Plant Archives Vol. 19 No. 1, 2019 pp.1578-1582 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF COMBINED APPLICATION OF SEA WEED EXTRACTS AND BIO-INOCULANTS AGAINST *R. SOLANI* AND *S. ORYZAE* OF RICE AT CAUVERY DELTA REGION OF TAMILNADU.

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Abstract

Sheath blight and Sheath rot are the major diseases of rice in Tamilnadu particularly in Cauvery delta region. Pot and field trials was conducted to evaluate the efficacy of consortia application of components *viz.*, Biocontrol agents and sea weed extracts against the sheath blight and sheath rot diseases and yield parameters during the samba season of 2017. Among the different combination of sea weed, bio inoculants, combined application of *Pseudomonas fluorescens* as seed treatments @ 10 g/kg, soil @10kg/ha at transplanting, foliar spray @10kg/ha at tillering and boot leaf stage and *Sargassum wightii* as seed treatment @ 10g/kg , foliar spray @ 20 lit/ha at tillering and boot leaf stage significantly reduced sheath blight incidence (11.67,14.52,17.72) and sheath rot diseases incidence (10.32,13.48,15.54) in field condition. The same treatment recorded the maximum grain yield (3712.80 kg/ha). All the components treated plants significantly decreased the disease incidence and increased the grain yield as compared to control.

Key words: Antagonistic micro-organisms, sea weed extracts, sheath blight, sheath rot.

Introduction

Rice (*Oryza sativa* L.) is one of the important food crop of the world both in terms of area 163.7 m ha and production 749.8 m tones. About 90 per cent of the world wide is grown and consumed in Asia and 60 per cent of world population were depends on the rice for their half of the calorie intake (FAO, Rice market monitor, 2015). Rice cultivation is often subjected to several biotic stresses of diseases like Sheath blight, Sheath rot, Blast, Brown leaf spot and Bacterial blight which are the important ones. Among different fungal diseases of rice, Sheath blight caused by *Rhizoctonia solani* Kuhn and sheath rot caused by *Sarocladium oryzae* (Sawada) Games and Haksworth are the major disease 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 diseases of rice yield loss varies from 9.6 to 85 % (Sakthivel., 2001). Many method of plant disease control are presently being used to control such as physical, chemical and cultural methods. Use of fungicides to control the diseases causes several adverse effect *i.e.*, Development of resistance in the pathogen, Residual toxicity, Pollution in environment, High cost etc.., The organic control of soil borne pathogens is a potential alternative to the use of chemicals (Rathore *et al.*, 2009). Marine products as sea weed provide a rich source of structurally diverse and biologically active secondary metabolites (Jeffrey Norrie *et al.*, 2014; Suthin raj *et al.*, 2016). Application of sea weeds extracts is proved to be better to decrease the foliar fungal diseases which ultimately increases its fertility and help the growth of plants (Jayaraj *et al.*, 2008). Biocontrol agents induce systemic resistance thereby contributing to disease control. Seed treatments with antagonistic bacteria resulted in increased root and shoot length of seedling. Foliar sprays with these antagonistic agents results in reduced sheath blight incidence (Sharma *et al.*, 2004). Hence, in the present study some biocontrol agents and marine products from locally available were tested *in-vivo* against *R. solani* and *S. oryzae.*

Materials and method

Isolation and Multiplication of pathogen

The diseased rice plant showing the typical symptom

of sheath blight disease were collected. The infected portion of the sheath was cut into small bits, surface sterilized into 0.1 per cent mercuric chloride solution for 30 sec washed in repeated changes of sterile distilled water and plated into PDA medium in sterilized Petri dishes. The plates were incubated for room temperature (28±2°C) for five days and were observed the fungal growth.

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 pathogens. From seven day old culture of the pathogens 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 (Anonmymus, 2012).

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 (Anonmymus, 2012).

Preparation Bio- inoculants

Pseudomonas fluorescens was isolates from the rhizosphere soil of healthy rice cultivating fields by serial dilution techniques and prepared as talc based formulation (Vidhyasekaran and Muthamilan, 1995).

Preparation seaweed extracts

Sargassum wightii and *Padina tetrastomatica* was collected from coromental coast of Bay of Bengal velankanni, Nagapattinam district, Tamil nadu. Each 1 kg of live, healthy and matured samples of each sea weed collected, washed thoroughly in sea water followed by tap water to remove extraneous particles and epiphytes. Then they were air dried under shade in laboratory for 3 days. The shade dried samples were chopped and pulverized. Each 50g powered samples was separately extracted for 7 days thrice in 500ml of 1:1 organic solvents using Erlenmyer conical flask under dark condition. The extract were pooled and concentrated by single flask evaporator under reduced pressure at 45ºC and weighed stored at 0ºC (Vallianayagam *et al.*, 2009).

Potculture experiment

The pot culture study was conducted with 8 treatments and three replication each at department of plant pathology, Annamalai university, Annamalai nagar. Fifteen kg of top soil collected from a rice growing field, was steam pasteurized and filled in 45 × 30 cm size cements pots. Thirty days old seedlings of rice variety ADT-36 was transplanted in pots. The components *P. fluorescens*, *Sargassum wightii* were tested against sheath blight and sheath rot diseases of rice.

The talc based formulation of *P. fluorescens* was used @ $2x10^{-8}$ $cfug^{-1}$. The seeds were treated @ 10g/kg of seed and dried in shade condition for four hours before sowing 0.2% conc. Of talc based formulation *P. fluorescens* was used as foliar application and talc based formulation of *P. fluorescens* was applied to the soil @10 kg /ha. *Sargassum wightii* @ 10g/kg was used for seed treatment and also used as foliar spray @20 lit/ ha. The chemical Tricyclazole was used for foliar spray @0.6g/ 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 plants were kept in the laboratory for 24 hrs to maintain a high relative humidity and subsequently moved to a green house maintained at 28±2ºC, 70-90% RH, under a light intensity of $85\mu mol^{-1}$ s^{-1}, 12 hrs photoperiod and subsequently transferred to pot culture yard. The treatments were designed on the basis of the above phenomenon and depicted in table. The diseases incidence was assessed at 30^{th}, 45^{th} and 60^{th} DAT.

Assessment of the disease severity

Twelve plants from each pot were randomly selected and tagged for grading the severity of diseases. The severity of two diseases *viz.*, sheath blight and sheath rot were recorded following IRRI recommended grading scale (Standard Evaluation system for Rice, 1980). The diseases severity was recorded in the three growth stage of the plants namely boot leaf stage, flowering stage and milking stage.

Field trial

The field trial was conducted during samba season at Krishnapuram village, cuddalore district, Tamilnadu in a field with sheath blight and sheath rot diseases incidence of rice. The trial were laid out in pots (4m × 4m) arranged in a RBD. Thirty day old seedling were planted into the field plots in rows with spacing of 15 × 10cm. Three replicated plots were maintained for each treatments. Treatments application details and experimental observation were the same as green house experiment. Regular cultivation practices were followed as per the recommendation.

Data were analyzed using GENSTAT computer statistical package for ANOVA to determine significant differences between treatments. Comparison between means was done using Duncan's multiple range test

(DMRT). A regression analysis was done to find out the correlation between the disease levels and percent loss in yield.

Results and discussion

Pot culture condition

All the components significantly reduced the sheath blight and sheath rot diseases incidence over the control (Table 1). Among the treatments, combined application of *P. florescens* as seed treatment @ 10g/kg of seed, soil @ 10kg/ha, foliar spray @ 10kg/ha at tillering and boot leaf stage and *Sargassum wightii* as seed treatment @ 10g/kg of seed, foliar spray @ 20lit/ha at tillering stage and boot leaf stage (T_4) significantly reduced severity of sheath blight incidence of 12.00, 14.28, and 16.45 percent at 30, 45 and 60 DAT and sheath rot disease incidence of 13.45, 15.76 and 17.38 percent 30, 45 and 60 DAT respectively and this treatment was superior than the standard chemical check Tricyclazole. Seed treatment @ 2g/kg and foliar application @0.6/lit of Tricyclazole at tillering and boot leaf stage (T_6) which recorded sheath blight disease severity of 13.22, 15.78 and 18.65 percent at 30, 45, 60 DAT and Sheath rot incidence of 15.67, 17.46 and 19.29 per cent at 30, 45, 60 DAT respectively. The treatments (T_4) recorded the maximum grain yield (34g/plant) than all other treatments. All components treated plants significantly increased the grain yield as compared to control.

Field trial

The field experiment revealed that combined application of *P. fluorescens* as seed treatment @10g/kg of seed, soil @ 10kg/ha, foliar spray @ 10 kg/ha at tillering and boot leaf stage and *Sargassum wightii* as seed treatment @ 10g/kg of seed, foliar spray @ 20lit/ha at tillering stage and boot leaf stage (T_4) significantly reduced the severity of sheath blight disease 11.67, 14.52 and 17.72 per cent at 30, 45, 60 DAT and sheath rot 10.32, 13.48 and 15.54 percent at 30, 45, 60 DAT (Table 2). It was significantly superior than the standard chemical check Tricyclazole (T_6), which recorded sheath blight disease severity 13.34, 16.28 and 18.47 per cent at 30, 45, 60 DAT and sheath rot disease severity of 13.45, 15.82 and 17.48 per cent at 30, 45, 60 DAT respectively.

Effect of *Sargassum wightii* and *Pseudomonas fluorescens* against sheath blight and sheath rot diseases of rice under pot culture condition.

Treatment	Sheath blight						Sheath rot						Grain yield (g/plant)
	Disease incidence (%)			Disease over control (%)			Disease incidence(%)			Disease over control (%)			
	30 DAS	45 DAS	60 DAS	30 DAs	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAs	45 DAS	60 DAS	
T_1	16.35 (28.06)	18.28 (26.62)	20.36 (26.07)	26.11 (17.71)	28.17 (29.64)	27.59 (34.31)	20.45 (29.17)	22.47 (30.48)	23.18 (28.94)	20.55 (16.10)	20.93 (24.79)	28.10 (30.08)	29
T_2	17.12 (24.44)	19.32 (23.14)	22.42 (23.85)	22.63 (28.40)	24.47 (33.32)	20.27 (30.73)	21.14 (27.37)	23.42 (25.91)	25.56 (26.88)	17.89 (25.02)	17.59 (30.52)	20.71 (26.95)	28
T_3	14.56 (29.03)	16.27 (28.26)	19.47 (25.93)	34.20 (34.45)	36.07 (31.68)	30.76 (40.10)	17.43 (32.21)	19.43 (30.37)	22.48 (29.52)	32.28 (29.78)	31.63 (32.01)	30.27 (42.75)	32
T_4	12.00 (24.62)	14.28 (25.31)	16.45 (22.20)	45.77 (32.05)	43.88 (41.48)	41.50 (38.05)	13.45 (27.47)	15.76 (28.29)	17.38 (23.39)	47.74 (27.22)	44.54 (41.86)	46.09 (38.38)	34
T_5	15.45 (20.26)	17.36 (21.32)	19.12 (22.43)	30.18 (42.57)	31.78 (39.38)	32.00 (35.79)	19.10 (21.51)	21.28 (23.31)	24.28 (24.67)	25.79 (43.70)	25.12 (38.71)	24.68 (34.62)	30
T_6	13.22 (23.40)	15.78 (23.78)	18.65 (30.29)	40.26 (36.91)	37.99 (15.80)	33.67 (26.75)	15.67 (24.69)	17.46 (26.15)	19.29 (30.33)	39.12 (34.22)	38.56 (18.69)	40.16 (27.07)	33
T_7	20.08 (26.18)	23.56 (32.02)	23.16 (30.10)	9.26 (35.46)	7.42 (33.68)	17.63 (24.82)	23.76 (28.78)	25.50 (24.63)	28.38 (26.05)	7.69 (39.32)	10.27 (33.38)	11.97 (20.24)	21
T_8	22.13 (26.82)	25.45 (23.92)	28.12 (25.58)	-	-	-	25.74 (28.30)	28.42 (32.19)	32.24 (34.59)	-	-	-	18
CD 3.830 (P=0.05) SEd= 1.785							CD 3.908 (P=0.05) SEd= 1.822						

T_1- Application of *Sargassum wightii* as seed treatment @ 10g/kg , foliar spray @ 20 lit/ha at tillering and boot leaf stage, T_2-Application of *Padina tetrastomatica*, as seed treatment @ 10g/kg , foliar spray @ 20 lit/ha at tillering and boot leaf stage T_3- Application of *P. fluorescens* as seed treatments @ 10 g/kg, soil @10kg/ha at transplanting, foliar spray @10kg/ha at tillering and boot leaf stage , T_4- T_1+T_3, T_5- T_2+T_3, T_6– Seed treatment @ 2g/kg, foliar application of Trycyclazole @ 0.6g/lit, T_7– Healthy control (without application of treatment and pathogen inoculation) and T_8– Inoculated control.

Effect of *Sargassum wightii* and *Pseudomonas fluorescens* against sheath blight and sheath rot diseases of rice under field condition.

Treatment	Sheath blight						Sheath rot						
	Disease incidence (%)			Disease over control (%)			Disease incidence(%)			Disease over control (%)			
	30 DAS	45 DAS	60 DAS	30 DAs	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAs	45 DAS	60 DAS	
T_1	19.42 (28.58)	22.12 (28.90)	25.45 (26.73)	15.15 (17.69)	14.06 (27.52)	10.57 (22.02)	18.54 (29.02)	21.72 (29.45)	24.46 (26.16)	21.47 (13.61)	15.25 (29.43)	14.17 (22.98)	3398.05
T_2	20.43 (26.87)	23.36 (25.74)	26.45 (26.14)	10.74 (19.13)	9.24 (24.77)	7.06 (22.90)	20.15 (26.67)	24.18 (24.97)	26.72 (25.50)	14.65 (22.50)	5.54 (29.65)	6.24 (27.60)	3267.80
T_3	16.66 (28.15)	19.55 (30.29)	21.56 (24.89)	27.21 (37.89)	16.23 (36.33)	24.24 (29.49)	15.62 (27.58)	17.72 (29.64)	19.35 (23.21)	33.84 (42.40)	30.86 (38.44)	32.10 (34.51)	3445.70
T_4	11.67 (28.06)	14.52 (22.39)	17.72 (23.79)	49.01 (41.31)	43.58 (37.31)	37.73 (23.75)	10.32 (27.77)	13.48 (21.54)	15.54 (23.43)	56.28 (43.51)	47.40 (38.21)	45.47 (33.74)	3712.80
T_5	18.87 (19.97)	20.24 (21.42)	22.26 (24.09)	17.56 (44.43)	21.36 (40.23)	21.78 (31.44)	17.83 (18.73)	19.44 (21.51)	21.44 (23.22)	24.48 (48.60)	24.15 (40.99)	24.77 (35.37)	3367.89
T_6	13.34 (26.24)	16.28 (30.48)	18.47 (30.95)	41.72 (15.40)	36.75 (27.82)	35.10 (18.97)	13.45 (24.89)	15.82 (30.41)	17.48 (31.12)	43.03 (14.46)	38.27 (29.84)	38.66 (22.11)	3532.48
T_7	22.89 (25.45)	25.74 (27.66)	28.46 (32.24)	-	-	-	23.61 (24.71)	25.63 (26.09)	28.50 (32.26)	-	-	-	2978.96
CD P(=0.05) 4.231 SEd = 1.972							CD (P= 0.05) 5.467 SEd = 2.509						

T_1- Application of *Sargassum wightii* as seed treatment @ 10g/kg , foliar spray @ 20 lit/ha at tillering and boot leaf stage, T_2- Application of *Padina tetrastomatica,* as seed treatment @ 10g/kg , foliar spray @ 20 lit/ha at tillering and boot leaf stage T_3- Application of *P. fluorescens* as seed treatments @ 10 g/kg, soil @10kg/ha at transplanting, foliar spray @10kg/ha at tillering and boot leaf stage , T_4- T_1+T_3, T_5- T_2+T_3, T_6– Seed treatment @ 2g/kg, foliar application of Trycyclazole @ 0.6g/lit, T_7– Control.

The study showed that most effective treatment (T_4) not only inhibited the disease severity but also recorded the maximum grain yield (3712.80 kg/ha) than all other treatments. All components treated plants significantly increased the grain yield as compared to control. The combined application of *P. fluorescens* mixed with organic manure formulation reduced sheath blight disease and also increased grain yield and grain weight of rice (Prashant Mishra *et al.*, 2009). This may be due to the production of an array of antifungal antibiotiocs such as 2, 4-diacetylphophloglucinol, oligomycin, Phenazine, Pyoluteorin, Pyrlnitrin and Pyocyanin by *P. fluorescens* (Gupta *et al.*, 2001). Sultana *et al.*, 2007, reported that brown, green, red sea weed were highly effective against *R. solani* in vitro and in vivo conditions. There are several workers have been reported on the efficacy of sea weed extract against fungal pathogens (Norrie *et al.*, 2002; Jaya raj *et al.,* 2008). This may be due to higher levels and early accumulation of phenolics and phytoalexins (Garcia- Mina *et al.*, 2004).

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