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Combined application of Azotobacter and Urea to improve growth of rice (Oryza sativum)

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Abstract: *Azotobacter is a free living nitrogen fixer and a plant growth promoting rhizobacteria. It is gram negative, motile, spherical bacteria. It promotes the plant growth by producing auxin, producing siederophores and solubilizing phosphate. In present study combine application of a biofertilizer and a chemical fertilizer is done. Azotobacter alone and with the combination of 100%, 75%, 50% and 25% urea is applied to rice seeds and effect on the seed germination and plant growth have been recorded. It is observed that coinoculation of chemical and biofertilizers improves the growth of plant.*

Key Words*: Auxin, Azotobacter, fertilizer, rice, urea*

I. Introduction

In modern agriculture practices to fulfill the soil with high nutrient content and to prevent the cop by the effect of pesticides there is an increase in the excessive use of chemical fertilizers and chemical pesticides. Due to this not only the fertility of soil get decreased but it also affect the growth of soil beneficial microorganism whose presence promote the growth of plants by many mechanisms. Bacteria are most abundantly occurring microorganism in rhizosphere. The group of bacteria which colonize plant root and promote the growth of plant is called as plant growth promoting rhizobacteria. Azotobacter is one of the plant growth promoting rhizobacteria which is proved to promote the growth of plant producing growth hormones. Azotobacter belonging to family Azotobacteraciae, is gram negative free living nitrogen fixer. It comprises of six species.(Tchan *et al*, 1984) Various crops in India have been inoculated with azotobacter and its application improves the yield of both annual and perennial crops.(Biswas *et al*). Azotobacter affects seed germination and seedling growth in plants and increase yield of crop plant upto 30%(Kloepper *et al* 1992, Shaukat *et al* 2006, Gholami *et al* 2009). It has been shown by many researchers that Azotobacter posses plant growth promoting properties such as plant growth hormone production, phosphate solubilization etc. It also shows antimicrobial activity. Joseph and co workers reported the IAA producing Azotobacter species from chick pea(Joseph *et al* 2007). *A. vinelandii* have ability to produce siederophores, solubilise phosphate and make it available for plants thus promote plant growth(Husen 2003). Urea is a chemical fertilizer most widely used for better growth of plants. In Chhattisgarh it is used in rice crop field to increase the avaibility of nitrogen. Although the use of urea increase the production of crop but it also causes harmful effect on the plant growth promoting rhizobacteria. In present study species of Azotobacter is isolated from rice field and different doses of a chemical pesticide urea is applied to Azotobacter, in vitro, to study the effect of chemical fertilizer on its growth.

II. Material and methods

2.1. Isolation of Azotobacter from Rice rhizosphere:-

Azotobacter is isolated from rhizospheric soil of rice plant by using the serial dilution plate techniques in Ashby's medium (Aneja). Rice plant were uprooted with some quantity of non rhizosphoric soil and place in sterilized bags and immediately brought to laboratory. The non rhizospheric soil was carefully removed and the rhizospheric soil is collected under aseptic condition. This rhizospheric soil is mixed with the distilled water through gentle shaking and the serial dilution technique is performed. An aliquot of the suspension is spread in the petriplate containing ashby's media. The plates were then allowed to incubate at 28ºC for 3 days. Fast growing Azotobacter colonies were streaked in another petriplate containing ashby's media and pure culture is maintained by subculturing the isolates.

2.2. Preliminary identification :-

Azotobacter isolated was preliminary identified on the basis of its morphology such as colony colour, elevation form, motality, gram's staining is performed and cell shape is noted. Biochemical test such as glucose, fermentation, urease hydrolysis, nitrate reductase, citrate utilization, indole production VP and MP reaction.

2.3. Growth under different temperature

The culture Azotobacter isolates were streaked on Ashby's agar plates and incubated at 10, 20, 28, 37, and 45ºC temperature. Growth was observed and recorded after 3 days of incubation.

2.4. Auxin productin

Azotobacter chroococcum is further tested for auxin production. Azotobacter isolate is grown in nutrient broth media containing tryptophan(100µg/l) and incubated at 28ºC for 5 days. 2ml culture suspension is then centrifuged 10000 rpm for 15 minutes. 2 drop of orthophospharic acid and 4 ml Salkowaski reagent is added to 1 ml of supernatant fluid. Appearance of pink color shows auxin positive test. Quantitative estimation of auxin is done in UV spectrophotometer at 530 nm after 30 min.

2.5. Effect of urea on growth of Azotobacter

In two different petriplates containing azotobacter growing agar media the recommended dose (A+100% U) and 50% of the recommended dose (A+50% U) of Urea is added in which azotobacter is inoculated and incubated to grow at 34ºC temperature for 48 hrs. Without urea media is considered as control(A)

2.6. Preperation of Broth inoculums

The inoculum was prepared by growing the isolated azotobacter strain in 250 ml flask containing nutrient broth media. This medium was incubated at 28± 1ºC for 48 hrs in rotary shaker. This is considered as T1. Another inoculum was prepared by growing the azotobacter isolate in nutrient broth media containing Chemical fertilizer urea. This is considered as T2. No inoculated nutrient broth media with azotobacter is designated as control i.e. T0.

2.7. Seed germination test and Pot experiment

The above treatment is applied in pot trial also. In pot trial the azotobacter inoculums were placed 2 cm below the soil in thin layer and after that the rice seeds were sown. Rice seeds were soaked in H2SO4 for 5 minutes and washed thrice with sterile water. Seeds were then treated with different treatment of T0, T1, T2. 20 seeds for each treatment were placed in sterile wet filter paper in in three different set of petriplates and kept for three 3 days in dark. Germination of seeds after three days is recorded. The same treatment of T0, T1, T2 is given to rice seeds for pot experiment. 10 seeds of each treatment were sown in pot containing sand.

2.8. Plant harvest and its analysis

Rice plants were harvested after 30 days of sowing. plants were separated from soil and washed thoroughly with water. Plant height and root length in cm for each plant were recorded in each treatment were recorded. Dry weight of shoot and root were recorded after drying in an oven for one day at 70ºC.

III. Results and Discussion

Azotobacter chroococcum is isolated from the rhizospheric soil of rice plant. It is gram negative, motile, rod shaped bacteria. It shows positive result for catalase, oxidase, urease and nitrate reductase test while it is negative for amylase test. It shows acid production from glucose, sucrose, lactose, mannitol, fructose, maltose, sorbitole and galactose. The amount of IAA is found to be 12.7 mg/l by *A. chroococcum* after 5 days of incubation. No growth is seen in 10ºC and 45ºC temperature which is considered as minimum and maximum temperature respectively foe the experiment setup. Slow growth is seen in 20ºC but very fast and good growth was observed in 28ºC and 34ºC temperature. The different concentration of urea applied to *A. chroococcum* in ashby's medium shows varied result. The growth of azotobacter in T1 is maximum than all other treatments but it is less than the growth in the control and as the concentration is increasing in the treatment the growth of *A. chroococcum* is seen decreasing. It implies that the urea is inhibiting the growth of Azotobacter.

IV. Conclusion

It is concluded from the present study that *Azotobacter* could be highly effective in improving the growth of plant and could be helpful in reducing the use of chemical fertilizers. It is also seen that azotobacter is urea adaptive bacteria. But there is a need of developing awareness among farmers to use biofertilizers.

V. Acknowledge

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Table 1. Biochemical characters of *Azotobacter chroococcum*

Characters	Results
Gram's reaction	-
Glucose	+
Amylase	-
Citrate utilization	-
Nitrate reductase	+
Urease	+
Catalase	+
Oxidase	+
H2S production	-
Acid production from glucose	-
Sucrose	+
Lactose	+
Mannitol	+
Galactose	+
Sorbitol	+
Fructose	+
Maltose	+

Table 2. Growth of azotobacter at different temperature

Temperature	Azotobacter growth
10ºC	_
20ºC	+
28ºC	++
34ºC	++
45ºC	_

Table 3.IAA production by Azotobacter chroococcum

Isolate	pH	IAA production after incubation of 5 days
Azotobacter chroococcum	5.6	12.7

Table 4. Effect of urea on azotobacter growth

Treatment	Growth of azotobacter
A	+++
(A+100%D)	-
(A+50%D)	++

-(no growth), ++(good growth), +++(very good growth)

Table 5. Seed germination test and pot experiments

Treatment	Seed germination in %	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)
To	73.04	6.9	4.1
T1	95.41	8.1	4.6
T2	96.59	8.8	5.1

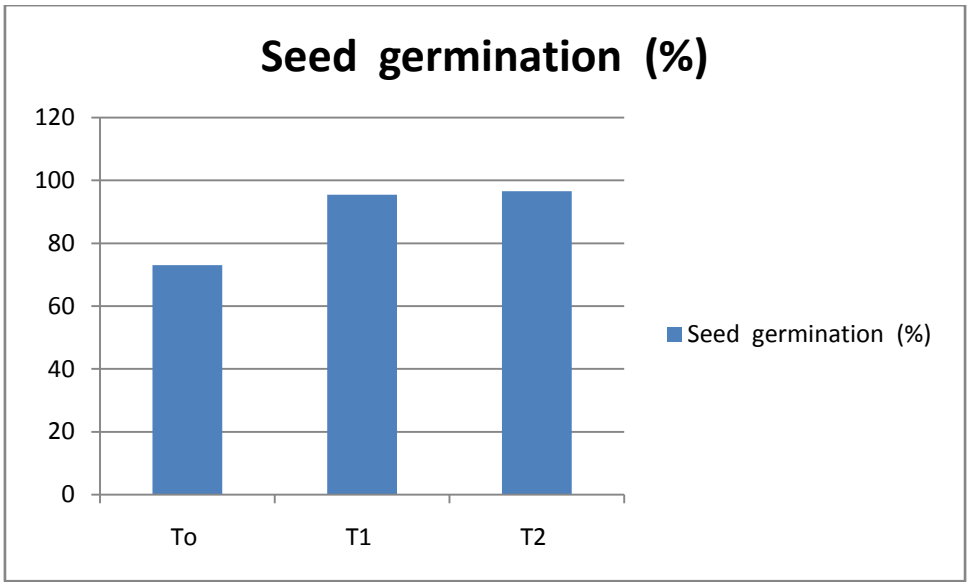


Fig. 1. Seed germination percentage

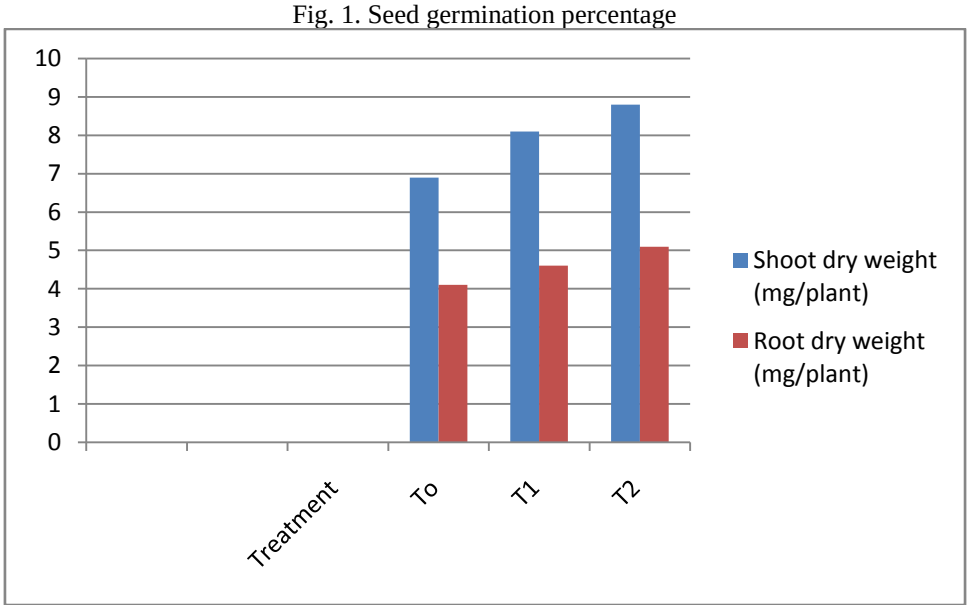
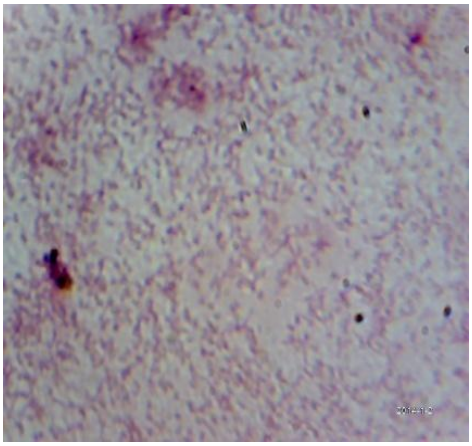


Fig. 2. Treatment of urea

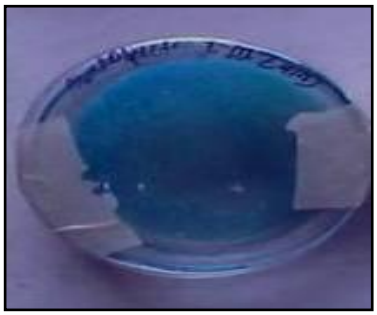
Picture 1. Azotobacter colony

Picture 2. Azotobacter Pure culture



Picture 3. Gram negative

Picture 4. Amylase +ve test



Picture 5. Citrate utilization +ve test

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