

Evaluation of Antifungal Properties of Certain Plants against *Drechslera Oryzae* Causing Brown Leaf Spot of Rice in Manipur Valley

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Abstract- Antifungal activity of aqueous extracts of locally available plants which are known for their medicinal values were tested *in vitro* against *Drechslera oryzae*, the causal organism of brown leaf spot of rice. The plants extracts were tested against the mycelial growth of *D. oryzae* at different concentrations of 5%, 10%, 15% and 20% using poisoned food technique. Among the plants extracts, *Acorus calamus* extract at 20% concentration alone showed 80.0% inhibition of mycelial growth whereas the other tested plants showed less inhibitory effect. In field trial, aqueous extract of *Acorus calamus* showed maximum percentage of disease control and reduced the disease incidence by 45.29% as compared with control plot.

Index Terms- Plant extracts, antifungal properties, *Drechslera oryzae*, brown leaf spot, rice.

I. INTRODUCTION

Brown leaf spot of rice caused by *Drechslera oryzae* (Breda de Haan) Subram. and Jain. is one of the major fungal disease of rice which occurs in almost all the rice growing areas [7]. The disease is of great importance in several countries and has been reported to cause considerable losses. It occurs, occasionally as an epidemic disease every year in mild or severe form. Several chemicals have been reported to control the disease [2]. The spraying of the fungicides have been reported to reduce the disease severity effectively [1]. However, the indiscriminate use of chemical fungicides to control the disease is not only hazardous to living beings but also adversely affects the environment [5]. This results to find out an alternative approaches which are economically feasible and eco-friendly like botanical pesticides or biological agents to control the disease [5]. The present investigation was therefore undertaken to test the antifungal activity of certain plant extracts against *D. oryzae*, the causal organism of brown leaf spot of rice.

II. MATERIALS AND METHODS

Ten locally available plants viz. *Acorus calamus*, *Allium hookeri*, *Artemisia vulgaris*, *Azadirachta indica*, *Centella asiatica*, *Eupatorium birmanicum*, *Lantana camara*, *Phlogocanthus thyrsoiflorus*, *Zanthoxylum alatum* and *Zingiber officinalis*, which are known for their medicinal values were selected to test their antifungal properties against *D. oryzae*. Aqueous extract of plant parts such as leaves, rhizomes were

prepared by using the standard method as given by Gerard Ezhilan et. al. [3]. The fresh plants parts were taken and washed with running tap water followed by sterile distilled water. It was then ground with sterile distilled water at the rate of 1 ml per gram of plant tissue (1:1 V/W) with mortar and pestle and filtered through double layered white muslin cloth. The filtrate so obtained formed the standard plant extract solution i.e.100 percent. The plant extract thus prepared were tested *in vitro* against the mycelial growth of *D. oryzae* using poisoned food technique. Potato Dextrose Agar (PDA) with 2% agar was used as culture medium. Varying amounts of the plants extract were added to the sterilized molten PDA to get a final concentration of 5%, 10%, 15% and 20%. The poisoned PDA was poured into sterile petriplates and allowed to set. Four replications were maintained for each concentration. Then the petriplates were inoculated with mycelium disc (4mm diameter) of the test fungus taken from the margin of five days old pure culture. The mycelium disc inoculated on PDA with no plant extracts but with only sterile water acts as control plate. The whole set up was incubated in inverted position at $26 \pm 1^\circ\text{C}$ in BOD incubator for 7 days. The radial growth of *D. oryzae* was measured after seven days of incubation and the percent inhibition of the fungal growth was calculated by using the formula given by Vincent [4].

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

where,

I = percent inhibition.

C = growth in control.

T = growth in treatment.

In vivo Tests:

In vivo tests were carried out at Langthabal, a small village located at the outskirts of the Imphal city for two consecutive growing years (2010 and 2011). The experiment was conducted in a sick field which shows disease incidence of brown leaf spot of rice during the last three years of cropping seasons. A susceptible rice variety (RCM10) was sown in a randomized block design in $3 \times 3 \text{ m}^2$ plots with three replications for each treatment. The 25 days old seedlings were transplanted @ 2-3 plants per hill at a spacing of 20 cm row to row and 15 cm plant to plant. The effective plant extract that showed high percentage of inhibition at 20% concentration in *in vitro* test viz. *Acorus calamus*, *Artemisia vulgaris*, *Centella asiatica*, *Lantana camara*, *Phlogocanthus thyrsoiflorus* were further determined to see their

effect in *in vivo* conditions. The plant extracts with 20% concentration were sprayed at weekly interval starting from tillering stage till flowering stage. In control plot only distilled water was sprayed. Data on disease incidence were recorded at weekly interval and calculated as per the formula given below:

Percent Disease Incidence,

$$(DI\%) = \frac{\text{Total no. of plants infected by a particular disease}}{\text{Total no. of plants assessed}} \times 100$$

The data so obtained was statistically analysed to ascertain the effectiveness of the treatments.

III. RESULTS

The result presented in Table 1 showed that the plant extracts were significantly effective against the mycelial growth

of *D. oryzae* as compared with the control plates. Among the plant extracts, aqueous extract of *Acorus calamus* (80.0%) at 20% concentration showed highest mycelial growth inhibition followed by *Artemisia vulgaris* (40.0%) and *Centella asiatica* (40.0%). Again, aqueous extract of *Eupatorium birmanicum*, *Lantana camara*, *Phlogocanthus thyrsoiflorus*, *Zanthoxylum alatum* and *Zingiber officinalis* showed 30% or more mycelial growth inhibition while *Allium hookeri* and *Azadirachta indica* showed less than 30% inhibition of mycelial growth.

The data presented in Table 2 showed that in *in vivo test*, the selected plants reduced the disease incidence during the two successive cropping seasons. Among the plants, maximum percent of reduction in disease incidence over control was observed in *Acorus calamus* (45.29%) followed by *Artemisia vulgaris* (26.44%), *Centella asiatica* (24.11%), *Lantana camara* (19.98%) and *Phlogocanthus thyrsoiflorus* (18.21%).

IV. TABLES

Table 1: Effect of plant extracts on radial growth of mycelium of *D. oryzae* at different concentrations.

Test plants	Parts used	Mycelial growth (in cm) at different conc ⁿ .				Mean	% inhibition of mycelial growth at different conc ⁿ .			
		5%	10%	15%	20%		5%	10%	15%	20%
<i>Acorus calamus</i>	Rhizome	1.8	1.6	1.3	0.9	1.4	60.0	64.4	71.1	80.0
<i>Allium hookeri</i>	Leave	4.4	4.3	3.8	3.4	4.0	2.2	4.4	15.6	24.4
<i>Artemisia vulgaris</i>	Leave	3.3	3.2	2.8	2.7	3.0	26.7	28.9	37.8	40.0
<i>Azadirachta indica</i>	Leave	3.5	3.4	3.4	3.3	3.4	22.2	24.4	24.4	26.7
<i>Centella asiatica</i>	Leave	3.2	3.0	2.8	2.7	2.9	28.9	33.3	37.8	40.0
<i>Eupatorium birmanicum</i>	Leave	3.7	3.4	3.3	3.1	3.4	17.8	24.4	26.7	31.1
<i>Lantana camara</i>	Leave	3.6	3.5	3.4	2.8	3.3	20.0	22.2	24.4	37.8
<i>Phlogocanthus thyrsoiflorus</i>	Leave	3.7	3.5	3.2	2.9	3.3	17.8	22.2	28.9	38.6
<i>Zanthoxylum alatum</i>	Leave	3.7	3.5	3.3	3.0	3.4	17.8	22.2	26.7	33.3
<i>Zingiber officinalis</i>	Rhizome	3.5	3.3	3.1	3.1	3.3	22.2	26.7	31.1	31.1
Control		4.5	4.5	4.5	4.5	4.5				
Mean		3.5	3.4	3.2	3.0					

CD (P = 0.05) Between plant extracts: 0.04
Between concentrations: 0.06

* Mean of four replications.

Table 2: Effect of plant extracts on the incidence of brown spot of rice in *in vivo tests*.

Test plants	D.I. %		Pooled mean	% Disease control
	2010	2011		
<i>Acorus calamus</i>	16.17 (23.71)	14.83 (22.65)	15.5	45.29%
<i>Artemisia vulgaris</i>	20.00 (26.57)	21.67 (27.74)	20.84	26.44%

<i>Centella asiatica</i>	20.17 (26.69)	22.83(28.54)	21.50	24.11%
<i>Lantana camara</i>	21.50 (27.62)	23.83(29.22)	22.67	19.98%
<i>Phlogocanthus thyrsiflorus</i>	22.33(28.20)	24.00(29.33)	23.17	18.21%
Control	27.33(31.52)	29.33(32.79)	28.33	

CD (P = 0.05) Between plant extracts: 12.9

* Mean of three replications.

* Values in parentheses are arcsine transformed values ($x = \sin^{-1} \sqrt{\hat{p}}$).

V. DISCUSSION

The application of chemical fungicides to control the disease has many drawbacks and is hazardous to the environment. Hence, to find out an alternative, locally available plants which possess medicinal properties were investigated. The use of plants extracts as antifungal substance against many fungal diseases has been reported by many workers. Bisht and Khulbe [2] reported maximum inhibition of mycelial growth of *D. oryzae* by *Juglans regia* (64.11%) in *in vitro* experiment. In the present study, aqueous extract of *Acorus calamus* alone showed 80.0% mycelial growth inhibition in *in vitro* experiment and 45.29% reduction of DI% in *in vivo test*. The fungicidal activity of *Acorus calamus* extract against *D. oryzae* might be due to the presence of antifungal compounds like α -asarone and β -asarone. Thingujam and Chhetry [6] also reported the effectiveness of the Tulsi leaf extract for the management of brown leaf spot disease of upland rice. The antifungal and anti microbial effect of *Acorus calamus* has already been reported by many workers.

VI. CONCLUSION

From the present study, it is concluded that locally available plant like *Acorus calamus* can be used to control the brown spot disease of rice in Manipur agro climatic condition without any harmful effect to the environment. Hence, more and more locally available plants need to be identified as an alternative means in

place of chemical fungicides for safe and effective control of plant diseases.

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