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IMPACT ANALYSIS OF FLY ASH ON CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF *BRADYRHIZOBIUN JAPONICUM*

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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 the morphological, cultural, and biochemical characteristics of bacterial strain obtained from selected legume i.e. *Glycine max. Rhizobia* inhabited in root nodules of plant, grown in fly ash amended soil (80% soil + 20% FA), *Rhizobia* was isolated and inoculated on Yeast Extract Mannitol Agar (YEMA) medium and it's morphological, cultural and biochemical characteristics were studied. It was observed that colonies were circular or irregular; light creamish, glistering, 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 bio chemical characteristics it was confirmed that isolated bacterial culture was of *Bradyrhizobium japonicum* and fly ash in the concentration of 20% does not have any negative effect on the characters of *Rhizobium*, our findings was supported by many earlier investigations.

Key word- *Bradyrhizobium japonicum, Glycine max,* Fly ash

Introduction

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. Glycin*e max a common legume plant of Hadoti region is selected for the study purpose. *Glycine max* is nodulated *by Bradyrhizobium japonicum*[1]. Fred et al., (1932)[2], recognized eight cross inoculants group in legumes. The genus *Rhizobium* was erected by Frank (1890)[3] 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 [4-7]. 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[8]. 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 [9-10]. 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 [11]. Although it contain almost all the plant nutrients but deficient in Organic Carbon i.e. N and P [12]. 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 *Bradyrhizobium japonicum* of *Glycine max*.

Material and Methods

Isolation and Purification: Isolation and Purification of *Rhizobium* strain was done as described by[4]. Healthy and mature pink colored nodules of selected plant grown over fly ash amended soil (20% Fly ash + 80% 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 [4] .

Characterization of isolates: The cultural and morphological as well as bio-chemical characteristics of the isolates were studied following the procedure given by [13].

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 [13]. To analyse the impact of fly ash on Biochemical characteristics of *the Bradyrhizobium japonicum*, of *Glycine max*, it was treated with 20% concentration of fly ash collected from Thermal Power Plant area.

Result and Discussion

The experimental results depict that *Glycine max* demonstrate that no marked difference in response to 20 % concentration of fly ash

Table 1. Cultural, Morphological and Biochemical Character of *Bradyrhizobium japonicum*

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

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-napthol 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 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* strain have been studied by investigators like [14-17]. Staining reactions of *Rhizobial* strains showed that *Rhizobium* is Gram negative.[18,4-7] Our findings were also supported these results but report of [15] showed that isolates of *Rhizobium* from tropical legume were gram positive. Similar to the work of various workers [4,19] results of our research indicates 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 Character of *Bradyrhizobium japonicum*. So that further study is required in this direction.

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