STUDY ON DIAZOTROPHIC AND IAA PRODUCING BACTERIA ISOLATED FROM DESERT SOIL SUBMIT AN APPLICATION FOR BIOFERTILIZER

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ABSTRACT: Biofertilizers are an alternative to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. The objective of this study wheat plant growth promoting by non symbiotic (Azotobacter spp.)Strains as biofertilizer on soil properties and seedling growth of wheat in soil, and the application treatments included the control (without bacteria inoculation), non symbiotic organism isolated saline desert soil in which studied soil all properties. Checked nitrogen fixing activity, IAA producing activity and studied activity against pesticide of all isolated and record results.

Keywords: Bacterial inoculation; plant growth promoting azotobacter spp; pesticide study

INTRODUCTION:

Biofertilizers are supposed to be a safe alternative to chemical fertilizers to minimize the ecological disturbance. Biofertilizers are cost effective, eco-friendly and when they are required in bulk can be generated at the farm itself. They increase crop yield up to 10-40% and fix nitrogen up to 40-50 Kg. The other plus point is that after using 3-4 years continuously there is no need of application of biofertilizers because parental inoculums are sufficient for growth and multiplication. They improve soil texture, pH, and other properties of soil. They produces plant growth promoting substances IAA amino acids, vitamins etc. They have 75% moisture and it could be applied to the field directly. Biofertilizers contained 3.5% - 4% nitrogen, 2% - 2.5% phosphorus and 1.5% potassium. In terms of N: P: K, it was found to be superior to farmyard manure and other type of manure (Mukhopadhyay, 2006)

Azotobacter and Azospirillium are nonsymbiotics bacteria it belongs to azotobacteriaceaes. It produces growth promoting substances which improve seed germination and growth of extended root system. It produces polysaccharides which improve soil aggregation. Azotobacter suppresses the growth of saprophytic and pathogenic micro-organism near the root system of crop plants.

The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable agriculture. Chemical agriculture has made an adverse impact on the health-care of not only soil but also the beneficial soil microbial communities and the plants cultivated in these soils. This eventually has lead to a high demand for organic produce by the present-day health conscious society and sporadic attempts are being made by farmers all over the world to detoxify the land by switching over to organic farming dispensing with chemical fertilizers, pesticides, fungicides and herbicides.

In present work isolated non symbiotic diazotrophic organisms from saline desert soil of little ran kachh in Gujarat. Studied soil physical and chemical property and carried out bio-chemical test, microscopic and macroscopic inspection of isolated.

2. Materials and methods:

1. Soil sampling(Analysis) and organism isolation:

Soil samples were collected from to layer of soil (1 to 20 cm deep) from different site near the desert of Kutch (little desert in Gujarat),sample were further characterized for soil properties like soil texture, water holding capacity ,chloride, organic carbon, organic nitrogen and total nitrogen concentration and total nitrogen concentration(pandey and sharma,1998).

Isolation of N_2 fixing organisms were carried out by enrichment isolation technique using nitrogen free mannitol broth (gm/lit) medium was inoculated with 1 gm soil sample and 48 hours incubation agar plates contains same composition were streaked .after 48 hours incubation at 37 c isolated colonies were taken and maintained as pure culture in same medium.

Mannitol broth :(gm/lit)
Mannitol : 15.0 gm
$K2HPO_4$:0.5 gm
$MgSO_4$:0.2 gm
$CaSO_4$:0.1gm
NaCl : 0.2 gm
$CaCO_3$:5.0gm
PH : 7.2

Bio-chemical analysis of isolates: isolated were inoculated in different sugar like glucose,ribose,maltose,lactose,xylose,mannitol,sucrose,the biochemical test were carried out with standard methods for urea utilization ,ammonia production ,nitrate utilization ,H_2S production & catalage and oxidaze activity.(Table: 1&2)

2. Analysis of N2 fixing activity of isolates:

K Jeeldal method (s k maiti e tal,2003)used for determine total nitrogen contents of biomass of cell in which three strong acid orthophosphric acid, sulfuric acid and nitric acid were taken for digested of cell.(Elbeltagy et at.,2001)(Table:3)

3. IAA production activity:

The bacteria were isolated from the soil on nitrogen free medium identification of isolated was carried out on basic of morphological culture characteristics on ASM by standard method (Hot et al, 1994) from IAA production axenic culture of the bacteria were grown in 100 ml Erlenmeyers flasks.IAA concentration determined by sprectophotometricaly method (Elbeltagy et at.,2000) (Table:4)

Medium Composition (IAA production (gm/lit))

Yeast Extract : 1gm
Mannitol : 10gm
$MgSO_4.7\ H_2O$: 0.20gm
$K2HPO_4$: 0.50gm
NaCl : 50gm
L-tryptophan : 3gm
pH : 7gm

6. Effect of pesticide on isolated,

The effect of 1% pesticide (2-4D, chlopyriphos, raft, triazophosphorous, monocrotophos, and hostathion) in acetone was investigated on different isolates with agar diffusions method. Zone of inhibition in mm to find out the effect of pesticide on growth of isolates. (Table: 5)

7. Biofertilizer formulation:

Formulation done by addition of sterile soil, charcoal, powder and active culture of isolates in the 5 liter beaker.

8. Plant growth promotion study:

Formulated biofertilizer were tested on wheat plant with the respect to the germination of wheat seeds.20 seeds were used in the experiment and using the direct observation quantitative measurement were done by calculating the ratio of germination at each successive day after one month's plant was harvested to find out dry weight and wet weight of wheat plant. (Figure: 1 &Table 6)

RESULT &DISCUTION:

Physicochemical analysis of soil indicated that soil salt concentration was higher and pH was alkaline, isolated had ability to grow and tolerated to higher pH and salt concentration. Three isolated organisms from "O" horizon of saline soil and studied by biochemical analysis it's indicated that organism may be non symbiotic azotobacter spp. (Table 1&2),nitrogen fixation activity of L-delta was 0.009% and IAA production of 36 µl /ml greater than other two isolated, (Table 3&4) .Pesticides studied were carried all isolates that indicate L-delta strain batter than other two, but remaining were not bed.(Table:5) studied of plant germination activity on wheat plant to prepare biofertilizer from isolated like mix culture and individual culture cheeked germination activity and compare with standard biofertiliger available in the market its provide positive results(figure 1) .

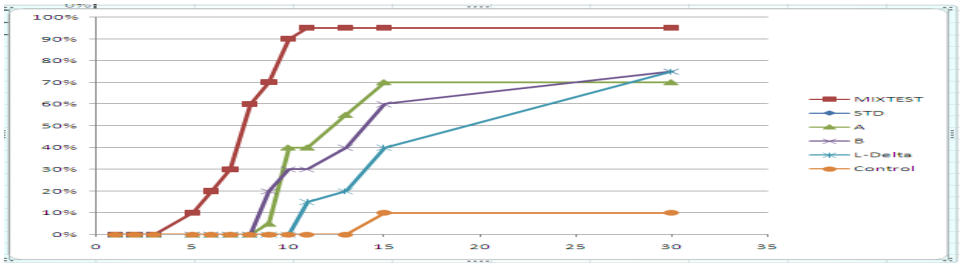

Figure: 1. Germination of wheat plant under treatment of microorganisms as biofrtilizer . (days---%(growth germination)

Table 1:Physico-chemical analysis of soil.

No.	Soil property	Sample L-delta	Sample A	Sample B
1	Texture	Sandy soil	Lomy soil	Sandy soil
2	Soil profile	O Horigen	O Horigen	O Horigen
3	pH	8.4%	8.0%	8.2%
4	Carbonate(co_3)	0.087%	0.020%	0.013%
5	Bicarbonate(Hco_3)	0.91%	0.42%	0.54%
6	chloride	2.95%	2.85%	2.82%
7	Calcium carbonate	85%	80%	79%
8	Available phosphate	0.102%	0.122%	0.111%
9	Available sulfur	41.70ppm	37.70ppm	36.21ppm
10	Available nitrogen	0.33%	0.32%	0.31%

Table:2. Biochemical analysis

No	Test	L-delta	A	B	No	Test	L-delta	A	B
1	ONPG	-	-	-	12	Malonate	+	-	
2	Lysine decarboxylase	-	-	-	13	Esculin		-	-
3	Omithine decarboxylase	-	-	-	14	Arbionose	+	-	-
4	Urease	-	-	-	15	xylose	+	-	-
5	Phenylalanine deamination	-	-	-	16	Adonitol	-	-	-
6	Nitret reduction	-	-	+	17	Rhmonse	-	-	-
7	H2S production	-	-	-	18	cellobiose	+	+	-
8	Citrate utilization	-	+	-	19	melibiose	+	+	-
9	V-P	-	-	-	20	saccharose		+	-
10	M-R	-	-	-	21	glucose	+	+	+
11	Indol		-	-	22	Lactose	+	+	+

Table:3. Nitrogen fixing activity of isolated

Strains	Nitrogen fixations (%)
L-delta	0.009
A	0.001
B	0.0045

Table:4. IAA production activity

strains	IAA(µl /ml)
L-delta	36
A	7
B	13

Table:5. Effect of pesticides

Strains	24D	Chlorpyriphos	Raff	Triazophosphoras	Monocrotophos	Hostathion	Endosalfan	Acetone
Ldelta	2	1	2	1	-	1	2	-
A	3	1	2	-	2	3	1	-
B	1	-	2	-	1	3	1	-

Table: 6. Plant growth promotion study on wheat plant after month.

weight	STD	MIXTEST	A	B	L-Delta	Control
Wet weight	3.24gm	3.40gm	1.46gm	1.73gm	0.82gm	0.1gm
Dry weight	0.33gm	0.33gmm	0.18gm	0.21gm	0.09gm	0.03gm
Total	2.91gm	2.92gm	1.28gm	1.52gm	0.73gm	0.07g

Conclusion: in present study demonstrate that wide occurrence of diastrophic haloalkalotolerant strain saline desert environment ability to grow diverse environment of pH and salt range, plant growth promotion was found by multiple characteristics fixing nitrogen, IAA production and pesticide active on isolated.mix culture is the best when it apply as biofertilizer at field level. Application of bifertilizer is eco-friendly approaches.

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