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Research article

IMPACT OF AM FUNGI ON DEHYDROGENASE ACTIVITY IN POLLUTED SOIL

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ABSTRACT: Rhizoremediation is a technique which uses the rhizospheric microbiota to remediate the pollutants. Dehydrogenase enzyme is considered to exist in soil as integral part of intact cells. Dehydrogenase activity in soils provide correlative information on the biological activity and microbial populations in the soil. When AM fungal spores were added to the phytoremediating legume plants the dehydrogenase activity tends to increase up to levels of control where no pollutant was present. The highest increase was found in *Cicer arientinum* on addition of AM fungal spores i.e. 16.4 µg INTF g^{-1} soil in control but rhizoremediated treatment gave increased activity up to 40.7µg INTF g^{-1} soil. *Phaseolus mungo* shows a very minimal difference in all three treatments.

Keywords: Rhizoremediation, microflora, AM fungi, dehydrogenase activity

INTRODUCTION

Awareness about harms of pollution in the scientific world has aroused in 1990s but now even general public is highly concerned about this issue. A cost effective and highly ethical method *Rhizoremediation* which involves the breakdown of contaminants in soil resulting from microbial activity that is enhanced in the plant root zone called rhizosphere, the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms can be used to remediate pollutants. Soil is a dynamic living resource and biologically mediated processes are central to its ecological function. Key soil microbial processes include degradation of organic residues, transformation of soil organic matter, mineralization and immobilization of nutrient and formation and stabilization of soil aggregates [1].

Microorganisms in soil are not only affected by the physical and chemical properties of the soil in general, but also by the moisture, temperature, pH and organic matter released from the roots in the rhizopheres of plants, all of which alter the microbial diversity and activity [2]. Due to extreme weather conditions like extreme variations in temperature and moisture, organically poor soils, with limited amounts of bioavailable inorganic nutrients, the plants are highly adaptable to dry as well as physiologically dry conditions (salinity) in the arid regions, the microbial flora also has high adaptability to cope with frequent droughts and various stresses such as starvation, high osmolarity, high temperature, and desiccation and variation according to the ecosystem of the desert [3].
Both plants and soil microorganisms have certain limitations with respect to their individual abilities to remove/breakdown organic compounds. A synergistic action by both rhizosphere microorganisms that leads to increased availability of hydrophobic compounds (act as biosurfactants) and seems to offer a favourable environment for co-metabolism of soil bound and recalcitrant chemicals [4,5] and plants that leads to their removal and/or degradation, may overcome many of the limitations, and thus provide a useful basis for enhancing remediation of contaminated environment.

AM fungi is plant microbe interaction where plant provides typical compounds exuded by the plant roots including amino acids, enzymes, proteins, organic acids, carbohydrates, and other cellular materials while AM fungi provides phosphate solubilization, increasing root surface area, water and mineral availability and also contribute by adding MHBs (mycorrhiza helping bacteria) to the rhizosphere. Soil microbes are the driving force behind many soil processes including transformation of organic matter, nutrient release and degradation of xenobiotics [6]. The measurement of enzyme activity of whole microbial community presents a useful means to evaluate the impact of pollutants on soils [7]. All the transformations of nutrients in biogeochemical cycles in soil are stimulated by enzymes by which they became available to plants and animals. The enzymes are frequently referred to as markers of soil environment purity [8]. Dehydrogenase enzyme is thought to be an indicator of overall microbial activity, because it occurs intercellularly in all living microbial cells, and is linked with microbial oxidoreduction processes [9]. It represents the oxidative activities of soil microbe by transferring hydrogen or electrons from substrates to acceptors and mineralizing capacity in soil and are suitable to assess broad- spectrum biological activity in short term [10]. Thus, the application of AM fungi as microflora to rhizosphere of phytoremediating legumes must show an increase in dehydrogenase activity, thereby, showing that the microbiota increases in polluted site when AM fungal population is exogenously added.

MATERIAL AND METHODS

Soil sampling for spore isolation

For the purpose of spore collection Rhizosphere soil samples (soil adhering to the roots) were collected at 30-90 cm depths along with root samples in five replicates from *P. cineraria* from Boranada industrial area of Jodhpur. Before sampling, the soils from the upper layer were scrapped off to remove foreign particles and litter. All the soil samples collected in self locking polyethylene bags from the rhizosphere of a particular plant species were homogenized replication wise before processing by sieving (< 2 mm mesh size) to remove stones, plant material and coarse roots. Sample of each soil was air dried to isolate the microbes present.

Spore isolation and mass culture

Spores from the rhizospheric soil were extracted by the Wet-sieving and Decanting technique [11] and sucrose centrifugation method [12]. The mass culture was done by growing the grass *Cenchrus ciliaris* in the soil collected from the polluted soil for six week duration. After that the plants were uprooted and roots were cleaned and chopped to 1 cm pieces and were used as inoculants (as a source of AM fungi). The presence of infection of AM fungi in roots was carried out by root staining method [13].

Soil treatment

The soil used was sterilized sand-soil mixture (1:1), with pH of 8.3, available P of 2.35 ppm, total N of 2.31 ppm, and organic carbon of 0.13 percent.

The medium crude oil (MCO) of 600 mg kg^{-1} concentration was utilized for contaminating the soil prepared above. Physical properties of MCO were as follows: specific gravity 0.87, API gravity 30.7 API degree, Reid vapor pressure 2.5 kPa, viscosity at 15 ^{0}C 21.4 CST, pour point -23 ^{0}C, and sulfur content 2.6 wt.%.

Healthy and viable seeds of five leguminous crop (*Dolichos diflorus*, *Cicier arientinum*, *Phaseolus vulgaris*, *Phaseolus aureus* and *Phaseolus mungo*) were surface sterilized. These seedlings were transplanted as five replicates each as;

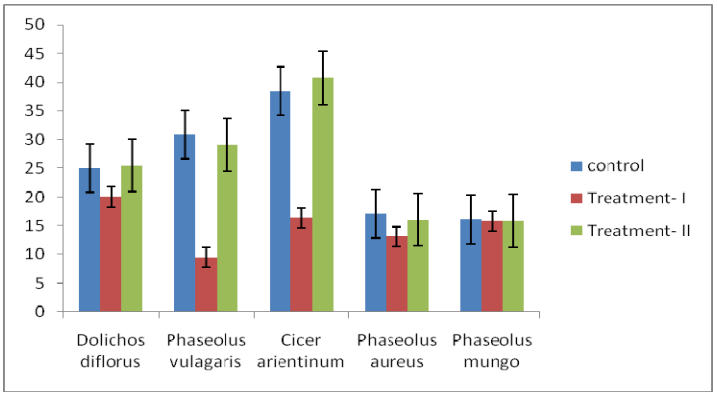
1) As control in non contaminated soil with one seedling per container
2) As phytoremediating legume plants treatment in soil with 2 kg of contaminated soil.
3) As rhizoremediating legume plants treatment in soil containing mixed population AM fungi which was added by using 40 g of roots of *Cenchrus ciliaris* as inoculants in each replicate.

Soil dehydrogenase activity

Soil dehydrogenase activity was estimated to check the active microbial activity in the rhizospheric soil. The measurement of soil dehydrogenase activity was done in the fifth week after planting the seedlings by iodonitrotetrazolium chloride (INT) method [14].

RESULT AND DISCUSSION

In the above experiment it was found that the control containing non contaminated soil and with AM fungal inoculation has higher degree of dehydrogenase activity in all the five replicates in each of the five leguminous plants (*Dolichos diflorus, Cicier arientinum, Phaseolus vulgaris, Phaseolus aureus* and *Phaseolus mungo*) as compared to the treatment where the 600 mg kg^{-1} concentration of MCO (medium crude oil) was used with the legumes. This observation shows that the stress in the polluting environment reduces the microbial growth as well as their activity. While, when the third treatment was done, where the crude oil contaminated soil-legume interaction was inoculated with the roots of *Cenchrus ciliaris* as AM fungal inoculants the activity again enhanced to the level of control in each case. Moreover, in *Cicer arientinum* the third treatment of rhizoremediation shows better enzyme activity (40.7 µg INTF g^{-1} soil) as compared to control (38.5 µg INTF g^{-1} soil). The chick pea also showed highest increase from the phytoremediating treatment (16.4 µg INTF g^{-1} soil). In the case of *Phaseolus mungo* the dehydrogenase activity did not show much changes in all three treatment. So, it is not a better option for remediation purpose. After cow pea, *Phaseolus vulgaris* was the most suitable phytoremediant showing increase of 29.1 µg INTF g^{-1} soil in treatment third (Am fungi and contaminant) as compared to 9.5 µg INTF g^{-1} soil in treatment second (only contaminant). The Histogram- 1 shows the results of dehydrogenase activity after statistical analysis performed by SPSS 16 software and comparison of means was done with Duncan test at 5% LEVEL. Thus, studies of dehydrogenase enzyme activity in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility and therefore, also enhances the rhizoremediation activity. Soil dehydrogenase activity is often used as a measure of any disruption caused by pesticides, trace elements or management. So, the experiment represented decrease when disrupted by MCO and again enhanced when stress was decreased by using AM fungi.



Histogram: 1: The dehydrogenase activity in the legumes in the fifth week of plantation

CONCLUSION

The dehydrogenase enzyme is produced in intact microbial cell and its activity denotes the active microbial population of soil. Rhizoremediation is a tool which copes with limitation of phytoremediation and microbial bioremediation. The above experiment proved that *Cicer arientinum* (a legume) is better performing rhizoremediation plant than the other four legumes followed by *Phaseolus vulgaris.* We can conclude that AM fungi reduces the abiotic stress which is result of pollution and enhances microbial population in rhizosphere due to almost similar results for control and rhizoremediation treatment.

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