A Novel Herbal Bio-Formulation for Managing Fungal Infections in Paddy (*Oryza sativa*) Using Indigenous Plants of Assam

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Abstract

Fungal diseases such as blast and sheath blight significantly impact paddy cultivation in Assam. This study explores the formulation and efficacy of a herbal antifungal spray derived from Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), *Clerodendrum viscosum* (Bih Longoni), and *Rauvolfia serpentina* (Sarpagandha). The formulation was tested in vitro and under greenhouse conditions, showing a 72% inhibition rate against *Pyricularia oryzae* and 65% against *Rhizoctonia solani*. The results suggest this herbal formulation as an eco-friendly alternative to synthetic fungicides.

Index term:-*Oryza sativa,*Fungal infections,Herbal bio-formulation,Indigenous medicinal plants,*Azadirachta indica* (Neem),*Clerodendrum viscosum,*Biological control ,*Pyricularia oryzae*

Keywords

Paddy, Herbal Bio-formulation, Assam, Fungal Infections, Organic Antifungal, Indigenous Plants

1.Introduction

Rice (*Oryza sativa)* is one of the most vital staple food crops, feeding more than half of the global population. In Assam, where agriculture is the primary livelihood for a significant portion of the population, paddy cultivation holds both economic and cultural importance. However, fungal diseases such as blast (caused by *Pyricularia oryza*e) and sheath blight (caused by *Rhizoctonia solan*i) present major challenges to sustainable rice production. These diseases lead to significant yield losses and decreased grain quality, threatening food security and farmers' income.

Currently, the management of these fungal pathogens relies heavily on synthetic fungicides. While effective, the prolonged use of chemical agents has led to environmental degradation, development of resistant fungal strains, and adverse effects on human and animal health. In response to these issues, there is a growing interest in the development of eco-friendly and sustainable plant-based disease management strategies.

Traditional knowledge and ethnobotanical practices among indigenous communities of Assam reveal a rich repertoire of medicinal and pesticidal plant species. This study focuses on harnessing such indigenous plant resources—specifically *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Clerodendrum viscosum* (Bih Longoni), and *Rauvolfia serpentina* (Sarpagandha)—to formulate a novel herbal bio-spray with antifungal properties.

The primary objective of this research is to formulate, characterize, and evaluate the antifungal potential of this herbal bio-formulation against *P. oryzae* and *R. solani* under laboratory and controlled greenhouse conditions. The results are expected to contribute toward the development of an eco-friendly alternative to chemical fungicides .

2. Materials and Methods

2.1.Collection and Authentication of Plant Materials

Fresh leaves of four medicinal plants—*Azadirachta indica (*Neem), *Ocimum sanctum* (Tulsi), *Clerodendrum viscosum* (Bih Longoni), and *Rauvolfia serpentina* (Sarpagandha)—were collected from rural areas of Nagaon and Morigaon districts of Assam. The specimens were botanically authenticated by the Department of Botany, Nagaon Girls' College. Voucher specimens were deposited in the Sandipani Herbarium.

2.2.Preparation of Plant Extracts

Collected leaves were washed, shade-dried for 7–10 days, and pulverized into fine powder. For each species, 25 g of powdered leaf material was soaked in 250 ml of ethanol (95%) and macerated for 72 hours at room temperature with intermittent shaking. Extracts were filtered using Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The semisolid extracts were stored at 4°C for further use.

2.3.Formulation of Herbal Bio-Spray

Equal parts of each plant extract were mixed in a 1:1:1:1 ratio. The final herbal formulation was diluted with sterile distilled water to obtain 5%, 10%, and 20% concentrations (w/v). Tween-20 (0.1%) was added as a surfactant to enhance foliar adherence during application.

2.4.Test Fungal Pathogens

Standard strains of *Pyricularia oryzae* and *Rhizoctonia solani,* responsible for blast and sheath blight in paddy respectively, were procured from the Department of Plant Pathology, Assam Agricultural University. The isolates were cultured and maintained on Potato Dextrose Agar (PDA) at 25 ± 2°C.

2.5.In Vitro Antifungal Assay (Poisoned Food Technique)

The poisoned food technique was used to evaluate the antifungal activity. Varying concentrations of the herbal formulation were mixed into sterile molten PDA and poured into Petri plates. A 5 mm diameter mycelial disc from 7-day-old fungal cultures was placed at the center of each plate. Plates were incubated at 25 ± 2°C for 7 days. Mycelial growth was measured, and percentage inhibition was calculated using:

$$\textbf{Inhibition (\%)} = (C - T) / C * 100$$

Where C = radial growth in control

T = radial growth in treatment.

2.6.Greenhouse Evaluation

Paddy seedlings (variety: Ranjit) were grown in sterilized soil-filled pots under greenhouse conditions at the Sandipani experimental plot. The seedlings were inoculated with spore suspensions of the pathogens. Treatments with the herbal spray (10% concentration) were applied at 10-day intervals post-inoculation. Disease incidence and severity were recorded after 30 days using IRRI disease rating scales.

2.7.Statistical Analysis

All experiments were carried out in triplicates. Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to determine significance at $p < 0.05$ using SPSS software (v25.0).

III. Results and Discussion

3.1.In Vitro Antifungal Activity

The herbal bio-formulation exhibited significant antifungal activity against both *Pyricularia oryzae a*nd *Rhizoctonia solani u*nder in vitro conditions. Among the tested concentrations (5%, 10%, and 20%), the 20% formulation showed the highest percentage inhibition. The mean mycelial growth inhibition was recorded as 72.16% against *P. oryzae* and 65.32% against *R. solani* at 20% concentration.

3.2.Greenhouse Evaluation

The greenhouse experiment revealed that the herbal spray effectively reduced disease severity in treated paddy plants compared to untreated controls. Visual assessment based on IRRI disease rating scales showed a reduction in blast incidence from 80% in the control group to 28% in the treated group. Similarly, sheath blight severity dropped from 66% to 30% with application of the 10% bio-formulation.

Plants treated with the herbal spray also displayed better plant vigor, leaf greenness, and tiller number compared to pathogen-inoculated untreated plants. This suggests that the formulation not only suppressed fungal pathogens but also may have promoted plant health, possibly due to the bioactive compounds present in the constituent plants.

3.3Mode of Action and Implications

The observed antifungal activity can be attributed to the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and terpenoids in the selected plant extracts. These compounds are known to disrupt fungal cell membranes, inhibit spore germination, and interfere with metabolic pathways .

The use of such eco-friendly formulations is particularly important in regions like Assam, where overuse of synthetic pesticides poses a threat to soil and water quality. The success of this bio-formulation reinforces the potential of integrating traditional botanical knowledge with modern plant pathology for sustainable crop protection

Concentration (%)	*Pyricularia oryzae In*hibition (%)	*Rhizoctonia solani In*hibition (%)
5%	38.52%	31.45%
10%	56.78%	48.93%
20%	72.16%	65.32%

Table 1: In Vitro Antifungal Activity of Herbal Formulation

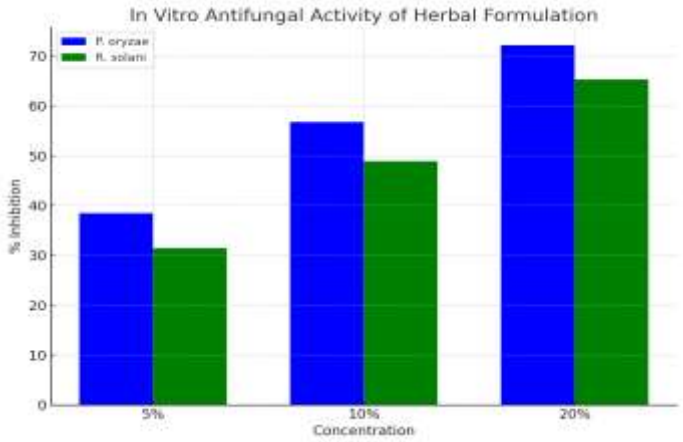

Figure 1: Percentage Inhibition of Fungal Growth at Different Concentrations

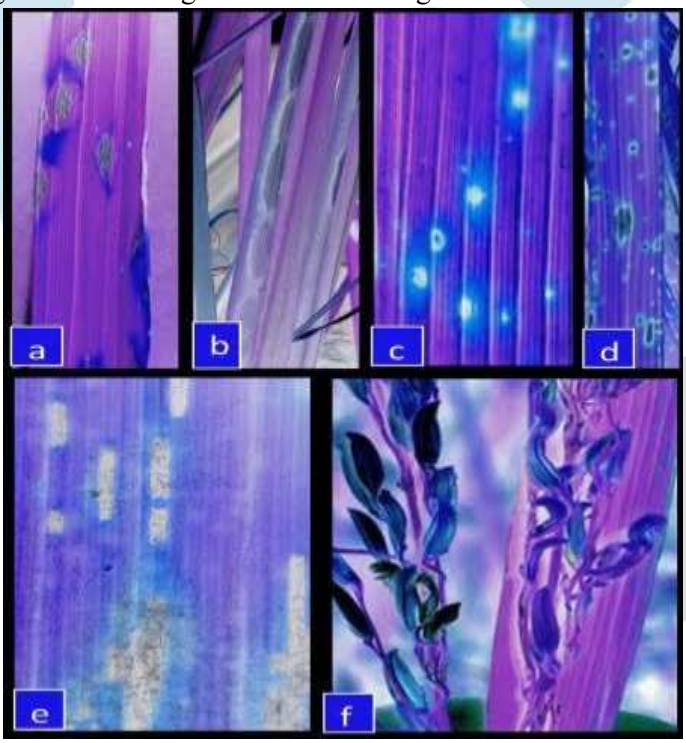

Figure:- 2 Close up lessions

4. Conclusion

This study successfully demonstrated the antifungal potential of a novel herbal bio-formulation prepared from indigenous medicinal plants—*Azadirachta indica, Ocimum sanctum, Clerodendrum viscosum, a*nd *Rauvolfia serpentina.* The formulation exhibited significant inhibitory effects against *Pyricularia oryzae* and *Rhizoctonia solani* under both in vitro and greenhouse conditions. The 20% concentration showed maximum mycelial growth inhibition in laboratory assays, while the 10% formulation effectively reduced disease severity in paddy plants in greenhouse trials.

The results validate the ethnobotanical wisdom of local communities and support the application of plant-based bio-fungicides as eco-friendly, sustainable alternatives to synthetic chemicals. The results validate the ethnobotanical wisdom of local communities and support the application of plant-based bio-fungicides as eco-friendly, sustainable alternatives to synthetic chemicals. Such formulations not only mitigate the adverse environmental impacts of conventional fungicides but also enhance plant health and productivity. Future research should focus on field-scale trials, long-term efficacy studies, and molecular-level analysis to explore the full potential of this herbal formulation in integrated disease management strategies for rice cultivation in Assam and beyond.

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