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EFFECT OF *Colletotrichum graminicola* ON APIGENINIDIN PRODUCTION, YIELD AND YIELD COMPONENTS IN RED SORGHUM (*Sorghum bicolor* (L.) Moench)

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ABSTRACT

This study was carried out to determine the effect of *Colletotrichum graminicola* on apigeninidin production, yield and yield components in four landraces of red sorghum. Foliar sprays of spore suspension on sorghum leaves infected with pathogen applied in the whorl were used as methods of inoculation in the screen house and field, respectively. General observation on pigment production on the leaves was made. Data were collected on agronomic traits, yield and apigeninidin content. Results show significant differences among the treatments for 100SWT, panicle length and apigeninidin content in the screen house. Significant differences ($P<0.05$) were observed between the treatments for number of leaves, plant height and seedling vigor on the field. In the screen house, '*Karan dafi*' had the highest grain yield of 3049.45kg/ha while '*Malog*' had the highest apigeninidin content (2158.6 mg/L). '*Malog*' had the highest yield of 5388.89kg/ha on the field while '*Karan dafi*' had the highest apigeninidin content of 1635.3 mg/L. '*Karan dafi*' showed stability in yield and apigeninidin content in both environments.

Keywords: Anthracnose; Apigeninidin; Landraces; Sickle cell Anaemia

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an annual plant that belongs to the family Poaceae. It is an important cereal crop grown in the semi–arid tropics of Africa and Asia due to its drought tolerance capacity. It is well adapted to a wide range of soil types and environmental conditions (Sudhakararao, 2011). It is the fifth most important cereal crop after rice, maize, wheat and barley, and is dietary staple of more than half a billion people in over 30 countries (ICRISAT, 2010). Sorghum species is one of the main foods that is widely consumed in Nigeria. Apart from its food value, sorghum is very rich in various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols and policosanols (Sudhakar *et al*., 2008). These phytochemicals have significant importance on human health. Mpiana *et al*. (2013) reported anti-sickling and anti-hemolysis activities of anthocyanins extract from red sorghum. The most common anthocyanin in sorghum is 3-

deoxyanthocyanindin (apigeninidin and luteolinidin). NIPRSAN is an antisickling drug made from 'Karan dafi' (red sorghum landrace) and other medicinal plants for the management of sickle cell anaemia in Nigeria by National Institute for Pharmaceutical Research and Development (NIPRD).

Anthracnose is one of the most damaging diseases of sorghum caused by *Colletotrichum graminicola*, which causes about 50% yield loss (Ali and Warren, 1992; Rekha and Singh, 2014). Development of varieties resistant to anthracnose is an economic approach to curtail diseases in crops. Identification of landrace(s) with more apigeninidin content will also be useful to pharmaceutical industries for the development of drugs used in the management of different ailments. The foliar infection occurs at any stage of plant growth and development, but the symptoms are observed after 40 days of seedling emergence (John *et al*. 2006). Research had shown that both resistant and susceptible sorghum varieties produce pigments around the sites of infection in response to fungal attack (Prom *et al*., 2009). Okubena *et al*. (2018) reported that apigeninidin was the predominant compound in the ethanolic extract of Jobelyn made from pigmented leaf sheath obtained from sorghum plants which accounted for 83.5% of the total amount of identified phenolic compounds. So, apigeninidin constitute the major phenolic compound in sorghum with anti-sickling, anti-haemolytic and anti-anaemic properties. Also, development of varieties resistant to anthracnose is an economic approach to curtail diseases in crops. This study was carried out to determine the effect of *C. graminicola* on apigeninidin production, yield and yield components in four landraces of red sorghum ('Karan dafi', 'Malog', 'Gumna' and 'jar dawa') in order to compare the quantity of apigeninidin produced by these landraces under different environmental conditions and to identify the best landrace(s) with the highest apigeninidin production as well as resistant landrace(s) that could be useful during drug development for the management of sickle cell anaemia and sorghum improvement program, respectively.

MATERIALS AND METHODS

Experimental Site

The experiments were carried out between 2017 and 2018 at Institute for Agricultural Research (IAR) screen house and research farm. The extracts obtained from leaf sheath were analyzed at Multi-user Laboratory in the Department of Chemistry and confirmation of compound produced was carried out at Department of Pharmaceutical Chemistry, Ahmadu Bello University, Samaru, Zaria. Samaru –Zaria is situated in the northern guinea savannah ecological zone of Nigeria (11^0 11^1N, 07^0 38^1E, 680 m above sea level). It has a mean annual rainfall of about 1045mm which is well distributed over the growing season of about 130-190 days between May and October.

Planting Material

Experimental materials comprising of '*Karan dafi', 'Malog', 'Gumna'* and *'jar dawa' (*Table l.0) were used. '*Karan dafi*' seeds were collected from farmers' field in Katsina State, Nigeria, '*Malog'* from Jos, Plateau State and the two other landraces collected by IAR from Niger and Kaduna States were multiplied for seed increase in IAR's field in 2016 raining

season. The seeds obtained from all the landraces were planted in the screen house and IAR's field in year 2017 and 2018, respectively.

Table 1: Brief description of the landraces and their sources

Landraces	Colour of the seeds	Sources
'Karan dafi'	Early maturing, red grain colour, semi-dwarf landrace	Katsina
'Malog'	Late maturing, maroon grain colour, tall landrace	Jos
'Gumna'	Early maturing, maroon grain colour, semi-dwarf landrace	Niger
'Jar dawa'	Late maturing, red grain colour, tall landrace	Kaduna

Screen House and Field Experiments

Four landraces were screened from June to November, 2017 in the screen house. Experimental pots ($0.09m^{2)}$ were laid out using Randomized Complete Block Design (RCBD) with three replications. Five seeds of each landrace were planted per pot and thinned to three stands/pot. For the field experiment, screening was done between July and November, 2018. Treatments were laid out using a split-plot design with infection as main plots and landraces as subplots with three replications. Each plot consisted of 2 ridges, 5m long with 0.6 m and 0.25 m inter and intra row spacing, respectively. Three to four seeds were planted per hole and thinned to two stands/hole. All the necessary agronomic practices for sorghum production were carried out. Weeding and Fertilizer application were done at 3 and 6 weeks after planting.

Inoculum Preparation

The media were prepared using the procedure described by Prom *et al*. (2009) with modification. Already prepared potato dextrose agar (30g) was dissolved in a litre of distilled water. The solution was boiled to congeal and allowed to cool for a while. 1.25g of streptomycin was added to the media to prevent bacteria from growing. The media was autoclaved at a temperature of 121^0C and pressure of 15pa for 25 minutes and it was removed and allowed to cool down to about15^0C. The media was poured into sterilized petri dishes in an inoculating chamber and allowed to solidify. After 24 hours, sorghum grains were sterilized using NaOCl for 5 minutes and rinsed with distilled water 3 times and plated in the media for 1 month till formation of conidia was observed.

Inoculation in the screen house

Three pots were used under infected and uninfected conditions, approximately 3-5 ml conidia suspension (PLATE I) was deposited on the leaves of each plant. Tween 20 (wetting agent) was added to the inoculum (0.5 ml/L) according to Prom *et al*. (2009), while the controls were not sprayed. After spraying, the plants were covered with polythene bags up to 24 hours to enhance colonization.

Field Inoculation

Sterilized sorghum seeds infected with *C. graminicola* were used for field inoculation. When about 7-10 leaves were fully developed; approximately 10 infected seeds were placed in each plant leaf whorl for proper inoculation (Prom *et al.*, 2009).

Extraction of Apigeninidin using Acidified Methanol

Dry leaf sheath obtained from different landraces were extracted with 1% HCl in methanol in accordance with Devi *et al.* (2011). 10ml of solvent was added to 0.5 g of samples in 50 ml of centrifuge tubes and the samples were shaken for 2 hours at low speed (75 rpm). Samples were stored at -20°C overnight in the dark to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then equilibrated to room temperature and centrifuged at 7,000 rpm for 10 min. Residues were rinsed with 10 ml volumes of solvent two times with shaking for 5 min, then centrifuging at 7000 rpm for 10 min. Finally, the extracts were mixed very well and stored at -20°C in the dark until further biochemical analysis (Joseph *et al*., 2004; Devi *et al*., 2011).

Preparation of Buffer

The following pH were prepared according to Association of Official Analytical Chemists (AOAC, 2005):

(**a**) pH 1.0 buffer (potassium chloride, 0.025M)- 1.86 g of potassium chloride was weighed into a beaker and 980ml of distilled water added. The pH was measured and adjusted to 1.0 with HCl. It was then transferred to 1.0 L volumetric flask and diluted to volume with distilled water.

(**b**) pH 4.5 buffer (sodium acetate, 0.4M). 54.43 g of sodium acetate was weighed into a beaker and 960 ml of distilled water was added. The pH was measured and adjusted to 4.5 with HCl. It was then transferred to 1.0 L volumetric flask, and diluted to volume with distilled water (AOAC, 2005).

Determination of Total Apigeninidin using UV/VIS Spectrophotometer

The pH differential method as reported by Fuleki and Francis (1968) was used for quantitative determination, carry 300 UV/VIS spectrophotometer was used for the absorbance measurement. Each of two 0.2 ml aliquots was diluted with 3.0 ml of pH 1.0 and pH 4.5 buffers but more dilution was made for some samples that were too concentrated to be read by the spectrophotomer. Total monomeric apigeninidin was determined by using its wavelength for absorbance determination (468 nm). Extinction coefficients for apigeninidin (18000 L/mol/cm)) was used using the formula described by NSF international, (2004) and AOAC (2005). Total Apigeninidin was calculated by using the formula below:

$$\text{Apigeninidin pigment content} = \frac{\text{A X MW X DF X 1000}}{\varepsilon \text{ X L}}$$

Where:

A= ((Absorbance λ vis-max- A700) pH 1.0 - (Absorbance λ vis-max-A700) pH 4.5)

Absorbance λ vis-max = Absorbance value using 468nm in pH1.0 buffer
A700 = Absorbance value using 700nm in pH1.0 buffer
Absorbance λ vis-max = Absorbance value using 468nm in pH4.5 buffer
A700 = Absorbance value using 700nm in pH4.5 buffer
ε= Apigeninidin molar absorptivity (18000 L/mol/cm)h
MW=Molecular weight of Apigeninidin (255.24 g/mol)
DF = dilution factor = 15
L = cell path length (usually 1 cm)

Data Collection

Observations were made on pigment production; colour of the pigment produced. The leaf sheaths were also sampled for extraction and quantification of 3- deoxyanthocyanindin.

Data were collected on seedling vigor, disease rating on a scale of 1-5, plant height, number of leaves, head count, 100seed weight, panicle length, glumes color, panicle weight and yield.

Seedling Vigor: the plants were scored on a scale of 1-5. 1(most vigorous) to 5 (least vigorous)

Disease Rating: The plants were rated for disease on a scale of 1-5 as described below according to (Prom *et al.*, 2009)

S/no	Status of plants after inoculation	Score
1.	Healthy plants	1
2.	Hypersensitive with local lesion	2
3.	Infected bottom leaves acervuli	3
4.	Middle to bottom infected leaves with acervuli	4
5.	Infected plant including flag leaf with acervuli	5

Days to 50% flowering: - the number of days from sowing to the time when 50% of the plants have produced flowers.

Number of leaves: - numbers of leaves from the base to the flag leaf of 3 randomly selected plants were taken from each row and their average recorded at physiological maturity

100 seed weight: the weight of 100 seeds after threshing was counted and recorded in grams

Panicle length: It was measured in cm using meter rule from the base of the panicle to the top of the panicle.

Glume's colour: the colour obtained from glumes of each landrace was taken as brownish or blackish in colour

Panicle weight: weight of panicle of the 3 randomly selected plant were measured in (g)

Grain yield/ha: after threshing and adequate drying, the grain weight/plot was taken in kilogramme (Kg) for field experiment and gramme (g) for the screen house experiment and converted to kg/ha

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2002). Means were separated using fisher's LSD at 0.05 probability level. Independent sample-t-test was also used for comparing mean differences between field and screenhouse values.

RESULTS AND DISCUSSION

Effect of Anthracnose on Pigment Production, Yield and Yield Components of Landraces of Red Sorghum

Pigment production was observed in this study on the infected leaves one week after inoculation (Plate II) under field and screen house conditions. Accumulation of phytoalexins associated with the hypersensitive reaction on inoculated leaves which was clearly visible during the wet season at 12 days after inoculation was also reported by John et al. (2006). The area of lesion was not as large under field condition as what was obtained under screen house condition (Plate II). This agreed with findings of John *et al.* (2006) who observed limited anthracnose lesion development at the site of infection during wet season. Accumulation of pigments around the sites of infection in response to attack was also reported by Prom *et al*. (2009). Mizuno *et al*. (2014) also observed production of the 3-deoxyanthocyanidins (apigeninidin and luteolinidin) at the sites of infection of sorghum plants. The colors obtained around the area of lesion were also similar to the colour of their seed coat except in 'Gumna' where reddish brown was observed around the area of lesion in the screen house while maroon colour was observed in the field (PLATE II) (Tables 2 & 3). Variations in color observed around site of infections were also reported by Mizuno *et al*. (2014). Acervuli were observed at the site infection in 'Gumna' after one week of inoculation. Formation of acervuli were also observed on the leaves of some uninfected plants of 'Gumna'. Productions of acervuli were observed on the infected leaves of 'Gumna', 'Malog' and 'jar dawa' after three weeks of inoculation. Little or no acervuli were observed on the infected leaves of 'Karan dafi' after third weeks of inoculation under field condition. Production of acervuli observed on the infected and uninfected leaves of 'Gumna' both in the screen house and on the field showed that it harbours conidia naturally.

No significant differences were observed between the treatments for most of the traits measured except 100SWT and panicle length under screen house condition, but significant differences were observed among the landraces used for all the traits measures except disease rating at 3rd week of Inoculation. Uninfected plot had higher 100SWT of 2.95g which was significantly different from what was obtained from infected plot (2.06g). Longer panicle of 28.82cm was obtained from uninfected plot which was significantly different from what was obtained from infected plot (18.00cm) as shown in Table 6.0

Analysis of variance showed significant differences ($P<0.05$) between the treatments for number of leaves, plant height, seedling vigour and disease rating at third week of inoculation but highly significant differences ($P<0.01$) were observed among the landraces tested for yield and yield components except seedling vigor and head weight on the field (Table 7).

Among the landraces tested ''Malog'' had the highest yield (5388.89kg/ha) which was not significantly different from what was obtained from 'Karan dafi' (4666.67kg/ha) but significantly different from what was obtained from ''Gumna'' (2166.67kg/ha) and 'Jar dawa' (2944.44 kg/ha) under the field condition. (Table 7) ''Gumna'' and 'Karan dafi' reached days to 50% flowering at the same time (56days) followed by ''Malog'' (75days), 'Jar dawa' was the last to flower. Number of leaves ranged from 8 for Karan dafi' and 'Gumna' to 14 for 'Jar dawa' Plant height ranged from 188 cm for 'Karan dafi' to 323cm for 'Jar dawa'. 'Malog' had 100SWT of 3.23g while the least was obtained from 'Karan dafi' (2.32g). The longest panicle was recorded from 'Jar dawa' (38cm) while 'Gumna' recorded

the least (19cm). The panicle is compact in nature for 'Karan dafi' and 'Gumna', spreading type for 'jar dawa' while in between for 'Malog'.

Significant interaction was observed between the treatment and landraces under the screen house condition for 100SWT, HWT, panicle length and yield (Table 8) but no significant interaction were observed between the treatments and landraces used under the field condition for most of the traits measured except for the disease rating at third week of inoculation (Table 8).

Based on the result obtained from this study, it was observed that 'Malog' and 'Jar dawa' are tall and late maturing while 'Gumna' and 'Karan dafi' are short and early maturing in nature. Karan dafi proved to be tolerant to anthracnose under both field and screen house conditions because reasonable amount of yield was produced in both environments. This can be attributed to it tolerance to infection or it early maturing in nature. 'jar dawa' produced low yield under the screen house condition which may be attributed to its susceptibility to infection or unfavorable environmental conditions while significant yield was obtained under field condition but the yield obtained was lower than what was recorded by 'Karan dafi' and 'Malog'. Effect of anthracnose stalk on yield reduction was said to be dependent on the environment reported by Callaway *et al*. (1992). Possible effect of genotype by environment interaction contribution to the variation in infection response observed within and between experiments for six accessions was reported by John and Louis (2006). The stability in yield and pigment production over the two environments in 'Karan dafi' was observed in this study.

Table 2: Effect of *Colletotrichum graminicola* on pigment production under screen house condition

Entry	Pigment production on the leaves	Pigment production on the leaf sheaths	Colour of the pigment produced	Glumes colour
'Karan dafi' infected	Pigment production observed	Pigment production observed	Reddish brown	Brown
'Malog' infected	Pigment production observed	Pigment production observed	Maroon pigment	Black
'Gumna' infected	Pigment Production observed	Pigment Production observed	Reddish brown	Brown
'jar dawa' infected	Pigment production observed	Pigment production observed	Reddish brown	-
'Karan dafi' uninfected	Dotted pigmentation observed	Pigment production observed	Reddish brown	Brown
'Malog' uninfected	Dotted pigmentation observed	Pigment production observed	Maroon	Black
'Gumna' uninfected	Dotted pigmentation observed	Pigment production observed	Reddish brown	Brown
'jar dawa' uninfected	Little or no pigment production observed	Little pigment production observed	Reddish brown	Light brown

Table 3: Effect of *Colletotrichum graminicola* on pigment production under field condition

Entry	Pigment production on the leaves	Pigment production on the leaf sheaths	Colour of the pigment produced	Glumes colour
'Karan dafi' infected	Pigment production observed	Pigment production observed	Red	Brown
'Malog' infected	Pigment production observed	Pigment production observed	Maroon	Black
'Gumna' infected	Pigment Production observed	Pigment Production observed	Red to maroon	Brown
'jar dawa' infected	Pigment production observed	Pigment production observed	Red	Brown
'Karan dafi' uninfected	Dotted pigmentation observed	Pigment production observed	Red	Brown
'Malog' uninfected	Dotted pigmentation observed	Pigment production observed	Maroon	Black
'Gumna' uninfected	Dotted pigmentation observed	Pigment production observed	Red to maroon	Brown
'jar dawa' uninfected	Dotted pigmentation production observed	Pigment production observed	Red	Brown

Table 4: Mean squares for yield and yield components of four landraces of sorghum under infected and non-infected conditions evaluated in 2017 in the screen house

Source of Variation	DF	50%DF	NOL	PH (cm)	SV	100SWT	HWT(g)	PL (cm)	DR1	DR3	Yield (kg/ha)
Rep	2	0.04	2.09	1617.25	4.29**	0.11	15.77	13.29	1.54**	0.13	407098.24
Trt	1	0.04	0.84	1074.68	0.38	4.68**	63.47	659.61**	0.67	0.04	113210.96
Variety	3	5604.38**	30.48**	6535.99**	1.93*	1.84**	2495.50*	338.37**	1.44*	0.38	4741275.2**
Variety*Trt	3	0.04	3.09	255.07	0.71	4.24**	2573.36*	418.20**	0.33	0.38	2158388.01*
Error	14	0.04	1.05	615.53	0.57	0.13	702.30	7.52	0.26	0.22	553014.60

Table 5: Mean squares for yield and yield components of four landraces of sorghum under infected and non-infected conditions evaluated in 2018 rainy season in IAR

Source of Variation	DF	50%DF	NOL	PH (cm)	SV	100SWT(g)	HWT(g)	PL (cm)	DR1	DR3	YIELD (kg/ha)
Rep	2	0.13	1.03	165.6	0.50	0.11	358.44	9.7	0.13	0.04	524305.56
Trt	1	0.00	5.85*	5691.84**	3.38*	0.01	625.26	1.23	0.67	2.04**	93750.00
Error (a)	2	8.17	0.70	363.10	0.50	0.03	372.12	4.71	0.04	0.04	513888.9
Entry	3	962.28**	36.79**	24248.64**	1.60	0.92**	797.44	467	0.78*	0.71	3337577.16**
Entryx trt	3	0.00	1.50	380.4	0.15	0.1	119.26	8.72	0.11	0.38	146219.14
Error (b)	12	2.19	0.69	379.5	0.50	0.06	279.44	8.4	0.19	0.04	403163.58

YD = Grain yield, 50%DF =days to 50% flowering, NOL= number of leaves, PH= Plant height, SV= seedling vigour, 100SWT= 100 seed weight, PL= panicle length, DR= disease rating. *= Significant ($p<0.05$), ** = Highly significant ($P<0.01$)

Table 6: Means for grain yield and other agronomic traits of four landraces of sorghum under infected and non-infected conditions evaluated in the screen house in 2017

Treatment	50%F	NOL	PH (cm)	SV	100SWT(g)	HWT(g)	PL (cm)	DR1	DR3	YD (kg/ha)
Infected	81.00	12.00	233.88	1.92	2.06	58.41	18.32	2.00	2.67	2023.81
Uninfected	80.00	12.00	247.26	2.17	2.95	61.66	28.82	1.67	2.58	2161.17
LSD	0.18	0.89	21.72	0.67	0.31	23.20	2.58	0.48	0.41	651.14
Variety										
‘Karan dafi’	55.00	10.00	198.53	1.50	2.73	78.61	24.13	1.33	2.50	3049.45
‘Gumna’	53.00	10.00	230.00	1.83	2.93	62.43	18.55	2.20	2.83	2408.42
‘Malog’	106.83	13.00	268.00	2.83	2.66	67.95	34.00	2.33	2.83	1978.02
‘jar dawa’	107.00	14.00	265.25	2.00	1.69	31.15	17.67	1.50	2.33	934.07
LSD	0.25	1.27	30.72	0.94	0.43	32.82	3.65	0.68	0.58	920.86
Interaction (Variety*Trt)					**	*	**			*
Mean	76.00	11.69	241.00	2.04	2.50	60.04	23.59	1.83	2.63	2092.49
CV (%)	28.74	9.03	9.77	32.29	14.11	42.54	13.37	30.05	17.9	35.31

YD = Grain yield, 50%DF =days to 50% flowering, NOL= number of leaves, PH= Plant height, SV= seedling vigor, 100SWT= 100 seed weight, PL= panicle length, DR= disease rating. *= Significant ($p<0.05$), ** = Highly significant ($P<0.01$),

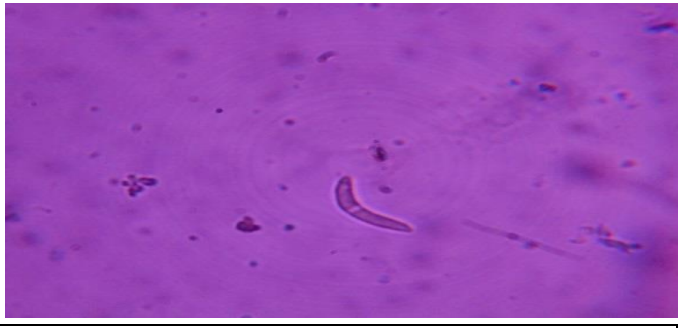
Table7: Means for grain yield and other agronomic traits of four landraces of sorghum under infected and non-infected conditions evaluated in 2018 rainy season on IAR field

Treatment	DF	NL	PH (cm)	SV	100SWT(g)	HWT(g)	PL (cm)	Drs1	Drs3	Yield kg/ha
Infected	66.75	10.00	237.83	1.75	2.77	54.70	28.39	2.17	2.83	3666.66
Uninfected	66.75	9.00	268.00	2.50	2.80	64.92	27.94	1.83	2.25	3916.66
LSD	0.10	0.74	17.33	0.63	0.22	14.86	2.58	0.39	0.18	956.95
Landrace										
'Karan dafi'	56.00	8.00	188.5	2.17	2.32	53.07	22.93	1.83	2.16	4666.67
'Gumna'	56.00	8.00	211.42	1.83	2.67	47.05	19.12	2.50	3.00	2166.67
'Malog'	75.17	12.00	290.5	2.83	3.23	69.71	31.93	2.00	2.50	5388.89
'jar dawa'	80.00	12.00	322.5	1.67	2.95	69.41	38.67	1.67	2.50	2944.44
LSD	1.43	1.05	24.51	0.89	0.31	21.03	3.65	0.55	0.26	1353.30
Interaction (Landrace x Trt)	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
Means	66.75	9.91	253.23	2.13	2.79	59.81	28.16	2.00	2.54	3791.67
CV	1.70	8.40	7.86	33.28	8.69	27.95	10.29	22.05	8.03	28.37

Table 8: Yield of sorghum and other traits as influenced by the interaction effects of different landraces and infection under the screen house condition Evaluated in the screen house in 2017

Landrace	100SWT (g)			HWT(g)			PL (cm)			Yield (kg/ha)		
	Infected	Uninfected	Mean	Infected	Uninfected	Mean	Infected	Uninfected	Mean	Infected	Uninfected	Mean
'Karan dafi'	2.71	2.76	2.73	87.05	70.16	78.61	24.50	23.78	24.13	3095.00	3003.66	3049.45
'Gumna'	3.04	2.81	2.93	62.64	62.24	62.43	16.88	20.22	18.55	2582.42	2234.43	2408.42
'Malog'	2..49	2.83	2.66	83.94	52.97	67.95	32.00	36.00	34.00	2447.58	1538.46	1978.02
'Jar dawa'	0.00	3.38	1.69	0.00	62.30	31.15	0.00	35.33	17.67	0.00	1868.13	934.07
Mean	2.06	2.95	2.50	58.41	61.66	60.04	18.32	28.82	23.59	2023.81	2161.17	2092.49
LSD	0.14	0.90		42.61	50.54		4.54	6.70		240.16	1932.4	

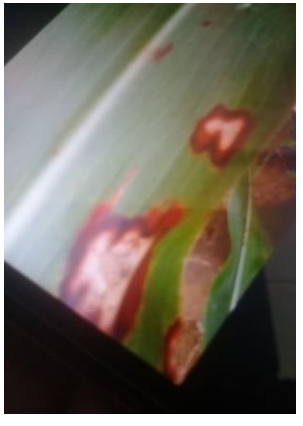
Plates of inoculum



Conidia isolated

Plate I: Plates of inoculum and conidia used for artificial inoculation of sorghum plants

Pigment production on the leaves of infected plants



Uninfected leaves

Plate II: Leaves of the different landraces infected with anthracnose under screen house and field conditions

Quantification of Apigeninidin from Four Landraces

Table 9 shows the quantity of apigeninidin obtained from four landraces of sorghum grown *in situ* under field and screenhouse conditions. Under disease condition, significant differences were observed among the treatment tested. In the screen house, uninfected plot recorded more apigeninidin content (1491.2 mg/L) which was significantly different from what was obtained from the infected plot (1154.4 mg/L) using the leaf sheath while under field condition, the infected plot recorded more apigeninidin content (1608.5 mg/L) which was significantly different from what was obtained from uninfected plot (1103.6 mg/L). This shows that, in addition to infection, there are other factors that affect apigeninidin production in red sorghum such as environment, among others. Among the landraces used no significant differences were obtained for apigeninidin content under field condition but significant differences were observed under screen house condition (Table 9.0), 'Karan dafi' recorded highest under field condition while least was recorded by 'Malog'. Under screen house condition 'Malog' recorded highest apigeninidin content (2158.6 mg/L) while the least was recorded by 'jar dawa' (704.5 mg/L). This indicates that in addition to infection and genetic make-up of each of the landraces, environments also have significant impact on apigeninidin production in red sorghum. Prom *et al*., (2009) reported that both resistant and susceptible varieties of sorghum produce pigments around the sites of infection in response to fungal attack.

Table 9: Quantity of Apigeninidin produced from *in situ* materials from four landraces of sorghum conducted at Samaru in 2018

Treatment	Conc. Field (mg/L)	Conc. Screenhouse (mg/L)
Infected	1608.5	1154.4
Uninfected	1103.6	1491.2
t- statistics	2.92	2.57
Critical t value (2.15)		
Landraces		
'Karan dafi'	1635.3	1098.4
'Gumna'	1200.6	1332.6
'Malog'	1101.1	2158.6
'Jardawa'	1487.4	704.50
LSD	632.6	440.50
Landraces*Trt	NS	NS
'Karan dafi' infected	1756.1	1048.0
'Karan dafi' uninfected	1514.5	1142.0
'Gumna' infected	1271.4	1174.7
'Gumna' uninfected	1129.7	1490.5
'Malog' infected	1412.2	1960.9
'Malog' uninfected	789.90	2356.4
'Jar dawa' infected	1994.30	433.40
'Jar dawa' uninfected	980.40	975.70
CV (%)	26.50	19.40
Mean	1356.10	1322.79
LSD	827.10	592.08

Conc. Field – concentration of apigeninidin obtained from the field materials, Conc. Screenhouse concentration of apigeninidin obtained from the screen house materials

Results obtained from this study showed that *C. graminicola* has effect on pigment production on sorghum leaves. This agree with findings of John *et al*. (2006) who observed accumulation of phytoalexins associated with the hypersensitive reaction on inoculated leaves while on the leaf sheath its influence was also observed but subject to other factors such as environment and genetic make-up of landrace (s) used. Also, the effect of the *C. graminicola* on yield can be attributed to amount of damage caused to the leaves and other photosynthetic activities of the attacked plants. 'Karan dafi' and 'Gumna' showed stability in apigeninidin content over the two environments. (Tale 9.0) under both infected and uninfected condition. This shows that 'Karan dafi' and 'Gumna' are naturally rich in phenolics compared to other landraces irrespective of environmental conditions.

CONCLUSION

Based on the results obtained from this study, *C. graminicola* has effect on pigment production on sorghum leaves while on the leaf sheath its influence was also observed but subject to other factors such as environment and genetic make-up of the landrace(s) used. Also, better yield was obtained from uninfected plots from both environments. Stability in yield and apigeninidin content were observed in 'Karan dafi' over the two environments tested irrespective of disease condition. This indicates its suitability for apigeninidin production and tolerance to *Colletotrichum graminicola.* Further research is required to validate its stability in apigeninidin production and yield over the multiple environments.

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