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Study on IAA Producing Rhizobacterial Isolates and Their Effect in Talc-Based Carrier on Some Plants

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Abstract

Compounds, known as hormones, are produced by rhizobacteria and are functionalized in growth promoting activity to the host plant. In this study, the production of Indole 3 acetic acid (IAA) by the plant growth promoting rhizobacteria (PGPR) was the main criterion in the strain selection to examine their plant growth promoting effects on plants. 12 bacterial strains were isolated from different fields of Kyaukse and Mandalay, Myanmar. Their biochemical characteristics were carried out by Bergey's Manual of Determinative Bacteriology. IAA production of bacterial isolates were tested by the Salkowski's method and analyzed by UV-Vis spectrophotometer. All isolates were found to produce IAA in a range from 30 ppm to 111 ppm and P6 produced the highest amount of IAA at 30th day. The talc based carrier formulation was used to extend the shelf life of selected isolates and this carrier maintained the bacterial population 10^5 cfu/g for 4 isolates and 10^4 cfu/g for the other isolates up to three months. According to the IAA productivity and one month shelf life data, P5, P6, P7, P8 and P10 were selected to determine their growth promoting activities of maize, betel and Kabuli chickpea plants. Among treatments, P7 isolate in talc based carrier which produced about 100 ppm IAA could enhance root formation significantly and P7 isolate also kept its high bacterial population in talc based carrier to 7×10^4 cfu/g up to four months.

Keywords

Rhizobacteria; IAA; Plant hormone; Talc based carrier; PGPR; Maize; Betel; Chickpea

Introduction

Bacteria that exert beneficial effects on plant development known as plant growth promoting Rhizobacteria (PGPR) have been reported widely. Evidently, PGPR holds enormous prospects in improved and sustainable plant production, including enhanced plant tolerance to stress, better plant nutrient uptake and reduced use of chemical inputs. The exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene [1,2], (ii) asymbiotic N2 fixation [3] (iii) antagonism against phytopathogenic microorganisms by production of siderophores [4] antibiotics [5] and cyanide [6], (iv) solubilization of mineral phosphates and other nutrients [7,8] .

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Plant growth hormone (PGH) is an organic bio-stimulant, that, when applied in small quantities, enhances plant growth and development such that the response cannot be attributed to application of traditional plant nutrients. IAA is involved in nearly every aspect of plant growth and development such as the formation of embryo in development, induction of cell division, stem and coleoptile elongation, apical dominance, induction of rooting, vascular tissue differentiation, fruit development, stimulation of ethylene synthesis, induction of adventitious roots on cuttings, and tropic movements. IAA is the naturally occurring auxin found in plants and diverse microorganisms inhabiting rhizosphere of various plants possess the ability to produce this hormone. The performances of PGPR for the plant growth promotion rely on their rhizospheric competition, ability to survive and colonization in the rhizospheric soil and the commercial use also requires inoculum that retains the high cell viability. To attain the huge population of interest PGPR at root zone, there are several approaches to develop the suitable formulations with appropriate carrier materials. Variety of materials used as carriers has been shown to improve the survival and biological effectiveness of inoculants by protecting bacteria from biotic and abiotic stresses [9].

Moreover, suitable carrier should be cheap, easily packed, transported, used, available, should have increased shelf life, should dissolve well in water and should release the bacteria, should tolerate adverse environmental conditions and should be compatible with other agrochemicals. Talc is a natural inert organic mineral and it supported the survival of inserted bacteria for several months' storage as described in many articles. In this study, the best IAA producer, five isolates were formulated with talc based carrier to improve their survival and their efficiency on plants as the impressive product.

Materials and Methods

Isolation of IAA producing bacteria from different fields

Fifteen soil samples were collected from paddy fields in Mandalay, Pa Leik, Sint Kaing, Han Myint Mo and near Kyaukse. Isolation of bacterial species was done on King's B medium [10] by serial dilution method. 1g of soil sample was added in 10 ml of normal saline and was shaken thoroughly. Further dilutions were made and that serially diluted sample was spread on King's B medium plate and incubated at 30°C for 24 hours.

Production of indole acetic acid (IAA)

A loopful of pure isolate was incubated in 10 ml King's B broth with at least 10^8 colony forming units (cfu/ml) at 30°C for 48 hrs. Cell concentration of 10^8 colony forming units (cfu/ml) was taken out and 3ml of inoculum was added to the 30 ml King's B liquid medium containing 0.5 g/l of tryptophan and incubated at 30°C about four weeks. And then, 2 ml of culture broth was collected individually on every 3 day interval and was centrifuged at 10000 rpm for 30 mins to separate the pellet and supernatant. The supernatant was taken and the pellet was discarded. The supernatant (1ml) was mixed with one drop of orthophosphoric acid and 2 ml of the Salkowski's reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink color indicated IAA production and was measured at 530 nm by UV-Vis spectrophotometer after 30 minutes [11].

Biochemical characteristics of isolated bacteria

Colonial and microscopic morphologies of 12 bacterial isolates were interpreted by Bergey's Manual of Determinative Bacteriology. Gram's staining; gelatin liquefaction, protease production, starch hydrolysis, casein hydrolysis, catalase production, indole production, methyl red and voges-proskauer, nitrate reduction and urease production tests were carried out.

Development of talc-based bio-formulation

The inert carrier used in the formulation was talc powder, 1% carboxymethyl cellulose (CMC) as binder and 1.5% $CaCO_3$ as buffer. A loopful of isolate was inoculated into the King's B broth and incubated to get the cell concentration of 10^8 CFU/ml. 1 kg of sterilized talc powder, 15 g calcium carbonate (to adjust the pH to neutral) and 10g carboxymethyl cellulose (adhesive) were mixed with 400 ml of fully grown bacterial inoculum for bio-formulation. The shelf life of isolates in bio-formulation was studied by a serial dilution technique and the samples were kept at room temperature (28 ± 2°C) for storage. Each of 1g of sample taken from each formulation at 1st, 2nd and 3rd month storage was mixed with 10 ml of sterile normal saline water and the number of colony forming unit (CFU) of bacteria was counted on King's B medium after 24 hrs plating.

Efficacy of bio-formulations and bacterial broth on maize plants by pot trial experiment

The broth culture of P5, P6, P7, P8 and P10 were incubated for fifteen days. The bacterial talc-based bio-formulation of each isolate was also prepared. The soil for the pot trial experiment was obtained from Tawtwin village, Kyaukse city, Myanmar and was dried under the sun. After that they were put into plastic cubs and were prepared in triplicates for the maize pot trial experiment. The maize seeds were washed with tap water and immersed in 2% sodium hypo-chloride for three minutes. Then they were washed five times with distilled water. Three seeds were sowed into each plastic cub containing the sterilized and prepared soil. After the germination and growing of three maize seeds, two plants of maize in each cap were taken out and removed to leave one plant of maize in each cap. After one week, 2 ml of each broth treatment was poured into the base of each plant for broth experiment to reach root area. For the talc-based carrier treatment, 1 g of talc-based bio-formulation was thoroughly mixed with 10 ml of water. Then, 2 ml of each talc-based carrier bio-formulation treatment was poured into the base of each plant for the talc-based carrier experiment to reach the root area. After two weeks, 2 ml of each prepared treatment was poured into the base of each plant.

Efficacy of bio-formulations on the betel cutting plants and Kabuli chickpea by pot trial experiment

About 18 cm long betel cutting plant was taken from a healthy mother vine using a sharp knife and all leaves were removed with the exception of the top 2 leaves. The cutting plant was folded into 3 loops and put in a plastic bag which was half filled with soil. 10 ml of treatment was poured onto every node of plant and then the bag was fully filled with soil and the plants were placed in a green house from 11 April to 28 May 2016. 10 replicates were done for each treatment and 1 g of bio-formulation which was dissolved in 10 ml of water was used for every replicate.

A bacterial talc-based bio-formulation of each of P5, P6, P7, P8 and P10 isolates was also prepared. The soil for the pot trial experiment was also taken from Tawtwin village, Kyaukse city, Myanmar. They were sterilized by drying under the sun and were put into plastic bags to prepare 10 replicates for the chickpea pot trial experiment. The chickpea seeds were washed with tap water and were immersed in 2% sodium hypo-chloride for three minutes. After that they were washed five times with distilled water. Three seeds of the washed chickpea were sowed into each plastic bag containing the sterilized soil. After growing, one plant of each bag was left by cutting two plants. For the talc-based carrier treatment, 1g of talc-based bio-formulation was thoroughly mixed with 10 ml of water. 10 ml of each talc-based carrier bio-formulation treatment was poured into the base of each plant to reach the plant's root area every two weeks.

Results

Isolation of IAA producing isolates

12 bacterial isolates were successfully isolated from 15 rhizospheric soil samples. Among the 12 bacterial isolates, 5 isolates produced fluorescent pigment while others did not.

Production of indole acetic acid

All 12 strains generally produced their highest amount of IAA at 21st, 30th and 33rd day in a range from 30 ppm to 111 ppm (Table 1). 3fluorescent pigment producing isolates were superior IAA producers in comparison with the others and P6 was the best IAA producer with 111.131 ppm IAA production. According to IAA productivity, 3 fluorescent pigment producing isolates (P6, P7 and P8) and other two isolates (P5, P10) which produced111.131 ppm, 101.511 ppm, 90.583 ppm, 90.275 ppm and 88.197 ppm respectively were chosen for the pot trial experiment.

Biochemical characteristics of bacterial isolates

Biochemical characteristics of bacterial isolates are showed in Table 2.

Shelf life of isolates in talc based carrier

The initial concentration of 10^8 cfu/ml for P3, P4, P9, P11, P12 and 10^9 cfu/ml for the other bacterial isolates were used for the preparation of the formulation. The decrease in population density was found from month to month and the five best bio-formulations (P5, P6, P7, P8 and P10) for greenhouse study were chosen according to the data of IAA production and the shelf life of the formulation stored up to 4 month. After 1 month storage, P7, P11 declined to 10^1 colony forming units compared with initial population count while 4 isolates (P1, P6, P8, P9) decreased to 10^2 cfu/ml and the other 6 isolates (P2, P3, P4, P5, P10, P12) decreased to 10 cfu/ml. The bio-formulations of the five isolates which were investigated in the greenhouse experiment had IAA production activity from 88 ppm to 111 ppm and these isolates also had the shelf life from 10^6 to 10^8 cfu/ml after 1 month storage. At the end of three months, the carrier which contained 4 bacterial potent isolates (P1, P2, P6 and P7) in the bio-formulation had the population of 10^5 cfu/ml and the other isolates had 10^4 cfu/ml. At the end of four months, the carrier which contained the bacterial strain P2 had maintained 10^5 cfu/ml, P4 in the bio-formulation had 10^3 cfu/ml, P12 in the bio-formulation did not survive anymore after the fourth month and the other isolates declined to 10^4 cfu/ml. P7 in the bio-formulation had the most bacterial concentration of 7×10^4 cfu/ml after fourth month (Table 3).

Efficacy of the bio-formulations and the bacterial broth on maize plants by the pot trial experiment

In that treatment, IAA effect of P6, P7, P8 and P10 in the broth culture showed better effect (0.196 g,0.160 g, 0.136 g and 0.148 g/plant)

in root weight respectively than water although that of P5 showed the same effect as the water effect (0.102 g/plant). And IAA effect of P6 showed the best activity among all treatments in broth culture. In talc-based carrier on maize plants' experiment, IAA effect of P5, P6, P7, P8 and P10 isolates showed significantly better effect (0.187 g, 0.207 g, 0.236 g, 0.150 g and 0.185 g/plant) in root weight. And IAA effect of P7 showed again the best root growth promoting activity among the five isolates as in betel growth. The effects of water, SS and FT on maize plants were 0.101 g/plant, 0.100 g/plant and 0.140 g/plant in root weight respectively (Table 4 and Figures 1 and 2).

Efficacy of bio-formulations on betel cutting plants and Kabuli chickpea plants by pot trial experiment

The survival rate for the betel cutting plants for all the treatments were higher than 60% and the variation of shoot and root numbers were significant between the treatments. But it was clearly found that there was no correlation between the number and the weight of roots or shoots and the shweseim treatment resulted in the lowest root weight while P6 and control showed very low shoot weight compared to other treatments. The total root weight of P7 and carrier without bacteria were

Table 1: IAA production of isolates (ppm).

Days / Strain	Concentration (ppm)												
	3	6	9	12	15	18	21	24	27	30	33	36	39
P1	4.69	5.38	5.92	9.92	16.92	28.62	34.94	45.56	56.33	56.95	77.19	61.33	53.87
P2	2.77	2.77	9.31	11.08	12.54	22.01	37.78	45.55	52.02	55.18	53.10	37.78	32.63
P3	13.31	14.46	15.62	16.62	18.62	19.54	20.47	21.54	23.01	45.71	52.33	43.17	41.17
P4	10.62	11.08	12.69	13.86	14.46	20.24	20.31	28.62	29.47	30.93	51.26	37.86	30.39
P5	22.39	31.70	35.32	39.79	49.25	50.25	56.02	61.65	67.64	90.27	64.87	62.95	60.11
P6	8.69	13.69	28.39	28.76	43.17	64.26	83.88	87.80	99.27	111.11	101.43	92.19	88.42
P7	6.15	16.47	29.09	33.31	37.63	56.64	73.19	84.42	90.19	92.73	101.51	98.12	91.50
P8	7.08	14.85	37.48	38.85	43.02	76.11	78.19	84.95	86.26	90.58	86.88	77.72	75.72
P9	19.85	22.70	23.85	24.62	28.63	34.24	47.63	59.18	55.18	59.41	64.26	57.10	53.64
P10	20.78	21.39	22.24	23.63	27.70	30.32	39.24	44.40	52.10	56.79	88.19	65.26	58.56
P11	14.93	15.08	17.23	18.39	24.46	27.47	30.55	27.39	24.32	22.31	21.01	21.39	16.47
P12	15.54	20.08	25.08	26.48	27.32	37.32	41.94	45.17	39.01	53.48	74.88	64.49	58.72

Table 2: Biochemical characterization of the best IAA producers.

Tests / Isolates	Results				
	P5	**P6**	**P7**	**P8**	**P10**
Gram reaction	-	-	-	-	-
Gelatin liquefaction	+	-	-	-	+
Protease production	+	-	-	-	+
Starch hydrolysis	+	-	-	-	+
Casein hydrolysis	+	-	-	-	+
Catalase production	-	+	+	+	-
Methyl red	+	+	+	+	+
VogesProskauer	-	-	-	-	-
Urease test	+	+	+	+	+
Indole test	+	-	-	-	+
Nitrate reduction	-	-	-	-	-
Citrate utilization	-	+	+	+	-

Table 3: Shelf life of selected isolates in talc based formulation.

Shelf life / Strains	(CFU/ml)	(CFU/g)			
	Initial count	**After one month**	**After two month**	**After three month**	**After four month**
P1	1.9×10^9	1.05×10^7	1.5×10^6	7.5×10^5	6.5×10^4
P2	1.15×10^9	9×10^6	1.4×10^6	4×10^5	2.5×10^5
P3	3.5×10^8	4×10^5	1×10^5	3×10^4	2×10^4
P4	1×10^8	2.5×10^5	1.5×10^5	6.5×10^4	8×10^3
P5	7×10^9	1.5×10^6	1.4×10^6	3×10^4	1.5×10^4
P6	1×10^9	3×10^7	8×10^6	2×10^5	1×10^4
P7	2×10^9	1.85×10^8	1.5×10^6	2×10^5	7×10^4
P8	1.55×10^9	2.45×10^7	3.5×10^4	2×10^4	2×10^4
P9	5.5×10^8	1×10^6	2.5×10^5	8×10^4	5×10^4
P10	2×10^9	1.25×10^6	5×10^4	5.5×10^4	1.5×10^4
P11	6.5×10^8	1×10^7	4×10^6	7.5×10^4	1×10^4
P12	7×10^8	4×10^5	3.5×10^5	4×10^4	None

Table 4: Effect of IAA producers in talc-based carrier and culture broth on maize plants.

Treatments	% Survival	No. of roots per plant	length of roots per plant(cm)	Weight of roots per plant (g)	length of shoots per plant(cm)	Weight of shoots per plant (g)
P5	30%	8	24.33	0.102	30.66	0.524
P6	30%	9	24.66	0.197	29	0.495
P7	30%	7.3	21	0.160	24.33	0.447
P8	30%	8	17.33	0.136	31	0.481
P10	30%	6	23.66	0.149	30.33	0.413
TP5	30%	8.3	19.33	0.187	23.33	0.422
TP6	30%	9.3	31.33	0.207	31	0.469
TP7	30%	8	40.33	0.256	28.66	0.689
TP8	30%	7.7	17.66	0.151	32	0.505
TP10	30%	7.7	32.33	0.185	32.33	0.508
FT	30%	7.7	31.66	0.141	30	0.250
SS	30%	5.3	24.66	0.100	27	0.543
W	30%	6.3	34.33	0.102	29	0.599

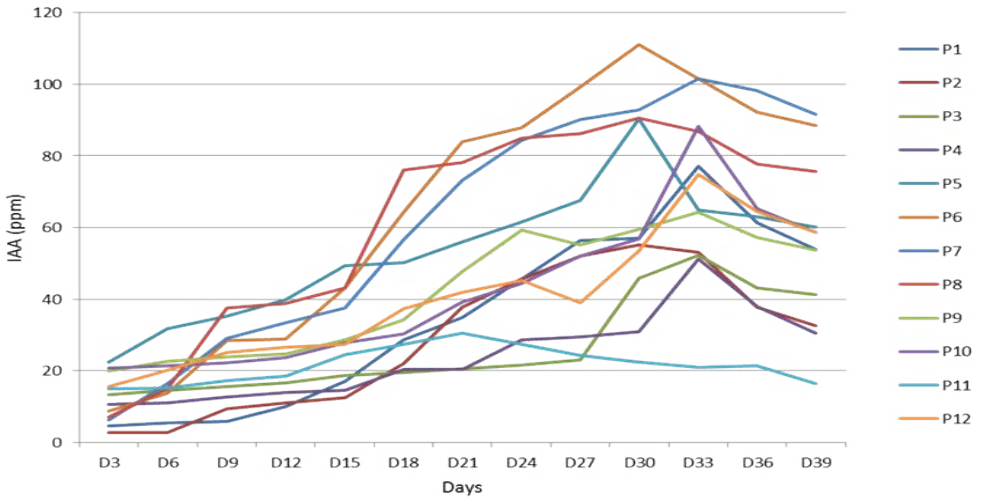

Figure 1: IAA production of 12 isolates by UV-vis spectrophotometer.

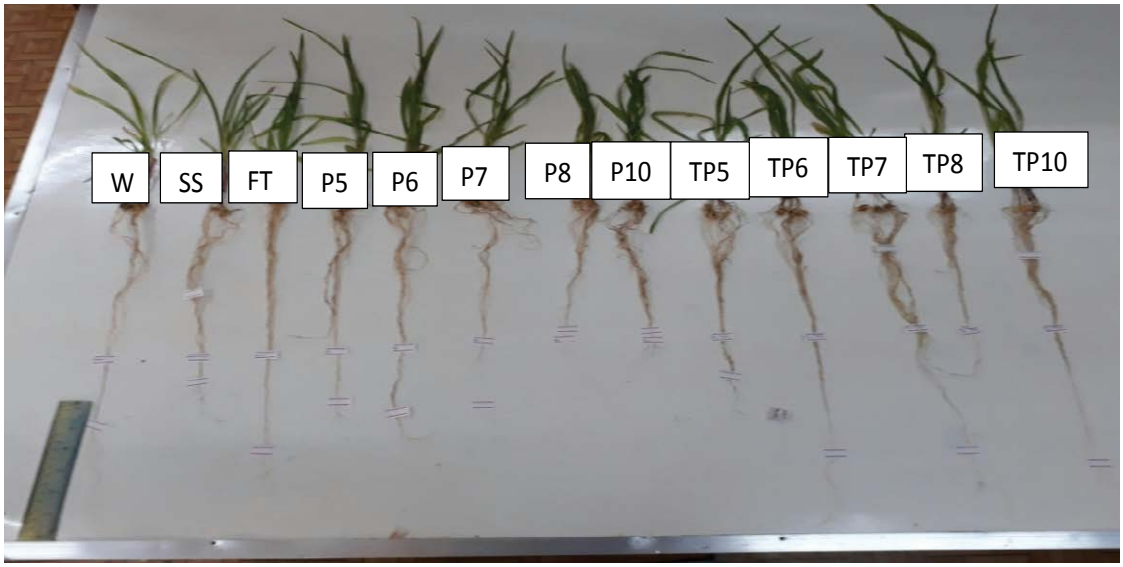

Figure 2: Evaluation of selected bio-formulations and culture broth on maize plants.

0.515 and 0.424 g respectively where the total root weight of others were from 0.301 to 0.358 g except shweseim treatment. Besides, the shoot weight of treatments were not so much different from each other (2.26-2.675 g) except P6 and control treatment while P10 and treatment with carrier without bacteria showed superior shoot weight 2.76 and 2.75 g respectively (Figure 1). The survival percent of all treatments is 100% in chick pea treatment. In chickpea treatment, IAA effect of P5, P6, P7, P8 and P10 showed better effect (0.526 g, 0.463 g, 0.395 g, 0.524 g and 0.588 g/plant) in root weight respectively. Shweseim showed the best effect (0.858 g/plant) among all treatments. The effect of FT and Talc were 0.503 g/plant and 0.547 g/plant. The effect of water on chickpea was 0.391 g/plant (Tables 5 and 6) (Figures 3 and 4).

Discussion

The plant rhizosphere is a dynamic ecological environment in soil for plant-microbe interactions [12,13]. Beneficial microbial allelopathies in the root zone are a key agent of change in soil ecosystems and affect crop health, yield and soil quality [14,15]. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil [16].

According to the present experiments, P5, P6, P7, P8 and P10 were gram negative bacteria isolated from paddy fields and the best IAA producing strains among 12 strains. P5, P6 and P8 produced 90.275 ppm, 111.131 ppm and 90.583 ppm of IAA after 30 days incubation

Table 5: Effect of IAA producing isolates in talc-based carrier on cutting betel plants.

Treatments	% Survival	No. of roots per plant	Weight of roots per plant (g)	No. of shoots per plant	Weight of shoots per plant (g)
TP5	60%	71.17	0.317	6.33	2.675
TP6	70%	62.29	0.311	2.57	1.951
TP7	80%	74.25	0.515	3.63	2.635
TP8	70%	75.86	0.358	3.71	2.638
TP10	70%	65.43	0.33	4.58	2.76
FT	80%	49.875	0.332	3.375	2.44
SS	70%	48.375	0.277	3.625	2.26
T	80%	49.857	0.424	4.71	2.75
W	90%	57.89	0.301	3.67	1.736

Table 6: The effect of IAA producing bacteria in talc based carrier on chickpea plants.

Treatments	% Survival	No. of roots per plant	Weight of roots per plant (g)	No. of shoots per plant	Weight of shoots per plant (g)
TP5	100%	42.5	0.526	22.4	0.810
TP6	100%	45.9	0.463	19.7	0.689
TP7	100%	39.9	0.395	23.4	0.706
TP8	100%	35.6	0.524	20.9	0.712
TP10	100%	33.1	0.588	20.1	0.664
FT	100%	43.5	0.503	19.1	0.753
SS	100%	44.3	0.858	26.5	0.887
T	100%	36.6	0.547	22.6	0.903
W	100%	45	0.391	19.3	0.711

P- Bacterial Isolate in culture broth

TP – Bacterial Isolate in the talc based carrier

FT – Ferti – Start (Rooting product)

SS – Shweseim (Chitosan based plant growth stimulator)

T – Talc based carrier without isolates

W – Water only

Figure 3: Evaluation of selected bio-formulations on cutting betel plants.

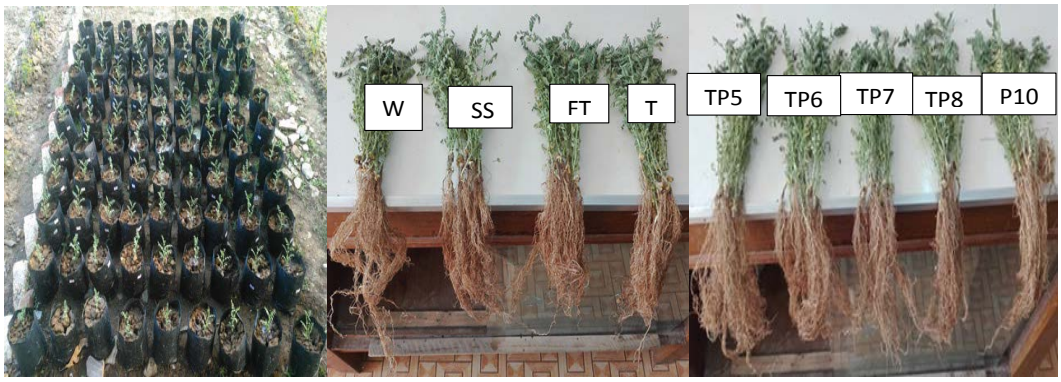

Figure 4: Evaluation of selected bio-formulations on chickpea plants.

period. P7 and P10 produced 101.511 ppm and 88.197 ppm of IAA after 33 days incubation period. Then, the shelf -life of isolated bacteria in talc-based formulation were checked one month after one month to fourth month. Shelf life of P5, P6, P7, P8 and P10 were1.5×10^4CFU/ML, 1×10^4CFU/ML, 7×10^4CFU/ML, 2×10^4CFU/Ml and 1.5×10^4 CFU/ML, respectively. So, P5, P6, P7, P8 and P10 in the talc-based formulation were chosen to carry out treatments on plants. Treatments on betel plants and chickpea plants were carried out by using P5, P6, P7, P8 and P10 in talc-based formulation. Treatments on the maize plants were carried out by using P5, P6, P7, P8 and P10 in talc-based carrier and P5, P6, P7, P8 and P10 in broth culture.

In the treatment on betel plants, IAA effect of five isolates in talc-based carrier showed better than effect and P7 showed significantly good effect on betel root formation and weight. In treatment on chickpea plants, IAA effect of five isolates in talc-based carrier showed better than water effect of water and P10 showed significantly better effect on chickpea root weight. In the maize plant pot trial experiment, IAA effect of five isolates in talc-based carrier and broth culture were carried out. In that treatment, P6, P7, P8 and P10 in broth culture showed better effect than water although P5 showed as the same effect as water. And P6 showed the best activity among all treatments in broth culture. In talc-based carrier on maize plants' experiment, effect of five isolates showed significantly better than water. And P7 also showed the best root growth promoting activity among five isolates. Then, IAA effect of isolates in talc-based carrier on maize root growth was more significant than that of isolates in broth culture on maize plants.

This study suggested that the use of PGPR isolate P7 as effective bio-formulation might also be beneficial for other crop cultivations. In addition, antagonistic microbes are suggested to be used in this carrier with some modification with chitin in many articles.

Conclusion

In the present study it was found that P7 strain produced high amount of IAA (101.511 ppm) and gave a good shelf life (7×10^4 CFU/ML) after 4 months. Moreover, P7 treatment significantly promoted root formation of betel cutting plants. In conclusion, IAA effect of isolates in broth culture and talc-based formulation showed good effect on three types of plants. IAA effect of P6 in broth culture showed the best root growth promoting activity. Moreover, IAA effect of isolates in talc-based carrier showed more significant than that of isolates in broth culture according to maize plant pot trial experiment. IAA effect of P7 and P10 in talc-based carrier were the best root growth promoting activity according to this study.

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