



IMPACT OF FLY ASH ON MORPHOLOGICAL, CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF *RHIZOBIUM MELILOTI* OF *MEDICAGO SATIVA*

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ABSTRACT

The present investigation is an attempt to study the impact of fly ash on *Rhizobium* bacteria of root nodules isolated from legume plants and to study morphological, cultural, and biochemical characteristics of bacterial strain obtained from selected legume i.e. *Medicago sativa*. *Rhizobia* inhabited in root nodules of *Medicago* plant, grown in fly ash amended soil (60% soil + 40% FA), *Rhizobia* was isolated and inoculated on Yeast Extract Mannitol Agar (YEMA) medium and its morphological, cultural and biochemical characteristics were studied. It was observed that colonies were circular or irregular; light creamish, glistening, gelatinous, convex with entire margins. The bacteria was gram negative, rod shaped, aerobic, non spore forming and slow moving bacteria arranged single, in pairs and in clusters. It showed negative chemical reaction for indole, methyl red, Voges-Proskauer and hydrogen sulphide, while showed positive reaction for citrate, catalase, urease and nitrate reduction. By the help of biochemical characteristics it was confirmed that isolated bacterial culture was of *Rhizobium meliloti* and fly ash in the concentration of 40% does not have any negative effect on the characters of *Rhizobium*, our findings was supported by many earlier investigations.

INTRODUCTION

Legumes are unique plants which have the ability to work with certain bacteria i.e. *Rhizobia*, (inhabit root nodules) to gather available nitrogen from the soil atmosphere and convert it into usable ammonia and make it available to the plant. Biological nitrogen fixation is a component of sustainable agriculture and Rhizobial inoculants have been applied frequently as bio-fertilizers. Each major legume group is nodulated by different species of *Rhizobium*. *Medicago sativa* - a common legume plant of Hadoti region is selected for study purpose. *Medicago sativa* also known as Alfalfa, Lucerne, Sativa (Family- Fabaceae) is an erect, ascending, smooth herbaceous legume that is much-branched glabrous perennial, 30-90 cm, with alternate trifoliate leaves. It is deep-rooted, (2-4m or more) in well-drained soils. (Ecocrop. FAO.Org). It is one of the highest yielding forage legumes and requires deep, well-drained fertile soils to maximize its potential. The inherent growth characteristics and good yield response to infrequent cutting make lucerne a highly suitable species for conservation as hay or silage. It is mainly grown as a fodder crop. It is grown as a cover crop to reduce erosion. A very unique character It is a nitrogen

fixer and estimates of annual rates range from 85 to 360 kg N/ha with a wide variation among sites. Similarly its fibres can be used in the manufacture of paper. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. A Deep-rooting ability is an important factor in drought tolerance and any adverse soil physical or chemical conditions, which restrict root growth, will reduce drought tolerance. Compared with many forage species, Lucerne is an efficient user of water supply largely as a result of its deep taproot system. During severe drought, plants become dormant but resume growth when moisture becomes available. So it is used as forage directly and as residues, this is used as manure and fuel. These properties make it a useful tool for agro forestry.

Medicago sativa is nodulated by *Rhizobium meliloti* (Subba Rao 1977). Fred et al. (1932) recognized eight cross inoculants group in legumes. The genus *Rhizobium* was erected by Frank (1890) based on its characters to form nodules on roots of legume plants. This property is the only valid test in the identification of the organism. Apart from it some diagnostic features of *Rhizobium* could be conveniently not only determine and identify the organism but also delineate

different species (Graham and Parker 1964, Vincent 1970, Gaur 1975, Mahana 1981). Review of literature indicates that during last decade there is decrease in soil quality of Kota region. It is also observed that due to lower content of various ions, production of legumes is also decrease. Due to decrease in legume production fertility of soil is badly affected. It was observed that fly ash which is generated from Thermal Power Station situated in Kota District, affect soil quality of study area.

The impact of coal residues on environment and health consequences has been reviewed extensively, conventional disposal methods for Fly ash lead to degradation of arable land and contamination of ground water (Jala and Goyal 2006). Now we move towards the utilization of fly ash in soil amendment and agronomy for wealth generation as well as pollution control because various previous researches and chemical analysis support that fly ash is a potential source of many macro and micro nutrients (Aggarwal and Gupta 1993, Singh et al. 1997). Whitish grey colour fly ash is mostly alkaline (pH 7.5-8.2) and hydrophilic in nature so that fly ash is a useful ameliorant that may improve the physico-chemical and biological properties of soil (Shridharan and Pandian 1998, Haynes 2009). Although it contain almost all the plant nutrients but deficient in Organic Carbon i.e. N and P (Rai et al. 2002). An integrated biotechnological approach to revegetation seems appropriate and should be investigated further. This problem may overcome by addition of organic manure and microbial inoculants in the fly ash and use of inoculated legumes to add N. Fly ash has impact on soil quality of study area. Soil quality may affect microbial population. In the light of above facts present research is undertaken to know the impact of fly ash on cultural, morphological and biochemical characteristics of *Rhizobium meliloti* of *Medicago sativa*.

MATERIALS AND METHODS

Isolation and Purification: Isolation and Purification of *Rhizobium* strain was done as described by Graham and Parker (1964). Healthy and mature pink colored nodules of selected Plant grown over fly ash amended soil (40% Fly ash + 60% soil) were collected and were washed thoroughly under tap water and surface sterilized with 0.1 % mercuric chloride and then 95% ethanol and crushed aseptically in sterile water blank. This nodule suspension was then serial diluted (10⁻⁵ to 10⁻⁷) streaked on the sterilized yeast extract mannitol agar (YEMA) medium plates containing Congo red

and incubated at 26 to 30°C temperature for 5-7 days. After incubation for 4-6 day transparent to white single colonies were transferred to YEMA slants described by Graham and Parker (1964).

Characterization of isolates:

The cultural and morphological as well as bio-chemical characteristics of the isolates were studied following the procedure given by Aneja (2008).

Cultural characteristics:

The shape, colour, opacity, margin and elevation of the colonies of the test isolates grown on standard YEMA plates were observed.

Morphological characteristics:

The shape, oxygen demand, motility, spore formation and Gram stain reaction of *Rhizobial* cells were observed under microscope using standard procedure.

Biochemical characteristics

Biochemical characteristics of the *Rhizobium* isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja (2008).

To analyse the impact of fly ash on Biochemical characteristics of *the Rhizobium meliloti*, of *Medicago sativa*, it was treated with 40% concentration of fly ash collected from Thermal Power Plant area.

RESULTS AND DISCUSSION

The experimental results depict that *Medicago sativa* demonstrate that no marked difference in response to 40% concentration of fly ash under pot conditions. Table-1 elaborate that colonies were circular or irregular; white creamish, gelatinous, opaque, convex with entire margins. The bacterium was gram negative, rod shaped, aerobic, non spore forming and motile. From Table-1 it was clearly observed that Indole was not produced after incubation of isolated *Rhizobial* inoculants in tryptophan broth. Similarly Methyl red and Voges-proskauer reaction were examined in glucose phosphate broth by adding methyl red and α -naphthol solution with KOH respectively. Citrate was utilized as a sole carbon source in Simon's citrate medium. Ammonia was produced by degradation of urea available in to the urea broth containing phenol red as an indicator by the bacterium inoculated. Catalase activity was observed by stirring the

Table 1. Cultural, Morphological and Biochemical Character of *Rhizobium meliloti*

Sr. No.	Characters	Result
1.	Shape	Circular
2.	Colour	White creamish
3.	Opacity	Opaque
4.	Margin	Regular/entire
5.	Elevation	Convex
6.	Shape	Rod shaped
7.	Oxygen demand	Aerobic
8.	Motility	Motile
9.	Spore formation	Non spore forming
10.	Gram's nature	Gram Negative
11.	Production of Indole from tryptophan	Negative
12.	Methyl red test	Negative
13.	Voges-Proskauer test	Negative
14.	Citrate utilization as source of carbon	Positive
15.	Production of ammonia from urea	Positive
16.	Catalase test	Positive
17.	Nitrate Reduction	Positive
18.	Production of Hydrogen peroxide	Negative

culture in a drop of hydrogen peroxide (10% by W/V). Nitrate was converted to nitrite by inoculants of Rhizobial strain. Production of hydrogen sulphide gas examined by SIM Agar method. It showed negative chemical reaction for indole, methyl red, voges-proskauer and hydrogen sulphide, while showed positive reaction for citrate, catalase, urease and nitrate reduction.

Morphological, cultural and biochemical characteristics of different *Rhizobial* strains have been studied by investigators like Allen and Allen (1981), Bisset (1959), Muthuswamy et al. (1973), Mahana (1981), Garg et al. (1991) and Oblisami (1974). Staining reactions of *Rhizobial* strains showed that *Rhizobium* is gram negative (Allen and Allen 1950, Graham and Parker 1964, Mahana 1981, Garg 1991). Our findings also supported these results but report of Bisset (1959) showed that isolates of *Rhizobium* from tropical legume were gram positive. Similar to the work of various workers (Graham and Parker 1964, Basak and Goyal 1980 and Garg 1991) we observed that the colonies of *Rhizobium* were circular, white glistening and attained normal growth within seven days growth when grown on YEMA medium. The morphological, cultural and biochemical characteristics of the purified *Rhizobial* strain which were purified after treatment with 40% of fly ash, indicate that there was no significant difference in *Rhizobial* strain collected from control. But it

may possible that higher concentration of fly ash may alter the cultural, morphological and biochemical characters of *Rhizobium meliloti*. So that further study is required in this direction.

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