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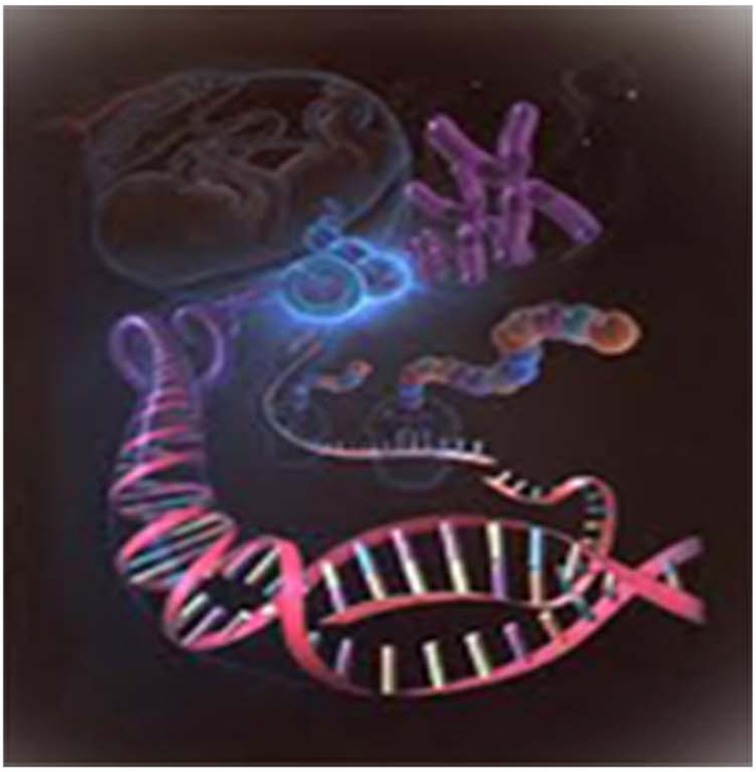

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Research Paper

NEUTRALIZATION OF HEAVY METALS TOXICITY BY *CENOCOCCUM GRANIFORME* IN THE ESTABLISHMENT AND SURVIVAL OF *PINUS KESIYA* (ROYLE EX GORDON) SEEDLINGS

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Pot experiment was carried out to study the effect of heavy metals toxicity by *Cenococcum graniforme* in the establishment and survival of *Pinus kesiya* seedlings. Different concentration levels, viz., 0 (control), 10, 50, 100, 200 and 500 ppm of Al, Pb, Zn, Cd, Ni and Cu, were added in pots separately containing three months old pine seedlings. The study was continued for one year. The presence of ectomycorrhizal symbiont showed a positive correlation between the mycorrhizal infection and all the growth parameters of the seedlings. Study revealed that, mycorrhizal colonization decreased with the increase of heavy metal concentration. In general, ectomycorrhizal fungi exhibited a resistance to different heavy metals resulting in better growth of pine seedlings than non-mycorrhizal ones.

Keywords: *Cenococcum graniforme*, heavy metals and *Pinus kesiya*

INTRODUCTION

Though studies on mycorrhizae have received enough attention during the past few years, yet their interaction in relation to environmental factors needs careful investigation particularly with respect to role of heavy metals. Due to various human activities, the heavy metals are enriched in soil. As a result, these heavy metals are transported to the environment by air and water and thereby increasing the metal concentration in the environment where they are bound. In this vast changing environment, mycorrhizae still function on a global scale under natural condition (Sharma and Ajungla, 2010). Some elements such as lead has a very short retention time in water, whereas zinc remain longer. Therefore, metals are concentrated in plant tissue depending on different types of the metals in different media (Forstner and Wittman, 1979).

Certain plants can resist high concentration of heavy metals on soils. Much has been investigated recently on the apparent role of mycorrhizae for their ability to survive in metal contaminated soil. Successful establishment and inoculation of isolated mycorrhizal fungi in field condition have been carried out with different plant

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species (Allen, 1974) and these species are able to grow in poor soil conditions due to their extensive mycorrhizal root systems (Koide and Lu, 1995). The growth and survival of the seedlings can also be related to the ways in which species can adjust their morphological and physiological characters to the environment (Imliyanger and Ajungla, 2012).

Several mycorrhizal fungi can protect their host against of heavy metals (Jones and Hutchinsons, 1986, 1988b). Toxic metals are numerous and vary and their action (Gadd, 1993). However many fungi show remarkable ability to survive and grow in high concentration of heavy metals. The survival of seedlings in contaminated sites determines its sensitivity to metal toxicity. Thus, mycorrhizal fungi may bind metals and detoxify them. The impact of this atmospheric change upon mycorrhizal function and the role, if any, of mycorrhizas in determining ecosystem responses to the perturbation are subjects of great importance (Smith and Read, 1997) which are beginning to receive attention.

MATERIALS AND METHODS

The experiment was performed with pine seedlings grown in pots containing sterile sand and soil mixture (1:3). In each pot, 15 seeds (surface sterilized with 5% NaOCl) were sown. After thirty days, the seedlings were inoculated with *C. graniforme* isolated from mature sporocarp tissues using modified Melin Norkan's (MMN) agar medium (Marx, 1969a). no fungal inoculums was added for control. After confirming the mycorrhizal establishment the seedlings were treated with different concentrations, viz., 0 (control), 10, 50, 100, 200 and 500 ppm of Zn, Pb, Cu, Ni, Cd and Al were treated (10 ml) in each pots. The study was continued for one year. The percentage of survival of seedlings was determined by

$$\text{Survivality (\%)} = \frac{\text{Total no. of seedlings at the time of harvesting}}{\text{Total no. of seedlings at the time of germination}} \times 100$$

Seedlings volume was calculated as D^2H or root colar diameter^2 X height (Marx, 1983)

$$\text{And the ectomycorrhizal (\%)} = \frac{\text{No. of lateral mycorrhizal rootlets}}{\text{Total no. of lateral rootlets}} \times 100$$

The data was processed by analysis of variance (ANOVA). Correlation coefficient was also calculated.

RESULTS

Inoculation of pine seedlings with *C. graniforme* consistently stimulated an increase in all the growth parameters studied. Seedlings exposed to metals treatment, specially 100 ppm and above showed reduced growth rate. However, mycorrhizal seedlings with any metal treatment exhibited vigorous growth.

Seedling roots treated with higher concentration of metals showed distorted primary root development and number of mycorrhizal roots were reduced and lateral root development was arrested. In lower concentration, 50 ppm and below there were no obvious effects on root morphology.

After a period of one year, seedlings showed maximum (78%) survival in Al treated seedlings (Table 1). While non-mycorrhizal seedlings ones showed lower (38%) survival especially Cd and Pb treated ones (Table 1).

Table 1. Establishment and Survival of 1 Year Old Pine Seedlings Inoculated With *C. Graniforme* and Treated With Heavy Metals

Metals conc. (ppm)	Shoot height (cm)	Seedlings vol. (cm^3)	Root colar dm (cm)	Survival (%)	Mycorrhizal infection (%)
0	8.2	0.512	0.25	93	88
Zn 10	6.3	0.333	0.23	88	64
Zn 50	6.0	0.317	0.23	80	53
Zn100	5.8	0.255	0.21	80	49
Zn200	5.6	0.246	0.21	73	46
Zn500	5.4	0.238	0.21	73	39
Ni 10	6.5	0.314	0.24	86	59
Ni 50	6.4	0.309	0.22	80	55
Ni100	6.0	0.264	0.21	80	54
Ni200	5.5	0.242	0.21	73	38
Ni500	5.0	0.162	0.18	73	36
Al 10	6.3	0.304	0.22	86	54
Al 50	6.2	0.300	0.22	86	45
Al100	6.0	0.290	0.22	84	41
Al200	5.5	0.140	0.16	71	32
Al500	5.4	0.138	0.16	71	32
Cd 10	6.6	0.238	0.19	86	63
Cd 50	6.3	0.227	0.19	80	61
Cd100	6.0	0.216	0.18	80	53
Cd200	5.9	0.206	0.20	78	42
Cd500	5.5	0.140	0.16	70	35
Cu 10	6.5	0.286	0.21	86	71

Mycorrhizal colonization was decreased as concentration of metals increased. The intensity of aberration was related to heavy metals. In general, ectomycorrhizal fungi exhibited a resistance to different heavy metals resulting in better growth of pine seedlings than non-mycorrhizal ones.

Table 2: Correlation Coeffecient (R) of Mycorrhizal Infection (%) With Various Parameters Of Mycorrhizal and Non-mycorrhizal Seedlings

Parameters	Mycorrhizal Infection (mycorrhizal)	Mycorrhizal infection (non-mycorhizal)
Shoot height	0.98*	NS
Seedlings volume	0.88**	NS
Root collar diameter	0.81**	NS
Survival	0.93*	NS

Note: * = significant at P<0.01 level; ** = significant at P<0.05 level

A positive correlation was found between mycorrhizal infection and shoot height, root length and survival (P<0.01) and seedlings volume and root collar diameter (P<0.05) in mycorrhizal seedlings (Table 2). There was a negative correlation between metal concentration and growth parameters.

A signification variation was found between sampling periods (P<0.01, P<0.05) of all growth parameters.

A signification variation was also found between mycorrhizal and non-mycorrhizal seedlings.

DISCUSSION

The ability of mycorrhizal fungi to grow in presence of heavy metals though to a much lesser extend was an important factor. It has been found that the ectomycorrhizal fungi increased the tolerance of pine seedlings to the treated heavy metals. At higher concentrations of metals however, inhibition of root extension as occurred. It is very likely that mycorrhizal fungi indirectly influenced the transport of heavy metals in plants. It appears the whole biomass in sporophore, the extrametrical mycelium as well as the mantle mycelia can complex a considerable amount of added metals and thus strongly reduced the toxic ions to the seedlings. It is also interesting to note that the greater root development in the seedlings treated with 50 ppm and below of heavy metals might be accounted for higher P accumulation. The metals primarily acted on the mycelium, the fungal tissue might also provide the host with protection against heavy metals depending upon the metal accumulation capacity of the mycelium (Ajungla *et al*., 2003). Cd, Pb and Ni are considered extremely toxic metals in plants causing disorders in photosynthetic machinery even at relatively low concentration resulting in growth reduction of seedlings (Tinker, 1981). The relation between mycorrhizae and heavy metal is quite complicated. The better growth, establishment and survival of mycorrhizal seedlings indicated that the mycorrhizal seedlings were more tolerant to the toxic effect of heavy metals. The severity of growth inhibition in non-mycorrhizal seedlings suggested that the metal accumulation is primarily responsible for seedlings damage.

CONCLUSION

Many seedlings are dependent on mycorrhizae for their growth, establishment and survival, one such example in the pine seedlings. Thus selecting a suitable ectomycorrhizal fungi for seedlings inoculums can be used in reforestation programs. Under stress environmental conditions, plants with mycorrhizal associations can adapt far better than the non-mycorrhizal plants (Sharma *et al.*, 2010), which is due to the fact that it can decrease the toxicity of heavy metals. Our results have shown that pine seedlings having mycorrhizal associations can tolerate toxicity more and even induce resistance up to a certain extent. Thus, ectomycorrhizal fungi (*Cenococcum graniforme*) play an important role in the growth of *Pinus kesiya* seedlings. The species of ectomycorrhizae used on various tree species in experimental inoculation program would show promise for eventual practical applications.

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