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IN VITRO ANTIMICROBIAL POTENCY OF PLANT EXTRACTS AGAINST *SAROCLADIUM ORYZAE*

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ABSTRACT

Studies were conducted to determine the effect ten plant extracts for the in vitro growth of *Sarocladium oryzae* cause of rice sheath rot disease. The results indicate that *Eucalytpus globules* leaf extract (10%) recorded the minimum mycelial growth (19.03 mm) and maximum per cent reduction of mycelial growth of pathogen (78.85%) over control. This was followed by *Allium cepae* and *Lantana camera* extract (27.24 mm, 69.73%; 30.57 mm, 66.03%). The lowest per cent inhibition of pathogen was observed by *Solanum nigrum* extract (49.95mm, 44.50%). The results also indicate that, extracts from *E. globules* exhibited highest seed germination, shoot length, root length and vigour index of rice seedlings by roll towel method. These plant extracts can thus be used for rice seed treatment to manage rice sheath rot disease.

Keywords **:** *Oryzae sativa, Sarocladium oryzae*, Plant extracts, Plant growth promotion

Introduction

Rice is a monocotyledonous annual grass and belongs to the family Graminae and the genus is *Oryza.* The major constraints are abiotic stresses (drought or excess of water, nutrient deficiencies and extreme temperatures) and biotic stresses (weeds, diseases and pests) (Saito and Matsukura, 2015). The crop is constantly subjected to various fungal, bacterial and viral diseases. Among the fungal diseases, sheath rot is considered as one of the most important emerging diseases of rice, causing yield losses from 20–30% up to 85% (Sakthivel, 2001; Bigirimana *et al.,* 2015; Mvuyekure *et al.,* 2017; Mvuyekure *et al.* 2018; Peeters *et al.,* 2021). This disease is mainly caused by the seed-borne fungus *S. oryzae* (Bigirimana *et al.,* 2015). Sheath rot, a prevalent disease, manifests in both rainfed and irrigated ecosystems, exerting its impact across all varieties of rice. The exclusive use of chemical pesticides for disease management poses significant risks, including health and environmental hazards, residue persistence, elimination of natural enemies, and the development of resistance. These concerns have spurred the development of eco-friendly and economically viable control methods, with plant extracts emerging as a promising alternative due to their known antimicrobial properties (Lalitha *at al.,* 2010). Plant extracts offer greater safety, biodegradability, and environmental compatibility compared to synthetic chemicals (Gurjar *et al*., 2012) (Enikuomehin, 2005) (Khan and Nasreen, 2010). Research by Gurjar *et al.* (2012) and others has demonstrated that extracts from plants such as *Azadirachta indica, Allium sativum, Eucalyptus globulus, Curcuma longa, Nicotiana tabacum*, and *Zingiber officinale* effectively inhibit pathogens like *Phytophthora infestans*, *Alternaria alternata*, *Rhizoctonia solani* and *Curvularia lunata*. These natural plant chemicals are cost-effective and readily accessible, particularly in developing countries where synthetic fungicides are often scarce and prohibitively expensive for resource-poor farmers (Mossini *et al.,* 2004). The main focus of the present study was to evaluate the efficacy of plant extracts against *S. oryzae in-vitro* and to assess their plant growth promoting activity by roll towel method.

Materials and Methods

Isolation and identification of pathogen

The pathogen was isolated from diseased rice sheaths exhibiting typical sheath rot lesios (Fig. 1). Small lesion edges were excised using a sterile knife, surface sterilized with 0.1% sodium hypochlorite solution for 30 seconds, rinsed thrice with sterile distilled water, and then plated on PDA medium in sterile Petri dishes. The plates were incubated at 28±2°C, and the hyphal tips radiating from the infected tissue were transferred to PDA slants for purification using the single hyphal tip method, following the protocol of Rangaswami (1972). The purified fungus was maintained on PDA slants for further studies (Fig. 2).

A total of 10 isolates (So1 to So 10) were obtained from infected sheath region of rice plants collected from different districts of Tamil Nadu. Based on the pathogenicity tests, the highly virulent isolate of So_5 alone was taken for subsequent experiments.

Preparation of aqueous extracts

Freshly collected plant leaf materials (Table 1; Fig. 2) were washed with tap water followed by distilled water to remove epiphytes and other debris, then dried under shade for three weeks. The dried leaves were ground into a fine powder (20 g dry weight) and soaked overnight in 100 ml of sterile distilled water. The resulting extract was strained through muslin cloth, filtered through Whatman No. 1 filter paper, and passed through a Seitz filter to eliminate bacterial contamination, forming a 100% standard plant extract solution (Shekhawat and Prasad, 1971). This extract was then diluted to the desired concentration with sterile distilled water, with all extracts used at 100% concentration to screen antifungal activity against *S. oryzae* in vitro. The plant species showed effectiveness in the preliminary screening alone were further diluted to different concentrations (5 and 10%) and tested against *S. oryzae*.

Efficacy of plant extracts on radial growth of *S. oryzae* (Poisoned food technique)

Efficacy of ten plant extracts were tested on the growth of *S. oryzae* by using poisoned food technique (Schmitz, 1930). The standard plant extract solution (100%) and the medium were prepared as already described. To the sterile PDA medium calculated quantity of the plant extracts were added through membrane filter so as to get the desired concentrations of the extracts in the medium and thoroughly mixed just before plating. A six-millimeter, 15-day-old PDA culture disc of *S. oryzae* was aseptically cut using a sterilized cork borer and placed at the center of Petri plates containing the medium, with three replications maintained. The plates were incubated at room temperature of 28±2°C for 15 days. The medium without plant extract served as control. The mycelial growth of the fungus was measured after 15 days. The per cent inhibition of mycelial growth was calculated.

Effect of seed treatment with different doses of selected antagonist on the incidence of rice sheath rot disease

25 rice seeds (variety RNR15048) were taken in a cloth bag soaked in 500 ml of water for 24 h and the excess water was drained. The seeds were allowed to sprout for 12 h. Whereas, two gram of *Eucalyptus* powder was suspended in 20 ml sterile distilled water in a 50 ml conical flask to obtain a 10% (w/v) concentration (Mbega *et al.,* 2012). Twenty-five rice seeds (var RNR 15048) were soaked in 10 per cent *Eucalyptus* powder leaf extract for 2 h. and then air dried for overnight in a sterile Petri plate.

Plant growth promotion (roll towel method)

All the ten plant extracts were assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Twenty-five treated seeds were placed on pre-soaked germination paper, covered with another pre-soaked paper strip, and gently pressed to hold the seeds in place. The paper and seeds were rolled in a polythene sheet and incubated in a growth chamber for 10 days, with three replications for each treatment. The root and shoot lengths of individual rice seedlings were measured, and the germination percentages were calculated. The vigor index was determined using the formula described by Abdul Baki and Anderson (1973).

Vigour Index = (Mean root length + Mean shoot length) x Germination (%)

Statistical analysis

The data were statistically analyzed using WASP version 2.0, developed by the Indian Council of Agricultural Research, Goa (Gomez and Gomez, 1984), with analysis of variance (ANOVA) performed at two significance levels ($P < 0.05$ and $P < 0.01$), means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Antifungal activity of plant extracts against *S. oryzae* (So5)

In the present investigations, the plant extracts showed varying degree of growth inhibition against *S. oryzae*. Among these, *E. globules* leaf extract (10%)

recorded the minimum mycelial growth (19.03 mm) and maximum per cent reduction of mycelial growth of pathogen (78.85%) over control (Table 2; Fig. 4). Similarly, Meera and Balabaskar, (2012) reported that *Eugenia caryophyllata* and *Eucalyptus globules* at 10% concentration were observed to be the most effective in inhibitory the mycelial growth, biomass production, spore germination and germ tube length of *S. oryzae*. Antifungal activity of *E. globules* may be due to the presence of eucalyptin, eucalyptol and elligatannin compounds (Gutiérrez *et al.* 1999). Besides several workers have studied the efficacy of various plant extracts against *S. oryzae* (Sharma and Sinha, 2013; Sunil Kumar and Patibanda, 2015; Shamsi and Chowdhury, 2016; Anandeeswari and Christopher 2020). All these earlier reports lend support to the present investigations.

Plant growth promotion

The current findings suggest that leaf extracts from *E. globules* extract recorded the maximum germination per cent (88.98%), shoot length (8.58cm), root length (12.05cm) and vigour index (1835.65) (Fig. 5). This was followed by *Allium cepae*, *Lantana camera, Abutilon indicum, Acalypha indica, Carica papaya, Azadiracta indica, Pongamia glabra* and *Solanum nigrum* in the decreasing order of merit (Table 3). The untreated control recorded the lowest values in terms of germination per cent (50.86%), shoot length (4.06 cm), root length (6.26 cm) and vigour index (524.87). Also, reports are that seed soaking with *A. sativum* and bulb extract increased the seed germination, shoot growth, root growth and vigour of various seedlings (Padmavathi, 1994; Sundarraj, 1994; Raja, 1995). In the study conducted by Nguefack et al. (2017), seed treatments utilizing ethanol extract and essential oil derived from *Callistemon citrinus* and *Ocimum gratissimum* demonstrated efficacy in enhancing field parameters, including emergence and yield, in tested rice varieties against *Bipolaris oryzae*. Among the natural products tested, garlic (20%) exhibited 95 per cent and Hinosan showed 98 per cent seed germination (Hubert *et al.* 2015) and for improving rice seedling growth (Jayaraj *et al.* 2018). The observed enhancement in seed germination and seedling growth by plant products can be attributed to various factors, including their fungitoxic action, which results in the eradication of pathogens both internally and externally within the seeds. Additionally, this effect may stem from the presence of hormone-synthesizing organisms within the phyllosphere population and the presence of auxins within the natural products. Antifungal activity of *E. globules* may be due to the presence of eucalyptin, eucalyptol and elligatannin compounds (Gutiérrez *et al.,* 1999). Besides several workers have studied the efficacy of various plant extracts against *S. oryzae* (Sunil kumar and Patibanda, 2015; Shamsi and Chowdhury, 2016; Anandeeswari and Christopher 2020). The above findings agree with present investigations. Identification and characterization of the antifungal compounds from recently tested plant extracts and their role in rice sheath rot disease control is also needed.

Fig. 1 : Sheath rot infected panicle

Fig. 2 : Axenic culture of *Sarocladium oryzae*

Fig. 3 : General view of different plant extracts

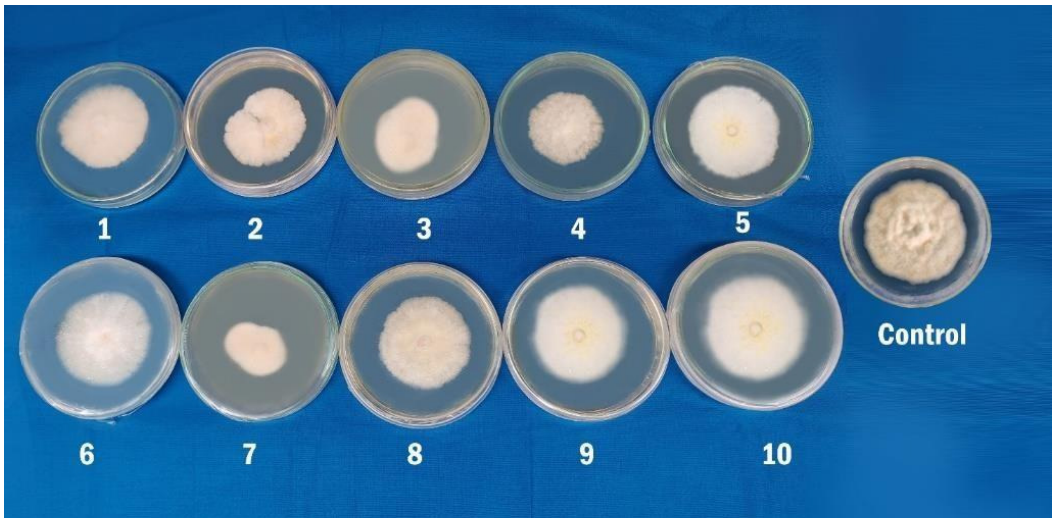

Fig. 4 : Antifungal activity of plant extracts against *Sarocladium oryzae*

1. Abutilon indicum *2. Acalypha indica* *3. Allium cepae* *4. Aloe vera*
5. Azadiracta indica *6. Catrica papaya* *7. Eucalyptus globules* *8. Lantana camera*
9. Pongamia glabra *10. Solanum nigrum*

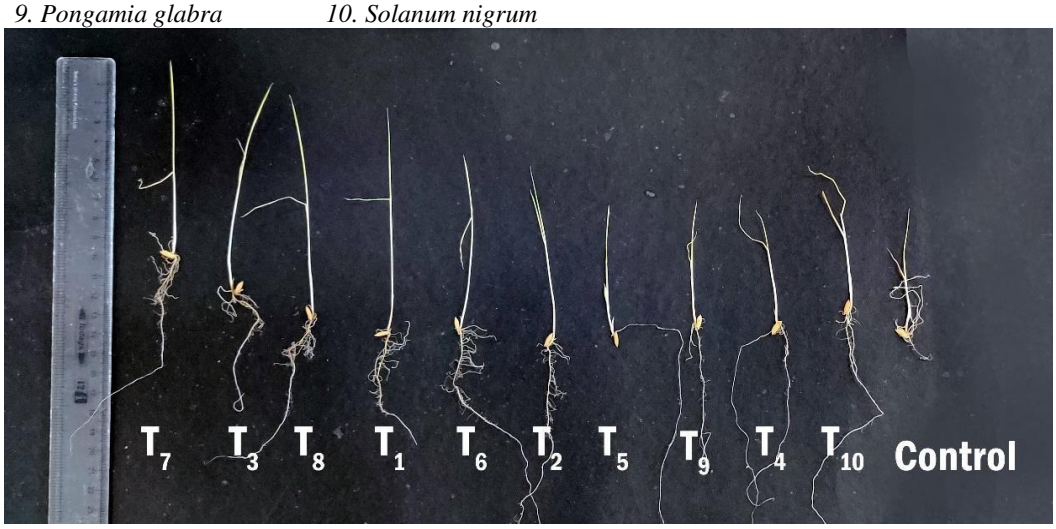

Fig. 5 : Effect of plant extracts on the growth of rice seedlings

1. Abutilon indicum *2. Acalypha indica* *3. Allium cepae* *4. Aloe vera*
5. Azadiracta indica *6. Catrica papaya* *7. Eucalyptus globules* *8. Lantana camera*
9. Pongamia glabra *10. Solanum nigrum*

Table 1 : List of plant extracts used for the present study

S.No	Plant extracts	Vernacular name	Parts used
1.	*Abutilon indicum*	Thuthi	Leaves
2.	*Acalipha indica*	Kuppaimeni	Leaves
3.	*Allium cepae*	Vengayam	Bulb
4.	*Aloe vera*	Katralai	Leaves
5.	*Azadiracta indica*	Vembu	Leaves
6.	*Carica papaya*	Papaya	Leaves
7.	*Eucalyptus globules*	Eucalyptus	Leaves
8.	*Lantana camera*	Road side weed	Leaves
9.	*Pongamia glabra*	Pungam	Leaves
10.	*Solanum nigrum*	Blackberry nightshade	Leaves

Table 2 : Antifungal activity of plant extracts against *S.oryzae* (So5)

S. No	Plant extracts	Linear growth of pathogen (mm)	Inhibition (%)
1.	*Abutilon indicum*	37.77^{d} (37.92)	58.03
2.	*Acalipha indica*	40.85^{f} (39.72)	54.61
3.	*Allium cepae*	27.24^{b} (31.46)	69.73
4.	*Aloe vera*	48.57^{i} (44.18)	46.03
5.	*Azadiracta indica*	43.98^{g} (41.54)	51.13
6.	*Carica papaya*	39.62^{e} (39.00)	55.97
7.	*Eucalyptus globules*	19.03^{a} (25.86)	78.85
8.	*Lantana camera*	30.57^{c} (33.56)	66.03
9.	*Pongamia glabra*	46.14^{h} (42.78)	48.73
10.	*Solanum nigrum*	49.95^{j} (44.97)	44.50
Control	-	90.00^{k} (71.56)	0.00
SE(d)	-	0.411	-
CD (0.05)	-	1.62 9	-

Values are mean of three replications.
Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 3 : Efficacy of seed treatment with plant extracts on seed germination and seedling vigour of rice by roll towel method

Plant extracts	Seed germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	Per cent increase over control
Abutilon indicum	68.26^{c}(55.69)	9.69^{de}	7.02^{e}	1140.62^{cd}	81.96
Acalipha indica	64.68^{d}(53.51)	9.88^{d}	7.89^{c}	1028.41^{d}	79.11
Allium cepae	71.00^{b}(57.41)	11.05^{b}	8.05^{b}	1733.91^{b}	85.97
Aloe vera	56.78^{gh}(48.87)	8.15^{g}	6.25^{fg}	817.63^{h}	65.98
Azadiracta indica	60.89^{ef}(51.27)	8.56^{ef}	6.25^{fg}	901.78^{f}	76.28
Carica papaya	62.66^{de}(52.31)	8.91^{e}	6.59^{f}	971.23^{e}	78.21
Eucalyptus globules	88.98^{a}(70.58)	12.05^{a}	8.58^{a}	1835.65^{a}	88.51
Lantana camera	69.58^{bc}(56.50)	10.11^{c}	7.39^{d}	1217.65^{c}	83.48
Pongamia glabra	58.89^{fg}(50.10)	8.21^{f}	6.89^{ef}	884.70^{g}	70.51
Solanum nigrum	55.43^{h}(48.09)	7.89^{h}	5.96^{g}	767.70^{i}	56.78
Control	50.86^{i}(45.47)	6.26^{hi}	4.06^{h}	524.87j	51.66
SE(d)	0.746	0.209	0.130	29.681	-
C.D(0.05)	2.515	0.541	0.227	192.183	

Values are mean of three replications. Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

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