Original Research Article
Efficacy of FluorescentPseudomonads isolates in nutrient limiting soil of easrterngangetic plains

ABSTRACT

Aims:

Excessive use of chemical fungicides cause environmental pollution and its residual effects are deleterious for human and animal consumption. So, application of biological control agents in IDM is an ecofriendly complement to manage the diseases as well as to protect the environment. PGPRs arefree living microorganismswhichhave antimicrobial properties as well as exert beneficial effects to plant growth.

Study design:

This present study has been designed to identify potential indigenous fluorescent psedomonads strains from different crop ecosystems under Eastern gangetic plains of West Bengal. Study of Plant growth promoting properties and antimicrobial properties were done under both *in-vitro* and *in vivo* condition.

Place and Duration of Study:

Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, Duration 03years

Methodology:

Under this experiment 22 bacterial isolates were isolated and purified from different rhizosphere. Isolates were identified based on different biochemical test like catalase, oxidase activity, citrate utilization. The major plant growth promoting properties like P, K and Zn solubilisation efficiency were tested. In-*vitro* antimicrobial properties were also tested like HCN production, Volatile and non volatile compounds production etc. Based on *in-vitro* performance, best performing isolate was

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selected for *in-vivo* in Lentil against *Stemphylium botryosum,* a major disease causing fungi of lentil.IDM practice was done in three stages like Seed treatment, Prophylactic spray and Therapeutic spray and three lentil variety WBL 77(Maitree), Pusa Ageti and MN12 were used.

Results:

Most of the isolates showed positive result in biochemical tests and performed well i.r.oPGP properties and antimicrobial properties. In field trial UBPF5 gave a promising result. PF5 isolate was identified through 16S rRNA based molecular method and sample showed 99.25% similarity with *Pseudomonas gessardii* based on nucleotide homology and phylogenetic analysis. In comparison to different combination of treatments cuprous chloride as seed treatment, *Pseudomonas* sp. as prophylactic spray and for therapeutic purpose Boscalid + Pyroclostrobin were found mostly efficient in respect of disease incidence as well as plant growth and yield. Prophylactic spray with *Pseudomonas* sp. in combination with biological seed treatment and therapeutic application of fungicide has resulted in 26-38% reduction in disease incidence and 28-32% increment in yield.

Conclusion: So, use of PGPR in combination with chemical fungicides is an eco-friendly tool to manage plant diseases in a sustainable manner.

Key Words: *PGPR, Antimicrobial, Pseudomonas, Lentil, Stemphylium, IDM*

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1. INTRODUCTION:

PGPR represent a wide range of rhizosphere-inhabiting bacteria which have the ability to colonize the root systems of plants and can stimulate plant growth by indirect or direct mechanisms. Direct mechanisms includebiofertilization by supplying essential nutrients like nitrogen, phosphorus, potassium, iron etc. to the plants, phytostimulation like production of different hormones Auxins, Gibberellins, cytokinine, ethylene, abscisic acid etc. Disease suppression, nutrient and niche competition, resistance induction, rhizoremediation, induction of stress tolerance come under indirect mechanisms. There are so many ongoing rigorous researches worldwide with greater aim to explore the wide range of PGPR possessing different novel traits like pesticide degradation, detoxification of heavy metals etc. Various plant growth promoting properties like P, K, Zn solubilisation, Ammonia

production, IAA production, antimicrobial properties like Volatile and non volatile compound production, rhizophere colonization, siderophore production, hydrolytic enzyme production, HCN production are under research. Beneficial rhizobacteria are free living or symbiotic in nature. Among free living rhizobacteria fluorescent psedomonads group of bacteria were emphasized. Beneficial effects of fluorescent psedomonads on plant growth and disease management have been reported since late '70S.

There are so many literatures available on antimicrobial and plant growth promoting properties of fluorescent psedomonads under both *in-vitro* and *in-vivo.*Among the fluorescent pseudomonads, primarily *Pseudomonas fluorescens* and *P. putida* were identified as important organisms with ability for plant growth promotion and effective disease management properties [1]. They have the capacity of production of Catalase, Oxidase enzymes and can utilize citrate as sole source of Carbon [2], [3]. Haas and Defago, 2005, mentioned about the production of antimicrobial compounds by fluorescent pseudomonads which protects plants against pathogenic microbes [4]. Some research studies proves the PGPR activity of *Pseudomonas* sp. by describing the reduction of pH from initial pH of 7.0±0.2 in bacterial culture after 96 hours of incubation along with solubilization of tricalcium phosphate by PSB strain KUPSB12 [5]. P.K and Zn Solubilisation capacity of *Pseudomonas* sp. is one of the most important features.

Under this study based on *in-vitro* performance, best performing strain of *Pseudomonas* sp. was applied under field condition.Soil of terrain zone is 3 to 6 feet deep acidic, sandy, sandy silt, silty, clayey silt in texture. Due to dominance of partially degradable organic matter, soil colour is black or grey. Nitrogen content and humus availability is low and presence of available phosphate and potash is medium to low. Micronutrient like B, Mo, and Zn deficiency is also prevalent in terrain zone. Due to prolonged rainfall from starting of March to starting of November, lime from the soil got leached with rain water. Leaching loss makes the soil more acidic and reduces the soil pH.Though different response was recorded in efficiency of treatment in plant disease management as well as plant growth promotion under field condition, strobilurin is known for its yield enhancing properties and included in IDM practices of Stemphylium leaf blight of Lentil [6]. To address these soil issues of Terai region, fluorescentpsedomonads were tested. Identification of region specific appropriate strains or mixture of strains in a host pathogen interaction is of utmost necessity to achieve sustainable package of practices.

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2.MATERIAL AND METHODS

2.1 Biochemical Characterization

2.1.1Isolation of Fluorescent pseudomonads-

Different strains of*Fluorescent pseudomonas*were isolated from different crop rhizosphere from different parts of Eastern Gangetic plains of West Bengal. Root rhizosphere of plants was collected and surface sterilized with 0.1% Mercuric Choride and washed with distilled water. These steps were repeated upto three times. After that sterilized roots were put on King's B broth medium for 24 hours at 28° C at BOD incubator. After 24 hours subculturing was done. For purification, single bacterial colony was taken from petriplate through sterilized loop and inoculated on King's B broth [7]. King's B media was proved to be most favourable for *Pseudomonas* sp. bacterial growth and King's B media is well known as *Pseudomonas* specific media [8].

2.1.2 Maintenance of Isolates-

50ml Glycerol was added to 50ml Distilled water thus 50% glycerol stock was prepared and twenty two strains of fluorescent pseudomonads were kept on 50% glycerol stock at -80°C. for maintenance further study.

2.1.3 Enzyme assay-

2.1.3.1 Catalase activity -Catalase test was performed by taking a drop of 3% hydrogen peroxide and added to 48 h old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicates positive catalase activity [9].

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2.1.3.2 Oxidase activity -To the 24 h old bacterial culture oxidase discs were placed on them. The isolates showing blue colouration of discs were considered as positive [9].

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2.1.4Citrate Utilization-

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Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2^{0}C for 24h. Change in colour from green to blue indicated the positive reaction for citrate utilization [9].

2.1.5 HCN Production-

HCN production by isolates was checked by method of Bakker and Schipper(1987) [10] on Kings B Medium. King's B agar was amended with 4.4 g/l glycine for HCN production test. Single isolates were streaked in each plate. Whatman no. 1 filter paper disc (9 cm in diameter) was soaked in 0.5% Picric acid and in 2% sodium carbonate. Soaked disc was placed in the lid of each Petriplate. As HCN is volatile in nature, Petriplates were sealed with parafilm and incubated at 30°C for 4 days. An uninoculated medium with the soaked filter paper was also kept as control for comparison of results.Appearance of light brown to dark brown color indicated HCN production.

2.2 Evaluation of antimicrobial traits of fluorescent pseudomonads against few plant pathogens

2.2.1 Koch's Postulate-

For in-vitro evaluation of antimicrobial property, isolated lentil leaf blight causing fungi*Stemphylium botryosum* was used. Before application koch's postulate was done to confirm the virulence or pathogenicity of the pathogen.

Fully developed fungal colony was inoculated on sterilized lentil seeds. After 15-20 DAI fungi was fully grown in the seeds. That colonized seeds were mixed with distilled sterile water and sprayed on the healthy lentil plants. After 10 DAI, disease symptoms were appeared on lentil leaves and after 15DAI entire plants including stems were infected.

2.2.2 Assay for non-volatile and volatile antimicrobial compound-

2.2.2.1 Assay for non-volatile antimicrobial compound- The antagonistic activity of all *Pseudomonas* isolates against test phytopathogenic *fungi Stemphylium botryosum*wasevaluated by dual culturing of the fungi and *Pseudomonas* isolates on Potato Dextrose Agar adjusted to pH 6.5. On each agar plate, individual Pseudomonas strain pre-cultivated overnight in King's B Broth were streaked at the periphery in straight lines of 30 mm and at a distance of 35 mm away from the centre of a 90 mm Petri dish and on the same day an agar plug of 6 mm diameter with growing fungal

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mycelium of test fungal pathogen was placed on the centre of the petriplate. Plates were incubated 28±1°Cuntil the growing fungal colony on a control plate without Pseudomonas inoculation reached comparable growth (5DAI).

2.2.2.1 Assay for volatile antimicrobial compound-The strains of fluorescent pseudomonads were streaked separately onto half of a two partition of thePetriplate containing Nutrient Agar medium. A 6 mm mycelial plug of fungal pathogen were placed on the other half of the partition petriplate containing PDA medium and the plates were immediately wrapped with parafilm to prevent the volatilization of volatile compounds be resealed and were incubated for 5 days at 28±1°C. Measurements of radial mycelial growth were taken after 5 days of incubation.

Percent of inhibition was calculated as per the formulae mentioned below

% Growth inhibition = [{(C-T)/C} X 100]

C= Radial mycelial growth in Control, T= Radial mycelial growth in Treatment

2.3 Evaluation of plant growth promoting attributes of fluorescent pseudomonads

2.3.1 Solubilization of P-

Pikovskaya's agar medium (glucose–10g, $Ca_3(PO_4)_2$–5g,$(NH_4)_2SO_4$–0.5g,KCl–0.2g,$MgSO_4$–0.1g,yeast extract–0.5g, agar–15g, distilled water–1000Ml) was used. One loopfull of the 24h broth culture was spot inoculated on the Pikovsakya culture plate. The plates were incubated at 28^0C for 96hoursand observed for the zone of clearance around the bacterial colony, which indicates solubilisation of P [11].

2.3.2Solubilization of K-

Bacterial cultures were inoculated onto Aleksandrov medium ($MgSO_4$- 0.5g, $CaCO_3$-0.1g, Potassium alumino silicate – 2g, Glucose-5g, $FeCl_3$-0.005g, $Ca_3(PO_4)_2$-2g, Agar-20g, pH-7.2±0.2) containing mica [12]. The plates were incubated at 30±1^0C for 3days and zone of clearance were observed.

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2.3.3Solubilization of Zn-

Mineral salts agar medium (Glucose–10g, $(NH_4)_2SO_4$–1.0g, KCl–0.2g, K_2HPO_4–0.1g, $MgSO_4$–0.2g and H_2O–1000mL with pH 7.0) amended with 0.1% of insoluble zinc oxide (ZnO) were used for Zn solubilisation test. The actively growing cultures were spot inoculated on to the medium, incubated at 28^0C and solubilization zone were measured 3days after inoculation [13].

Zone of Clearance = Diameter of Total Zone – Diameter of bacterial colony

Bacterial Efficiency =

$$\frac{\text{Clear Zone}}{\text{Bacterial Colony Zone}}$$

2.4 Molecular identification-

Best performing variety was identified based on partial 16S rRNA gene sequence analysis and submitted in NCBI.

2.5Integrated Disease Management-

In the University farm to formulate a specificIDM practice for management of Stemphylium blight in lentil a field trial was done. Three varieties of lentilWBL 77(Maitree), Pusa Ageti and MN12 were selected. For seed treatment suitable *Rhizobium, Trichoderma* sp andPhosphate solubilizer as consortium, produced in the Bio-control Laboratory of the University(Uttar Banga Krishi Viswavidyalaya) and cuprous chloride as immuno-modulator targeting systemic acquired resistance were used. During the next stage i.e. prophylactic application of fungicide (Chlorothalonil 75 WP) or biological (Fluorescent pseudomonas, UBPF5, Population 10^8 @ 5g/lit) was evaluated. Two fungicide mixtures were used at final phase of intervention, first one was a combination of pyraclostrobin (12.8%, Quinone outside inhibitor in complex III of the cytochrome bc1 complex) and boscalid (25.2%, succinate dehydrogenase inhibitor in complex II of mitochondrial electron transfer chain) and the second one was a combination of Azoxystrobin (8.3%, Quinone outside inhibitor in complex III of the cytochrome bc1 complexand Mancozeb (66.7%, multisite activity interferes with enzymes containing sulfhydryl groups).At the final stage two therapeutic fungicideswere tried to find the best possible

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combination of crop protection measure. These three stages of treatment with three lentil varieties has been described in **Table No. 1**

Table-1: Details of the treatments obtained during 3 different phases

STAGE-1	STAGE-2	STAGE-3	
Treatments	**Treatments**	**Treatments**	
A_1V_1	$A_1B_1V_1$	$A_1B_1C_1V_1$	$A_2B_1C_1V_2$
A_1V_2	$A_1B_2V_1$	$A_1B_1C_2V_1$	$A_2B_2C_1V_2$
A_1V_3	$A_2B_1V_1$	$A_1B_2C_1V_1$	$A_2B_1C_2V_2$
A_2V_1	$A_2B_2V_1$	$A_1B_2C_2V_1$	$A_2B_2C_2V_2$
A_2V_2	$A_1B_1V_2$	$A_2B_1C_1V_1$	$A_1B_1C_1V_3$
A_2V_3	$A_1B_2V_2$	$A_2B_2C_1V_1$	$A_1B_2C_1V_3$
Control	$A_2B_1V_2$	$A_2B_1C_2V_1$	$A_1B_1C_2V_3$
	$A_2B_2V_2$	$A_2B_2C_2V_1$	$A_1B_2C_2V_3$
	$A_1B_1V_3$	$A_1B_1C_1V_2$	$A_2B_1C_1V_3$
	$A_1B_2V_3$	$A_1B_2C_1V_2$	$A_2B_2C_1V_3$
	$A_2B_1V_3$	$A_1B_1C_2V_2$	$A_2B_1C_2V_3$
	$A_2B_2V_3$	$A_1B_2C_2V_2$	$A_2B_2C_2V_3$
	Control	Control	

3.RESULTS AND DISCUSSION-

3.1 Biochemical Characterization

3.1.1Isolation of Fluorescent pseudomonads-

Different strains of fluorescent Pseudomonads were isolated from different parts of West Bengal and Sikkimhaving diverse edapho-climatic condition and from the rhizoplane of different cereals, pulses, oilseeds, vegetables, leafy vegetable, spice crop, ornamental and medicinal plants- Fruit, Plantation crop and weeds etc. After 24hrs of incubation of sterilized roots, greenish or yellowish and sometimes pinkish pigmentation in broth under UV light appeared which proves the existence of fluorescent Pseudomonads. Twenty two strains of fluorescent pseudomonads were

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isolated. Islam *et al.,* 2016 [14] also isolated endorhizospheric bacteria like *Pseudomonas* sp, *Bacillus* sp. from the roots of Cucumber. In **Table No. 2** collection site and crop plants from which bacterial strains were isolated has been presented. Isolation of strains has been presented in **Fig No. 1.**

Table 2. Isolates of *fluorescent*Pseudomonadsfrom different locations of West Bengal

Sl. No.	Isolates	Source	Location	Latitude	Longitude
1	**UBPF1**	Rhizosphere of wheat	Pundibari ,Coochbehar	26.5243°N	89.1075°E
2	**UBPF2**	Rhizosphere of maize	Hoglabari ,Coochbehar	26.4151°N	59.3867°E
3	**UBPF3**	Rhizosphere of mungbean	Khairabari.Coochbehar	26.3665°N	89.4092°E
4	**UBPF4**	Rhizosphere of potato	Madhupur.Coochbehar	26.3641°N	89.3779°E
5	**UBPF5**	Rhizosphere of tea	Pundibari,Coochbehar	26.5243°N	89.1075°E
6	**UBPF6**	Rhizosphere of ginger	Pundibari,Coochbehar	26.5243°N	89.1075°E
7	**UBPF7**	Rhizosphere of brinjal	Dinhata, CoochBehar	26.1291°N	89.4695°E
8	**UBPF8**	Rhizosphere of rice	Pundibari,Coochbehar	26.5243°N	89.1075°E
9	**UBPF9**	Rhizosphere of ladiesfinger	Pundibari,Coochbehar	26.5243°N	89.1075°E
10	**UBPF10**	Rhizosphere mustard	Pundibari,Coochbehar	26.5243°N	89.1075°E
11	**UBPF11**	Rhizosphere of wheat	Pundibari,Coochbehar	26.5243°N	89.1075°E
12	**UBPF12**	Rhizosphere of lentil	Pundibari,Coochbehar	26.5243°N	89.1075°E
13	**UBPF13**	Rhizosphere of citrus	Satmile, Coochbehar	26.3300°N	89.2200°E
14	**UBPF14**	Rhizosphere of weed (Chenopodium)	Cooch Behar	26.3452°N	89.4482°E
15	**UBPF15**	Rhizosphere of patharkuchi	Alipurduar	26.4918°N	89.5271°E
16	**UBPF16**	Rhizosphere of Periwinkle	Mohanpur, Nadia	23.6565°N	88.2254°E
17	**UBPF17**	Rhizosphere of weed	Sikkim	27.3516°N	88.3239°E
18	**UBPF18**	Rhizosphere of Coriander	Kaliganj, Cooch Behar	26.3452°N	89.4482°E
19	**UBPF19**	Rhizosphere of Ginger	Kalimpong	27.0594°N	88.4695°E
20	**UBPF20**	Rhizosphere of Ginger	Kalimpong	27.0594°N	88.4695°E
21	**UBPF21**	Rhizosphere of Ginger	Kalimpong	27.0594°N	88.4695°E
22	**UBPF22**	Rhizosphere of wheat	Malda	25.0108°N	88.1411°E

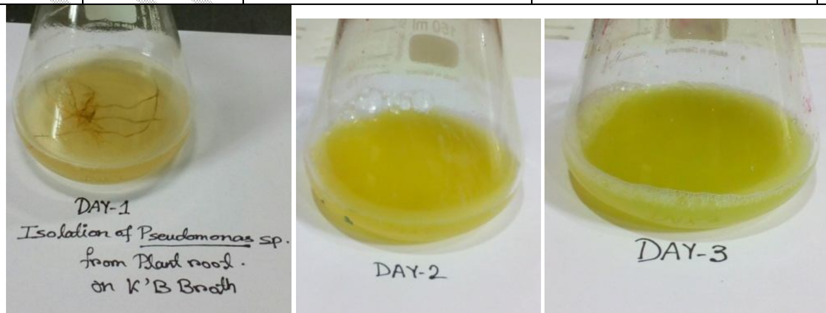

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Fig No. 1 Steps of Isolation of fluorescent Pseudomonads and Isolated 22 strains in Broth and solid media of King's B

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3.1.2 Enzyme assay-

3.1.2.1 Catalase activity -

Isolates were screened for their ability to produce Catalase enzyme. Presence of Catalase denoted by Positive (+) and absence was denoted by Negative (-) **(Table No. 3)**. Table indicates the catalase activity of each bacterial isolates.

Out of 22 isolates 18 isolates showed positive (UBPF1, UBPF3, UBPF4, UBPF5, UBPF7, UBPF9, UBPF11, UBPF12, UBPF13, UBPF14, UBPF15, UBPF16, UBPF17, UBPF18, UBPF19, UBPF20, UBPF21, UBPF22) and four isolates showed negative catalase activity (UBPF2, UBPF6, UBPF8, UBPF10).**(Fig No. 2)**

In Catalase activity assay 3% H_2O_2 was added in 48hrs old bacterial culture. Presence of bubbles or effervescence denotes positive Catalase activity. As Catalase enzyme breaks the H_2O_2 into O_2 and H_2O which causes formation of bubbles. Negative results denote the isolates are unable to produce catalase.

As per available literature it has been shown that out of 30 isolates under state condition of Telangana, each and every isolates have showed positive test result for Catalase activity [2].

3.1.2.1 Oxidase activity -

Oxidase is one kind of enzyme which catalyzes the oxidation reaction by oxygen and it is a characteristics feature of various bacterial isolates. Oxidase helps bacteria to thrive well under low oxygen condition.

UBPF1, UBPF3, UBPF4, UBPF5,UBPF6, UBPF7, UBPF8, UBPF9, UBPF10, UBPF11, UBPF12, UBPF13, UBPF15, UBPF16, UBPF17, UBPF18, UBPF19, UBPF20UBPF21, UBPF22- 20 isolates exhibit positive result and rest 2 isolates UBPF 2 and UBPF 14 showed negative result.**(Table No. 3)**. Positive results denote production of Oxidase and negative result indicates low level production of Oxidase or inability to produce oxidase enzyme.**(Fig No. 3)**

In the study by Vinay *et al.,* 2016 [15] it was also observed that out of 30 isolates of fluorescent pseudomonadsall showed positive result for oxidase activity.

Cytochrome C Oxidase enzyme plays an important role in Electron transport chain of respiratory system in bacterial physiology. It transfers electrons to oxygen during respiration in cell. Cytochrome C oxidase accelerate the oxidation of N,N,N'chloride ,N'- tetramethyl-p-phenylenediamine dihydrochloride by oxygen. Oxidised compound changes the color of the disc from white to purple which denotes the positive enzymatic assay.

3.1.3 Citrate Utilization-

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Citrate utilization is one of the important test for differentiation of various isolates under fluorescentPseudomonadsbased on ability of utilization of citrate as sole source of carbon and growth of bacterial isolates on Simmons citrate agar media. Simmons citrate agar media contains Sodium citrate as sole source of carbon. Bromothymol blue was used as pH indicator. **Table No. 3** provides the information about citrate utilization capacity by different isolates of fluorescent *Pseudomonads*. 22 isolates showed positive result i.e. capable of utilizing citrate. **(Fig No. 4)**

The isolates showing positive result contain citrate permease enzyme. This enzyme helps to transport the citrate into cell and inside bacterial cell citrate undergoes several enzymatic reactions. After enzymatic reactions due to production of alkaline byproducts , pH of the media get increased which leads to change in the colour from green to blue and Bromothymol blue helps in this. Concept of citrate utilization was studied by Kachurka*et al.,* 2018 [16].

3.1.4HCN Production-

Production of HCN as secondary metabolites is one of the most important features of fluorescent Pseudomonads. In **Table No. 3** the qualitative analysis of level of HCN production has been indicated as "-", "+", "++" and "+++". "-"denotes negative, "+" weak, "++" moderate and "+++" strong producer of Hydrogen cyanide. Colour conversion of filter paper from Yellow to Yellowish orange, Orange or Brownish orange denotes the qualitative production of HCN. After 4 days of incubation yellow colour denotes negative result, which means inability of producing of HCN by the isolate. In **Table No. 3** Yellow is denoted by "-", Light Brown is denoted by "+", Brown colour by "++" and Reddish Brown colour by "+++".UBPF7, UBPF12, UBPF17, UBPF 18, UBPF21, UBPF22 showed higher HCN production, followed by UBPF4, UBPF10, UBPF13, UBPF14. UBPF2, UBPF3, UBPF5, UBPF6, UBPF8, UBPF11, UBPF15, UBPF19 and UBPF20 showed moderate HCN production and UBPF1, UBPF9 and UBPF16 showed no HCN production. HCN production by *fluorescent* Pseudomonads group of bacteria involves various enzymatic conversion of Precursors like Glycine into HCN. Glycine is the major precursor of HCN production,**(Fig No. 5).** Production and function of HCN in the physiology of biological control agent has also been reported by Haas et al., 2005 [17]. .

Glycine + 2NADH + H^+ + O_2 HCN + NH_3 + NAD +H_2O_2

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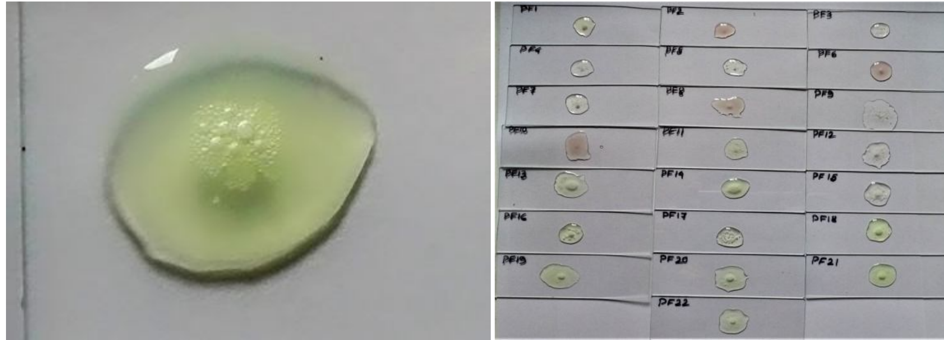

Fig No. 2 Catalase Production by isolates

Table 3. Enzyme Assay, Citrate Utilization and HCN Production by different isolates

Isolates	Catalase	Oxidase	Citrate	HCN
UBPF1	+	+	+	-
UBPF2	-	-	+	+
UBPF3	+	+	+	+
UBPF4	+	+	+	++
UBPF5	+	+	+	+
UBPF6	-	+	+	+
UBPF7	+	+	+	+++
UBPF 8	-	+	+	+
UBPF 9	+	+	+	-
UBPF 10	-	+	+	++
UBPF 11	+	+	+	+
UBPF 12	+	+	+	+++
UBPF 13	+	+	+	++
UBPF 14	+	-	+	++
UBPF 15	+	+	+	+
UBPF 16	+	+	+	-
UBPF 17	+	+	+	+++
UBPF 18	+	+	+	+++
UBPF 19	+	+	+	+
UBPF 20	+	+	+	+
UBPF 21	+	+	+	+++
UBPF 22	+	+	+	+++

'+' Denotes Positive Reaction, '-' Denotes Negative Reaction, In HCN production: '-' Yellow, '+' Light Brown, '++' Brown, '+++' Reddish Brown

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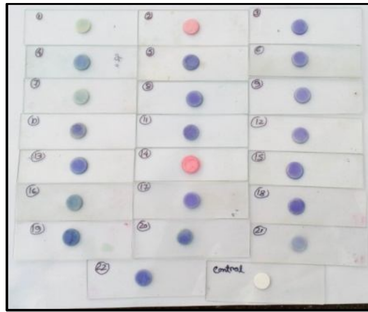

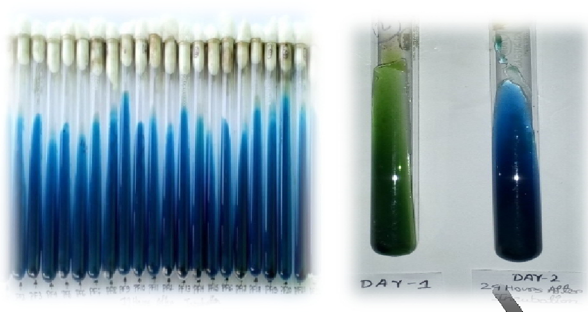

Fig No. 3 Oxidase Production by isolates

Fig No. 4 Citrate Utilization by isolates

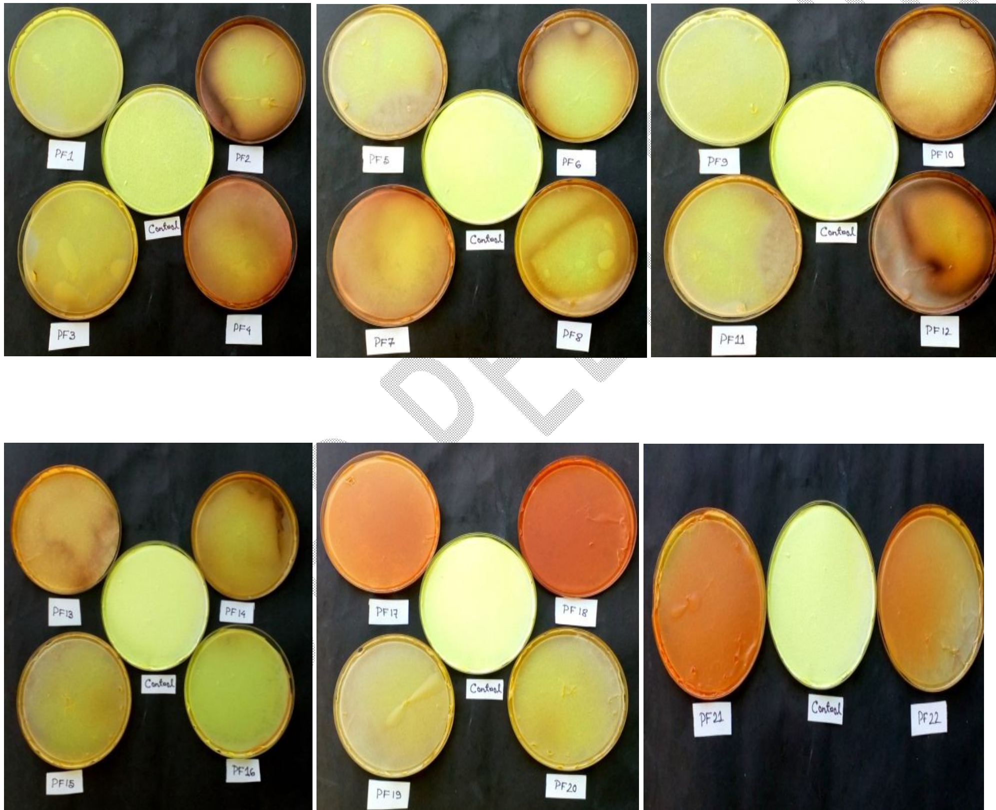

Fig No. 5HCN Production by isolates

3.2 Evaluation of antimicrobial traits of fluorescent pseudomonads against few plant pathogens

3.2.1 Koch's Postulate-

Result of Koch's postulate was successfully achieved which proves pathogenicity of the test pathogens. From the artificially inoculated leaves which were showing symptoms fungus was isolated. Disease symptoms and fungal spores are presented in **Fig No. 6.**AppearanceofDisease symptoms have been presented in **Fig No. 7.**

Fig No. 6 Infected Plant Parts, Fungus Culture on Solid PDA Media, Fungal Spore

Fig No. 7 Uninoculated Plants, Day of Inoculation, 10DAI, 15DAI respectively

3.2.2 Assay for non-volatile and volatile antimicrobial compound-

3.2.2.1 Assay for non-volatile and volatile antimicrobial compound-

22 isolates of *fluorescent* Pseudomonads were used against *Stemphylium* sp. isolated from leaf blight effected part of Lentil. Percent Growth inhibition of *Stemphylium* sp. by different isolates of fluorescent Pseudomonads by producing non volatile compounds are presented in **Table No. 4.** Among 22 isolates UBPF5 was found most effective with 62.38% inhibition of growth of *Stemphylium* sp. followed by UBPF7 (57.01% of Inhibition), UBPF16 (55.74% of Inhibition) and UBPF17 (51.64% of Inhibition). UBPF14 was found least effective against *Stemphylium* sp. under *in-vitro* condition with 26.80% of inhibition followed by UBPF10 (31.96% inhibition). **(Fig No. 8,9).**

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After coming in contact to the plant pathogenic fungi, fluorescent Pseudomonads group of bacteria produce various antimicrobial compounds like non volatile compound (Pyoverdine), Mupirocin antibiotic which inhibits bacterial isoleucyl-tRNA synthetase [18].As per available literature under *in-vitro* condition *Pseudomonas fluorescens* showed 84.4% mycelia growth inhibition of *Stemphylium vesicarium*where asRidomil gold plus showed 99.6% inhibition , *Trichoderma harzianum* No. 1 inhibited 78.1% mycelial growth and *Bacillus subtilis* No. 1inihibited 79.6% [19]..

Like non volatile compound production assay, volatile compound production assay was done using 22 isolates of *fluorescent* Pseudomonads against *Stemphylium* sp. Percent Growth inhibition of *Stemphylium* sp. by different isolates of fluorescent Pseudomonads are presented in **Table No. 4.** Data presented in the table reveals that 22 isolates varied in their capacity of growth inhibition of the test pathogens by producing volatile compounds.

Among 22 isolates UBPF13 was found most effective in volatile compound production with 85.17% inhibition of growth of *Stemphylium* sp. followed by UBPF19 (80.37% of Inhibition), UBPF10 (79.87% of Inhibition) and UBPF02 (72.28% of Inhibition). UBPF07 was found least effective against *Stemphylium* sp. under *in-vitro* condition with 38.24% of inhibition followed by UBPF09 (42.57% inhibition). **(Fig No. 10, 11)**

Table No.4 Evaluation of Antimicrobial properties of different isolates of *fluorescent Pseudomonads*

Isolates	% of inhibition by non volatile compounds	% of inhibition by volatile compounds
UBPF 1	44.92^{abcd}	56.88^{de}
UBPF 2	32.61^{cd}	72.28^{bc}
UBPF 3	44.12^{abcd}	48.75^{efgh}
UBPF 4	49.60^{abc}	52.18^{efg}
UBPF 5	62.38^{a}	45.41^{fgh}
UBPF 6	37.00^{bcd}	48.28^{efgh}
UBPF 7	57.01^{ab}	38.24^{h}

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UBPF 8	34.38^{cd}	51.17^{efg}
UBPF 9	47.77^{abc}	42.57^{gh}
UBPF 10	31.96^{cd}	79.87^{ab}
UBPF 11	43.88^{abcd}	53.06^{defg}
UBPF 12	41.24^{bcd}	48.28^{efgh}
UBPF 13	45.24^{abcd}	85.17^{a}
UBPF 14	26.80^{d}	52.66^{efg}
UBPF 15	46.87^{abcd}	50.72^{efg}
UBPF 16	55.74^{ab}	54.08^{def}
UBPF 17	51.64^{abc}	51.61^{efg}
UBPF 18	42.86^{abcd}	71.78^{bc}
UBPF 19	45.63^{abcd}	80.37^{ab}
UBPF 20	47.43^{abc}	46.38^{efgh}
UBPF 21	37.23^{bcd}	45.41^{fgh}
UBPF 22	44.04^{abcd}	64.06^{cd}
SEm	**3.7919**	**2.0888**
LSD	**10.808**	**5.953**

As per literature available volatile fatty acid disrupts fungal cell membrane. Dimethyl disulfide, Methyl-1-butanol has antifungal property. Volatile compounds 2,3-Butanediol can induce systemic resistance against plant pathogens [20].

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Fig No. 8 Pictorial Presentation of Non Volatile Compound Production

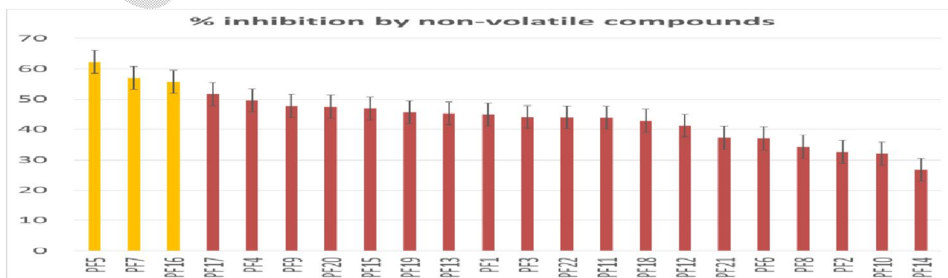

Fig No. 9 Graphical Presentation of Non Volatile Compound Production, X Axis- Isolate, Y axis % Inhibition

Fig No. 10 Pictorial Presentation of Volatile Compound Production

Fig No. 11 Graphical Presentation of Volatile Compound Production, X Axis- Isolate, Y axis % Inhibition

3.3 Evaluation of plant growth promoting attributes of fluorescent pseudomonads

3.3.1 Solubilization of P, K, Zn-

Solubilization of Phosphorus, Potassium and micronutrient Zinc in the soil is one of the important features of Plant growth promotion of *fluorescent pseudomonads.* As the experiment was done with the aim to apply the biocontrol agent *fluorescent pseudomonads* to the field condition in Cooch Behar under Terrai Tista Alluvial agroclimatic zone, the major soil problems under terrain tista zone were emphasized.

For testing phosphorus solubilization in pikovskaya's media tricalcium phosphate acted as a sole source of phosphorus in insoluble form. 22 isolates were tested for phosphorus solubilization property. Among 22 isolates, highest efficiency of phosphorus solubilization was tested in UBPF22 (efficiency 0.71), followed by UBPF7 (0.67). UBPF1, UBPF2, UBPF6, UBPF8, UNPF10, UBPF13, UBPF19, UBPF20, UBPF21 showed no phosphorus solubilization.**(Table No. 5)(Fig No. 12,13)**

Among 22 isolates, highest efficiency of potassium solubilization was tested in UBPF11 (efficiency 2.56), followed by UBPF8 (2.1). Zero potassium solubilization efficiency was observed in UBPF1 and followed by UBPF21 with efficiency 0.24. **(Table No. 5)(Fig No. 14,15)**

Highest efficiency of zinc solubilization was tested in UBPF17 (efficiency 5.63), followed by UBPF22 (4.63). Zero zinc solubilization efficiency was observed in UBPF6 and followed by UBPF2 with efficiency 0.97. **(Table No. 5)(Fig No. 16,17)**

Pseudomonas sp. produces various organic acids like citric acid, gluconic acid; lactic acid, oxalic acid, acetic acid and these acids reduce pH of the media and dissolve the insoluble form of phosphates. These organic acids gets attached with cations like Al^{3+}, Ca^{2+}, and Fe^{3+} and phosphate ions get free in a soluble form which is easily accessible to the plants [21]. By reducing pH of the media *Pseudomonas* sp. breaks down silicate minerals like mica or feldspar and dissolve the insoluble form of potassium and release K^+ ion and make it available to plants. Chelating compounds produced by biocontrol agents get attached to zinc ion which prevents formation of insoluble zinc compounds.

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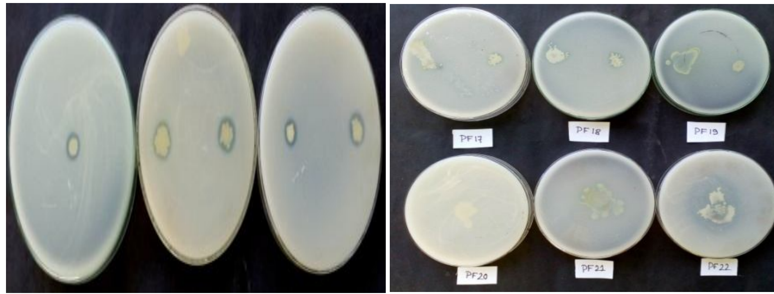

Fig No. 12P- Solubilization

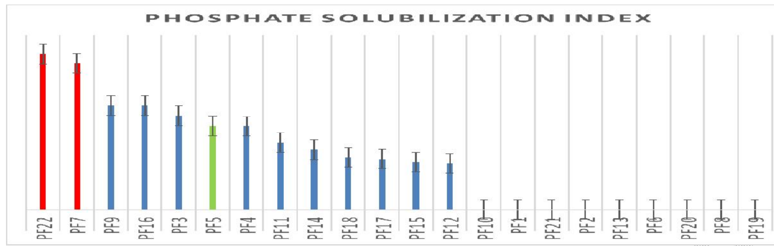

Fig No. 13 Graphical Presentation P-Solubilization Efficiency, X axis- Isolates, Y axis Efficiency

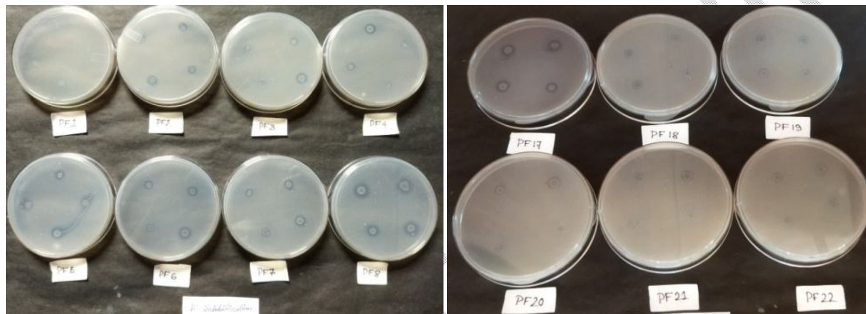

Fig No. 14K- Solubilization

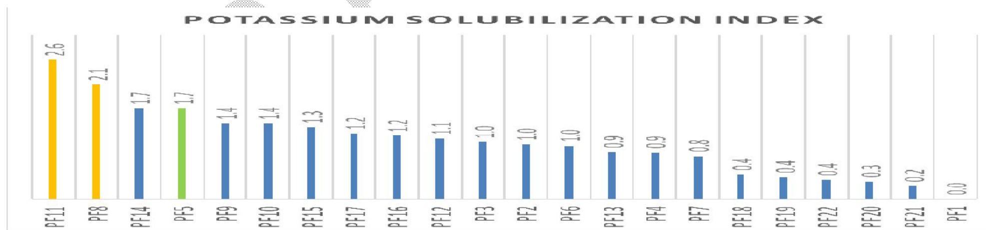

Fig No. 15 Graphical Presentation K- Solubilization Efficiency, X axis- Isolates, Y axis Efficiency

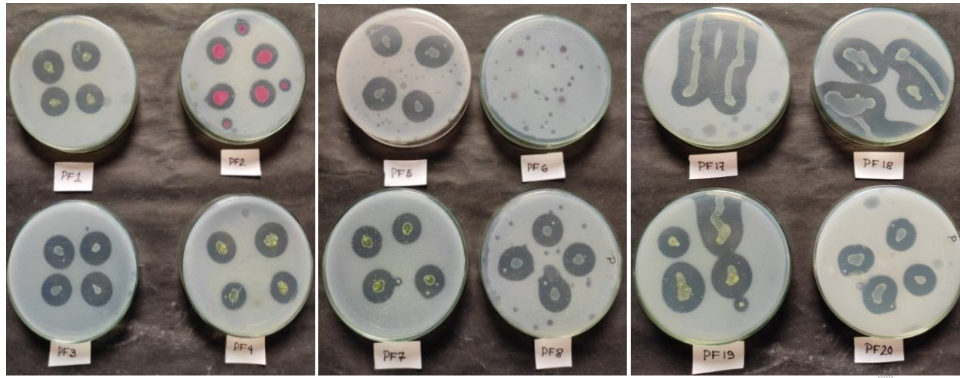

Fig No. 16 Zn- Solubilization

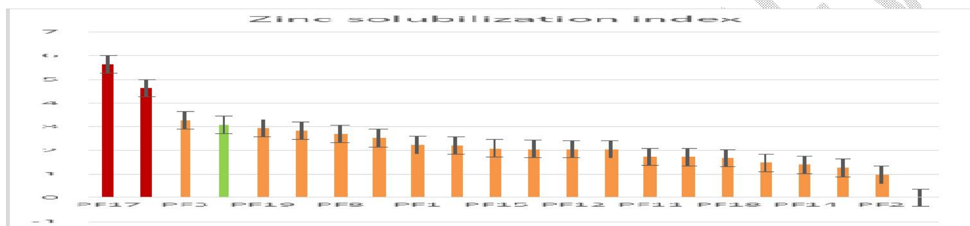

Fig No. 17 Graphical Presentation Zn- Solubilization Efficiency, X axis- Isolates, Y axis Efficiency

Table No. 5 Plant growth promoting attributes of fluorescent pseudomonads

Isolates	P-solubilization efficiency	K-solubilization efficiency	Zn-solubilization efficiency
UBPF 1	0^{e}	0^{e}	2.24^{cde}
UBPF 2	0^{e}	1^{bcde}	0.97^{ef}
UBPF 3	0.43^{bcd}	1.04^{bcde}	3.27^{bc}
UBPF 4	0.38^{cd}	0.85^{cde}	1.72^{cdef}
UBPF 5	0.38^{cd}	1.67^{abc}	3.08^{bcd}
UBPF 6	0^{e}	0.97^{bcde}	0^{f}
UBPF 7	0.67^{ab}	0.78^{cde}	2.06^{cde}
UBPF 8	0^{e}	2.1^{ab}	2.69^{bcde}
UBPF 9	0.48^{abc}	1.38^{bcd}	2.52^{cde}
UBPF 10	0^{e}	1.38^{bcd}	1.47^{cdef}
UBPF 11	0.31^{cd}	2.56^{a}	1.73^{cdef}
UBPF 12	0.21^{de}	1.11^{bcde}	2.06^{cde}
UBPF 13	0^{e}	0.86^{cde}	2.21^{cde}
UBPF 14	0.27^{cd}	1.67^{abc}	1.39^{cdef}
UBPF 15	0.22^{de}	1.32^{bcd}	2.09^{cde}

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UBPF 16	0.48^{abc}	1.16^{bcde}	1.25^{def}
UBPF 17	0.23^{de}	1.2^{bcd}	5.63^{a}
UBPF 18	0.24^{cde}	0.44^{de}	1.66^{cdef}
UBPF 19	0^{e}	0.4^{de}	2.94^{bcde}
UBPF 20	0^{e}	0.32^{de}	2.04^{cde}
UBPF 21	0^{e}	0.24^{de}	2.83^{bcde}
UBPF 22	0.71^{a}	0.36^{de}	4.63^{ab}
SEm	**0.04468**	**0.21646**	**0.36998**
LSD	**0.127**	**0.617**	**1.055**

3.4 Molecular identification-

On the basis of Antimicrobial and PGPR property best performing isolate was selected and that strain of Pseudomonas showed 99.25% similarity to *Pseudomonas gessardii*based on nucleotide homology and Phylogenetic analysis. The same was submitted to NCBI and accession no. against the submitted bacterial sequence is PPO25825.1.

3.5Integrated Disease Management-

Differential response by different varieties was evident from the data with higher efficiency for yield enhancement in MN 12 followed by Maitree and low efficiency in comparatively susceptible variety PusaAgeti. In MN 12 highest efficiency for yield was in treatment Cuprous chloride ►Fluorescent pseudomonas ►Boscalid+pyraclostrobin, however they were closely followed by Cuprous chloride ►Fluorescent pseudomonas ►Azoxystrobin+Mancozeb and Cuprous chloride ►Chlorothalonil ►Boscalid+pyraclostrobin without significant difference. The same in PusaAgeti was too low with less than 30% efficiency and MN12 had shown an efficiency of 50% with Cuprous chloride ►Fluorescent pseudomonas ► Boscalid+pyraclostrobin treatment. Cuprous chloride ►Fluorescent pseudomonas ► Boscalid+pyraclostrobin treatment was found to be most efficient treatment irrespective of any variety however the treatment was at par with two other treatments Cuprous chloride ►Fluorescent pseudomonas ► Azoxystrobin+Mancozeb and Cuprous chloride ►Chlorothalonil ►Boscalid+pyraclostrobin. **(FIG No. 18).**

Best combination for disease management for different varieties differed based on their degree of resistance but, irrespective of any variety the best combination for management of the disease was **Cuprous chloride ►Fluorescent pseudomonas ►Boscalid+pyraclostrobin.**

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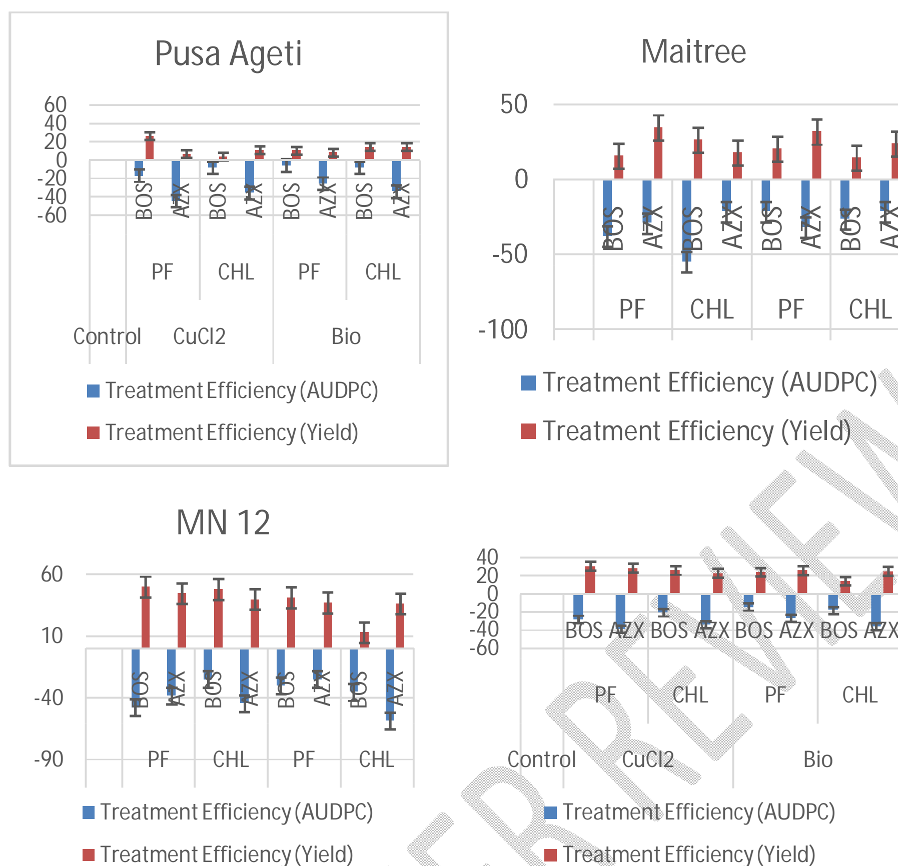

Fig No. 18 Treatment efficiency for reducing disease and enhancing yield for Maitree, PusaAgeti, MN12 and irrespective of any variety. (±SEm)

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4. CONCLUSION-

Under this experiment 22 isolates were taken into consideration. Most of them showed positive response for Catalase, Oxidase production, Citrate utilization test and HCN production. Mostprominent characteristic symptoms was yellowish green or pinkish fluorescent pigment under UV light. Based on *in vitro* Anti microbial and PGPR property best performing variety was selected and identified based on 16S rRNA and showed 99.25% similarity to *Pseudomonas gessardii*.

Under *in-vivo* IDM practices it was observed that cuprous chloride as seed treatment, *Pseudomonas* sp. as prophylactic spray and for therapeutic purpose Boscalid + Pyroclostrobin were found mostly efficient in respect of disease incidence as well as plant growth and yield. Prophylactic spray with *Pseudomonas* sp. in combination with biological seed treatment and therapeutic application of fungicide has resulted in 26-38% reduction in disease incidence and 28-32% increment in yield. So,

this can be concluded that biological control agents may be a wise choice in IDM for improvement of yield as well as reduction of disease incidence. This biocontrol agent is also effictive for nutrient deficient soil of Terai region.

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