academicJournals

Vol. 8(25), pp. 3289-3293, 4 July, 2013
DOI: 10.5897/AJAR12.2199
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of *Stoechospermum marginatum* (Ag). Kutz extract on controlling blast disease in rice caused by *Pyricularia oryzae* under natural field condition

G. Flora* and S. Maria Victorial Rani

St. Mary's College (Autonomous), Thoothukudi-628 001, Tamilnadu, India.

Accepted 14 June, 2013

Foliar application of aqueous concentrate of *Stoechospermum marginatum* in reducing the severity of fungal blast of rice caused by *Pyricularia oryzae* was investigated. The results indicated that, the seaweed concentrate significantly reduced the severity of the disease. Present study also revealed that, the peroxidase activity, phenylalanine ammonia-lyase activity and catalase activity were 184, 197, and 192% higher than the control (without any application) respectively. Seaweed concentrate enhanced sugar, amino acid, ascorbic acid, protein, phenol, flavonoid, and tannin content in 60th day by 123, 150, 110, 162, 145, 120, and 178%, respectively in comparison with control where as these were decreased in 90th day of assay. All the parameters were also analysed with foliar application of Bavistin, a common fungicide considered as reference control and the results were compared and discussed.

Key words: Foliar application, fungal blast of rice, bavistin.

INTRODUCTION

Rice is a staple food crop of India, providing 43% of calorie requirement for more than 70% of population. The productivity of rice is low in India (3,000 kg ha^{-1}) when compared to the world average of 40004 kg ha^{-1} and leading rice growing countries like China 6,289 kg ha^{-1} (FAO, 2006). Incidence of disease at the critical growth period reduces the growth and yield. Bacterial leaf blight, bacterial leaf streak, fungal diseases such as blast, black smut (bunt), false smut, leaf spot, sheath blight, fungo-viral disease and white tip caused by nematodes are the common diseases in paddy causing great loss in yield. Blast of rice is caused by *Pyricularia grisea* also named as *Pyricularia oryzae* and *Dactylaria oryzae*, it was reported to occur in every rice growing area of the world. In India, the disease may cause damage up to 75% and the success depends on continuous input of fertilizers, pesticides and other energy using practices. So an alternative strategy is to be practiced to achieve success in crop protection and yield. Hence a trial was made to bring out the effect of concentrate of *Stoechospermum marginatum* (SWC) on growth, yield and control of fungal blast disease in *Oryza sativa.*

MATERIALS AND METHODS

Field experiment was conducted at Kuppanapuram, Thoothukudi District, Tamil Nadu in 2009. The soil type was loamy sand with pH 7.1. The experimental plot of 10 feet length and 6 feet breadth was orderly designed as control, reference control and experimental in 3 replicates. Healthy seeds obtained from June were planted during August prior to monsoon to prevent wash off of the SWCs from the leaves. After 30 days of growth, application of bavistin and SWC was done regularly once in 10 days with a hand sprayer until the leaves wet completely and runs down during the morning hours. Few plants were randomly selected for measurements and the

*Corresponding author. E-mail: floranarayanan@yahoo.com. Tel: 9976940601.

Table 1. Impact of *S. marginatum* concentrates on infection index.

S/N	Treatment	Infection index (%)
1	Control	58.6 ± 1.2
2	Bavistin	21.2 ± 1.8
3	*S. marginatum*	19.6 ± 1.3

Table 2. Impact of *S. marginatum* concentrate on growth parameters in *O. sativa* with blast disease in field condition.

S/N	Treatment	Number of leaves	Leaf area cm^2	Spot per leaf let	Shoot length	Root length
1	Control	38 ± 1.5	395.2 ± 2.1	26 ± 0.2	25.7 ± 1.8	11 ± 5
2	Bavistin	40 ± 1.6	720 ± 4.54	8 ± 0.45	35.5 ± 1.6	15 ± 4
3	*S. marginatum*	62 ± 2.5	1209 ± 2.48	4 ± 0.12	40.9 ± 2.46	16 ± 4

Aqueous concentrate obtained from *S. marginatum* were applied as foliar spray on 30 days old plants expressing disease symptoms (experimental) in field condition. Control- infected plants retained without any treatment, bavistin-reference control. All the parameters were recorded on 90^{th} day. Data are the mean of 10 replicates ± SD.

leaves were immediately analyzed for various enzyme activities.

The newly formed shoots were used for all the analyses. These evaluation of leaf spot disease (spot per leaf), growth parameters (number of leaves, leaf area, shoot length and root length), (Table 2) infection index, biochemical (protein, sugar, aminoacids, lipid, ascorbic acid, phenol, flavanoid and tannin) and enzymes (peroxidase, penylalanine ammonia-lyase, catalase, polyphenol oxidase, nitrogen reductase enzyme) were done on 90^{th} day after treatment and the data were tabulated.

RESULTS AND DISCUSSION

The results indicated that infection index (Table 1) was greatly reduced in SWC applied plot (19.6%) when compared with the control (58.6%) and reference control (21.2%) plots. Similarly number of spots per leaf (26) was also increased in both the controls. Overall growth was highly stimulated in SWC applied plants than others. The shoot length was 82 and 17% more in SWC and bavistin applied plants, respectively than the control. Leaf area was remarkably more in SWC treated (205%) than the control. However both the treatments had negligible influence on root growth.

The increased shoot growth, root growth, number of leaves, leaf area and decreased diseases incidence in the SWC treated plants observed in the present study would possibly be due to the seaweed components such as minerals nutrients, amino acids, vitamins, cytokinins, auxins and abscisic acid. These components when absorbed through leaves/petiole would affect the celluar metabolism leading to enhancement of all the growth parameters (Crouch and Van Staden, 1992; Reitz and Trumble, 1996; Durand et al., 2003; Stirk et al., 2003; Ordog et al., 2004: Rayorath et al., 2008; Maria et al., 2009). The polysaccharides, laminarin and fucoidan exhibit wide ranges of biological activities (Rioux et al., 2007). Sulfated fucoidans from brown algae have evinced biological activities in mammalian systems (McClure et al., 1992). Laminarin has been shown to stimulate natural defense responses in plants and is involved in the induction of genes encoding various pathogenesis-related (PR) proteins with antimicrobial properties (Van Loon and Van Strien, 1999). Laminarin induced the activation of JA signaling pathway and protection against pathogens in tobacco plants (Aziz et al., 2003). Combined use of *Pseudomonas aeruginosa* with seaweeds this include, *Melanothanus afaghusainii, Padina tetratromatica, Cystoclonium purpuraeum,* and *Hypnea valentiae* significantly reduced the infection of *Macrophomia phaseolina* and *Rhizochonia solani* on mash bean and sunflower (Shahnazdawar et al., 2007).

Biochemical constituents like soluble protein, total soluble sugar, free amino acid and lipids were enhanced by the SWC (Table 3). Total chlorophyll, chlorophyll-a, and chlorophyll-b were more in reference control and SWC treated leaves and lesser in the control. The amount of total chlorophyll was 7 and 6% higher than control and reference control respectively (Table 4). The secondary metabolite phenol was estimated at 56 mg catechol equ/g fw in SWC sprayed ones which was 145% higher than the control. However, same amount of phenol (56 mg catechol equ/g fw) was present in the reference control. Similar to phenol, quantity of flavonoid and tannin were found to be more or less same in reference control and experimental leaves but the amount was lesser in the control (Table 5).

This showed the post inflectional increase in phenolic contents, which could be due to enhanced synthesis of phenols or translocation of phenols (Sharma et al., 1983). Increase in phenols after infection was also observed in banana infected by *Cercospora musae* and *Heminthosporium gibberosporium* (Jaypal and Mahadevan, 1968) and in ground nut infected by *Phaeoisariopsin personata* (Jyosthna et al., 2004).

Table 3. Impact of *S. marginatum* concentrate on chlorophyll content in *O. sativa* with blast disease in field condition.

S/N	Treatment	Total chlorophyii (mg/g)
1	Control	1.079 ± 0.04
2	Bavistin	1.771 ± 0.08
3	*S. marginatum*	1.858 ± 0.08

Table 4. Impact of *S. marginatum* concentrate on biochemical components in *O. sativa* with blast disease in field condition.

S/N	Treatment	Soluble protein (mg/g/f.w)	Total soluble Sugar (mg/g/f.w)	Free amino acids (mg/g/f.w)	Lipid (mg/g/f.w)
1	Control	21.9 ± 0.95	16.40 ± 0.70	14.45 ± 4.09	13.6 ± 0.25
2	Bavistin	20.34 ± 9.49	19.29 ± 5.99	18.10 ± 4.45	15.4 ± 1.54
3	*S. marginatum*	35.72 ± 4.101	20.28 ± 5.28	22.28 ± 0.39	14.0 ± 0.64

Table 5. Impact of *S. marginatum* concentrate on phenol, flavonoid and tannin content in *O. sativa* with blast disease in field condition.

S/N	Treatment	Phenol (mg GAE Equ/g)	Flavonoid (mg Qt/g)	Tannin (mg CE/g)
1	Control	40.11 ± 5.48	3.1 ± 0.5	11.5 ± 0.05
2	Bavistin	56 ± 5.38	3.2 ± 0.6	16 ± 0.94
3	*S. marginatum*	56 ± 4.35	3.51 ± 0.01	19 ± 0.02

Aqueous concentrate obtained from *S. marginatum* were applied as foliar spray on 30 days old plants expressing disease symptoms (experimental) in field condition. Control- infected plants retained without any treatment, vistin-reference control, data are the mean of 5 replicates ± SD.

Table 6. Effect of *S. marginatum* concentrate on antioxidative enzyme activities in *O. sativa* with blast disease in field condition.

S/N	Treatment	Peroxidase activity (OD_{420}/g^{-min})	Penylalanine ammonia- Lyase activity (μmol transcinnamic acid $g.FW^{-1}min^{-1}$)	Catalase activity (OD_{240}/g^{-min})	Polyphenol oxidase activity (OD_{495}/g^{-min})
1	Control	0.47 ± 0.04	0.60 ± 0.04	0.15 ± 0.05	0.82 ± 0.044
2	Bavistin	0.64 ± 0.08	0.98 ± 0.06	1.60 ± 0.04	1.62 ± 0.08
3	*S. marginatum*	0.75 ± 0.07	1.15 ± 0.05	1.81 ± 0.04	1.97 ± 0.011

Presence of catechin in strawberry leaves inhibited infection by *Alternaria alternata*, lectins in higher concentration cause lysis and growth inhibition of many fungi. Tannin and some fatty acid like compounds such as dienes in high concentration in cells of young fruit, leaves or seeds affords them resistance to fungal pathogens (Sharma, 2006). Thus, it was evident that, the phenol, flavonoid, and tannin were synthesized by the host plant, *O. sativa* as a protective response against *P. oryzae*. Peroxidase activity in leaves treated with bavistin was enhanced in comparison with control. In SWC sprayed leaves, the activity was 184% higher than the control plots. Phenylalanine ammonia lyase activity was highly increased with SWC application and it was 8 fold higher than the control. Catalase activity was also higher in SWC treated leaves. Bavistin treatment also showed greater influence (Table 6). Similar trend could be noted in polyphenol oxidase activity, revealing that these antioxidative enzymes were related to host parasite interaction resulting in induced immunity to *C. arachidicola*.

Grain yield is directly related to nitrate reduction and a

Table 7. Influence of *S. marginatum* concentrate on nitrate reductase activity in *O. sativa* with blast disease in field condition.

S/N	Treatment	Nitrate reductase enzyme µmoles NO^-_2/gfw/h
1	Control	0.87 ± 0.04
2	Bavistin	1.28 ± 0.08
3	*S. marginatum*	1.82 ± 0.07

Table 8. Effect of SWC application on yield parameters in *O. sativa* with blast disease in field condition.

S/N	Seaweed sprayed	Number of panicles	Number of grains Per panicle	Total grain weight/plant(g)
1	Control	4 ± 0.2	55 ± 1.8	13 ± 1.1
2	Bavistin	7 ± 0.4	75 ± 2.1	20 ± 1.5
3	*S. marginatum*	9 ± 0.6	95 ± 2.8	24 ± 5.7

Aqueous concentrate obtained from *S. marginatum* were applied as foliar spray on 30 days old plants expressing disease symptoms (experimental) in field condition. Control- infected plants retained without any treatment, bavistin-reference control, data are the mean of 10 replicates ± SD.

cascade of enzymatic reactions lead to protein synthesis and its allocation to seeds. Assessment of nitrate reduction in terms of nitrate reductase activity and estimation of photosynthetic pigments foretell the yield potential of plants. Effect of SWC and bavistin application on nitrate reductase activity is presented in Table 7. On the 90th day NR activity was only 0.87 µmoles NO_2^-/gfw in the control while it was 1.28 µmoles NO_2^-/gfw and 1.82 µmoles NO_2^-/gfw, respectively in reference control and experimental plant. This shows that, nitrate metabolism was highly disturbed and affected in the infected (control) plants.

Effect SWC treatment on yield of rice was also studied. The yield parameters included number of panicles, number of grains per panicle and weight of grains per plant. The number of panicles formed depends on number of tillers. In SWC treated plants number of tillers associated with number of panicles, were more than the control.

SWC treated plants showed the highest number of panicles (9) while the control had only 4 panicles (Table 8). All the SWC treated plants produced more grains per panicle (75 to 95) than the control (55). Consequently the highest grain weight was observed in plants treated with SWC (24 g) in comparison with control (13 g) Application of bavistin also enhanced the grain yield, but the extent of enhancement was lesser than experimental ones.

It is evident that, seaweed application improved the yield by reducing the disease incidence and enhancing photosynthesis and nitrate assimilation. Pathogenesis related protein, a structurally diverse group of plant protein have been shown to be toxic to invading fungal pathogens. Under normal conditions they are present in trace amounts in plants, but produced in much greater concentrations following attack by pathogen or under stress. Such proteins exist inside the cells as well as in intercellular spaces in different plant parts; leaf, root, and seed. There are more than fourteen families of pathogenesis–related proteins recognized, some of which are β 1, 3 - glucanases, chitinases, lipoxygenases, proteinases, peroxidases, cystein- rich protein and glycine - rich protein (Ng, 2004) (Data are not shown).

Conclusion

The present study clearly indicates that, resistance or tolerance induced by SWC was highly effective in field condition. Also the study establishes that, mechanisms involved in inducing tolerance are complex, those suggested include synthesis of more secondary metabolites-phenols, flavonoids enhanced peroxidase, phenyl alanine lyase, catalase, and poly phenol oxidase activities.

ACKNOWLEDGEMENT

The authors wish to record their sincere thanks to the Department of Botany, St. Mary's College (Autonomous), Thoothukudi, Tamil nadu, India for providing adequate facilities to carry out the present work.

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