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ROLE OF DEFENSE ENZYME INDUCED BY THE ECOFRIENDLY COMPONENTS AGAINST SHEATH BLIGHT (*RHIZOCTONIA SOLANI*) KUHN AND SHEATH ROT (*SARACLADIUM ORYZAE*) SAWADA GAMES AND HAKSWORTH DISEASES OF RICE

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Abstract

Rice is one of the most important staple food crop which is predominantly grown in the worldwide. Rice is contributed about 45 percent in total cereal production of India and is main food source for more than 60 percent population of the country. In Tamil Nadu rice crop is predominantly grown in the Cauvery delta region which is also known as rice bowl of Tamil Nadu. The efficacy of different ecofriendly components were evaluated under pot culture condition for access the induction of defense enzyme. Among the different ecofriendly components combined application of *P. fluorescens* (Seed treatment @10 g/kg of seed + prophylactic spray @ 0.5% at 20, 40 and 60 DAS) + Fortified lignite fly ash (Soil application 40 kg at the time of sowing + foliar dust @40 kg/ha +Annamalai mixture foliarspray @20 l/haat 30 and 60 DAS) shows highest induction of defense enzyme *viz*., peroxidase (PO), polyphenol oxidase (PPO) L-phenylalanine ammonia-lyase (PAL) and β-1,3 glucanase.

Key words **:** Ecofriendly components, PO, PPO, PAL, β-1,3 glucanase and rice diseases

Introduction

Rice is one of the most important staple food crop which is predominantly grown in the worldwide. Rice is contributed about 45 percent in total cereal production of India and is main food source for more than 60 percent population of the country. In Tamil Nadu rice crop is predominantly grown in the Cauvery delta region which is also known as rice bowl of Tamil Nadu. Many biotic factors affect the rice production especially fungal disease causes huge economic losses. Among the fungal disease sheath blight caused by *Rhizoctonia solani* Kuhn and sheath rot caused by *Saracladium oryzae* (Sawada) Games and Haksworth are the major diseases in all rice growing areas in the world. The disease sheath blight can cause yield loss of 5.2 - 50 per cent depending on environmental conditions and crop stages (Rajan 1987; Sharma *et al.*, 1994). Sheath rot disease of rice yield loss varies from 9.6 to 85% (Sakthivel, 2001). This diseases of rice are being controlled by several approaches like chemical, cultural, and ecofriendly management among them the ecofriendly management is found more economically and avoid environment pollution as well as increase the production of pesticide free rice. Application of biocontrol agents along with animal products and fortified lignite fly ash bring about induced systemic resistance fortifying the physical and mechanical strength of cell wall and changing physical and biochemical reaction of host leading to synthesis of defense chemical against the challenge of pathogen (Jayalakshmi *et al.,* 2009). The aim of the present study was to check the efficacy of companied application of bioinoculants along with animal product and fortified lignite fly ash on suppression of sheath blight and sheath rot and their effect on induction of defense enzyme *viz*., peroxidase (PO), polyphenol oxidase (PPO) L-phenylalanine ammonia-lyase (PAL) and β-1,3 glucanase.

Materials and Method

Isolation and Multiplication of Fungal Pathogens

The isolate was obtained from rice variety ADT-36. The infected portion of the leaf sheath along with healthy tissue was cut into small pieces. Infected tissues were separately surface-sterilized by washing with sterilized water and then immersing in 10% bleach solution for 2 min. The samples were rinsed twice with sterile water and blotted dry. The blotted dry leaf sheath of each sample were placed on 2-3 layers of moist blotting paper in Petri dishes and incubated at 21° ± 1°C under 12-h alternate exposure to near ultraviolet light and darkness for 3-4 days. The fungus developed and sporulated on the infected leaf tissue. Using a low-power stereomicroscope, a few conidia were removed with a needle spread onto potato dextrose agar (PDA) medium. The inoculated Petri dishes were then placed in

an incubator at 28°C for 3 days (Chowdhury *et al.,* 2015). After establish of the culture in the media and compared with fungal description of Sharma *et al.* (1994) and identify were confirmed.

Rice hull and rice grain were added proportionately and thoroughly mixed, transferred to open mouthed bottles and closed with a cotton wool plug. The desired quantity of water was added. The bottles were sterilized at 15 psi for 2 hr for two successive days. The medium was used to grow *R. solani* pathogen. From seven days old culture of the pathogen grown in PDA, six discs of nine mm were taken and inoculated into each bottle. The bottles were then incubated at room temperature (28ºC) for 14 days and the inoculum thus prepared was used for subsequent studies (Anonymus, 2012). The isolate of *S. oryzae* was also isolated and multiplied as described above. The isolate was identified according to fungal description by Mew and Mishra (1994).

Inoculation of the Pathogens

The isolates *R. solani* and *S. oryzae* spore coated Rice hull and rice grain are inserted in the sheath region of the plant at early boot stage (Anonymus, 2012).

Preparation of Ecofriendly Components

Preparation of bio inoculants

P. fluorescens and *B. subtilis* were isolated from the rhizosphere soil of healthy rice cultivating fields by serial dilution technique. The *P. fluorescens* was identified by starch hydrolysis test **(**Cappucino, 1983) and B. *subtilis* was identified by starch hydrolysis test **(**Cappuccino and Sherman 2002**)** prepare as talc based formulation (Vidhyasekaran and Muthamilan 1995).

Preparation of Annamalai Mixture

The extracts of cow urine, cow dung, sheep dung, poultry litter and neem cake at 100% concentration were taken and mixed thoroughly at the ratio of 1:1:1:1:1(Kurucheve *et al.*, 1999).

Preparation of Fortified Lignite Fly Ash

New formulation developed by us. Strain of *P. fluorescens* and *B. subtilis* were grown in nutrient broth for 48 hrs as a shake culture in rotary shaker at150 rpm. At room temperature (25±2°C). Lignite fly ash (class F) was collected from Neyveli lignite corporation, Neyveli. One kg of lignite fly ash was added with 10gofcarboxymethylcellulose and mix well. These carriers were autoclaved for 30 min of two consecutive days. One kg of carrier material was added with Four hundred ml of the each bacterial suspension, containing 9×10^8 colony forming units (CFU) plus 20 ml of molasses and mixed well and shade dry for 2 hrs under sterile conditions. The above mentioned product is named as Fortified lignite fly ash. Then packed in polythene bags, sealed and stored at room temperature (25±2ÚC). The population of antagonistic was estimated at monthly interval for 3 month by using serial dilution technique.

Induction of Defense Mechanism and Experimental Design

The pot culture experiment was conducted with 8 treatments and three replications each at Department of Plant Pathology, Annamalai University, Annamalainagar from June to August 2018. Fifteen kg of top soil, collected from a rice growing field, was steam pasteurized and filled in 45 x 30 cm size cement pots. Thirty days old seedling of rice ADT 36 was transplanted in pot to access the induction of defense enzyme by ecofriendly components *viz*., *P. fluorescens*, *B. subtilis*, Annamalai mixture, and Fortified lignite fly ash were tested against challenge inoculation of *R. solani* and *S. oryzae*. The talc based formulation of *P. fluorescens* and *B. subtilis* were used @ 2 x 10^{-8} CFU g^{-1}. The seeds were treated @ 10 g/kg of seed and dried in shade condition for four hours before sowing, 0.2% conc. of talc based formulation *P. fluorescens* and *B. subtilis* were used as foliar application and talc based formulation *P. fluorescens* and *B. subtilis* were applied to the soil @ 10 kg/ha. Newly formulated lignite fly ash was applied to the soil @ 40 kg/ha at the time of transplanting in dust formulation and applied to the foliar @ 30 kg/ha during the late booting stage as dust formulation. Twenty per cent conc. of Annamalai Mixture was used for seed treatment and also used as foliar spray during the tillering stage @ 20 lit/ha. The chemical Tricyclazole was used for foliar spray @ 0.6 g/lit as standard chemical check. The artificial inoculation of *R. solani* and *S. oryzae* by insertion placement with spore coated Rice hull and rice grain method into the rice sheath. The inoculated plant were kept in the laboratory for 24 hours to maintain a high relative humidity and subsequently moved to a green house maintained at 28±2º C, 70 to 90% relative humidity, under a light intensity of 85 µmol m-1 S-1, 12 hour photoperiod and subsequently transferred to experimental yard.

Enzyme Extraction

The plant tissues collected from rice plants were immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. Plighting extract prepared from leaves was used for the estimation of peroxidase (PO), polyphenol oxidase (PPO) L-phenylalanine ammonia-lyase (PAL) and β-1,3glucanase.

Assay for Peroxidase (PO) (Hammerschmidt *et al*., 1982)

Peroxidase activity was assayed spectrophotometrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2 which was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 ηm was recorded at 30 sec. interval for 3 min and the boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} on fresh weight basis.

Assay for Polyphenol Oxidase (PPO) (Mayer *et al*., 1965)

The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 µm min^{-1} g^{-1} fresh weight of tissue.

Assay for Phenylalanine Ammonia-Lyase (PAL) (Ross and Sederoff, 1992)

The assay mixture containing 100 µl of enzyme, 500 µl of 50 mm Tri HCl (pH 8.8) and 600 µl of 1mML-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later, 1.5 ml of toluene was added and vortexed for 30 sec. the centrifuged (1000 rpm, 5 min) toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured at 290 ηm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as ηmoles of cinnamic $acidmin^{-1}$ g fresh $tissue^{-1}$.

Assay for β-1, 3-Glucanase (Pan *et al*., 1991)

Crude enzyme extract of 62.5 µl was added to 62.5 µl of 4 per cent laminarin and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid (DNS) and heated for 5 min on boiling waterbath (DNS prepared by adding 300 ml of 4.5 per cent NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartarate). The resulting coloured solutions were diluted with distilled water, vortexed and the absorbance was read at 500 µm. The crude extract preparation mixed with laminar in at zero time incubation served as blank. The enzyme activity was expressed as µg equivalents of glucose min^{-1} g fresh $weight^{-1}$.

Result and Discussion

The pot culture experiment was carried out to access the induction of defense enzyme by application of eco friendly components *viz*., *P. fluorescens* and *B. subtilis,* Annamalai mixture and Fortified lignite fly ash in rice plants challenged with pathogens sheath blight (*R. solani)* and sheath rot (*S. oryzae)*. All the ecofriendly components significantly inducethe defense enzymes than the control (Table1). Among the treatments high amount of PO (14.96), PPO (0.95), PAL (234.4) and β-1, 3-Glucanase (14.96) were observed in the combined application of *P. fluorescens* and *B. subtilis* as seed @ 10g/kg of seed, soil @ 10 kg/ha at transplanting, foliar spray @ 10 kg/ha at tillering and boot leaf stage plus spraying Annamalai Mixture @ 20 lit /ha at tillering stage along with Fortified lignite fly ash as soil @ 40 kg/ha at transplanting and foliar dust @ 30 kg/ha at boot leaf stage (T_5). This was followed by the standard chemical check Tricyclazole seed treatment @ 2g/kg and foliar application @ 0.6g/lit of Tricyclazole, at tillering and boot leaf stage (T_6). The rice plants inoculated with the pathogen alone recorded an increase in the activity of PO (13.90), PPO (1.240), PAL (0.870) and β-1, 3-Glucanase (213.9) than the rice plants either treated with pathogen or treated with antagonists. The induction of PO, PPO, PAL and β-1, 3-Glucanase gradually increases up to fifth day of pathogen inoculation and thereafter decline slowly. Several authors have reported the induction of defense enzymes in crop plants treated with biocontrol agents and animal products (Ramamoorthy *et al.,* 2002; Singh *et al.,* 2003; Kachroo *et al.,* 2006; Tiwari *et al.,* 2011) ISR is a systemic response, expressed in both roots and shoots, that can affect a wide range of organisms.

Table 1 : Induction of defense enzyme activity in rice plants treated with ecofriendly components against *R. solani* and *S. oryzae* under pot culture condition

Treatments	β- 1, 3 glucanase activity in plants Time interval (days)					PO activity in plants Time interval (days)					PPO activity in plants Time interval (days)					PAL activity in plants Time interval (days)				
	1	3	5	7	9	1	3	5	7	9	1	3	5	7	9	1	3	5	7	9
T1	45.90	78.40	139.4	179.9	145.9	7.110	8.110	9.180	11.00	10.08	0.290	0.510	0.810	1.020	1.000	0.460	0.620	0.640	0.710	0.690
T2	47.40	83.80	149.7	190.9	157.8	7.263	8.620	9.560	11.53	10.90	0.300	0.540	0.850	1.090	1.050	0.480	0.650	0.690	0.790	0.770
T3	46.50	82.50	145.1	187.8	151.9	7.150	7.663	9.030	10.55	9.90	0.257	0.500	0.790	1.030	0.960	0.460	0.590	0.630	0.680	0.660
T4	49.50	97.70	162.9	210.3	168.5	7.860	8.657	10.35	12.16	11.54	0.320	0.810	0.100	1.190	1.150	0.500	0.720	0.770	0.820	0.800
T5	51.90	99.90	170.1	234.4	180.1	7.990	9.930	12.57	15.27	14.96	0.340	1.140	1.210	1.410	1.303	0.520	0.790	0.860	0.950	0.940
T6	50.00	98.40	163.5	213.9	171.6	7.900	9.030	11.01	13.90	12.95	0.330	0.950	1.000	1.240	1.190	0.510	0.740	0.810	0.870	0.820
T7	19.60	25.00	28.4	31.90	20.66	6.960	7.120	7.960	8.08	7.57	0.190	0.210	0.220	0.260	0.240	0.440	0.460	0.470	0.480	0.470
T8	17.60	19.70	20.07	20.01	20.01	6.910	7.010	7.010	7.01	7.01	0.180	0.190	0.190	0.190	0.190	0.440	0.450	0.450	0.450	0.450
CD (p=0.05)	0.173	0.173	0.176	0.162	0.440	0.044	0.495	0.017	0.017	0.306	0.034	0.017	0.017	0.041	0.057	0.017	0.017	0.017	0.017	0.017

T_1 – Combined application of *P. fluorescens* and *B. subtilis* as seed @ 10g/kg of seed, soil @ 10 kg/ha at transplanting, foliar spray @ 10 kg/ha at tillering and boot leaf stage, T_2 – Application of Annamalai Mixture as seed, foliar spray @ 20 lit /ha at tillering stage and boot leaf stage, T_3 – T_1 +T_2, T_4– Application of fortified lignite fly ash as soil @ 40 kg/ha at transplanting and foliar dust @ 30 kg/ha at boot leaf stage, T_5– T_3+ T_4, T_6– Seed treatment @ 2g/kg, foliar application of Trycyclazole @ 0.6g/lit. at tillering and boot leaf stage, T_7– Healthy control and T_8– Inoculated control.

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