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Original Article

Microfloral Studies in Rhizosphere of Plant *Cassia Angustifolia* Vahl Grown in Different Soil Treatments

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ABSTRACT

The present study was carried out on the isolation, quantitative and qualitative analysis of microflora from the rhizosphere soils of *Cassia angustifolia.* The plants were grown in pot culture experiments with three treatments in black soil, treatment No. 1 is a control soil without any addition, treatment No. II is soil introduced with Cadmium 10ppm, chromium 20ppm, nickel 16ppm, treatment No. III is soil with 1% of calcium hydroxide along with heavy metals and were grown up to productivity levels. The fungal population in (treatment II) heavy metal treated soil showed minimum number of cfu/gm soil as compared to other two treatment types. Fungal species composition is almost same in control soils whereas heavy metal + $Ca(OH)_2$ treated soil has increased number of fungal species. The (treatment II) heavy metal treated soil showed bacterial population in 10^3dilution and heavy metal + $Ca(OH)_2$ treated (treatment III) and normal soil showed high bacterial population when compared to heavy metal treated soil type

Key words: *Cassia angustifolia*, microflora, fungi, bacteria, rhizosphere soil.

1. INTRODUCTION

Cassia angustifolia Vahl is an important medicinal plant [1] commonly known as senna or cassia senna. It is distributed from India to Eritrea. Senna is one of the most widely used herbal laxatives [2] and is grown under different climatic and growing conditions. Senna is a branching shrub with a height up to 1.8m found in abundance throughout south India and other parts of the country .The leaves are large, compound and

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pinnate, the fully grown leaf lets are bluish green to pale green in colour, the flowers are bright yellow in colour many flowered racemes, the pods are slightly curved 3.5 to 6.5 cm long up to 1.5 cm broad. The plant contains sennosides A, B, C,and D. It also contain beta-sterol (0.33%) flavonols, kaempferol, kaempferin and isorhamnitin. Senna is used in medicine as a cathartic. It is especially useful in habitual constipation. The medicinal action of senna can be attributed mainly to the anthraquinone glycosides, especially Sennosides A and B[3] dried leaves and pods contain up to 7% sennosides.[4, 5, 6]

Among heavy metals to pollutants nickel is considered as an essential micronutrient for plants but is strongly phytotoxic at higher concentrations.[7] Cadmium can inhibit root and shoot growth, effects nutrient uptake and also frequently accumulated in agriculturally important crops.[8] Studies on long term exposure of heavy metals showed decrease in microbial diversity and metabolic processes.[9] Plants roots and soil microbes and their interaction improve metal bio availability in rhizosphere.[10] The role of natural plant population and multi species communities remains poorly understood.[11] It is a habitat for a vast interactive community of rhizosphic micro organisms whose activities largely determine the physcio-chemical properties of the rhizosphere soil.[12] Soil as a living system inhabits assorted cluster of living organisms, both micro flora (fungi, bacteria, algae and actinomycetes) and micro fauna (protozoa, nematodes, earthworms, moles, ants).[13] Keeping in view the importance of this environment friendly micro flora, the present work was under taken in selected plant of *Cassia angustifolia*. The present study helped us in obtaining more meaningful and realistic knowledge of distribution, diversity and composition of nickel, chromium and cadmium tolerant microbial communities associated with the above plant.

2. MATERIALS AND METHODS

2.1 Soil Preparation and collection

Plants were grown in pot culture experiments with three treatments in black soil at Botanical Garden, Department of Botany, Osmania University, Hyderabad and India.

Treatment no. I - A control without any addition to the soil. Treatment no. II – Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil. Treatment no. III – 1% of Calcium hydroxide was also added along with heavy metals to soil, and were grown up to the productivity levels[14]. The rhizosphere soil samples were collected by digging out soil roots of *Cassia angustifolia* plants grown in pots. Randomly selected plants were uprooted carefully and the excess of soil was removed by gentle shaking and the soil adhering to roots formed composite samples. The collected samples were placed in plastic bags and kept at 4°C in the laboratory until processed. Three such samples were collected for each pot culture and pooled and mixed together into a single.

2.2 Isolation of fungi and bacteria from Rhizosperic soils

Soil samples were collected at random, minimum five samples from each experimental pots mixed thoroughly to make a composite sample for micro biological analysis. Fungi and bacteria were isolated from the rhizosphere soil samples of *Cassia angustifolia* grown in three different experimental pots by serial dilution plate technique. Potato Dextrose Agar media was used for isolation of fungi and incubated at 27°C for 2-7 days. Nutrient Agar media was used for isolation of bacteria and incubated at 27°C for 1 day. Isolated colonies were counted and (cfu) forming units per gram of soil was calculated.

Cfu or viable cells/gm of dry soil = mean plate count X dilution factor/Dry weight of soil taken.

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All the isolates were maintained at 4°C in equal volumes of nutrient broth and 30% glycerol.

The isolated fungi were identified on the basis of colony morphological characters up to genus level by following standard manuals.[15] Gram stain was used for isolated bacteria and all are gram negative. The details of fungal and bacterial population in different soil types and fungal species in three different soil types were clearly shown in table-1 and table-2 respectively.

Table 1: Fungal and bacterial population in different soil types

	Treatment I (control soil)		Treatment II (soil+ heavy metal		Treatment III (soil + heavy metal+1% $Ca(OH)_2$	
Organism	**Dilution**	**cfu per gm of soil**	**Dilution**	**cfu per gm of soil**	**Dilution**	**cfu per gm of soil**
Fungi	10^3	$48.86X10^3$	10^3	$45.45X10^3$	10^3	$50X10^3$
	10^4	$54.54X10^4$	10^4	$51.13X10^4$	10^4	55.6810^4
Bacteria	10^5	$56.81X10^5$	10^5	$48.86X10^5$	10^5	$54.54X10^5$

Table 2: Fungal species in different soil types

Fungal species	Treatment I (Control soil)	Treatment II (Soil+Heavy metal)	Treatment III (Soil+Heavy metal+1% $Ca(OH)_2$)
Aspergillus	+	+	+
Rhizopus	+	-	-
A.niger	+	-	+
A.flavus	+	-	+
Rhizopus nigrecns	+	+	-
Aspergillus sp's	+	-	+
Aspergillus flavipus	-	-	+

3. RESULTS AND DISCUSSION

Bacteria: The bacterial population in 10^{-5} dilution of treatment-I soil was $56.81X10^5$ cfu/gm soil, whereas in treatment-II soil, the bacterial population in 10^5 dilution was $48.86X10^5$ cfu/gm, in contrast to this the soils of treatment-III the bacterial population was 54.54 X 10^5 cfu/gm. The heavy metal treated soil supported low population in 10^5 dilution compared to the other treatment soil types. Soils of treatment I and treatment III shown the bacterial population was near to same. The bacteria isolated from three soil types were gram negative.

Fungi: The fungal population in treatment- I soil types is $48.86x10^3$and $54.54x10^4$cfu/gm soils. In treatment-II soils, the fungal populations are $45.45x10^3$and $51.13x10^4$cfu/gm soils. In treatment-III soil shown $50x10^3$and $55.68x10^4$cfu/gm soils. Treatment-II soils support low fungal population ($45.45x10^3$and$51.13x10^4$)in both the dilutions. The treatment-I and treatment-III soils shown nearly equal number of populations.

In the present soil types a total of 7 genera and two mycelia sterile were isolated. 5Species were Aspergillus, 2 Species of rhizopus 1 Species each Aspergillus, A.niger, A.flavus A.flavipus, Aspergillus sp. Rhizopus nigrecans, Rhizopus. In treatment-III soil, the Aspergillus, A.niger, A.flavus, A.flavipus sp. are present. In all the three soil treatments only Aspergillus sp. are present.

4. CONCLUSION

The fungal population in (Treatment II) soils showed minimum no. of cfu/gm soil as compared to other two soil types. Fungal species composition is almost near in treatment-I and treatment-III soils. The treatment-II soil showed minimum bacterial population in 10^5dilution compared to the other soil types. Treatment-I and Treatment-III showed nearly equal to maximum bacterial population. Finally the heavy metals reduced the fungal growth, it may be one of the reason expected to decrease the fungal growth

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