Int.J.Curr.Microbiol.App.Sci (2019) *8*(8): 2846-2853

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.808.327

Assessment of Method of Application of Microbial Antagonist for the Management of Sheath Rot Disease of Rice under Green House Condition

B. Bora* and M. S. Ali

Department of Plant Pathology, Biswanath College of Agriculture, Biswanath Chariali, Assam, India

**Corresponding author*

Keywords

Microbial antagonist, bio-formulation, Talcum powder

Article Info

Accepted:
22 July 2019
Available Online:
10 August 2019

A B S T R A C T

Sheath rot disease caused by *S. oryzae* has emerged as one of the major diseases of rice inflicting yield losses ranging from 9.6 to 85% depending on the weather conditions. Pesticides are mostly used for its management and application of these chemicals over a long period may result in development of resistance, pesticide residue risk, health and environmental issues which necessitates an alternative economically feasible eco-friendly method for its management. The present investigation was undertaken to evaluate the effect of method of application of microbial antagonists in suppression of sheath rot disease of rice under green house condition. Talcum powder based bio-formulation of *P. fluorescens* (10^9 cfu/ml) was found most effective when applied in combination as seed treatment, seedling root dip treatment and foliar spray at boot leaf stage in reducing maximum DI (68.24%) and PDI (57.26%) over control and also found to increase the yield attributing characters and grain yield to the tune of 13.19 % over control under greenhouse condition.

Introduction

Rice (*Oryza sativa L.*) is one of the principal staple food for more than half of the world's population and approximately 90 per cent of the world`s rice is grown and consumed in Asia and 60 per cent of world`s population were depends on rice for their half of the calorie intake. Rice contributed about 45 per cent of total cereal production in India and is the main food source for more than 60 per cent population of the country. In India, the crop is cultivated in about 43.19 million hectares area with an annual production of 110.15 million tonnes and productivity of 2550 kg/ha (Anonymous, 2017). In Assam rice is grown in an area of 24.67 Lakh hectares with a production of 51.27 Lakh tonnes and productivity of 2078 kg/ha (Anonymous, 2016) which is very low as compared to national average. The hot and humid climate prevailing in Assam during the long and heavy

monsoon season provides the most favourable agro-ecological environment for rice cultivation as well as for disease development. Sawada (1922) first reported the occurrence of sheath rot disease of rice from Formosa, Taiwan and was subsequently reported from different rice-growing ecosystems of the world. Agnihothrudu (1973) reported the disease for the first time in India and later several workers reported it from different parts of the country.

The crop is constantly subjected to various diseases fungal, bacterial and viral diseases. Among the various diseases, sheath rot disease caused by *Sarocladium oryzae* (Sawada) Gams and Hawksw has gained the status of major diseases of rice inflicting considerable yield losses all over the rice growing areas of the world especially due to the introduction of high yielding cultivars (Reddy and Gosh, 1985). The yield losses due to sheath rot disease vary from 9.6 to 85% depending on the weather conditions during the crop growth period (Phookan and Hazarika, 1992).The disease causes empty grain production (Kulwant and Mathur, 1992) and glume discolouration (Sachan and Agarwal, 1995) and seed discolouration (Reddy *et al.,* 2000). It also causes poor grain filling and reduction in seed germination (Vidyasekaran *et al.,* 1984).

Chemical pesticides are exclusively used for the management of the disease but not considered as a long term solution because it may lead to health and environment hazards, residue persistence and elimination of natural enemies and development of resistance. Increasing public concerns about the quality of food grains has accelerated the development of ecofriendly and economically feasible control methods.

The use of microorganisms as biological control agents to control plant disease has emerged as powerful alternative method (Kulkarni *et al.,* 2007). Antagonistic fungi have been used exclusively as biocontrol agents to control plant diseases with 90% of applications being made using different strains of *Trichoderma* e.g. *T. harzianum*, *T. virens*, *T. viride* (Benitez *et al.,* 2004). Plant Growth Promoting Rhizobacteria, *Pseudomonas fluorescens* also plays an important role as biocontrol agent in management of several soil borne pathogens (Sakthivel and Gnanamanickam, 1987). They reported that seed bacterization with *P. fluorescens* (Pfc-strain) on rice plants (cv. TKM 9) under green house and field studies could enhance plant height (12-27%) and substantially reduced the sheath rot infection thereby enhanced (3-16%) grain yield. Looking to the above figure and facts, the present study was undertaken evaluate the effect of method of application of microbial antagonists in suppression of sheath rot disease of rice under greenhouse condition.

Materials and Methods

Collection of diseased specimens

Rice (*Oryzae sativa*) plants showing typical symptoms of sheath rot disease were selected for collection of disease specimen. The boot leaf sheath showing the typical symptoms of sheath rot disease were collected from the experimental farm, Regional Agricultural Research Station, North Lakhimpur. The samples were brought to the laboratory for critical observation and for further studies.

Isolation and purification of the causal organism

The diseased specimens showing typical symptom were first washed thoroughly with tap water and then rinsed with distilled water for further studies. Small portion of infected parts containing healthy and diseased tissues were cut in to 0.5cm pieces with the help of

sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite solution for 2 minutes and rinsed aseptically in three changes of sterilized distilled water to remove traces of the chemical and dried in sterilized blotting paper. The surface sterilized pieces were then transferred aseptically to petri dishes containing 2 per cent sterilized Potato Dextrose Agar (PDA) and incubated at 28±2°C for 7-8 days. The petri dishes were examined at regular time intervals for fungal growth and then transferred aseptically to potato dextrose agar slants. The fungal culture was purified by single spore isolation method and the fungus isolated during the present study was identified based on the characteristics of the colony, mycelium, conidiophores, and conidia with the help of standard reference books (Hollidays, 1980; Manibhushan Rao, 1996).

Pathogenicity test

Susceptible rice variety Mahsuri were grown in the greenhouse to perform the pathogenicity test of the pathogen. At booting stage, tillers were inoculated with pure culture of *S. oryzae* grown on rice grains following standard grain inoculum technique (Sakthivel and gnanamanickam, 1987). A total of 25 tillers were inoculated with the isolate of *S. oryzae*. Observations were made regularly for the appearance and development of symptoms. After 14 days of inoculation, symptoms of sheath rot disease occur (International Rice Research Institute, Philippines, 1988)

Pot experiment

A Pot experiment was conducted at Krishi Vigyan Kendra, Lakhimpur during 2016-17 with three replications following the Completely Randomized Block (CRB) to evaluate the effect of method of application of *Pf* formulation against sheath rot disease. 30 days old seedlings of rice variety Mahsuri were transplanted in pots @ three hills per pot and all the production practices recommended in the packages and practices jointly published by the department of Agriculture, Government of Assam and Assam Agricultural University, Jorhat were followed. The following treatments were undertaken for the pot experiment.

T_1: Seed Treatment (ST) with bio-formulation (*Pf*)

T_2: Seedling Root Dip treatment (SRDT) with bio-formulation (*Pf*)

T_3: Foliar Spray (FS) at boot leaf stage with bio-formulation (*Pf*)

T_4: T1+T2

T_5: T1+T3

T_6: T2+T3

T_7: T1+T2+T3

T_8: Control

Preparation of Antagonist bio-formulation

Pseudomonas fluorescens, the most effective microbial antagonist against *S. oryzae* as observed in the dual culture technique *in vitro* was selected for preparation of bio-formulation and talcum powder based bio-formulation of *P. fluorescens* was prepared by following the standard procedures (Bora and Bora, 2008; Bora *et al.*, 2013).

Preparation of Nursery bed

Seed Treatment

Seeds of variety Mahsuri were treated with the *Pf* formulation @ 10gm per kg of seed for one

litre of water which served as one of the treatments. Two lots of seeds were soaked separately one treated with *Pf* formulation and another without formulation for 24 hours and incubated for 48 hours at room temperature for sprouting.

Raising of nursery bed

Nursery beds were prepared at experiment farm KVK, Lakhimpur following the recommended agronomic practices of AAU, Jorhat. Two nursery beds were prepared separately, one for treated seed and another for untreated seeds.

The sprouted seeds were shown uniformly in two separate seed beds after application of FYM to increase the seedling vigour and ensured adequate supply of water as and when needed.

Seedling Root Dip treatment

Seedlings of rice variety Mahsuri were uprooted and washed in trap water and then dipped in a solution of the talc based bio-formulation of the microbial antagonist (*Pf*) @ 10 gm per litre of water for 1 hour before transplanting which served as one of the treatments.

Inoculation of Pathogens

Artificial inoculation of the fungus (*S. oryzae*) into the healthy rice plants was done by following the single grain insertion method given by Sakthivel and Gnanamanickam (1987). In this method, 15 days old mass culture of the fungus (*S. oryzae*) grown on rice grains were used for inoculation to the healthy rice plants of variety Mahsuri grown on pots at boot leaf stage by gently inserting the single grain in between the flag leaf sheath and unemerged panicle, with the help of sterilized forceps and covered with polythene bags for development of symptoms.

Foliar spray of bio-formulation (*Pf*)

Foliar spray of the talcum powder based bio-formulation of the microbial antagonist (*Pf*) was given two days after artificial inoculation of the sheath rot pathogen @ 0.2% at boot leaf stage which served as one of the treatments.

Assessment of Disease Incidence and Percent Disease Index

Inoculated plants will be regularly observed for first appearance of sheath rot symptoms. The percent disease incidence will be calculated after 25 days of inoculation by counting the total number of infected tillers and then dividing it by total number of inoculated tillers and multiplied by 100 as described by Mukherjee *et al.,* (1981). Observations on the percent disease severity will be calculated by using standard evaluation system rice (IRRI, 1996) rating scale (Table 2)

The numerical values will be further used for the calculation of PDI (Percent disease index) using the formula:

$$\text{PDI} = \frac{\text{Sum of individual rating}}{\text{No. of leaves examined}} \times \frac{100}{\text{Maximum disease rating}}$$

DI and PDI were calculated by following the standard formula. The yield attributing characters such as plant height, no of total, effective tiller per hill, panicle length, no of total, healthy grains per panicle, no of discoloured and chaffy grains per panicle, 1000 grain weight and grain yield per hill were recorded

Statistical Analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for Completely Randomized Design

and Randomized Block Design and interpretation of data was carried out in accordance with Gomez and Gomez (1984). The treatment means were compared by Duncan's Multiple Range Test (DMRT).

The package used for analysis was IRRI-Stat version 92- a developed by International Rice Research Institute, Biometrics Units, The Philippines.

Results and Discussion

Effect of Method of application of microbial antagonist against S. oryzae

Data presented in Table 3, 4 revealed that the combined application of talcum powder based bio-formulation of *P. fluorescens* as seed treatment (ST), seedling root dip treatment (SRDT) and foliar spray (FS) was found to be the most effective treatment combination recording lowest DI (9.82 %) and PDI (14.57 %) respectively among all the treatments and also recorded the maximum reduction of DI and PDI (68.24%, 57.26 %) over control followed by ST+ SRDT (57.41 %, 48.60%) and ST+ FS (52.66, 38.49%) respectively. This treatment combination (ST +SRDT + FS) was also found to be most effective recording highest number of tillers (13.67) per hill, highest number of effective tillers (12.34) per hill, highest plant height (135.24 cm) and panicle length (24.96 cm), highest number of total grains (238.27), healthy grains (222.27) per panicle, lowest number of discoloured grains (10.22), chaffy grains (6.41) per panicle and highest 1000 grains weight (17.64 g) and grain yield per hill(51.24 g).

The possible mechanism for reduction of sheath rot disease incidence and percent disease index and also enhancement of yield attributes in the present study might be due to increased growth rate of rice plants because of *P. fluorescens,* which is a plant growth promoting rhizobacteria and also may be due to induction of systemic resistance against *S. oryzae* which is in conformity with the findings of (Weller and Thomashow, 1994) who observed that Growth promotion occurs as a result of direct stimulation of plant growth, induction of host plant systemic resistance, or suppression of plant pathogens.

Table.2 Standard Evaluation System Rice Rating Scale (IRRI, 1996)

Scale Grade	Description
0	No lesion/spot on flag leaf sheath
1.	Spot visible on the tillers upon very careful examination (< 1 % flag leaf sheath area covered).
3	Spot visible on the tillers upon very careful examination (1-5 % flag leaf sheath area covered).
5	Spots easily visible on the tillers (6-25 % flag leaf sheath area covered)
7	Spots present on almost whole the tillers parts (26-50 %) flag leaf sheath area covered, damage conspicuous.
9	Spots very common on whole the tillers parts (51-100%) flag leaf sheath area covered), death of plants common, damage directly reduce severe yield loss.

Table.3 Effect of method of application of *Pf* formulation against sheath rot disease of rice under greenhouse condition

Treatments	Total no of tillers /hill	No of effective tillers/hill	DI (%)	Reduction of DI over control (%)	PDI (%)	Reduction of PDI over control (%)
T_1=ST	10.67^{c}	8.67^{d}	18.84 $(25.68)^{cd}$	39.14	26.53 $(31.0)^{d}$	36.96
T_2=SRDT	$9.34d^{e}$	7.34^{ef}	21.48 $(27.60)^{b}$	30.59	37.35 $(37.66)^{bc}$	25.77
T_3=FS	8.67^{e}	6.67^{f}	23.18 $(28.75)^{ab}$	25.09	39.09 $(38.69)^{b}$	14.48
T_4=ST + SRDT	12.67^{b}	11.12^{b}	13.18 $(20.98)^{de}$	57.41	19.23 $(25.98)^{e}$	48.60
T_5=ST+FS	11.34^{c}	9.67^{c}	14.65 $(22.29)^{cde}$	52.66	20.89 $(27.18)^{e}$	38.49
T_6=RDT+ FS	9.67^{d}	7.67^{e}	20.76 $(27.08)^{bc}$	32.93	34.43 $(35.93)^{c}$	28.96
T_7=ST+SRDT+FS	13.67^{a}	12.34^{a}	9.82 $(17.93)^{e}$	68.24	14.57 $(22.42)^{f}$	57.26
T8= Control	7.67^{f}	5.34^{g}	30.95 $(33.35)^{a}$		45.0 $(42.13)^{a}$	
SEd ±	0.47	0.44	2.3		1.19	
CD(P=0.05)	1.08	0.94	4.92		2.56	

ST= Seed Treatment, SRDT= Seedling Root Dip Treatment, FS= Foliar Spray, *Pf= Pseudomonas fluorescens* DI= Disease Incidence, PDI= Percent Disease Index, Data within parenthesis are angular transformed values

Table.4 Effect of method of application of *Pf* formulation on the yield attributing characters of rice under greenhouse condition

Treatments	Plant height (cm)	Panicle length (cm)	Total no of grains/ panicle	No of healthy grains/ panicle	No of chaffy grains/ panicle	No of discoloured grains/ panicle	1000 grain weight(g)	Grain yield/hill (gm)
T_1=ST	128.21^{cd}	23.42^{b}	221.65^{c}	190.19^{d}	13.12^{de}	19.48^{c}	16.78^{bc}	48.74^{bc}
T_2=SRDT	125.56^{de}	23.24^{bc}	210.98^{d}	172.13^{f}	17.25^{bc}	22.19^{bc}	16.12^{cd}	47.56^{cd}
T_3=FS	124.26^{e}	23.18^{bc}	196.95^{e}	152.16^{g}	20.14^{b}	25.12^{b}	15.46^{de}	46.173^{de}
T_4=ST + SRDT	132.52^{ab}	24.36^{ab}	230.65^{b}	210.31^{b}	8.15^{tg}	13.25^{de}	17.27^{ab}	50.64^{ab}
T_5=ST+FS	130.12^{bc}	24.253^{ab}	224.53^{bc}	198.29^{c}	11.14^{ef}	16.12^{d}	17.16^{ab}	50.38^{ab}
T_6=RDT+ FS	126.45^{de}	23.28^{b}	214.94^{d}	180.13^{e}	15.41^{cd}	20.13^{c}	16.32^{c}	48.323^{c}
T_7=ST+SRDT+FS	135.24^{a}	24.96^{a}	238.27^{a}	222.27^{a}	6.41^{g}	10.22^{e}	17.64^{a}	51.24^{a}
T8= Control	120.78^{f}	21.86^{c}	170.62^{f}	117.47^{h}	24.15^{a}	30.12^{a}	15.14^{e}	44.48^{e}
SEd ±	3.32	0.65	2.92	2.91	1.68	1.45	0.36	0.89
CD(P=0.05)	1.56	1.4	6.24	6.22	3.61	3.1	0.76	1.91

ST= Seed Treatment, SRDT= Seedling Root Dip Treatment, FS= Foliar Spray, *Pf= Pseudomonas fluorescens,* Data are mean of three replications.

Antifungal metabolites produced in situ by PGPR have key roles in the suppression of soil borne pathogens (Lugtenberg *et al.*, 2002; Van Loon *et al.*, 1998). Similar trends of results were also observed by several earlier workers (Nayar, 1996; Jeyalakshmi *et al.*, 2010; Manonmani *et al.*, 2008; Mathivanan *et al.*, 2005).

Combined application of *P. fluorescens* bio-formulation as seed treatment (ST), seedling root dip treatment (SRDT) and foliar spray (FS) was found to be the most effective among all the treatments recording lowest DI (9.82 %) and PDI (14.57 %) and also increased the yield attributing characters of rice under greenhouse condition.

Acknowledgements

The authors are thankful to Head, Department of Plant Pathology, Dean, College of Agriculture and Director of Post Graduate Studies, Assam Agricultural University, Jorhat for providing all the necessary facilities for conducting the experiment.

References

Agnihothrudu, V. (1973). *Acrocylindrium oryzae* Sawada- Sheath rot on paddy. *Kavaka*, 1:69-71.

Anonymous (2016). Directorate of Agriculture, Department of Agriculture and Horticulture, Government of Assam, Guwahati

Anonymous (2017). Directorate of Economics and Statistics, Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India, New Delhi, pp.88

Benítez, T.; Rincón, M.A.; Limón, M.C. and Codón, C.A. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiol*, 7: 249-260.

Bora, L. C. and Bora, P. (2008). Mass culture of saprophytic antagonists and their application for management of bacterial wilt and rhizome rot of ginger. *J. Mycol. Pl. Path*., 38(1): 245-251.

Bora, P.; Bora, L. C. and Begum, M. (2013). Ecofriendly management of bacterial wilt disease in brinjal through application of antagonistic microbial population. *Journal of Biological Control*, 27(1): 29-34.

Gomez, K. A. and Gomez, A. A. (1984). A Statistical Procedure for Agricultural Research. John Wiley and Sons, New York

Holliday, P. (1980). Fungus Diseases of Tropical Crops. CMI. Kew, Cambridge University Ross, Cambridge. 605 p.

IRRI. (1988). Annual Report for 1979, Los Banos. Philippines, 123-128.

IRRI. (1996). *Annu. Rep. Rice*. p. 25.

Jeyalakshmi, C.; Madhiazhagan, K. and Rettinassababady, C. (2010). Effect of different methods of application of *Pseudomonas fluorescens* against bacterial leaf blight under direct sown rice. *Journal of Biopesticides*, 3(2): 487 – 488.

Kulkarni, M.; Chaudhari, R. and Chaudhari, A. (2007). Novel tensio-active microbial compounds for biocontrol applicatins. In: *General Concepts in Integrated Pest and Disease Management* (eds. A. Ciancio and K.G. Mukerji). Springer, 295-304.

Kulwanth, S. and Mathur, S.B. (1992). Further evidence of transmission of *Sarocladium oryzae* through rice seeds and its quarantine significance. *Indian Phytopath*, 45(1): 454 456.

Lugtenberg, B.J.J.; Chin-a-Woeng, T.F.C. and Bloemberg, G.V. (2002). Microbe-plant interactions: principles and mechanisms *Antonie van Leeuwenhoek*, 81: 373–383

Manibhushanrao, K. (1996). Sheath Rot Disease of Rice. Daya Publishing

House, Delhi. p. 71.

Manonmani, K.; Jayasekhar, M. and Muthusamy, M. (2008). Biocontrol efficacy of bacterial antagonists in the management of sheath rot disease of rice. *Indian J. Agric. Res*., 42(3): 212-215.

Mathivanan, N.; Prabavathy, V.R. and Vijayanandraj, V. R. (2005). Application of talc formulations of *Pseudomonas fluorescens* migula and *Trichoderma viride* pers. ex. S.f. gray decrease the sheath blight disease and enhance the plant growth and yield in rice. *Journal of Phytopathol.* 153: 697-701.

Mukherjee, P.; Singh, B.B. and Rahman, F. (1981). Testing of indigenous germ plasm of rice against sheath rot artificial inoculation. *Indian Phytopath*, 34: 287-290.

Nayar, K. (1996). Development and evaluation of a biopes ticide formulation for the control of foliar pathogens of rice. Ph.D Thesis, TNAU, Coimbatore, India.

Phookan, A. K. and Hazarika, D.K. (1992). Distribution of sheath rot (ShR) in six agroclimatic zones of Assam, India. *IRRN*, 17: 16.

Reddy, C.S. and Ghosh, A. (1985). Sheath rot incidence and yield losses in rice due to the joint infection of rice tungro virus and sheath rot fungus. *Indian Phytopath*., 38(1): 165.

Reddy, M.M.; Reddy, C. S. and Gopal, S.B. (2000). Effect of sheath rot disease on quality characters of rice grain. *J. Mycol. Plant Pathol*., 30(1):68-72.

Sachan, I.P. and Agrawal, V.K. (1995). Seed discoloration of rice: location of inoculum and influence on nutritional value. *Indian Phytopath*., 48(1): 14-20.

Sakthivel, N. and Gnanamanickm, S.S. (1987). Evaluation of *P. fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice (*Oryza sativa* L.). *Appl. Environ. Microbiol*., 53(9):2056-2059.

Sawada, K. (1922). Descriptive Catalogue of Formosan fungi II. *Rep.Govt.Res.Inst. Dep. Agric. Formosa*, 2: 1-135.

Van Loon, L.C.; Bakker, P.A.H.M. and Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol*., 36: 453-483.

Vidhyasekaran, P.; Ranganathan, K. and Rajamanickam, J. (1984). Quality of rice grains from sheath rot affected plants. *IRRN*, 12(1):17.

Weller, D.M. and Thomashow, L.S. (1994). Current challenges in introducing beneficial microorganisms into the rhizosphere. In: Molecular ecology of rhizosphere microorganisms (O'Gara F. Dowling D.N. Boesten B., Eds.), pp.1–18 VCH, Weinheim

How to cite this article:

Bora B. and Ali M. S. 2019. Assessment of Method of Application of Microbial Antagonist for the Management of Sheath Rot Disease of Rice under Green House Condition. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 2846-2853. doi: https://doi.org/10.20546/ijcmas.2019.808.327