International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 1477-1480

Received: 08-05-2020
Accepted: 10-06-2020

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Isolation and *in vitro* evaluation of fungicides against anthracnose of sorghum caused by *Colletotrichum graminicola*

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DOI: https://doi.org/10.22271/chemi.2020.v8.i4n.9820

Abstract
Sorghum is subjected to several diseases at all the stages of its development. Sorghum belongs to the family poaceae. It is one of the most important cereal crops in india, it stands fifth most important world cereal crop after wheat, rice, maize and barley. Among all the diseases infecting sorghum, anthracnose caused by *Colletotrichum graminicola* is one of the most destructive foliar diseases in sorghum. According to present investigation under *in vitro* conditions, followed by eight fungicides i.e, (five systemic and three non-systemic) were used at different concentrations 100ppm, 500ppm, 1000ppm were tested by Poison Food Technique to know their inhibitory effect on the growth of anthracnose of *Colletotrichum graminicola.* According to the present investigation Mancozeb, Propiconazole, Carbendazim and Bavistin shows high inhibiting growth of mycelium, where as the fungus inhibiting growth was recorded very poor in Hexaconazole, Chlorothalonil, Propineb and Difenconazole.

Keywords: Sorghum¸ anthracnose¸ *Colletotrichum graminicola* mycelium growth¸ fungicides.

Introduction
Sorghum [*Sorghum bicolar* (L) Moench] is one of the yields that are cultivated in marginal regions, where low oil and moisture and high encompassing temperatures are the fundamental limiting abiotic factors [wenzel and van Rooyen 2001, Machado *et al.*, 2002, Gebeyehu *et al.*, 2004] sorghum is a tropical plant having a place with the poaceae family and it is accepted to have started in north east Africa, where it was tamed around 3000-5000 years back [Pederson *et al.*, 2003] the principle races of developed sorghum are bicolar, vulgare, c audatum, kafir, guinea and durra [deu *et al.*, 1994] sorghum positions fifth among oats in world production, which was 57 million tons in 2006 with the creating scene representing 84% of this all out [Fao,2008] over 35% of the sorghum delivered is straightforwardly utilized for human utilization. The rest is utilized fundamentally for animal fields, production of liquor and industrial items [awika and roney, 2004]
Its substitute uses are poultry feeds and liquor. In china, sorghum is aged and refined to create maotai, which is viewed as one of the nation's most popular alcohol. Sorghum was ground and the flour was the primary option in contrast to wheat in north china for quite a while. The harvest is hereditarily fit to hot and dry agro-ecologies where it is hard to develop other food grain crops. Sorghum is really a double reason crop where both grain and Stover are profoundly esteemed items. In larger parts of the developing world, Stover contributes up to half of the complete estimation of the harvest. Among the rural people who remain alive on oats and millets sorghum is the principle wellspring of calories. Another sort of sorghum, sweet sorghum is developed for the production of syrup. Sorghum is developed broadly all through the parched, semi-bone-dry tropics and mild districts inside the scope of 45ºN to 45ºS. Its exceptional nature of withstanding dry season [Ross and webster, 1970] makes it a possible option in dry land and downpour took care of conditions in semi-dry tropics [SAT].
Sorghum is developed more than 90 nations on the planet, Asia 29% and Africa 52% of 42.8 million of all out world region. In Asia India accounts 84%, china8% and Thailand 1.4% of the complete 12.5 million HA territory. The world evaluated sorghum creation is 59 million, with

efficiency of 1.4 t ha. The significant states in India where the oat grain is created are Maharashtra, Karnataka, Gujarat, Madhya Pradesh, Andhra Pradesh, Rajasthan Uttar Pradesh. Maharashtra produces most extreme sorghum in India.

Materials and methods.

Isolation of *Colletotrichum graminicola*

In the present investigation the diseases samples were collected from the field. Small tissues from infected stem or roots (5mm) along with the healthy tissue were cut with sterile scalpel. The tissues were surface sterilized with 0.1% mercury chloride for 30 seconds. The tissues were subsequently washed in three changes of sterile distilled water to eliminate mercury ions. The surface sterilized tissues were transferred on to the PDA and incubated at 25 ± 2°C in BOD incubator and growth was observed periodically.

3.4.1. *In-vitro* evaluation of fungicides against *Colletotrichum graminicola* causing anthracnose of sorghum

The relative efficacy of eight fungicides including systemic and non-systemic fungicides were evaluated under *in vitro* conditions at three different concentration levels i.e. 100, 500, and 1000 ppm by using poisoned food technique (Nene and Thapliyal, 1979). From the stock double strength potato dextrose agar medium, different lots each containing 50ml double strength potato dextrose medium in a conical flask (150ml) were sterilized at 15 psi (1.05 kg/cm^2) pressure at 121.6ºC for 20 minutes. Simultaneously, concentrations of different fungicides were also prepared in equal amount (50 ml) of sterilized distilled water so as to get the desired concentration of fungicides after mixing the fungicide solutions in the double strength media.

Fungicides solution were added separately to equal quantities of double strength PDA medium aseptically before pouring in Petri plates. The culture discs (6 mm) cut from the margin of 7 days old vigorously growing culture of the test pathogen were placed in the center of each prepared Petri plate. A control was also maintained in which only plain sterilized water was added to the double strength medium. Each treatment was replicated four times and the inoculated plates were incubated at 25 ± 2°C in BOD incubator. The colony diameter of the test pathogen was recorded until the control plates were full of mycelial growth of the test pathogen. The per cent inhibition of mycelial growth at different test concentrations in relation to control was calculated by using the formula (Vincent, 1947) given as below:

$$I = \frac{(C-T)}{C} \times 100$$

Where,

I = Percent inhibition

C = Radial growth in check in cm

T = Radial growth in treated plates in cm

Table 1: The following fungicides were used for *in vitro* evaluation are given below

S. No.	Fungicides	Test Concentration (ppm)
1.	Carbendazim	100, 500, 1000
2.	Propiconazole	100, 500, 1000
3.	Bavistin	100, 500, 1000
4.	Mancozeb	100, 500, 1000
5.	Hexaconazoloe	100, 500, 1000
6.	Chlorothalonil	100, 500, 1000
7.	Propineb	100, 500, 1000
8.	Difenconazole	100, 500, 1000

Results and discussion

***In-vitro* evaluation of fungicides against *Colletotrichum graminicola* causing anthracnose of sorghum**

Eight fungicides among which there are five systemic and three non-systemic fungicides were tested against *C. graminicola* under *in vitro* conditions through poison food technique. The data regarding the percent growth inhibition by different fungicides at different concentration levels 100, 500 and 1000 ppm. Variable inhibition reactions of different fungicides against *C. graminicola* were described in the report.

Systemic fungicides were found more inhibitory as compared to non-systemic fungicides even at equal levels of concentration against the pathogen. Irrespective of different concentration levels of fungicide tested against mycelial growth of *C. graminicola.* Carbendazim, bavistin, mancozeb and propiconazole proved most efficacious providing inhibition of colony diameter at 100, 500 and 1000 ppm followed by Difeconazole, chlorothalonil, propineb and hexaconazole also resulted in significant growth inhibition of the pathogen. The inhibitory responses being only moderate in fungicides.

The data regarding the growth inhibition by eight fungicides (five systemic and three non-systemic) are as follows, Among the systemic fungicide, carbendazim, propiconazole and bavistin produced significant growth inhibition at the concentration level of 500 ppm and at the concentration level of 1000 ppm. Hexaconazole and difenconazole provided statistically similar inhibition at different dosage levels proved next efficacious. Among the non-systemic fungicide, mancozeb produced significant growth inhibition at the concentration level of 500 ppm and at the concentration level of 1000 ppm. Chlorothalonil and propineb provided statistically similar inhibition at higher (1000 ppm) dosage level proved next efficacious.

Table 2: *In-vitro* evaluation of carbendazim (systemic fungicide) against *colletotrichum graminicola* causing anthracnose of sorghum

Fungicide concentrations	Carbendazim					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	16.00	23.75	32.00	40.50	51.50
500 PPM	6.00	11.75	15.75	21.50	28.75	35.00
1000 PPM	6.00	6.00	7.50	9.00	11.00	14.00
Control	14.25	24.25	43.00	64.75	77.00	90.00
C.D.	1.17	1.23	1.31	1.25	1.54	1.35
SE(m)	0.38	0.40	0.42	0.40	0.50	0.43

Table 3: *In-vitro* evaluation of propiconazole (systemic fungicide) against *Colletotrichum graminicola* causing anthracnose of sorghum.

Fungicide concentrations	Propiconazole					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	15.00	21.75	30.00	38.50	55.50
500 PPM	6.00	12.75	16.75	22.50	29.75	38.00
1000 PPM	6.00	6.00	7.50	10.00	12.00	14.00
Control	14.25	24.25	43.00	64.75	77.00	90.00
C.D.	1.17	1.23	1.31	1.25	1.54	1.35
SE(m)	0.38	0.40	0.42	0.40	0.50	0.43

Table 4: *In-vitro* evaluation of Bavistin (systemic fungicide) against *Colletotrichum graminicola* causing anthracnose of sorghum

Fungicide concentrations	Bavistin					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100M	6.00	20.00	27.00	35.75	43.00	59.00
500M	6.00	13.00	25.75	32.00	40.00	45.25
1000	6.00	8.25	10.25	11.25	14.00	16.00
Control	14.00	25.00	34.25	53.25	73.50	90.00
C.D.	0.64	1.17	15.75	1.29	1.49	1.17
SE(m)	0.20	0.38	5.05	0.42	0.48	0.38

Table 5: *In-vitro* evaluation of Mancozeb (Non-systemic fungicide) against *Colletotrichum graminicola* causing anthracnose of sorghum.

Fungicide concentrations	Mancozeb					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 M	6.00	22.00	36.00	45.75	51.00	63.00
500 M	6.00	14.00	27.75	33.00	41.00	48.25
1000	6.00	8.25	10.25	11.25	14.00	18.00
Control	14.00	25.00	34.25	53.25	73.50	90.00
C.D.	0.64	1.17	15.75	1.29	1.49	1.17
SE(m)	0.20	0.38	5.05	0.42	0.48	0.38

Table 6: *In-vitro* evaluation of Hexaconazole (systemic fungicide) against *Colletotrichum graminicola* causing anthracnose of sorghum.

Fungicide concentrations	Hexaconazole					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	23.25	38.00	48.00	54.00	65.75
500 PPM	6.00	17.00	28.00	39.25	43.00	52.00
1000 PPM	6.00	10.25	13.00	15.00	20.75	25.00
Control	14.00	26.25	45.00	53.00	70.00	90.00
C.D.	0.64	1.44	1.27	1.33	1.33	1.17
SE(m)	0.20	0.46	0.41	0.43	0.43	0.38

Table 7: *In-vitro* evaluation of Chlorothalonil (Non-systemic fungicide) against anthracnose of sorghum caused by *Colletotrichum graminicola*

Fungicide concentrations	Chlorothalonil					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	25.00	39.00	49.00	54.25	67.00
500 PPM	6.00	18.00	29.75	40.00	45.00	55.50
1000 PPM	6.00	10.75	13.75	17.00	25.00	29.75
Control	14.00	24.75	43.00	58.00	80.00	90.00
C.D.	0.64	1.33	1.39	1.27	1.33	1.25
SE(m)	0.20	0.43	0.45	0.41	0.43	0.40

Table 8: *In-vitro* evaluation of Propineb (Non-systemic fungicide) against anthracnose of sorghum caused by *Colletotrichum graminicola*

Fungicide concentrations	Propineb					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	22.00	41.00	52.00	54.75	70.00
500 PPM	6.00	19.00	30.50	39.00	47.00	57.25
1000 PPM	6.00	11.00	16.75	19.00	26.00	30.25
Control	13.00	25.00	41.50	60.00	76.50	90.00
C.D.	0.64	1.17	1.39	1.27	3.08	1.23
SE(m)	0.20	0.38	0.45	0.41	0.99	0.40

Table 9: *In-vitro* evaluation of Difenconazole (Systemic fungicide) against anthracnose of sorghum caused by *Colletotrichum graminicola.*

Fungicide concentrations	Difenconazole					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
100 PPM	6.00	20.00	36.25	44.00	54.00	70.00
500 PPM	6.00	15.00	30.50	39.00	47.00	59.25
1000 PPM	6.00	10.25	16.75	19.00	26.00	34.25
Control	13.00	25.00	41.50	60.00	76.50	90.00
C.D.	0.64	1.17	1.39	1.27	3.08	1.23
SE(m)	0.20	0.38	0.45	0.41	0.99	0.40

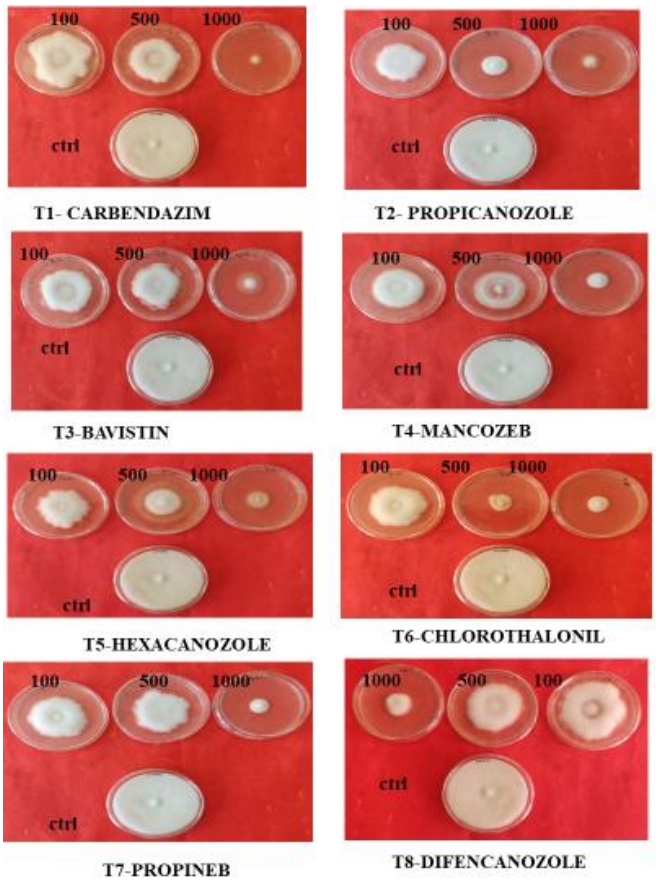

Plate 1: *In vitro* effects of different fungicides against mycelial growth of *Colletotreichum graminicola* causal agent of anthracnose of sorghum.

High efficacies of Carbendazim, bavistin, mancozeb and propiconazole have also been recorded against *Colletotrichum graminicola* by Vijaya N.L, Patel D.S and Maheshwari K.D (2019) [11]. While evaluating different fungicides Rekha, (2013) reported that bavistin as the most promising fungicides at higher concentrations against the mycelial growth of *Colletotrichum graminicoloa*. Similarly, Narendra kumar P.G, (2006). reported mancozeb as most effective against *Colletotrichum graminicola* followed by chlorothalonil. Vijaya N.L *et al.* (2019) [11] recorded carbendazim,

propiconazole, copperoxy chloride and mancozeb as potent fungicides against mycelial growth of *Colletotrichum graminicola* causing anthracnose of sorghum. Chandra Sekhar J, *et al.*, 2020 [3] who also used same fungicides at concentration levels against the pathogen and similar results were observed.

Acknowledgement
It's my heartfelt gratitude to the chairman of my Advisory Committee, Dr. Jai Prakash Mishra (Principal, Deptt. of Plant pathology), Asst-Professor Dr. Rajandra Prasad (Deptt. of Plant pathology). Special thanks to my seniors Mr. Vedukola Pulla Reddy, Mr. Sunil Kumar, Mr. J Chandra sekhar, Mr. G Anil Kumar Reddy and V.K Kiranmayee for all your extensive support, guidance, continuous encouragement, innovative ideas, valuable suggestions and needful help during the entire course of my study and always corrected me while I was finishing my research works.

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