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DEVELOPMENT AND USE OF DIFFERENT FORMULATIONS OF *PSEUDOMONAS FLUORESCENS* SIDEROPHORE FOR THE ENHANCEMENT OF PLANT GROWTH AND INDUCTION OF SYSTEMIC RESISTANCE AGAINST *PYRICULARIA ORYZAE* IN LOWLAND RICE

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ABSTRACT

The application effect of different formulations of *Pseudomonas fluorescens* siderophore *viz*., control, *Pseudomonas fluorescens* cells alone, *Pseudomonas fluorescens* + Fe + siderophore complex, Siderophore alone, Fe + siderophore complex alone and challenge inoculation of *Pyricularia oryzae* on the enhancement of growth and yield parameters and ISR mediated biocontrol against *Pyricularia oryzae* in lowland rice cv.IR-50 was studied under pot culture condition. It was observed that the application of different formulations *P. fluorescens* siderophore augmented the growth and yield parameters *viz*., plant height, shoot and root dry weight, chlorophyll content and reduced the blast disease incidence in lowland rice crop to a marked level, but with variation among them. Interestingly, the application of *P. fluorescens* cells along with their Fe + siderophore complex augmented the above said parameters to a higher level followed by Fe + siderophore complex alone, siderophore alone, *P. fluorescens* cells alone treatments. Moreover, the same treatment altered the biochemical constituent *viz*., total and OD phenol content, reducing and non-reducing sugar levels and physiological activities *viz*., PO and PPO levels of rice plant to a higher level when compared to other treatments and suggesting the induction of systemic resistance in the host plant against the phytopathogen *viz*., *Pyricularia oryzae*. The results of the present study clearly revealed the role of physiological status of the microorganism in siderophore mediated plant growth stimulation and induction of systemic resistance against *Pyricularia oryzae* in lowland rice.

KEYWORDS: Lowland rice, *Pseudomonas fluorescens*, PGPR, Siderophore, ISR, *Pyricularia oryzae.*

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INTRODUCTION

Rice (*Oryza sativa* L.) is the foremost cereal of the world and is the staple food for more than 60 per cent of world's population. India has the largest area under rice crop and ranked second in the production, next to China. Among the different rice production systems of India, the irrigated lowland ecosystem is the first and foremost one in terms of area and production, but with least productivity. Among the several biotic and abiotic factors, poor nutrient availability and incidence of diseases are considered to be the major constraints that eventually lead to the low productivity in lowland rice[1]. Iron, as an essential trace element, plays a crucial role in plant-microbe interactions. The trace element is often used as a co-factor in various enzymes and also plays a structural role in microorganisms. Despite the most abundant element on earth's crust, the availability of iron is limited by the low solubility of Fe^{3+}, a predominant state of iron in lowland rice rhizosphere[2]. Moreover, the incidence of blast disease, caused by *Pyricularia oryzae*, is one of the ubiquitous and most destructive fungal diseases of lowland rice and causing an yield loss upto 90 per cent [3]. Hence, the lowland rice productivity (yield/ha) must be greatly increased by providing additional nutrient inputs and through effective control of phytopathogens.Now-a-days, lowland rice production strategies mainly focus on the enormous use of synthetic fertilizers and pesticides to enhance the per hectare yield of the crop. The consistent uses of these chemicals are too expensive and cause undesirable changes in the environment. Hence, the use of plant growth promoting rhizobacteria (PGPR), as a biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemicals. Moreover, the biological approach has a great potential in supplying "Fe" nutrition and biocontrol against phytopathogens by the production of iron chelating compounds such as, siderophores that are secreted and used by many microorganisms under condition of iron limitation [4, 5]. The occurrence and activities of fluorescent pseudomonads, as PGPR, in lowland rice rhizosphere have been frequently reported [6,7,8,9]. *P. fluorescens*, as a member of fluorescent pseudomonads, has an ubiquitous occurrence in lowland rice rhizosphere and involved in plant growth stimulation and ISR mediated biocontrol against *Pyricularia oryzae*[10,11,12,13,14]. *P. fluorescens*, as PGPR, has a fast growth in lowland rice rhizosphere when compared to other bacteria and produced a variety of secondary metabolites, including siderophore. The positive role of microbial siderophores on plant growth stimulation and biocontrol of phytopathogens in different crop plants has been reported by many authors [15,16,17,18]. However, there were no earlier reports on the positive role of microbial siderophores, produced by *P. fluorescens*, on plant growth stimulation and ISR mediated biocontrol against *Pyricularia oryzae* in lowland rice, available. Hence, the present study has been undertaken with aim to exploit the positive role of different formulations of siderophore, produced by *P. fluorescens*, on plant growth stimulation and ISR mediated biocontrol against *Pyricularia oryzae* for the maximization of lowland rice productivity.

MATERIALS AND METHODS

P. fluorescens (PF-5), an efficient isolate obtained from the rhizosphere of lowland rice cv.IR-50, grown at C.Veerachozhagan, Cuddalore district, Tamil Nadu state, India, was used for the present study. The isolate was maintained in King's "B" agar slants at 30 ± 2°C with monthly transfer. *P. fluorescens* (PF-5) was grown in King's "B" broth under shaking culture condition at 30 ± 2ºC for 24 h. Then, the medium was centrifuged at 5000 × g for 10 min to harvest the log phase cells and the pellets were washed three times with 0.1 M phosphate buffer (pH 6.8). Finally, the cells were resuspended in the same buffer to a cell concentration of 1×10^7 CFU/ml by measuring the OD at 420 nm and used as inoculum.*Pyricularia oryzae* AU-1 (provided by Department of plant pathology, Annamalai University) was maintained in Oat Meal Agar (OMA) medium and used for the challenge inoculation purpose. Thick spore suspension of 10 days old culture of *Pyricularia oryzae*

was prepared in sterile distilled water 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 spores/ml^{-1}) was prepared and used for challenge inoculation purpose.

(i) Seed bacterization

Seed bacterization of *P. fluorescens* isolate (PF-5) was done at a concentration of 1 x 10^7 cells/seed.

(ii) Preparation of P. fluorescens + Fe-siderophore complex

One ml culture of *P. fluorescens*, (PF-5) was inoculated into 100ml sterile King's 'B' broth, maintained in 250ml Erlenmeyer flask and inoculated at 30 ± 2˚ C for 5 d under static culture condition. After the incubation period, the iron siderophore complex was obtained by adding iron (1mM $FeCl_3$) to the bacterial suspension maintained in King's 'B' broth.

(iii) Preparation of siderophore

One ml culture of *P. fluorescens* (PF-5) was prepared in phosphate buffer (pH 6.8) and inoculated into 100 ml of King's "B" broth supplemented with NH_4Cl (0.1%, w/v) maintained in 250 ml Erlenmeyer flask under sterilized condition. The flasks were incubated for 5 days at 30 ± 2˚C under static condition. After the incubation period, the *P. fluorescens* cells were removed by centrifugation at 7000 x g for 20 min from the broth which was then dialysed against water.

(iv) Preparation of Fe-siderophore complex

This was prepared as same for siderophore preparation together with addition of 1mM $FeCl_3$ after dialysis.

POT CULTURE EXPERIMENT

A pot culture experiment was conducted at Annamalai University experimental farm, Annamalai Nagar, India, during Aug – Nov, 2011 to study the application effect of different formulations of *P. fluorescens* siderophore and challenge inoculation of *Pyricularia oryzae* on plant growth stimulation and ISR (Induced Systemic Resistance) mediated biocontrol against *Pyricularia oryzae* in lowland rice crop cv.IR-50.Rectangular cement pots with 18" X 12" X 12" size were filled with 45 kg paddy field soil, flooded with water for two days and brought into puddle condition. Seeds of rice variety cv.IR-50 were loosely packed separately in small gunny bag and soaked in water for 12 hrs. Then, the bags were subsequently kept in dark place after covering with wet gunny bags to ensure optimum condition for germination. The pre-germinated seeds of cv.IR-50 rice were sown in rows in pots separately. On the 5th day after sowing, the seedlings were thinned out in order to get 50 numbers per pot. The seedlings were raised under lowland conditions and the age was counted from the time of sowing. The experiment was arranged in a randomized block design (RBD) and three replications were maintained with following treatments *viz*., Control, *P. fluorescens* cells alone, *P. fluorescens* cells with Fe-siderophore complex, siderophore alone and Fe-siderophore complex alone. During the experimental period, the annual mean minimum and the maximum temperature of experimental area is 25ºC and 39ºC, respectively and the mean highest and lowest humidity were 96 and 78 percent, respectively. The mean annual rain fall of this area is 1500 mm. A fertilizer schedule of 30: 15: 15 NPK/ac was followed.Rice plants were challenge inoculated by spraying the *Pyricularia oryzae* spore suspension (50,000 spore/ml inoculum level) on 10th DAS with an atomizer and the control plants was sprayed with sterile water. High humidity was created by sprinkling the water frequently in the poly house. The crop was given a hand weeding on 30th DAS and well protected against pests and diseases. The experiment was maintained as per the conditions prevailing in lowland rice ecosystem. Five representative samples of plant hills in each pot were pegmarked for periodical observations. The plant height, shoot and root dry weight, chlorophyll content[19], phosphorous content[20] of lowland rice was recorded on 45 DAS. The reducing and non- reducing sugar was estimated according to Mahadevan and Sridhar[19] whereas, the total phenol content was assayed according to Malik et al.[21]. The defence enzyme activities, such as, peroxidase (PO), Polyphenol oxidase (PPO) was assayed

according to Putter[22] and Ester-Bauer[23], respectively on 0,7,14 and 21 DAS.

RESULTS AND DISCUSSION

The effect of different formulations of *P. fluorescens* siderophore *viz*., control, *P. fluorescens* cells alone, *P. fluorescens* + Fe + siderophore complex, Fe + siderophore complex alone, siderophore alone on plant growth stimulation and ISR mediated biocontrol against *Pyricularia oryzae* in lowland rice cv.IR-50 was studied under pot culture condition. Among the different treatments, the treatment of *P. fluorescens* cells along with their iron-siderophore complex was found to augment the plant height, dry weight of root and shoot, chlorophyll content and grain and straw yield of lowland rice to a maximum level followed by Fe + siderophore complex alone, siderophore alone, bacterial suspension alone and control treatments (Table 1).

Table 1

Application effect of different formulations of P. fluorescens and its siderophore application on growth and yield parameters of rice cv.IR-50 during Pyricularia oryzae incitation

Treatments *	Plant height(cm)	Dry weight		Chlorophyll content (mg/g of leaf)	Disease Incidence (%)	Grain Yield (Kg/ha)	Straw Yield (Kg/ha)
		Root (g/Plant)	Shoot (g/plant)				
Control	53.40[e]	0.282[e]	1.124[e]	2.51[e]	-	5.65[e]	9.10[e]
Pseudomonas fluorescens cells alone	60.42[d]	0.310[d]	1.286[d]	2.60[d]	31.11[d]	5.72[d]	9.70[d]
Pseudomonas fluorescens cells + Fe + siderophore complex	69.86[a]	0.413[a]	1.510[a]	2.93[a]	17.00[a]	6.20[a]	10.56[a]
Siderophore alone	63.10[c]	0.335[c]	1.382[c]	2.72[c]	23.62[c]	5.80[c]	10.00[c]
Fe + Siderophore complex alone	65.36[b]	0.369[b]	1.460[b]	2.86[b]	18.52[b]	5.97[b]	10.30[b]
LSD (P=0.05)	2.73	0.02	0.06	0.07	3.18	0.09	0.25

**** - Average of three replication (at 1 x 10^7 CFU / ml inoculum level).***

The results of the present study clearly revealed that the increased growth and yield parameters of lowland rice due to the application of live cells of *P. fluorescens*, live cells of *P. fluorescens* along with Fe + siderophore complex, siderophore alone and siderophore-Fe complex alone treatments when compared to control. However, the effect was more pronounced during the application of live cells of *P. fluorescens* along with its Fe + siderophore complex than the other treatments. The result clearly revealed the importance of plant-bacteria interaction in increasing the growth and yield parameters of lowland rice rather than mere the application of microbial siderophore and microbial siderophore + iron complex. Siderophores are well known for their plant growth promoting characteristics and as phytopathogenic suppression agents[24,25]. Many authors confirmed the ability of siderophores to reduce the chlorosis and enhance the growth of plants grown in iron deficient nutrient solutions[26, 27,28,29]. Reid et al.[28] and Becker et al.[29] discussed the importance of plant-bacteria interactions in augmenting the growth and yield parameters of different crop plants apart from the application of microbial siderophore and iron + microbial siderophore complex treatments. Bansal et al.[30] reported the plant growth stimulation and yield of wheat crop due to *Azotobacter chroococcum* and Fe-siderophore complex inoculation. In the present study, different formulations of siderophore, produced by *P. fluorescens*, and challenge inoculation of *Pyricularia oryzae* augmented the biochemical changes *viz*., increasing level of total and OD phenol, reducing level of reducing and non-reducing sugar and increasing activities of phenol oxidizing enzymes *viz*., PO and PPO in lowland rice (Fig 1 to Fig 6).

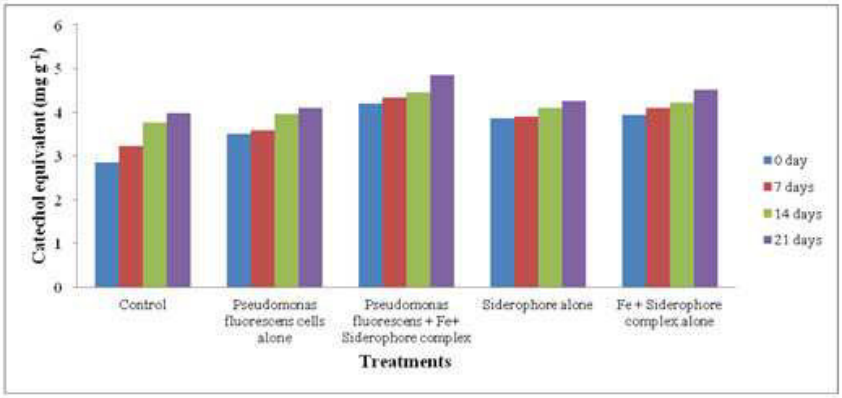

Figure 1

Changes in total phenol content of IR-50rice as influenced by the application of different formulations of Pseudomonas fluorescens siderophore during Pyricularia oryzae incitation

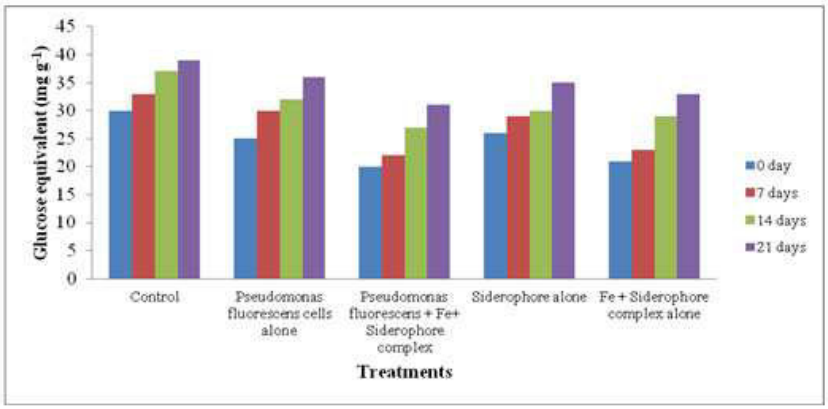

Figure 2

Changes in reducing sugar content of IR-50rice as influenced by the application of different formulations of Pseudomonas fluorescens siderophore during Pyricularia oryzae incitation

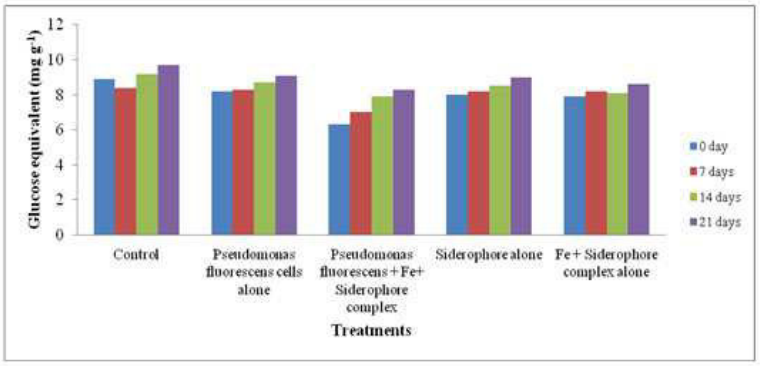

Figure 3

Changes in non-reducing sugar content of IR-50 rice as influenced by the application of different formulations of Pseudomonas fluorescens siderophore during Pyricularia oryzae incitation

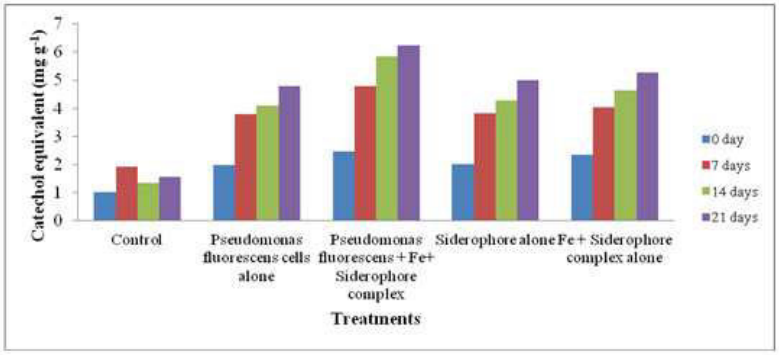

Figure 4

Changes on Ortho-dihydroxy phenol (OD phenol) content of IR-50 rice as influenced by the application of different formulations of Pseudomonas fluorescens siderophore during Pyricularia oryzae incitation

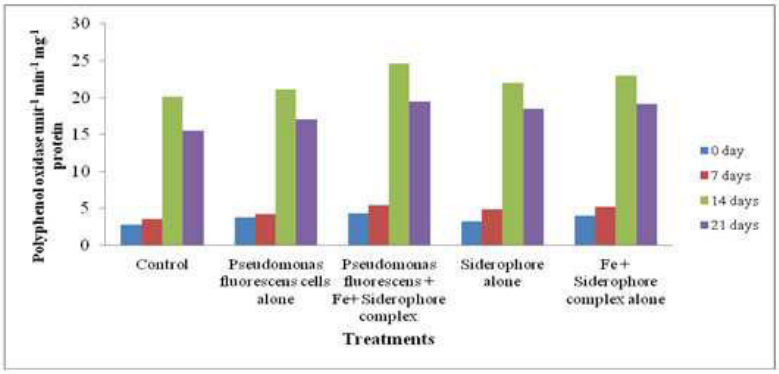

Figure 5

Changes in Polyphenol oxidase content of IR-50 rice as influenced by the application of different formulations of Pseudomonas fluorescens siderophore during Pyricularia oryzae incitation

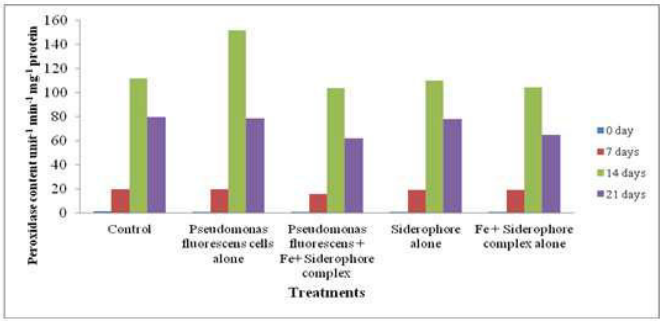

Figure 6

Changes in Peroxidase content of IR-50 rice as influenced by the application of different formulations of Pseudomonas fluorescens and its siderophore during Pyricularia oryzae incitation

Aver-Yanor and Lapikova[31] and LakshmiNarayana[32] reported the positive role of microbial siderophore in augmenting the total and OD phenol content and PO and PPO activities in rice plant during the incitation of *Pyricularia oryzae*.

CONCLUSION

The results of the present study are also confirmed the positive role of *P*. *fluorescens* cells together with its siderophore iron complex on the enhancement of phenol content and phenol oxidizing enzymes content in rice plant which imparts systemic resistance to rice plant against the incitation of *Pyricularia oryzae*.

REFERENCES

1. Mandal DK, Mandal C, Raja P and Goswami SN, Identification of suitable areas for aerobic rice cultivation in the humid tropics of eastern India. Current Science, 99: 227-231, (2010).
2. Epstein E, Silicon. Annual Review. Plant physiology and Plant Molecular Biology, 150: 641-664, (1999).
3. Lal R and Soil C, Sequestration impacts on global climatic change and food security. Science, 304: 1623-1627, (2004).
4. Neilands JB, Siderophores: Structure and Function of Microbial Iron Transport compounds. Journal of Biological Chemistry, 270: 26723-26726, (1995).
5. Pal RB and Karuna Gokaran, Siderophores and Pathogenicity of microorganisms. Bioscience and Technology, 1: 127-134, (2010).
6. Glick BR, The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology, 41: 109-117, (1995).
7. Saveetha K, Karthiba L, Muthuraj R, Saravanakumar D, Balasubramanian P, and Samiyappan R, Understanding the molecular basis of plant growth promoting effect of *Pseudomonas fluorescens* on rice through protein profiling. Proteome Science, 7: 47, (2009).
8. Viveros OM, Jorquera MA, Crowley DE, Gajardo G, and Mora ML, Mechanisms and practical considerations involved in plant growth promotion by rhizobacterial. Journal of Soil Science and Plant Nutrition, 10: 293-319, (2010).
9. Ramezanpour MR, Biochemical characteristics and genetic diversity of fluorescent *Pseudomonas* isolated from rice rhizosphere in North Iran. *American-Eurasian* J. Agri. Environ. Sci, 10: 180-185, (2011).
10. van Peer R, Niemann GJ and Schippers B, Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas sp* strains WCS 417r. Phytopathology, 81: 728-734, (1991).
11. Leeman M, Van Pelf JA, Den Ouden FM, Heinsbroek M, Bakker PAHM and Schippers B, Introduction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology, 85:1021-1027, (1995).
12. Wei G, Kloepper JW, Tuzun S, Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology, 86: 221-224, (1996).
13. Chen C, Belanger RR, Benhamou N, and Paulitz TC, Defence enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiology and Molecular Plant Pathology, 56:13-23, (2000).
14. Bakker PAHM, Ram LX, Pieterse CMS, and Loon VC, Understanding the involvement of rhizobacteria-mediated induction of systematic resistance in biocontrol of plant disease. Canadian Journal of Plant Pathology, 25: 5-9, (2003).
15. Bakker PAHM, van Peer R, and Schippers B, Biological Control of Soil-Borne Plant Pathogens, CAB International, Wallingford: 131-142, (1990).
16. Walker TS, Basis HP, Grotewold E, and Vivanco JM, Root exudation and

rhizosphere biology. Plant Physiology, 132: 44-51, (2003).
17. Djavaheri M, Mercado-Blanco J, Versluis C, Meyer JM, Van Loon LC, Bakker PAHM, Iron-regulated metabolites produced by *Pseudomonas fluorescens* WCS374r are not required for eliciting induced systemic resistance against *Pseudomonas syringae* pv. tomato in Arabidopsis. MicrobiologyOpen, 1(3): 311–325, (2012).
18. Sandheep AR, Aju K, Asok Ans Jisha MS, Biocontrol of *Fusarium* wilt of vanilla (*Vanilla planifolia*) using combined inoculation of *Trichoderma sp* and *Pseudomonas sp*. International Journal of Pharma and Bio Sciences, 3(3): 706-716, (2012).
19. Mahadevan and Sridhar R, Methods in physiological plant pathology, 3rd Edition, Sivakami publications: 82. (1986).
20. Watanable FS and Olsen SR, Test of an ascorbic acid method for determining Phosphorus in water and $NaHCO_3$ extracts from Soil. Soil Science Society of America Proceedings, 29: 677-678, (1965).
21. Malik KA, Rackshanda B, Mehnaz S, Rasul G, Misra MS, and Ali S, Association of nitrogen-fixing plant growth promoting rhizobacteria (PGPR) with kallan grass and rice. Plant and Soil, 194: 37-44,(1997).
22. Putter J, Methods of Enzymatic Analysis, 2 Ed Bergmeyer Academic Press, New York: 635, (1974).
23. Ester-Bauer HE, Schwarzl and Hayn M, Analytical Biochemistry: 477-486, (1977).
24. Clavente V, de Orellano, Sansone ME, Benuzzi G, and Sanz de Tosetti MI, Effect of nitrogen source and P^H on siderophore production by *Rhodotorula*, strains and their application to biocontrol of phytopathogenic moulds. Journal of Industrial Microbiology and Biotechnology, 26: 226-229, (2001).
25. Schroth MN and Hancock JG, Disease suppressive soil and root colonizing bacteria. Science, 216: 1376-1381, (1982).
26. Kloepper JW, Schroth MN, and Miller TD, Effect of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathology, 70:1020-1082, (1980).
27. Cline GR, Patrick Reid CP, Paul PE, and Szaniszlo PJ, Effect of hydroxamate siderophore on iron absorption by sunflower and sorghum. Plant Physiology, 76: 36-39. (1984).
28. Reid CPP, Crowley DE, Kim HJ, Powell PE, and Szaniszlo PJ, Utilization iron by oat when supplied as ferrated synthetic chelate or as ferrated hydroxamate siderophore. Journal of Plant Nutrition, 71: 437-447,(1984).
29. Becker J, Messens O and Hedges RW, Influence of agrobactin on the uptake of iron by plants. FEMS Microbial, Ecol, 31: 171-175, (1985).
30. Bansal RK, Dahiya RS, Lakshminarayana K, Sunjena S, Anand RC, Narula N, Effect of rhizosphere bacteria in plant growth of wheat infected with *Heterodera avenae*. Namatologia mediterranea, 27: 311-314, (2000).
31. Avar-Yanor AA and Lapikova VP, Peroxidase activity of excretions of healthy and blast infected rice leaves. Doklady Biological Science, 340: 81-42, (1995).
32. Lakshiminarayanan J, Evaluation of efficiency of “WANIS” against *Pyricularia oryzae* Cav. and *Helminthosporium oryzae* (Breda de Hann) Subram and Jain, the incidents to blast and brown spot disease of paddy, Annamalai University, Annamalai nagar, India, (2001).