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In vitro Efficacy of Plant Extracts against *Bipolaris setariae* causing Brown Top Millet Leaf Blight

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A B S T R A C T

Twelve botanicals were evaluated against *Bipolaris setariae* causing leaf blight on brown top millet under *in vitro* conditions at ICAR-AICRP on small millets. University of Agricultural Sciences, GKVK, Bangalore by poison food technique at 10, 20 and 30 per cent (w/v). Among the 12 botanicals Agave showed maximum inhibition of mean mycelial growth (81.52 %) and sporulation and also agave showed cent percent inhibition at 30 per cent concentration followed by datura and eucalyptus which accounted 40.62 and 34.32 per cent mean mycelial growth inhibition. Whereas, Calotropis was unableto inhibit the fungal growth and sporulation as evidenced by 0 per cent inhibition of mycelial growth. Similarly, Pongamia (1.48 %), turmeric (1.77 %) and Gliricidia (9.46 %) extracts exhibited less inhibition of mycelial growth.

Introduction

Millets have been considering super grains since ages. With their nutritional value they became boon crops for human suffering with cardiac, diabetic and obese problems. They have recognized not only as smart crops for their capability of photo insensitivity, drought tolerance and climate resilience but also smart food because of their richness in vitamins, fibre and mineral content. Due to their importance FAO have approved and declared 2023 as International year of millets also India announced 2018 as national year of millets. Though the importance of millets is remarkable, the production accounts only 2 per cent of worlds cereal production where Asia accounts 40 per cent of millet production of the world.

As a cause of climate resilient and hardy nature and owing to their superior nutritional qualities, millets are gaining importance over cereals which were hitherto predominant (Anju and saritha, 2010). Browntop millet is native to India but was introduced to United States in 1915 for cultivation purpose (Oelke *et al.*, 1990). It was recently included into millets system in India as one of the small millet for food and fodder purpose. Being

different from other small millets by having characters like shortest growth period, shade tolerant, suppress root knot nematode and because of their sharp and stiff leaf structure it obstructs the entry of rats in to fields and also serves as cover crop and reduces soil erosion (Sujata *et al.*, 2018).

As the millets are hindering from their potential yield production by the attack of several diseases like blast, blight or brown spot, downy mildews, smut, rust and other bacterial and viral diseases. Among them, blight or brown spot caused by *Helminthosporia* are most devastating next to blast. Browntop millet was first reported to be attacked by *Bipolaris setariae* causing leaf spot in Bihar (Misra and Prakash, 1972). In cropping system, sever incidence of leaf bight symptoms were noticed in the genotype evaluation trial (ICAR-AICRP on small millets) during *Kharif* 2018 and the disease was identified as *Helminthosporium* leaf blight (Anonymous, 2018-19).

Later, causal organism was identified as *B. setariae* using molecular markers. By considering the severity and prevalence of the disease in millet growing areas present study was undertaken as one of Integrated disease management (IDM) component to evaluate different botanicals against *B. setariae* under laboratory conditions.

Materials and Methods

In vitro evaluation of twelve plant extracts at three different concentrations *viz.,* 10, 20 and 30 per cent were studied for their effect on growth and sporulation of *B. setariae* by using the poison food technique (Nene and Thapliyal, 1973; Sharvelle, 1961).

The different botanicals with plant parts used for extraction are listed in table 1. Extraction was done after washing the leaves of each plant thoroughly with running tap water and surface sterilized with 0.1 per cent sodium hypochlorite solution. The stock solutions of 100 per cent botanicals were made based on weight per volume (w/v) and stock solution of each plant extract were filtered through a muslin cloth.

An appropriate amount of botanical stock solution was added to the PDA medium, to obtain a required concentration of 10, 20 and 30 per cent and the conical flasks were gently shaken to completely disperse the botanical solution. About 15-20 ml of poisoned media was poured into 90 mm Petri dishes. 5 mm disc from 10 days old culture was taken by using an aseptic 9 mm cork borer and transferred to the centre of each Petri dish containing the poisoned medium.

A control was maintained in which the fungal pathogen was grown under similar conditions on agar medium without any plant extract. The inoculated plates were incubated at 27±1 °C for 12 days and radial growth of the test pathogen was recorded in three directions and the average diameter was calculated. The percentage of inhibition of growth over control was calculated with the formula (Vincent, 1947).

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

Where., I = Per cent inhibition. of mycelium

C= Growth of mycelium in control
T = Growth of mycelium in treatment

Results and Discussion

Twelve plant extracts were tested at three concentrations (10, 20 and 30 %) using poisoned food technique to know the efficacy to inhibit the mycelial growth and sporulation of *B. setariae* (Table 2, Plate 1 and Fig. 1). Among the botanicals, highest significant

mean per cent inhibition of mycelial growth was recorded in agave (81.52 %) whereas no inhibition was noticed in calotropis. In the other plant extarcts, the inhibition was moderate and variable. Amongst the concentrations, 30 per cent showed maximum (26.95 %) inhibition of mycelial growth with the least in 10 per cent (10.08 %).

With respect to interaction of botanicals and concentrations, complete mycelial growth inhibition was recorded in agave at 30 per cent. Agave was observed to be effective even at lower concentration than other botanicals at higher concentration.

Calotropis at all the concentrations, turmeric at 10 and 20 per cent and *Aloe vera* at 10 per cent showed no inhibition. Inhibition of mycelial growth was possibly due to inhibition of spore production, inhibition of spore germination and deformed conidia. Those botanicals that didn't affect the mycelial growth of *B. setariae* probably affected the conidia or sporulation.

Jatoi *et al.*, (2015) recorded maximum inhibition of mycelial growth of *H. oryzae* in the ginger, garlic and datura extracts, but in our studies these extracts resulted only in moderate inhibition, with agave as the best. Subedi *et al.*, (2019), Nayak and Hiremath (2019), Kumar *et al.*, (2009), Sandeep (2015) and Gurjar *et al.*, (2012) reported that neem (*A. indica*) and garlic were effective against *Helminthosporium* sp. in different crops.

These results were not in accordance present study where neem and garlic were found to be ineffective against *B. setariae*.

Table.1 List of Botanicals used for *in vitro* evaluation against *B. setariae* infecting browntop millet

Sl. No	Common name	Scientific name	Family	Plant part used
1	Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome
2	Garlic	*Allium sativum* L.	Amaryllidaceae	Bulb
3	Ginger	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome
4	Aloe vera	*Aloe vera* (L.) Burm. f.	Asphodelaceae (Liliaceae)	Succulent leaves
5	Agave	*Agave americana* L.	Asparagaceae	Leaves
6	Calotropis	*Calotropis gigantea* (L.) Dryand	Apocynaceae	Leaves
7	Gliricidia	*Gliricidiasepium* (Jacq.) Kunth ex walp.	Fabaceae	Leaves
8	Eucalyptus	*Eucalyptus obliqua*L'Her.	Myrtaceae	Leaves
9	Datura	*Datura metel* L.	Solanaceae	Leaves
10	Neem	*Azadirachta indica* A. Juss.	Meliaceae	Leaves
11	Congress grass	*Parthenium hysterophorus* L.	Asteraceae	Leaves
12	Karanj	*Pongamia pinnata* (L.) Pierre	Fabaceae	Leaves

Table.2 *In vitro* efficacy of botanicals against *B. setariae* infecting browntop millet

Sl. No.	Botanicals	Per cent inhibition over control*			Mean	Microscopic observation
		10 % (w/v)	20 % (w/v)	30 % (w/v)		
1	Turmeric	0.00 (0.29)	0.00(0.29)	5.31 (13.21)	**1.77 (4.60)**	Sporulation (-) and deformed hyphae
2	Garlic	9.01 (17.44)	25.18 (30.12)	36.29 (37.04)	**23.45 (28.20)**	Sporulation (-)
3	Ginger	3.94 (11.44)	7.40 (15.71)	20.98 (27.25)	**10.77 (18.13)**	Sporulation (-)
4	Aloe vera	0.00 (0.29)	9.38 (17.74)	22.71 (28.44)	**10.70 (15.49)**	Sporulation (-)
5	Agave	60.74 (51.20)	83.83 (66.30)	100.00 (89.71)	**81.52 (69.07)**	Sporulation (-)
6	Calotropis	0.00 (0.29)	0.00 (0.29)	0.00 (0.29)	**0.00 (0.29)**	Sporulation (+++) but deformed shape
7	Gliricidia	0.49 (2.52)	10.86 (19.21)	17.03 (24.35)	**9.46 (15.36)**	Sporulation (+) but spore is 2-5 celled
8	Eucalyptus	20.98 (27.25)	25.80 (30.52)	56.17 (48.55)	**34.32 (35.44)**	Sporulation (-)
9	Datura	23.95 (29.30)	43.83 (41.45)	54.07 (47.34)	**40.62 (39.36)**	Sporulation (-)
10	Neem	7.03 (15.36)	12.72 (20.89)	11.85 (20.13)	**10.53 (18.80)**	Sporulation (++++)
11	Congress grass	4.94 (12.77)	19.00 (25.85)	21.48 (27.60)	**15.14 (22.07)**	Sporulation (-)
12	Karanj	0.00 (0.29)	0.00 (0.29)	4.40 (12.10)	**1.48 (4.22)**	Sporulation (++)
	Mean	**10.08 (12.98)**	**18.31 (20.69)**	**26.95 (28.95)**	**19.98**	
		Botanicals (B)	Concentration (C)	B × C		
	S.Em ±	0.40	0.20	0.70		
	CD (P 0.01)	1.14	0.55	1.98		

Note: * Mean of three replications; -: no sporulation; +: 1-15 conidia; ++: 16-45 conidia; +++: 46-70 conidia; ++++: >70 conidia per microscopic field. Figures in parenthesis are angular transformed values

Fig.1 *In vitro* efficacy of plant extracts on mycelial growth inhibition of *B. setariae*

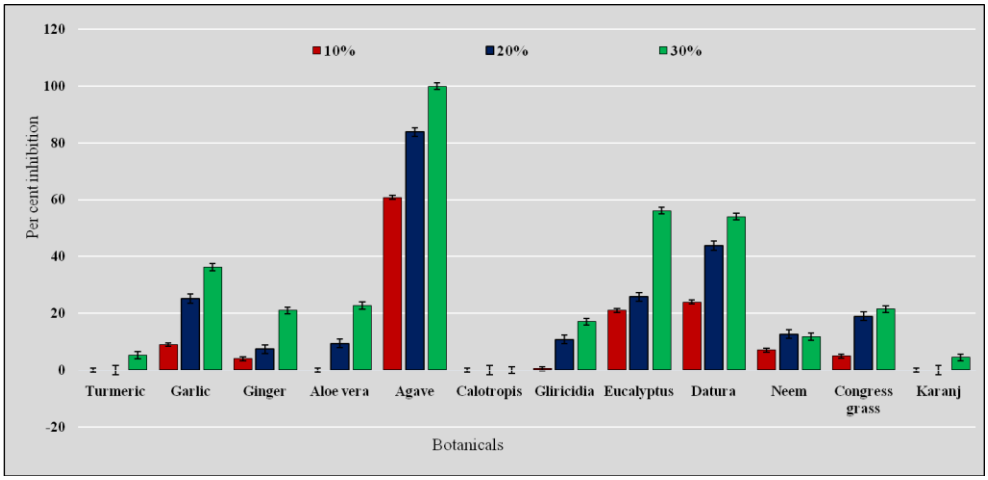
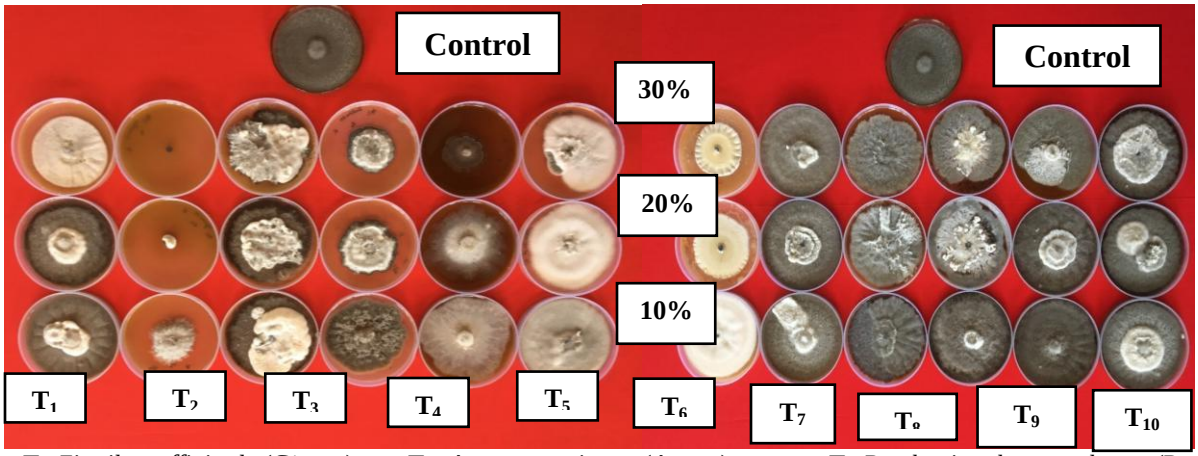


Plate.1 *In vitro* efficacy of different botanicals against *B. setariae*



T_1-*Zingiber officinale* (Ginger) T_2- *Agave americana* (Agave) T_3-*Parthenium hysterophorus* (Parthenium)
T_4- *Datura metel*(Datura) T_5-*Eucalyptus globulus* (Eucalyptus) T_6- *Aloe vera*
T_7- *Allium sativum* (Garlic) T_8-*Calotropis gigantea* (Calotropis) T_9-*Azadirachta indica* (Neem)
T_{10}-*Gliricidiasepium*(Gliricidia) T_{11}-*Curcuma longa*(Turmeric) T_{12}-*Pongamia pinnata* (Pongamia)

In conclusion among the 12 botanicals, significant mean highest percent inhibition of mycelial growth was noticed in agave (81.52 %) whereas no inhibition was observed in calotropis. Agave showed complete (100 %) inhibition at 30 per cent concentration. Sporulation was also not observed in Agave treatment where abundant sporulation in Calotropis treatment. The present study revealed that agave (*Agave americana*) as one of the promising botanicals against emerging leaf blight disease of browntop millet under *in vitro* conditions. However, efficacy of agave needs to be evaluated under field conditions in disease hot spot areas.

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