Effect of Carbon, Nitrogen and Sulphur on Growth and Sporulation of *Colletotrichum graminicola*

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Abstract

The sorghum anthracnose fungus, *Colletotrichum graminicola* is a long established cause of reduced yield in sorghum globally including Nigeria. An *in vitro* study was carried out to assess the responses of *Colletotrichum graminicola* to different carbon, nitrogen and sulphur sources using Czapek dox broth as the basal medium. Sucrose was replaced with glucose, maltose and soluble starch; sodium nitrate was replaced with urea, potassium nitrate and ammonium sulphate; while magnesium sulphate was replaced with sodium sulphate, copper sulphate and zinc sulphate. Among the various carbon sources tested, glucose, sucrose and maltose supported good growth and sporulation but the optimum growth was observed in glucose. Potassium nitrate was found to be the best source of nitrogen for growth of *Colletotrichum graminicola* while NH_4SO_4 was the best for sporulation. The best sulphur source for growth was $MgSO_4$; all other sources were less effective than the control.

Keywords: *Colletotrichum graminicola,* carbon, nitrogen, sulphur, growth & sporulation

Introduction

Anthracnose of sorghum caused by *Colletotrichum graminicola* was first reported in Togo (West Africa) in 1902 (Sutton, 1980). Since then, the disease has been reported in most sorghum growing regions of the world and has been a major limiting factor to its production in Nigeria (Pande *et al.,* 1991). The disease is so severe because all parts of the plant may be affected and various phases of the disease may occur in the field. The pathogen can cause leaf blight, stalk rot and root rot of seedlings (Gwary et al., 2004). *C. graminicola* infects all aboveground parts of the sorghum plant-stem, leaf, peduncle, inflorescence and grain. Peduncles, panicles and the grains may be infected separately or together (Sutton, 1980).

According to Kiyawa et al., (2013), *Colletotrichum* can spread to affect up to 80% of the crop. Fungi possess an ability to utilize a wide range of nutrients as a source of energy with carbon and nitrogen occupying the premier position amongst the essential elements required (Tasiwal and Benagi, 2009). Nutrients are substances used in biosynthesis and energy release and therefore serve as cardinal impetus towards the viability, survival and sustenance of any organism (Safavi

et al., 2007). Sulphur being a compound of the sulphahydryl or thiol group of many enzymes, co-enzymes and vitamins affect various other vital processes of fungi (Tasiwal, 2008). The responses of *Colletotrichum species* to different nitrogen and carbon sources have been investigated by different researchers (Ding *et al.,* 2007; Tasiwal, 2008; Zubbair, 2009; Deshmukh *et al.,* 2012). This study was carried out to determine the most readily utilizable source of carbon, nitrogen and sulphur for growth and sporulation of *Colletotrichum graminicola.*

Materials and Methods

Selecting Czapek's Dox liquid medium as the basal medium, the nitrogen, carbon and sulphur sources were substituted with other sources.

Nitrogen utilization

Sodium nitrate (2.0g) was substituted with an equivalent amount of urea, potassium nitrate and ammonium sulphate which were the sources of nitrogen used for this study. 5 mm mycelial disc of the fungus cut from the growing edge of a 5 day old colony was placed in the media. Each treatment was replicated five times. (Ding *et al*, 2007)

Carbon utilization

In this case, the sucrose in the liquid medium was replaced by glucose, maltose, and soluble starch. 5 mm mycelial disc of the fungus cut from the growing edge of a 5 day colony was placed in the media. Each treatment was replicated five times. (Ding et al, 2007)

Sulphur utilization

In this experiment, magnesium sulphate in the liquid medium was replaced by sodium sulphate, copper sulphate, zinc sulphate. 5 mm mycelial disc of the fungus cut from the growing edge of a 5 day colony was placed in the media. Each treatment was replicated five times. (Ding et al, 2007)

The controls were similarly prepared except that in this case, the composition of Czapex Dox liquid medium remained unchanged (sodium nitrate (2.0g), Potassium dihydrogen phosphate (1.0g), Magnesium sulphates (0.5g), Potassium chloride (0.5g), Ferrus sulphates (0.01g) and sucrose (30g)).

All flasks were incubated at room temperatures (28 ± 2^0C). After 10 days of incubation, mycelia of the fungus were harvested, dried and weighed. Spore count and measurement were also done by means of 3 haemocytometer readings recorded for each replicate. For each treatment, spore length and width of 50 randomly selected spores were measured.

Statistical Analysis

Data was subjected to analysis of variance appropriate to CRD. Mean comparisons were carried out using DMRT. Statistical package used =

SAS (2002) software version 9.1 and means were separated using DMRT at 5% level of significance.

RESULTS

Utilization of carbon sources

Effect of carbon sources on mycelial weight of *C. graminicola*

The growth of the pathogen differed on the different carbon sources tested (Table 1). The pathogen grown on glucose and sucrose recorded higher mycelial weight followed by maltose. Starch was the least utilized by the fungus. All carbon sources differed significantly from the control.

Table 1: Effect of carbon sources on mycelial weight of *C. graminicola*

Carbon source	Mycelial weight (mg)
Glucose	383.50[a]
Sucrose	372.25[a]
Maltose	325.75[b]
Starch	293.50[c]
Control	228.75[d]
S. E. ±	5.105

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Effect of carbon sources on number of conidia of *C. graminicola*

C.graminicola sporulated heavily on glucose and maltose (Table 2). Sporulation was also abundant on sucrose while the least sporulation was observed when starch was used as sole carbon source. There was very poor sporulation on medium without carbon source (control).

Table 2: Effect of carbon sources on number of conidia of *C. graminicola*

Carbon source	**Conidial count (x10^5 conidia/ml)**
Glucose	23.97[a]
Sucrose	20.36[b]
Maltose	23.27[a]
Starch	18.34[c]
Control	0.03[d]
S. E. ±	0.317

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Effect of carbon sources on conidial size of *C. graminicola*

Table 3 shows that carbon sources have significant effect on the length of the conidia. The longest spores were observed on sucrose, followed by those on maltose. Among the carbon sources, the shortest conidia were recorded in sucrose which was not significantly different from those recorded under control.

Table 3: Effect of carbon sources on conidial size of *C. graminicola*

Carbon source	Conidial size (µm)	
	Length	**Width**
Glucose	18.29^{c}	2.56^{a}
Sucrose	20.31^{a}	2.45^{a}
Maltose	19.85^{ab}	2.53^{a}
Starch	19.23^{ab}	2.55^{a}
Control	18.59^{bc}	2.48^{a}
S. E. ±	0.478	0.062

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test

Utilization of nitrogen sources

Effect of nitrogen sources on mycelial weight of *C. graminicola*

Mycelial weight of the fungus was influenced by all the nitrogen sources tested (Table 4). Maximum growth of the fungus was observed where KNO_3 was used as nitrogen source (484.75mg), followed by $NaNO_3$ (355.75mg) and NH_4SO_4 (325.25mg). There was a marked significant difference between the nitrogen sources tested and the control.

Table 4: Effect of nitrogen sources on mycelial weight of *C. graminicola*

Nitrogen source	Mycelial weight (mg)
KNO_3	484.75^{a}
$NaNO_3$	355.75^{b}
NH_4SO_4	325.25^{c}
Urea	228.75^{d}
Control	195.00^{e}

S. E. ±	6.012

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Effect of nitrogen sources on number of conidia of *C. graminicola*

All the nitrogen sources supported sporulation of the fungus. Maximum sporulation was observed on NH_4SO_4, followed by $NaNO_3$ and then KNO_3. No sporulation was observed in the control experiment (Table 5).

Table 5: Effect of nitrogen sources on number of conidia of *C. graminicola*

Nitrogen source	**Conidial count (x10^5 conidia/ml)**
KNO_3	19.31^c
$NaNO_3$	21.12^b
NH_4SO_4	28.78^a
Urea	10.72^d
Control	0.00^e
S. E. ±	0.279

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Effect of nitrogen sources on conidial size of *C. graminicola*

The results show that the nitrogen sources had no significant effect on the size of the conidia, though the conidia were larger in KNO_3 and in NH_4SO_4 than in $NaNO_3$ (Table 6).

Table 6: Effect of nitrogen sources on conidial size of *C. graminicola*

Nitrogen source	**Conidial size (µm)**	
	Length	**Width**
KNO_3	21.08^a	2.56^a
$NaNO_3$	19.88^a	2.46^a
NH_4SO_4	20.39^a	2.57^a
Urea	19.91^a	2.51^a
Control	0.00^b	0.00^b
S. E. ±	1.056	0.098

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Utilization of sulphur sources

Effect of sulphur sources on mycelial weight of *C. graminicola*

Of the four sulphur sources tested, $MgSO_4$ supported the maximum growth. Interestingly, the fungus grew well in plates with no sulphur source (control) and mycelial weight was significantly different from growth observed in $NaSO_4$, $ZnSO_4$ and $CuSO_4$ (Table 7).

Table 7: Effect of sulphur sources on mycelial weight of *C. graminicola*

Sulphur source	Mycelial weight (mg)
$MgSO_4$	383.75[a]
Na SO_4	335.50[c]
Zn SO_4	224.00[d]
Cu SO_4	184.75[e]
Control	366.25[b]
S. E. ±	4.701

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test; NS = Not significant

Effect of sulphur sources on number of conidia of *C. graminicola*

All the sulphur sources supported sporulation of the pathogen (Table 8). Maximum sporulation was observed where $MgSO_4$ was the sole sulphur source followed by NaSO4. The least sporulation was in $CuSo_4$. The differences in number of conidia on different sulphur sources were significantly different from one another.

Table 8: Effect of sulphur sources on number of conidia *C. graminicola*

Sulphur source	Conidial count (x10^5 conidia/ml)
$MgSO_4$	13.16[a]
Na SO_4	12.06[b]
Zn SO_4	8.05[d]
Cu SO_4	6.64[e]
Control	11.10[c]
S. E. ±	0.130

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test; NS = Not significant

Effect of sulphur sources on conidial size of *C. graminicola*

The effect of sulphur sources on sporulation showed that except for $CuSO_4$ which had the least spore size, conidia size did not vary significantly on the different sulphur sources (Table 9).

Table 9: Effect of sulphur sources on conidial size of *C. graminicola*

Sulphur source	Conidial size (µm)	
	Length	**Width**
$MgSO_4$	19.77[a]	2.46[a]
Na SO_4	18.63[ab]	2.44[a]
Zn SO_4	17.81[b]	2.58[a]
Cu SO_4	15.40[c]	2.51[a]
Control	19.12[ab]	2.52[a]
S. E. ±	1.534	0.165

Means in each column with same superscript are not significantly different at P ≥ 0.05 using Duncan's Multiple Range Test.

Discussion

Colletotrichum graminicola varied in its ability to utilize different carbon sources. Maximum growth was observed where glucose was used as the carbon source followed by sucrose and then maltose. Growth and conidial production was least in starch. There was however no significant difference between growth in glucose and sucrose. These results matched with earlier findings by Saifullah and Ranganathaiah (1990) who reported good growth of *C.graminicola* in hexoses and disaccharides. According to Garraway and Evans (1994), the utilization of glucose maybe due to ease with which this sugar was metabolised to produce cellular energy. A similar trend was observed by Zubbair (2009) who reported maximum growth of *Colletotrichum hibisci* on sucrose followed by glucose then maltose and starch. Sucrose being a major component of photosynthetic plants is generally utilized as good source by most of the plant pathogenic fungi (Lily and Barnett, 1951). The utilization of various carbon compounds may depend either on the activity of the fungus to utilize certain simpler forms or on its power to convert the complex carbon compounds into simpler forms which may be easily utilized (Tasiwal, 2008). This may be the case in this present investigation. Although growth in starch was minimal, it indicates the presence of enzymes having the ability to breakdown starch into simple

sugars. Sporulation occurred on all carbon sources tested but was best on glucose. This is in conformity with the findings of Zubbair (2009) and Maqsood *et al.,* (2014).

The present study revealed that *C.graminicola* is capable of utilizing a variety of nitrogen sources. Mycelial weights of the fungus in the different nitrogen sources tested were significantly different from each other. Potassium nitrate supported maximum growth of the pathogen in terms of the mycelial weight; the least mycelial weight was recorded in urea. All the nitrogen sources however significantly improved growth of the fungus above the control. Similar findings were reported by Tasiwal (2008), Deshmukh *et al.,* (2012) and Kushwaha (2015) who reported the superiority of potassium nitrate over all other nitrogen sources tested. Compared to ammonium sulphate and magnesium nitrate, potassium nitrate (KNO_3) was a good enough source for vegetative growth but not for sporulation; NH_4SO_4 was the best nitrogen source for sporulation followed by $NaNO_3$, $KNO_3$and urea. There was no sporulation in the control indicating that nitrogen is required for sporulation in *C. graminicola.* This is not the first report of this trend. Lily and Barnett (1951) reported that certain sources of nitrogen favour the sporulation of some fungi which are not necessarily the same as those which are favourable for the growth. Kumara and Rawal (2008) reported maximum growth on aspartic acid and abundant sporulation on potassium nitrate with *C. gloeosporioides*. Kushwaha (2015) explained that the reason for good growth of *C. capsici* could be due to the fact that nitrates are not toxic and utilized more easily. Similarly, Manjunatha and Rawal (2002) reported ammonium nitrate as a better source for growth and sodium nitrate better for sporulation. Growth in urea was poor in comparison to the other sources tested. According to Cochrane (1958), urea breaks down to ammonia during autoclaving and ammonia in high concentration is toxic to fungi.

Nitrogen sources in the present experiment however does not seem to affect the size of the conidia; there are no significant differences between the various sources of nitrogen with regards to conidial size. There was no sporulation in the control hence could not be compared with the treatments.

Maximum mycelial weight and sporulation of *C.graminicola* was obtained in magnesium sulphate and least in copper sulphate. Similar findings were reported by Tasiwal (2008) working with *C.gloesoporioides.*

Conclusion

In the overall evaluation, *Colletotrichum graminicola* preferred glucose for growth and sporulation followed by sucrose and then maltose. Among the nitrogen sources, the pathogen was observed to prefer potassium nitrate for growth and ammonium sulphate for

sporulation. Magnesium sulphate was found to be the best source of sulphur for growth of the fungus.

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