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Effect of Pesticide on Nitrogen fixing ability of *Azotobacter* species

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ABSTRACT

In the present study, four isolates of *Azotobacter paspali* were isolated from rhizosphere of maize and wheat plants and tested their Nitrogen fixing ability after treatment with Mancozeb. It was observed that the lower concentration of Mancozeb (1gm/L) increase growth of *Azotobacter* species while at a concentration of 4gm/L, all cells died. The concentration of 2gm/L and 3gm/L decreased the no. of cells and Nitrogen fixing capacity of *Azotobacter* was decreased by 50%.

Key Words - Mancozeb, Rhizosphere, *Azotobacter*, Nitrogen fixing ability.

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INTRODUCTION

Nitrogen is an essential macronutrient for the development and growth of plants. Plants cannot utilize free Nitrogen from atmosphere. They need Nitrogen in the form of inorganic nitrogen compounds like Nitrate or Nitrite. A no. of microbes are able to fix atmospheric nitrogen. This process of Nitrogen fixation is known as Biological Nitrogen fixation. Nitrogen fixing microbes include free living or symbiotic bacteria like *Azotobacter, Azospirillum, Nitrosomonas, Bacillus, Pseudomonas, Serratia, Rhizobium, Bradyrhizobium,* Cyanobacteria like *Nostoc, Anabaena, Rivularia, Calothrix, Mycorrhiza,* Actinomycetes like *Frankia* (Ravi Kumar *et al.*,2007).

Azotobacter are free living soil bacteria capable for fixing atmospheric Nitrogen. *Azotobacter chroococcum* can fix 20kg N/Ha/Yr (Kizilkaya -2009). *Azotobacter* may be used as biofertilizer for the production of better yield in crops (Nagananda *et al.*, 2010).

In recent era, pesticides are used in agriculture system to protect crops bacterial and fungal pathogens as well as from insects. These pesticides have adverse effect on targeted and non-targeted microbes (Purushothaman *et al.*, 1976).

Application of pesticides in agriculture field may affect the no. of microbes in soil, biochemical activity of microbes and may change structure of microbial community (Smith *et al.*, 2000; Chen *et al.*, 2001; Cycon, 2006).

In the present study, effect of Mancozeb was tested on nitrogen fixing ability of *Azotobacter* species.

MATERIAL & METHOD

Soil samples were collected from Rhizospheres of Maize and Wheat plants. Collected soil samples were diluted up to the 10^{-5} dilution with distilled water. Diluted samples were inoculated in Waksman medium and Ashby's medium. The culture was inoculated at 30°C temperature for 24 hours. Single colonies were culture were transferred on fresh medium and sub-cultured to purify the bacteria. Cell and Colony morphology, Gram staining, Biochemical test and Sugar fermentation test were examined for all isolates. On the basis of these tests four isolates of

Azotobacter paspali were identified as described in Bergey's manual of Systematic Bacteriology (2012).

Estimation of Nitrogen fixation:

Effect of different concentration of Mancozeb was tested on Nitrogen fixing ability of different isolates. Mancozeb was supplemented in Ashby's broth at a concentration of 1gm/L, 2gm/L, 3gm/L and 4gm/L. Isolates were inoculated in each concentration and the culture was incubated at 30°C for 72 hours. Survival cells were subcultures 3-4 times in same medium. For the estimation of Nitrogen fixation, survival cells were inoculated in fresh medium and incubated at 30°C for 10 days. One set of culture was kept without inoculation of isolates. One set of untreated cell were also inoculated in Ashby's medium and incubated at 30°C for 10 days. Percentage Nitrogen was determined for both treated and untreated cells by Kjeldahl method.

After 10 days incubation, 250ml isolate containing broth was transferred in Kjeldahl microflask. Digestion salt mixture and 3ml Conc. Sulphuric acid was added and digested on sand bath. After digestion, 10ml distilled water was added and cooled (Digestion salt mixture contain Potassium sulphate, Copper sulphate and metallic selenium in a ratio of 50:10:01).

Digested sample was added in Kjeldahl distillation apparatus. 10ml 40% NaOH was added in distillation flask. 10ml 40% Boric acid and 3 to 4 drops Bromo-cresol reagent was taken in a conical flask. The conical flask was connected to the condenser of Kjeldahl apparatus. After distillation, the content of conical flask was titrated against HCl. Appearance of Pink color showed end point. Similar experiment was conducted with Ashby's broth without isolate which was the blank. Percentage Nitrogen was estimated by the formula:-

$$\%\, Nitrogen = \frac{Sample\ titre - Blank\ titre}{Volume\ of\ sample} x\ \frac{Normality}{of\ HCl}\ x\ 14\ x\ 100$$

RESULT

Four isolates RAP1, RAP2, RAP3 and RAP4 of *Azotobacter paspali* were identified from different soil samples.

The result of cell and colony morphology, gram staining, biochemical test, sugar fermentation test is mentioned in Table No. 01, 02 and 03. It was observed that the lower concentration of Mancozeb (1gm/L) increased the growth of isolates but in higher concentration (2gm/L & 3gm/L) decreased the growth. At a concentration of 4gm/L of Mancozeb, all cells of all isolates died. Survival rate is mentioned in Table No. 04.

Nitrogen fixation ability of both treated and untreated isolates were observed. Maximum Nitrogen fixation was observed in isolate No. AP4. The Nitrogen fixing ability of treated cells decreased up to 50%. The result is mentioned in Fig No. 01.

Table 1- Gram staining, Cell and colony morphology of isolates of *Azotobacter paspali*

Isolate No.	Cell Shape	Colony Morphology						Gram stain
		Size	Shape	Margin	Texture	Color	Appearance	
RAP1	Oval	Moderate	Irregular	Entire	Rough	Dark brown	Mucoid	-
RAP2	Rod	Moderate	Irregular	Entire	Rough	Dark brown	Mucoid	-
RAP3	Oval	Moderate	Irregular	Entire	Rough	Dark brown	Mucoid	-
RAP4	Spherical	Moderate	Irregular	Entire	Rough	Dark brown	Mucoid	-

Table 2- Bio-chemical tests of isolates of *Azotobacter paspali*

Sl. No.	Isolate No.	Cat	Oxi	Ind	MR	NR	Citrate	VP
1	RAP1	+	+	-	-	-	+	-
2	RAP2	+	+	-	-	-	+	-
3	RAP3	+	+	-	-	-	+	-
4	RAP4	+	+	-	-	-	+	-

Table 3- Sugar fermentation test of isolates of *Azotobacter paspali*

Isolate No.	Glucose	Fructose	Sucrose	Lactose	Mannitol	Raffinose	Arabinose
RAP1	+ve	+ve	+ve	-ve	+ve	__	__
RAP2	+ve	+ve	+ve	-ve	+ve	__	__
RAP3	+ve	+ve	+ve	-ve	+ve	__	__
RAP4	+ve	+ve	+ve	-ve	+ve	__	__

Table 4- Effect of different concentration of Mancozeb on Survival of different isolates of *Azotobacter paspali*

Isolate No.	Concentration	Total Colony	Dead Colony	Survival Colony	% survival
RAP1	1gm/L	17	0	17	100%
	2gm/L	17	8	9	52.94%
	3gm/L	17	11	6	35.29%
	4gm/L	17	17	0	0%
	Control	15	0	15	100%
RAP2	1gm/L	13	0	13	100%
	2gm/L	13	5	8	61.53%
	3gm/L	13	9	4	30.76%
	4gm/L	13	13	0	0%
	Control	10	0	10	100%
RAP3	1gm/L	15	0	15	100%
	2gm/L	15	7	8	53.33%
	3gm/L	15	10	5	33.33%
	4gm/L	15	15	0	0%
	Control	12	0	12	100%
RAP4	1gm/L	16	0	16	100%
	2gm/L	16	7	9	56.25%
	3gm/L	16	11	5	31.25%
	4gm/L	16	16	0	0%
	Control	14	0	14	100%

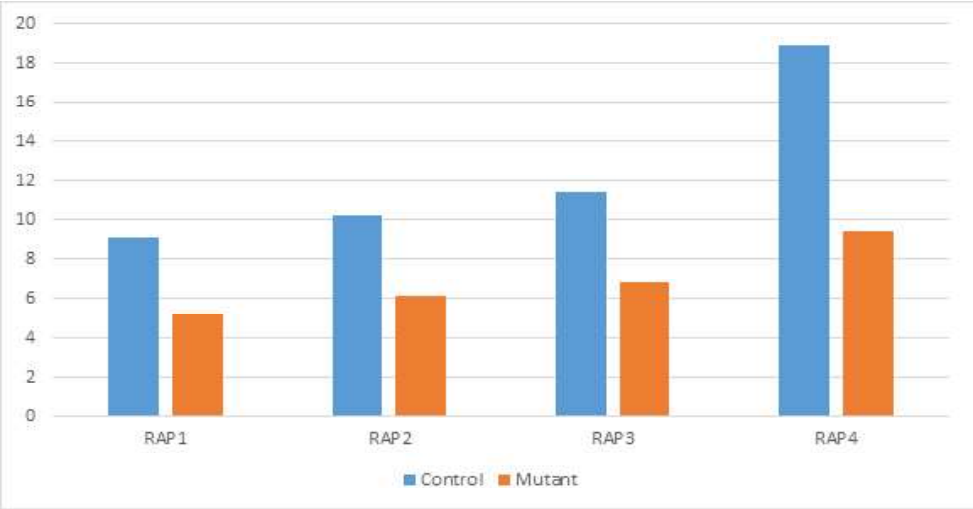

Fig. 1- Percentage Nitrogen content of mutant and control isolates of *Azotobacter paspali* (Mancozeb)

CONCLUSION

Azotobacter is able to utilize pesticides as carbon source and degrade them at lower temperature. Thus this bacterium may be used for bioremediation. In the present study, it was observed that low concentration of pesticide increase the growth of *Azotobacter* isolates but higher concentration decrease their growth and nitrogen fixing ability. Mancozeb was used at a concentration of 1gm/L, 2gm/L, 3gm/L and 4gm/L. At a concentration of 1gm/L, no. of cells increased but at a concentration of 2gm/L about 40% cells died and 3gm/L about 67%-70% cells died and a concentration of 4gm/L all cells died. Thus, it is clear that low concentration of pesticide is beneficial for *Azotobacter* species while higher concentration decreases no. of cells and Nitrogen fixing ability.

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