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Population Dynamics, Siderophore Production and Disease Suppression during Different Seasons of *Pseudomonas aeruginosa* from Acidic Paddy Fields

Surabhi Sankar[1], C. Dileep[2]*

[1,2]Dept. of Post-Graduate Studies and Research in Botany, Santana Dharma College Alappuzha, University of Kerala, 688003, Kerala, India

**Corresponding Author:drcdileep@gmail.com, Mobile No.: 9446264129*

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Abstract- Applications of bio inoculants (PGPRs) are one of the most successful, non-chemicals, eco-friendly approach in agriculture. The bio inoculants were collected from the acidic paddy fields of Champakkulam in 3 different seasons. There were 120 strains isolated from the acidic conditioned paddy fields of Champakkulam. Among them KS1 is exhibited maximum zone of inhibition, siderophore production and root colonization than other strains and control. These were herbicide and pesticide resistant strains were used against domestic phyto pathogen. Root colonization of these bacterial strain showing various changes on these different seasons .In summer season strain produced maximum absorbance in UV mediated spectrophotometry .KS1 (MK 894011) ,PS2 (MK 894012), PS3 (MK 894013), RS1 (MK 894010), KNP (MK 894014) were identified as *Pseudomonas aeruginosa by* biochemical characterization as well as 16 s ribosomal RNA sequence. Data were subjected to t-test, and ANOVA. All treatments are compared with FPO4 **(**KF500003).The experiments having 5 replicates in each treatments. This study mainly focused on antagonistic effect, siderophore production, disease resistance and root colonization different seasons in different area of Champakkulam village.

Keywords- Antagonistic, bio inoculants, *Pseudomonas*, spectrophotometry, siderophore.

I. INTRODUCTION

PGPRs play a pivotal role in the bio control of phyto pathogens **[5]** .Biological molecules of these PGPRs having wide applications in various fields like Agriculture improvement and nutrient enrichment of soil. These can control the pest by bio methods **[14].** Hence these bio inoculants also known as bio remedial agents too **[13].** Rice cultivation of these area based on a special master plan. Over 160 years they practiced in the bio saline farming. Salinity is one of the major problems in this area. Agriculture practices are mainly depended on seasons and flash floods wash away the whole agriculture crops. For more profit all farmers use chemical contained fertilizers on their crops within a less period of cultivation. Due to this unexpected change in climate the characteristic of this agriculture land changes at every season. The soil contains many factors suitable for agriculture with enormous number of microorganisms which augment the farming product . Soil analysis and research works were point out its possibilities. Especially *Pseudomonas fluorescens* species **[6**] Rhizobacteria strains can promote the plant growth **[10]; [20]; [9]** and control the diseases. Seasonal changes and soil factors influence the mass production of these microbes. Maximum microbial multiplication results the metabolite production through root colonization **[23];[11]**which increases the siderophore level in Ecto rrhizosphere .As a results the plant growth rate improved and reduce the wide spreading of pathogens **[3].** Rhizobacteria diversity, their colonization ability, mechanism of action, formulation and application all are able to facilitate their environment and plant growth.

Seasonal changes affects the siderophore production. Maximum siderophores observed in summer season and it calculated through the intensity of siderophores in succinate (iron deficient media) by UV Spectrophotometry. Antagonism against common domestic fungi checked. Pant growth efficacy of bio inoculants checked by seed bacterisation. Root colonization of bio inoculants calculated from the serial dilution of soil samples. ISR resistance (24) of disease confirmed from field study of Paddy cultivation for a 3month interval of time. Recent research reports are focused the ecofriendly treatments of crops using these bio inoculants.

II. RELATED WORKS

Growth promotion efficacy of these PGPR proved in many studies. These fluorescent Pseudomonads are

siderophore producing microbes .Rhizopheric and rhizoplane bacteria are rich in this type of PGPR. Fluorescent Pseudomonad strains FPC 32 and FPO4 were isolated from the rhizoplane of *Capsicum* spp.L and *Oryza sativa* L. showed growth promotion ,yield, and root colonization in Bhindi, Paddy, Peanut. Both strain were effective to suppress collar rot of fungi, Aspergillus *niger*..Sumandu *et al*.,2018 reported tolerant isolates PRS3 and PRP5 from acidic paddy fields have great inhibitory property against *Rhizoctonia solani* enhance growth of paddy plant and reduce the sheath blight of paddy . *Pseudomonas aeruginosa sps.*KS1 and PS2 isolated from the acidic paddy fields below sea level also potential against pathogenic fungi like *Fusarium moniliforme* and *Rhizoctonia solani* **[28]** reported earlier from our studies **[27]** .Herbicidal tolerant *Pseudomonas aeruginosa spp* RP12, MIP, H1,and RRK were also highly potential isolates for growth promotion of plant **[26**].Isolates from the Bamboo *Pseudomonas fluorescens* and *Bacillus polymixia* release enzymes and antibiotics that can inhibits the growth and development of pathogenic microbes reported from the studies of Y.H Muhammed **[25].**

III. METHODOLOGY

Isolation, Identification of samples: A total of 120 isolates were collected from the 10 agro based land of Champakkulam village. Selected and identified strains are KS1,PS2,PS3,KNP, and RS1. Both rhizoplane and rhizosphere were collected. All strain are from rhizoplane of paddy roots. The strains were marked with chlorpyriphos pesticide (resistance with 650ppm) and it taken for further studies.

Fungal pathogens : *Aspergillus flavus (MTCC 183), Aspergillus tubigensis (MTCC 2425), Aspergillus niger (MTCC 2756), Aspergillus fumigatus (MTCC 3376) , Rhizoctonia solani (MTCC 4634), Fusarium moniliforme (MTCC 6985*) are used for this study. All were procured from IMTECH (CSIR), Chandigarh, India.

Medium: KB Medium, Succinate medium , NA medium and PDA medium were used in this study.

***In vitro* Antibiosis study**: It's a biological interaction study using *Pseudomonas* and with different fungal isolates. KB Medium contained petriplates were streaked the 24 hr. Old *Pseudomonas* inoculam (2 cm from the periphery) in the form of 4 curves in and it incubated at 36℃ ± 2℃ .After 48 hr. old fungal disc (4mm) were inoculated on the centre of 4 curves of *Pseudomonas* strain. The petriplates were kept under 36℃ ± 2℃ in BOD incubator. Inhibition zone (in mm) will be measured after 3 days onwards till 5th day of inoculation. Petriplates without fungus served as control.

Soil pH and soil analysis: pH of soil samples were checked in lab. Analysis of this agriculture land was done at soil testing lab, Alappuzha. pH is different in each stations of this village and it recorded.

Seasons: Monsoon, winter and summer seasons were noted. Mainly pigment production and root colonization were checked on these periods.

Seed bacterisation study: Onam (local) variety of Paddy (*Oryza sativa*) seeds was used in this study. It collected from Onattukara Regional Agriculture Research centre, Kayamkulam. They are washed with tap water 4 or more times, then surface sterilized with sodium hypochlorite for 3 or 4 minutes. Rinsed it in distilled water and dried it in sterile air for 24 hrs. Then sterilized seeds about 100 gm. were mixed bacterial suspension already prepared by mixing 48 hr. old bacterial culture scrapped from KB AND 1% CMC (Carboxy Methyl Cellulose) .Then it allow to dry in sterile air stream for 1 day. Seeds with CMC and without bacterial suspension treatment considered as control.

Treated seeds were sown in pots containing common garden soil pH is 7.4.Pots were kept under shade house and maintaining watering.100 days require for its maturity. Bacterial suspension treatment continuing after 7days of sprouting. Growths of plant measured at 7 days of interval till the 42nd day of plant maturation. Data were subjected to ANOVA, and DMRT.

Root colonization study:
Plants were *Pseudomonas* treated soil were dug out and removed the soil particles by gentle shaking and then carefully cut it in to small pieces using sterile knife and placed in sterile distilled water 100mlfor 5 minutes to release the rhizosphere bacteria. Shake it well. Serial dilution were done and root washing were made and plated on KB (King's B) medium containing petriplates .Similarly 1 ml. of aliquot was poured on KB medium contained plates taken as control. Both plates were kept under 36℃ for 48 H in BOD. Obtained colonies counted in KB medium are introduced bacterial strain and others in medium is TARB (Total Aerobic Rhizobacteria).These root colonization checked in all three seasons in both rhizosphere and rhizoplane condition.

Disease suppression study:
From the antagonistic observation and plant growth improvement study , selected bacterial and fungal strains were selected to field study. So the effective potential strains KS1 and PS2 were considered as bio inoculants from the *Pseudomonas spp.*Two Disease, Sheath blight (*Rhizoctonia solani*) and Bakanae (*Fusarium moniliforme*) .In this study *Fusarium moniliforme* taken as pathogen against both KSI and PS2 strains in field and also were selected to check the disease severity. Less symptoms and improved root colonization were noted as the result.

IV.RESULTS AND DISCUSSION

Isolation of *Pseudomonas* strains:
Study areas were lying under Kuttanad Taluk. This is lying in two districts. There are 10 agro based stations in Champakkulam village taken for this study. Farming occurs under 3 m. below sea level. A special agro climatic condition was noted in this region. Soil samples were randomly collected in three seasons. Totally 120 strains were collected. From the primary screening most of them are less siderophore productive. That measured through the UV spectrophotometry. Light absorption and peak formation noted and it selected to the identification process. Rhizosphere and rhizoplane soils were taken to the isolation .

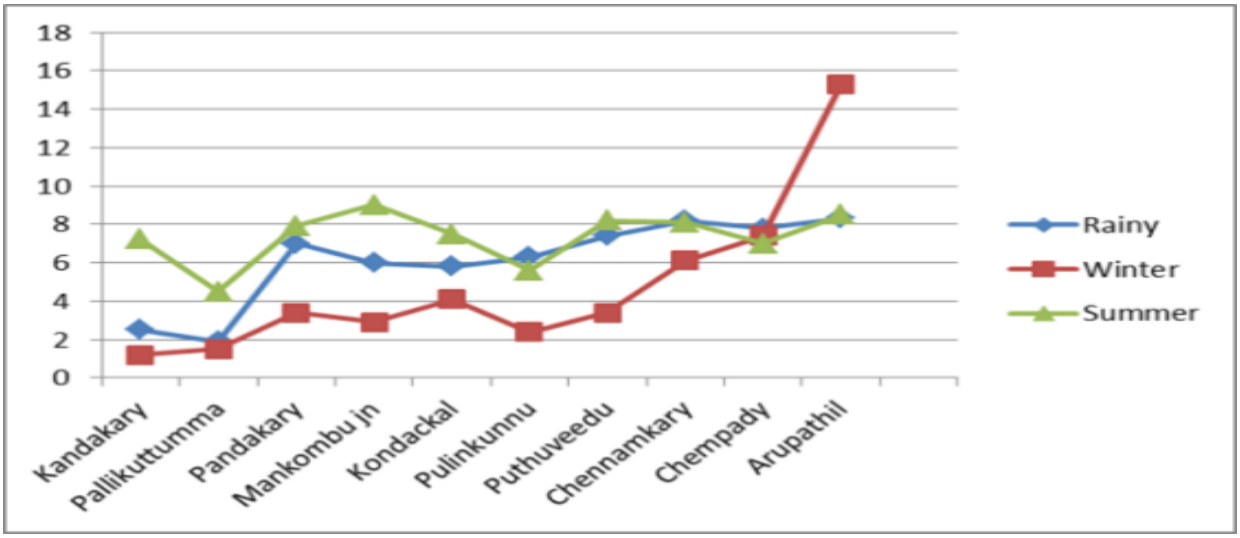

Fig . 1 Graphical representation of Total colony population in organism from rhizoplane in all three season in KB Medium .

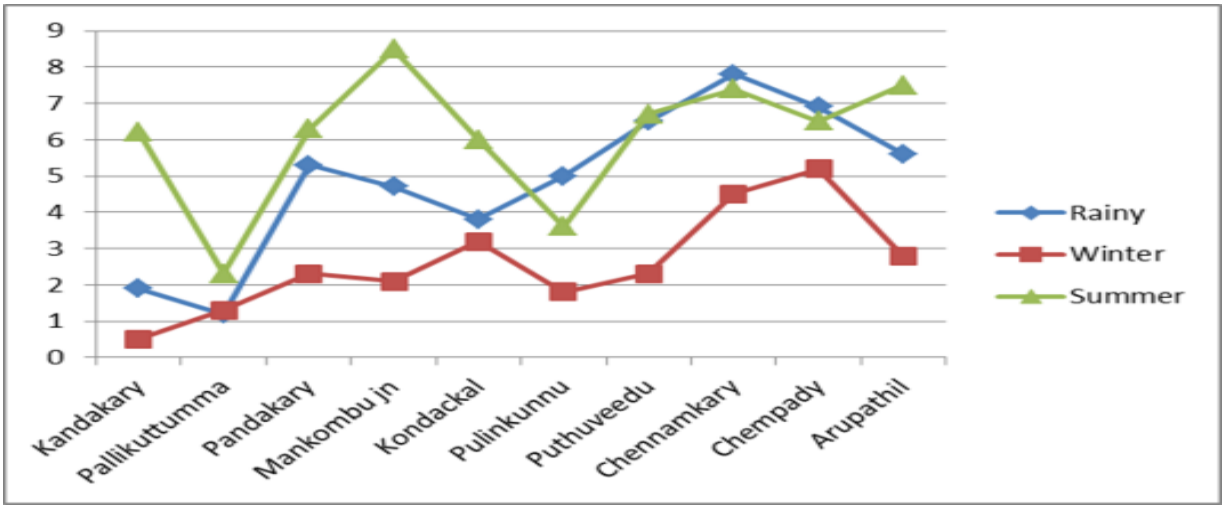

Fig. 2 Graphical representation of Total colony population in rhizosphere organism from in all three season in NA Medium

Rhizoplane isolated organisms were most pigment productive and more colony formation in both NA and KB medium. So we collect paddy rhizoplane for further isolation and selection. Collect the samples randomly and stair case method throughout the three different seasons.

Characterization of isolates:
Selected acidic strains KS1, PS2, PS3, KNP, and RS1 were confirmed as *Pseudomonas aeruginosa* partial sequence with the 16 s rRNA primer sequence. It was done at RGCB Thiruvananthapuram.

Marking with chlorpyriphos pesticide: Isolates were checked its resistance with this pesticide.650 ppm resisted strains taken for the studies.

***In vitro* antagonistic study of bacterial strains:** In this biological interaction between two organisms bacteria and fungi, that means one is detrimental to another due to their metabolic compounds .Here bacterial siderophores are effective against disease causing pathogens. Common phyto pathogens were taken to check the efficacy of best performing acidic isolates.

Table 1.. Showing the Antagonism test results. Inhibition zone (in mm) width measured after the 3days of inoculation.

		Bacterial strains						F-value (P-value
		PS2		KS1		FPO4(Control)		
Fungal strains	**N**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
A.niger	**5**	**1.56[b]**	**0.09**	**2.07[b,c]**	**0.27**	**1.04[a]**	**0.53**	
A.flavus	**5**	**1.46[a,b]**	**0.18**	**1.72[a,b]**	**0.16**	**1.23[a,b]**	**0.15**	
A.fumigatus	**5**	**1.37[a,b]**	**0.18**	**1.56[a]**	**0.11**	**1.50[a,b,c]**	**0.20**	
A.tubingensis	**5**	**1.37[a,b]**	**0.19**	**1.51[a]**	**0.20**	**1.90[c]**	**0.12**	**27.8 (<.01)**
F.oxyspourm	**5**	**1.37[a,b]**	**0.13**	**2.44[c]**	**0.27**	**1.62[b,c]**	**0.10**	
F.moniliformae	**5**	**1.36**	**0.15**	**3.50[d]**	**0.49**	**1.94[d]**	**0.58**	
R.solani	**5**	**1.29[a]**	**0.13**	**3.32[d]**	**0.49**	**2.70**	**0.43**	
Total	**35**	**1.39[p]**	**0.16**	**2.22[r]**	**0.70**	**1.70[q]**	**0.61**	
F-value (P-value)		**1.63 (.167)**		**33.4 (<.01)**		**11.7 (<.01)**		

a,b,c ,d are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test(among fungi).p,q,r are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test(among strains)

ANOVA shows there is no significant difference in among different fungal strains in PS2, but significantly different in KS1 at 1% level of significance (p-value<.01). The DMRT shows that highest observed in *F.moniliforme* in KS1 bacterial strain and which is significant.

The average values in each bacterial strain shows highest in KS1 and which is significantly different from others.

Plant growth efficacy of acidic isolates in Paddy plant: Onam local variety of Paddy seeds were taken to study.120 days required for it harvest. Root length, Shoot length, fresh weight, and dry weight are the growth parameters selected for the study.

Table .2 Datas about the growth parameters of tests plants.

Strains	**N**	**Mean of growth parameters**			
		Shoot length	**Root length**	**Fresh weight**	**Dry weight**
KS1	**25**	**52.80[c]**	**13.43[b]**	**12.64[c]**	**2.67[c]**
PS2	**25**	**34.44[b]**	**10.59[a]**	**9.00[b]**	**2.08[b]**
Control	**25**	**29.16[a]**	**9.55[a]**	**5.40[a]**	**0.81[a]**
Total	**75**	**38.80**	**11.19**	**9.01**	**1.85**
F-value (p-value)		**51.34 (<.01)**	**24.76 (<.01)**	**45.71 (<.01)**	**31.76 (<.01)**

a,b,c are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test

ANOVA shows there is significant difference in shoot length , Root length, Fresh Weight , Dry Weight among different strains at 1% level of significance (p-value<.01). The DMRT shows that all are mutually significant.

Population density of microorganism in nursery condition treated plant in rhizosphere (10^6dilution).

More efficient strains were found from nursery treatment of these plants .Chlorpyriphos resistance strains were identified in root colonization studies.

Table 3. Shown the root colonization of introduced bacterial population around the root and rhizosphere.

Strains	N	cfu After 28thweek of cultivation	TARB	F-value (p-value)		
		Mean	SD	Mean	SD	
PS2	5	21.2[b]	0.8	25.4[c]	1.1	
PS3	5	16.4[a]	1.1	16.8[a]	1.6	
KS1	5	41.6[c]	2.9	34.0[d]	1.0	
KNP	5	16.6[a]	1.1	21.8[b]	1.5	
FPO4	5	22.0[b]	1.9	24.8[c]	1.3	0.197 (.659)
Total	5	23.6	9.6	24.6	5.9	
F-value (p-value)	179.29 (<.01)	110.75 (<.01)				

a,b,c ,d are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test(among strains)

ANOVA shows there is significant difference in population density among different strains in CFU after

28th day of cultivation and TARB at 1% level of significance (p-value<.01). The DMRT shows that highest observed in KS1 in both cases and which is significant.

Crop production of acidic isolates from nursery fields:
From the harvest of this nursery farming got a good result from paddy plants. Comparatively maximum production reported in KS1 treated paddy plants. Chaff is less in this KS1 treated plants than PS2.

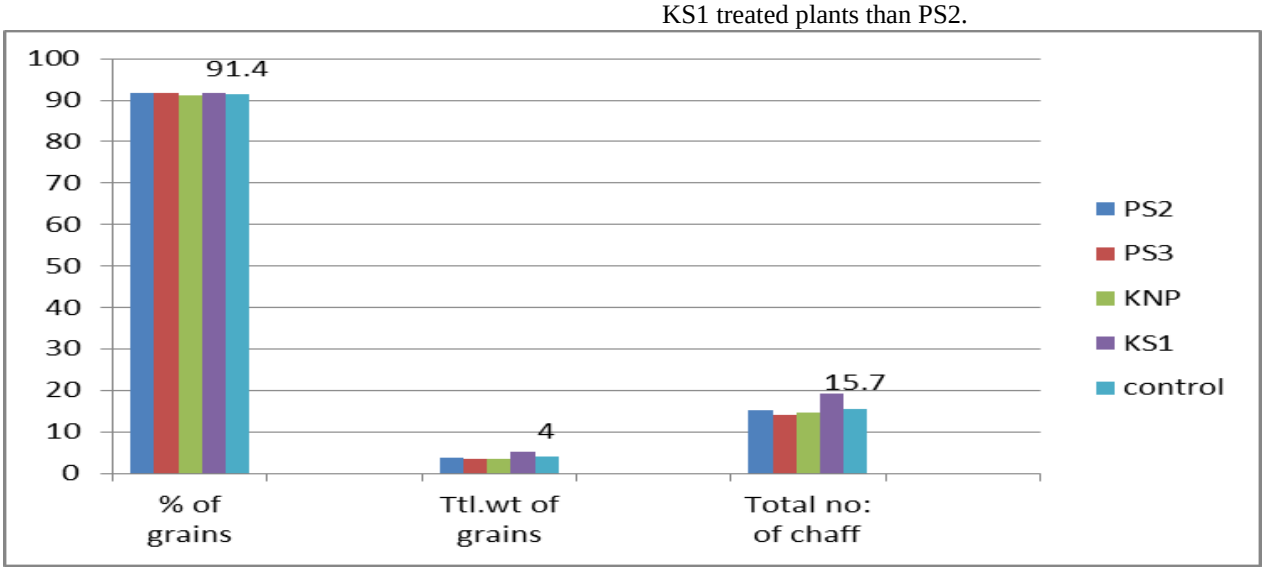

Fig. 3 Graphical representation of crop yield of nursery conditioned farming.

ISR (Indused Systemic Resistance) study with Fungal pathogen (*Fusarium moniliforme*):
Disease suppression studies mainly focused on Bakanae disease control measures on paddy. *Fusarium* wilt or root decay is severe to paddy plants. It affects the area, decrease the crop production, and also their growth of the plant. Seed bacterisation done with pathogen, non –treated seeds as control and were taken for field. When sprouting started after 7 days of saw. Apply the bacterial suspension to the base of plants continuing till the harvest of paddy plants.

Bakanae of Paddy is a root decaying disease .So selects this drenching method for the application of bacterial treatment suspension of *Pseudomonas aeruginosa.* Continuing the bacterial treatment until the plant showing less symptoms and seen healthier than other treated KS1 and non-treated control. Chlorpyriphos resistant strains were used to check its identification

.

Table.4 Datas were given till the 90 days of its harvest.a,b,c ,d,e are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test(among days)

Days	N	KS1+*Fm* treated		PS2+ *Fm* treated		Control		F-value (p-value)
		Mean	SD	Mean	SD	Mean	SD	
7	5	20.0^{a}	1.6	13.8^{a}	1.3	18.0^{a}	1.0	11.49 (<.01)
14	5	22.8^{a}	1.9	15.2^{a}	1.9	15.0^{b}	1.0	
28	5	32.2^{b}	2.5	17.8^{a}	1.8	15.0^{a}	1.0	
45	5	36.4^{c}	1.1	23.6^{b}	4.4	28.0^{c}	1.0	
60	5	45.4^{d}	2.7	28.0^{b}	5.7	35.0^{d}	1.0	
90	5	62.6^{e}	5.9	42.6^{c}	3.3	36.0^{d}	1.0	
Total	30	36.6^{q}	14.9	23.5^{p}	10.5	24.5^{p}	9.1	
F-value (p-value)	132.05 (<.01)	48.92 (<.01)		477.5 (<.01)				

p,q,r are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test (among strains).

ANOVA shows there is significant difference in number of bacterial colonies among different time slots in each strain at 1% level of significance (p-value<.01). The DMRT shows that highest observed in 90 days significantly higher than other time slots. Overall the highest observed in KS1 on 90 days.

The average value in each strain shows highest in KS1(36.6) and which is significantly higher than in other strains(DMRT).

Pull out the up rooted plant and serial dilution done. Plate the diluted aliquot and kept under the BOD at 36℃ for 24 H. Count the colony number in each plate. This is the introduced 650 ppm

resistant *Pseudomonas aeruginosa* bacterial colonization check with other domestic microbes (TARB). Repeat it in each week after application of *Pseudomonas culture*.

From the field study we got a good result. Comparatively best plant growth and less symptoms noted in KS1 strain treated plants. So KSI is more efficient than other two treatment

Less symptoms showing the treated plants isolates against *Fusarium moniliforme* in large field:

Table 5.Showing the datas of less symptomized plants among the beds.a,b,c are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test

.Strains	N	Mean	Std. Deviation	F-value (p-value)
KS1	25	31.6^{a}	5.9	40.75 (<.01)
PS2	25	56.4^{b}	9.7	
Control	25	76.2^{c}	7.4	
Total	75	54.7	20.2	

ANOVA shows there is significant difference in Dry Weight among different strains at 1% level of significance (p-value<.01). The DMRT shows that all are mutually significant.

Production of siderophores in KS1 and PS2:

Siderophores are secondary metabolic compounds of microorganisms. Under iron restricted condition many bacteria emulsify the iron chelating compounds called siderophore. There are many variation are seen in the structure of siderophore based on their type of organisms.KS1 and PS2 were the best strains from acidic isolates. Which shown a good peak absorbance in 409 nm in their spectrometric analysis at 15,000 rpm. Checked it in different temperature level. Datas were shown in the table below.

Table.6 is showing the siderophore production at different temperature level of isolates KS1 and PS2.a,b,c,d are homogeneous subsets formed by Duncan's Post hoc Multiple Comparison Test.

Temp	Mean ± SD	
	KS1	PS2
25C	2.33^{b}±0.25	1.91^{b}±0.46
30C	3.64^{d}±0.24	2.83^{c}±0.26
35C	2.81^{c}±0.09	$2.46^{b,c}$±0.71
40C	2.23^{b}±0.16	2.06^{b}±0.06
45C	1.44^{a}±0.15	0.54^{a}±0.17
Total	2.49±0.76	1.96±0.88
F-value (p-value)	91.74 (<.01)	23.17 (<.01)

ANOVA shows there is significant difference in Production of siderophores at different temperature levels in both the strains KS1 and PS2 at 1% level of significance (p-value<.01). The DMRT shows the value at 30℃ is highest and significantly different from others in KS1. In PS2, the value is highest at 30° C but is not significantly different from at 35℃.

Paired Sample t-test (Comparison between KS1 and PS2)

Table 7 is the results of sample t-tests of KS1 and PS2

Strains	Mean	Std. Deviation	t-value (p-value)
KS1	2.49	0.76	5.4 (<.01)
PS2	1.96	0.88	

The value is high in KS1. The pair wise t-test shows that the Production of siderophores is significantly different in between the strains KS1 and PS2 at 1% level of significance.

Most of the fluorescent *Pseudomonads* rhizobacterial strains were inhibitory to phyto pathogens. Their metabolic product siderophore having a role in this antagonism .The isolate KSI shown a great inhibitory capacity to pathogens compared with other isolates. This is siderophore mediated antagonism. *P.fluorescens* and *P.putida* reported as bio control agents among rhizobacterial strains **[4] ; [1]);[19].**Antagonitic nature and disease suppressive nature of bacterial inoculants against fungi help to place a role in agriculture improvement. Maximum inhibitory nature shows the strain KSI against *Fusarium moniliforme.*

Root colonization and the population density of the introduced organisms results the plant growth with the roots. KSI shown the best results in both studies. Crude siderophores are a source of boosting agents for plant

growth. Some times their ability may hide in structure of compound. Separate the compounds and use their efficacy in appropriate way.

Seasonal changes also imparts role in the growth of organisms and their siderophore production. Isolates collected in summer season showed more metabolite production than other season. It can measured from UV spectrometric readings.

V.CONCLUSION FUTURE STUDY

Bio-efficacy of all test samples was effective in agriculture improvement. Antifungal metabolites of *Pseudomonas aeruginosa* tested against soil –borne pathogens. A completely eco-friendly treatment gets from the mass multiplication culture process of these isolates. Moreover *Pseudomonas spp.* are known for their survival in extreme conditions like low or high pH, temperature, and salinity .The studies confirmed production of siderophores ,which further confirmed plant growth under saline water. All the parameters taken for investigation shown an enhancement confirms the role of siderophores under abiotic stress condition. The work also shows in the production of siderophores in the test salt level condition for productivity improvement and disease management.

These free-living natural rhizobacteria can change the polluted environment through their microbial actions. So protect the soil micro biome and conserve these isolates and use their potentials for a better future. The detailed characterization and structure determination of siderophore and other antifungal metabolites can make a big chance to explore bio chemical industry. The use of these PGPRs can used as bio pesticides or bio growth agents instead for chemical fertilizers.

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AUTHOUR PROFILE

SURABHI SANKAR is a research scholar in the Department of PG studies and Research in Botany,Sanatana Dharma College Alappuzha, University of Kerala,since 2015.She presented research papers in National and International symposiums.She has one 1st prize in the paper presentation in ISMPP National conference held at Dharward University Karnataka.

Dr.C.DILEEP

HOD and Asst. Professor of Dept. of PG Studies and Research in Botany, Sanatana Dharma College ,Alappuzha, University of Kerala. He has more than 20 Research papers,2 books were published. Acted as Member of PG board of Studies (Botany),Mahatma Gandhi University Faculty of Science, Kerala and Chair man (Msc.Botany exam).Kerala University. Areas of Research Microbiology, Phyto bacteriology and PGPR.

