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Response of Sweet Potato to Application of Pgpr and N Fertilizer

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ABSTRACT

This study was conducted to evaluate the response of sweet potatogrowth and yield to theapplication of PGPR and Nitrogen fertilizer**.**The field experiment was established under a design of Randomized Complete Block Designwith 3 replications.The inoculation for this study was comprised of 4 treatmentsand a control (*Klebsiella* sp. UPMSP9, *Erwinia* sp. UPMSP10,*Azospirillumbrasilense* SP7, *Bacillus sphaericus* UPMB10 and Uninoculated control) combinations with 3 levels of N fertilizer (0, 33, and 100 kg N ha^{-1}).The results indicated that the inoculation of the *Klebsiella* and supplied with 33kg Nha^{-1}increased sweet potato yield significantly compared to control. Similarly, the application of the bacterium *Klebsiella* and supplied with 33kg Nha^{-1} recorded higher uptake of N, P and K compared to control. Soil P, K, Ca and Mg Concentrations were higher with PGPR and N application of 33kg N ha^{-1} compared with the 100kg N ha^{-1}. The concentrations of IAA in soils inoculated with rhizobacterial isolates were significantly higher than uninoculated control. Highest IAA was observed with *Klebsiella* inoculation at33kg Nha^{-1} fertilization rate. These findings showed that PGPR could be a potential inoculant at a reduced rate of N fertilizer for sweet potato production.

Keywords: Sweet potato, Indole-acetic acid, *Klebsiella* sp., *Erwinia* sp., *Azospirillumbrasilense, Bacillus sphaericus,*Nitrogen fertilizer,

INTRODUCTION

Sweet potato (*Ipomoea batatas L*.) is an important root crop worldwide and is considered as a main source of food. It is the most promising food commodity around the world as it is ranked as the seventh most important food crop after rice and maize(FAO, 2009). Sweet potato is also considered to have a very high amount of carbohydrate which serves as a great source of energy. Besides being a wide range of food source, many developed countries have utilized sweet potato as raw material for various industries including fermentation, textiles and cosmetics (Vosawai *et al*., 2015).In Malaysia, many areas in the states of Terengganu, Perak and Kelantan are involved in sweet potato production and it is known as the second highest tuber crop producer after cassava (Hanim*et al*., 2014).

Generally, sweet potatorequires high amount of fertilizer for commercial cultivation which can lead to increased production cost and environmental pollution due to the over usage of chemical fertilizers. This indirectly contributes to the negative impact on the sustainability of the ecosystem. Biofertilizer is globally popular as an alternative source

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of chemical fertilizer which improves plant growth through increased uptake of water and mineral nutrients(Saravanakumar*et al*., 2007, Umair*et al*., 2018). In the environment, plants and bacteria has a natural relationship existence that plays a vital role in the growth and the health of plants (Abdisa*et al*., 2012).

Plant growth promotingRhizobacteria (PGPR) is used as biofertilizer and bioenhancer for different crops as an alternative source of chemical fertilizer.PGPRencompasses all microorganism that inhabit and colonize plant roots and exert positive effects on plant improvement by various mechanisms, ranging from direct growth promotion, such as increased solubilization and uptake of nutrients or production of plant growth regulators, to indirect growth promotion, such as pathogen suppression in biological control and production of phytohormones(Dey*et al*., 2004, Naseri*et al*., 2013).*Azospirillum* and other groups of PGPR bacteria have been found to produce and release a broad spectrum of plant growth regulators, such as auxin, gibberellin and cytokinin. Among these indole-3-acetic acid (IAA) is considered as the most physiologically active auxin in plants and involved on diverse plant growth and IAA is well known to stimulate both rapid responses (e.g. increases in cell elongation) and long term (e.g. cell-division and differentiation) in plants (Kumar *et al*., 2002, Glick, 2012). Eighty percent of microorganisms isolated from the rhizosphere of various crops have the ability to produce auxins as secondary metabolites which help in stimulating plant growth (Adesemoye*et al*., 2008).Nutrients are required for plant growth similarly sweetpotato requires high amounts of nutrients especially nitrogen. Nitrogen is one of the most abundant elements in plants andanimals, as it is a major component of proteins. it is also a highly demanding key element for sweetpotato and other root crop yield (Canbolat*et al*., 2006, Adeyeye*et al.*, 2016).

Thereare environmental factors under field conditions that can affect the activities of PGPR. Theconcentration of IAA in soildepends on bacterial population.The variations in bacterial population due to rapid wetting and drying of soil, rainfall distribution, temperature variations, pest and disease could affect the performance of PGPR (Beneduzi*et al.,* 2012).However, the survival and performance of these bacteria in the presence of added fertilizers under normal agronomic cultural condition need to be evaluated.Therefore, thefield studieswereconducted to evaluateperformance of the PGPR with different levels of nitrogen fertilizer on growth and yield of sweet potato.

MATERIALS AND METHODS

The field experiment was conducted at UPM experimental plot. The experiment was comprised of 5treatments(*Klebsiella* sp. UPMSP9*, Erwinia* sp. UPMSP10,*Azospirillumbrasilense* SP7*, Bacillus sphaericus* UPMB10 and Uninoculated control) combinations with three levels of N fertilizer (0, 33, and 100 kg N ha^{-1}). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The cuttings of sweetpotatoshoot were inoculated with four rhizobacterial isolatesas per treatment. The cuttings without inoculation were used as control.The cuttings were planted slanting with 1/3 buried in the soil. The soil was covered with plastic mulch to control weeds, combat insects, prevent soil loss during heavy rains and maintain soil moisture. The interspaces between planting rows were weeded manually

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when necessary. The crop was irrigated regularly. Each plant was inoculated with the respective inoculum at planting and one month after planting with 20 mL inoculum per plant (approximately 10^9 CFU mL^{-1}).The plants were harvested after4 months of planting. Driedshoot samples were digested with concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) according to micro-kjeldahl method (Thomas *et al.,* 1967).N, P, K concentrations were determined using autoanalyzer (Technicon II, Technicon Ltd.) and Ca, Mg by using Atomic Absorption Spectrophotometer(Perkin-Elmer, 5100 PC, Perkin Elmer).

Leaf chlorophyll content was measured based on SPAD value of youngest fully expanded leaf using a chlorophyll meter The chlorophyll content was determined using the acetone extraction method as described by Coombs *et al*., (1985).Fresh soil samples were collectedfor soil pH and nutrient analysis (N, P, K, Ca, Mg, IAA). Soil pH was measured with a glass electrode pH meter (PHM 210, Metrolab) in a 1:2.5 soil-water suspension. The total nitrogen was determined following the micro-Kjeldahl method (Thomas *et al.*, 1967). The available phosphorus was measured by Bray-2 method (Bray and Kurtz, 1945).Total N and the available P were analyzed by autoanalyzer. Concentrations of exchangeable K, Ca and Mg were determined using the shaking method (Schollenberger and Simon, 1945) and their concentrations were measured using Atomic Absorption Spectrophotometer. A modified method of Sarwar*et al*., (1992) wasused to determine the concentrations of IAA in soil.

Data wereanalyzedbyStatistical Analysis System (SAS,1989).Differences among treatment means were determinedusing Tukey'sStudentized Rangetest (HSD) comparison method at p=0.05.

RESULTS AND DISCUSSION

Sweet potato growth parameters

There was a significant response of PGPR inoculation, N fertilizationand interaction of both factors on sweetpotatoyield and shoot-storage root ratio (Table 1).Preciselysweetpotatoyield and shoot-storage root ratio increased with increasing N rates andinoculated plants showed higher sweetpotatoyield and shoot -storage root ratio compared to the uninoculated control. The action of PGPR has improved the plant growth and yield by altering the root architecture and converting nutrients from unavailable to available form through various biological processes(Radziah and Zulkifli, 2003).Nitrogen is known to be the most essential components for plant growth. Nitrogen is present in a high amount in the atmosphere, approximately 78% but it remains unavailable to plants as there is no plants which are available to convert atmospheric dinitrogen into ammonia and to be used directly for plant growth and development. Nitrogen starvation in plant resulted in loss of vegetative vigor and depletion of growth hormone. Growth hormone concentration varies with nitrogen supply and growth vigor(Koodi *et al*., 2017). In general, low concentration of hormone is required for plant growth. Studies have found that application of higher N usually showed reduction in growth and yields of sweetpotato. Thus, reduction of N by 1/3 normal N rate to inoculated plant gave

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comparable yields with the full fertilizer application consequently presenting a 67% saving in N fertilizer (Vosawai *et al*., 2015).

Only nitrogen fertilization rates significantly (P<0.05) influenced the leaf chlorophyll content. The highest chlorophyll content was observed at plants applied with 33kg Nha^{-1} (Table 1).Thus, application of N increased the available N for increased chlorophyll content of leaves which is important in photosynthesis.Kalam*et al*.,2017, had suggested inoculation of wheat plants with biofertilizers, *Bacillus polymyxa* or *Azospirillumbrasilinse*as that produced auxin significantly increased the chlorophyllas compared with uninoculatedtreatment. The rhizobacterial inoculation has been proven to directly and indirectly stimulate the plant growth. The indirect ways include triggering the systemic resistance in plants to combat broad spectrum plant pathogen**(**Khalid *et al*., 2004,Vurukonda*et al*., 2016).

Table 1: Rhizobacterial inoculation and N fertilization effects onchlorophyll contentof shoot, sweet potato yield and shoot to storage rootratio

Treatments		**mg Clp/mg LFW**	**Sweet potato yield ($t\ ha^{-1}$)**	**Shoot/storage rootratio (S/R)**
Bacterial Isolates	**N Fertilizer ($N\ ha^{-1}$)**			
Control	0 kg	0.009d	4.41g	2.86c
	33kg	0.014ab	12.70b	3.10bc
	100kg	0.012bcd	8.32cde	3.90a
***Klebsiella*sp**	0 kg	0.010cd	8.97cd	3.70ab
	33kg	0.015a	17.69a	3.88a
	100kg	0.013ab	16.27a	3.79a
***Erwinia* sp.**	0 kg	0.009d	7.97def	3.70ab
	33kg	0.014ab	14.13b	3.94a
	100kg	0.014ab	13.55b	3.43abc
***Azospirillum*sp.**	0 kg	0.010cd	6.31f	3.38abc
	33kg	0.015a	13.68b	3.72 ab
	100kg	0.013ab	9.76c	3.86 a
***Bacillus*sp**	0 kg	0.010cd	7.09ef	3.60 ab
	33kg	0.015ab	13.34b	3.66 ab

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	100kg	0.013ab	9.26cd	3.79 a
Significance due to PGPR.		NS	*	*
N Fert		*	*	*
PGPR * N Fert		NS	*	*

Note: *Significant (P<0.05), Means in column followed with same letter (s) are not significantly different (P>0.05). mg Clp/ mg LFW: mg chlorophyll / mg leaf fresh weight

Nutrient uptake in shoots

PGPR inoculation and N fertilization rate significantly (P<0.05) influenced the shoot nutrient uptake (Table 2). Plants inoculated with*Klebsiella* and supplied with33kg Nha^{-1} recorded higher uptakeof N, P and K compared to control. There were also significant interaction effects of PGPR inoculation and N fertilizer onN, P, K, Ca and Mg uptake. The increased plant growth could have increased the uptake of minerals and increased the content of nutrients in plant.

Researchers have found that the inoculation with PGPR can increase the yield and N,P and K uptake in non-leguminous crops (Baset*et al.,*2010, Souza *et al*.,2015).Combined inoculation of *Azospirillumbrasilense* and thephosphate–solubilizing bacteria *Pseudomonas strica* or *Bacillus polymyxa* on field grown sorghum significantly increased grain and dry matter yields and N and P uptake as compared with single inoculation of individual organisms.Beneficial effects of PGPR can affect the mineral nutrition of plants by changing root uptake characteristics of different crop plants (Okon and Itzigsohn, 1995, Farzana*et al*., 2020). Inoculation of PGPR may improve sweetpotato plant growth through production of growth promoting substances and enhanced mineral uptake by the roots (Umesh*.,*2014).

One of the important phytohormones synthesized by PGPR is indole acetic acid (IAA) which is considered to be the most physiologically active auxin in plants. IAA induce plant growth by stimulating cell division and differentiation and increases root hair for higher nutrient uptake (Richardson *et al*., 2009 and Farzana*et al*., 2017). The inoculation with PGPR can increase the yield and N, P, K content in plants by changing root uptake characteristics of different crop plants (Dinesh *et al*.,2015).

Vegetable crops like tomato, cucumber and pepperinoculated with various strains of PGPR having IAA producing ability showed significantly increased in growth parameter(Kidoglu*et al.,* 2007). In case of rice crop IAA producing PGPR confirmed promising effects on plant growth and N,P, K uptake in plants (Ahemad and Kibret., 2014).

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Table 2: The effect of Rhizobacterial inoculation and N fertilization on sweet potato shoot nutrient uptake

Treatments		Nutrient Uptake (g plant^{-1})				
Bacterial Isolates	**N Fertilizer (N ha^{-1})**	**N**	**P**	**K**	**Ca**	**Mg**
Control	0 kg	1.83h	0.57f	3.88h	0.65h	0.30h
	33 kg	6.49c	1.38c	10.54ef	1.76d	0.88e
	100 kg	5.03def	1.10de	8.81efg	1.40 def	0.51gh
*Klebsiella*sp.	0 kg	5.34de	1.08de	9.12ef	1.38ef	0.61efg
	33 kg	9.86a	2.63a	19.29a	2.63ab	1.99a
	100 kg	8.65b	2.19b	16.19bc	2.55abc	1.49bc
Erwinia sp.	0 kg	4.44ef	1.10de	8.64fg	0.95gh	0.59fgh
	33 kg	8.43b	2.18b	16.88b	2.80a	1.73ab
	100 kg	6.43c	1.47c	12.59d	2.25c	1.30cd
Azospirillum sp.	0 kg	3.40g	0.91e	6.87g	1.12fg	0.46gh
	33 kg	8.39b	2.11b	16.73bc	2.54abc	1.49bc
	100 kg	5.66cd	1.23cd	10.69de	1.51de	0.81ef
Bacillus sp.	0 kg	4.19fg	0.91e	6.80g	1.09fg	0.58fgh
	33 kg	8.41b	2.00b	14.71c	2.35bc	1.28cd
	100 kg	5.58cd	1.39c	10.14ef	1.58de	1.18d
Significance due to PGPR		*	*	*	*	*
N Fert		*	*	*	*	*

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PGPR * NFert	*	*	*	*	*

Note: NS: non significance, and *: significant difference at (P<0.05).Means in column followed with same letter (s) are not significantly different(P>0.05).

Soil pH and nutrient concentration

There was a significant effect of PGPR and nitrogen fertilizer onsoil P, Ca and Mg, but not the soil pH,and N and K concentrations.The results showed that soil pH was almost neutral in all treatments. The interaction effect of PGPR and nitrogen fertilization significantly influenced the soil P, Ca and Mg.Plants inoculated with PGPR and 33kg N ha^{-1}showed higher Nconcentration compared to the control plants without N fertilizer (Table 3).Concentrations of P, K, Ca and Mg were higher with PGPR and N application of 33kg N ha^{-1} compared to the 100kg N ha^{-1}(Table 4).

The effects of PGPR applied alone on in combination with fertilizers on sensitive biochemical indices reflecting soil quality (Gravel*et al*.,2007 and Kavino*et al*.,2010). The present study focuses chemical parameters that reflect the fertilizers and activity of Rhizobacterial processes. Chemical properties are more sensitive to environmental stress, degradation and provide soil quality. Soil nitrogen varied with the rate of N applied. The N in soil at harvest was low due to uptake by plants and some probably lost through leaching.

The biochemical parameters are different because it is related to microbial activity. The activities of enzymes are involved in the N and P cycles in soil. (Dinesh *et al*., 2013). Similarly, the soil enzymes were activated to varying degrees by PGPR and NPK applied alone or in combination.The stronger effects of PGPR + NPK fertilization positively influenced soil enzymes might be due to the greater metabolism by soil microorganisms.

Most of the biofertilizers belongs to several groups such as nitrogen fixation, phosphate solubilization and cellulolyzation. The phosphate solubilizing bacteria secrete various organic acids which enhances the phosphorus absorption by dissolving the rock phosphate anduptakingtricalcium phosphates in soil (Datoniya*et al*., 2016).In Malaysia, biofertilizers are being utilized in a very large scale predominantly for the plant nutrient supply, reducing the toxic effect of the soil contaminants and thus improving soil fertility and moisture.

Table 3: Effect of Rhizobacterial inoculation and N fertilization on soil pH and nitrogen concentration

Treatments		**Soil pH**	**N (%)**
Bacterial	**N Fertilizer**		

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Isolates	(N ha^{-1})		
Control	0 kg	6.72	0.07
	33 kg	6.53	0.09
	100 kg	6.66	0.08
Klebsiella sp.	0 kg	6.76	0.08
	33 kg	6.62	0.12
	100 kg	6.72	0.10
*Erwinia*sp.	0 kg	6.71	0.09
	33 kg	6.62	0.11
	100 kg	6.83	0.10
Azospirillum sp.	0 kg	6.54	0.09
	33 kg	6.46	0.12
	100 kg	6.56	0.10
Bacillus sp.	0 kg	6.64	0.07
	33 kg	6.40	0.11
	100 kg	6.66	0.08
Significance due to PGPR	NS	NS	
N Fert.	NS	*	
PGPR * N Fert.	NS	NS	

Note: NS: non significance, and *: significant difference at (P<0.05). Means in column followed with same letter (s) are not significantly different (P>0.05).

Table 4: Effect of Rhizobacterial inoculation and N fertilization on soil nutrient concentration

Treatments		Nutrient concentration			
Bacterial Isolates	**N**	**P (mg**	**K cmol (+)**	**Ca cmol(+)**	**Mg cmol(+)**

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	Fertilizer (N ha^{-1})	kg^{-1})	kg^{-1}	kg^{-1}	kg^{-1}
Control	0 kg	19.27e	0.25	9.71fg	0.47b
	33 kg	38.62bc	0.29	11.13def	0.59b
	100 kg	30.48cd	0.27	12.00bcde	0.57b
Klebsiella sp.	0 kg	29.40d	0.26	10.65defg	0.52b
	33 kg	48.31a	0.30	16.18a	0.73b
	100 kg	46.06a	0.29	15.65a	0.60b
*Erwinia*sp.	0 kg	33.15cd	0.27	9.23g	0.60b
	33 kg	44.33ab	0.28	15.49a	1.18a
	100 kg	41.61ab	0.30	13.11bc	0.68b
Azospirillum sp.	0 kg	28.31d	0.27	10.40efg	0.56b
	33 kg	42.86ab	0.28	12.05bcd	0.69b
	100 kg	32.59cd	0.30	11.50cde	0.64b
Bacillus sp.	0 kg	27.11de	0.25	10.71defg	0.57b
	33 kg	42.36ab	0.29	13.36b	0.67b
	100 kg	30.96cd	0.30	12.23bcd	0.64b
Significance due to PGPR	*	NS	*	*	
N Fert.	*	*	*	*	
PGPR * N Fert.	*	NS	*	*	

Note: NS: non significance, and *: significant difference at (P<0.05). Means in column followed with same letter (s) are not significantly different (P>0.05).

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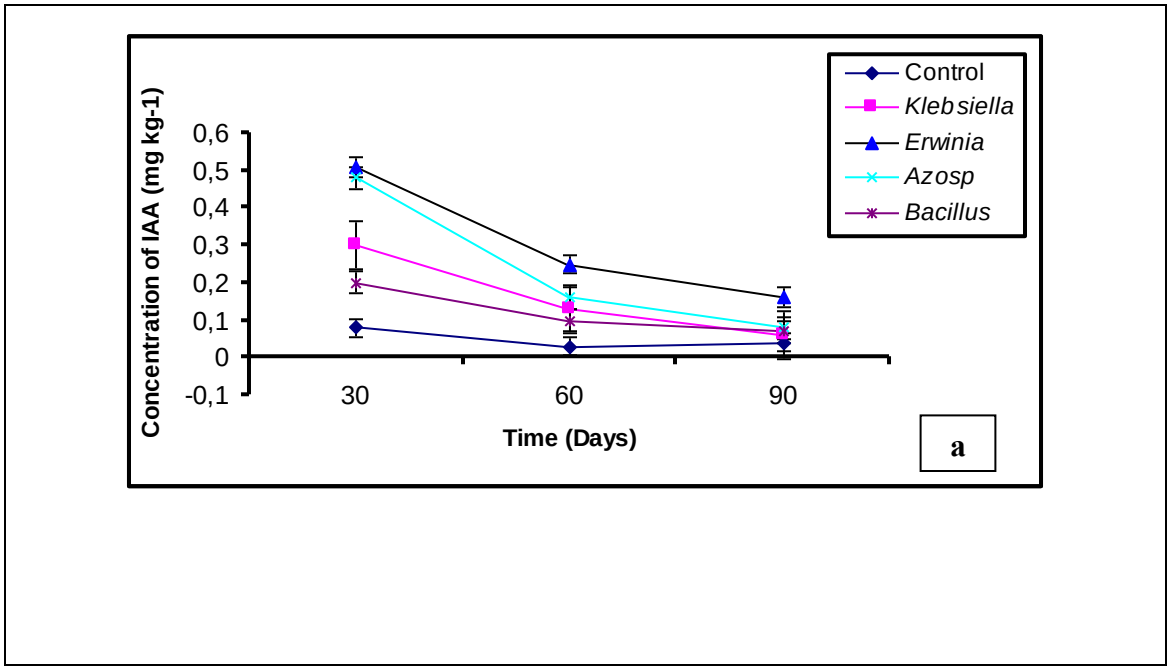
Concentrations of IAA in soil

PGPR inoculation and N fertilization influenced the concentration of IAA in soil (Fig.1a, b, c). There was significant (P≤0.05) interaction between PGPR and N fertilization on IAA concentration. In general, the concentration of IAA in soil decreased with increased in growth period. Theconcentrations of IAA in soils inoculated with rhizobacterial isolates were significantly higher than uninoculatedcontrol. Highest IAA was observed with *Klebsiella* inoculation at33kg Nha^{-1} fertilization rate.

PGPR inoculation and nitrogen fertilization significantly increased the IAA like compounds in soil. The increased IAA like compounds in soilcould be due to IAA synthesized by the bacterial inoculant. The bacteria probably synthesized IAA throughTRP pathways by utilizing L-TRP excreted from the root. It has been reported that up to 85% of Rhizobacteriaare able to synthesize indole acetic acid (IAA) which colonize seed or root is able to induce the cell proliferation and enhancement of IAA in soil (Bashan *et al*.,2014).

Different soils have been reported to vary in their native auxin content depending on the microbial population and other environmental factors such as substrate concentration, carbon source, temperature, aeration, pH (Khalid *et al.,* 2004,Gupta *et al.,* 2015). BesidesIAA, PGPR is known to produce plant-growth substances such as ethylene and cytokinins which are also important in improving plant growth and causing some physiological events. The increase instorageroot yield in this study could probably due to the production of IAA by the introduced isolates.



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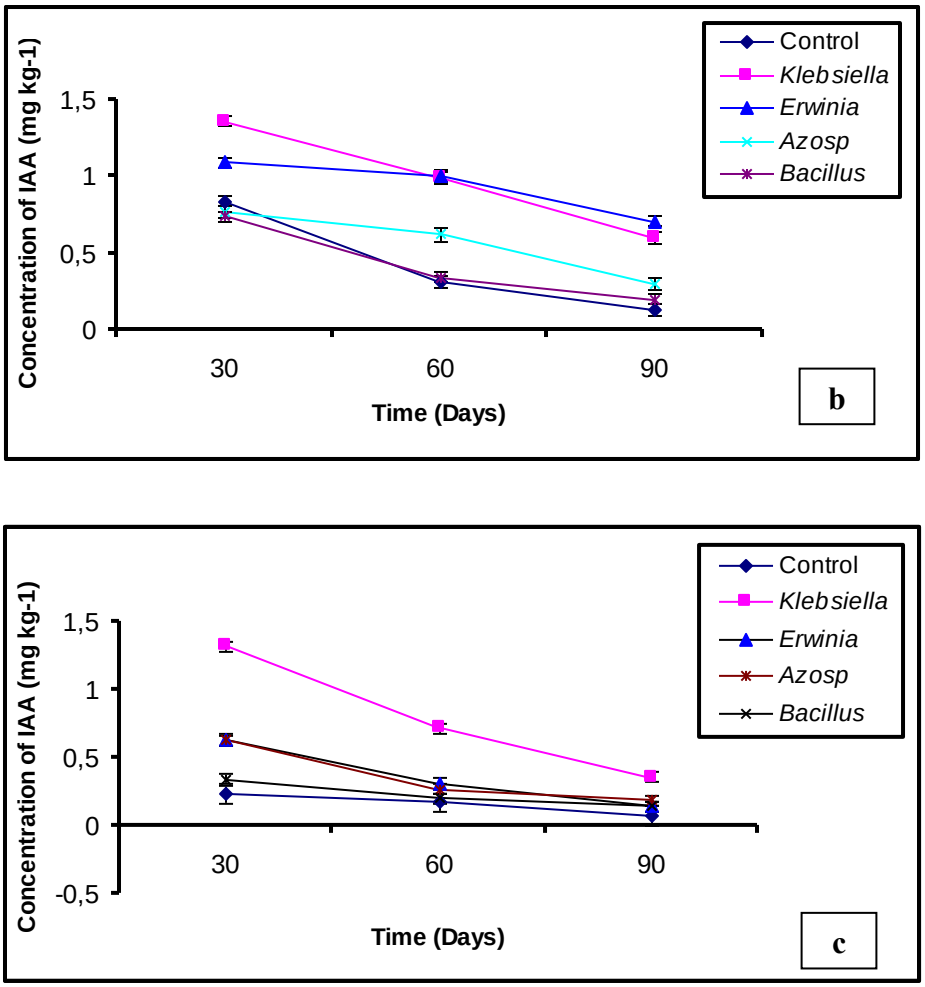

Figure 1: Effect of Rhizobacterial inoculation and nitrogen on concentration of IAA in soil at different sweet potato growth stages;(a)0 kg Nitrogen,(b) 33kg Nitrogenand (c)100 kg Nitrogen.

CONCLUSION

We can conclude that a considerable amount of nitrogen fertilizer could be saved by substituting it with *Klebsiella*inoculum, which may be equally effective as one-third of the recommended nitrogen fertilizer used.Application of 33 kg Nha^{-1} generally increased yield but at higher application of 100kg Nha^{-1}yield was reduced. Field inoculation of *Klebsiella* with 33kg N ha^{-1} enhancedthe nutrient uptake and produces IAA, thus, stimulate growth of sweetpotato and improve soil chemical properties. There is a potential in developing bacterial inoculant for use in commercial sweetpotato production. This can be incorporated into the agricultural sector for the promising growth and yield of sweet potato.

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