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EFFECTS OF HEAVY METALS ON GROWTH AND NODULATION OF *RHIZOBIUM SP.* IN *VIGNA RADIATE* (L.) WILCZEK

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Abstract

Nowadays heavy metal contamination is widespread in the lithosphere as well as in the hydrosphere. Huge territories of land have been debased with substantial metals because of the utilization of pesticides, manures, and fertilizer squanders, and furthermore because of overwhelming metal discharge from purifying industries and metalliferous mines. To avoid the contamination of heavy metals from polluted water and soil phytoremediation process is used, which includes plants like some floating hydrophytes and legumes. *Rhizobium* is a genus of gram-negative soil bacteria that fix nitrogen by symbiotic association with leguminous plants and usually form root nodules. Treatment with $CdCl_2$ and $NiCl_2$ had the most harmful impact on the growth of Rhizobium. Nodulation boundaries e.g. “number of plants nodulated, number of nodules per plant, number of nodules per gram fresh weight of the roots, nodule dry weight per plant, and weight of the nodule were inhibited, the hindrance being fixation subordinate”. The impact was indistinguishable in all the overwhelming metal salts tried. Inhibitory consequences for nodulation have been evaluated because of the restraint of the development of Rhizobium by the impact of the overwhelming metal salts. These Rhizobia can be utilized as inoculants for crop legumes under ominous ecological states of agrobiological systems or as of late recovered desert.

Key words: Agrobiological, Heavy metals, Legumes, Nodules, Rhizobium

Introduction

Metals with generally high densities, atomic weights, or atomic numbers are recognized as substantial metals. They may be characterized on the basis of their chemical behavior. Accumulation of these heavy metals puts negative impacts on the growth of plants and its physiological activities. Some metals (heavy metals) are also there physically in the soil (Giller, et al., 1998). Many geologic and anthropogenic activities increasing the concentration of these elements to that much amount that they became harmful to both plants and animals. Growth reduction is the result of changes in biochemical as well as physiological activity in plants that grow on heavy metal contaminated areas. These heavy metals have also toxic effects on many free living microorganisms. The free living nitrogen fixing bacteria also effected which leads to fluctuation in nitrogenase activities and growth. This results in the inhibition of nodulation and growth of leguminous plants. If the reduction in plant growth continues that will lead tofood insecurity. That’s why the remediation of heavy metal is needed. The present examination was embraced to research the job of Cd^{+2} (cadmium) and Ni^{+2} (nickel) on growth of *Rhizobium* as well as on nodulation in *Vigna radiata* belongs to family Fabaceae (Aloui et al., 2011; Jali et al., 2019).

Materials and methods

Collection of soil and compost

Vigna radiata seeds are planted in four different pods containing 1.25kg of soil and 0.25 kg of compost. Each pod contains 5 to 7 seeds. The seeds ought to be surface cleaned with 0.1% $HgCl_2$ solution for 10 min. followed by washing with distilled water and incubated for 1 hour with a one-night culture of *Rhizobium* sp. at 30° C. Treaded crops were planted in the pods. The pots were irrigated with water on an alternative day and with supplement arrangement after every 15 days. Then they are allowed to grow with proper watering and care under control condition till the formation of root nodules (Zhigang et al., 2006).

Heavy metal application

After conforming about the formation of root nodules in each plants, the plants of one pod are allowed to grow under control condition and the plants of other three pods are applied with different heavy metals like $NiCl_2$ and $CdCl_2$. The heavy metal concentrations were determined on a soil weight premise. The soil was altogether blended and permitted to equilibrate for seven days. The treated soil was then placed in the cases. The heavy metals are firstly diluted with water at the ratio 5mg/10 ml. The plants both under ordinary control conditions and applied with heavy metals are then permitted to develop in the nursery. Following 30 days of development, the plants were expelled from the units alongside the soil (Edwards et al., 1982, Handique et al., 2009).

Figure 1. Plants treated with heavy metals: Nickel Chloride ($NiCl_2$) and Cadmium Chloride ($CdCl_2$)

Weighing of components

Soil is taken in a container from the lower part of the plant grown in control condition. The soil is diluted with distilled water for several times. Then the preparation of nutrient medium should be done by adding proper components of it. The components like nutrient agar, sucrose, etc. added in proper ratio by the help of weighing machine and mixed with 100ml of distilled water (Turnau, 1998).

Bacterial culture

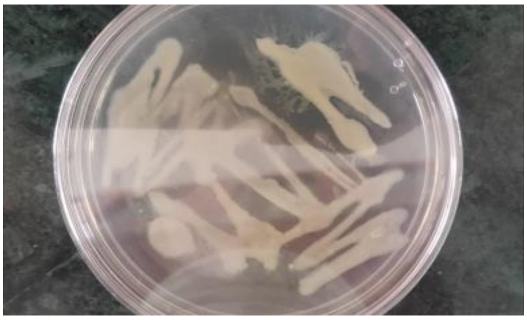
Then the media stored in flask and autoclaved at 120 pressure. Other equipment and container used should also be sterilised in autoclave. Then the media poured in sterilised petridishes and allowed to cool. Then the soil sample taken and bacteria are cultured by streak plate method over the prepared medium. It is done over the cooled solid nutrient agar medium with the help of sterilised loop. Then the bacteria are allowed to grow overnight at room temperature (37º C). All the container and media should be kept in laminar air flow to avoid contamination. After one day (24 hours) it can visualize the grown bacterial colony over the solid medium (Mahalik et al., 2017; Behera et al., 2020).

Figure 2. Bacterial colonies grown in nutrient agar

Rhizobial colony grown in YEMA medium

After that the colonies grown in the nutrient agar medium are taken and spreaded on the YEAM medium by streak plate method by sterilised loop. Then in the culture medium rhizobium bacteria are allowed to by incubation of 3/4 days (Garg,and Aggarwal, 2012).

Figure 3. Growth of *Rhizobial* colony in YEMA medium



Addition of heavy metals to the medium

As this medium is specific for rhizobium growth then the bacteria grown are identified as rhizobium sp. "After that different concentrations of Nickel Chloride ($NiCl_2$) and Cadmium Chloride ($CdCl_2$) were added into 50 ml of the YEMA bacterial growth medium after autoclaving. The cultures were inoculated with 1 ml of an overnight bacterial culture (Rhizobium sp.)" (Garg,and Aggarwal, 2012).

Results and discussion

After adding of heavy metal in the media, the growth of bacteria seen. And also the plants treated with heavy metals ($NiCl_2$, $CdCl_2$) also put several impacts on number nodules and nitrogenase activity of it. The expansion of heavy metal to the 4 days old culture media it was observed that there is radically decreased the development of the Rhizobium colony (Table 1.). $CdCl_2$ was the most harmful of the overwhelming metal salts tried. All the substantial metal salts tried diminished the boundaries e.g. the number of plants nodulated and the number of nodules per plant, the number of nodules per gram fresh weight of root (Table 2), nodules dry weight per plant, and weight per knob (Table 2).

Table 1. Growth of *Rhizobium* sp. in 4 days old culture media in different concentration of heavy metals (OD A_{560})

ppm	0	25	50	75	100
$NiCl_2$	1.15	0.25*	0.01*	0.012*	0.016*
$CdCl_2$	1.16	0.03*	0.02*	0.03*	0.04*

N.B. * Indicated significant difference from the control at 1% level

Effects on nodulation

The root nodules per plant decreases , there was a higher activation of the stores to the rest of the root nodules, prompting an enlarge in the root nodules dry weight. The decline in the number of nodules per plant brought about by these heavy metal salts might be because of the injurious impact of these metals on the development of *Rhizobium*, on the other hand, as the root development be hindered by these overwhelming metals, fewer destinations were accessible for the contamination procedure. It might be conceivable that both of these variables together reason a restraint of the nodulation procedure. The heavy metal i.e. $CdCl_2$, utilized in this investigation decreased the acetylene reduction action of the nodules. “Restraint of the acetylene reduction movement with heavy metal salt treatment has been accounted for in a few legume *Rhizobium* symbioses”. The system of the hindrance of acetylene decrease movement isn't clear and potentially includes a few elements like direct obstruction with the enzyme protein and lower accessibility of the photosynthate. Heavy metals are known to repress or inhibited photosynthesis.

Effects on legume Rhizobium symbiosis

Vegetable *Rhizobium* advantageous interaction is profoundly touchy to heavy metal harmfulness. With respect to the outstanding situation of nickel, it appears to be qualified to take note of that nickel has end up being a fundamental component in Fabaceae being a segment of urease, a catalyst engaged with the preparation of nitrogen stockpiling substances in vegetable seeds. Further investigations are required to assess the current inconstancy as far as metal harmfulness for this procedure just as to grow heavy metal resilience for advantageous nitrogen fixation.

Rhizobium beneficial interaction is profoundly delicate to heavy metal poisonousness. With respect to the remarkable situation of nickel, it appears to be qualified to take note of that nickel has proved up being a basic component in Fabaceae being a segment of urease, an enzyme engaged with the preparation of nitrogen stockpiling substances in legume seeds. Further experiments are required to assess the current fluctuation regarding metal toxicity for this procedure just as to grow heavy metal resilience for advantageous nitrogen fixation.

Table 2. Effect of heavy metals on fresh weight and dry weight of nodules

A- Fresh weight						B- Dry weight				
ppm	0	25	50	75	100	0	25	50	75	100
$NiCl_2$	45	34.0*	27.6*	15.9*	5.2*	6.1	7.0	7.2	11.5*	5.4
$CdCl_2$	45	28.8*	27.3*	3.7*	0.3*	6.1	6.0	7.5	7.0	5.0*

N.B. * Indicated significant difference from the control at 1% level

Conclusion

The present investigation revealed that the natural nodulation of wild legumes are drastically affected by habitat. The *Rhizobium* isolated from root nodules of plant and grown harsh environmental conditions differs from rhizobia from cultivated legumes. The extreme presence of heavy metals is harmful to crop production. Thus extreme accumulation of these heavy metals in soils must be prohibited. There are some species of *Rhizobium* that they can survive in heavy metal condition. This *Rhizobium* could be valuable in agricultural practice, specifically in the inoculation of crop legumes grown under unfavourable conditions or in the new reclaimed soil. Now a days the legumes grown in the industrial soil can also help for phytoremediation as well as economic value of the country.

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