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ROLE OF DEFENCE ENZYMES ACTIVITY IN RICE AS INDUCED BY IDM FORMULATIONS AGAINST SHEATH BLIGHT CAUSED BY *RHIZOCTONIA SOLANI*

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ABSTRACT

Integrated disease management (IDM) of rice sheath blight is gaining momentum and encompasses all the available control methods with each method compensating the deficiencies of others. Among the available IDM practices, combined use of chemical, cultural, biological and host plant resistance is a common phenomenon.Combined applications of bio agent with chemical fungicides are an important IDM package against sheath blight disease. The use of fungal bioagents in controlling rice sheath blight in an IDM is gaining more importance.The combination of seaweeds, Annamalai mixture with P. fluorescenswas used for the Integrated Disease Management.Induction of defence enzymes in crop plants treated with bio-control agents and challenged with the pathogen. Defense reaction occurs due to accumulation of peroxidase, phenylalanine ammonia lyase and PR-protein like β-1, 3-glucanase etc. by estimating theses enzyme activity helpful to study the role of these enzymes in defence against the plant pathogen. Among the various treatment, the plants treated with S. wightii, P. fluorescens and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with R. solani (T_6) recorded a maximum induction of β -1,3-glucanase activity 76.2 µg of Glucose released/min/g of fresh tissue on 7th day after pathogen inoculation. Among the various treatment, the plants treated with S. wightii, P. fluorescens and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with R. solani (T_6) recorded maximum induction of Peroxidase activity (61.11 changes in absorbance/min/g of fresh tissue) at 7th day after pathogen inoculation. Application ofS. wightii, P. fluorescensand Annamalai mixture (seed,prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with R. solani (T_6) recorded maximum induction of PPO activity(3.50 changes in absorbance/min/g of fresh tissue) at 7th day, which decreased further. PAL activity was found to increase significantly in plants treated with S. wightii,P. fluorescensand Annamalai mixture (seed,prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with R. solani (T_6) recorded maximum induction of PAL activity (5.20 changesin absorbance/min/g of fresh tissue) at 7th day there after it decreased. In conclusion, prior treatment of combined application ofS. wightii, P. fluorescensand Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) (T_5) followed by challenge inoculation with R. solanitriggered the plant mediated defense mechanism that in turn reduce the sheath blight incidence in rice.

Keywords: Seaweeds, Integrated disease management, bio control, Rice sheath blight

I. INTRODUCTION

1.1. Integrated disease management

Integrated disease management (IDM) of rice sheath blight is gaining momentum and encompasses all the available control methods with each method compensating the deficiencies of

others. Among the available IDM practices, combined use of chemical, cultural, biological and host plant resistance is a common phenomenon. However, host plant resistance to sheath blight range only from very susceptible to moderately susceptible levels in rice (Groth and Bond, 2007), thus chemical management has become a necessary component for an effective IDM. Combined applications of bio agent with chemical fungicides are an important IDM package against sheath blight disease. The use of fungal bioagents in controlling rice sheath blight in an IDM is gaining more importance.

Combined applications of bio-agent with chemical fungicides are an important IDM package against sheath blight disease. Application of bioagents with soil organic amendements such as FYM, wheat straw, daincha (*Sesbaniaaculeata*), saw dust and neem cake worked effective in managing rice sheath blight disease and also in increasing the grain yield(Khan and Sinha, 2006). Combined field applications of *T. viride* (5kg) and validmycin (2L)/ha was found to be effective in controlling sheath blight and sheath rot diseases of rice besides enhancing crop yield (Daroga *et al.,* 2007).Spraying of the spore suspension of *T. viride* (Tv3235) along with carbendazim (0.1%) and soil applications of FYM (1%) + saw dust (1%) showed maximum reduction in sheath blight severity, per cent disease incidence and significant increase in grain yields over control (Sundarraj *et al.,*1996).

Green house and field studies against rice sheath blight pathogen with different bacterial bioagents isolated from farmyard manure, rice seed, rice phyllosphere and rice rhizosphere proved that three bacteria, PF-9 *(Pseudomonas fluorescens*), B44 (*Bacillus* sp), and Chb-1 (chitinolytic bacterium) are compatible with carbendazim (Bavistin) at 500 and 1000 ppm concentrations. Among the three bioagents, PF-9 was most effective in reducing disease severity either alone or in combination with one spray of 0.1% Bavistin @ 0.1 per cent, followed by combination of PF-9 and B-44 (Laha and Venkataraman, 2001).

Other IDM packages that were found effective against sheath blight are combined use of botanicals, fungicides and organic amendments. Janki Kandhari (2007) reported that Achook (Azadirachtin), a neem based chemical performed better with a sheath blight disease incidence of 65% compared to control (83%). Asbhrafuzzaman *et al.,*(2005) reported that sheath blight disease development was least and mean filled grains per panicle, 1000 grain weight, straw and grain yields were higher in pot culture studies with combined doses of ash, bleaching powder, poultry manure and bavistin over control.

1.2. Induction of systemic resistance

The bio-control agents bring about induced systemic resistance (ISR) fortifying the physical and mechanical strength of cell wall as well as chemical, physiological and biochemical reaction of host leading to synthesis of defense chemicals against pathogens. Defense reaction occurs due to accumulation of peroxidase, phenylalanine ammonia lyase and PR-protein like β-1, 3-glucanase etc. Several authors have reported the induction of defense enzymes in crop plants treated with bio-control agents and challenged with the pathogen (Suthin raj *et al*.,2008).

1.3. Induction of defense enzymes activity

1.3.1. Changes inperoxidase activity (Po)

Peroxidase (PO), part of the PR-9 family have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross linking, indole acetic acid oxidation, cross linking of extensin monomers, oxidation of hydroxyl cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran, 1997).

Bradley *et al.,* (1992) reported that increased PO activity has been correlated with resistance in many plants including barley, cucurbits, cotton, tobacco, wheat and rice and this enzyme is involved in the polymerization of proteins and lignin or suberin precursor into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls or movement

through vessels. Increase in PO activity associated with induced systemic resistance was observed in cucumber (Nandi *et al.,* 2013).

Increased PO and PPO on treatment with cow dung water extract might have induced systemic resistance against *X. oryzae* pv. *oryzae* (Sible, 2000). Cow and horse manure compost increased the PO activity of treated cucumber plants (Venkatesh *et al.,*2013).

1.3.2. Changes in polyphenol oxidase activity (PPO)

Polyphenol oxidase (PPO) usually accumulates upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult because the quinonoid reaction products of PPO covalently modify and cross link the enzyme. The increased activation of PPO could be detected in the cucumber leaf in the vicinity of lesions caused by some foliar pathogens. Moreover PPO can be induced by octadecanoid pathway (Constabel *et al.,,* 1995).

Cucumber seeds treated with *Brevibacillus brevis* showed a higher activity of PPO 12 days after pathogen inoculation (Liang *et al.,* 2013).

Accumulation of PPO was higher in the combination of *Pseudomonas* strains treated banana plants (Harish, 2005) and in rice plants pretreated with PGPR isolates (Nandi *et al.,* 2013).

1.3.3. Changes in phenylalanine ammonia-lyase activity (PAL)

Phenylalanine ammonia-lyase (PAL) is a defense gene activated in the incompatible interaction, catalyzing the determination of L-phenylalanine to produce cinnamic acid; a substrate feeding to several biosynthetic routes to various classes of phenylpropanoid derived secondary plant products (Halbrook and Sheel, 1989). PAL activity provide precursors for lignin biosynthesis and other phenolics that accumulate in response to infection, for example; salicyclic acid (SA) a molecule that is essential for systemic acquired resistance (Klessig and Malamy, 1994). Mauch-Mani and Slusarenko (1996) reported that the major function of PAL is production of SA precursors in the resistance of *Arabidopsis* to *Peronospora parasitica.*

PAL is the key enzyme in inducing synthesis of salicylic acid (SA) which induces systemic resistance in many plants. The gene was cloned and transgenic rice plants expressing PAL showed systemic resistance against rice pathogens (Lamb *et al.,*1997). Seed treatment and seedling root dipping with PGPR induced early and enhanced levels of PAL in rice plants (Nandi *et al.,* 2013). Plants treated with *Pseudomonas* strains initially showed higher levels of PAL and PO in susceptible rice seedlings pretreated with the *T. harzianum* strain NF 9 at 16, 96 and 120 h. after pathogen inoculation (Venkatesh *et al.,*2013).

Suthin Raj *et. al* (2008) reported that cotton seeds treated with endophytic bacterial bioagents challenged with *R. solani* resulted in increased activity of PAL when compared to uninoculated control.

1.3.4. Changes in β-1, 3 glucanase

Evidence of β-1,3-glucanases in disease resistance was first reported by Kauffimann *et al.,*(1987). In dicots, β-1, 3-glucanase genes are considered to constitute a part of the general array of defense genes induced during pathogenesis (Mauch *et al.,* 1988). β-1,3-glucanases especially in conjunction with chitinase are capable of hydrolyzing fungal cell walls *in vitro* (Mauch *et al.,*1988). Both of these enzymes are co-induced in response to pathogen attack (Vogeli *et al.,*1988).

Induction of β-1, 3-glucanases was demonstrated in barley and other monocots like wheat, rice and sorghum in response to infection by the necrotrophic pathogen *Bipolaris sorokiniana* (Jutidamrongphan *et al.,*1991). Daugrosis *et al.,*(1992) reported rapid induction of β-1, 3-glucanases in bean with challenge inoculation of *Colletotrichum lindemuthianum.* Purified fungal elicitor also induced these enzymes in the bean host. Purified acidic β-1,3-glucanases from cucumber had antifungal activity against *R. solani* (Ji and Kuc, 1996). Increased resistance to *Peronospora*

tabacina and *Phytophthora parasitica* var. *nicotinae* in transgenic tobacco expressing β-1,3-glucanase cDNA coding for the PR-N was reported by (Venkatesh *et al.,*2013). Anita and Samiyappan(2012) found an 8-fold increase in β-1, 3-glucanase in bean in response to PGPR treatment and such treatment offered protection against pathogenic *R. solani.*

II. MATERIALS AND METHOD

2.1. Induced systemic resistance

2.1.1. Sample Collection

A glasshouse experiment was laid out in completely randomized design using the ADT-36 variety to assess the induction of defense enzymes by IDM formulation against challenge inoculation of *R. solani* with following treatments.

T_1 – Application of *Sargassum wightii*(Seed treatment (10g/kg) + prophylactic spray at 20, 35 and 50 DAT)
T_2 – Application of Annamalai mixture (Seed treatment (10ml/kg) + prophylactic spray at 20, 35 and 50 DAT)
T_3 – Application of *P. fluorescens*(Seed treatment (10g/kg) + prophylactic spray at 20, 35 and 50 DAT)
T_4 – $T_1 + T_2$
T_5 – $T_1 + T_3$
T_6 – $T_1 + T_2 + T_3$
T_7 – Seed treatment with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)
T_8 – Inoculated control
T_9 – Healthy control

Twenty days after transplanting, the plants were challenge inoculated with a conidial suspension of *R. solani* with a spore load of 1×10^6 ml^{-1}. The samples of the above treated plants were collected at different time intervals (1, 3, 5, 7 and 9 days) after pathogen inoculation.Three replications were maintained in each treatment. Fresh plant samples were used for analysis.

2.2 Enzyme extraction

The plant tissues collected from 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.Plightingextract prepared from leaves was used for the estimation ofperoxidase (PO), polyphenol oxidase (PPO) and L-phenylalanine ammonia-lyase (PAL).

2.3 Spectrophotometric assay

2.3.1 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 wasexpressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} on fresh weight basis (Fig 3).

2.3.2. 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 (Fig 4).

2.3.3 Phenylalanine ammonia-lyase (PAL) (Ross and Sederoff, 1992)

The assay mixture containing 100 µl of enzyme, 500 µl of 50 mM Tris 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} (Fig 5).

2.3.4. 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 water bath (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} (Fig 6).

III. RESULTS

3.1. Induction of defense enzymes

Green house study was conducted to test the inductionof defense enzyme on rice plants with different application of IDM formulation.

3.1.1. β-1, 3-glucanase

β- 1, 3-glucanase activity was observed in the leaf samples of rice at different day's interval. Among the various treatment, the plants treated with *S. wightii*, *P. fluorescens* and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_6) recorded a maximum induction of β -1,3-glucanase activity 76.2 µg of Glucose released/min/g of fresh tissue on 7th day after pathogen inoculation. It was followed by the plants treated with hexaconazole (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_7) recorded 74.1 µg of Glucose released/ min/g of fresh tissue on 7th day after pathogen inoculation. The enzyme activity was significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 15 and Figure 3).

3.1.2 Peroxidase (PO)

The activity of PO was observed in leaf sample of rice at different day's interval. Among the various treatment, the plants treated with *S. wightii*, *P. fluorescens* and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_6) recorded maximum induction of Peroxidase activity (61.11 changes in absorbance/min/g of fresh tissue) at 7th day after pathogen inoculation. It was followed by the plants treated with hexaconazole (seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_7) recorded a maximum induction of 60.50 changes in absorbance/min/g of fresh tissue respectively at the 7th day after pathogen inoculation. Theenzyme activity was significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 16& Figure 4).

Table 1. β- 1,3 glucanase activity* in rice plants treated with different IDM formulations under greenhouse condition

Treatments	β-1,3 glucanase activity * in plants				
	Time interval (days)				
	0	1	3	5	7
T_1 – Application of *Sargassum wightii*(Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	46.2	82.9	158.9	220.2	166.9
T_2 – Application of Annamalai mixture (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	44.5	79.0	155.3	208.9	163.8
T_3 – Application of *P. fluorescens* (seed treatment + prophylactic spray at 20, 35 and 50 DAT)	45.9	80.5	157.5	210.5	164.2
T_4 – $T_1 + T_2$	47.8	93.8	159.8	230.9	168.2
T_5 – $T_1 + T_3$	47.0	90.30	159.1	225.6	167.2
T_6 – $T_1 + T_2 + T_3$	49.5	96.8	162.6	233.2	170.5
T_7 – Seed treatment with Hexaconazole + spraying 20,35 and 50 DAT)	48.2	64.3	160.9	174.1	159.2
T_8 – Inoculated control	18.5	18.6	18.9	19.0	18.9
T_9 – Healthy Control	18.5	22.9	27.8	30.8	19.9

CD for Treatment: 0.06. CD for time interval (Day's): 0.08.
CD for interaction between Treatment × Time interval (Days): 0.17.
*µg of glucose released/min/g of fresh tissue
**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P=0.05)

3.1.3 Polyphenol oxidase (PPO)

Application of *S. wightii*, *P. fluorescens*and Annamalai mixture (seed,prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_6) recordedmaximuminductionofPPOactivity(3.50 changes in absorbance/min/g of fresh tissue) at 7th day, which decreased further. Without inoculation of pathogen and IDM combination recorded a minimum poly phenol activity when compared to all other treatments. In all the treatments enzyme activity was increased up to 7th day there after declined (Table 17& Figure 5).

3.1.4. Phenylalanine ammonialyase (PAL)

PAL activity was found to increase significantly in plants treated with *S. wightii*,*P. fluorescens*and Annamalai mixture (seed,prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_6) recorded maximum induction of PAL activity (5.20 changesin absorbance/min/g of fresh tissue) at 7th day there after it decreased.It was followed by the application of hexaconazole(seed, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R.solani*(T_7) recorded a maximum induction at the 7th day of 5.09 changes in absorbance/min/g of fresh tissue respectively. The enzyme activity was significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 18& Figure 6).

Table 2.Peroxidase activity* in rice plants treated with different IDM formulations under greenhouse condition

Treatments	PO activity * in plants				
	Time interval (days)				
	1	3	5	7	9
T_1 – Application of *Sargassumwightii* (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	6.92	17.10	34.26	58.66	43.30
T_2 – Application of Annamalai mixture (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	6.19	16.15	32.50	57.86	42.10
T_3 – Application of *P.fluorescens* (seed treatment+ prophylactic spray at 20, 35 and 50 DAT)	6.80	16.98	33.81	58.10	42.90
T_4 – $T_1 + T_2$	7.02	17.91	35.54	59.95	44.60
T_5 – $T_1 + T_3$	7.00	17.55	34.95	59.10	44.06
T_6 – $T_1 + T_2 + T_3$	8.20	19.16	37.80	61.11	47.25
T_7 – Seed treatment with Hexaconazole + spraying 20,35 and 50 DAT)	7.75	18.58	36.52	60.50	45.80
T_8 – Inoculated control	3.36	6.91	9.10	11.56	9.60
T_9 – Healthy Control	4.80	8.80	26.20	40.06	31.52

CD for Treatment: 0.06. CD for time interval (Day's): 0.08.
CD for interaction between Treatment × Time interval (Days): 0.17.
*Changes in absorbance/min/g of fresh tissue
**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 3. Polyphenoloxidase activity* in rice plants treated with different IDM formulations under greenhouse condition

Treatments	PPO activity * in plants				
	Time interval (days)				
	1	3	5	7	9
T_1 – Application of *Sargassum wightii* (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	0.27	0.93	2.11	2.97	2.11
T_2 – Application of Annamalai mixture (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	0.24	0.90	2.01	2.90	2.14
T_3 – Application of *P. fluorescens* (seed treatment+ prophylactic spray at 20, 35 and 50 DAT)	0.26	0.91	2.06	2.92	2.00
T_4 – $T_1 + T_2$	0.29	0.98	2.27	3.16	2.20
T_5 – $T_1 + T_3$	0.28	0.95	2.19	3.07	2.17
T_6 – $T_1 + T_2 + T_3$	0.32	1.12	2.48	3.50	2.66
T_7 – Seed treatment with Hexaconazole + spraying 20,35 and 50 DAT)	0.30	1.01	2.31	3.36	2.45
T_8 – Inoculated control	0.11	0.18	0.58	0.82	0.68
T_9 – Healthy Control	0.16	0.22	0.85	1.16	0.91

CD for Treatment: 0.05. CD for time interval (Day's): 0.07.
CD for interaction between Treatment × Time interval (Days): 0.16.
*Changes in absorbance/min/g of fresh tissue
**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 4. Phenylalanine ammonia-lyase activity* in rice plants treated with different IDM formulations under greenhouse condition

Treatments	PAL activity * in plants				
	Time interval (days)				
	1	3	5	7	9
T_1 – Application of *Sargassum wightii* (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	0.45	0.90	2.27	4.96	4.45
T_2 – Application of Annamalai mixture (Seed treatment + prophylactic spray at 20, 35 and 50 DAT)	0.41	1.84	2.14	4.81	4.26
T_3 – Application of *P. fluorescens* (seed treatment+ prophylactic spray at 20, 35 and 50 DAT)	0.43	0.86	2.20	4.90	4.35
T_4 – $T_1 + T_2$	0.47	0.98	2.30	5.00	4.10
T_5 – $T_1 + T_3$	0.46	0.95	2.29	4.98	4.27
T_6 – $T_1 + T_2 + T_3$	0.50	1.12	2.66	5.20	4.41
T_7 – Seed treatment with Hexaconazole + spraying 20,35 and 50 DAT)	0.48	1.05	2.42	5.09	4.22
T_8 – Inoculated control	0.24	0.37	0.82	1.21	0.90
T_9 – Healthy Control	0.27	0.40	1.02	1.85	1.26

CD for Treatment: 0.05. CD for time interval (Day's): 0.06.
CD for interaction between Treatment × Time interval (Days): 0.15.
*Changes in absorbance/min/g of fresh tissue
**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P=0.05)

IV. DISCUSSION

Plants are bestowed with various defense related genes and it is well known that the defense genes are sleeping genes and appropriate stimuli or signals are needed to activate them. Inducing the plants own defense mechanisms by prior application of biological inducer is thought to be a novel plant protection strategy. Inductions of systemic resistance by *P. fluorescens* have been reported earlier by several workers (Ngullie*et al.,* 2010). Seed treatment with *P. fluorescens* suppressed the foliar pathogen by inducing systemic resistance (Wei *et al.,* 1991 and 1996).

In the present study, rice, plants pretreated with *S. wightii, P. Fluorescens*and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) (T_5) significantly induced the synthesis and accumulation of β-1,3-glucanase, PO, PPO and PAL, against*R. Solani*when compared to all the treatments. Besides, *P. fluorescens* strain P. f- 1 effectively increased defense related enzymes β-1,3-glucanase, PO, PPO and PAL in rice plants in host against the pathogen.

The enzyme activity significantly increased from7^{th} day after the pathogen inoculation and then activity was declined in all the treatments. Similar results were reported by Nakkeeran*et al.,* 2006, who reported that peat soil based formulation of *P. chlororaphis* strain PA 23 and *B. subtilis*strain BSCBE4 enhanced the maximum induction of PR protein than the application of individual bio-agents in hot pepper against *Pythiumaphanidematum*. Application of *Ascophyllumnodosum*+ chlorothalonil treatment was recorded maximum induction of PO, PPO, PAL, β-1,3-glucanase, and total phenols than the application of *A. nodosum*alone in carrot against *Alternaria* leaf spot disease(Jayaraj*et al.,* 2008). Isolates of *P. fluorescens*GB 10 or GB 27 applied in combination

with *T. viride*pq 1 recorded the maximum induction of β-1,3-glucanase and chitinase than the *T. viride*alone in groundnut against stem rot (Jetiyanon and Kloepper, 2002). *P. fluorescens* enhanced the induction of PR protein (Anand*et al.,* 2010;). Usha Rani *et al.*, 2008 revealed that the bacterized seed + soil application with *P. fluorescens* led to accumulation of PO and PPO in tomato fusarial wilt.

Increase in PO and PPO activity at a later stage may contribute to cross linking of hydroxyproline rich glycoproteins (HRGPs), lignifications that will act as barriers against pathogen entry. PO-generated hydrogen peroxide may function as an anti-fungal agent in disease resistance. Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals that are antimicrobial in nature (Chen *et al.,* 2000). PO is a key enzyme in the biosynthesis of lignin and other oxidised phenols (Bruce and West, 1989). PAL plays an important role in the biosynthesis of phenolics and phytoalexins(Daayf*et al.,* 1997). The increase in PAL activity indicates the activation of the phenyl propanoid pathway.

The product of PAL is trans-cinnamic acid, which is an immediate precursor for the biosynthesis of salicylic acid; a signal molecule in systemic acquired resistance (SAR) (KlessigandMalamy, 1994). PAL is a key enzyme of phenylpropanoid metabolism which leads to the synthesis of phenols (Massala*et al.,* 1980). Hydrolytic enzymes such as chitinase and β-1,3-glucanase at the site of penetration of fungal hyphae of *F. oxysporum*f. sp. *pisi*. These enzymes act upon the fungal cell wall resulting in degradation and loss of inner contents of cells (Benhamou*et al.*, 1996). The enzymatic degradation of the fungal cell wall may release non-specific elicitors (Hammerschmidt *et al.,* 1982) which in turn elicits various defense reactions. The fungal cell wall elicitors have been reported to elicit various defense reactions in green gram (Ramanathan*et al.*, 2000). The phenomenon of the free-proline accumulation in plants exposed to diverse environmental and biological stresses have considerable physiological significance.

Prior treatment of combined application of *S. wightii*, *P. fluorescens*and Annamalai mixture (seed, prophylactic spraying at 20, 35 and 50 DAT) (T_5) followed by challenge inoculation with *R. solani*triggered the plant mediated defense mechanism that in turn reduce the sheath blight incidence in rice.

VI. ACKNOWLEDGMENT

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