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Full Length Research Paper

Effects of some herbicides on the survival of *Colletotrichum graminicola* (Cesati) Wilson

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The aim of this investigation was to evaluate the effects of some herbicides (Butachlor and Glyphosate) on the survival of *Colletotrichum graminicola*, the causal organism of soft rot of most fruits, tubers and vegetables. Three different concentrations of the herbicides and a check (20%, 50%, 80%, and 0% or water) were prepared and amended with PDA medium, and each was replicated five times. At least 50 fungal spores of *Colletotrichum graminicola* were germinated on each of herbicide- PDA medium and incubated for a period of seven days. The results of the study has shown that 50% concentration of both Butachlor and Glyphosate produced the highest inhibitory effect on the spore germination of the fungal isolate while 20% concentration of both herbicides produced little or no effect on spore germination. Similarly, both at 50% and 80% concentrations of the two herbicides, there was significant ($P < 0.05$) reduction in the mycelia diameter of the fungus. This suggests that both butachlor and glyphosate at 50% and 80% could confer resistance to sorghum against leaf anthracnose caused by *Colletotrichum graminicola*.

Keywords: herbicide, *Colletotrichum graminicola*, spore germination, mycelial length

INTRODUCTION

Sorghum leaf and stalk anthracnose also known as red stalk rot induced by the fungus *Colletotrichum graminicola* (Cesati) Wilson is an important disease of sorghum in Nigeria and all sorghum producing areas of the world (Rao *et al.*, 2002). Anthracnose affects all plant parts including the stem, leaf, peduncle, inflorescence and grain (Gwary *et al.*, 2002). Foliar anthracnose occurs in most areas where Sorghum is grown including North, South and Central America, West Indies, Asia and Africa (Freidericksen, 1982), including Nigeria where it is a major disease of

sorghum (Marley *et al.*, 2000). It affects a number of the (Poaceae) grass family members among them some varieties of the genus *Sorghum bicolor* (Ali and Warren, 1992). Among the susceptible varieties, differences in severity of the disease occurred depending on the environment in which the varieties are grown (Bergquist, 1973; Warren, 1996). Leonard (1974) reported that an unimportant pathogen (*Colletotrichum graminicola*.) considered as minor disease was found most damaging pathogen observed on corn in North Carolina in 1972 and 1973. Lodging and premature death of plants from the top caused by *C. graminicola* was considered most common in the coastal plain. Mbwaga *et al.* (1993) carry out a survey between 1986 and 1990 crop season which indicated that

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diseases viz., grain moulds, grey leaf spot (*Cercospora sorghi*), anthracnose (*Colletotrichum graminicola*), rust (*Puccinia purpurea*), leaf blight (*Excerohilum turcicum*), ladder leaf spot (*Cercospora tusimaculans*), sooty stripe (*Ramulispora sorghi*) and zonate leaf spot (*Gleocercospora sorghi*) of sorghum were in high frequency. Mathur *et al.* (2001) conducted a field survey in rainy season of 1994 where a popular commercial sorghum hybrid CSH-9 was showing anthracnose (*C. graminicola*) symptom in several farmers' fields in Maharashtra state of India. Marley *et al.* (2002) conducted a field survey in some states of northern Nigeria, throughout the sorghum (*Sorghum bicolor*) growing regions to identify the constraints against sorghum production in Nigeria. They found sorghum anthracnose as a major disease of sorghum in the region. Ngugi *et al.*, (2002) conducted a two year survey (July, 1995 and 1996) to assess the prevalence and severity of sorghum anthracnose, in 91 and 109 farmer's field, respectively. Fourteen foliar and six panicle diseases were observed, with limited variation in disease prevalence and severity between the 2 years. The most common foliar disease observed was anthracnose with prevalence ranging from 44-65%.

Although leaf lesions are the first symptoms of infection in the field, there are four separate phase of the disease occurring in seedling, root rot, leaf (foliar). Stalk rot and seed mold and they may all occur in Sorghum within single growing seasons (Crouch, 2010). The most common symptoms include brownish discoloration to irregular patches of discoloration boarded by veins. Spores masses of fungus can sometimes be found in lower leaf surfaces along if veins during extended moist conditions (Stephen *et al.*, 2002). The disease most often develops during the warm, humid weather. The infection process takes place rapidly when warm wet weather condition prevail (Murty and Record, 2010). The disease is usually seed-borne (Saifulla and Ranganathan, 1999) hence control of the disease could be achieved traditionally by rotational cropping and use of cultivars and hybrids that are resistant to infection (Mutege, 2010). Alternatively however, sorghum leaf anthracnose can be controlled by the use of seed treatment fungicides (Gwary *et al.*, 2002). Recent researches have shown that anthracnose of sorghum and many cereal crops caused by *Colletotrichum graminicola* is completely eradicated by the use of systemic seed treatment fungicides such as metalaxyl, carboxin and thiabendazole. Similarly, systemic fungicides have been indicated in the control of some seed-borne pathogenic fungi in the soil especially when applied before seeding (Gale, 2002; Stephen *et al.*, 2002; Kutama *et al.*, 2011). Typical of these herbicide is the glyphosate. Glyphosate is a widely used broad-spectrum herbicide. Recent studies in glyphosate-resistant (GR) crops have shown that, in addition to its herbicidal activity, it exhibits activity against fungi, thereby providing disease control benefits (Stephen *et al.*, 2002). Laboratory studies confirmed earlier observations that glyphosate has activity against *Colletotrichum graminicola*. The

results showed that glyphosate at rates between 0.84 and 1.68 kg/ha delayed the onset of the pathogen.

The aim of this work is to evaluate the effect of different levels of concentration of the two herbicide on the growth and sporulation of *Colletotrichum graminicola* isolate *in-vitro*.

MATERIAL AND METHODS

Sample Collection and Preparation of the Media

Infected sorghum leaves showing characteristics features of anthracnose were collected in polythene leather to avoid contamination and reduce mortality of the organism and was carried directly to the laboratory for analysis.

The media was prepared and taken to the laboratory for sterilization at 100 °C for 15mins. Chloramphenicol was added before the sterilization to inhibit the growth of bacteria. The media was allowed to cool and 20ml was poured on each plate and allowed to solidify.

Inoculation of the Sample

Using new sterilized needle the infected leaves was scratched and dropped on each plate. This was then kept at room temperature for 5 days. Observation was done every day to see the growth of the fungi.

Isolation of Pure Culture

After the growth of the fungi was obtained, the same procedure was followed to prepare another media for pure culture. The original sample was observed to have four different fungal colonies. Each colony was then scratched and dropped on a separate plate after which it was kept at room temperature for 5 days to observe another growth. On the fifth day, another media was prepared for another pure culture (Pande *et al.*, 1991)

Microscopy and identification of fungal isolate

Microscopic identification of *Colletotrichum graminicola* was carried out as described by Pande *et al.* (1991) and Kutama *et al.* (2010)

Preparation of PDA – Herbicide Medium

Three different concentrations were made in 20%, 50% and 80% of glyphosate and butachlor as followed.

1 Ltr-----200L

50ml-----2000ml

20%= 2.5ml (herbicide) ----- 100ml (water)

50%= 1ml (herbicide) ----- 40ml (water)

80%= 0.6ml (herbicide) ----- 25ml (water)

After all the three different concentrations were prepared 5ml of each was collected using separate 5 ml syringe and

Table 1. Effect of different concentrations of Butachlor and Glyphosate on the germination of *Rhizopus stolonifer* spores seven days after incubation

	Germination % of <i>Colletotrichum graminicola</i> five days after incubation in:	
	Butachlor	Glyphosate
20	50.3	53.2
50	23.5	24.2
80	16.2	20.1
0 (water)	83.9	87.1
Mean	43.48	46.15
S.E	1.73	1.65

Table 2. Effect of different concentrations of Butachlor and Glyphosate on the mycelia growth (sporulation) of *Colletotrichum graminicola* spores seven days after incubation

	Germination % of <i>Colletotrichum graminicola</i> seven days after incubation in:	
	Butachlor	Glyphosate
20	4.43	2.15
50	1.81	1.21
80	1.93	2.47
0 (water)	6.02	5.00
Mean	3.55	2.70
S.E.	2.93	2.34

applied on each PDA plate. At least 50 spores of *Colletotrichum graminicola* were inoculated on each herbicide –PDA medium. The experiment was replicated twenty times and some control plates were left to stand without the addition of any herbicide. Observation was made daily for five days and data was collected and recorded.

Data Collection and analysis:

Data was collected using thread and ruler to measure the length of each mycelium before and after the application of herbicide and then the mean value and standard error was calculated.

RESULTS AND DISCUSSION

Among the systemic herbicides *viz.*, butachlor and glyphosate was successful in reducing the diseases level at 50% concentration inhibiting the growth of *C. graminicola* at two stages of the concentrations. Similarly Motpkhaye (1983) studied the efficacy of some herbicides against germination of *C. graminicola* in the laboratory. The

best results were given by butachlor and glyphosate. Mo *et al.* (2008) found that, sorghum seeds when treated with butachlor prior to sowing and then six herbicides sprays of either glyphosate or butachlor, reduced the infection of *C. graminicola*. Jhamaria and Sharma (2002) reported that glyphosate was the best treatment in minimizing the disease severity of *Rhizoctonia solani* Kühn. causing web blight. Davidse (1986) reported that butachlor induced nuclear instability by disturbing the mitosis and meiosis process. The various concentrations of the herbicides significantly inhibited mycelial growth was compared with the control. However, butachlor and glyphosate had the greatest inhibitory effect. Mycelial growth increasingly reduced with increasing concentration of the herbicide. The results of earlier works and the present studies indicated that the herbicides are highly effective in reducing the growth of the fungus.

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