Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 3083-3086

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In vitro evaluation of botanicals against *Colletotrichum graminicola* causing Anthracnose of sorghum

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Abstract
Effect of aqueous extracts (leaf, rhizome and bulb) of nine botanicals were tested evaluated *in vitro* (each @ 10 and 20 %) against Anthracnose of sorghum caused by *Colletotrichum graminicola* under *in vitro* condition. Among the nine botanicals tested were found fungistatic against *C. graminicola* and the results obtained on its mycelial growth and inhibitions. Results revealed that all the nine botanicals evaluated were found fungistatic against *C. graminicola* and recorded significantly reduced mycelial growth and increased mycelial inhibition of the test pathogen over untreated control. The mycelial growth was found to be decreased and its inhibition was increased with increase in concentrations of the botanicals tested. However, significantly highest average growth inhibition was recorded with *A. indica* (70.73%), followed by *Z. officinale* (62.58%), *A. cepa* (54.43%), *P. hystrophorus* (49.81%) and *P. pinnata* (42.95%).

Keywords: *Colletotrichum graminicola, A. indica, P. pinnata, P. hystrophorus*

Introduction
Sorghum (*Sorghumbicolor* (L.)Moench, is an important cereal crop in India popularly known as 'Jowar' and large size of among other grain millets is called 'Great millet'. In India the production is concentrated in the four states Maharashtra, Karnataka, Andhra Pradesh and Gujarat; it is next in importance to rice and wheat and is planted on nearly 5.84 million hectares with an annual production of 5.90 million tones (Anonymous, 2013). Maharashtra contributes 23.81 lakh hectares and 8.82 lakh hectares areas with production of 11.19 and 13.25 lakh tonnes in *Rabi* and *Kharif* respectively (Anonymous, 2013).Powell *et al.* (1977) reported that grain yield was reduced by 70% and more than half the yield loss resulted from incomplete grain fill as verified by 42% decrease in 1000-seed mass and 17.2% decrease in seed density. Uttarakhand has been identified as hot spot for the anthracnose disease(Singh and Singh 2008). Anthracnose of sorghum was first reported from Togo in 1902 (Mughogho, 1988).

Materials and Methods
Aqueous extracts of nine botanicals were evaluated *in vitro* against *C. graminicola*, applying Poisoned food technique. Aqueous extracts of the test botanicals were prepared by grinding with mixture-cum grinder. The 100 gm. washed leaves/ bulbs/rhizomes of each of the test botanicals were macerated in 100 ml distilled water (w/v) separately and the macerates obtain were filtered through double layered muslin cloth. Each of the filtrate obtained was further filtered through Whatman No. 1 filter paper using funnel and volumetric flasks (100 ml cap.). The final clear extracts /filtrates obtained formed the standard aqueous extract of 100 per cent concentration. These were evaluated (@ 10 and 20% each) *in vitro* against *C. graminicola*, applying Poisoned food technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium.

An appropriate quantity of each test aqueous extract (100%) was separately mixed thoroughly with autoclaved and cooled (40^0C) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations (@ 10 and 20%). The PDA medium amended separately with the test aqueous extract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each test botanical extract and their respective concentrations, three plates / treatment / replication were maintained and all the treatments were replicated thrice. Upon solidification of the PDA (amended), all the treatment plates were aseptically inoculated by placing in the center a 5 mm mycelial disc obtained from a week old actively growing pure culture of *C. graminicola*. Plates containing plain PDA without any botanical extract and inoculated with mycelial disc of the test pathogen served as untreated control. All these plates were then incubated at 28 ± 2^0C temperature for a week or till the

untreated control plates were fully covered with mycelial growth of the test pathogen.

Experimental details
Design : CRD
Replications: Three
Treatments : Nine

Treatment details

Tr No.	Treatments (Botanicals)		Plant part used	Concentrations used (%)	
	Common Name	Scientific Name			
T_1	Bougainvillea	*B. spectabilis*	Leaves	10	20
T_2	Garlic	*A. sativum*	Cloves	10	20
T_3	Neem	*A. indica*	Leaves	10	20
T_4	Onion	*A. cepa*	Bulb	10	20
T_5	Karanj	*P. pinnata*	Leaves	10	20
T_6	Drumstick	*M. oleifera*	Leaves	10	20
T_7	Parthenium	*Physterophorus*	Leaves	10	20
T_8	Sorghum	*S. bicolor*	Stem	10	20
T_9	Sorghum	*S. bicolor*	Root	10	20

Observations on radial mycelial growth/colony diameter of the test pathogen were recorded treatment wise at 24 hours interval and continued till mycelial growth of the test pathogen was fully covered in the untreated control plates. Per cent inhibition of mycelial growth of the test pathogen over untreated control was calculated (Vincent, 1927)

Results and Discussion
Results (Table-1) revealed that all the nine botanicals evaluated were found fungistatic against *C. graminicola* and recorded significantly reduced mycelial growth and increased mycelial inhibition of the test pathogen over untreated control (PLATE-I). The mycelial growth was found to be decreased and its inhibition was increased with increase in concentrations of the botanicals tested.

Radial mycelial growth
At 10 per cent concentration, radial mycelial growth of the test pathogen was ranged from 32.00 mm (*A. indica*) to 70.00 mm (*M. oleifera*). However, significantly least mycelial growth was recorded with *A. indica* (32.00). This was followed by the botanicals *viz., Z. offiacinale* (36.33 mm). The botanicals *A. cepa* (44.33 mm), *P. hystrophorus* (50.00 mm), *P. pinnata* (56.66 mm) and *B. spectabilis* (59.66 mm) record moderate mycelial growth. Whereas, the botanicals *viz., S. bicolor* (root extract), *S. bicolor* (leaf extract) and *M. oleifera* recorded comparatively maximum mycelial growth 65.33, 67.33 and 70.00 mm, respectively.

At 20 per cent concentration, radial mycelial growth of the test pathogen was ranged from 20.66 mm (*A. indica*) to 62.33 mm (*M. oleifera*). However, significantly least mycelial growth was recorded with *A. indica* (20.66 mm) This was followed by the botanicals *viz., Z. offiacinale* (31.00 mm). The botanicals *A. cepa* (36.66 mm), *P. hystrophorus* (40.33 mm), *P. pinnata* (46.00 mm) and *B. spectabilis* (46.66 mm) both were at par, record moderate mycelial growth which. Whereas, the botanicals *viz., S. bicolor* (root extract), *S. bicolor* (leaf extract) and *M. oleifera* recorded comparatively maximum mycelial growth 50.33, 54.66 and 62.33 mm, respectively.

Average radial mycelial growth of the test pathogen was ranged from from 26.33 mm (*A. indica*) to 66.16 mm (*M. oleifera*). However, significantly least mycelial growth was recorded with *A. indica* (26.33 mm). This was followed by the botanicals *viz., Z. offiacinale* (33.66 mm). The botanicals *A. cepa* (40.49 mm), *P. hystrophorus* (45.16 mm), *P. pinnata* (51.33 mm) and *B. spectabilis* (53.66 mm) record moderate mycelial growth. Whereas, the botanicals *viz., S. bicolor* (root extract), *S. bicolor* (leaf extract) and *M. oleifera* recorded comparatively maximum mycelial growth 57.83, 60.99 and 66.16 mm, respectively.

Table 1. *In vitro* bioefficacy of plant extracts against *C. graminicola*

Tr. No.	Treatments	Col. dia.*(mm) at Conc.		Av. (mm)	% Inhibition *(mm) at Conc.		Av. (%) inhibition
		10 %	20 %		10 %	20%	
T_1	Bougainvillea (*B. spectabilis*)	59.66	46.66	53.66	33.70 (19.69)	48.14 (20.77)	40.92(20.23)
T_2	Garlic (*Z. officinale*)	36.33	31.00	33.66	59.62 (36.59)	65.55 (40.95)	62.58 (38.77)
T_3	Neem (*A. indica*)	32.00	20.66	26.33	64.44(40.11)	77.03 (50.58)	70.73 (45.34)
T_4	Onion (*A. cepa*)	44.33	36.66	40.49	50.73 (30.49)	58.14 (37.95)	54.43 (34.22)
T_5	Karanj (*P. pinnata*)	56.66	46.00	51.33	37.03 (21.73)	48.88 (29.26)	42.95 (25.49)
T_6	Drumstick (*M. oleifera*)	70.00	62.33	66.16	22.22 (12.83)	30.77(17.92)	26.49 (15.37)
T_7	Parthenium(*P. hystrophorus*)	50.00	40.33	45.16	44.44 (26.38)	55.18 (33.48)	49.81 (29.93)
T_8	Sorghum leaf(*S. bicolor*)	67.33	54.66	60.99	25.18 (14.58)	39.25 (23.11)	32.21 (18.84)
T_9	Sorghum root (*S. bicolor*)	65.33	50.33	57.83	27.40 (15.90)	44.07 (26.15)	35.73 (21.02)
T_{10}	Control	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E. ±	0.59	0.51	0.55	0.60	0.70	0.65
	C.D. (P=0.01)	1.76	1.51	1.63	1.77	2.07	1.92

*-Mean of three replications, Dia.: Diameter, Av.: Average, Conc.: Concentration, Figures in parentheses are angular transformed values

2 My celial inhibition
Results obtained on mycelial growth inhibition of the test pathogen with the botanicals tested at various concentrations are presented in the Table-1 and depicted in the (Table-1, PLATE-I and Fig.-1).

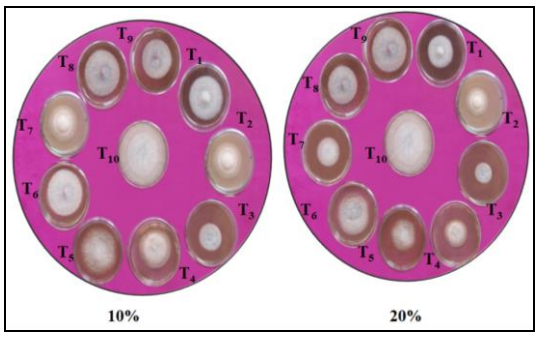
Results (Table-1, Fig.-1) revealed that all the botanicals tested (@ 10 and 20 % each), significantly inhibited mycelial growth of the test fungus over untreated control (00.00%). Further, it was found that per cent mycelial growth inhibition of the test pathogen was increased with increase in

concentration of the botanicals tested **(**PLATE-I).

At 10 per cent, mycelial growth inhibition ranged from 22.22 (*M. oleifera*) to 64.44 (*A. indica*) per cent. However, significantly highest mycelial growth inhibition was recorded with the botanicals *A. indica* (64.44%). This was followed by the botanicals *viz., Z. officinale* (59.62%), *A. cepa* (50.73%), *P. hystrophorus* (44.44%), *P. pinnata* (37.03%) and *B. spectabilis* (33.70%). Botanical *S. bicolor* (root extract), *S. bicolor* (leaf extract) and *M. oleifera* were found less effective with significantly less mycelial growth inhibition of 27.40, 25.18, 22.22 per cent, respectively.

At 20 per cent, mycelial growth inhibition ranged from 30.77 (*M. oleifera*) to 77.03 (*A. indica*) per cent. However, significantly highest mycelial growth inhibition was recorded with the botanicals *A. indica* (77.03%). This was followed by the botanicals *viz., Z. officinale* (65.55%), *A. cepa* (58.14%), *P. hystrophorus* (55.18%), *P. pinnata* (48.88%) and *B. spectabilis* (48.14%) both are at per. Botanicals *S. bicolor* (root extract), *S. bicolor* (leaf extract) and *M. oleifera* were found less effective with significantly less mycelial growth inhibition of 44.07, 39.25 and 30.77 per cent, respectively.

Average mycelial growth inhibition ranged from 26.49 (*M. oleifera*) to 70.73 (*A. indica*) per cent. However, significantly highest mycelial growth inhibition was recorded with the botanicals *A. indica* (70.73%). This was followed by the botanicals *viz., Z. officinale* (62.58%), *A. cepa* (54.43%), *P. hystrophorus* (49.81%), *P. pinnata* (42.95%) and *B. spectabilis* (40.92%). Botanicals *S. bicolor* (root extract), *S. bicolor* (stem extract) and *M. oleifera* were found less effective with significantly less mycelial growth inhibition of 35.73, 32.21and 26.49 per cent, respectively.



In vitro* efficacy of the botanicals against mycelial growth and inhibition of *C. graminicola

T1: Bougainvillea (*B. spectibilis*)
T2 : Garlic (*A. sativum*)
T3 : Neem (*A. indica*)
T4 : Onion (*A. cepa*)
T5: :Karanj (*P. pinnata*)
T6 : Drumstick (*M. oleifera*)
T7 : Parthenium (*P. hystrophorus*)
T8:Sorghum Leaf (*S. bicolor*)
T9 :Sorghum Root (*S. bicolor*)
T10 :Control

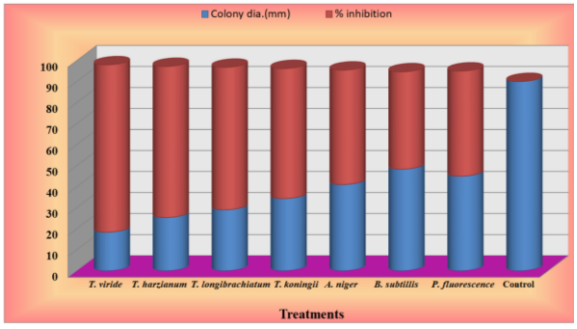


Fig. 1: *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *C. graminicola*

Conclusions

Anthracnose of sorghum has been reported as a serious threat to bean production in a major sorghum growing region of India and therefour serve as a guide for further field testing in the future. *In vitro* all the nine botanicals tested were found fungistatic against *C. graminicola*. However, significantly highest average growth inhibition was recorded with *A. indica* (70.73%), followed by *Z. officinale* (62.58%), *A. cepa* (54.43%), *P. hystrophorus* (49.81%) and *P. pinnata* (42.95%).

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