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In vitro evaluation of plant extracts against *Pyricularia oryzae* (Cav.) causing Rice Blast disease

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Invitro studies of plant extracts against rice blast disease caused by *Pyricularia oryzae* (Cav.). The plant species evaluated were *Azadirachta indica* (Juss.)*, Eucalyptus globulus* (L.)*, Jatropha curcas, Ocimum sanctum* (L.)*, Tegetes erecta, Allium cepa and Allium sativum.* Antifungal activity was tested at concentration of 5, 10 and 15% of plant extracts, using the poisoned food technique. Among the extracts of seven botanicals, highest per cent inhibition of mycelial growth of fungus was recorded in garlic clove extract (89.73, 98.27 and 98.57%) at all the concentration tested with mean of 95.52 per cent followed by onion bulb extract with the inhibition of 89.10, 93.13 and 97.40 per cent respectively with mean of 93.21 per cent. Minimum inhibition was observed in marigold leaf extract, with 25.37, 28.8 and 29.53 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 27.90 per cent. In general, the inhibition of radial growth of fungus increased with increase in concentration of each plant extracts.

Key words: Antifungal attributes, mycelial growth, plant extracts, *Pyricularia oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops belonging to the family Poaceae. It is a staple food crop of 60 per cent of world's population. There is a growing demand for rice due to increment in human population. Mostly, it is being consumed after cooking with water. Other edible uses include rice flakes, puffed rice, rice wafers and canned rice. It is also used in starch and brewing industries. The byproducts of rice milling i.e. rice husk and bran are used as cattle and poultry feed and for oil extraction. Rice straw is good cattle feed and also used in making hats, mats and ropes. Rice contributes nutritionally significant amounts of thiamine, riboflavin, niacin, carbohydrates and zinc to the diet, but smaller amounts of other micronutrients.

The world's estimated rice production is 719.7 m.t. during 2015 (Anon., 2015). India is the largest rice growing country accounting for about one third of the world acreage under the crop. In India, rice is grown in an area of 43.9 m.ha. with an annual production of 106.5 m.t. and the average productivity of 2424 kg ha^{-1} (Anon., 2015). Rice is grown throughout India in all the states. The major rice growing states of India are West Bengal, Uttar Pradesh, Bihar, Madhya Pradesh, Odisha, Andhra Pradesh, Karnataka and Chhattisgarh.

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia oryzae* Cavara (teleomorph = *Magnaporthe grisea* (Hebert) Barr Comb nov.). Losses due to the blast disease may range up to 90 per cent depending upon the component of the plant infected. *M. grisea* infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality.

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The pathogen may infect all the above ground parts of a rice plant at different growth stages *viz.,* leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped.

MATERIALS AND METHODS

Fresh sample of each test plant (Table 1) were collected and washed first in tap water and then in distilled water. 100 g of fresh sample was crushed in a surface sterilized Pestle and Mortar by adding 100 ml sterile distilled water (1:1 w/v). The extract was used as stock for the study.

To study the anti-fungal activity of plant extracts, the poison food technique was followed. To prepare 5, 10 and 15 per cent concentrations of plant extract, five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten PDA medium respectively. The medium was thoroughly shaken for uniform mixing of the extract. Twenty ml of molten media was poured into 90 mm sterilized petriplates. Each plate was inoculated with 5 mm mycelia disc taken from the periphery of seven days old *P. oryzae* culture and incubated at 28+1^{0}C till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the centre of the petriplate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration.

Three replications were maintained in each treatment. Suitable control plates were maintained where in culture discs were incubated into the centre of potato dextrose agar plates without plant extracts. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and per cent inhibition of mycelial growth over control was calculated by using the formula :

$$I = \frac{(C-T)}{C} x100$$

where, I = Per cent inhibition, C= Growth of the fungus in control, T= Growth of the fungus in treatment.

RESULTS AND DISCUSSION

Efficacy of seven botanicals *viz.,* Neem leaf extract, Eucalyptus leaf extract, Jatropha leaf extract, Tulsi leaf extract, Garlic clove extract, Onion bulb and Marigold leaf extract (Table 1) were tested at three different concentrations (5, 10 and 15%) each by following poisoned food technique as explained in 'Material and Methods'. The per cent inhibition over control was worked out based on the test fungal growth in control plate. The results thus obtained are presented in Table 2 and depicted in Fig. 1 and 2.

Among the extracts of seven botanicals, highest per cent inhibition of mycelial growth was recorded in garlic clove extract (89.73, 98.27 and 98.57%) at all the concentration tested (5, 10 and 15%) with mean of 95.52 per cent followed by onion bulb extract with the inhibition of 89.10, 93.13 and 97.40 per cent at 5, 10 and 15 per cent concentration respectively with mean of 93.21 per cent. In eucalyptus leaf extract 28.10, 31.80 and 42.80 per cent inhibition at 5, 10 and 15 per cent concentrations with mean 34.23 per cent was recorded respectively. In tulsi leaf extract 29.13, 32.83 and 38.47 per cent inhibition with mean 33.44 per cent was recorded at 5, 10 and 15 per cent concentrations respectively. In neem leaf extract 27.63, 30.50 and 41.53 per cent inhibition with mean 33.22 per cent was recorded at 5, 10 and 15 per cent concentrations respectively; 25.40, 32.50 and 39.20 per cent inhibition was observed in jatropha leaf extract at 5, 10 and 15 per cent concentration with mean 32.34 per cent respectively. Minimum inhibition was observed in marigold leaf extract, with 25.37, 28.80 and 29.53 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 27.90 per cent.

Table 1 : List of plants used for *in vitro* evaluation against Rice Blast

Botanical Name	Common name	Plant parts (Extract from)
*Azadirachta indica (*Juss.)	Neem	Leaf
Eucalyptus globulus (L.)	Eucalyptus	Leaf
Jatropha curcas	Jatropha	Leaf
Ocimum sanctum (L.)	Tulsi	Leaf
Allium sativum	Garlic	Clove
Allium cepa	Onion	Bulb
Tegetes erecta	Marigold	Leaf

Disease management through botanicals is very important aspects to minimize the cost of cultivation. Among extracts of seven botanicals, garlic bulb

Table 2 : *In vitro* evaluation of plant extracts against Rice Blast fungus *Pyricularia oryzae*

Treatments	Extracts of Botanicals	Mean per cent mycelial inhibition			
		Concentration of botanicals (%)			
		5	10	15	Mean (%)
T1	Neem leaf	27.63	30.50	41.53	33.22
T2	Eucalyptus leaf	28.10	31.80	42.80	34.23
T3	Jatropha leaf	25.40	32.50	39.20	32.34
T4	Tulsi leaf	29.13	32.83	38.47	33.44
T5	Garlic bulb	89.73	98.27	98.57	95.52
T6	Onion bulb	89.10	93.13	97.40	93.21
T7	Marigold leaf	25.37	28.80	29.53	27.90
		Botanicals	Concentrations	B X C	
	SEm±	0.42	0.28	0.73	
	C.D at 1%	1.62	1.06	2.80	
	C.V %	2.54			

extract recorded maximum mycelia inhibition of (89.73, 98.27 and 98.57%) at all the concentration (5, 10 and 15%) tested with mean of 95.52% followed by onion bulb extract with the inhibition of 89.10, 93.13 and 97.40% at 5, 10 and 15 per cent concentration respectively with mean of 93.21%.

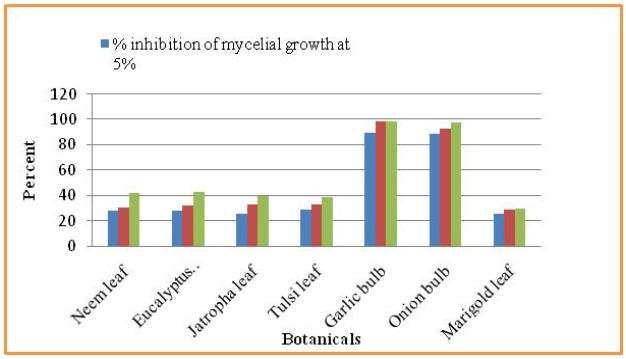

Fig. 1 : Effect of botanicals on the mycelia growth of *Pyricularia oryzae*

Minimum inhibition was observed in Marigold leaf extract, with 25.37, 28.8 and 29.53 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 27.90 per cent. Sireesha.O. and Venkateswarlu (2013) recorded that the *Allium sativum* L. was found toxic to *P. oryzae*. Amadioha (2000) and Pandey (2015) observed that neem extract (*Azadirachta indica*) caused the maximum inhibition of mycelial growth of *P. oryzae*.

However, in the present study garlic bulb extract showed maximum mycelial inhibition which is attributed to presence of phytochemicals and alkaloids which have inhibitory effect on *Pyricularia oryzae.*

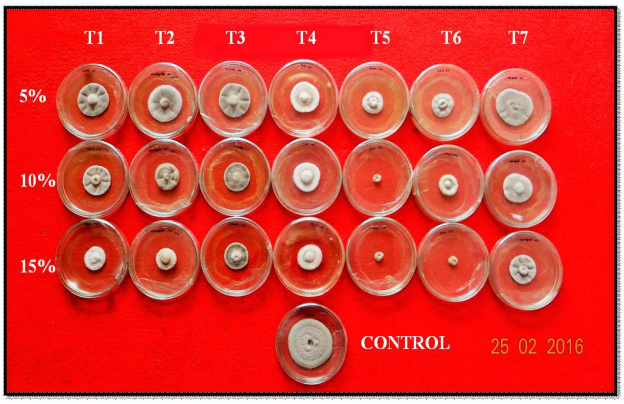

Fig. 2 : Effect of botanicals on the mycelia growth of *Pyricularia oryzae.* T1: Neem leaf extract, T2: Eucalyptus leaf extract ,T3: Jathropa leaf extract T4: Tulsi leaf extract T5: Garlic clove extract T6: Onion bulb extract T7: Marigold leaf extract.

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