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Development of bioformulation and delivery system of *Pseudomonas fluorescens* against bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*)

P.P.Jambhulkar[1]* and P. Sharma[2]

[1]Agricultural Research Station, (MPUAT, Udaipur), Banswara-327 001, India

[2]Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110 012, India

*Corresponding Author's Email : ppjambhulkar@gmail.com

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Abstract

Antagonistic potential of *Pseudomonas fluorescens* isolate RRb-11 has been evaluated against bacterial leaf blight (BLB) pathogen of rice *in vitro, in vivo*, microplot and field tests. RRb-11 isolate mass multiplied in substrates like talc and kaolinite powder and bran of barley, soybean and wheat to prepare suitable bioformulation. The maximum shelf life of *P. fluorescens* was recorded in talc based bioformulation up to 150 days after storage. In rhizosphere competence study, the root rhizosphere of talc, kaolinite and barley based bioformulation treated plants showed good survivability and competence even up to 90 days after treatment. In field study, the talc based bioformulation was applied and the best results were obtained when talc based bioformulation of *P. fluorescens* RRb-11 was applied as seed treatment, seedling root dip and soil application in combination which reduced the disease by 92.3 and 88.5% over control in the year 2009 and 2010, respectively. This treatment also produced maximum yield of 3.88 t ha^{-1} *i.e.*, 61% greater than control.

Key words

Bioformulation, *Pseudomonas fluorescens*, Rhizosphere, Shelf life, Survivability

Introduction

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922) is the most important bacterial disease in terms of economic loss (Swings *et al.*, 1990). In India millions of hectares of paddy crops are severely affected by BLB causing yield loss of 6-60% (Shanthi *et al.,* 2011). It is also an important disease at Banswara district of Rajasthan, where the field trial was conducted. The disease causes straw coloured lesions either on one or both margins of leaves. It starts from tip and goes downwards causing drying of leaf tips, inward rolling and twisting of infected portion. It produces three types of symptoms *viz.*, kresek, leaf blight and pale yellow phase (Mew *et al.*, 1993). The kresek phase is most severe and causes drying of shoots. Environment friendly cultivation practices emphasize the need to maintain the microbial balance in the rhizosphere by application of plant growth promoting rhizobacteria (PGPR) and thereby, limit the density of seed and soil borne pathogens to a minimum level for disease control (Adesemoye *et al.,*2009). Among PGPR, fluorescent Pseudomonads are the most exploited bacteria for biological control of soil-borne and foliar plant pathogens. In addition to disease control, it promotes the growth and development of crop plants.

A bioformulation can improve product stability, shelf life and also protect bacteria against different environmental conditions and provide initial food source. Application of PGPR either to increase crop health or to manage plant diseases depends on the development of commercial formulations with suitable carriers that support the survival of bacteria for a considerable length of time., it is imperative to evaluate the survival of the immobilized bacteria in different carriers, and also their ability to retain attributes for plant growth promotion (Aeron *et al.,*2011).

Earlier studies indicated that seed treatment and soil application of *P. fluorescens* prevented the pathogen infection

and reduced the disease incidence in many crops (Ramamoorthy and Samiyappan, 2001). But the study on efficacy of *P. fluorescens* bioformulation against *X. oryzae pv. oryzae* is limited (Jeyalakshmi *et al*., 2010). In the present study, cell viability of *P. fluorescens* RRb 11 was evaluated in various carrier materials for determining its survivability, rhizosphere competence and was evaluated for inhibitory action against *Xanthomonas oryzae* glasshouse, microplot and field study.

Materials and Methods

Plant material and bacterial culture inoculation : Pusa Basmati 1, a susceptible variety of rice to bacterial leaf blight pathogen *Xanthomonas oryzae*. *oryzae* (Xoo) was selected for the experiment. The highly virulent culture of *X. oryzae* pv. *oryzae* isolated from Haryana, India was used. Actively growing 24 hr old bacterial growth was suspended in sterile distilled water and optical density of suspension was adjusted to 0.45 (10^8 cfu ml^{-1}) at 610 nm with using UV-visible spectrophotometer. Succulent and vigorously growing 21-day-old paddy (Pusa Basmati 1) seedlings were transplanted in the pots and micro plot (1x1 m^2). Inoculation of pathogen on leaf was done at maximum tillering stage by leaf clip method (Kauffman *et al.,* 1973). In this method leaf tips of 5-week-old-rice plant (Pusa Basmati-1) were clipped off by sterilized scissor and then the cut end of the leaves were submerged in the Xoo suspension.

PGPR strain isolation and *in vitro* antagonism : Rice Rhizosphere bacteria (RRb-11) was isolated from twenty rhizospheric soil samples of basmati rice grown in the field of IARI, New Delhi and Almora and it was earlier characterized biochemically and identified as *Pseudomonas fluorescens.* For testing antagonistic activity, 1ml *Xoo* suspension (0.1 OD) was mixed with 25 ml of melted and cooled (40-45°C) NSA (nutrient sucrose agar) and poured into Petriplates. The solidified plates were spot inoculated with 24 hr old culture of RRb-11 isolate. After 2 days of incubation at 28°C, the plates were examined for antagonistic action indicated by appearance of inhibition zone at the site of antagonistic growth and width of clear zone was measured. Control plates contained only Xoo and were not inoculated by RRb-11. experiment was conducted in triplicate and was repeated twice.

Development of bioformulation : The formulation was developed with slight modification in a process as described by Amer and Utkhede (2000) using different carriers. *Pseudomonas fluorescens* strain RRb-11 was grown in liquid Nutrient broth for 48 hr as shaker culture in shaker incubator at 150 rpm at 28ºC temperature. The carboxy methyl cellulose (CMC), carrier and bacterial suspension in broth (10^8 c.f.u. ml^{-1}) were used in 1:50:4 ratio. The bioformulation was prepared as: talc powder (5.0g carboxy methyl cellulose (CMC) + 250 g talc powder (autoclaved at 121ºC at 15 p.s.i. for 30 min) + 20 ml of bacterial suspension in broth; kaolinite powder (5.0g CMC + 250 g autoclaved kaolinite powder + 20 ml of bacterial suspension in broth); wheat bran (5.0g CMC + 250 g autoclaved wheat bran + 20 ml of bacterial suspension in broth) ; barley bran (5.0g CMC + 250 g autoclaved barley + 20 ml of bacterial suspension in broth); soybean bran (5.0g CMC + 250 g autoclaved soybean bran + 20 ml of bacterial suspension in broth) and 20 ml of bacterial broth suspension alone as a control. The bioformulations were dried overnight aseptically at room temperature. The materials were stored in sealed plastic bags at room temperature. Three independent samples were analysed with three replications for each analysis.

Survival of PGPR strain : The survivability of *P. fluorescens* RRb 11 cells was determined in five bio-inoculant preparations. 10 g samples were drawn at 0, 30, 60, 90 120 and 150 days after storage from four bags of each carrier material at each time and bacterial population was assessed by dilution plate method on nutrient agar (NA) medium aseptic conditions. Suitable dilutions were spread plated on NA medium, amended with antibiotics (rifampicin and streptomycin—50 µg ml^{-1} each), and incubated at 28±1°C for 48 hr. The bacterial population (CFU g^{-1}) was enumerated. The experiment was conducted in triplicate and one bag of each carrier from each replicate was investigated for survivability after every 30 days interval.

Glass house trial : The efficacy of different carrier based powder formulation in controlling bacterial leaf blight of rice was assessed in glass house condition in 2007 and 2008. The temperature and relative humidity was ranged from 25 to 29ºC and from 70-90% RH respectively during glass house trials. Eight seedlings were maintained in a pot containing sand, soil and manure in the ratio 2:1:1. Each treatment was replicated thrice. The design of the experiment was Completely Randomised Block Design (CRD). treatment was replicated three times.

Microplot trial : The efficacy of powdered formulation of *P. fluorescens* RRb-11 was evaluated in microplot trial at Research farm of Indian Agricultural Research Institute (IARI), New Delhi during 2007 and 2008 *kharif* seasons. The size of each microplot was 1 X 1 m^2. The powdered formulation was applied by using different delivery methods *viz.* seed bacterisation (5 g kg^{-1} seeds), seedling root dip (5 g l^{-1} water) and soil treatment (5 g l^{-1} of water) through soil drenching and were sown to raise nursery according to the treatments. The nursery was raised for 21 days and the seedlings were transplanted by following different methods of delivery system to test the efficacy of different carrier based bio formulation. The plants were inoculated with pathogen at 36 days after sowing and disease intensity was assessed 15 days after inoculation. The experiment was repeated once.

Effect of carriers on rhizosphere competence of *P. fluorescens* RRb-11 isolate : The bacterial population densities in the rhizosphere of rice plants, grown during glasshouse and field trials were analyzed at every 30 days intervals on Nutrient Agar (NA) supplemented with streptomycin and rifampicin (50

µg/ml each). Sampling involved squeezing the pots initially to loosen the soil. The seedlings were then removed in their entirety from the loose soil, keeping as much of the root mass intact as possible. Plants were uprooted from field plot at every 30 days intervals. The root mass was placed in a universal bottle containing 10 ml SDW (Sterile Distilled Water). The closely adhering rhizosphere soil was washed off by agitating the bottles by hand, and the roots were then removed from the universal bottle. The rhizosphere soil suspension was serially diluted and plated in triplicate onto Nutrient Agar medium with antibiotics, incubated at 28±1°C for 48 h assay the population density of bacterial isolate RRb-11.

Evaluation of talc based bioformulation in field condition : The talc based bioformulation was found best in glass house and microplot study was evaluated in field condition by using different methods of delivery system in the year 2009 and 2010 at Agricultural Research Station, Banswara, Rajasthan. A susceptible variety of Mahi Sugandha was taken for the experiment. Plot size for the experiment was 3x2 m^2. The bio formulation of *P. fluorescens* RRb-11 was applied as seed bacterisation, seedling root dip and soil treatment either alone or in combination. Seed treatment was applied as 5 g kg^{-1} seeds, seedling root dip as 5 g l^{-1} water and soil treatment as 5 g l^{-1} water. The plants were inoculated with the pathogen at 36 days after sowing and diseases intensity was assessed 15 days after inoculation. The experiment was designed in Randomised Block Design. The experiment was repeated once.

Statistical analysis : The disease intensity data was arcsine transformed before analysis of variance (ANOVA). The package used for analysis was Web Agri Stat Package 2.0 (WASP 2.0) developed by ICAR Research Complex for Goa, India.

Results and Discussion

Among the fluorescent pseudomonads isolated from twenty rhizospheric soil samples, four isolates from Delhi and Almora were found to inhibit growth of Xoo. All the four isolates were identified as *Pseudomonas fluorescens*. Their efficacy to inhibit Xoo varied widely and RRb-11 was found very effective for control of Xoo (Table 1).

The shelf life of bacteria varies depending upon bacterial genera, carriers and their particle size. The bacterium *P. fluorescens* survived well in talc, kaolinite and barley bran even up to 150 days after storage (DAS). But wheat and soybean bran did not support the growth after 60 DAS. Among all the carriers tested, the bacterium survived best in talc powder (Table 2). The initial population of *P. fluorescens* RRb-11 increase up to 30 DAS and reached its peak and thereafter starts declining as the period of storage increased. The maximum survivability of *P. fluorescens* RRb-11 was observed in talc based bioformulation even up to 150 DAS with viable population of 30.1 X 10^7 cfu g^{-1}. may be because

Table 1 : Efficacy of *Pseudomonas fluorescens* isolates in inhibiting growth of Xoo under *in vitro* conditions

Rice rhizobacteria (RRb)	Zone of inhibition* in (mm) after 48 hr at 27°C
RRb-11 (Delhi)	*20.00
RRb-103 (Almora)	14.00
RRb-15 (Delhi)	6.00
RRb-101 (Almora)	8.20
SEm±	1.03
CD at 5%	3.2

*Mean of three replications

talc has very low moisture equilibrium, relative hydrophobicity, chemical inertness, reduced moisture absorption and prevent the formation of hydrate bridges that enable longer storage period these results are in strong agreement with previous findings of other researchers (Bora *et al.,* 2004; Sivakumar *et al*., 2012). Survival of *P. fluorescens* in talc, kaolinite and barley bran was more as they were having relatively smaller particle size which increased the survival rate than in wheat and soybean bran with bigger particle size. The carriers with smaller particle size have increased surface area, which increased resistance to desiccation of bacteria by increased coverage of bacterial cells (Nakkeeran *et al*., 2005).

The bacterial isolate *P. fluorescens* RRb-11 tended to decrease in number in rhizosphere soil over the 90 days of sampling period. RRb-11 survived well on roots or in the rhizosphere of Pusa Basmati 1 plants. This isolate was found to have an efficient colonization ability of 5.8 X 10^6cfu $g^{-1}$and 6.5 X 10^6cfu g^{-1}in seeds treated with talc based bioformulation at 30 DAS on rice roots in both glasshouse and field plot studies respectively (Table 3). The root rhizosphere colonisation tended to increase population of *P. fluorescens*RRb-11 at increasing rate up to 60 DAS thereafter the population reduced and finally declined at 90 DAS, in all the treatments. But appreciably highest population of *P. fluorescens* RRb-11 was observed in talc based bioformulation treatment at 60DAS. Rhizosphere competence confers the microorganisms required capability to be most effective at the plant root–soil interface where in addition to utilization of exuded compounds, roots can also absorb transformed molecules readily (El-Tarabily, 2008).

All the methods of delivery systems viz. seed treatment, seedling root dip and soil drenching not only reduce disease intensity but also increased yield. Previously, many agricultural products have been tested for their capacity to serve as substrates for mass production of biocontrol microbes; these include rice bran, wheat bran, broken rice, paddy husk, neem cake, sesame cake, peeled banana and coconut, mesocarp fiber of oil palm and soybean oils (Tewari and Bhanu, 2003; Sawangsri *et al*., 2007). In the present study, *P. fluorescens* was multiplied in

Table 2 : Survival of *P. fluorescens* population in different carriers

Formulation	Survival of population (1 X 10^7 cfu g^{-1})					
	Days after storage					
	0	30	60	90	120	150
Wheat	173.4	5.8	0	0	0	0
Soybean	157.8	47.4	0	0	0	0
Barley	170.1	196.3	213.3	90.3	12.1	6.9
Talc	171.6	308.6	236.4	123	33.8	30.1
Kaolinite	177	247.6	200.3	87.4	14.6	11.3
SEM ±	4.03	20.13	15.14	12.36	6.67	4.01
CD at 5%	11.4	59.6	39.4	34.1	17	10.7

Table 3 : Effect of different carriers on rhizosphere competence of *Pseudomonas fluorescens* RRb11 population

Treatment	Glass house rhizosphere population 1 X 10^6 cfu g^{-1}			Field plot rhizosphere population 1 X 10^6 cfu g^{-1}		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Wheat + RRb 11	3.1	2.9	0	1.7	2.2	0.0
Soybean + RRb11	2.5	2.8	0.4	2.3	2.7	1.8
Barley + RRb11	4.9	5.1	3.5	5.3	5.6	2.4
Talc + RRb 11	5.8	5.9	5.0	6.5	6.8	3.3
Kaolinite + RRb11	4.3	4.8	4.1	4.9	5.2	2.7
Control	1.5	1.4	1.3	1.8	1.9	1.3
SEM ±	0.31	0.29	0.48	0.45	0.40	0.23
CD at 5%	0.9	0.8	1.4	1.3	1.1	0.6

*Average of three replications; The initial number of cells for each treatment was 1 X 10^8 cfu ml^{-1}; DAS: Days after sowing

nutrient broth and mass multiplied in various carriers such as talc, kaolinite, barley bran, soybean bran and wheat bran. The application of fluorescent Pseudomonads by seed treatment (Niranjana *et al*., 2009), seedling root dip (Verma, 2009) and soil drenching (Jayalakshmi *et al*., 2010) has also been attempted by many workers to control diseases in various crops. The efficacies of these various carrier based bioformulations were tested in unity and in combination. In glass house study, seed treatment, seedling root dip and soil drenching individually showed minimum disease intensity of 8.47, 13.79 and 19.26% respectively (Table 4) which clearly revealed that seed treatment with talc based bioformulation alone was most effective for the management of bacterial leaf blight of rice. All carriers, the talc gave the most promising results on residual cell viability, similar to that recommended for free living soil bacteria by the Bureau of Indian Standards: Specification for Inoculants -2000, and proved to be most suitable as a carrier material for *P. fluorescens* RRb 11. Wheat and soybean based carriers were, however, established and laterly have proved to be inferior carrier materials. A drastic reduction in cell populations of *P. fluorescens* RRb-11 was observed in wheat and soybean bran carriers which proved unsuitable carrier as the residual cell population was several times less than the recommended population (10^7cfu g^{-1}) as per Bureau of Indian Standard (2000). Kaolinite and barley bran were found to be average carrier materials, the viability of *P. fluorescens*RRb-11 initially decreased; however it was well within the permissible limit of standards. Seeds treated with talc based bioformulation gave maximum grain yield of 3.66 t ha^{-1} as compared with seedling root dip (3.59 t ha^{-1}) and soil drenching (3.48 t ha^{-1}). In combined application, talc showed maximum compatibility with kaolinite to reduce disease intensity and increase grain yield. As the survivability of *P. fluorescens* in wheat and soybean bran was very low, the bioformulation made from these carriers neither reduced disease intensity nor increased yield. In microplot study, seed treatment, seedling root dip and soil drenching methods of bioformulation treatment responded similarly and maximum reduction in disease intensity as well as increase in yield was found on application of talc based bioformulation alone (Table 5). The results are in conformity with Nandakumar *et al.* (2001) who reported reduction in sheath blight of rice and *Fusarium* wilt of pigeon pea on application of *P. fluorescens* formulation in greenhouse and field conditions.

The present study clearly indicated that treatment with talc based bioformulation significantly reduced disease intensity and increased yield as compared with other treatments. Moreover, the talc based bioformulation gave very good response with seed treatment and seedling root dip as compared with soil drenching method of delivery system. Although a single delivery method was sufficient to reduce the disease intensity of bacterial leaf blight of rice, but when the combination of two or three delivery methods were tested, the most effective control was

Table 4: Effect of application of various bioformulations using different methods of delivery system on disease intensity of BLB and yield of rice in glass house experiment

Treatments	Glass house											
	Seed treatment				Seedling root dip				Soil drenching			
	%DI	%ROC	Yield (t ha^{-1})	% IOC	%DI	%ROC	Yield (t ha^{-1})	% IOC	%DI	%ROC	Yield (t ha^{-1})	% IOC
Kaolinite powder	*12.66 **(20.44)	75.9	3.53	29.78	15.94 (23.27)	69.09	3.47	36.61	23.91 (29.05)	51.76	3.39	23.72
Soybean bran	31.66 (33.74)	39.81	2.94	8.10	26.52 (30.52)	48.57	2.86	12.60	36.16 (36.75)	27.05	2.51	-8.40
Barley bran	17.48 (23.90)	66.76	3.27	20.22	24.51 (29.46)	52.47	3.09	21.65	28.89 (32.29)	41.72	3.17	15.69
Wheat bran	32.98 (34.62)	37.30	2.81	3.31	32.95 (34.81)	36.11	2.71	6.70	41.87 (40.17)	15.53	2.58	-5.83
Talc Powder	8.47 (16.82)	83.87	3.66	34.56	13.79 (21.52)	73.26	3.59	41.34	19.26 (25.49)	61.14	3.48	27.00
Soybean bran + Barley bran	31.00 (33.59)	41.06	3.15	15.81	29.12 (32.26)	43.53	3.06	20.47	33.00 (34.65)	33.43	2.97	8.3
Soybean + wheat bran	37.54 (37.42)	40.11	3.03	11.40	35.35 (36.12)	31.45	2.78	9.45	41.54 (39.97)	16.20	2.55	-6.93
Barley bran + Talc powder	22.67 (28.22)	56.90	3.37	23.90	18.61 (25.38)	63.91	3.23	27.16	24.19 (29.21)	51.20	3.28	19.71
Soybean bran+ Kaolinite powder	29.38 (32.59)	44.12	3.03	11.40	31.24 (33.60)	39.42	3.07	20.87	27.71 (31.58)	44.10	3.10	13.14
Barley bran+ Wheat Bran	34.88 (35.86)	33.67	3.07	12.87	31.7 (33.67)	38.53	2.98	17.32	35.18 (36.00)	29.03	2.76	0.73
Soybean bran+ Talc powder	33.34 (34.83)	36.61	3.11	14.34	29.88 (32.78)	42.06	3.07	20.87	30.45 (33.06)	32.52	3.07	12.04
Barley bran+ Kaolinite powder	24.07 (28.85)	54.23	3.34	22.79	21.34 (27.34)	58.62	3.19	25.60	22.74 (28.28)	54.12	3.20	16.79
Wheat bran+ Talc powder	31.18 (33.78)	40.72	3.05	12.13	25.13 (29.57)	51.27	3.11	22.44	33.74 (35.32)	31.93	2.98	8.75
Wheat bran+ Kaolinite powder	29.96 (32.68)	43.04	3.09	13.60	28.4 (31.51)	44.93	3.07	20.87	32.32 (34.30)	34.78	2.85	4.01
Talc+ Kaolinite powder	13.47 (21.00)	74.37	3.57	31.25	16.44 (23.65)	68.12	3.43	35.04	21.08 (26.62)	57.47	3.25	18.61
Control	52.60 (46.35)		2.72		51.57 (45.80)		2.54		49.42 (44.57)		2.74	
CV	11.32		3.81		9.38		2.26		6.37		2.40	
CD (P=0.05)	5.83		0.31		4.80		0.24		3.56		0.21	

*Average of three replications;**Figures in parentheses are arcsine transformed values; DI- Disease intensity; ROC-Reduction over control; IOC-Increase over control

obtained. Thus, a different experiment was framed which involved treatment of talc based bioformulation using different methods of delivery system in unity and in combination. Among all the treatments, the talc based bioformulation was applied using single delivery system, seed treatment with talc based bioformulation was found more efficient in colonising rhizosphere bacteria. Any combination of treatment together with seed treatment increases the efficacy of the rhizobacteria as compared with seed treatment alone. But when seed treatment was combined with seedling root dip or soil treatment, the seed treatment along with seedling root dip was found very effective and reduced disease intensity by 85.7 and 76.8 % in 2009 and 2010, respectively. Among all the delivery systems tested and observed, the combination of all methods such as seed treatment, seedling root dip and soil drenching was found to reduce the BLB disease by 92.3 and 88.5% over the control in the year 2009 and 2010, respectively. This treatment also produced maximum mean yield of 3.88 t ha^{-1} (61.0%) more than control (Table 6). Similarly, Meena and Muthuswamy (1998) assessed the efficacy *P. fluorescens* against *Rhizoctonia solani* in green house. They applied seed treatment, seedling root dip and foliar spray in unity and in combination. Among all the commercial

Table 5: Effect of application of various bioformulations using different methods of delivery system on disease intensity of BLB and yield of rice in microplot experiment

Treatments	Microplot											
	Seed treatment				Seedling root dip				Soil drenching			
	%DI	%ROC	Yield (t ha^{-1})	% IOC	%DI	%ROC	Yield (t ha^{-1})	% IOC	%DI	%ROC	Yield (t ha^{-1})	% IOC
Kaolinite powder	*10.52 **(18.87)	78.97	3.85	34.85	13.25 (21.27)	74.83	3.71	35.35	24.17 (29.40)	51.55	3.22	22.01
Soybean bran	33.21 (35.18)	33.62	2.93	2.63	25.23 (30.13)	52.08	2.96	8.1	32.83 (34.94)	34.20	2.86	8.39
Barley bran	14.01 (21.85)	71.97	3.56	24.80	26.54 (31.00)	49.59	3.21	17.18	31.36 (34.05)	37.14	2.71	3.0
Wheat bran	32.14 (34.52)	35.75	2.53	-11.21	33.82 (35.55)	35.76	2.85	4.08	39.46 (38.92)	20.90	3.33	26.22
Talc Powder	7.52 (15.76)	84.97	4.13	44.73	9.36 (17.75)	82.22	3.84	40.27	14.25 (22.13)	71.43	3.65	38.59
Soybean bran+ Barley bran	29.58 (32.94)	40.87	2.98	4.55	31.12 (33.88)	40.89	3.14	14.63	35.23 (36.40)	29.38	3.01	14.30
Soybean+ wheat bran	29.24 (32.72)	41.55	2.85	-0.14	32.56 (34.78)	38.16	2.85	3.97	40.12 (39.29)	19.58	2.96	12.48
Barley bran+ Talc powder	20.33 (26.72)	59.36	3.54	24.17	21.00 (27.24)	60.11	3.41	24.55	23.45 (28.95)	52.99	3.32	25.92
Soybean bran+ Kaolinite powder	30.14 (33.28)	39.75	3.38	18.32	30.27 (33.37)	42.50	3.31	20.83	29.68 (32.99)	40.51	3.03	14.80
Barley bran+ Wheat Bran	35.21 (36.39)	29.62	3.10	8.62	34.54 (35.99)	34.39	3.10	13.10	34.12 (35.73)	31.60	2.96	12.44
Soybean bran+ Talc powder	33.48 (35.35)	33.08	2.96	3.85	30.05 (33.22)	42.92	3.12	14.00	28.75 (32.42)	42.37	3.13	18.59
Barley bran+ Kaolinite powder	24.10 (29.40)	51.82	3.34	17.0	23.64 (29.07)	55.10	3.35	22.11	24.66 (29.75)	50.57	3.41	29.49
Wheat bran+ Talc powder	30.25 (33.36)	39.54	2.94	3.01	24.13 (29.41)	54.17	3.00	9.48	31.23 (33.97)	37.40	3.00	14.04
Wheat bran+ Kaolinite powder	30.22 (33.33)	39.60	3.24	13.66	29.45 (32.83)	44.06	3.26	18.78	31.42 (34.07)	36.42	2.80	6.26
Talc + Kaolinite powder	11.26 (19.52)	77.49	3.86	35.37	17.55 (24.73)	66.67	3.65	33.24	19.63 (26.28)	60.65	3.37	27.70
Control	50.03 (45.01)		2.86		52.65 (46.52)		2.74		49.89 (44.94)		2.63	
01)				(46.52)				(44.94)				
CV	6.24		3.81		4.2		2.26		4.0		2.40	
CD (P=0.05)	3.15		0.13		2.3		0.16		2.20		0.12	

*Average of three replications;**Figures in parentheses are arcsine transformed values; DI- Disease intensity; ROC-Reduction over control; IOC-Increase over control

Table 6 : Effect of different methods of application of talc based bioformulation of *P. fluorescens* RRb-11 on management of BLB of rice

Delivery system of talc based bioformulation	% Disease intensity (2009)	% ROC	% Disease intensity (2010)	% ROC	Mean yield (t ha^{-1})	% increase over control
Seed treatment	*9.36**(17.75)^{de}	84.0	10.45(18.80)^{c}	78.6	3.65	51.45
Root dip	14.5(22.38)^{c}	75.3	13.25(21.27)^{c}	72.9	2.61	8.30
Soil drenching	17.8(24.95)^{b}	69.6	19.80(26.40)^{b}	59.5	2.47	2.43
Seed treatment+Root dip	8.35(16.71)^{e}	85.7	11.32(19.47)^{c}	76.8	3.62	50.20
Seed treatment+Soil drenching	11.35(19.64)^{d}	80.6	13.21(21.23)^{c}	73.0	3.71	53.94
Seed treatment+Root dip+Soil drenching	4.53(12.28)^{f}	92.3	5.63(13.58)^{d}	88.5	3.88	61.00
Control	58.61(49.98)^{a}	-	48.90(44.37)^{a}	-	2.41	-
CV	4.96		10.81			
CD (P=0.05)	2.06		4.54			

*Average of three replications; **Values in parentheses are arcsine transformed values

formulation treatments, the combination of seed treatment, seedling root dip and foliar spray afforded the highest disease reduction (75%) over the check treatment.

From this study, it is concluded that talc based bio formulation of *P. fluorescens* RRb-11 isolate showed maximum shelf life and survivability in rhizosphere to reduce disease intensity of bacterial blight of rice and thereby increase yield when applied as seed treatment, seedling root dip and soil drenching in combination.

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