

## Efficacy of Aqueous Leaf Extract of Medicinal Plants against Blast and Brown Spot Disease of Rice

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### ABSTRACT

#### Keywords

Phytochemicals, Rice, Leaf blast, Brown spot, Leaf extracts.

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Aqueous extract of leaves of ten medicinal plants viz. *Calotropis gigantea*, *Adhatoda vasica*, *Achyranthus aspera*, *Daucuscarota*, *Calendula officinalis*, *Aloe vera*, *Momordica monodelpha*, *Moringa pterygosperma*, *Eclipta alba* and *Ricinus communis* was tested in-vitro at 0.20% and 0.50% concentration using poisoned food technique for antimicrobial activity against mycelial growth of *Pyricularia oryzae* (Syn. *Magnaporthe oryzae*) causing leaf blast and *Helminthosporium oryzae* (syn. *Bipolaris oryzae*) causing brown spot diseases in rice. The leaf extracts of all the ten medicinal plants were found significantly effective in reducing mycelial growth of the pathogens. The result reveals that the aqueous leaf extract of all the medicinal plants at the concentration of 0.50% was found most effective in minimizing the mycelial growth of both the pathogens.

### Introduction

Phytochemicals are chemical compounds that occur naturally in the plant kingdom. Some are responsible for the organoleptic properties of the natural sources in which they are present. The term is generally used to refer to those chemicals that may have biological significance, for example carotenoids, flavonoids, coumarins or chromones, but not all are established as essential nutrients. There may be as many as 4,000 different phytochemicals having potential activity against several diseases such as cancer and metabolic or degenerative diseases. Before the sixteenth century, most of the mainstream

medical systems were based on the idea that one should work with nature and that the body's own healing capacity could be strengthened and complimented by the right herbs (Active constituents of herbs, 2015; Venkateshwer Rao). Plant chemistry includes the miracle of photosynthesis, plant respiration, structure, growth, development, and reproduction. Much of the chemical basis of life is common to both plants and animals. From a holistic perspective the whole of the plant must be respected as an integrated biologically evolved unit that is beyond the analytical comprehension of science.

Physiologically active plant constituents are usually classified by their chemical structure rather than specific actions and include Alkaloids, Anthocyanins, Anthraquinones, Cardiac Glycosides, Coumarins, Cyanogenic Glycosides, Flavonoids, Glucosilates, Phenols, Saponins and Tannins.

Rice is one of the staple food crops and grown in about 11% of the total cultivated land area globally and serves as the major food for nearly half of world's population (Rout and Tiwari, 2012a). Many biotic stresses hamper rice production and specifically, fungal diseases cause huge economic losses (Kumar *et al.*, 2009). Among these the seed-borne diseases mainly leaf blast caused by *Magnaporthe oryzae* represents a serious threat to global rice production (Xue *et al.*, 2014), and brown spot caused by *Bipolaris oryzae* reduces rice yield and shows substantial global impact (Nejad *et al.*, 2014) to the crop. The losses due to these diseases in developing countries are estimated to be 60-80% higher than in industrialized countries and conservatively estimated that they cause losses in the order of 50 million ton of food annually (Vishunavat, 2012). The uses of fungicides to control the disease have been effective. However, the excessive use of these synthetic chemicals has caused environmental contamination and toxicity to living organisms. It has also increased costs to growers (West *et al.*, 2003), and their repeated use over decades has disrupted natural biological systems, and sometimes resulted in development of fungal resistance along with producing undesirable effects on non-target organisms, and fostered environmental and human health concerns (Yoon *et al.*, 2013). Therefore merits attention of all concerned to look into the potential of integrating in the management of economically important diseases, the products prepared from green plants should be preferred as they are environmentally non-

pollutive and non-hazardous in preparation and use (Rout and Tiwari, 2012b). The secondary components of some plants contain medicinally active fractions of plant tissue that are toxic to pathogens (Gurjar *et al.*, 2012) and thus can be utilized in plant disease management programme. Earlier several workers have successfully reported the effective control of rice diseases using plant extracts (Sena *et al.*, 2013; Harlapur *et al.*, 2007; Dutta *et al.*, 2004; Madhusudan, 2004; Raji, 2004). Therefore the present research aimed to study the efficacy of aqueous plant extracts of ten medicinal plants in managing the leaf blast and brown spot pathogens in rice.

### **Materials and Methods**

In the present investigation infected leaves were collected showing the typical lesions of blast and brown spot in the field for single spore isolation. The diseased leaves were cutted into small pieces using sterilized blades. These pieces were surface sterilized by dipping in mercuric chloride solution (1:1000) for one minute and were washed by sterilized water for several times. Potato Dextrose Agar medium (Riker and Riker, 1936) was taken as basal medium for in vitro studies. The pieces were inoculated in sterilized petri discs containing Potato Dextrose Agar medium amended with streptomycin sulphate under aseptic condition. The pure fungal culture was maintained for further study.

The fresh leaves of ten medicinal plant species viz. *Calotropis gigantea*, *Adhatoda vasica*, *Achyranthus aspera*, *Daucas carota*, *Calendula officinalis*, *Aloe vera*, *Momordica monodelpha*, *Moringa pterygosperma*, *Eclipta alba* and *Ricinus communis* were collected. These leaves were washed with tap water and sterilized water. They were air dried and their powder sample was prepared. The cold water

extract were obtained by adding 20 (0.20%) and 50 g (0.50%) of the powder of each plant leaf to 100 ml of sterile distilled water. The extracts were filtered by cheese cloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Whatman's No. 1 filter paper.

The leaf extracts of each plant species were taken at concentration of 0.50% and 0.20% and amended with poisoned food Potato Dextrose Agar media and were poured in sterilized petri disc. 5 mm mycelium disc of the test fungus were cutted from the margins of the old test culture and were placed centrally in each of the petri discs. The inoculated plates were incubated at room temperature and the colony diameter was recorded at an interval of 24 hours. The results obtained have been presented in Table 1.

## Results and Discussion

Antimicrobial activity of ten medicinal plants leaf extracts was assayed and results on effect of leaf extracts on mycelial growth of *Magnaporthe oryzae* (*Pyricularia oryzae*) and *Bipolaris oryzae* (*Helminthosporium oryzae*) have been presented in Table 1. The data revealed that the plant extracts were found significant ( $P < 0.05$ ) in suppression of mycelia growth at higher concentration over untreated check in both the fungal pathogens. However, the suppression rate decreases with the passage of time.

The result reveals that the leaf extract of *Calotropis gigantea*, *Achyranthus aspera*, *Calendula officinalis*, *Moringa pterygosperma*, *Eclipta alba* and *Ricinus communis* at the concentration of 0.50% was found most effective in minimizing radial growth of the *Magnaporthe oryzae* (*Pyricularia oryzae*) (28.36mm, 29.55mm, 29.50mm, 28.35mm, 29.25mm and 29.15mm

respectively) after 96 hrs which gradually increased to 37.25, 38.95, 52.15, 37.015, 37.35, 37.25 and 37.81mm respectively and 38.00mm after 196 hrs of incubation, respectively. The leaf extract of *Adhatoda vasica*, and *Momordica monadelpha* showed a more or less similar pattern of growth suppression in both the fungi. Similarly, the leaf extract of *Daucus carota* and *Aloe vera* exhibited more or less same response of growth suppression in both fungi.

The suppression of mycelial growth of leaf brown spot pathogen *Bipolaris oryzae* (*Helminthosporium oryzae*) follows similar pattern in all the tested plant extracts (Table 1). The rate of suppression of radial growth of *B. oryzae* was comparably more than *M. oryzae* (*Pyricularia oryzae*).

The highest concentration of aqueous leaf extracts were more pronounced compare to low concentration in reducing the radial growth of the fungus as observed in case of blast pathogen. Among the treatments leaf extract *C. gigantea*, *C. officinalis*, and *E. alba* at concentration of 0.50% was found highly effective in reducing the radial growth of the fungus (27.15 mm, 30.17mm, 30.15mm respectively) after 96 hrs. of incubation which gradually inclined to 37.25, 37.15 and 37.205mm 196 hrs of incubation, respectively. Similarly, the leaf extract of *A. vera* and *D. carota* showed a more or less similar suppression in radial growth in the range 40.15mm and 39.25mm respectively after 192 hours of incubation.

Leaf extract of *Adhatoda vasica*, and *Momordica monadelpha* exhibited similar growth suppression response to *B. oryzae* that caused the reduction in radial growth to 38.95mm and 37.35mm respectively after 192hrs of incubation. The leaf extract of *A. aspera* and *E. alba* showed a more or less similar growth inhibition pattern in *B. oryzae*.

**Table.1** Effect of aqueous leaf extract of medicinal plants on mycelia growth of *M. oryzae* (*Pyricularia oryzae*) and *Bipolaris oryzae* (*Helminthosporium oryzae*)

Leaf extract	Concentration %	Periods of incubation in hrs					
		96		120		192	
		<i>P.oryzae</i>	<i>H. oryzae</i>	<i>P.oryzae</i>	<i>H. oryzae</i>	<i>P.oryzae</i>	<i>H. oryzae</i>
<i>C. gigantia</i>	0.50	28.36	27.15	37.07	34.95	38.20	37.25
	0.20	34.95	33.94	47.25	45.15	52.15	51.65
<i>A.vasica</i>	0.50	30.25	31.25	38.10	38.26	41.25	38.95
	0.20	36.55	38.25	49.16	49.35	55.35	55.20
<i>A.aspera</i>	0.50	29.55	30.15	37.61	35.35	38.65	37.85
	0.20	38.45	37.35	37.25	38.24	46.34	52.15
<i>D.carota</i>	0.50	32.25	29.60	38.70	36.35	39.45	39.25
	0.20	40.15	38.25	49.36	47.65	54.25	54.15
<i>C.offic.</i>	0.50	29.50	30.17	37.65	35.41	38.15	37.15
	0.20	38.50	37.41	31.35	38.25	47.10	52.21
<i>A.vera</i>	0.50	32.35	29.65	38.72	37.37	40.10	40.15
	0.20	40.15	38.30	50.12	47.15	55.12	55.10
<i>M.mona.</i>	0.50	30.15	31.10	37.55	35.45	38.25	37.35
	0.20	38.25	34.45	31.42	38.30	47.25	52.25
<i>M.pteryg.</i>	0.50	28.35	27.20	37.12	34.15	38.21	37.35
	0.20	35.10	34.25	47.30	45.12	52.10	51.60
<i>E.alba</i>	0.50	29.25	30.15	37.15	35.25	38.27	37.25
	0.20	38.35	37.45	31.40	38.35	47.15	52.25
<i>R.comm.</i>	0.50	29.15	30.25	37.15	35.36	38.10	37.80
	0.20	38.36	37.41	37.15	38.25	46.34	52.26
Control		47.35	48.50	60.00	59.25	67.75	67.25
SEm±		0.23	0.11	0.24	0.03	0.15	0.17
CD (p<0.05)		0.85	0.41	0.83	0.08	0.65	0.63

The mycelial growth of *M. oryzae* decreases with the increasing concentration of all plant extracts tested. The findings are similar to Amadioha (2000) who reported that the cold water extract of neem compared favourably with Cardendazim at 0.1% a.i.in controlling the rice blast in vivo. The observations supports the findings of Sireesha and Venkateswarlu (2013) who found the efficacy of plant parts extract of Neem seed kernel, Neem oil, *Pongamia* spp. The present findings also gain support from the work of Sandeep (2015) who observed the similar pattern of growth suppression in blest and brown spot pathogens using leaf extract of

Neem (*Azadirachta indica*), Emblica (*Emblica officinalis*), Karanj (*Pongamia glabra*) and Babool (*Acacia nilotica*). Extracts Panchagavya and *Asafoetida* spp. extract in descending order against rice blast fungus. Hajano *et al.*, (2012) studied efficacy of the extracts of garlic, neem and calatropisby food poisoning method, and observed that only higher dose of garlic completely inhibited the mycelial growth of *M. oryzae*. Gohel and Chauhan (2015) reported that Neem leaf extract were found effective but comparably less significant than standard fungicides and bio-agent in minimizing leaf blast intensity in rice. Hubert

*et al.*, (2015) observed that extracts from *C.arabica*, *N. tabacum*, *A. vera*, *A. indica*, were found significant to manage rice blast disease *in-vitro* and *in-vivo*. The research is also in accordance with Khoa *et al.*, (2011) who observed that foliar spray of aqueous extracts of herbal plants have been found effective in reducing rice blast severity. Results on suppression of *Bipolaris oryzae* under various concentration of plant extracts were significant. The results are similar with the findings of Al-Hazmi (2013) who reported that Neem leaf extract were mostly affective in growth retardation of the *Helminthosporium sp.* fungi when applied at the highest concentration (1:1, v:v).

The findings of the research are also in close conformity with Ahmed *et al.*, (2002) who found that the plant extracts of neem and garlic were the most effective against *Bipolaris oryzae* at 1:1 dilution. Similarly, Devi and Chhetry (2013) reported that the plants extracts of *Acorus calamus*, *Centella asiatica*, *Artemisia vulgaris* and *Azadirachta indica* shows reduction in the mycelial growth of *D. oryzae* at different concentrations.

The findings are also in accordance with Farooq *et al.*, (2015) observed that seed treatment with Neem and Almandia leaf extract shows effective reduction in brown spot incidence but comparatively less significant than standard fungicides and hot water treatment at flowering, milking and maturity stage of seed growth *in vivo*. Harish *et al.*, (2008) observed that under glasshouse conditions, post-infectious spraying of rice plants with neem cake extract and *N. oleander* leaf extract were significantly effective however comparably less than *T. viride* in reducing the incidence of brown spot of rice. Thus it can be concluded that plant extracts were found most effective in reducing mycelial growth of leaf blast and brown spot

fungi and therefore used for the management of the diseases in rice. Moreover, they are nature friendly, reduces chemical hazards and are economical and feasible thus easily accessible to the growers.

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