicides were evaluated at lower concentrations of 0.25 and 0.13mg/ml a.i., Apron star was effective in inhibiting mycelial spread at 0.13 mg/ml a.i (Thiamethoxam + metalaxyl +difenoconazole). Effects of the fungicides on mycelial growth of *C. graminicola* were summarized in Table 2.

**DISCUSSION**

The effect of fungicides on mycelia growth of *C. graminicola* has demonstrated that all the fungicides assessed *in-vitro* had significant inhibitory effects on mycelia growth of the organism. The potent fungicidal activities of these chemicals had been reported previously by other workers. Lim (1980) reported that some mango isolates of *Glomerella cingulata* (*C. gloeosporoides*) were extremely sensitive to benzimidazole compounds – a group to which benomyl and carbendazim belong.
Table 2: Effects of different concentrations (µg/ml) of three fungicides on the mycelia growth of *Colletotrichum graminicola* in-vitro.

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Concentration (mg/ml a.i)</th>
<th>Mycelial growth diameter (cm)</th>
<th>Growth as % of control</th>
<th>% Mycelial growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benomyl (Benlate)</td>
<td>6.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.6g</td>
<td>9.0g</td>
<td>91.0a</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>1.0f</td>
<td>14.9f</td>
<td>85.1b</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>2.3c</td>
<td>34.3c</td>
<td>65.7c</td>
</tr>
<tr>
<td>Thiamethoxan + metaloxyl + difenoconazole (Apron star)</td>
<td>4.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>1.4d</td>
<td>20.9d</td>
<td>79.1b</td>
</tr>
<tr>
<td>Mancozeb (Dithane M-45)</td>
<td>25.00</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>12.50</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>0.0h</td>
<td>0.0h</td>
<td>100.0a</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>0.9f</td>
<td>13.4f</td>
<td>86.6ab</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.2e</td>
<td>17.9e</td>
<td>82.1b</td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>3.9b</td>
<td>58.2b</td>
<td>41.8d</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>6.7a</td>
<td>100a</td>
<td>0.0e</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different, according to DMR Test ($P \leq 0.05$).

Alabi *et al.* (1986) demonstrated that benomyl and carbendazim + maneb were the most effective of eleven fungicides screened against the cowpea brown blotch pathogen, *C. capsici*. In a study conducted by Akpa and Tsammani (1993), carbendazim + Maneb did not show complete inhibitory effect on *C. graminicola*. The effectiveness may be attributable to the direct close contact between the fungus and the fungicides in the agar plate. However, the ineffectiveness of a fungicide in inhibiting
mycelial growth \textit{in-vitro} may not necessarily be the same on the field. It has been demonstrated by Davis and Diamond (1952) that some systemics which are fungicidally inactive \textit{in-vitro} could initiate antifungal effect \textit{in-vivo}. Apron star contains both systemic and broad spectrum pesticides as a mixture of thiamethoxam, metalaxyl-M and difenoconazole, which has been employed in the control of fruit and vegetable diseases in South West Nigeria. Delp (1980) has recommended the use of companion fungicides with different mode of action as the most effective method of controlling diseases so as to avoid the emergence of resistant strains of a fungus.

The inhibitory effect of benomyl on fungal mycelia growth had been reported in \textit{Sclerotium rolfsii} and \textit{C. gloeosporoides} (Shimfe, 1984), \textit{Botrytis cinerea} (Grindle, 1982), \textit{Merotherium roridum} (Sohi and Kalra, 1982) as well as on germination and spore survival of \textit{C. lindemuthianum} (Tu and Jarvis, 1979). In contrast to these reports, benomyl has been reported by some workers to have little or no fungicidal effect on mycelia growth and spore germination of some fungi (Edginton et al., 1971; Malham et al., 1975). An \textit{in-vitro} study of fungitoxic effects of three benzimidazole compounds, Edginton et al. (1971 and 1980) found that \textit{Curvilaria} and \textit{Drechslera} spp. were insensitive to benomyl, a result obviously in contrast with its effect on \textit{C. graminicola}.

It is apparent from this study that all the fungicides assessed \textit{in-vitro} could control the sorghum anthracnose pathogen on the field. It could also enhance better performance on the moderately resistant sorghum cultivars if they are used as seed dressing chemicals. It was also observed from a research work in which foliar sprays of mancozeb and Carbendazim + Maneb significantly reduced the severity of sorghum anthracnose in northern Nigeria (Akpa et al., 1992). However, there is need for further evaluation of these fungicides on the experimental field since foliar spraying of fungicides is not widely practiced in sorghum production. Therefore, sorghum seed dressing with these fungicides should be evaluated on the field to ascertain their efficacies in integrated approach to disease management.

REFERENCES


