EFFECT OF HEAVY METALS ON THE STRUCTURE AND FUNCTION OF ECTOMYCORRHIZAE OF PINE (*Pinus kesiya* Royle Ex. Gordon)

ABSTRACT

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THESIS SUBMITTED
IN FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN BOTANY

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1999

Abstract

The present investigation was undertaken to study the toxicity of heavy metals on the structure and function of ectomycorrhizal fungi and their ability to colonize the pine seedlings.

Four mycorrhizal fungi forming ectomycorrhizae with Khasi pine (*Pinus kesiya* Royle ex Gordon) were selected. Six heavy metals were applied separately in pots as well as in field condition to the mycorrhizal and non-mycorrhizal pine seedlings. The study sites were located in pine forest stand of permanent campus, North Eastern Hill University, Shillong (1500, m msl). One site was located 1 m higher than the other. Pine seedlings inoculated with mycorrhizal fungi and treated with different concentrations were studied for one year to study the structure of mycorrhizae, enzyme activity in root region and nutrient uptake by the seedlings.

The fungal colonization was maximum in seedlings treated with lower concentration of metals as compared to higher concentration.

Heavy metals concentration showed negative correlation with mycorrhizal colonization.

Heavy metals were toxic to the development of ectomycorrhizae. High concentration of metals was specially toxic to the external mycelium, which ultimately resulted in reduction of ectomycorrhizal development. All the four ectomycorrhizal fungi could withstand the heavy metal toxicity upto 50 ppm.

However, the high concentration of metals limited the production of mycelium and its colonization. It was observed that non-mycorrhizal pine seedlings were more sensitive to the toxic effect of heavy metals than ectomycorrhizal ones.

Considerable differences were found in the levels of metals deposition in different ectomycorrhizal fungi inoculated seedlings. Significantly greater quantity of heavy metals was analysed in non-mycorrhizal roots than in mycorrhizal ones. Maximum concentration of Zn and Cu as compared to Cd, Pb, Ni and Al was observed. Cd and Pb were most toxic to ectomycorrhizal fungi than other heavy metals. The response of ectomycorrhizal fungi to heavy metals varied and that the response dependent on the ectomycorrhizal fungal species and the metal concentrations. The accumulations of metals in the mycelium and protecting root against metal toxicity was an important factor. All mycorrhizal fungi tested had an ameliorating affect on the reduction of metal concentration in the root tissue. There was a significant variation in the metal contents ($P < 0.01$) between mycorrhizal and non-mycorrhizal seedlings.

Heavy metals had deleterious effect on the activities of urease, dehydrogenase and phosphatase in the rhizosphere soil and root surface of pine seedlings. The enzyme activity was lower in rhizospheric soil than the root surface.

There was a decrease in urease, dehydrogenase and phosphatase activity when treated with high concentration of heavy metals. The degree of inhibition was dependent on the amount of metals added and the contents of soil organic matter. The degree of inhibition of enzymes was smaller in ectomycorrhizal roots

than those of non-mycorrhizal ones.

Dehydrogenase activity was highest during summer and lowest during winter. The activity of dehydrogenase was more in root surface than in the rhizosphere soil in all the four seasons. Cu and Zn did not reduce the dehydrogenase activity significantly in *Boletus* sp. inoculated seedlings. Highest percentage of inhibition of dehydrogenase was observed in Cd, Pb and Ni treated rhizosphere soil. Among the four ectomycorrhizal fungi, *S. aurantium* inoculated roots showed the least seasonal variation but highest activity. Pb and Ni showed maximum inhibition in dehydrodenase activity rhizosphere soil and the root surface. In non-mycorrhizal rhizospheric soil, Zn and Ni caused upto 50% inhibition and Pb and Cu caused upto 44% inhibition on root surface dehydrogenase activity. It was observed that in absence of ectomycorrhizae, heavy metals drastically reduced the enzyme activity. Dehydrogenase activity was more in mixed mycobionts inoculated roots and the rhizosphere soil than in single inoculum treated root surface and rhizospheric soil. Highest percentage of inhibition in enzyme activity was observed in Al and Pb treated root surface and its rhizosphere soil.

The urease activity was apparently less at 100 ppm and above of Zn, Cu, Ni, Cd, Pb and Al treated pots. Soil urease activity was less as compared to the root surface. The highest activity of urease was observed in autumn and lowest during winter. Ni and Cd were found more toxic to urease activity than rest of the metals. Highest percentage of inhibition of urease activity was observed in *S. aurantium* treated seedlings.

The degree of inhibition of urease activity was more on non-mycorrhizal root surface and its rhizosphere soil than in mycorrhizal ones.

The urease activity in root surface and rhizosphere soil was higher in mixed mycobionts inoculated seedlings than the single fungus inoculated ones. In Zn and Cu treated seedlings, root surface and rhizosphere soil showed maximum and minimum inhibition of urease activity respectively.

In two seasons, phosphatase activity was discernable, one with a higher activity in autumn and summer and another with a markedly low activity in winter and spring.

Increased inhibition in phosphatase activity was observed at higher concentration of heavy metal treated seedlings. The highest percentage of inhibition of phosphatase activity was observed in *S.aurantium* inoculated soil and Pb treated root surface followed by Ni treated rhizosphere soil. Minimum inhibition was observed in *Boletus* sp. inoculated seedlings treated with Cd and Cu.

Higher activity and lower inhibition percentage of phosphatase activity were observed in seedlings inoculated with mixed mycobionts. Maximum inhibition was observed in Zn treated seedlings and minimum in Cu treated seedlings.

There was a positive correlation between mycorrhizal infection and the activity of urease, phosphatase and dehydrogenase ($P < 0.01$). A negative correlation was found between the metal concentration and the activity of urease, phosphatase and dehydrogenase. A significant variation between the sampling periods ($P < 0.05$) and between mycorrhizal and non-mycorrhizal ($P < 0.01$)

was also found.

Insignificant difference in the growth of seedlings between the four ectomycorrhizal fungi inoculated seedlings was observed. The non-mycorrhizal seedlings showed significantly lower growth as compared to ectomycorrhizal seedlings. However, inspite of heavy metal contaminations, *C. graniforme*, *Boletus* sp. *S. luteus* and *S. aurantium* did significantly ($P < 0.01$ and $P < 0.05$) improve growth seedlings than non-mycorrhizal ones. There was a positive correlation between mycorrhizal infection and the growth of pine seedlings.

Inoculation of pine seedlings with ectomycorrhizal fungi consistently stimulated an increase in shoot height, number of needles, needle length, seedlings volume, root colar diameter, lateral root length and mycorrhizal infection throughout the growing seasons as compared to non-mycorrhizal ones. The growth of both mycorrhizal and non-mycorrhizal seedlings without any heavy metal treatment was vigorous as compared with that of plants exposed to heavy metals.

Shoot height was improved in *S. luteus* inoculated seedlings with an average of 7 cm followed by *Boletus* sp. and *C. graniforme* treated seedlings as compared to 4.5 cm in non-mycorrhizal seedlings. Minimum shoot height was observed in Pb and Al treated seedlings.

Root length was reduced by 100 ppm and more concentrations of all the heavy metals treated seedlings. *C. graniforme* infected seedlings treated with Cu showed maximum root length. Minimum root length was observed in Zn treated non-mycorrhizal seedlings.

Seedling volume was maximum in Zn and Cu treated and

S. luteus infected seedlings. Number of needles was more in ectomycorrhizal seedlings compared as to non-mycorrhizal ones. *C. graniforme* inoculated seedlings showed more number of needles and minimum in *S. aurantium* inoculated seedlings.

Length of needle did not vary significantly in different treatments of heavy metals in ectomycorrhizal and non-mycorrhizal seedlings.

Mycorrhizal infection was maximum in *S. luteus* inoculated and treated with Pb seedlings and minimum was noticed with *C. graniforme* inoculated and treated with Al.

C. graniforme inoculated seedlings showed highest percentage of survival followed by *Boletus* sp. and *S. aurantium* inoculated seedlings.

Seedlings survival and the growth were higher in mixed mycobionts inoculated seedlings as compared to single fungus inoculum seedlings. The percentage of inhibition of mycorrhizal infection also increased with increase in the metal concentration. Shoot height, needle length, number of needles, seedlings volume and seedlings survival were higher in mycorrhizal treated seedlings than non-mycorrhizal ones.

A trend in decrease in nutrient concentration of seedlings applied with different concentration of heavy metals was observed. The concentrations of nutrients as a whole were restricted by the presence in heavy metals.

More nitrogen contents was found in pine seedlings infected with *C. graniforme, S. luteus, S. aurantium* and *Boletus* sp. treated with Cd, Cu, Ni, Zn, Pb and Al compared to non-mycorrhi-

zal ones. The concentration of nitrogen decreased with the increase in heavy metals.

Seedlings infected with mixed mycobionts exhibited maximum uptake of nitrogen. Pb and Ni treated seedlings showed less nitrogen content as compared to Zn, Cu, Al and Cd treated seedlings.

P-contents in pine seedlings was also decreased with increasing concentration of heavy metals. P-contents was noticed maximum in *Boletus* sp. inoculated seedlings, while non-mycorrhizal seedlings showed lowest p-contents. Seedlings infected with mixed mycobionts showed more P-contents than single inoculum inoculated seedlings.

K-contents in pine seedlings was also low at high concentration of heavy metals. Ectomycorrhizal seedlings had more K-contents than non-mycorrhizal ones.

Insignificant variation in the contents of N, P and K between the various mycobionts was observed . However, a significant variation was found in the contents of nitrogen ($P < 0.01$), potassium and phosphorus ($P < 0.05$) between mycorrhizal and non-mycorrhizal ones and also between pots and field condition ($P < 0.01$).