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The Role of Cadmium Tolerant Strain of *Rhizobium*sps. On Promoting the Growth of Plant *Cicer arietinum*

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Abstract- The higher plants are able to take up heavy metals depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentration of other elements. The *Rhzobium*sps.is used as microbial inoculants to the cicer arietinum (chana) plant. Here, cadmium resistant bacterium is developed. The *Rhizobium sps*.Adapt resistance tolerant strain at 0.35 mg/l in Norris and Date medium on 7 day.The present study report clearly shows that Cd-t strain of *Rhizobium sps*.at0.35mg/l suitable for the growth and developmentof *Cicer* plant. The application of this strain is not only helpful to accumulation of the cadmium but also enhance the growth of the crop.

Key words: Heavy metal, cadmium,*strain, Rhizobium, Cicer*

1. Introduction

Cicer arietinum is important annual agricultural cash crop widely grown in India, Africa and America. The seeds are high in fiber, protein and are a good source of iron, phosphorus and folic acid. *Rhizobium* is Nitrogen fixing bacteria occurs in soil. This bacteria enhance the growth and yield of leguminous plant. Many soil bacteria are tolerant

to heavy metals and play important roles in mobilization or immobilization of heavy metal [1]. Plants require both macro and micro nutrients for their growth. However, in case of any metal deficiency, plants can regulate that deficiency,increase the metal availability in the root environment. The polluted soil with heavy metals resulted in a significant decrease of the activities of various soil enzymes like acid phosphatase and urase of microbes [2]. Structural changes of soil microbial community and the level of metal tolerance were increased in response to even slight metal contamination of the soil.

Cadmium and its compounds are water soluble and easily absorb and enter in an ecosystem. No physiological role of cadmium in human cellular metabolism has been reported so far and it is extremely toxic in minute quantity [3]. Several chemical and physical methods are used to remove cadmium from industrial effluent prior to release the effluent into the environment but all these methods are expensive and less effective [4]. Use of living and dead microbial biomass removes heavy metal ions including Cd from the environment. Along with metal toxicity, they are often additional factors that limit plant growth in contaminated soils including arid conditions, a lack of soil structure, low water supply and nutrient deficiency. Therefore improvement of plant growth under stressed conditions is determined. Microorganisms develop more resistance to heavy metals and adopt particular conditions containing toxic metal in their media [5].

In this investigation the *Rhizobium*sps.isolate and developed Cd- tolerant bacteria strain and determine its associations with roots and shoots accumulation and growth parameters of *Cicer- arietinum.*The creation of such metal tolerant plant microbes' relations or associations improving and promoting the growth of *Cicer* plant in heavy metal stressed soils.

2. Materials and methods

2.1 Selection and the determination of Tolerance Index Concentration (TIC) for *Rhizobium sps.*

In the present investigation *Rhizobium* sps.taken for metallic studies.An inhibitory level of organism of Cd was determined by selecting the range of 0.01 - 20 mgl^{-1}in Norris and

Date medium.Cd was added after the transfer of inoculums in the medium at the time of inoculation. The flasks were maintained at 26- 30°C with photon flux density 20-30 µ mol photon M^{-2} S^{-1} for 5-6 days. A strongly inhibitory level of organism was determined it under microscope after every 2,4,6 and 8 days against original inoculums.

2.2 Production of New Metal Resistant Strains on the basis of following observations -

i) Maximum concentration of metal causing no lag in bacterial growth.
ii) Minimum concentration of metal causing some inhibition of bacteria.
iii) Maximum concentration of metal at which bacteria is alive.
iv) Minimum concentration of metal at which bacteria is killed.

The tolerance index concentration was calculated as $(i.\ ii.\ iii.\ iv)^{1/4}$

2.3 Serial Sub culturing and developed Cd Strains

The media for toxicity were prepared and pH was adjusted. Metal solutions and the media were autoclaved separately and metal solutions were added after the transfer organisms. An experiment was performed in quadruplicate. The lowest concentrations of the metal solutions used in the present experiment were those of inhibitory level. Out of four test tubes of the respective concentrations, the test tube showing rapid growth was used as an inoculums for the further subculture.The process was repeated many times leading to gradually increasing strongly inhibitory level.

The inhibitory level of cadmium obtained by repeated culture upto 50 generations at concentration of the metal sufficient to cause a marked reduction in growth rate.The level of heavy metal just permitting detectable growth of *Rhizobium*sps.was raised from $0.10mgl^{1}$ to 0.50 mgl^{-1}.Theadapted strain isCd-t 0.35.

2.4 Biometric observations and sampling Technique

Plants were selected in four replication, labeled and biometric observation were recorded as-

a. Final plant stand was recorded by taking actual count of plants at harvest stage.

b. Plant Height was measured from the base of the stem upto the base of the growing tip. Theobservations were recorded at harvest.

c. Number of branches per plant-The number of branches arising from main stem was recorded on selected plants. The average number of branches per plant was calculated.

d. Number of developed pods per plant-The mature pods from observation plants was counted.

e. Grain weight per plant- Plants from each net treatment were threshed and grains separated and weighed. The average grain weight per plant was calculated.

f. Test weight-A random sample of 10 or 100 grains from the net produce were drawn and its weight was recorded as test height.

3. Results and Discussion

3.1 Selection and the determination of Tolerance Index Concentration (TIC) for *Rhizobium sps.*

The phenomenon of balanced growth simplifies the task of the measuring the growth rate of bacterial culture,since all components of the population is the same[6]. The bacterial cells are capable of binding large quantities of different metals [7].The growth as well as survival rate of Cd-t strain bacteria was high in medium as shown in table1.The strain shows tolerance and maximum concentration at 0.35mg/l.It is clear from the Fig.1 and table 2, day 7 showed the maximum growth of Cd-t strain. The growth of *Psudomonas* sp. decrease with the increasing Cd concentration upto 7ppm and increases growth in a Cd concentration above 7 ppm resulted in a proportional increase in the rate of growth [8]. The growth of metallic strain of Cu-t *Azotobacte*r and *Bacillus* were found at 0.36 and 0.40mg/l respectively [9].

Table1 Growth of W-strain and Cd-t (0.35) strain of *Rhizobium* sps. in Norris and Date medium

Amount of medium	OD of W-strain	OD of Cd-strain
10 ml	6.98	8.60

Table 2 Optical Density of W-strain and Cd-t (0.35) strain of *Rhizobium* sps. in Norris and Date medium

Day	OD W-strain	OD Cd- strain
1	0.121	0.124
2	1.52	1.64
3	1.99	2.04
4	2.93	3.11
5	3.45	3.92
6	5.18	5.88
7	6.98	8.60
8	7.80	7.78
9	5.40	6.56
10	4.21	5.01
11	3.18	4.68
12	2.55	3.72
13	2.18	2.97
14	1.60	2.01
15	0.88	1.89

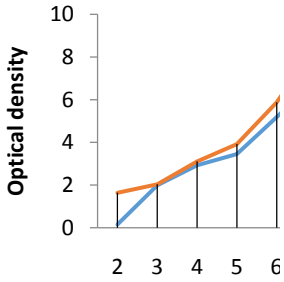

Fig. 1 Growth Curve of W-strain and Cd-strain of *Rhizobium*sps. in Norris and Date Medium

3.2 Effect of Wild and cadmium strains of *Rhizobiumsps. onCicer arietinum*

The individual effect of wild and cadmium strains of bacteria, the maximum height, number of pods and branches, grain yield/plant and test weight/100 seeds were obtained when cadmium strainis applied to this crop followed by with or without combination wild strain of *Rhizobium*sps as shown in table 4. There were slightly significant effect as compared to the W-strain of *Rhizobium sps*.Cadmium concentrations in normal plants ranged from 0.1-2.4 ppm [10], but at higher concentration it has been shown by adverse effect to plant growth and dry matter yield [11]. The zinc strain of biofertilisers significantly influence over all parameters as compared to single or combined used of wild strain of *Azotobacter* and *Bacillus* [12]. [13] shows that adding Zn

and Cd individually into a hyperaccumulator plant (P. griffithii) accumulation of both metals in roots, petioles and leaves was significantly increased.Cd- contamination resulted in increased total chlorophyll content in tomato and decreased total biomass [14]. However, Cd-tolerant PGPR are present in the root zone of metal accumulating plant B. juncea grown in soils contaminated with heavy metals and originating from different geographical regions [15]. The dissolved soil organic substances have significant effects on heavy metals transformations by increasing the solubility of heavy metal, root growth, and plant uptake [16] [17].A remarkable result was obtained by [18]. Who reported a partial loss of Cd-t tolerance of *H. lanatus*after cultivation of an uncontaiminated soil for 6 yrs, indicating a phenotypic plasticity for Cd-resistance [19].

Table 4 Application of Wild and cadmium (0.35) strains of *Rhizobium sp*. On *Cicer arietinum* Plant

Treatment	Control	W-strain	Cd-t strain (0.35mg/l)
Plant height(cm)	20.5	25.0	27.7
Number of branches per plant	10	14	14
Number of pods per plant	20	26	28
Grain yield per plant (gm)	4.0	5.98	6.4
Test Weight per 100 seeds (gm)	21.12	23.42	23.60

4 Conclusion

The bacterial cells are capable of binding large quantities of different metals. Adsorption equations may be useful for describing bacterium-metal interactions with metals such as Cd or Cu[7]. *Rhizobium sps*. Can adapt and tolerate cadmium. Under stress environment due to the industrial effeluents etc. the non essential toxic metals are releae and contaminated water used by the farmers to their plant. So the application of Cd tolerant bacteria can help not only to improve the quality of the crops but also the enhance the soil microflora. The use of this Cd-t strain on cicer plant shows that there is no adverse effect on the yield of crop.

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