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In vitro antifungal potency of neem and weed extracts against *Rhizoctonia solani*, the causal agent of sheath blight of rice

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Abstract
The antifungal activity of aqueous leaf extract of *Azadiracta indica*, *Fimbristylis miliacea, Arenaria serpyllifoia, Mitracarpus villosus, Mianthium stellatum, Croton sparsiflorus, Ocimum sanctum, Lepidium sativum, Cassia auriculata* and *Corchorus olitorius* was investigated against *Rhizoctonia solani*. At 10% concentration, the maximum antifungal potential was observed with the extracts of *Azadiracta indica*, which revealed 57 percent inhibitory activity against *R. solani*, followed by leaf extract of *Fimbristylis miliacea* (54%) and *Arenaria serpyllifoia* (51%). The application of botanical extracts for disease management could be less expensive, less polluting and ecofriendly.

Keywords: Sheath blight, *Rhizoctonia solani,* neem, weeds

1. Introduction

Rice (*Oryza sativa* L.) is an important food crop and it is the staple diet of over three billion people around the world. Rice is susceptible to various biotic and abiotic stresses during its cultivation (Plodpai *et al.*, 2013) [7]. Among the biotic stresses, the loss inflicted by pathogens, insect pests and nematodes are considerably significant. Rice crop is suffered by more than 40 diseases among which blast, bacterial blight, sheath blight, sheath rot and grain discoloration causes significant damage to rice crop (Islam and Monjil 2016) [3].

Sheath blight is one of the most important disease of rice incited by soil borne fungal pathogen *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris*), first reported by Paracer and Chahal (1963) [6]. The disease was first reported in Japan in 1909 and thereafter in Taiwan in 1912, Philippines in 1918 and India in 1920. In terms of severity and economic importance, sheath blight is considered as the second most devastating disease next to rice blast (Donayre and Dalisay 2015) [2]. The disease causes an annual yield loss of upto 50% in intensive rice production systems, especially in tropical Asia. A crop with a high plant density and closed canopy associated with high nitrogen application favours the disease incidence (Kagale *et al.*, 2011) [4]. The symptoms of sheath blight are usually observed near the water level, as elliptical or oblong greenish grey water soaked lesions and brown margins on leaf sheaths and causes stem lodging (Singh *et al.*, 2004) [14]. High temperature (22°C to 35°C) and high relative humidity favours the disease development. The pathogen survives as mycelia or resting structures known as sclerotia. Sclerotia are encapsulated, tight hyphal clump that protect and preserves the fungus during unfavourable condition in plant debris and on weeds to complete the disease cycle and it spreads through irrigation (Salim *et al.*, 2017) [9].

Management of this disease is difficult due to viability of sclerotia in the soil for several years. The durability of recently developed rice varieties with resistance to *R.solani* is uncertain due to the pathogen's wide host range and variability in terms of interaction with its host and environmental conditions. Several broad spectrum fungicides have been recommended for control of sheath blight. The indiscriminate and disproportionate use of chemicals might cause development of resistance in the pathogen, residual toxicity and environmental pollution (Choudhury *et al.*, 2017) [1]. Hence, the development of novel and safe plant protectants which interfere with the fungal pathogenicity factors is the need of the hour (Karthika *et al.*, 2017) [5].

In recent years, plant extracts mainly neem derivatives gaining importance for the control of the plant diseases due to their antifungal and antibacterial properties (Sehajpal *et al.*, 2009) [11].

Weeds cause considerable yield reduction in rice cultivation. Some weedy species may serve as a source of biologically active extract in plant protection, which exhibit antifungal properties. Identification of such weed/plant species which are enriched with antimicrobial principles, bio-active compounds and its application to crop plants provides disease resistance capacity by direct inhibition of growth of the pathogen and inducing defense responses. Hence, using abundant weeds in controlling plant pathogens is the result of both weeds and plant disease management. This approach is considered as eco-friendly, cheaper, bio-degradable and aptly called as zero cost technology.

In this paper, we evaluated the antifungal activity of neem and 39 weed extracts against rice sheath blight pathogen, *R.solani* under laboratory condition.

2. Materials and Methods

2.1. Collection and isolation of the pathogen

The diseased rice plant showing the typical symptoms of sheath blight disease were collected from Eachangkottai village. The sheath blight infected tissue was cut using sterile scalpel. The tissue bits were then surface sterilized with 1% sodium hypochloride for few seconds and subsequent washing with sterile water thrice and inoculated in sterilized Petri plates containing Potato Dextrose Agar (PDA) medium amended with streptomycin. The plates were incubated at 27±2 ^{o}C. The growth of hyphae from the tissue were observed after 48 hrs and maintained by subsequent subculturing for screening.

2.2. Collection of weeds and preparation of extract

The weeds were collected from the AC&RI, Thanjavur. The freshly collected weed species were used for the preparation of extract. The weeds were washed with fresh water to remove the dirt and finally rinse with sterile water. These were ground in a pestle and mortar at 1g/ ml of sterile water. Then the macerated tissues were filtered through double layered muslin cloth and finally through Whatman No.1 filter paper. The plant extracts were filtered through Seitz filter to get rid of bacterial contamination. This formed the 100% standard plant extract.

2.3. Screening by poison food technique

The efficacy of the extract on *R.solani* was studied by poison food technique. From the standard plant extract 10ml were added to 90ml of sterilized and warm PDA medium and thoroughly mixed by shaking, which gives 10% concentration. 20ml of this mixture was poured in a sterile Petri plate and allowed it to solidify. A 9mm actively growing culture disc of *R. solani* was aseptically placed onto the medium at the centre of the plate which serves as treatment and the PDA medium with the test fungus alone acts as a control. The plates were incubated at room temperature for 48 hrs. The percent inhibition was measured using the formula,

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Where, Dc = Average diameter of fungal growth (cm) in control

Dt = Average diameter of fungal growth (cm) in treatment

3. Results and Discussion

By screening neem and thirty nine weeds by poison food technique, nine weeds significantly inhibits the pathogen as shown in table 2. Neem leaf extract shows 57.14% inhibition against the pathogen. With respect to weeds, the more inhibition was observed in the leaf extract of *Fimbristylis miliaceae* (54.28%) and the least inhibition was observed in the leaf ectract of *Corchorus olitorius* (17.14%). The remaining thirty weeds does not showed any inhibition to the pathogen *R. solani.* Our results are in accordance with previous findings by (Sharma *et al.*, 2018) [12] who have demonstrated botanicals against *R.solani* under *in vitro.* Sehajpal *et al.*, (2009) [11] reported that *A. indica* have moderate effect against the pathogen *R.solani* and its fungitoxic activity is due to the presence of azadirachtin containing desactylimbin. A study conducted by San Aye and Matsumoto (2011) [10] found that the neem leaf extract inhibited the growth of *R.solani* by 87.5%. A study conducted by Sifat and Monjil (2017) [13] reported that the neem leaf extract inhibited (96.75%) the *Rhizoctonia sp.* by indigenous medicinal plant extract. Also, the *Ocimum sanctum* inhibited the sheath blight pathogen in our study which is in accordance with (Islam and Monjil 2016) [3]. According to (Rahman *et al.*, 2012) [8] the neem and tulsi leaf extract significantly increased the grain yield. Thus, weeds are the unexplored and potent source of antifungal compounds with high biomass can be utilized for disease control. Furthermore, weeds or plant extracts have a potential value to inhibit the mycelial growth, malformation and surprisingly it also inhibits the development of sclerotia thereby inhibits the perpetuation of pathogen to further seasons. Considering the effectiveness and economics in controlling the sheath blight disease of rice, neem leaf extract is the suitable option for the farmers which is more ecofriendly and safe to environment over the chemicals.

Table 1: List of plants used for screening *in vitro*

S. No.	Common name	Scientific name	Family
1.	Neem	*Azadiracta indica*	Meliaceae
2.	Tropical rose mallow	*Hibiscus vitifolicus*	Malvaceae
3.	Thoth (florida keys Indian mallow)	*Abutilon hirtum*	Malvaceae
4.	Indian copper leaf (Kuppaimeni)	*Acalypha indica*	Euphorbiaceae
5.	Devils horsewhip (Naiyuruvi)	*Achyranthus aspera*	Amaranthaceae
6.	Slender amaranthus	*Amaranthus viridis*	Avaranthaceae
7.	False Solomon’s seal	*Mianthium stellatum*	Asparagaceae
8.	Purple chloris (mayilkondai)	*Chloris barbata*	Poaceae
9.	Spindle pod (Naikadugu)	*Cleome viscose*	Capparidaceae
10.	Asian pigeon wings (sangu poo)	*Clitoria ternatea*	Fabaceae
11.	Jews mallow (Punnakupoondu)	*Corchorus olitorius*	Tilliaceae
12.	Haspan flat sedge	*Cyperus haspan*	Cyperaceae
13.	Crow foot grass (kakakal pul)	*Dactyloctenium aegyptium*	Poaceae
14.	Asthma herb (Amman pacharisi)	*Euphorbia hirta*	Euphorbiaceae
15.	Goats foot	*Iopomeapes-caprae*	Convolvulaceae

16.	Common leucas (thumbai)	*Leucas aspera*	Convolvulaceae
17.	Tropical girdle pod	*Mitracarpus villosus*	Rubiaceae
18.	Stone breaker (Keezhanelli)	*Phyllanthus niruri*	Euphorbiaceae
19.	Ivy god (kowai)	*Coccinia grandis*	Cucurbitaceae
20.	Common wire weed	*Sida acuta*	Malvaceae
21.	Nightshade	*Solanum xanthocarpum*	Solanaceae
22.	Horse purslane (saranai)	*Trianthema protulacastrum*	Aizoaceae
23.	Rail poondu	*Croton sparsiflorus*	Euphorbiaceae
24.	Tanners cassia	*Senna auriculata*	Fabaceae
25.	Tulsi	*Ocimum sanctum*	Lamiaceae
26.	Native bryony	*Diplocyclos palmatus*	Cucurbitaceae
27.	Hog weed	*Boarhaevia diffusa*	Nyctaginaceae
28.	Grass-like fimbry	*Fimbristylis miliacea*	Cyperaceae
29.	Chinese violet	*Asystasia gangetica*	Acanthaceae
30.	Bougainvilla	*Boungaillea glabra*	Nyctaginaceae
31.	Sirukanpoolai	*Aerva lanata*	Amaranthaceae
32.	Lemon basil (Nai thulasi)	*Ocimum americanum*	Lamiaceae
33.	Bind weed	*Convolvulus arvensis*	Convolvulaceae
34.	Pepper grass	*Lepidium sativum*	Brassicaceae
35.	Common vetch (Patasu)	*Vicia sativa*	Fabaceae
36.	Spinach	*Basella alba*	Basellaceae
37.	Narrow leaf	*Aerva javanica*	Amaranthaceae
38.	Spurge	*Euphorbia microphylla*	Euphorbiaceae
39.	False daisy	*Eclipta alba*	Asteraceae
40.	Red tassel flower	*Emilia sonchifolia*	Asteraceae

Table 2: Efficacy of different plant extracts against *Rhizoctonia solani* under *in vitro*

S. No.	Scientific name	Mycelial growth of the pathogen (mm)*	Per cent reduction over control (%)
1.	*Azadirachta indica*	38.57	57.14 (48.74)**
2.	*Fimbristylis miliacea*	41.15	54.28 (46.57)
3.	*Arenaria serpyllifoia*	43.72	51.42 (45.94)
4.	*Mitracarpus villosus*	46.29	48.57 (44.85)
5.	*Mianthium stellatum*	59.15	34.28 (35.20)
6.	*Croton sparsiflorus*	64.29	28.57 (32.53)
7.	*Ocimum sanctum*	64.29	28.57 (32.99)
8.	*Lepidium sativum*	66.86	25.71 (30.90)
9.	*Cassia auriculata*	74.57	17.14 (24.04)
10.	*Corchorus olitorius*	74.57	17.14 (24.79)
11.	Control	90.00	0.00 (0.49)
CD (P=0.05)		2.45	1.94

*Mean of three Replications

** Values in the parentheses are arc sin transformed value

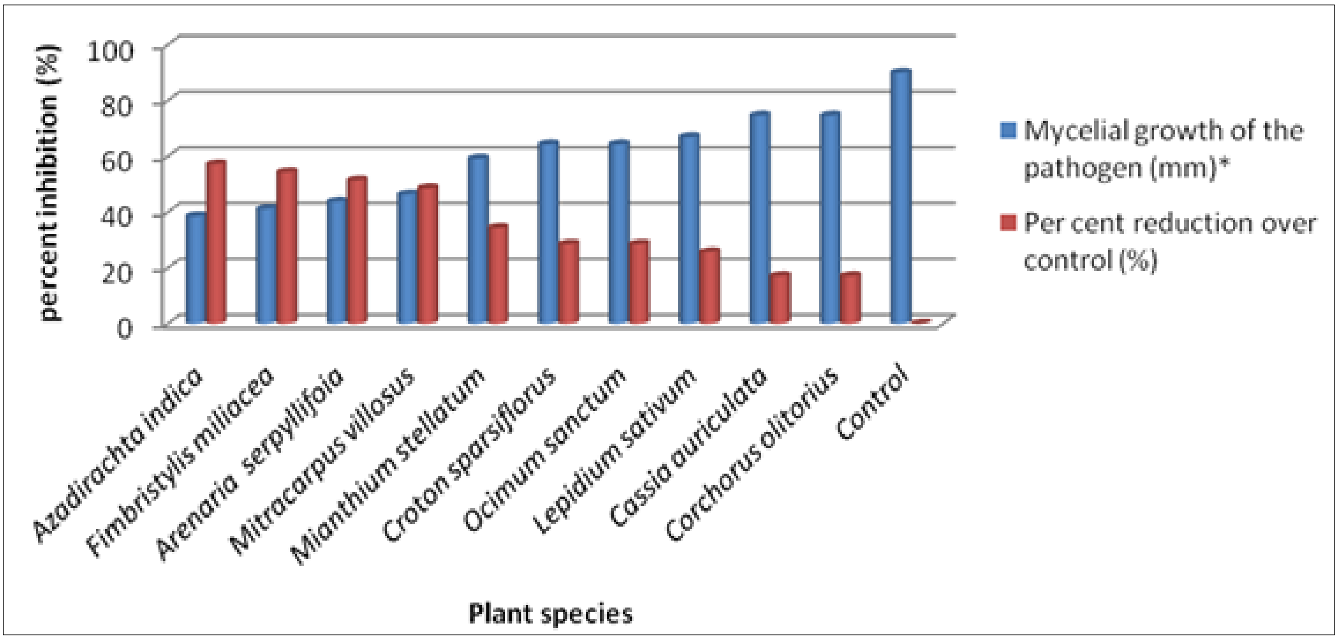

Fig 1: Efficacy of different plant extracts against *Rhizoctonia solani* under *in vitro*

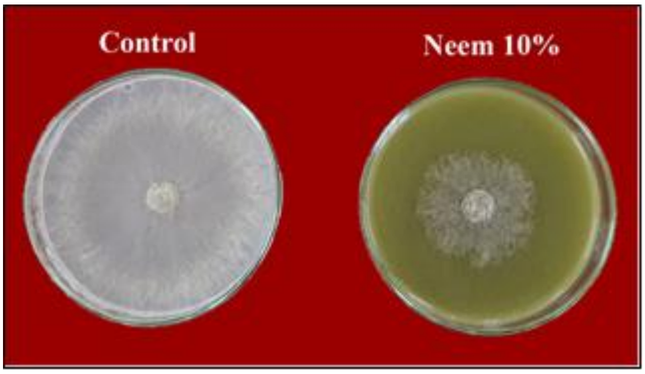

Fig 2: Mycelial growth reduction of the pathogen at 10% concentration of Neem leaf extract over control

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