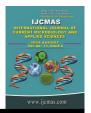


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Evaluation of Botanical Extracts against *Pyricularia oryzae***, A Causal Agent of Blast of Paddy**

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ABSTRACT

Rice is one of staple food of India and is only second most consumed cereal crop around the world. Blast of paddy is one of the economically important diseases caused by the phytopathogen *Pyricularia oryzae*. The present investigation carried out to evaluate the potentiality of extract of locally available botanicals namely, *Catharanthus roseus*, *Duranta repens*, *Solanum nigrum*, *Crotalaria trichotoma*, *Oxalis latifolia*, *Leucas aspera* and *Parthenium hysterophorus* against *Pyricularia oryzae in-vitro*. The experiment was carried out using 5% aqueous extracts of botanicals by poisoned food technique. Out of the seven tested aqueous botanical extracts *D. repens* has shown highly significant results with 100% growth inhibition of pathogen which is on par with fungicide mancozeb, followed by *C. roseus* (31.36%), *S. nigrum* (30.76%), *C. trichotoma* (25.44%), *O. latifolia* (24.14%) and least inhibition was observed in *L. aspera* (15.38%) and *P. hysterophorus* (10.05%).

Introduction

Oryza sativa belongs to the family Gramineae (Poaceae), is one of the major food crops of the world and forms the staple diet of half of the world's population. Asia is the leader in rice production accounting for about 90% of the world's production. Globally, India stands first in the area covered in cultivation and second in production after China (FAOSTAT, 2021). With the current status of the production of rice the demand projected to be increases 30 % by 2050 (Mohanty and Chavez, 2010; FAO-UN, 2015). Rice is prone to several diseases which in turn reduces yield of the crop and affects the economy of farmers specially in developing countries. The crop is susceptible to many viral, bacterial and fungal diseases.

Blast of paddy caused by the phytopathogen *Pyricularia* oryzae, is an economically important and widely distributed phytopathogen throughout the world and it occurs in mild to severe form in different parts of India.

Rice blast is one of the most catastrophic diseases which occur on all above-ground parts of the rice plant. Rice blast caused by *Pyricularia oryzae* was firstly described by Cavara in 1891, which attack the leaf sheath, rachis, the joints of the culm, and even the glumes. Infection to the culms causes greater damage than leaf infection. Severe leaf infection occurring in the post-transplant stage may, however, lead to total destruction of the foliage (Ou, 1985). Natural plant products and their analogous are an important source of new agricultural

chemicals which are gaining more importance in the research area and are used as organic methods in control of plant diseases.

There are many reports on use of botanicals in managing plant diseases both in vitro and in vivo. Botanicals like Azardirachta indica, Solanum torvum, Hyssopus officinalis, Crotalaria trichotoma, Citrus aurantifolia, Asafoetida sp. and Pongamia sp. Melia azadirachta, sativum, Allium cepa, Curcuma longa, Caryophylius aromaticus are known to be effective in controlling phytopathogens like Pyricularia oryzae, Alternaria alternata, Bipolarize oryzae, Curvalaria lunata, Fusarium oxysporum, Fusarium moniliformae, Fusarium solani, Xanthomonas oryzae, Pyrenophora avenae, Alternaria solani, Rhizoctonia solani, Alternaria triticina (Amadioha, 2000; Letessier et al., 2001; Shervin, 2012; Pal and Kumar, 2013; Ravikumar and Rajkumar, 2013; Chakrapani et al., 2020 and Puja et al., 2021).

The present investigation focused on evaluating the efficacy of locally available botanicals in managing blast of paddy *in vitro*, which is an effort to demonstrate ecofriendly approach in plant disease management by using locally available resources and also cost effective compared to synthetic fungicide.

Materials and Methods

Collection and Preparation of plant extracts

Botanicals were selected on the basis of three criteria i.e., (i) Presence of antimicrobial toxins. (ii) Presence of preinfection defense bio-chemicals which provide resistance to plants against pathogens. (iii) Availability of plants materials throughout the year with very little or no commercial value as compared to their chemical alternatives.

Such seven plants with family and plant parts used are listed in Table 1. Fifteen grams of each selected seven plants was macerated with 30 mL of distilled water in mortar and pestle.

The macerated biomass was kept overnight in culture tubes for exudation of biochemicals. The biomass was filtered through muslin cloth, followed by through Whatman No. 1 filter paper. The filtered extracts were stored at 4°C as 50% stock solutions for further experiment.

Determination of efficacy of plant extracts against *Pyricularia oryzae* by poison food technique

Efficacy of plant extracts on the growth of Pyricularia oryzae in vitro was determined by poison food technique. Plant extract of 2mL from the each stock solution (50% concentration) was added to 18 mL of sterilized potato dextrose agar (PDA) medium in a conical flask to make final concentration of 5%, mixed thoroughly, and autoclaved. A 5 mm diameter disc of actively growing 6-7 days old culture of *P. oryzae* was placed in the centre of the Petri plates. The Petri plates containing medium with 0.2% mancozeb fungicide (Indofil® mancozeb 75% WP) served as positive control and plates with medium amended with 2mL distilled water served as negative control. Three replicates were maintained for each treatment. Radial growth of mycelium was measured after seven days of incubation. The results were compared with negative control. Percentage of inhibition was calculated with the following formula (Singh and Tripathi, 1999).

Percentage of inhibition (%) =
$$\frac{dc-dt}{dc} \times 100$$

Where, dc = Mycelia growth in control

dt = Mycelia growth in treatment

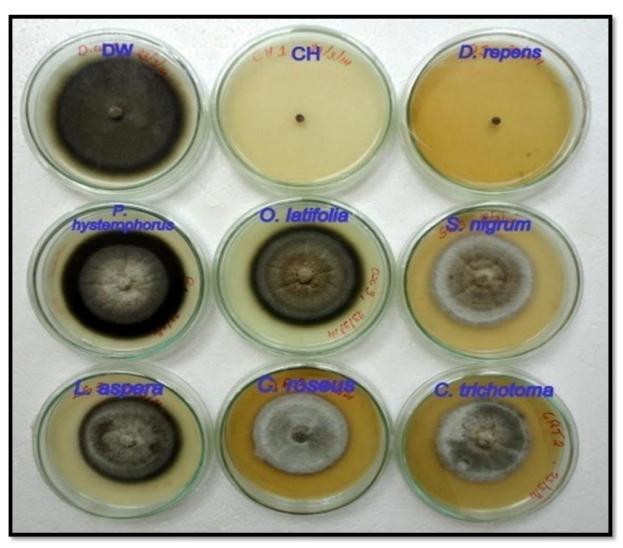
Results and Discussion

In the present investigation seven aqueous extracts of the selected botanicals (Table 1) used to assess their efficacy against phytopathogen P. oryzae. Among the seven botanicals used, D. repens showed highly significant activity with complete inhibition (100%) of the mycelial growth of the pathogen at 5% of concentration which is on par with synthetic chemical fungicide, followed by C. roseus (31.36%), S. nigrum (30.76%), C. trichotoma (25.44%), O. latifolia (24.14%), L. aspera (15.38%) and P. hysterophorus (10.05%) (Table 2 and Fig. 1 and 2) which corroborates with earlier reports that plant extracts of different families have shown inhibitory activity against many fungal pathogens (Choi et al., 2004; Sohbat et al., 2014; Puja et al., 2021; Julián et al., 2015; Madhu et al., 2014; Manasi and Ajit, 2014; Pandukur and Amienyo, 2016; Malarkodi and Manoharan, 2013; Meera and Balabaskar, 2012; Mehajabeen, 2011; Bolatito et al., 2023).

Table.1 Name of Botanicals and parts used for aqueous extraction

SL. No.	Scientific name	Family	Plant part used for extraction
1	Crotalaria trichotoma Linn.	Leguminoceae	Leaves
2	Leucas aspera	Lamiaceae	Leaves
3	Duranta repens Linn.	Verbenaceae	Leaves
4	Parthenium hysterophorus Linn.	Asteraceae	Leaves
5	Catharanthus roseus Linn.	Apocynaceae.	Leaves
6	Solanum nigrum Linn.	Solanaceae	Leaves
7	Oxalies latifolia	Oxalidaceae	Leaves

Figure.1 Inhibitory activity of Botanical aqueous extracts at 5% concentration compared to Chemical (0.2%) and Distilled water

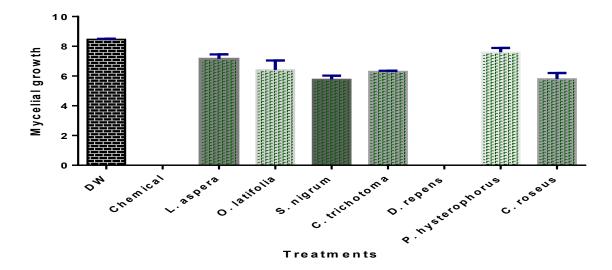


Labic-2 initionity activity of aqueous plant extract	Table.2 Inhibitor	y activity of	aqueous r	olant extract
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Sl.No.	Treatment	Treatment of aqueous extract in %	Colony diameter (in cm)	% of Mycelial inhibition
1	Distilled water	5	8.45 ± 0.033	0
2	Chemical (Mancozeb)	0.2#	$0.0 \pm 0.0 ***$	100 %
3	Leucas aspera	5	7.15 ± 0.30	15.38 %
4	Parthenium hysterophorus	5	7.6 ± 0.39	10.05 %
5	Oxalis latifolia	5	6.41 ± 0.62 *	24.14 %
6	Crotalaria trichotoma	5	6.3 ± 0.05 *	25.45 %
7	Catharanthus roseus	5	$5.8 \pm 0.45 **$	31.36 %
8	Duranta repens	5	0.0 ± 0.0 ***	100 %
9	Solanum nigrum	5	5.85 ± 0.31 *	30.76 %

#Recommended dose

Figure.2 Graphical representation of Mycelial inhibition of Pyricularia oryzae by various treatments



Control of crop diseases and managing phytopathogens which are responsible for causing diseases is one of the most economically challenging tasks to both farmers and scientists. As the diseases of the crop plants progressed, the research over such diseases and study of causal agents responsible also increased which resulted in discovery of chemical fungicide to control and manage the disease caused by phytopathogens. However, overuse of chemical fungicides eventually led to harmful effects on humans and other animals (Sanjay *et al.*, 2018; Jochen *et al.*, 2019). This forced the researchers to find alternative approaches in managing plant diseases and inclined towards natural botanicals to find the antimicrobial substances.

The Aqueous extract of *D. repens* with numerous biochemical compounds was significantly effective which is on par with fungicide. The aqueous extracts of *D. repens* with significant activity against tested pathogen *P. oryzae* proved that, even crude extracts of botanicals are capable of controlling the pathogens equivalent to purified chemical fungicides.

Hubert et al., (2015) also found that plant extracts of Coffee arabica, Nicotiana tabacum, Aloe vera and Azadirachta indica were effective in mycelial inhibition of Pyricularia grisea. The similar results were obtained with aqueous plant extracts of Azadirachta indica, Aleo vera, Nicotiana tabacum, against Magnaporthe oryzae

^{***} Data was analyzed by Tukey's post-test at P ≤ 0.05 level of significance using Graph pad prism 5 software

as reported by Falade and Asowata-Ayodele (2022). Based on the results from the present findings it is found that biological control of phytopathogens and diseases is becoming more and more evident and gaining more importance as they are not hazardous to the farmers, soil microbial flora and fauna. Thus from the present findings *D. repens* can be used in control of *P. oryzae*.

Further, *D. repens* could be developed as an alternative bio-formulation to fungicide which can be used directly by the farmers with proper guidelines and hence can reduce the dependency on fungicides reducing the contamination of soil.

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Author Contributions

M. C. Ravikumar: Investigation, formal analysis, writing—original draft. G. S. Nandeesha: Validation, methodology, writing—reviewing. Rajkumar H. Garampalli:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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