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Isolation of Indole Acetic Acid Producing Bacteriafrom Fig fruit decomposed soils and Their effect on plant growth promotion

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Abstract :

Production of growth hormones has been the major criteria of bacteria in plant growth promotion. Indole acetic acid (IAA) production is a major property of rhizosphere bacteria that stimulate and facilitate plant growth. Indole acetic acid (IAA) is one of the most important phytohormones enhances the root structure and plant growth. The present work deals with to isolate the bacteria having Indole Acetic Acid (IAA) producing activity from soil under fig fruit waste decomposed soils. Isolates were characterized on the basis of staining techniques and biochemical tests such as oxidase, catalase, indole, TSI, methyl red, Voges – Proskauer, citrate utilization, urease and starch hydrolysis test. The bacterial strains were screened for their IAA production, the major growth promoting trait helpful for the sustainable agriculture. IAA production from different bacterial strains was estimated using salkowski reagent Out of sixteen isolated bacteria five isolates were selected as efficient producers of Indole acetic acid. All five isolates were observed for the production of IAA, among them FFA16 isolate was comparatively recorded with high competence. All the isolates were observed for their effect on maize and paddy seeds plant growth and found increased plant growth with the isolates when compared to control. In conclusion the study suggests the IAA producing bacteria as efficient bio fertilizer inoculants to promote plant growth.

Keywords: *Plant growth-promoting microbes (PGPM), Indole acetic acid (IAA), Biochemical tests, Bio fertilizer*

Introduction:

The use of plant growth-promoting microbes (PGPM) is a potentially advantageous technique for improving crop productivity, food quality and security in more sustainable and eco-friendly agricultural systems (Souza et al., 2015; Abhilash et al., 2016; Mimmo et al., 2018; Asghari et al., 2020; Etesami, 2020). Rhizosphere fungal and bacterial community can harbor beneficial organisms known as PGPM. These organisms have the ability to colonize plant roots providing benefits to their hosts, by modulating the production of phytohormones, increasing the availability of soil nutrients, and the resistance against pathogens. Besides, minimizing the use of chemical fertilizers, mitigating biotic and abiotic stresses, and increasing plant production (Abhilash et al., 2016; Asghari et al., 2020; Etesami, 2020). The microorganisms used to increase agriculture productivity are *Azospirillum, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Pseudomonas, Rhizobium, Frankia, Klebsiella, Clostridium, Trichoderma, Beauveria, Serratia* and *Streptomyces* (Abhilash et al., 2016; Oosten et al., 2017; Gouda et al., 2018).

Plant growth promoting bacteria (PGPB) are free living soil bacteria found in the rhizosphere soil. They help in the fixation of atmospheric nitrogen (Zehr et al., 2003), production of siderophores (Machua and Milagres, 2003), solubilization of soil minerals (Tilak et al., 2005) and synthesis of phytohormones (Chopade et al., 2008). The synthesis of plant hormones such as auxins, gibberellins, cytokinines and polyamines by rhizosphere microbes are considered to be specific to the microbe-host rhizospheric bacteria synthesize auxins in order to perturb host physiological process for their own benefit (Ahmed et al., 2008; Cassan et al., 2009; Tien et al., 1979; Yang et al., 2009). Indole -3- Acetic Acid (IAA) is the principle and first auxin sequestered from plants (Levean and Lindow, 2005; Aziz et al., 2015). Indole 3- acetic acid been characterized as the most predominant, physiologically active, naturally occurring auxin, produced in larger quantities than any other related compounds (Hariharan et al.,2014). Different stages of plant growth could be effected by the IAA production from rhizospheric bacteria includes seed germination (Hariharan et al., 2014), root elongation (Hariharan et al., Sadaf et al.,2009, Farah 2005, Loper1986), growth of root hairs (Sadaf et al., 2009), shoot length, shoot length, weight (Hariharan et al.,2014).

Various microorganisms present in soil capable of producing IAA include various bacterial and fungal species. Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as PGPR. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics (Arshad *et al.,* 1992). The microorganisms isolated from rhizosphere region of various crop have an ability to produce Indole acetic acid as secondary metabolites due to rich supply of substrates. IAA has been implicated in virtually all aspects of plant growth and development including the production of longer roots with increased number of root hairs and root laterals which are involved in the nutrient uptake (Datta and Basu, 2000).

In this regard, the present study was conducted to isolate potential IAA producing bacteria from decomposed fig fruit waste soils of Andhrapradesh.

The objective of this study was to isolate and screen indigenous Indole acetic acid producing bacteria from fig fruit waste decomposing soils and effect of isolates on plant growth

2.Materials and Methods

2.1. Isolation of IAA producing bacteria from fig fruit waste decomposing soils

Collection of samples

Samples were collected from soil of fig fruit waste soils. soil samples were taken and kept in a sterile zip lock polyethylene bag. Samples were taken to the laboratory maintaining the aseptic conditions. Ten gram of rhizosphere soil was transferred in a 250 ml sterile Erlenmeyer flask and 90ml sterile distilled water was added. Tenfold serial dilution was carried out with the soil mixture.0.1 mL of diluted sample was plated on sterile Luria Bertani (LB) agar medium (Himedia, India) and incubated for 3 days at 28 °C. Single colonies were picked up and streaked on sterile LB agar plates to get pure culture. Well isolated colonies were observed for morphological characterization.

Total 16 isolates were obtained from fig fruit waste decomposed soils. The isolates were further screened for IAA production.

2.2. Identification of isolates

The isolates based on morphological observation and biochemical characterization were identified. The tests involved, were Gram staining, amylase and gelatinase, catalase like enzyme production, citrate utilization, indole test, Vogus Proskaur test, methyl red test, H_2S production, sugar fermentation etc. (Aneja, 2001).

2.3. Screening of bacteria

Isolates were screened for auxin production by using Salkowski reagent. Broth cultures were centrifuged and supernatants were mixed with Salkowski reagent in a ratio of 1:2. Salkowski reagent was prepared by mixing 150 ml of concentrated H_2SO_4, 250ml of distilled water and 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$ (Patten and Glick, 2002). The mixture was allowed to stand for 30 minutes at room temperature in dark for color production. Isolates showing pink to red color were selected as IAA producers and were used in further experiments.

2.4. Quantitative determination of IAA production from bacteria

To determine the amounts of IAA produced by each isolate, a colorimetric technique was performed with Van Urk Salkowski reagent using the Salkowski's method (Ehmann, 1977). All pure isolates were inoculated into 250ml conical flasks containing 50ml LB broth media with tryptophan as precursor. The liquid cultures are incubated on shaker incubator at 180rpm for 2days at 280 ± 10 c. Cultures were centrifuged at 12000rpm for 10 min. Supernatant was reserved and 1ml was mixed with 2ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution) and kept in the dark. The development of pink color indicated the IAA production. The optical density (OD) was recorded at 530 nm after 30 min

2.5 Effect of IAA producing isolates on plant growth by pot assay

To study the effect of IAA producing rhizospheric isolates on plant growth, Highest auxin producing bacterial isolate (FFA16) was used in maize and rice pot assay. Local Maize and paddy seeds were used for seed coating. The seeds were surface sterilized by immersing in 95% ethanol for 30 s and mercury chloride (0.2%) for 3 min. Then further to remove traces of mercury chloride, the disinfected seeds were washed 5 times by sterile distilled water. 0.1ml overnight grown culture (0.5 OD) was applied on seed surface for seed coating. Seeds were dried and sowed into sterile soil as carrier. Six seeds were sown in each pot used per pot at equal distance and experiment was performed in triplicates for each isolate. The uncoated seeds were used as control. Triplicates were maintained for all the isolates. After appearing seedlings of soil 0.1 g of Tryptophan per kg soil after dissolving in water was added to every pot. Pots were irrigated with sterile distilled water every day and kept in sunlight. At the interval of every 5th day, plant was uprooted and seedlings were measured for shoot and root length and number of root hair of individual seedling was measured in maize and paddy plants and the mean value was compared with the control.

3, Results and Discussion

3.1. Isolation and Identification of rhizospheric isolates

Sixteen bacterial isolates were successfully isolated from rhizosphere soil among which eight were selected based on IAA production ability. The isolates were identified based on morphological observation and biochemical characterization (Table 1). Bergey's manual of determinative of bacteriology was used as a reference to identify the isolates (MacFaddin, 2000).

	Isolates				
Characteristics	FFA 2	FFA 5	FFA 8	FFA 9	FFA16
Colony colour	off white	white	white	Off white	white
shape	cocci	rod	rod	rod	rod
Gram staining	Gram positive	Gram positive	Gram negative	Gram negative	Gram positive
motility	Non Motile	Motile	Motile	Motile	Motile
Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Capsule staining	+	+	+	+	+
Acid fast	-	-	-	-	+
endospore	-	-	+	+	+
Indole	-	+	-	+	+
Methyl red	+	+	+	+	-
Vogus proskaur test	-	-	+	+	-
Citrate utilisation	+	+	+	+	+
glucose	+	+	-	+	+
Sucrose	+	+	+	-	-
Mannitol	+	+	-	-	+
Mannose	+	+	-	-	+
Fructose	+	+	-	+	+
Lactose	-	+	+	+	_-
H2S Production	-	+	+	+	+
Catalase	-	+	+	-	+
Oxidase	-	-	-	-	+
Pectinase	+	+	+	-	+
Amylase	+	+	+	+	+
Urease	-	-	+	+	+
Starch hydrolysis	+	+	+	+	+

Table 1. Morphological and Biochemical characterization of IAA producing Isolates

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. Eight isolates are positive for IAA production but among those five isolates FFA2, FFA5, FFA8, FFA9 and FFA16 were selected as potential IAA producers. Hence for further characterization these isolates were selected.

Most of studies from the earlier work showed that IAA producing organisms are Gram negative (Lindow et al., 1998; Datta and Basu, 2000). Few Gram positive strains belong to Bacillus strain known to produce IAA (Wahyudi et al., 2011). Present study showed that three IAA positive strains were Gram positive and two strains were Gram negative.

3.2. Quantitative determination of IAA production from bacteria

All five isolates were able to produce moderate to high amount of IAA under laboratory condition. IAA production was checked with use of Salkowski reagent. Colour development was first visible at the highest IAA concentration within minutes and continued to increase in intensity for a period of 30 min. Hence optical density was measured after 30 min. Among the isolates FFA16 found to be the best producer of IAA. On the other hand, FFA2, FFA5, FFA 8, FFA9, were found to be a medium producer of IAA as shown in Figure 1.

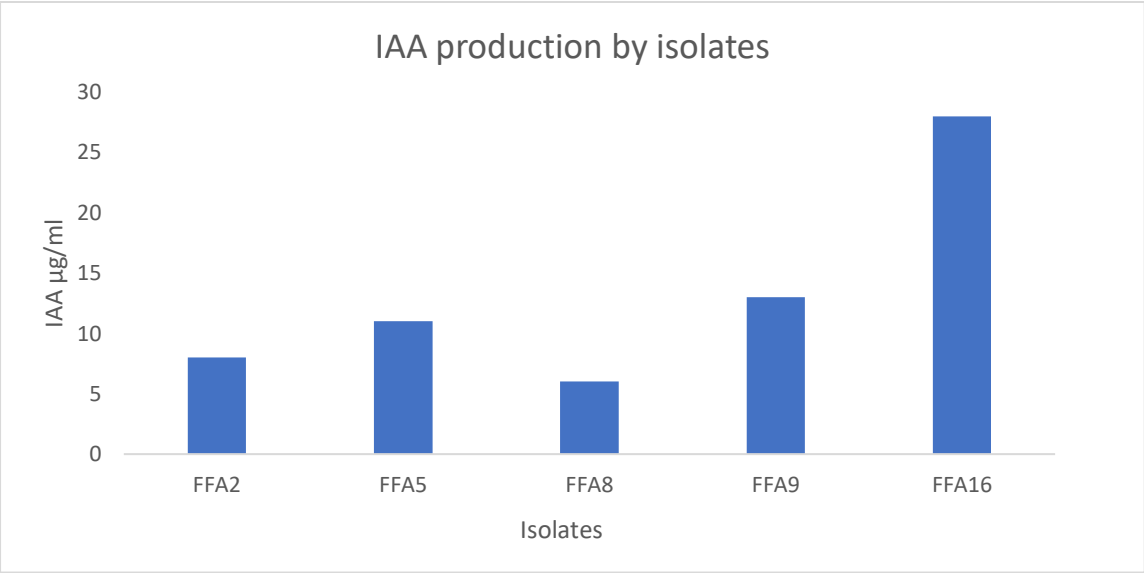

Fig 1: Quantitative determination of IAA production from bacteria

The use of the technique for the detection of IAA using the Van Urk Salkowski reagent is an important option for qualitative and semi-qualitative determination that assure the presence of the hormone in the supernatant of bacterial cultures or liquid formulations of biological inoculants. The amount of IAA produced by the bacteria was within the detection limits of Salkowski reagent (Ehmann, 1977). The reagent gives reaction with IAA and does not interact with L-tryptophan and Na-Acetyl-L-tryptophan and used by and large (Vaghasiat et al., 2011).

3.3. Biological feasibility of isolates for plant growth by pot assay

The property of synthesizing IAA is considered as effective tool for screening beneficial microorganisms suggesting that IAA producing bacteria have profound effect on plant growth (Wahyudi et al, 2011). Inoculation with IAA producing bacteria induces the proliferation of lateral roots and root hairs. Fatima et al. (2009) also showed that germination rate, roots, shoot growth of plant were increased by IAA and PGPR. Therefore, these isolates were studied for their effect on plant growth under controlled conditions. There was a significant increase in root and shoot elongation and number of roots in pot assay of maize and rice seeds by highly IAA producing strains. Both maize and paddy seeds treatment with all isolates FFA2, FFA5, FFA8, FFA9 and FFA 16 strains increased root length, shoot length and root number. Among all IAA producers FFA16 showed highest plant growth promoting activity as shown in Figure 2

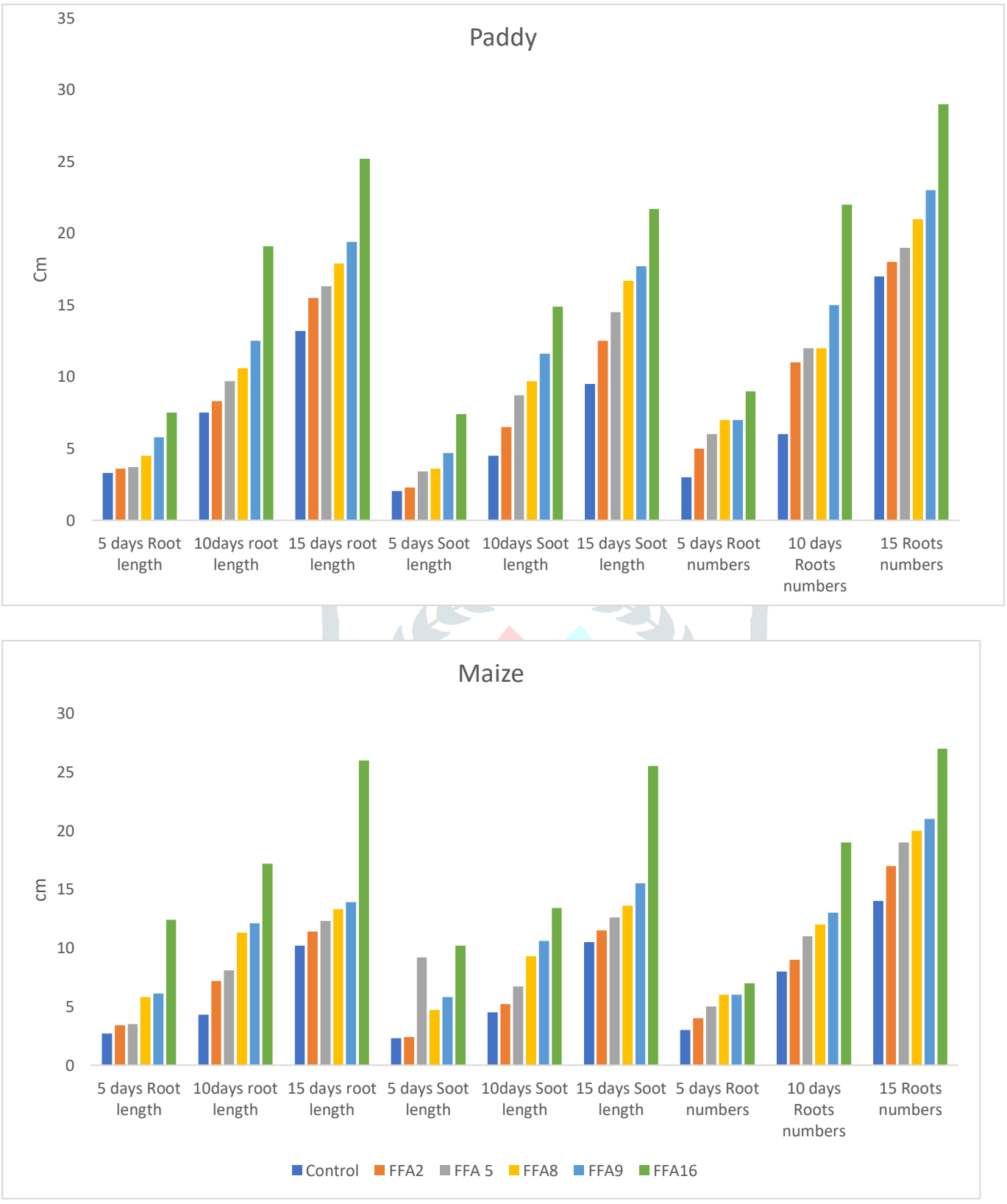

Figure 2. Growth stimulatory effect of bacterially synthesized IAA on paddy and maize root and shoot length and root numbers compared with untreated control

Data obtained from pot experiment on plant growth are summarized in Table 2

Paddy (Mean ± SD)									
	Root length			Shoot length			Root number		
Isolates	**5th day**	**10 th day**	**15 th day**	**5 th day**	**10 th day**	**15 th day**	**5 th day**	**10 th day**	**15 th day**
Control	3.33±0.15	7.53± 0.30	13.23± 0.11	2.03± 0.05	4.50± 0.36	9.53± 0.15	3.06± 0.11	6.06± 0.05	17.13± 0.15
FFA2	3.66± 0.25	8.36± 0.49	15.50± 0.10	2.33± 0.30	6.60± 0.20	12.50± 0.26	5.50±0.366	11.13±0.15	18.16± 0.15
FFA5	3.73± 0.15	9.76± 0.15	16.33± 0.41	3.43± 0.20	8.70± 0.20	14.53± 0.20	6.56± 0.41	12.06± 0.11	19.16± 0.15
FFA8	4.53± 0.15	10.60± 0.36	17.90± 0.40	3.63± 0.20	9.76± 0.15	16.70± 0.10	7.16± 0.11	12.06± 0.11	21.06± 0.11

FFA9	5.83± 0.12	12.56± 0.30	19.30± 0.17	4.767± 0.153	11.66± 0.15	17.70± 0.10	7.40± 0.43	15.00± 0.10	23.00± 0.10
FFA16	7.53± 0.35	16.10± 0.26	25.26± 0.46	7.46± 0.11	14.96± 0.37	21.76± 0.66	9.10± 0.10	22.03± 0.95	29.667± 0.681
	Maize (Mean±SD)								
Isolates	Root length			Shoot length			Root number		
	5 th day	**10 th day**	**15 th day**	**5 th day**	**10 th day**	**15th day**	**5th day**	**10 th day**	**15 th day**
Control	2.70± 0.20	4.30± 0.55	10.26± 0.60	2.30± 0.10	4.53± 0.05	8.57± 0.06	3.66± 0.57	8.66c 0.57	14.40± 0.50
FFA2	3.40 0.400	7.23± 0.25	11.46± 0.11	2.46± 0.11	5.26± 0.47	10.56± 0.15	4.16± 0.28	9.33± 0.57	17.56± 0.51
FFA5	3.56± 0.15	8.13± 0.15	12.30± 0.52	3.50± 0.30	6.70± 0.26	11.63± 0.37	5.667± 0.57	11.66± 0.89	19.10± 0.17
FFA8	5.83±	11.06±	13.36±	4.73±	9.36±	13.56±	5.66±	11.93±	20.66±

	0.05	0.11	0.15	0.15	0.15	0.35	0.57	0.11	0.57
F**FA9**	6.13± 0.11	12.16± 0.15	15.66± 0.40	5.80± 0.17	10.63± 0.05	15.53± 0.20	6.33± 0.57	13.60± 0.52	21.3± 0.577
F**FA16**	12.43± 0.40	17.20± 0.20	25.30± 0.52	10.03± 0.32	13.40± 0.26	20.23± 0.81	6.33± 0.70	19.26± 0.64	27.13± 0.90

Table 2. pot experiment on plant growth for "Paddy" and "Maize" with mean and SD values of isolates showing significant difference

From the pot experiment assay, it is proved that, the isolates were significantly augment the root length, shoot length and number of roots of maize and paddy seedlings ,when compared with control. In earlier report, root elongation was found to occur in *Sesbania aculeata* by inoculation with *Azotobacter* spp. and *Pseudomonas* spp., in *Brassica campestris* by *Bacillus* spp (Ghosh *et al.*, 2003), in *Vigna radiata* by *Pseudomonas putida* (Patten and Glick, 2002) and in *Pennisetum americanum* by *Azospirillum brasilense* (Tien *et al.*, 1979). This indirectly confirms the involvement of bacterial isolates in enhancing the plant growth by synthesizing IAA

4. Conclusion

In conclusion, rhizosphere soil is a huge source of nutrient for microbes. There is a complex mechanism between plant root and soil microbes. Bacterial IAA proved to be an efficient metabolite affecting plant growth promotion by the bacterial isolates. From this study, it is clear that rhizospheric soil can provide a rich source of IAA producing bacteria and has the ability to produce a significant amount of IAA. These five isolates were characterized by microscopic observation and biochemical test. These isolates produced maximum amount of IAA and have positive response in plant growth in terms of root shoot growth. So it can be stated that presence of such growth promoting bacteria are responsible for the beneficial effects on plant growth and they can be used as bio fertilizer in the field to prevent environmental pollution instead of industrial chemical.

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