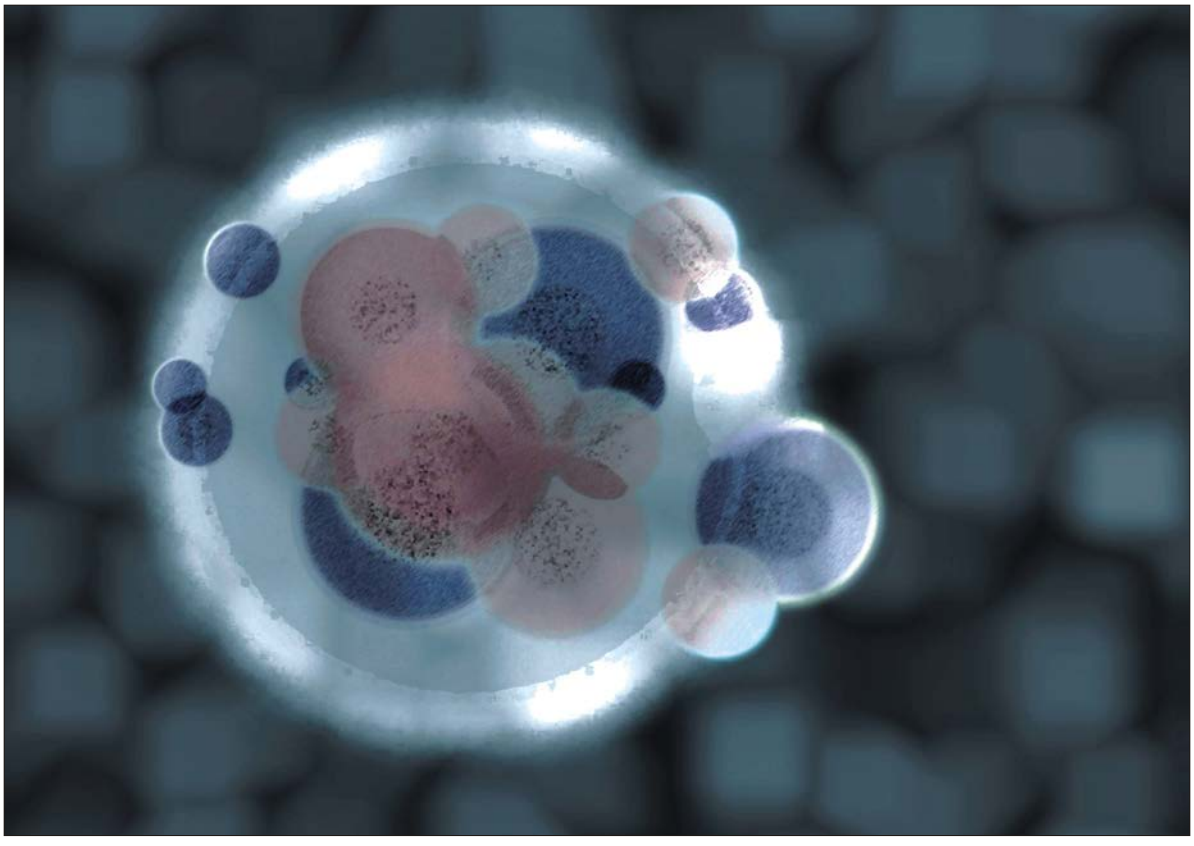

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Mycotoxic Effect of Seed Extracts Against *Helminthosporium oryzae* Breda de Hann, the Incitant of Rice Brown Spot

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Abstract: Rice brown spot caused by *Helminthosporium oryzae* Breda de Hann is a major constraint for rice production. Fifteen seed extracts were evaluated at 10% concentration under laboratory condition against *Helminthosporium oryzae*. Among them rhizome extract of turmeric (*Curcuma longa* L.), seed extracts of sundavathal (*Solanum indicum* L.) and Vedpalai (*Wrightia tinctoria* Roxb.) exerted the maximum inhibition of the mycelial growth and spore germination of *H. oryzae* proving the antifungal activity against the pathogen. Thus the plant extract with mycotoxic effect can be well exploited in future for the control of *H. oryzae* in rice.

Key words: Rice, brown spot, *Helminthosporium oryzae*, mycelial growth, spore germination

INTRODUCTION

Rice (*Oryza sativa* L.) ranks second in the world in terms of both area and production among the crops grown. Of the various diseases affecting rice crop, brown spot caused by *Helminthosporium oryzae* Breda de Hann is an important disease. The disease occurs in all rice growing areas of the world, especially under semi dry conditions[1]. Although, chemicals are available for the management of the disease, continuous use of chemicals is known to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution etc. Extracts of certain plants contain alkaloids, tannins, quinones, coumarins, phenolic compounds, phytoalexins which are known for antifungal activities[2]. A number of plant species have been reported to possess natural substances which were toxic to many fungi causing plant diseases[3,4]. Therefore, an ecofriendly approach ameliorating all these ill effects is needed. Plant extracts were therefore, used in the present study against *H. oryzae* under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of pathogen: Rice leaves of different cultivars viz., ADT 36, ADT 37, ASD 16, ASD 18, IR 50, CO 37 and TKM 9 showing the typical symptoms of brown spot caused by *Helminthosporium oryzae* Breda de Hann were collected from different rice growing areas of Madurai, Theni, Trichy, Thanjavur, Thiruvarur, Nagapatinum, Cuddalore, Ariyalur and Tirunelveli districts of Tamil Nadu. The diseased tissue was washed in water and cut into small pieces (3 mm) using a sterilized scalpel and surface sterilized in 0.1% mercuric chloride solution for 30 sec followed by washing in three changes of sterile distilled water. Sterilized PDA (Potato Dextrose Agar) medium was poured into sterile Petri plate @ 15 ml per plate amended with 100 ppm of streptomycin sulphate (to avoid bacterial contamination) and surface sterilized bits were placed at equidistance. All these were carried out under aseptic condition. The plates were incubated at room temperature (28±2°C) for seven days and observed for the fungal growth . The fungus was purified by single spore isolation technique[5] and identified based on the description given by Ou[1]. The pure culture was maintained on PDA slants.

Preparation of seed extracts[6]: The fresh seed materials as mentioned in Table 1 were washed in sterile water. These were ground in a pestle and mortar using sterile water at the rate of one ml g^{-1} of the material. The extract was then passed through two layers of muslin cloth and finally through sterilized Whatman No. 1 filter paper. The extract was then passed through seitz filter to free it from bacterial contamination. This formed the standard seed extract solution (100%). This was further diluted to the required concentration using sterile distilled water/sterile medium. All the extracts were used at 10% concentration.

***In vitro* evaluation of seed extracts on the growth of *H. oryzae*:** Efficacy of seed extracts on the growth of

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Table 1: Seed extracts (10% water extracts) screened for antifungal activity against *H. oryzae*

Vernacular/ common name	Scientific name	Botanical family
Kuppaimeni	*Acalypha indica* L.	Euphorbiaceae
Vagai	*Albizzia lebbeck* Benth.	Leguminae
Amaranthus	*Amaranthus viridis* L.	Amaranthaceae
Mustard	*Brassica campestris* L.	Cruciferae
Turmeric	*Curcuma longa* L.	Zingiberaceae
Arali	*Nerium oleander* L.	Apocynaceae
Gulmohar	*Delonix regia* L.	Caesalpinaceae
Veliparuthi	*Pergularia extensa* (Forssk.) Chiov.	Asclepiadaceae
Keelanelli	*Phyllanthns niruri* L.	Euphorbiaceae
Kodukapuli	*Pithecolobium dulce* L.	Mimosaceae
Castor	*Ricinus communis* L.	Euphorbiaceae
Sundavathal	*Solanum indicum* L.	Solanaceae
Ponnarali	*Thevitia neerifolia* Juss.	Apocynaceae
Vedpalai	*Wrightia tinctoria* Roxb.	Apocynaceae
Ginger	*Zingiber officinale* Rose.	Zingiberaceae

H. oryzae was evaluated by using poisoned food technique[7]. The standard seed extract solution (100%) was mixed with PDA medium at the required quantity so as to get the required concentration of the seed extract and sterilized. Twenty ml of this mixture was poured into sterilized Petri dishes and allowed to set. A 9 mm actively growing PDA culture disc of *H. oryzae* was taken by means of a sterilized cork borer and placed at the centre of the medium. The plates were incubated at room temperature (28±2°C). PDA without plant extract served as control. For comparison mancozeb (0.2%) was used by following the same procedure. Three replications were maintained. The radial growth of the mycelium was measured seven days later or when the fungus was grown fully in the control plate which ever was earlier. The results were expressed as per cent growth inhibition over control.

Efficacy on spore germination was assessed by using spore germination assay[8]. One drop of the seed extract (10%) was placed in the cavity of the depression slide and allowed to air dry. A drop of the conidial suspension ($4x10^6$ spores/ml) of *H. oryzae* prepared in sterile distilled water was added to the dried extract and thoroughly mixed. The cavity slide was incubated in Petri dish- glass bridge chamber. Three replications were maintained for each treatment. For comparison mancozeb (0.2%) was used. The spore suspension in sterile distilled water served as control. The spores were observed for germination in three different microscopic fields and recorded after 48 h to calculate per cent germination.

Statistical analysis: The data generated from various experiments of this study were statistically analysed following the procedure described by Gomez and Gomez[9]. The data with per cent values were subjected to arc sine transformation and the means were compared by Duncans Multiple Range Test.

RESULTS

Among the 15 seed extracts (in water) tested against *H. oryzae*, 10% extract of turmeric (*Curcuma longa*) and sundavathal (*Solanum indicum*) showed significantly the highest reduction in the mycelial growth of the pathogen. The diameter of the growth was 2.6 cm (70.45% growth reduction) and 3.13 cm (64.43% growth reduction) in these seed extracts as against 8.80 cm growth in the control. Ten per cent extracts of Vedpalai (*Wrightia tinctoria*) and kodukapuli (*Pithecolobium dulce*) were on par showing mycelial growth of 3.70 (57.95% growth reduction) and 3.77 cm (57.16% growth reduction), respectively. Mancozeb (0.2%) used for comparison recorded significantly the highest (93.18%) growth reduction with the minimum mycelial growth of 0.6 cm (Table 2).

Table 2: *In vitro* assay of seed extracts against the growth of *H. oryzae* (Poisoned food technique)

Seed extracts (10%)	Mycelial growth (cm)*	Growth inhibition (%)
Turmeric rhizome	2.60b	70.45
Sundavathal	3.13c	64.43
Vedpalai	3.70d	57.95
Kodukapuli	3.77d	57.16
Castor	3.90de	55.68
Vagai	3.90de	55.68
Arali	3.97de	54.89
Mustard	4.23e	51.93
Kuppaimeni	4.87f	44.66
Keelanelli	4.90f	44.32
Ponnarali	5.07f	42.39
Amaranthus	5.83g	33.75
Ginger	6.00g	31.82
Gulmohar	6.07g	31.02
Veliparuthi	6.17g	29.89
Mancozeb (0.2%) (Control)	0.60a	93.18
Control (without seed extract)	8.80h	-

*Mean of three replications.
Means followed by a common letter(s) are not significantly different at 5% level

Table 3: *In vitro* assay of seed extracts against the spore germination of *H. oryzae*

Seed extracts	Spore germination (%)*	Germination inhibition (%)
Turmeric rhizome	26.61(31.06)b	70.25
Sundavathal	28.70(32.40)c	67.92
Vedpalai	30.05(33.24)d	66.41
Kodukapuli	31.93(34.41)e	64.30
Castor	37.30(37.64)f	58.30
Vagai	39.17(38.75)g	56.21
Arali	40.55(39.55)h	54.67
Mustard	43.39(31.20)I	51.49
Kuppaimeni	45.44(42.39)j	49.20
Keelanelli	47.35(43.48)k	47.07
Ponnarali	49.50(44.72)l	44.66
Amaranthus	52.23(46.28)m	41.61
Ginger	54.05(47.33)n	39.58
Gulmohar	57.15(49.11)o	36.11
Veliparuthi	59.43(50.43)p	33.56
Mancozeb (0.2%) (control)	6.94(15.28)a	92.24
Control (without seed extract)	89.45(71.05)q	-

* Mean of three replications,
Data in the parentheses are arc sine transformed values. Means followed by a common letter(s) are not significantly different at 5% level

The seed extract (10%) of turmeric (*Curcuma longa*), sundavathal (*Solanum indicum*), vedpalai (*Wrightia tinctoria*) and kodukapuli (*Pithecolobium dulce*) showed significantly the highest inhibition of spore germination of the pathogen to the extent of 70.25, 67.92, 66.41 and 64.30%, respectively. The spore germination in response to these seed extracts were 26.61, 28.70, 30.05 and 31.93%, respectively as against 89.45% germination in the control. Mancozeb (0.2%) used for comparison recorded significantly the highest (92.24%) inhibition on the spore germination by recording the minimum of only 6.94% spore germination (Table 3).

DISCUSSION

Plant extracts as potential antifungal agents were being exploited against several plant diseases[10,11]. In the present study among the various seed extracts rhizome extract of turmeric was observed to be the most effective in inhibiting the mycelial growth and spore germination of the pathogen under *in vitro* condition. Natural products from many plants were known to control plant pathogens[12,13] including *Drechslera oryzae*[14,15]. Ganguly[16] reported the efficacy of leaf extract of *Vinca rosea* and *Azadirachta indica* against the mycelial growth of *H. oryzae*. Earlier, Selvaraj and Narayanasamy[17] reported that seed extracts of *Tribulus terrestris* was effective against rice brown spot pathogen. The anti fungal properties of turmeric rhizome powder against *H. oryzae* was reported by Sitansu and Deb[18]. Srijib Gangopadhyay[19] demonstrated the antifungal effect of turmeric against seed borne rice diseases and none of the turmeric treated plants developed disease symptoms. The antifungal activity of leaf extract of *Lawsonia inermis* on *D. oryzae* tested at 1:40 dilution (EC_{50} concentration) exhibited a gradual decrease in growth, total DNA, RNA, protein synthesis and oxygen uptake. The inhibition may be attributed to the loss of energy or in other words due to inhibition of respiration. The antifungal factor contained in the leaf was identified as 2-hydroxy-1, 4 naphthoquinone (Lawsone) which may be responsible for the fungitoxic nature[20]. Spraying of spinach and rhubarb leaf extracts induced systemic resistance in cucumber to anthracnose and the resistance appeared to be a host mediated response[21]. Rai *et al.*[22] reported that *Adenocallima alliaceum* completely inhibited germination of spore of *Alternaria alternata* and *Fusarium oxysporum* in undiluted extract. Rhizome extract of *Curcuma longa* exhibited maximum reduction of aflatoxin B1 in paddy which may be due to the presence of certain anti fungal substances like ethyl p-methoxy cinnamate in Curcuma[23].

Though mancozeb (0.2%) was observed to be the most effective treatment, the easy availability of the plant species coupled with its less phytotoxicity make them as a potential alternative. It is concluded that the rhizome extract (10%) of turmeric (*Curcuma longa* L.) and seed extract of sundavathal (*Solanum indicum* L.) exhibited the maximum inhibition of the mycelial growth and spore germination of *H. oryzae*. Fungitoxicity of botanical products were considered to be the safe means of plant disease control[24,25]. Due to their readily available source, non phytotoxicity and easily biodegradable nature fungitoxic principles from plants will have a promising role in managing the disease. However it will be desirable to isolate the active principle from the extract of these plants for testing its efficacy against plant diseases under field conditions.

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