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RESEARCH **P**APER

Isolation and functional characterization of cadmium resistant strains RCP 1 using agronomical parameter of Chaulai (*Amaranthus tricolor* L.)

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Resistance and tolerance are arbitrarily defined terms frequently interchangeable and after based on weather a given isolate can grow in the presence of selected heavy metal concentration in laboratory media. Total bacterial strains were isolated and screened on the basis of their heavy metal tolerance ability for cadmium and their PGPR activity. It was estimated that they have good tolerance power for cadmium the heavy metals and are plant growth promoting rhizobacteria. If we study morphological aspects we can say that Gram negative rods are more efficient in tolerating heavy metals. In case of treatment I, fresh weight was increased 22.22 per cent and dry weight was increased 20.73 per cent compared to positive control. In case of treatment II, fresh weight was increased 42 per cent and dry weight was increased 20 per cent compared to negative control. In case of negative control, fresh weight was decreased 56.52 per cent and dry weight was decreased 50 per cent compared to positive control. By performing experiment it was proved that the cadmium concentration reduced significant percentage of chaulai fresh weight and dry weight but increased when treated with strain RCP 1.

Key words : Rhizobacteria, Tolerance, Resistance, Functional characterization, Cadmium

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INTRODUCTION

Heavy metal is natural constituent of the earth crust and is present in varying concentration in all ecosystems. Heavy metal is chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Copper, chromium, cadmium are known to be the most commonly metal used and more widespread contaminants of their environment (Patterson, 1977; Aksu, 1998; Doenmez and Aksu, 2001). Heavy metal could have long term hazardous impact on health of soil micro-organism and adverse influence on soil biological process (Khan *et al*., 2009).

Cadmium is one of the most toxic pollutants of the surface soil layer, released into the environment by mining and smelting activities, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels (Tang *et al*., 2006).

Cadmium is heavy metal of no nutrition function, it is a natural element in the earth crust and usually found

as a mineral combined with other element such as oxygen (cadmium oxide), chlorine(cadmium chloride), sulfur(cadmium sulfate) and is a toxic agent to microorganism, but some are microbial strain resistant to the metal . The present investigation was carried out to isolate and functional characterization of cadmium resistant strains RCP 1 and its effect on chaulai plant in cadmium contaminated soil.

RESEARCH METHODOLOGY

Collection of soil samples :

Soil sample was collected from the rhizospheric soil of plants from Panki power house. The collected soil samples and strains were stored at 4°C. The details of soil samples collected from rhizosphere are:

Sr. No.	Soil sample	Source	Plant	Colour	Texture	Arran-gement	pH
1.	RCP	Rhizo-sphere	*Calotropis - procera* (Madar)	Dark brown	Rough	Clumpy	9

Isolation of sample

Soil sample (2 g) was grounded and suspended in 20 ml sterile water. Then isolation of the sample was done by serially diluting it and 0.1 ml of diluted suspension was placed on nutrient agar medium and spread evenly on solid plates. The plates were incubated at suitable temperature for different time intervals. The screenings of the strains were done on the basis of their tolerance power with cadmium concentration (Ahmad *et al.*, 2008).

Heavy metal tolerance :

Stock solution of cadmium was made (1 g in 25 ml distilled water). Different concentration of cadmium in media was pipetted and tolerance power of the strains was determined (Mark, 1987).

Identification of bacteria :

Bacterial colonies were identified by gram staining, starch hydrolysis test, gelatin hydrolysis test, indole test, methyl red test, voges porskauer test, citrate utilization test, triple sugar iron agar test, urease test, catalase test and H_2S production tests (Verma, 2013).

Plant growth promoting activity :

Phosphate solubilization:

The phosphate solubilization by the rhizosperic microbes was detected and estimated by the method (Tank and Saraf, 2010).

HCN production :

HCN production was performed by the method of (Bakker *et al.*, 1987).

Siderophore production :

Siderophore production by selected bacterial cultures was detected (Schwyn and Neillands,1987). Both qualitative and quantitative determination of siderophore was done.

Screening of physiological characterization of bacterial strains :

Temperature and pH were determined for screening of physiological characterization of bacterial strains.

RESEARCH FINDINGS AND ANALYSIS

Soil microbial population is known to play a key role in maintaining soil fertility by recycling of plant nutrients and also for removal of metals in contaminated soils. In this context various approaches have been used to study microbial activities in soil. As it is difficult to elucidate soil microbial activity using single monitoring approach, therefore, for a better insight and complete depiction of soil microbial situation, different approaches need to be used. In present investigation total of 4 bacterial isolates were isolated from rhizospheric soil of *Calotropis procera* (Madar) from Panki power house and tentatively identified on the basis of morphological and biochemical characterization. All bacterial isolates belonged to the Gram negative groups.

Table 1 : Heavy metal tolerance of soil micro-organism

Sr. No.	Rhizosphere	Strains	Cd (µg/ml)	Incubation time	Incubation temp.	Media
1.	*Calotropis procera* (Madar)	RCP 1	1400	18-24 h	28°C	Nutrient agar
		RCP 2	600	18-24 h	28°C	
		RCP 3	700	18-24 h	28°C	
		RCP 4	490	18-24 h	28°C	

Heavy metal tolerance :

Among all these strains maximum tolerance for cadmium was shown by strain RCP 1 as shown in Table 1.

Characterization of bacteria :

Specific soil strains were isolated from soil sample along with concentration of cadmium on nutrient agar medium. These isolates were tentatively identified on the basis of their staining, behaviour, morphological and cultural characteristics as shown in Table 2.

The histogram showed the comparative analysis between root length in presence and absence of cadmium. In case of treatment I (Media + seeds + strain RCP 1) root length was increased 30.55 per cent compared to positive control (Media + seeds). In case of treatment II (Media + seeds + strain RCP 1 + Cd Conc.) root length was increased 39 per cent compared to negative control (Media + seeds + Cd conc.) In case of negative control (Media + seeds + Cd conc.), root length was decreased 44.44 per cent compared to positive control (Media + seeds) as shown in Fig. 1 and 2 and Table 4.

The histogram showed the comparative analysis of root and shoot length in presence or absence of cadmium.

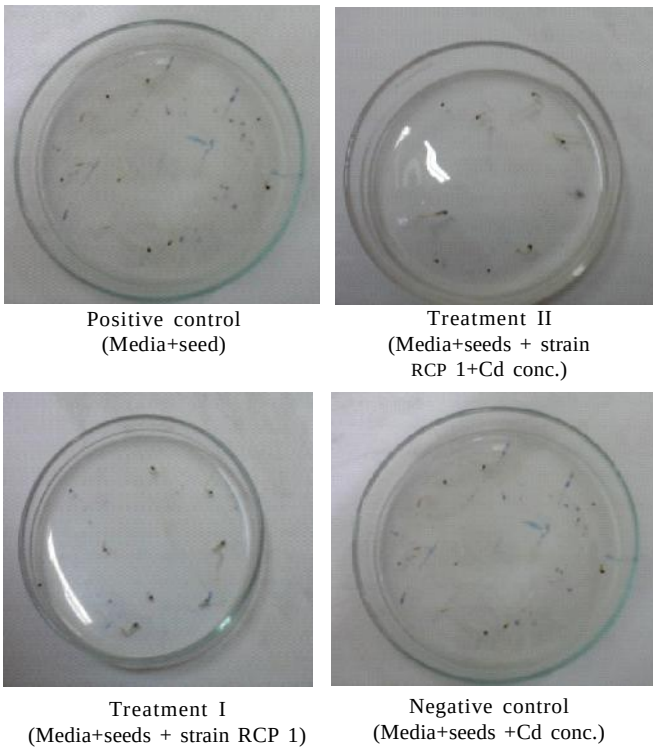

Fig. 1: Effect of microbial treatment of strain RCP 1 on chaulai seeds germination in presence or absence of cadmium: *In vitro* experiment

Table 2 : Morphological characterization of all isolated strains

Strains	Gram staining
RCP 1	Gram negative, rod shaped, fluorescent.
RCP 2	Gram negative, rod shaped.
RCP 3	Gram negative, rod shaped, fluorescent.
RCP 4	Gram negative, rod shaped.

Biochemical characterization of all isolated strains

Table 3: Biochemical characterization of all isolated strains

Sr.No.	Srains	Starch hydrolysis	Gelatinase	Indole production	MR	VP	Citrate utilization	Triple sugar iron agar test	Urease	Catalase	H_2S production
1.	RCP 1	+	+	+	+	+	+	+	-	+	-
2.	RCP 2	-	-	-	+	+	-	+	-	+	-
3.	RCP 3	-	-	-	-	-	-	+	-	+	-
4.	RCP 4	-	-	-	+	+	-	-	-	+	-

Functional characterization

Table 4: Effect of microbial treatment of strain RCP 1 on chaulai seeds germination in presence or absence of cadmium: *In vitro* experiment

Treatments	Root length (in cm)
Positive control (Media + seeds)	1.80
Treatment I (Media + seeds + strain RCP 1)	2.35
Treatment II (Media + seeds + strain RCP 1 + Cd conc.)	1.39
Negative control (Media + seeds + Cd conc.)	1.00

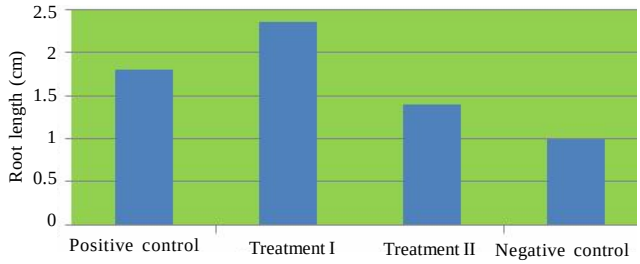

Fig. 2: Effect of microbial treatment of strain RCP 1 on chaulai seeds germination in presence or absence of cadmium: *In vitro* experiment

In case of treatment I (Soil + seeds + strain RCP 1), root length was increased 22.22 per cent and shoot length was increased 20.73 per cent compared to positive control (Soil + seeds). In case of treatment II (Soil + seeds + strain RCP 1 + Cd conc.), root length was increased 35.66 per cent and shoot length was increased 46.05 per cent compared to negative control (Soil + seeds + Cd conc.). In case of negative control (Soil + seeds + Cd conc.), root length was decreased 31.46 per cent and shoot length was decreased 30.06 per cent compared to positive control (Soil + seeds), similar results also reported by Rajkumar *et al.* (2006) and Belimova *et al.* (2005) (Fig. 3 and Table 5).

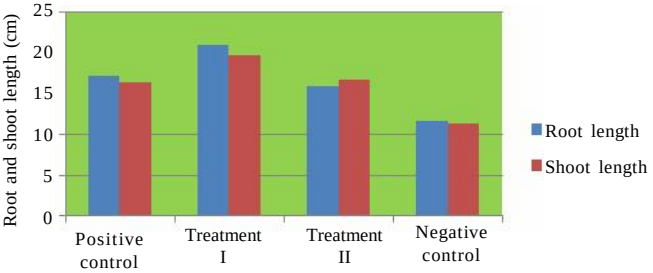

Fig. 3: Effect of microbial treatment of strain RCP 1 on chaulai seeds in pot experiment in presence and absence of cadmium (*In vivo*)

The histogram showed the comparative analysis of fresh and dry weight in presence or absence of cadmium. In case of treatment I (Soil + seeds + strain RCP 1), fresh weight was increased 13.04 per cent and dry weight was increased 23.33 per cent compared to positive control (Soil + seeds). In case of treatment II (Soil + seeds + strain RCP 1 + Cd conc.), fresh weight was increased 42 per cent and dry weight was increased 20.0 per cent compared to negative control (Soil + seeds + Cd conc.). In case of negative control (Soil + seeds + Cd conc.),

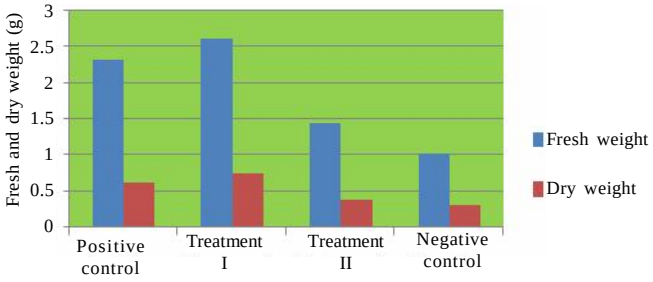

Fig. 4: Effect of microbial treatment of strain RCP 1 on chaulai seeds in pot experiment in presence and absence of cadmium (*In vivo*)

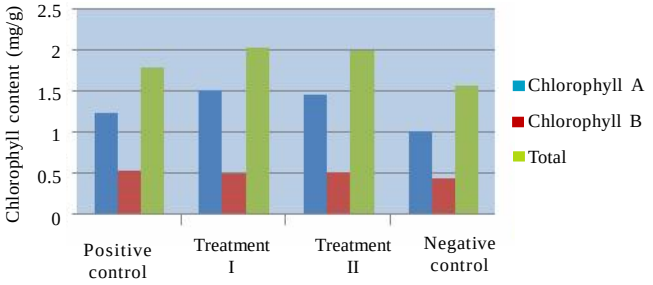

Fig. 5: Effect of strain RCP 1 on chlorophyll estimation of chaulai plant

Table 5 : Effect of microbial treatment of strain RCP 1 on chaulai seeds in pot experiment in presence and absence of cadmium (*In vivo*)

	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Positive control (Soil + seeds)	17.10	16.30	2.30	0.60
Treatment I (Soil+ seeds + strain RCP 1)	20.90	19.68	2.60	0.74
Treatment II (Soil + seeds + strain RCP 1 + Cd conc.)	15.90	16.65	1.42	0.36
Negative control (Soil + seeds + Cd conc.)	11.72	11.40	1.00	0.30

Table 6 : Effect of strain RCP 1 on chlorophyll estimation of chaulai plant

	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total (mg/g)
Positive control (Soil + seeds)	1.2303	0.5360	1.7852
Treatment I (Soil+ seeds + strain RCP1)	1.5012	0.5007	2.0243
Treatment II (Soil + seeds + strain RCP 1 + Cd Conc.)	1.4575	0.5061	1.9859
Negative control (Soil + seeds + Cd conc.)	1.0132	0.4401	1.5705

1 = Positive control (Soil + seeds) | 2 = Treatment I (Soil+ seeds + strain RCP 1) | 3 = Negative control (Soil + seeds + Cd conc.) | 4 = Treatment II (Soil + seeds + strain RCP 1 + Cd conc.)

Fig. 6: Effect of microbial treatment of strain RCP 1 on chaulai seeds in pot experiment in presence and absence of cadmium: *In vivo* experiment

1 = Positive control (Soil + seeds) | 2 = Treatment I (Soil+ seeds + strain RCP 1) | 3 = Negative control (Soil + seeds + Cd conc.) | 4 = Treatment II (Soil + seeds + strain RCP 1 + Cd conc.)

Fig. 7: Effect of microbial treatment of strain RCP 1 on chaulai seeds in pot experiment in presence and absence of cadmium: *In vivo* experiment

fresh weight was decreased 56.52 per cent and dry weight was decreased 50 per cent compared to positive control (Soil + seeds). By performing experiment it was proved that the cadmium concentration reduced significant percentage of chaulai fresh weight and dry weight but increased when treated with strain RCP 1 (Tripathi *et al.*, 2004 and Ahmed *et al*., 2008) as shown in Fig. 4, 6, 7 and Table 5.

Chlorophyll content increased due to inoculation of strain RCP 1. It provided various function in the plant as nitrogen fixation, synthesizing siderophores, solubilizing minerals as phosphate and other PGPR characteristics. But in presence of heavy metal, the chlorophyll content was decreased.

Heavy metal contamination of soil is often associated with iron deficiency in a range of different plant species. The low iron content of plans due to the presence of heavy metals generally results in chlorosis, since iron deficiency inhibits both chloroplast development and chlorophyll biosynthesis (Imsande, 1998 and Parekh *et al*., 1990). In the presence of metal there was significant reduction in percentage of chlorophyll content (Table 6 and Fig. 5).

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