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Growth enhancement in *Arachis hypogaea* at salinity stress by bacterial SP

[1]Rajiv Gandhi, [2]Meganathan B and [3]Sankar R

[1, 2]Research Scholar, Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India.

[3]Assistant Professor, Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

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Abstract

Salinity stress is an important environmental problem that adversely affects crop production by reducing plant growth. The impact of rhizobacterial strains to alleviate salinity stress on the germination of *Arachis hypogea* seeds were assessed using different concentrations of NaCl. Plant growth hormones, also known as phytohormones, regulate plant development and evoke a specific physiological response in plants, at very low concentration. Indole acetic acid (IAA) is one of the most important physiologically active auxins.IAA is a general product of L-Tryptone metabolism produced by many microbes including Plant Growth Promoting Rhizobacteria (PGPR). Tryptophan is an essential amino acid that can undergo oxidation by the action of the bacterial enzyme tryptophanase. Not all bacteria possess this enzyme and so this test can be used as a biochemical differentiators this tryptophanase is mainly use in production of plant growth hormones using rhizobacteria. The most important effect of indole acetic acid is to promote development of roots and stems, through stretching of the newly formed cells in the meristem. Effect of IAA producing *Rhizobium*, sp and *Serratia*, sp bacteria on plant growth was studied by pot culture experiments by using sterilized air dried soil and viable ground nut seed. Both bacteria demonstrated increase in root length, shoot length, root and shoot fresh and dry weight, on bacterial inoculated ground nut seeds over control. The main goal of this study is to isolate and screen indigenous Indole acetic acid-producing bacteria from different rhizospheric soil.

Keywords: Growth Enhancement, Arachis hypogaea, Salinity Stress and Bacterial SP

Introduction

Salinity is one of the major abiotic stresses that adversely affect modern agriculture and constitutes a problem everywhere in the world. More than 6% of the world's total land area is salt-affected; most of this salt-affected land has arisen from natural causes and the accumulation of salts over long period of time in arid and semiarid zone (Rengasamy, 2002) [19]

Soil salinity stresses, plants in two ways, high concentration of salts in the soil make it harder for roots to extract water and high concentrations of salts with in the plant can be toxic. Salts on the outside of the roots have an immediate effect on cell growth and supplementary metabolism; toxic concentrations of salts take time to gather inside plants, before they affect plant function. (Munns &Tester, 2008) [16]

One approach to solve the salt stress problem is the use of plant growth-promoting bacteria (PGBP). Many gram –positive and negative PGBP have been reported to colonize the plant rhizosphere and confer beneficial efforts by various direct and indirect mechanisms, which can be correlated with their ability to form biofilms, chemo taxis, and the production of exopolysaccharide. Indole -3-acetic acid (IAA) and amino cyclopropane-1-carboxylate (ACC) deaminase. (Glick, 1995) [10] A major challenge towards world agriculture involves production of 70% more food crop for an additional 2.3 million people by 2050 worldwide (FAO; 2009) [6] Salinity is a major stress limiting the increase in the demand for food crops. More than 20 % of cultivated land worldwide (~ about 45 hectares') is affected by salt stress and the amount is increasing day by day.

Plant growth promoting rhizobacteria are free living, soil –borne bacteria, which enhance the growth of the plant either directly or indirectly. The direct mechanism involve nitrogen fixation, phosphorus solubilisition, HCN production, production of phytohormone such as auxin, cytokinin and gibberellins and lowering of ethylene concentration (Glick 1995; Glick *et al.*, 1999) [9, 10]

There are several plant growth promoting rhizobacterial (PGPR) inoculants that seems to promote plant growth through different mechanism such as plant growth hormone production, nutrient acquisition and plant disease suppression PGPB are inhabiting rhizosphere are capable of producing plant growth regulators such as auxin, gibberellins and ethylene. Indole-3-acetic acid is a naturally occurring auxin which involves in cellular development and physiological process in plants. Different soil microorganisms including bacteria, fungi, and algae are capable of producing physiologically active quantities of auxins which may exert prominent effects on plant growth and development (Stein *et al*., 1990; Finnie &Van staden, 1985; Rifat Hayat *et al*., 2010) [7, 23]

Plant growth hormones also known as phytohormones, regulate plant development and evoke a specific physiological response in plants, at very low concentration. Auxin were the first class of plant growth regulators to be identified. Auxin have many regulatory functions in plants, including stimulating cell enlargement, cambium cell division, differentiation of phloem and xylem, rapid establishment of roots and lateral root formation. Auxins also help in regulating flowering and fruiting. Auxins mediate the tropism response to gravity and light (Patten *et al.*, 2002:. Lwin *et al.*, 2012) [18, 11]

The plant growth promoting rhizobacteria (PGPR) are involved in the production of diverse microbial metabolites like

siderophores, plant growth enhancement through Indole acetic acid (IAA) production and HCN production.

Indole acetic acid (IAA) is one of the most physiologically dynamic auxins, IAA is a common product of L- Tryptophan metabolism fashioned by several microorganisms including plant growth – promoting rhizobacteria (PGPR) (Lynch,1985) [13]

The microorganisms isolated from rhizosphere region of innumerable crop have an facility to produce Indole acetic acid as secondary metabolites due to rich supply of substrates. Indole acetic acid helps in the construction of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Datta and Basu, 2000) [4]

IAA is primarily a product of secondary metabolism and L-Tryptophan apparently becomes more available for IAA synthesis once primary metabolism has slowed (Brandl, *et al*, 1996) [14]. IAA is the principle and first auxin isolated from plants. IAA is a commercially important and one of the most widely studied plant growth promoting hormone. IAA is known to control organogenesis, tropic responses, cellular responses such as cell enlargement, division and differentiation, gene regulation and responses to light and gravity

Ground nut (*Arachis hypogaea*. L) Is an annual legume and the 13th salient oil seed crop of the world. In India, groundnut is an important oil seed food and feed crop grown in an area of 6.45 million ha with the total production of 6.57 million tons based on an average of the last 5 years (FAO, 2005) [5]

The different growth stages of these crop is often subjected to various types of abiotic stress like drought, salinity, high-temperature etc. which may cause yield loss. Soil salinity, spread in about 2.0 m ha of coastal and saline areas in the major groundnut growing states of India, is one of the most important abiotic factors that significantly affect seedling, vegetative and reproductive growth, seed quality and productivity. Groundnut yields have been reported to be severely affected with an increase in soil and water salinity (Nithila *et al*, 2013) [17].

Today bio fertilizers have emerged as a highly potent alternate to chemical fertilizers due to their eco-friendly, easy to apply, non-toxic and cost-effective nature. Also, they make nutrients that are naturally profuse in soil or atmosphere, usable for plants and act as improvements to agrochemicals. Bio fertilizers is a substance which contains living microorganisms which when applied to seed, plant surfaces, or soil colonizes rhizosphere or the interior of the plant and plant and promotes growth by increasing the accessibility of primary nutrients to the host plant (Ahsan ML, *et al*., 2012)

In this present study to enhance, crop production, the IAA growth hormone has increased plant growth in saline condition. The saline water concentrations has made the crop productivity in higher level by the IAA production enhanced, by microbial biomass.

Materials and Methods

Isolation of IAA producing bacteria

The soil sample were collected from rhizosphere region and root nodules of groundnut plant by using sterile container. Collected soil samples were stored at 4ºCat refrigerator for the further studies.

IAA producing bacteria were isolated from the collected soil samples by using serial dilution technique (10^{-2} to 10^{-9} dilution). The diluted samples plated on sterile nutrient and YEMA agar petriplates and kept for incubation at 37º C for 48 hours. The nutrient agar and YEMA agar plates were observed for growth of microorganisms after 48 hours of incubation. The isolated bacterial strains were maintained at sterile condition for the further studies.

Characterization of IAA production

Primary screening

IAA production was detected by the method with some modification. 25 ml nutrient broth was inoculated with freshly grown cultures and kept at 37 degree C for 36 h at 120 rpm in an incubator shaker. Cultures were centrifuged at 10,000 rpm for 15 min. at room temperature. Carefully 1 ml supernatant was pipetted out and 2 ml Salkowski r eagent (2% 0.5 M $FeCl_3$ in 35% per chloric acid) added. Further, two drops of orthophosphoric acid added to it and kept in dark for color formation. The optical density was documented at 530 nm after 2 h. IAA concentrations were determined using the standard plot of IAA.

Standard graph preparation

Standard IAA was purchased from HI Media and different concentrations 1 mg / 100 ml were prepared in distilled water. Each tubes were noted like (0.2, 0.4, 0.6, 0.8, 1 ml) can be make up with distilled water. For color formation, Salkowski reagent was added to different dilution of IAA (1:2) followed by the addition of two drops of orthophosphoric acid and kept in dark. Spectrometry measurements were complete at 530 nm and standard curve was plotted.

Identification of IAA producing bacteria

The morphological characterization was done for the isolated bacteria to identifying their shape, size, and arrangement of cell by the gram staining, capsular staining, spore staining.

Biochemical characterization

The above identified two IAA producing bacteria were subjected to various biochemical tests oxidase test, catalase test, indole test, methyl red test, voges proskaur test, citrate utilization test, nitrate test were done.

Salt tolerance assay and PBPR properties under salt stress:

The two bacterial strains of *Serratia, Rhizobium,* which were maintained in 50% (v/v) glycerol at -70 degree C were re-cultivated in nutrient broth (NB) for 12-18 hours. The bacterial cells were collected by centrifugation, washed with 0.85 %(w/v) sodium chloride (Nacl) and re-suspended with deionized water to obtain a suspension equal to 1.0 at optical density of 600 nm. The cell suspension was 10 fold serial diluted to10-4 and 10-5 before being used as bacterial samples. The salt tolerance study was carried out by spreading 0.1 ml of each sample on nutrient agar (NA) (HIMedia, India) and pseudomonas agar F(PAF medium) (Difco, USA), with each modified by 2%,4%,6%,8% or 10%(w/v) Nacl. Plates were incubated at ambient temperature (28+_2degree C) for 3 days. The tolerant bacteria at 8% (w/v) Nacl were selected and preserved on PAF-medium containing 8% (w/v) Nacl.

Bioassays for plant growth promoting traits

IAA production

The selected antagonistic bacterial strains was inoculated in 100 ml conical flask containing sterile nutrient broth (NB) supplemented with 0.1% of tryptophan .The flask were kept for enrichment by incubating at room temperature for 24 hours . IAA was determined invitro by the method of Salkowski. From each of the enriched flasks,1.5 ml of culture was taken in centrifuge tube and centrifuged for 5 min at 8000 rpm,1ml of supernatant was taken and mixed with 2 ml of Salkowski's reagent (2% 0.5 M FeCl 3 in 35% per chloric acid) and incubated for 30 min in dark for development of pink colour indicate positive reaction and yellow colour indicate negative reaction, spectrophotometric analysis of each sample was done

for IAA detection which showed higher absorbance at 520 nm and their IAA concentrations were measured according to standard graph values were considered as efficient IAA producers and chosen for further experimental procedure

Optimization of IAA production

IAA production and optimization of production parameters: PH is one of the most important physicochemical parameters for IAA production.100 ml of sterile (NB + 0.1% tryptophan) was prepared and distributed in sterile test tubes and the pH adjusted to 5, 6, 7, 8 and 9, respectively. The tubes prepared according to respective pH were inoculated with isolates and labeled according to culture inoculated and kept for incubation at room temperature for 24h. All the incubated samples were weighed for measuring IAA concentration by Salkowski's method after each 24 h. Temperature is also an important parameter for IAA production since the growth of bacteria precious by low or high temperature and IAA production is dependent upon the correct growth of microorganism. Thus, its fabrication was maintained at 25, 30, 35, 37, 40 and 45º C at 120 rpm. Five different sugars *viz*. Dextrose, mannitol, sucrose, mannose and starch at different concentrations of 0.5%, 1.0%, 1.5%, 2% and 2.5% were used as a carbon source for the IAA production. Different nitrogen sources *viz*. beef extract, soybean meal, urea, glycerine, nicotinic acid, ammonium nitrate and potassium nitrate at varying concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% were used for the study. Quantification of IAA production was performed through the standard plot. The salt level is very important for plant growth. The salt concentration are the 2%, 4%, 6%, 8% and 10% were used for optimization process.

Extraction of IAA

After incubation, cells were separated from the supernatant by centrifugation at 10,000 rpm for 30 min. The supernatant was acidified to pH 2 with 1N HCl and extracted twice with ethyl acetate. Extracted ethyl acetate fraction was evaporated in a rotator evaporator at 40 °C. The extract was dissolved in 1ml of methanol and kept at 20 °C.

Characterization of IAA by FTIR

FTIR Analysis for Confirmation of IAA Production in order to confirm the production of IAA by bacterial isolate based on information about its chemical bonds and molecular structure, FTIR analysis was carried out. Methanolic extract was finally dried and mixed with spectral grade potassium bromide, and FTIR spectral analysis of IAA was verified at the transmission mode from 400–4000 cm−1.

Pot experiments

Experiments were carried out in pots filled with 2 kg soil, previously heat sterilized in metal buckets of 100 °C for 1 hour on each of the three successive days. This is a recognized technique for soil sterilization since some spore forming bacteria may tolerate high temperatures, spores may germinate on the second or third day. However, soil and seeds were initially sterilized in order to eliminate possible contamination by resident Rhizobia, later plants were kept under non-sterile conditions. Pots were sterilized by swabbing thoroughly with 95% (v/v) C_2H_5OH. Seeds of *Arachis hypogeae.L* was surface sterilized by rinsing in C_2H_5OH (90% v/v) and soaking for 5 minutes in H_2O_2 (3% v/v) followed by three washings in sterile distilled water. Seeds were germinated in sterilized dishes encompassing sterile damp filter paper. Sterile distilled water was supplementary at intervals to keep the filter paper and germinating seeds wet. Seeds were incubated at 30 °C for 2-3 days until radicals were 2-3 cm long and root hairs appeared. Three or four seedlings were inoculated with *Rhizobium* culture.

Preparation of inoculums

The isolated IAA producing microbial culture were used for pot experiments. The bacteria were grown in 250 ml Erlenmeyer flasks containing 40 ml yeast-extract mannitol (YEM) broth (Somasegaran and Hoben, 1985) [22] in a shaking incubator for 3 days. The cultures were shaken only for 8 hrs each day at 28°C. One mille liter (containing 106-7 cells) of the bacterial culture at their logarithmic stage of growth was inoculated just after seedlings were transferred into the pots. Three days later, seedlings were re-inoculated in order to confirm root hair infection by Rhizobia.

Salt stress treatment

After transplanting the sterile seedlings into pots, the inoculated plants were irrigated by the nutrient solution described above. Treatments were executed when plants were 21 days old. Preliminary experiments showed that plants can form active N2-fixing nodules at that age, accordingly, treatments in the present study started after module formation on plant roots. In addition to controls, four levels of salinity were applied where NaCl was added to the basic nutrient solution as follows:
S1: 40 mMNaCl S2: 80mM NaCl
S3: 120 mMNaCl S4: 160 mMNaCl
S5: 200 mMNaCl.

As NaCl is known to be absorbed by several legumes,50 ml of each level as applied once in every 10 days and salt treatments continued for 9 weeks. At the end of each period (10 days), the pots were flushed thoroughly with non-saline nutrient solution to avoid salt precipitation around roots. As plants grew in size with healthy nodules, the volume of liquid added was increased. The proportional difference between salt treatments was kept constant. Determination of morphometric characters of plants, fresh and air-dry weight of nodules Plants grown for the salt stress studies were harvested at 10, 25, 40 and 55 days after treatments. Each plant was decapitated and the root length, shoot length, leaves length and width were measured. The root systems were washed gently under tap water. Roots were blot dried and nodules from each individual root were collected, counted and air-dried. Fresh weight of nodules was estimated. Nodules were air-dried at room temperature for 4 h or until their weight was constant.

Growth measurement

Each plant was decapitated and the root length were measured. All the plantlets of batch 1 and batch 2 were removed gently from pots and each root,shoot of each plantlet was measured manually using scale. The average root measurement for each pot of both the batches was calculated. The plants were washed gently under tap water. And the fresh weight of the plants were measured by using the electronic balance in gram. The plants were decapitated and the plant leaves length and width were measured by using the measuring scale in cm.

Results and Discussion

Isolation of Bacteria

The serial dilution technique was employed to isolated bacteria from soil samples. In totally, 10 morphological different bacterial strains were isolated and it was named as A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10. The plates were stored in refrigerator for further use.

The isolates were identified based on morphological observation and biochemical characterization. Bergeys manual of determinative of bacteriology was used as a reference to identify the isolates (Macfaddin, 2000) [15] It has been reported that IAA production by bacteria can vary among different

species and strains, and it is also influenced by culture condition, growth stage and substrate availability. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992) [21]

Identification of isolated bacteria

The bacteria were isolated from soil. It was enriched in nutrient agar and yeast mannitol salt agar plate. The bacterial strain was named as strain A4 strain A5. The bacterial strain A_4 which isolated is identified by morphological characteristics that shows Gram negative, motile and spore positive. Further, the isolate was identified by biochemical analysis that shows positive for Indole, Methyl red, Citrate, Catalase, Oxidase and shows negative for Voges proskauer, Starch hydrolysis, Casein hydrolysis, Hydrogen sulphide mentioned it in Table

The results of the morphological and biochemical characterization performed on the isolated strain. The strain form white, smooth, on Yeast mannitol salt agar plate. The strain A4 was identified as *Rhizobium.* The bacterial strain A5 which isolated is identified by morphological characteristics that shows Gram negative, motile and spore positive. Further, the isolate was identified by chemical analysis that shows positive for Voges proskauer, Citrate, Catalase, Gelatin hydrolysis, Casein hydrolysis, Starch hydrolysis and shows negative for Indole, Methyl red, Hydrogen sulphide, Oxidase.

The results of the morphological and biochemical characterization performed on the isolated strain. The strain form red, smooth, on nutrient agar plate. The strain A5 was identified as *Serratia* sp.

Table 1: Identification of isolated bacteria

Test								
				A4			A5	
Morphological characters								
Gram staining				-			-	
Spore staining				+			+	
Motility test				+			+	
Shape				Rod			Rod	
Biochemical characterization	Indole test	MR test	VP test	Citrate utilization	Catalase test	Oxidase test	Starch hydrolysis test	Gelatin hydrolysis test
A4	+	_	+	+	+	+	_	_
A5	-	_	+	+	+	_	_	+
Biochemical characterization	Triple sugar iron test	Hydrogen sulfide production test	Urease test	Lipid hydrolysis	Protein hydrolysis	Nitrate reductase test	Carbohydrate fermentation test	
A4	+	_	+	+	+	+	+	*Rhizobium* sp.
A5	+	_	_	+	+	_	+	*Serratia* sp.

Screening method

Primary screening: The IAA production index was correlated with the quantity measured by the method. It was observed that the rate of diffusion of IAA on the agar plate was directly proportional to the diffusion potential of the IAA which represented their concentration: therefore the diameter of halo zone may be represented for the amount of IAA produced. Higher the amount of IAA showed larger the size of halo zone diameter in a definite time. The test was also made by spot inoculation by the diluted inoculum on solid agar plate and after appearance of colonies IAA reagent was over layered, such type of pink coloured halozones also appeared around colonies in IAA producers whereas no pink halo zone around IAA production lacking strains.

Table 2: Primary screening of IAA production

S.no	Bacterial strains	IAA production (µg/mg)	Halo zone Diameter (cm)	IAA production index
1	A1	5.35	7	0.76
2	A2	6.57	8	0.73
3	A3	2.3	3	0.77
4	A4	6.80	10	0.96
5	A5	6.69	9	0.84
6	A6	1.92	3	0.71
7	A7	4.67	6	0.64
8	A8	5.32	7	0.73
9	A9	6.42	8	0.78
10	A10	3.48	5	0.79

Salt tolerance assays & PGPR properties under salt stress

The salt tolerance of the 2 isolates *Rhizobium sp* & *Serratia sp* was determined by total number of colony on nutrient agar medium supplemented with 0- 10 % (w/v) Nacl. The results showed that the bacteria were able to grow on Nutrient agar plus 0-8% (w/v), Nacl, The highest percentage of Nacl to 10% (w/v) suppressed the growth of the bacteria on the Nutrient agar medium. Therefore, the population range of the three bacteria cultivated on Nutrient agar medium plus 6,8,10 % (w/v) Nacl was investigated to confirm their tolerance, *Rhizobium , sp* and *Serratia, sp* were able to grow well in 8 %(w/v) Nacl, Therefore, the 2 bacteria could be classified as halo tolerant bacteria. The result was showed in Table.

Table 3: Salt tolerance test

Salt Concentration	No. of Colonies In *Rhizobium* sp.	No. of Colonies In *Serratia* sp.
2% Nacl	TNTC	TNTC
4% Nacl	306	779
6% Nacl	178	250
8% Nacl	84	106
10% Nacl	23	75

Optimization for IAA production

Optimization for IAA production at various PH by *Rhizobium* sp. and *Serratia* sp.

Lower pH limits the growth of plants, as concentration of metal ions could reach toxic levels in the soil at low pH. A number of

physiological and metabolic process taking place in the rhizosphere can be affected by soil pH and metal cations present in the vincinity, therefore, impact of pH range of 5-9 was only checked for IAA production *Rhizobium* sp was able to produce good amount of IAA throughout the pH range tested, which varied from 5-9 PH. *Rhizobium* sp has a higher biomass of IAA production in PH 6, and *Serratia* sp has a higher IAA production in PH 8. In this study *Rhizobium* sp shows high IAA production in pH 6 (5.5 µg/100 ml).

Optimization for IAA production at various temperature by *Rhizobium* sp. and *Serratia* sp.

Optimization of temperature for better growth was analysed. The effect of temperature was studied in the range 25-45 ºC where by maximum yield was observed. It was observed that *Serratia* sp. showed maximum activity. *Serratia* sp. optimized at 40 ºC shows an maximum IAA production pattern compared to *Rhizobium* sp. From this study *Serratia* sp. has a higher IAA production capacity in 40 ºC (1.95 µg/100 ml). The effect of different ranges of temperature (20 -40 °C) was studied. The optimum temperature for IAA production was 30 ºC. According to Sudha *et al.* (2012) [24] 37 ºC temperature was optimum for *Rhizobium* and *Bacillus* spp. for IAA production.

Optimization for IAA production at various carbon source by *Rhizobium* sp. and *Serratia* sp.

The carbon sources that are used in production of secondary metabolites have profound effect on the overall efficiency of biosynthesis. Five different sugars were used in the study to check their effect of IAA production. Maximum IAA production was obtained at 1% concentration of about 3.65 µg/100 ml while IAA maximum rate shows at 2.5% concentration of about 4.35 µg/100 ml in *Serratia sp* in Glucose optimization. *Rhizobium* sp. shows maximum rate at 2% concentration of about 2.15 µg/100 ml while IAA maximum rate shows at 0.5% concentration of about 1.35 µg/100 ml in *Serratia* sp. in Mannitol optimization. *Rhizobium* sp shows a maximum rate IAA production at 2.5% of about 2.3 µg/100 ml while IAA maximum rate shows at 1.5% concentration of about 0.9 µg/100 ml in *Serratia* sp. in Sucrose optimization

Monosaccharides were better sources than disaccharide and polysaccharide. Therefore, glucose was found as the best sugar source for IAA production.

Optimization for IAA production at various nitrogen source by *Rhizobium* sp. and *Serratia* sp.

Effect of nitrogen source is used for better growth analysed. It was observed *Rhizobium* sp. showed activity in ammonium nitrate. *Rhizobium* sp. showed maximum IAA production activity compared to *Serratia sp.* Ammonium nitrate was chosen as the principle nitrogen source due to its widespread usage as a cheap source of nitrogen for IAA production. From this study, Maximum IAA production was obtained at 1.5% concentration of about 2.35 µg/100 ml while IAA maximum rate shows at 1% concentration of about 1.95 µg/100 ml in *Serratia* sp. in Beef extract. *Rhizobium* sp. shows maximum rate at 1% concentration of about 1.45 µg/100 ml while IAA maximum rate shows at 0.5% concentration of about 0.75 µg/100 ml in *Serratia* sp. in Ammonium nitrate. *Rhizobium* sp. shows a maximum rate IAA production at 1% of about 2.1 µg/100 ml while IAA maximum rate shows at 2% concentration of about 0.75 µg/100 ml in *Serratia* sp. in potassium nitrate.

The suitable nitrogen source for IAA production was different with the isolate type as $NaNO_3$ for br1, KNO_3 and peptone for br2. KNO_3 and peptone for br3 and mr2 while $NaNO_3$ and peptone for wr2. There was a significant difference between the concentrations of IAA which indicates the effect of nitrogen. Basu and Ghosh (2001) [1] have reported that Glucose and KNO_3 as the best carbon and nitrogen sources of IAA production by *Rhizobium* spp.

Optimization for IAA production at various various salt concentration by *Rhizobium* sp. and *Serratia* sp.

Salinity adversely affect the crop development, growth and decrease yield quality. Therefore, the different concentration of salt was optimized for maximum production of IAA. Maximum production of IAA was obtained at 1% concentration of salt (1.15 µg/100 ml) in *Rhizobium* sp. whereas *Serratia* sp. produce maximum rate of IAA at 2.5% concentration of salt (0.6 µg/100 ml).

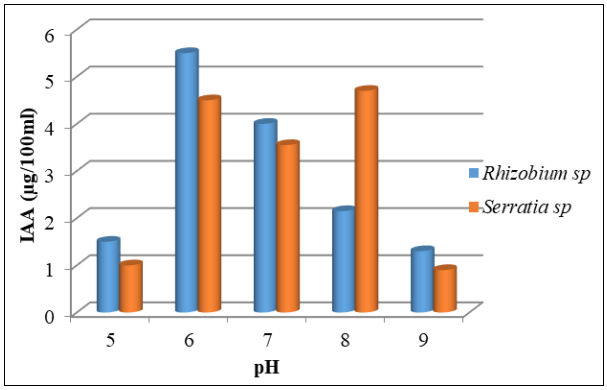

Fig 1: pH

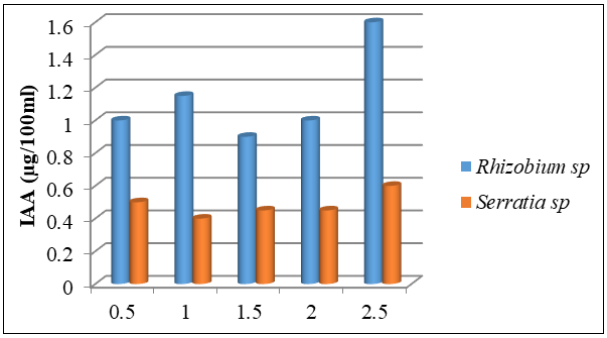

Fig 2: Salt concentration (%)

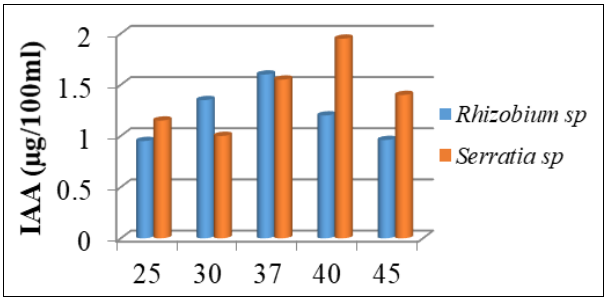

Fig 3: Temperature

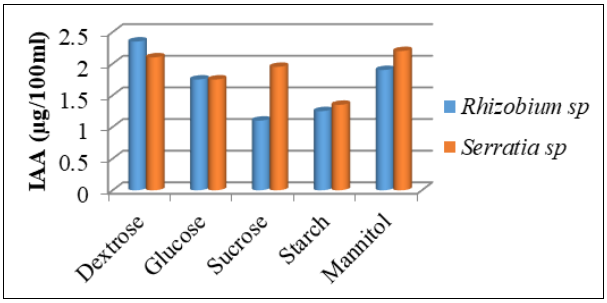

Fig 4: Carbon source

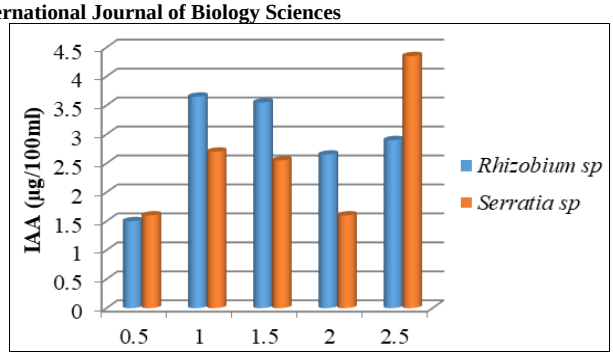

Fig 5: Concentration of Glucose (%)

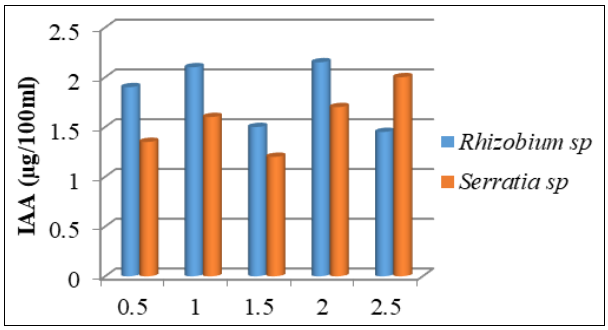

Fig 6: Concentration of Mannitol (%)

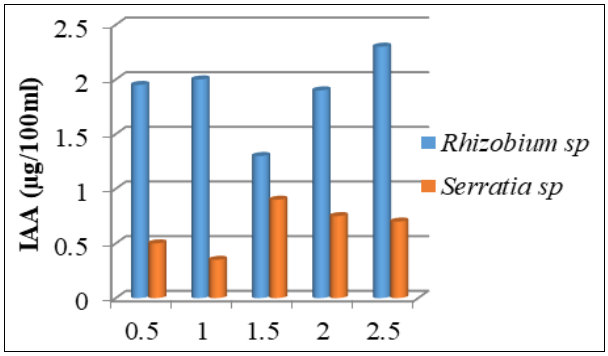

Fig 7: Concentration of Sucrose (%)

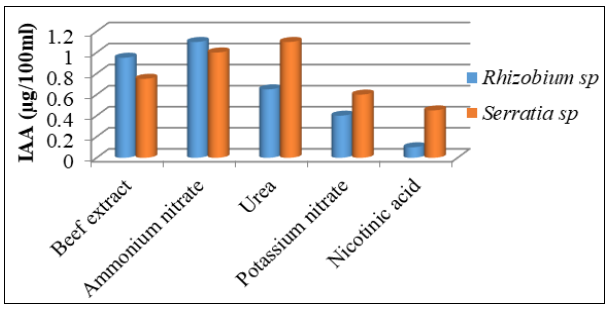

Fig 8: Nitrogen source

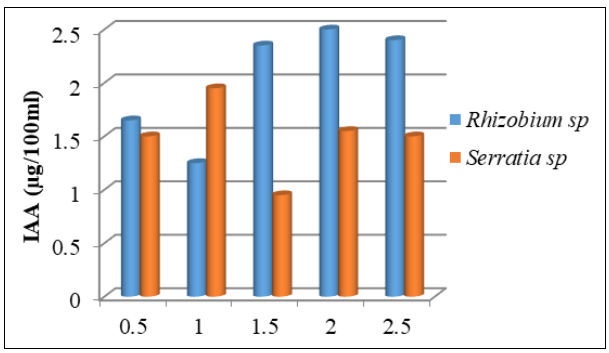

Fig 9: Concentration of Beef extract (%)

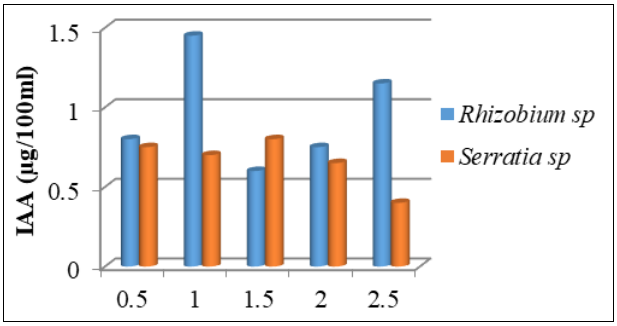

Fig 10: Concentration of Ammonium Nitrate (%)

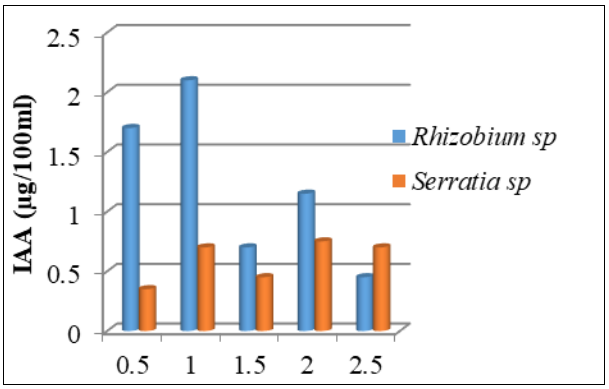

Fig 11: Concentration of Potassium Nitrate (%)

Extraction of IAA

IAA production isolates treated with Di ethyl ether this solution is shaken well for about 30 -45 min. The IAA gets dissolved in the ether. The fraction of ether is separated from the separating funnel and its volume is reduced to1/4 of the original of ether.in di ethyl ether solution and IAA producing media mixed equal amount after extraction produce IAA were 8 g and 35 mg.

IAA analysis by FTIR

The FT-IR analysis of pure IAA, isolated from the strain *Rhizobium* sp. The functional group of the extracted Indole acidic acid were identified as C-O group by FT-IR spectroscopy the results of FT-IR spectroscopy are shown in figure 5.6. The absorption bands at 1080.14 cm-1 which related to primary alcohol OH group. 2059.98 and 2021.40 cm-1 related to Isothiocyanate group. 1735.93 cm-1 related to aldehyde C=0group. 1635.64 cm-1 related to conjugated ketone C=O group. 1404.18 cm-1 related to carboxylic acid O=H group. 1080.14 cm-1 related to primary alcohol C-O group. group. The peaks showing presence of primary alcohol, Isothiocyanate group, aldehyde, conjugated ketone and carboxylic acid group which are found in the structure of IAA are depicted in Figure. From the figure, the characteristic peaks observed were 1080.14, cm-1 (primary alcohol group), 2059.98, 2021.40, 1735.93, 1635.64, and 1404.18 cm-1 Hence, the FTIR analysis confirmed the presence of IAA in the extract by showing the presence of characteristic functional groups of IAAs.

The FT-IR analysis of pure IAA, isolated from the strain *Serratia* sp. The functional group of the extracted IAA granules were identified as C- O group by FT-IR spectroscopy the results of FT-IR spectroscopy are shown in figure 5.7. The absorption bands at 1080.14, and 1141.86 cm-1 which related to alcohol C-O group. 2075.41,2021.40 cm-1 related to isothiocyanate N=C=S group. 1728.22 cm-1 related to alpha, beta unsaturated ester C=O group. 1635.64 cm-1 related to alkene C=C group. 1404.18 cm-1 related to alcohol O-H group. 1141.86 cm-1 related to secondary alcohol C-O group. 1080.14 cm-1 related to primary alcohol C-O group. The peaks showing presence of

isothiocyanate, alpha, beta unsaturated ester, alkene, alcohol, primary and secondary alcohol which are found in the structure of IAAs are depicted in Figure. From the figure, the characteristic peaks observed were 1080.14,1141.86 cm-1 (C-O group), 2075.41, 2021.40, 1728.22, 1635.64, and 1404.18 cm-1 (Prasanna *et al.*, 2011). Hence, the FTIR analysis confirmed the presence of IAA in the extract by showing the presence of characteristic functional groups of IAAs.

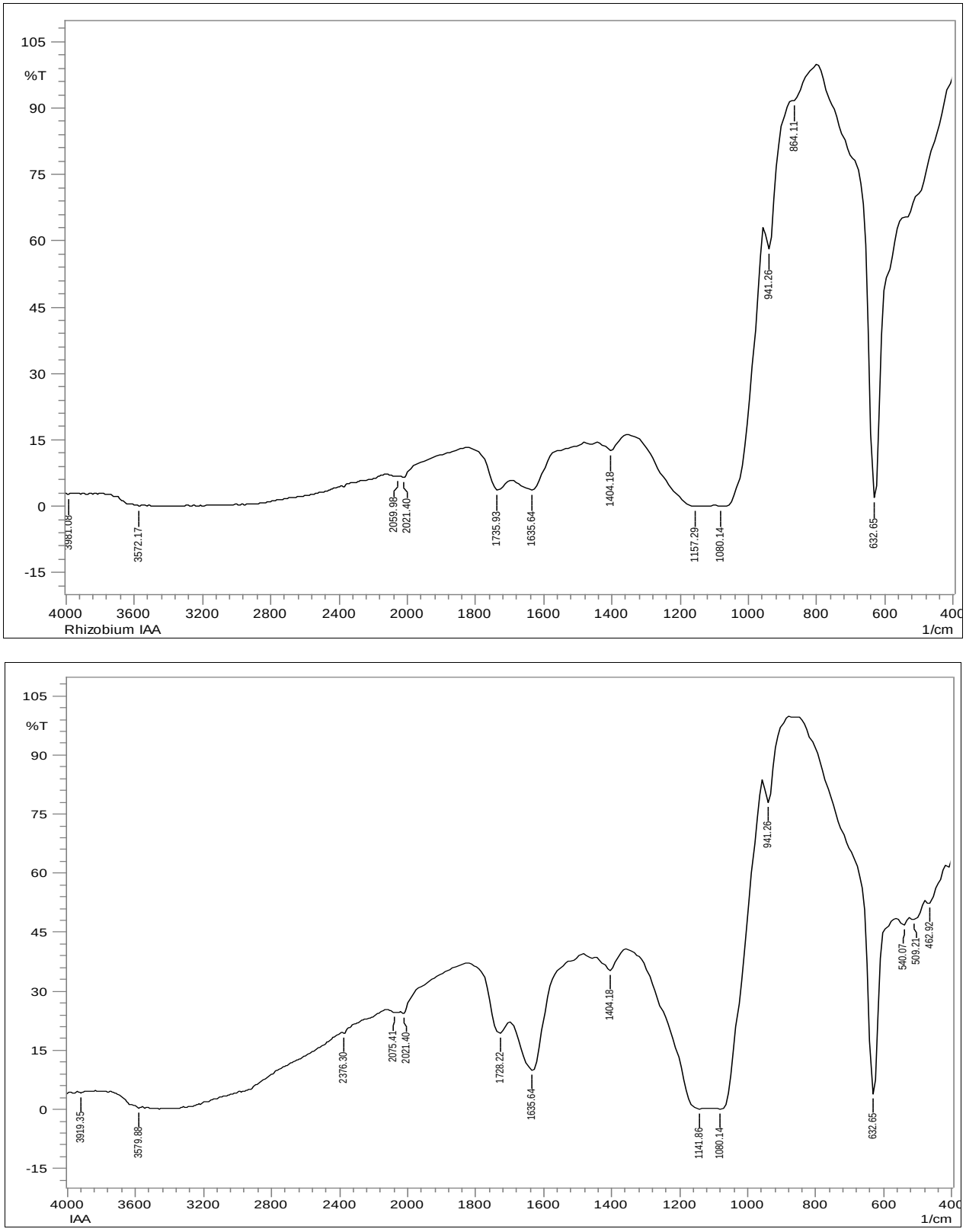

Fig 12: FTIR Analysis of *Rhizobium* FTIR Analysis of *Serratia*

Plant growth measurements

Root, Shoot length (cm)

Data presented in table showed that the effect of different salt concentration on the root length of ground nut plant. The highest root length was recorded in *Rhizobium* sp (5.9 cm) treated plants were compared with *Serratia* sp (2.9 cm) and bio control (2.6 cm). Significantly higher shoot length was produced in *Rhizobium* sp (27.4 cm) compared to *Serratia* sp (20.4 cm) and control (12.8 cm)

Root and Shoot fresh weight of plant (g)

The data pertaining to the effect of salt concentration on shoot fresh weight of groundnut plant are depicted. The shoot fresh weight of groundnut plants was significantly high in *Rhizobium*

sp, (4.8 g), compare to *Serratia sp* (4.2 g). The least weight of whole plant was observed in the uninoculated control (2.48 g). The fresh root weight on groundnut plants was significantly high in *Rhizobium sp*, (0.960 g) compared to *Serratia sp*,(0.701 g) the least weight of whole plant was observed in the uninoculated control (0.342 g)

Root and Shoot dry weight of plant (g): The results of the dry weight of shoot groundnut under to different biological control on recorded. Shoot dry weight of the plant was highest in the *Rhizobium* sp (0.940 g) and *Serratia sp*, (0.928 g) the least dry weight was seen in the control plants (0.400 g)**.** The results of the root dry weight of on a under two different biological control on recorded. root dry weight of plants was highest in the *Rhizobium sp* ,(0.920 g) and *Serratia sp* (0.461 g). The least dry weight was seen in the control plants (0.022 g).

Fig 13: Pot experiment for *Rhizobium* sp. Pot experiment for *Serratia* sp.

Fig 14: Plant growth measurement for *Rhizobium* sp. *Serratia* sp.

Conclusion

The main objective of this study was to isolate stain with maximum IAA producing capability from soil. A total of 10 bacterial stains were isolated from rhizospheric soil collected from rhizosphere region .out of 10 isolates 2 were observed to be IAA producers. *Rhizobium* was only isolate capable of producing high concentration (170 µg/100 ml) and *Serratia*, sp produced about (130 µg/100 ml) which is lower than the *Rhizobium* strains by utilizing L-Tryptophan as a precursor. IAA production was analyzed and confirmed by FTIR. The isolated strains from rhizospheric soil is used for agriculture used as plant growth promoting rhizobacteria and to increase the awareness about chemical fertilizer-based agriculture product or practice. These rhizobacteria are used to promote the growth to the salinity affected plant in the salinity stress area because the salinity stress limit the crop production, IAA producing rhizobacteria are used to enhance the growth of plants. In this present study the pot experiment don with use of *rhizobium* sp and *Serratia* sp., the production of IAA in various salt concentration was higher in *rhizobium* sp. compare to *Serratia* sp. from this observation.

References

1. Ashan ML, Ali A, Ahmed L. Bio-fertilizer: A highly potent alternative to chemical fertilizers; uses and future prospects. J Chem. Eng. Biol. Sci. 2012;6(4):10-23.
2. Basu PS, Ghosh AC. Production of indole acetic acid in culture by *Rhizobium* species from root nodules of a monocotyledonous tree, *Roystonea regia*. Acta Biotechnol. 2001;21:65-72.
3. Basu PS, Ghosh AC. Production of extracellular polysaccharides by *Rhizobium* sp. of *Roystonea regia*, the

only root nodule forming monocotyledon. Indian J Exp. Biol. 1999;37:487-490.
4. Datta C, Basu PS. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol Res. 2000;155:123-127.
5. FAO. 2005. [Online]. Available: http://www.faostat.org.
6. FAO. High-level expert forum – how to feed the world in 2050. Economic and Social Development, Food and Agricultural Organization of the United Nations, Rome, Italy; c2009.
7. Finnie JF, van Staden J. Effect of seed weed concentrate and applied hormones on *in vitro* cultured tomato roots. J Plant Physiol. 1985;120:215-222.
8. Girdhar IK, Bhalodia PK, Miara JB, Girdhar V, Dayal D. Performance of groundnut, *Arachis hypogaea* L., as influenced by soil salinity and saline water irrigation in black clay soils. J Oilseeds Res. 2005;22(1):183-187.
9. Glick BR, Patten CL, Holguin G, Penrose DM. Biochemical and genetic mechanisms used by plant growth-promoting bacteria. Imperial College Press, London, UK; c1999.
10. Glick BR. The enhancement of plant growth by free-living bacteria. Can J Microbiol. 1995;41:109-117.
11. Lwin KM, Myint MM, Tar T, Aung WZM. Isolation of plant hormone (indole-3-acetic acid-IAA) producing rhizobacteria and study on their effects on maize seedlings. Eng. J. 2012;16(5):137-144.
12. Kloepper JW, Lifshitz R, Zablotowicz RM. Free-living bacterial Inocula for enhancing crop productivity. Trends Biotechnol. 1989;7:39-44.
13. Lynch JM. Origin, nature, and biological activity of aliphatic substances and growth hormones found in soil. In: Vaughan D, Malcolm RE, editors. Soil Organic Matter and Biological Activity. Martinus Wijhoff by Dr. W. Junk Publishers, Dordrecht, Boston, Lancaster; c1985. p. 151-174.
14. Brandl M, Clark EM, Lindow SE. Characterization of the indole acetic acid (IAA) biosynthetic pathway in an epiphytic strain of *Erwinia herbicola* and IAA production *in vitro*. Can. J Microbiol. 1996;42:586-592.
15. MacFaddin JF. Biochemical tests for identification of medical bacteria. Williams and Wilkins, London; c2000.
16. Munns R, Tester M. Mechanisms of salinity tolerance. Ann Rev Plant Biol. 2008;59:651-681.
17. Nithila S, Durga DD, Velu G, Amutha R, Rangaraju G. Physiological evaluation of groundnut (*Arachis hypogaea* L.) varieties for salt tolerance and amelioration for salt stress. Res J Agric Forestry Sci. 2013;1(11):1-8.
18. Patten CL, Glick BR. Role of *Pseudomonas putida* indole-3-acetic acid in development of the host plant root system. Appl Environ Microbiol. 2002;68:3795-3801.
19. Rengasamy P. Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: An overview. Aust J Exp Agr. 2002;42:351-361. doi:10.1071/EA01111.
20. Rifat Hayat, Safdar Ali, Ummay Amara, Rabia Khalid, Iftikhar Ahmed. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann. Microbiol. 2010;60:579-598.
21. Sarwar M, Kremer RJ. Determination of bacterially derived auxins using a microplate method. Lett Appl Microbiol. 1992;20:282-285.
22. Somasegaran P, Hoben HJ. Handbook for Rhizobia: Methods in Legume-*Rhizobium* Technology. New York: Springer-Verlag; 1994.
23. Stein A, Fortin JA, Vallee G. Enhanced rooting of *Picea mariana* cuttings by ectomycorrhizal fungi. Can J Bot. 1990;68:492-498.
24. Sudha M, Shyamala GR, Prbhavati P, Astapritya P, Yamuna Devi Y, Saranya A. Production and optimization of indole acetic acid by indigenous microflora using agro waste as substrate. Pak. J Biol. Sci. 2012;15:39-43.