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Biological Control of Rice Sheath Blight Disease using Medicinal Plant Extract and *Pseudomonas fluorescences*

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ABSTRACT

Rice sheath blight caused by *Rhizoctonia solani* khun. Biological method is more ecofriendly and easily managing the fungal disease. This method would be more effective and less time consuming, gaining popularity, and cheaper strategy for managing the fungal disease. Plant extracts are more effective control of plant diseases is gaining importance due to their antifungal and antibacterial properties. In the present study, seven medicinal plant product and biocontrol agent were evaluated viz., *Azadirachta indica, Vitex negundo, Justicia adhatoda, Curcuma longa, Aloe barbadensis miller, Ocimum tenuiflorum, Allium sativum,* and *P. fluorescens* were screened against *R. solani*, the rice sheath blight pathogen. The biocontrol agent was *P. fluorescens* and plant extracts showed most effective inhibition of radial growth of the pathogen but most effective plant extracts was *Allium sativum* (garlic) extract. The combination of and *P. fluorescens* and plant extract expectedly inhibited the pathogen growth of *R. solani* for *in vitro* and *in vivo* condition.

***Key words*:** Rice, *Rhizoctonia solani*, Medicinal plant extracts, *P. fluorescens*

Rice is a predominant crop and a mainstay for the rural population and their food security. The rice production will be achieved by 2020, has been 128 MT to feed on the growing population in India [1]. Also, [2] reported that 50% of yield loss due to the incidence of sheath blight disease. Rice sheath blight caused by *Rhizoctonia solani* Kuhn. Initially the disease was reported by Miyake from Japan in 1910 while in India, it was reported by [3] from Gurdaspur and Punjab. Generally, chemical methods is widely used for combating the sheath blight incidence but continuous uses of fungicides leads susceptibility of crop and develop the resistance on pathogen. So alternative source of biological method. Plant extracts are more effective control of plant diseases is gaining importance due to their antifungal and antibacterial properties. The antimicrobial compounds work effectively even against the phytotoxins secreted by plant pathogens [4]. The antifungal efficacy of plant extracts against many fungal pathogens. In this component controlling the complete inhibit the mycelial growth and sporulation of fungi [5]. The natural products can improve the ability of crop which may be very useful and reduce the diseases severity. Various plant product used *in vitro* and *in vivo* condition. The present study was undertaken to investigate the effect of biocontrol agent and medicinal plant product for managing rice sheath blight disease.

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MATERIALS AND METHODS

Isolation of P. fluorescencs

Rhizoplane-colonizing *P. fluorescens* was isolated from fresh roots of paddy grown in different locality of Cuddalore district and were designated as PF_1 to PF_7. The soil particles loosely adhering to the roots were teased out and used for the isolation of *P. fluorescens*. A soil suspension was prepared from each rhizosphere sample by shaking one g of soil sample in 10 ml of sterile dist. water and serial dilutions were made. One ml of soil suspension from aliquot dilutions (10^{-5} to 10^{-8}) was aseptically added to sterile Petri dishes containing twenty ml of sterile King's B medium and incubated at 28 ± 2°C for 48 h after incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4°C for further use.

Dual culture technique of P. fluorescens against R. solani

The antagonistic activity of *P. fluorescens* against *R. solani* was tested by dual culture technique [6]. Isolates of *P. fluorescens* was streaked (one cm away from the edge) containing PDA. A 9mm mycelial disc from seven days old PDA culture of *R. solani* was placed at the opposite side of

Petri dishes perpendicular to the respective bacterial and fungal antagonist and incubated at 28±2°C for 15 days. Petri dishes inoculated with fungal discs alone served as control. Three replications were maintained for each isolate. Observation on width of inhibition zone and mycelial growth of test pathogen was recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent [7].

$$I = C – T / C \times 100$$

Where;
I - Per cent inhibition
C- Mycelial growth of pathogen in control
T- Mycelial growth of pathogen in dual plate.

Preparation of plant extract

The fresh leaves were collected separately washed with tap water, then with alcohol and finally with repeated changes of sterile distilled water. They were separately ground in sterile distilled water at the rate of one ml/g of leaf tissue in a sterilized pestle and mortar. The extract was stained through two layer of muslin cloth subsequently filtered through and centrifuged @ 1500 rpm for 10 min. And formed the standard plant extract solutions (100%) [8].

Poisoned food technique

Potato dextrose agar PDA medium mixed separately with extracts of different plant species at different concentrations viz., 5, 10, 15, 20% poured into sterile Petri dishes, allowed to cool and solidify. The mycelial disc (9mm) of 15 days old culture of test pathogen placed at the center of the petri dishes and incubated at 25±2°C for 10 days. The PDA medium with the same concentration of sterile distilled water alone served as control. The experiments was replicated thrice and the per cent inhibition of mycelial growth if any was determined by the formula:

$$PI = C – T / C \times 100$$

Where;
C= diameter *R. solani* control
T = Diameter *R. solani* treated

Agar well method

The antimicrobial activity of plant leaf extracts against *R. solani* was tested by Agar well method. 20 ml of PDA medium was seeded with 3 ml of spore suspension (5X 10^{-5}). Wells made on the agar surface with a 5mm cork borer. 1 ml of medicinal plant extracts was poured separately into the well using a sterile syringe at different concentrations viz. 5,10,15 and 20%. The plates were incubated at 28±2°C for seven days and observe for fungal growth. Three replications were maintained for each treatment. The plates were observed for zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Paper disc method

The desired concentration of medicinal plant leaf extract were impregnated into sterile filter paper discs (5 mm dia.) the impregnated extract discs were placed on seeded agar plates and incubated at room temperature 28±2°C. Three replications were maintained for each treatment. The inhibition zone of fungal growth around the treatment sterile filter paper discs was measured and recorded.

Effect of P. fluorescence (PF_5) and garlic extract of (20%) on the management of rice sheath blight ADT-36 (Filed trial)

Separate field study was conducted to test the efficacy of *P. fluorescence* (PF_5) and garlic extract of (20%) for assessing their influence on the incidence of sheath blight of rice. The same combination of treatments mentioned in the pot culture experiment were evaluated in field experiments conducted during Kuruvai 2019 at Sivapuri, Cuddalore district, Tamil Nadu. The sheath blight susceptible variety ADT 36 was used for the study. The experiments were conducted in a randomized block design with three replications for each treatment and a suitable control. Also, the fertilizer application was done following the blanket schedule of N:P:K (150:50:50) recommended by the State Agricultural department. A plot size of 5×4 m was maintained for each treatment and the crop was raised with a spacing of 12.5 × 10 cm and all the standard agronomic practices as recommended by the State Agricultural Department were followed. The fungicide carbendazim 50% WP @ 0.1 per cent was used for comparison. The rice crop was harvested at maturity, thrashed, winnowed and cleaned plot wise, dried and the yield was recorded and expressed as kg/ha. In the field trial the observations on disease incidence and biometric attributes was assessed on a randomly selected set of 25 hills per plot at the time of maturity.

Treatment schedule

T_1	:	Soil application with PF_5 @ 2.5kg/ha
T_2	:	Seed treatment (@ 10 g/kg) + Soil application (@ 2.5kg/ha) with PF_5
T_3	:	Foliar application with garlic extract @ 20% at 30 DAT
T_4	:	Foliar application with garlic extract @ 20% at 30 and 45 DAT
T_5	:	$T_1 + T_3$
T_6	:	$T_1 + T_4$
T_7	:	$T_2 + T_3$
T_8	:	$T_2 + T_4$
T_9	:	Carbendazim
T_{10}	:	Control

RESULTS AND DISCUSSION

Evaluation of isolates of P. flourescens on the growth of R. solani under in vitro (dual culture)

All the isolates significantly reduced the mycelial growth of the pathogen over control under dual culture technique. In present study, PF_5 was recorded the maximum growth inhibition of *R. solani* (71.31 per cent). The least growth inhibition of the pathogen (PF_1) was recorded at **(**56.93 per cent). The results of the current study agreement with finding of [9]. The secondary metabolites like 2,4, diacetylphloroglucinol, oligomycin, oomycinA, phenazzine, pyoluterin, pyrolnitrin, pyocyanin, iturin, hydrogen cyanide, antibiotics and lytic enzymes, β 1,3-glucanase and chitinase were reduced by *P. fluorescence* [10-11].

Antifungal activity of medicinal plants on the growth of R. solani (RS_6) (Poisoned food technique)

Among the seven plant extracts tested against *R. solani*, garlic extract at 20 per cent concentration was found to be the best as it completely inhibits the growth of the test pathogen at 20 per cent conc. It was followed by neem @ 20

per cent recorded 18.92 per cent. The plant extracts produce flavonoids, alkaloids, phenolic compound and glycosides, saponins, tannins, terpenoids [12]. *R. solani* findings are well endorsed by earlier workers [13-14].

Table 1 Evaluation of isolates of *P. fluorescence* on the growth of *R. solani* (RS_6) under *in vitro* (Dual culture)

Isolates	Linear growth of *R. solani* (mm)		Percent growth inhibition
	Inhibition zone	Radial growth	
PF_1	51.24 (45.71)	38.76 (38.50)	56.93^{g}
PF_2	56.18 (48.54)	33.82 (35.55)	62.42^{d}
PF_3	60.13 (50.84)	29.87 (33.12)	66.81^{b}
PF_4	54.31 (47.47)	35.69 (36.68)	60.34^{f}
PF_5	64.18 (53.23)	25.82 (30.53)	71.31^{a}
PF_6	58.32 (49.78)	31.68 (34.25)	64.80^{c}
PF_7	55.56 (48.19)	34.44 (35.93)	61.73^{e}
Control		90.00 (71.56)	-

Table 2 Antifungal activity of medicinal plants on the growth of *R. solani* (Poisoned food technique)

Medicinal plants	Mycelial growth of *R. solani* (mm)							
	5%	% Inhibition	10%	% Inhibition	15%	% Inhibition	20%	% Inhibition
Neem	40.21	55.32^{b}	34.17	62.03^{b}	28.03	68.85^{b}	18.92	78.97^{b}
Nochi	66.11	26.54^{f}	60.33	32.96^{e}	54.82	39.08^{f}	43.56	51.60^{f}
Garlic	37.15	58.72^{a}	29.56	67.15^{a}	18.73	79.18^{a}	8.99	90.01^{b}
Adathoda	72.61	19.32^{g}	69.36	22.37^{f}	58.19	35.34^{f}	52.12	42.08^{e}
Turmeric	44.32	50.75^{c}	39.12	56.53^{c}	30.97	65.58^{c}	24.67	72.58^{c}
Aloe	60.17	33.14^{e}	57.20	36.44^{e}	42.03	53.30^{e}	39.92	55.64^{e}
Thulasi	56.21	37.54^{d}	48.24	46.40^{d}	40.06	55.48^{d}	35.71	60.32^{d}

Table 3 Evaluation of medicinal plants on the growth of *R. solani* (Paper disc assay and agar well method)

Plant product	Inhibition zone (mm)									
	Paper disc method					Agar well method				
	5%	10%	15%	20%	mean	5%	10%	15%	20%	Mean
Neem	37.09	39.61	41.27	42.81	40.19^{a}	36.30	37.18	39.52	40.15	38.28^{a}
Nochi	25.49	26.43	29.31	34.43	27.91^{d}	23.16	24.61	25.09	26.83	24.92^{d}
Garlic	39.21	40.75	43.03	45.52	42.12^{a}	38.03	39.08	41.92	43.24	40.56^{a}
Adathoda	20.15	22.04	23.67	25.19	22.76^{e}	19.52	20.96	21.64	23.72	21.46^{e}
Turmeric	35.71	37.92	39.14	40.16	38.23^{b}	34.67	36.43	37.75	39.27	37.03^{b}
Aloe	28.24	29.31	32.61	34.18	31.08^{c}	27.80	28.15	29.19	30.52	28.89^{c}
Thulasi	30.52	31.20	33.06	35.47	32.56^{c}	29.91	30.14	32.31	40.15	38.28^{c}

Table 4 Effect of *P. fluorescence* (PF_5) and Garlic extract of (20%) extract on the management of rice sheath blight ADT-36 (Field trial)

Treatments	PDI (%)	Per cent disease reduction	Germination (%)	Plant height (cm)	No. of productive tillers	1000g weight	Yield (t/ha)
T_1: Soil application with PF_5 @ 2.5kg/ha	10.74^{e}	68.60	76.62	105^{e}	12^{e}	19^{e}	4.35^{e}
T_2: Seed treatment (@ 10 g/kg) + Soil application (@ 2.5kg/ha) with PF_5	10.53^{e}	69.21	75.10	104^{e}	11^{e}	18^{e}	4.37^{e}
T_3: Foliar application with garlic extract @ 20% at 30 DAT	12.32^{g}	63.98	70.38	101^{g}	8^{g}	15^{g}	4.13^{g}
T_4: Foliar application with garlic extract @ 20% at 30 and 45 DAT	11.43^{f}	66.58	73.29	103^{f}	10^{f}	17^{f}	4.18^{f}
T_5: $T_1 + T_3$	9.34^{d}	72.69	78.38	107^{d}	13^{d}	21^{d}	4.41^{d}
T_6: $T_1 + T_4$	8.78^{c}	74.33	80.49	109^{e}	15^{e}	22^{e}	4.48^{e}
T_7: $T_2 + T_3$	7.56^{b}	77.90	82.56	110^{b}	16^{b}	24^{b}	4.53^{b}
T_8: $T_2 + T_4$	6.42^{a}	81.23	84.78	113^{a}	18^{a}	26^{a}	4.62^{a}
T_9: Carbendazim	6.28^{a}	81.64	72.43	102^{f}	9^{f}	16^{f}	4.23^{f}
T_{10}: Control	34.21^{h}	-	67.38	97^{h}	14^{h}	19^{h}	3.12^{h}

Effect of Pseudomonas fluorescence and garlic extract against sheath blight of rice under pot and field condition

In present study, application with bulb extract of *Allium sativum* and *P. fluorescens* on rice plants significantly reduced the incidence of *R. solani* in both pot and field experiments. The combined application *P. fluorescent* (seed treatment @ 10g/kg + soil application @ 2.5kg/ha) with (T_2) with foliar application of garlic extract @ 20% at 30 and 45 DAT (T_4) (i.e., $T_8 = T_2 + T_4$) were effectively controlled disease incidence and reduced the damage by directly

affecting the growth of the pathogen. The application of bioagents and botanicals was found to be effectively reduced the incidence of sheath blight under both pot and field trials [15]. Currently new researchers reported PGPR and induced defence enzyme control the soil borne diseases both *in vitro* and *in vivo* conditions [16-17].

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