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RESEARCH ARTICLE

BIO-INOCULATION EFFECT OF *B. mucilaginosus* AND ORGANIC RESIDUES SUPPLEMENTATION AND ENHANCEMENT OF INDUCED SYSTEMIC RESISTANCE (ISR) AGAINST *Pyricularia oryzae* ON GROWTH AND YIELD OF LOWLAND RICE *var.*, IR-50

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ABSTRACT

The bioinoculation effect of two different formulations of COI and biofloc of *B. mucilaginosus* isolates *viz*., SSB-8, SSB-11 and SSB-17 together with supplementation of rice residues *viz*., rice straw, rice husk, and black ash on the induction of systemic resistance against *Pyricularia oryzae* in lowland rice was studied under pot culture conditions. It was observed that the individual application of *B. mucilaginosus* cells *viz*., either COI or biofloc altered the biochemical and physiological parameters *viz*., reducing and non-reducing sugar levels, total phenol content and defense enzymes activities, such as peroxidase (PO), polyphenol oxidase (PPO) of rice plant to a higher level when compared to control. However, the application of *B.mucilaginosus* cells as bifoloc was found to augment the above parameters to a higher level when compared to co-inoculation of the same isolates of *B.mucilaginosus* isolates. Interestingly, the effect was more pronounced during the supplementation of rice residues along with *B.mucilaginosus* inoculation. Among the different rice residues tested, rice straw, supplementation augmented the systemic resistance of lowland rice against *Pyricularia oryzae* to a higher level when compared to rice husk and black ash supplementation, the treatment comprising of biofloc formulation of *B.mucilaginosus* isolates together with supplementation of rice straw @ 5 t ha^{-1} induced the systemic resistance of lowland rice against *P. oryzae* to the highest level when compared to other treatments. The effect was more pronounced during the supplementation of silicon rich, rice residues along with the application of *B.mucilaginosus* bioflocs. Hence, it is proposed that the application of SSB isolates, as biofloc formulation, together with supplementation of rice straw @ 5 t ha^{-1} might be followed for the maximization of ISR in rice *P. oryzae* pathosystem under lowland condition. It has been postulated that the EPS biosynthesis of SSB cells during the biofloc processes might act as an elicitor for the enhancement of ISR in rice *P. oryzae* pathosystem.

INTRODUCTION

Silicon (Si) is the second most abundant mineral element in soil comparing 28 per cent of the earth's crust, approximately (Epstein, 1999). Silicon is also one of the most dominant elements in the ash of plants, especially, grasses and cereals. Among the different agriculturally important plant species, rice is a siliceous plant and found to be the largest uptaker of silicon from soil (Basak *et al*., 2009). Eventhough, silicon present in different forms in soil, monosilicic acid (H_2SO_4) and polysilicic acid are the main forms of the same. Silicon in rice is usually deposited in various organs which include the husks, leaves leaf sheath, culm and roots. It has been observed that supplementation of silicon improved the yield and reduced the plant biotic stresses of crop plants including blast caused (Epstein, 1994). Although silicon is not considered an essential element for rice, it is responsible for strengthening the leaves which contribute to high disease and pest resistance/ tolerance (Meyer and Keeping, 2001). The application of Si to rice plants under controlled conditions increased the systemic resistance to blast as well as increased Si content in rice. The Si content in rice straw and husks were proportional to the amount of Si is applied to the soil, and that the severity of blast on panicles was inversely proportional to the amount of the Si in rice tissues (Kawashima, 1927). The role of different bacterial genera in the dissolution of silicate minerals in soil has been reported, frequently (Datnoff *et al*., 1997; Belanger *et al*., 1995). The possible role of *B.mucilaginosus* on silicate solubilization in soil has been well documented (Vonder Weid, 2000) reported that the dissolution of silicate minerals by *B.mucilaginosus* was directly proportional to the promotion of organic acid produced by bacteria. The acidolysis and ligand degradation are the main mechanisms of *B.mucilaginosus* in dissolving silicate minerals. The formation of bacterium mineral complexes is the necessary condition for the bacterial weathering of silicate minerals and extracellular polysaccharides also played in important role in bacterium-mineral interaction processes by forming bacterium-minor physiochemical properties of micro environment (Mo Binbin and Lan Bin, 2011). Straw, husk and

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black ash are the important residues of rice with high silicon content. If this residue is not returned to soil this may cause mining of soil for silicon nutrient leading to negative balance in rice crop. This is one of the reasons for the yield decline in lowland rice system. Thus, there are urgent needs to usage these residues of rice crop for sustainability and stability of the system (Mandal *et al*., 2004). In the present study, the bio-inoculation effect of two different formulations of *B.mucilaginosus* cells and rice residues supplementation on the enhancement of induced systemic resistance (ISR) against *Pyricularia oryzae* in lowland rice has been studied under pot culture condition.

MATERIALS AND METHODS

A pot culture experiment was conducted to study the effect of two different formulations of *Bacillus mucilaginosus* cells *viz.*, co-inoculation and biofloc application on the enhancement of growth and yield in lowland rice with special emphasis to ISR mediated biocontrol against blast disease (*Pyricularia oryzae*) incidence. The study was conducted during Rabi season (Sep to Jan. of 2011). Rectangular cement pots of size 18"x12"x12" were filled with 45 kg of paddy field soil flooded with water for 2 days and brought to fine puddle condition. Seeds of the rice variety IR 50 were loosely packed separately in small gunny bag and soaked in water for 12 h. Then, the bags were subsequently kept in dark place after covering with wet gunny bags to ensure optimum condition for germination. The seeds germinated within 24 h. after soaking. The pre-germinated seeds of rice (cv. IR 50) was treated with SSB isolates and bioflocs of the same was sown in rows in pots separately. On the 5^{th} day of sowing, the seedlings were thinned to get 50 numbers per pot. The age of the seedlings were counted from the time of sowing. The experiment was arranged in randomized block design (RBD) with three replications and its following were

Treatment

T_1 - Control
T_2 - Co-inoculation (COI) of SSB isolates alone
T_3 - COI + rice straw
T_4 - COI + rice husk
T_5 - COI + Black ash
T_6 - Biofloc (Natural) of SSB isolates alone
T_7 - Biofloc (N) + rice straw
T_8 - Biofloc (N) + rice husk
T_9 - Biofloc (N) + black ash

During the experimental period, the annual mean minimum and maximum temperature of experimental area is 25°C and 39°C, respectively and the mean highest and lowest relative humidity were 96 and 78 per cent respectively. The mean annual rainfall of this area is 1500 mm. The water level was maintained at 5-10 cm level throughout the crop growth. Gap filling was done after 10 days of planting. Weeding was done manually twice during crop growth. The soil samples were collected, processed and analysed for silica and potassium content by the following methods. A fertilizer schedule of 100:50:50 NPK ha^{-1} was followed. Regarding the N fertilization, 50% of the same was given as basal dose, while other 50% was given as top dressing in two split doses. The rice residues like straw, husk and black ash were added @ 5 t ha^{-1} prior to planting. The entire P and K were applied basally. The pots were inoculated with SSB.

Challenge inoculation of rice plants with *Pyricularia oryzae*

The inoculum of *P. oryzae* which was maintained in oat meal agar (OMA) medium was used for the inoculation purpose. Thick spore suspension was prepared with sterile distilled water from 10 day old culture maintained in OMA medium and strained through double layer muslin cloth so as to get a free suspension of conidia. The population was adjusted with the help of Haemocytometer and a spore suspension with optimum spore concentration (50,000 spore's ml^{-1}) was prepared. Then, the spore suspension was added with few drops of Tween-80 which increased the adherence capacity of the spores and acts as a sticker. Before spaying, the plants were pre-incubated for sometime by covering with thick, water sprayed polyethylene sheets to preserve moisture and increase the leaf cells more prone to the pathogen. Then, sheets were removed and spraying of spore suspension was done late in the evening. Control plants were also sprayed with sterile distilled water. After spraying, the plants were covered with polyethylene bags again for about 72 h to maintain the humidity.

Composition of oat meal agar medium (Rangaswami, 1960)

Quaker oats	- 100.0 g
Agar	- 15.0 g
Distilled water	- 1000 ml
pH	- 6.0 - 6.5

Sampling

For biochemical analysis, the samples were collected at 0, 7, 14 and 24 d after challenge inoculation of *P. oryzae*. The plant height, shoot dry weight, root dry weight, chlorophyll content (Mahadevan and Sridhar, 1986), IAA production (Tien *et al*., 1979), silica content (Sizumi and Yoshida, 1962), grain and straw yield of lowland rice was recorded on 45^{th} DAS. The reducing and non-reducing sugar was estimated according to Mahadevan and Sridhar, 1986) whereas the total phenol content was assayed according to Malik *et al*. (1997). The defence enzyme activities such as peroxidase (PO), polyphenol oxidase (PPO) was assayed according to Putter (1974) and Ester Baier (1977) respectively.

RESULTS AND DISCUSSION

The effect of two different bioformulation *viz.*, co-inoculation + biofloc application of silicate solubilizing bacterial cells *viz.*, *Bacillus mucilaginosus* together with rice residues supplementationon the growth yield parameters *viz.*, plant height, root and shoot dry weight, silica, potassium, IAA and chlorophyll content, grain and straw yield of lowland rice cv. IR 50 was studied under pot culture condition. It was observed that application of two different formulations of SSB cells could augment the growth and yield parameters of lowland rice cv. IR 50 when compared to control (without any bio-inoculation). These observations clearly revealed the positive effect of SSB cells inoculation in augmenting the growth and yield parameter of lowland rice. Regarding the two different formulations of SSB cells, the application of biofloc formulations consisting of *Bacillus mucilaginosus* isolates could augment the growth and yield parameters of lowland rice to a higher level when compared to co-inoculation of SSB isolates. However, the effect was more

Table 1. Effect of different formulation of *Bacillus mucilaginosus* cells on the enhancement of growth and yield parameter in lowland rice (*Oryza sativa*) cv. IR 50

Treatments	Plant height (cm)	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Silica content (kg ha^{-1})	Potassium content (kg ha^{-1})	Chlorophyll content (mg g^{-1} of leaf)	IAA content (%)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Control	51.2^{i}	0.341^{i}	0.86^{i}	118.2^{i}	28.5^{i}	4.00^{i}	11.40^{i}	5.80^{i}	8.64^{i}
COI + alone	56.4^{h}	0.361^{h}	1.00^{h}	125.5^{h}	325^{h}	1.34^{h}	15.11^{h}	5.87^{h}	8.92^{h}
COI + rice straw	68.0^{d}	0.421^{d}	1.52^{d}	157.2^{d}	352^{d}	1.66^{d}	16.75^{d}	6.32^{d}	10.00^{d}
COI + rice husk	65.4^{e}	0.390^{e}	1.40^{e}	142.6^{e}	350^{e}	1.59^{e}	16.70^{e}	6.26^{e}	9.75^{e}
COI + black ash	62.4^{f}	0.381^{f}	1.31^{f}	135.7^{f}	341^{f}	1.50^{f}	15.75^{f}	6.16^{f}	9.34^{f}
Biofloc (N) alone	59.4^{g}	0.370^{g}	1.18^{g}	130.2^{g}	330^{g}	1.43^{g}	15.50^{g}	6.05^{g}	9.04^{g}
Biofloc (N) + rice straw	77.4^{a}	0.500^{a}	1.90^{a}	169.7^{a}	451^{a}	1.79^{a}	18.25^{a}	6.75^{a}	11.44^{a}
Biofloc (N) + rice husk	73.5^{b}	0.468^{b}	1.78^{b}	167.5^{b}	428^{b}	1.74^{b}	17.96^{b}	6.48^{b}	10.84^{b}
Biofloc (N) + black ash	70.2^{c}	0.437^{c}	1.69^{c}	164.5^{c}	408^{c}	1.70^{c}	17.35^{c}	6.40^{c}	10.36^{c}
LSD (p=0.05)	3.05	0.019	0.59			0.70	0.775	0.165	0.335

[a]Average of three replications
[b]Values followed by different letters are significantly differed at five per cent level according student 't' test

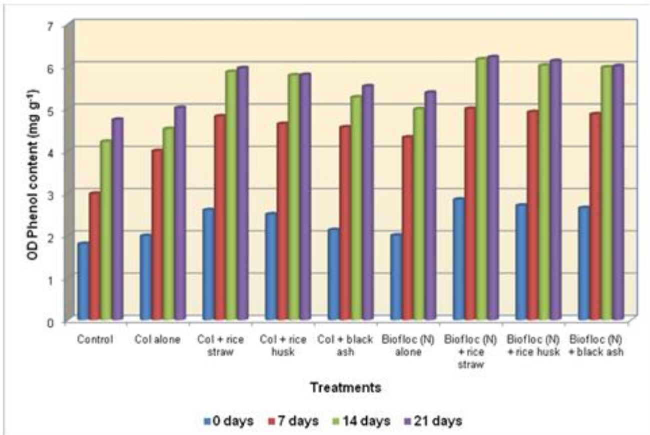

Fig. 1. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of Pyricularia oryzae on OD phenol content of lowland rice

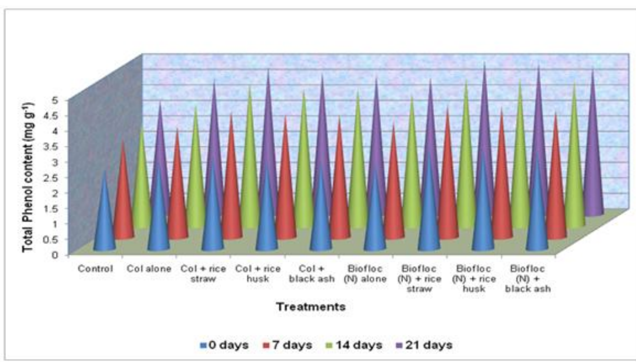

Fig. 2. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of Pyricularia oryzae on Total phenol content of lowland rice

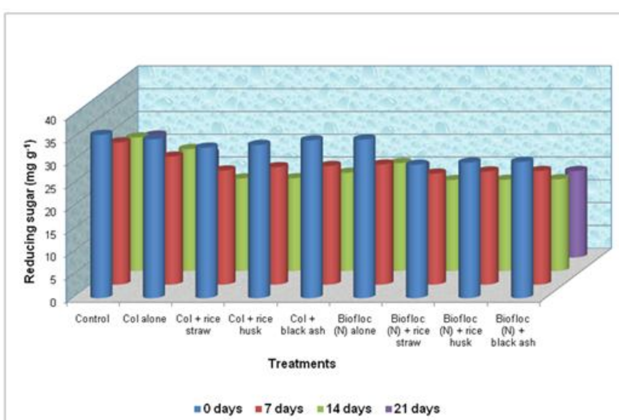

Fig. 3. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of pyricularia oryzae on Reducing sugar content or lowoland rice

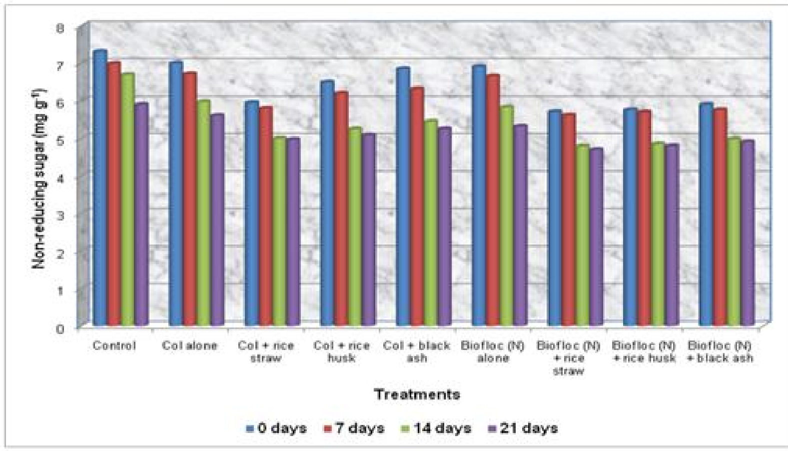

Fig. 4. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of phyricularia oryzae on non-reducing sugar content of lowland rice

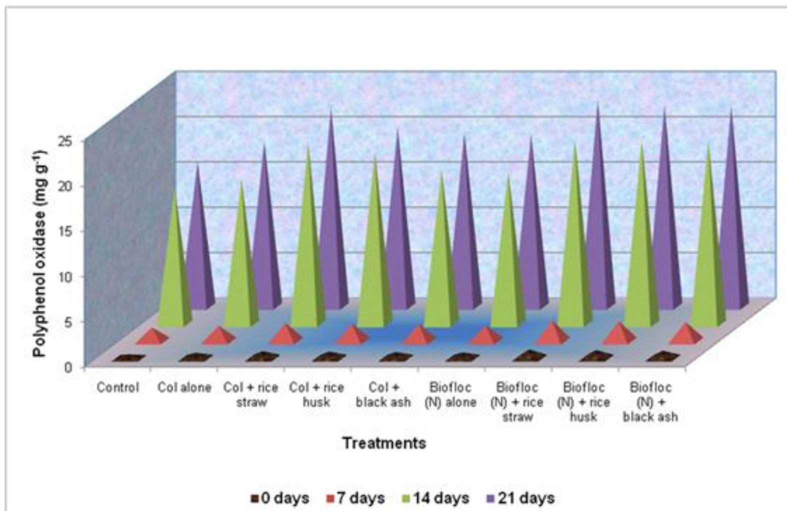

Fig. 5. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of Pyricularia oryzae on polyphenol oxidase content of lowland rice

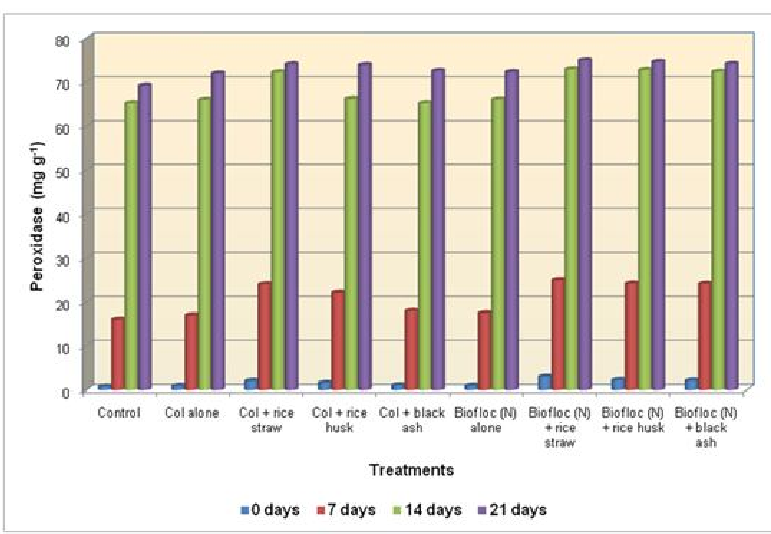

Fig. 6. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of Pyricularia oryzae on peroxidase content of lowland rice

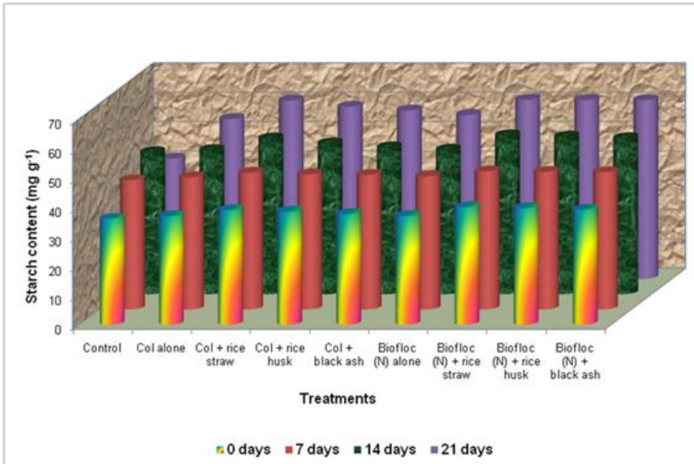

Fig. 7. Bio-inoculation effect of two different formulations of SSB isolates together with rice residues supplementation and challenge inoculation of Pyricularia oryzae on starch content of lowland rice

pronounced during the supplementation of rice residues along with bio-inoculation of SSB isolates. Greater plant height of rice due to the inoculation of *Pseudomonas* and *Paenibacillus* has been reported by Agarwal and Singh (2000). Increase in dry matter production, silica content, chlorophyll content, grain and straw yield of rice has been reported (Ding *et al*., 2005; Selvakumari *et al*., 2000; Nadeen *et al*., 2006). The positive role of biofloc application in augmenting the growth and yield parameters in upland rice reported by Umamaheswari and Sekar (2011). However, the application effect of SSB bioflocs *viz*., *B. mucilaginosus* to rice crop has not been reported so far. This is the first comprehensive report regarding the positive role of SSB bioflocs in augmenting the growth and yield parameters in lowland rice. Regarding the effect of two different bioformulations of SSB cells along with rice residues application on the enhancement of ISR mediated biocontrol of *P. oryzae* with special emphasis to biochemical and physiological aspects revealed the highest performance of SSB biofloc application together with the rice straw application @ 5 t ha^{-1} in augmenting the phenol metabolism *viz*., total phenol content and orthodihydroxy phenol, carbohydrate metabolism *viz*., reducing and non-reducing sugar level and defense enzyme activities *viz*., peroxidase (PO) and polyperoxidase (PPO) of lowland rice plant whenc compared to other treatments (Fig. 1 to 7). The application of SSB co-aggregates consisting of *B. mucilaginosus* isolate along with rice straw supplementation augmented the total phenol, OD phenol and PPO activities of lowland rice plant to a higher level whereas a reduction in reducing and non-reducing sugar levels, observed.

Farkas and Kiraly (1962) correlated to phytopathogens. It was known that OD phenols are the most active forms of phenol and their oxidation products are more toxic than phenol. The oxidation mediated by the enzyme PO and PPO and resulting quinines are effective inhibitors of S-H group of enzymes which may be inhibitory to the pathogens (Goodman *et al*., 1967). Ushrani (2005) reported the induction of phenolics content of rice plant due to *B. mucilaginosus* inoculation and challenge inoculation of *P. oryzae*. Mishra *et al*. (2006) reported the *Rhizobium* mediated induction of phenolics in rice plant during the challenge inoculation of *P.oryzae*. Nanthakumar (1998) correlated the ISR with two fold increase in peroxidase activity against rice sheath pathosystem (*Rhizoctonia solani*) in rice plant. The sugar content in healthy and pathogen inoculated plants very often correlated with resistance mechanism (Horsfal and Diamond, 1957). In the present study also, the reducing and non-reducing sugar levels were found to decrease with bioflocs application together with challenge inoculation of *P. oryzae*. The higher rate of reduction in the level of reducing and non-reducing sugars may be one among the vital phenomena contributing resistance to plant. The result of present study clearly envisaged the positive role of SSB biofloc application consisting of *B. mucilaginosus* isolates along with rice straw supplementation in augmenting the ISR against *P. oryzae* in lowland crop. However, the mechanism of SSB bioflocs mediated ISR against *P. oryzae* in rice plant is still unclear and the subject needs further elaborate research.

REFERENCES

Basak, B.B. and D.R. Biswas. 2009. Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by Sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. J. Plant and Soil, 317: 235-255.

Belanger, R.R., P.A. Bowen, D.L. Ehret and J.G. Menziers. 1995. Soluble silicon: Its role in crop and disease management of green house crops. Plant Disease, 79: 329-336.

Datnoff, L.E., C.W. Deren and G.H. Snyder. 1997. Silicon fertilization for disease management of rice in Florida. Crop Prot., 16: 525-531.

Epstein, E. 1994. The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci., USA, 91: 11-17.

Epstein, E. 1999. Silicon Annu Rev. Plant Physiol. Plant Mol. Biol., 50: 641-664.

Ester-Bauer,H,E.,Schwarzl and M.Hayn.1997. Anal Biochem., pp.477-488.

Forkas, G.L. and Z. Kiraly. 1962. Role of phenolic compounds in the physiology of plant disease and disease resistance. Phytopathol., 44: 105-150.

Goodman, R.N., Z. Kiraly and M. Zaitlin. 1967. The biochemistry and physiology of plant diseases. D.Van Nostrand Co. Inc., Princeton, New Jersey, USA, p. 354.

Horsfall, J.G. and A.E. Diamond. 1957. Interaction of tissue sugar, growth substances and disease susceptibility, Z. Pflanzenchu., 64: 415-421.

Kawashima, R. Influence of silica on rice blast disease. Japanese Journal of Soil Science and Plant Nutrition, 1: 86-91.

Krishna, G. Mandal, Arun K. Mishra, Kuntal M. Hati, Kali K. Bandyopadhyay, Prabir K. Ghosh and Manoranjan Mohanty. 2004. Rice residue management options and effects of soil properties and crop productivity. Food, Agriculture and Environment, 2(1): 224-231.

Mahadevan, A. and R. Sridhar. 1986. Methods of physiological plant pathology III Eds. Sivakami Pub., Madras, p. 82.

Malik, K.A., B. Rackshanda, S. Mehnaz, G. Rasul, M.S. Misra and S. Ali. 1997. Association of nitrogen-fixing plant growth promoting rhizobacteria (PGPR) with Kallan grass and rice. Plant and Soil, 194: 37-44.

Mayer, J.H. and M.G. Keeping. 2001. Past, present and future research of the role of silicon for sugarcane in southern Africa. In: Silicon in agriculture edited by L. Datnoff, G. Korndorfer and G. Synder, New York, Elsevier Science, pp. 257-275.

Mishra, R.P.N., R.K. Singh, H.K. Jaiswal, Vinothkumar and S. Maurya. 2006. *Rhizobium* mediated induction of phenolics and plant growth promotion in rice (*Oryza sativa* L.). Curr. Microbiol., 52: 383-389.

Putter, J. 1974. In methods of enzymatic analysis 2 Ed. Bergmeyer Academic Press, New York, p. 635.

Umashankari, J. and C. Sekar. 2011. Comparative evaluation of different bioformulations of PGPR cells on the enhancement of induced systemic resistance (ISR) in rice *Pyricularia oryzae* pathosystem under upland condition. Curr. Bot., 2(3): 12-17.

Usharani, G. 2005. Studies on the development of rhizobacteria (PGPR) and biocontrol agent of rice (*Oryza sativa* L.) in Cuddalore district, Tamil Nadu. Ph.D. Thesis, Annamalai University, India.

Vonder Weid, I.A., E. Paiva, A. Nobrega, J.D. Van Elsas and L. Seldin. 2000. Diversity of *Paenibacillus polymyxa* strains isolated from the rhizosphere of maize planted in --------- soil. Res. Microbiol., 151: 369-381.

Yoshida, S., Y. Ohnishi and K. Kitanishi. 1962. Chemical forms, mobility and deposition of silicon in rice plant. Japanese J. Soil Sci. and Plant Nutr., 8: 107-111.

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