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KITCHEN WASTE USED FOR POTASSIUM SOLUBILISING BIOFERTILISERS

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Abstract: Potassium (K) is the third major essential macronutrient for plant growth. Potassium helps to grow healthy plants by promoting green brawny stems on deep roots. It helps strengthen plants' abilities to resist disease and plays a significant role in increasing crop yields and overall quality. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble forms. It's necessitating for enzyme activation within the plant, which affects protein, starch and adenosine triphosphate (ATP) production. The use of plant growth promoting Rhizobacteria (PGPR), including phosphate solubilizing and potassium mobilizing bacteria as biofertilizers, was suggested as a sustainable solution to improve plant nutrient and production. Potassium solubilizing bacteria (KSB)such as *Pseudomonas*, which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates. Rhizosphere bacteria have been found to dissolve potassium from insoluble K- bearing kitchen waste. In this study, bacterial isolate was obtained from Rhizosphere by modification with Aleksandrov medium containing kitchen waste such as banana waste, ginger peel and else potassium source. The *Pseudomonas* can be easily immobilized into carrier like peat soil, charcoal, sawdust and talcum powder. The carrier mixed culture was used to check its efficiency and effectiveness and it showed that the level of K was increased by using this modification. Significant differences in the root length and shoot length of chick pea plants was observed at 15 days of plant growth due to various inoculation treatments and fertilizer application. The treatment receiving inoculation of *pseudomonas* isolate recorded maximum root length (28 cm) and (27 cm) in babna peel and ginger peel respectively whereas shoot length (33.16cm) and (30.75cm) in banana peel and ginger peel respectively. Which were significantly superior over the all other inoculated treatments and the absolute control.

Index Terms **- PGPR, Potassium solubilizing Bacteria, chick pea plant, Kitchen Waste**

1. INTRODUCTION

India being basically an agricultural country; one third population depends on agriculture sector directly or indirectly. Agriculture continues to be the main part of the Indian economy. Indian agriculture contributes to the national Gross Domestic Product about 25 per cent.

The fertilizers can be categorized into three types depends on the production process: chemical, organic and biofertilizer. Using of chemical fertilizer or organic fertilizer has its merits and demerits in the context of nutrient supply, crop growth and environmental quality. The advantage need to be consolidated in order to make maximum use of each type of fertilizer and achieve balanced nutrient management for crop growth (Jen-Hshuan, 2006). Soil microbes have great importance in cycling nutrients such as carbon (C),nitrogen (N), phosphorus (P), and sulphur (S). Not only do they control the forms of these elements (e.g. specialized soil bacteria convert ammonium N (NH_4^+) to nitrate(NO_3^-) they can regulate the quantities of N available to plants. Aside from their effects on the availability of nutrients the bacterial soil life prevents the assimilation of several harmful ions. The use of living bacteria (biofertilizer) stimulates mineralization of organic residues in soil, therefore makes the nutrients more available. At the same time due to effect of living bacteria from biofertilizer, the uptake of heavy metals decreases (Lévai *et al*, 2008). Recent study have proved that the used ofbiofertilizer by combining 25% of chemical fertilizer bring a good result for plants'growth in long term period (Kramany et al., 2007).

Biofertilizer is known as a substance which having living microorganisms who help with expansion of the root system and better seed germination. The microorganisms containing biofertilizers can be the device we could change apply of chemical fertilizers. Biofertilizers are products having living cells of different types of microorganism, which have an ability to convert nutritionally important elements to available form by biological processes. In recent years, biofertilizers have developed as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies (Marianna *et al*, 2005). It is a great interest in creating novel associations between higher plants and various N2-fixing microorganisms (Al-Khiat, 2006).

Biofertilizers include mainly the nitrogen fixing, phosphate solubilizing, potassium solubilizing and plant growth-promoting microorganisms. Amidst, biofertilizers profiting the crop production are *Azotobacter*, *Azospirillum*, *blue green algae*, *Azolla*, *P-solubilizingmicroorganisms*, *mycorrhizae* and *sinorhizobium* (Selvakumar *et al*, 2009). For examples, Nitrogen-fixer such as *Azotobactor chroococcum* can supply nitrogen by mending the nitrogen from atmosphere and convert the nitrogen into ammonium ion for plants'uptake. Besides, *Basillus megaterium* is one of the phosphorus solubilizer that apply in biofertilizer to solubilize phosphorus soil and rock in form of phosphate ion. Then, KSB is a reason *Basillus mucilaginosus* to solubilize potassium rock and can stimulate plant growth through synthesis of growth promoting substance.

The need for dependency on fertilisers arises from the geographical distribution of potassium across Indian agricultural soils. As for as potassium fertilizer is concerned, India depends entirely upon imports from foreign countries and the demand for potassium fertilizer is increasing about 8.5% per annum. In the year 2006, India will have to spend about 40,480 cores of rupees for importing estimated demand of four million tonnes of potash. Indian soils have sufficient quantity of potassium to support crops raised in it, but ironically, this potassium content in soil is in unavailable form to plants. Identifying a suitable solution to convert this unavailable form of potassium (accounts for 90 to 98% of total potassium in all soils) in to available form can resolve this problem and will bolster balanced nutrition and sustainable soil health. Potassium solublizing bacteria would be a novel solution to convert insoluble form of soil potassium in to soluble (available to plant) form. While China is already exploiting the potentials of potassium solubilizing bacteria as biofertilizer, in our country no such efforts are taken.

While potassium solubilizing bacteria 2% of soils cultivated in India definitely require external potassium fertilizer supplementation (Rehanul Hasan 2002; Ramamurthy and Bajaj, 1969). While soils in India contain about 90 to 98 % of total potassium in insoluble forms, short fall in soluble forms of potassium application makes poor yield, decrease in quality of the products and deterioration of soil fertility due to lack of balanced fertilizer application (N:P:K application). Unavailable form of potassium can be converted to available form by utilizing the potential of "Potassium Solublizing Bacteria". As parent material for soils are different from each other, hence, potassium solubilizing bacteria may also differ based upon parent materials of soils. Thus it becomes absolute necessary to screen different soil types for potassium solublizing bacteria.

potassium solubilizing bacteria (KSB) are able to solubilize primary K minerals, such as mica, illite and orthoclases, through production and of organic acids into available form. A combine of application of rock P and K materials with co-inoculation of bacteria which solubilize them might give a faster and continuous supply of P and K for optimal plant growth (Han and Lee, 2005). Using of plant growth promoting rhizobacteria (PGPR), including K solubilizing and potassium mobilized bacteria as biofertilizers, was suggested as a feasible solution to improvement of plant nutrient and production (Vessey 2003).

2. MATERIAL AND METHODS

2.1. Isolation of potassium solubilizing bacteria

2.1.1. Collection of soil samples: The rhizosphere soil of different crops plants was collected in the areas of agronomy farm, collage of agriculture, pune. The samples were brought in polythene bag.

2.1.2. Isolation and purification of potassium solubilizers: Potassium solubilizing bacteria were isolated from collected soil samples by serial dilution plate count method using Aleksandrov medium (Hu *et al.*, 2006) which is a selective medium for isolation of potassium solubilizers. Agar medium containing insoluble potassium bearing kitchen waste (banana peel, waste of ginger). The plates were incubated at room temperature (30±1°C) for 3 days and the colonies exhibiting clear zones were selected, purified by four-way streak plate method. The zone of solubilization was measured and the selected isolates were preserved on agar slants for further use

2.2. Identification and characterization of the bacterial isolate

2.2.1. General characterization: The selected isolate was examined for the colony morphology, cell shape, gram reaction and ability to form spores as per the standard procedures given by Anonymous (1957) and Barthalomew and Mittewer (1950).

2.2.2. Biochemical characterization: The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992).

2.3. Quantitative estimation of K released from insoluble K bearing kitchen waste

The isolates showing zone of solubilization on Aleksandrov agar were further examined for their ability to release K from broth medium (supplemented with 1% kitchen waste). One ml of overnight culture of each isolate was inoculated to 25 ml of Aleksandrov broth (Hu *et al.*,2006) in three replicates. All the inoculated flasks were incubated for two weeks at 28±2°C. The amount of K released in the broth was estimated at 7, 15, and 20 days of incubation from triplicates flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 minutes in the remi microcentrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry (Sugumaran and Janarthanam, 2007). One ml of the culture supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with the distilled water and mixed thoroughly. After that solution fed to flame photometer using standards. Simultaneously, a standard curve was prepared using various concentrations from 2 ppm KCl solution. The amount of potassium solubilized by the isolates was calculated from the standard curve of potassium Chloride .

2.4. Preparation of biofertilizers

The production of carrier based potassium solublising biofertilizers involves five stages-

- Culturing of microorganisms- starter culture and inoculum production
- Processing of carrier material
- Mixing the carrier and broth culture
- Packing
- Proper storage

2.5. Pot Experiment

A pot culture experiment was conducted using potassium solubilizing biofertilizer, to study their performance in enhancing the growth K uptake and yield of chick pea plants as detailed below.

2.5.1. Treatments: The treatments fixed for pot culture experiment presented in Table 2.1 were used to record observation on plant growth parameters for chick pea plant after 15th days of plant growth.

Table 2.1: Details of the treatments used for pot culture experiment

Sr no.	Treatment (for banana peel)	(for waste of ginger)
T_1	control	control
T_2	Biofertilise r + Peat soil	Biofertilise r + Peat soil
T_3	Biofertiliser + Charcoal	Biofertiliser + Charcoal
T_4	Biofertiliser + Shaw dust	Biofertiliser + Shaw dust
T_5	Biofertiliser + Telco am	Biofertiliser + Telco am
T_6	Biofertiliser + peat soil + rhizobium	Biofertiliser + peat soil + rhizobium
T_7	0.1 gm b.f + 0.62gm seed	0.1 gm b.f + 0.66gm seed
T_8	0.5 gm b.f + 0.57 gm seed	0.5 gm b.f + 0.67 gm seed
T_9	1 gm b.f + 0.61 gm seed	1 gm b.f + 0.67 gm seed
T_{10}	2 gm b.f + 0.45 gm seed	2 gm b.f + 0.66 gm seed

2.5.2. Inoculation of the Seeds: The seeds which are used for pot culture, they are first rolling in the accasia gum slurry(40% accasia, is used as a adhesive agent and not toxic to the biofertiliser) to properly coating of biofertiliser to seeds. Then seeds were showing in pot. About 4 seedsper plant were used. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pests and diseases.

2.5.3. Observations: Observations on plant growth parameters were recorded at 15 days after sowing, where as growth, yield and yield parameters were recorded. The Growth Parameters The next parameters, root height (cm), number of branches, shoot height (cm), numbers of leaves were measured.

3. RESULTS AND DISCUSSION

In this study attempts were made to isolate potassium solubilizing bacteria from rhizosphere plants. The isolate was examined for their ability to solubilize insoluble potassiuml. The selected isolate was characterized and tentatively identified upto genus level based on morphological and biochemical properties. The efficient K soluiblizers was further subjected for their ability to release K from potassic kitchen waste, mechanisms involved in K solubilization. Highly efficient K solubilizing strains were also tested for their influence on growth and nutrient uptake of chick pea plant under pot culture conditions. The results obtained in these studies are presented here under.

3.1. Isolation of potassium soluiblizing bacteria (ksb) from rhizosphere soil

The rhizosphere soil sample was collected and used for the isolation of KSB. The Isolate was purified, identified and maintained for further use.

3.2. Identification of ksb isolates

The selected isolate of KSB was identified upto genus level based on their morphological and biochemical characters and the results are presented in Table 3.1 The isolate was gram negative rod belongs to genera ***Pseudomonas***.

3.2.1. General Characterization

3.2.1.1. Colony morphology : The colony characteristics were studied for the isolate and recorded (Table 8a). The isolate developed Creamy, smooth, raised, small colonies.

3.2.1.2. Gram Staining and Microscopic examination: The microscopic observation of the gram stained slides clearly indicated the presence of Gram positive and Gram negative organisms. The isolate was gram negative pink colored rod shaped (Fig: 3.1).

3.2.1.3. Test for Sporulation : The spore forming isolate produced endospores that stained green and those which did not produce spores stained red indicating the absence of spores. The isolate stained red indicating the absence of spores (Fig: 3.2).

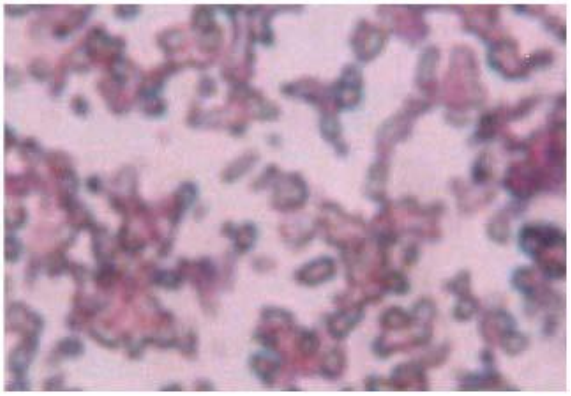


Fig: 3.1 Gram staining of Phosphate (K) solubilizing isolate

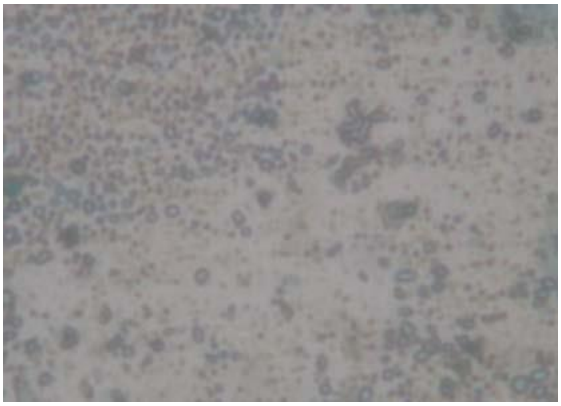


Fig: 3.2 Spore staining of Phosphate (K) solubilizing isolate

3.2.2. Characterization of potassium solubilizers:

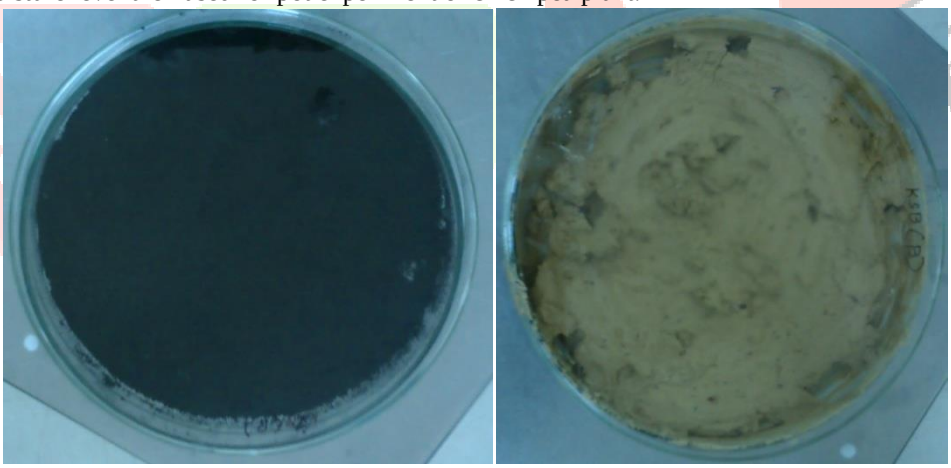
The biochemical characterization of the isolates was carried out and results were detailed as below (Table: 3.1):

Table: 3.1 Morphological and biochemical characteristics of the potassium solubilizers (+) =positive; (-) =negative.

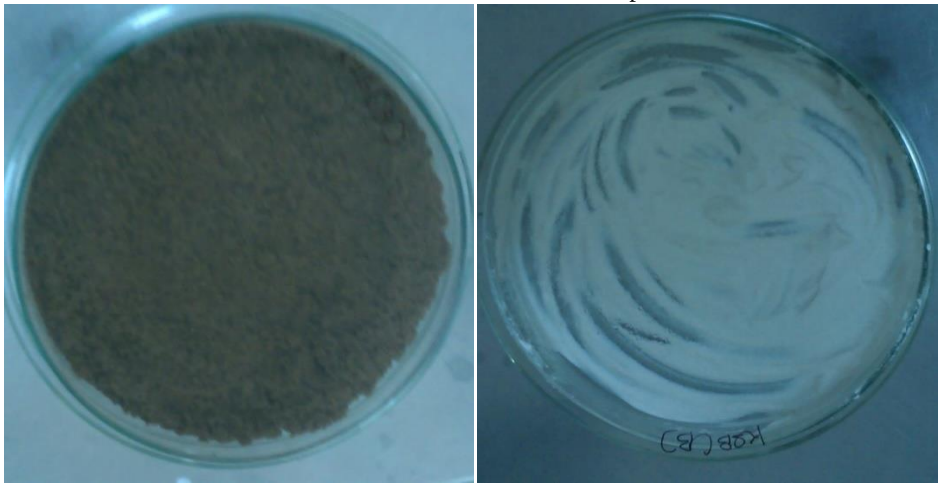
General Characterization	Colony Characteristics	Creamy, smooth, raised, small
	Gram reaction & cell shape	-Ve, rod
	Spore formation	+
biochemical characteristics	starch hydrolysis	-
	Acid production	+
	catalase test	-
	citrate utilisation test	+
	H_2S production test	-
	Indol production test	-
	Urease test	-
	Gelatin liquification test	+
	Oxidase test	+
	MR-VP test	-
Carbon source utilization	Glucose	+
	Sucrose	+
	Lactose	+
Probable genus	***Pseudomonas***	

3.3. Preparation of biofertiliser:

For the production of carrier based potassium solubilising biofertiliser, the inoculums size was about 2.48 x 10^{10} cfu/ml ,it is measured by spread plate colony count method. After that broth was mixing with different carrier material such as charcoal, peat soil, telcoampowder and shaw dust which are low cost and easly available, in 1:1 ratio and left it for 2 -3 days for curing and having 40 – 50 % moisture level then used for pot experiment of chick pea plant.



Charcoal + ksb peat soil + ksb



Shaw dust + ksb telcoam powder + ksb

Fig: 3.3 Curing of carrier mixed with biofertiliser(ksb)

3.4. Pot Experiment:

To study the effect of inoculation isolate of K solubilizing bacteria on growth, yield and K uptake of chick pea plants and wheat palnt , a pot culture experiment was conducted and the results recorded at 15 DAS and at harvest are presented in Table 3.2.

3.4.1. Root length

Significant differences in the root length of chick pea plants was observed at 15 days of plant growth due to various inoculation treatments and fertilizer application (Table 3.2). The treatment receiving inoculation of *pseudomonas* isolate recorded maximum root length (28 cm) and (27 cm) in babna peel and ginger peel respectively. However, all the other inoculated treatments showed significant increase in root growth over absolute control.

3.4.2. Shoot length

All the treatment receiving inoculation of bacteria increased the shoot length of chick pea plants significantly over absolute control. Among the inoculated treatments ,the maximum shoot length of chick pea plant at 15 days of growth (33.16cm) and (30.75cm) in banana peel and ginger peel respectively. which were significantly superior over the all other inoculated treatments and the absolute control.

3.4.3. Number of leaves

Among the inoculation treatments highest number of leaves was recorded with Treatment of T_2 (64.75leaves/plant) and treatment T_2 (82.25 leaves/plant) in Banana peel and ginger peel respectively. No significant differences existed between the levels of K with respect to number of leaves per plant.

3.4.4. Number of branch

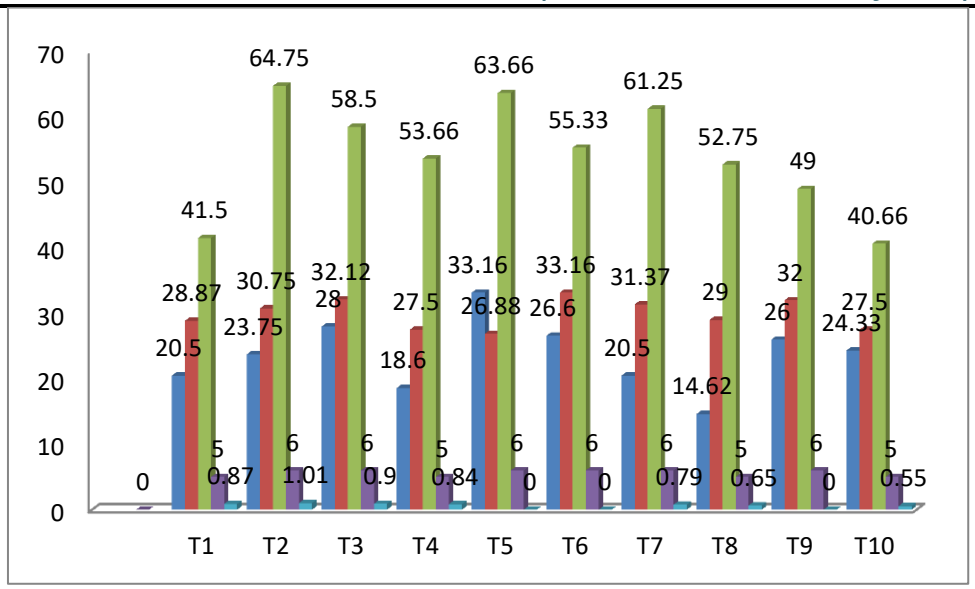
Among the inoculation treatments highest numbers of branches were recorded but no more differences were observed. Mostly the numbers of branch were 5 to 6 in chick pea plant with ksb biofertiliser.

No significant differences existed between the levels of K with respect to number of leaves per plant.

Fig: 3.4 General view of pot culture experiment

Table: 3.2 Effect of efficient potassium solubilizing bacteria (KSB) on growth of chick pea plant at 15 DAYS (For Banana peel).

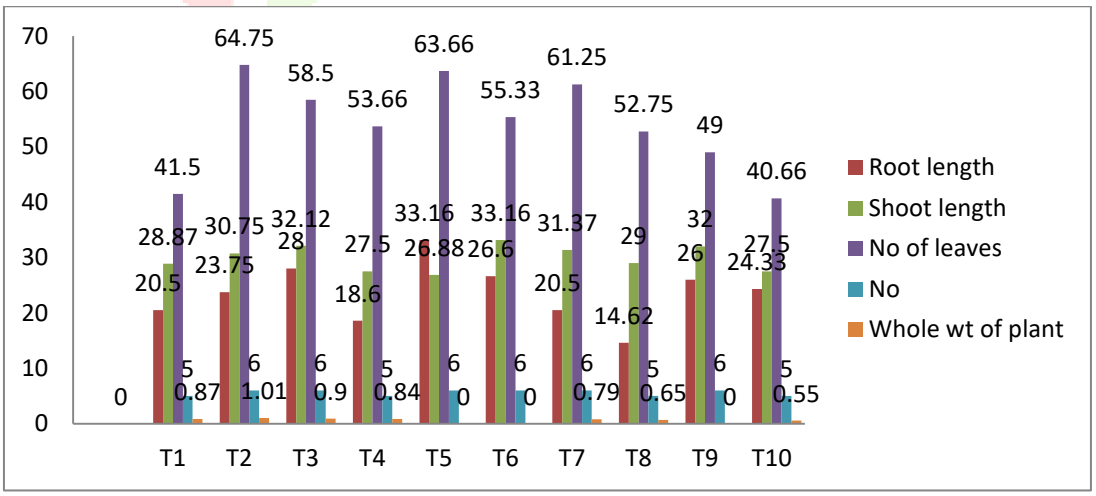
Sr no.	Treatment (for banana peel)	Root length	Shoot length	No of leaves	No of branch	Whole wt of plant
T_1	Control	20.5	28.87	41.5	5	0.87
T_2	Biofertiliser + Peat soil	23.75	30.75	64.75	6	1.01
T_3	Biofertiliser + Charcoal	28	32.12	58.5	6	0.90
T_4	Biofertiliser + Shaw dust	18.6	27.5	53.66	5	0.84
T_5	Biofertiliser + Telco am powder	33.16	26.88	63.66	6	o.86
T_6	Biofertiliser + peat soil + rhizobium	26.6	33.16	55.33	6	o.87
T_7	0.1 gm b.f + 0.62gm seed	20.5	31.37	61.25	6	0.79
T_8	0.5 gm b.f + 0.57 gm seed	14.62	29	52.75	5	0.65
T_9	1 gm b.f + 0.61 gm seed	26	32	49	6	o.45
T_{10}	2 gm b.f + 0.45 gm seed	24.33	27.5	40.66	5	0.55



Graph: 3.1 Effect of efficient potassium solubilizing bacteria (KSB) on growth of chick pea plant at 15 DAYS (For Banana peel).

Table: 3.3 Effect of efficient potassium solubilizing bacteria (KSB) on growth of chick pea plant at 15 DAS (For ginger peel).

Sr no.	Treatment (for ginger peel)	Root length	Shoot length	No of leaves	No of branch	Whole wt of plant
T_1	control	20	31.5	72.25	5	1.31
T_2	Biofertiliser + Peat soil	24.37	30	82.25	3	1.16
T_3	Biofertiliser + Charcoal	25.12	32.75	59	6	1.035
T_4	Biofertiliser + Shaw dust	27	30	73	6	1.00
T_5	Biofertiliser + Telcoam powder	25.5	30.75	81.25	6	1.06
T_6	Biofertiliser + peat soil + rhizobium	14.75	29.37	59	6	1.27
T_7	0.1 gm b.f + 0.62gm seed	28	28	54.5	6	0.84
T_8	0.5 gm b.f + 0.57 gm seed	10.5	28.6	67.6	5	0.81
T_9	1 gm b.f + 0.61 gm seed	9.5	27.5	54.5	5	0.79
T_{10}	2 gm b.f + 0.45 gm seed	15	27	50	4	0.47



Graph: 3.2. Effect of efficient potassium solubilizing bacteria (KSB) on growth of chick pea plant at 15 DAYS(For ginger peel).

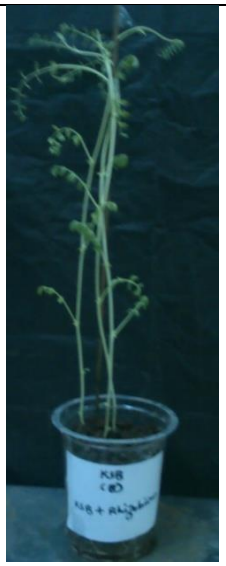
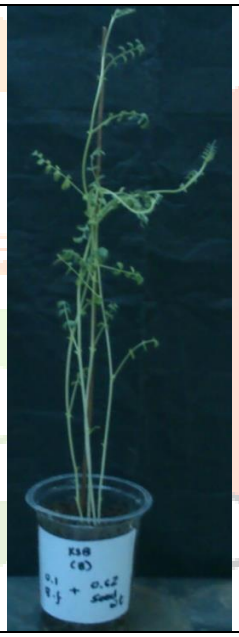
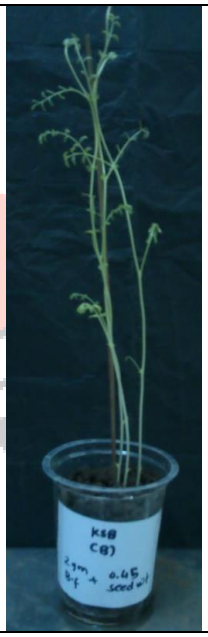
				
T1	T2	T3	T4	T5
control (No inoculation, no fertilizer K)	Biofertiliser + Peat soil	Biofertiliser + Charcoal	Biofertiliser + Shaw dust	Biofertiliser + Telcoam powder
				
T6	T7	T8	T9	T10
Biofertiliser + peat soil + rhizobium	0.1 gm b.f	0.5 gm b.f	1 gm b.f	2 gm b.f

Fig: 3.5 Effect of KSB on plant growth at 55 DAYS

Conclusion:

In present study, bacterial isolate was obtained from rhizosphere on modified Aleksandrov medium containing kitchen waste such as banana waste, and ginger peel and potassium source. Bacterial strain, among culture tested, showed significant potassium solubilization. The *Pseudomonas* can be easily immobilized into carrier like peat soil, charcoal, sawdust and talcum powder. The carrier mixed culture was used to check its efficiency and effectiveness and it showed that the level of K was increased by using this modification. Significant differences in the root length and shoot length of chick pea plants were observed at 15 days of plant growth due to various inoculation treatments and fertilizer application.

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