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Influence of copper and formalin on the mycorrhiza of pine (*Pinus kesiya* Royle ex Gordon)

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Various concentrations of copper sulphate and formalin were tested for their effect on the efficiency of mycorrhizal functioning in pine seedlings. Low and higher doses of copper applied to the container grown seedling exhibited a less stimulatory effect than nedium doses. When applied in higher concentrations, the formalin caused mortality in young pine seedlings. The seedling yield and phosphate uptake was found maximum in 100 ppm applied concentration of copper, while slow growth and lower phosphate concentration was observed in the seedlings not given any copper treatment. Formalin at 50 ppm concentration slightly improved the seedling growth and phosphate uptake in mycorrhizal seedling as compared with untreated ones. Variation in the development and spread of ectomycorrhiza on the surface of roots of pine seedlings was also recorded in responses to copper and formalin treatments.

INTRODUCTION

Investigations on mycorrhiza during last two decades have clearly revealed its contribution towards the enhancement of survival and establishment of forest tree seedlings (Marx 1980). Phosphorus uptake (Harley 1969; Marks, Kozlowski 1973; Mikola 1980), uptake of other nutrients (Bowen 1973; Gray, Gerdemann 1973; Sanders et al. 1975; Cooper, Tinker 1978; Rhodes, Gerdemann 1978) and water uptake (Safir et al. 1971) are enhanced by mycorrhiza. Ectomycorrhiza is also known to produce phytohormones (Slankis 1973) and provide physical, mechanical and chemical barriers to root pathogens (Marx 1969). It determines the behaviour and strategies of plants (Malloch, Malloch 1981; Wallace et al. 1982) and determines the successional pattern of an ecosystem Miller 1980; Reeves et al. 1979). It plays a crucial role in forestry practices (Trape 1981; Sharma, Mishra 1981). Efficiency and development of mycorrhiza may be altered by the increased use of fungicides in the forest management practices. Therefore, the present study was undertaken to investigate the effect of copper and formaline, commonly used in plant management practices.

MATERIALS AND METHODS

Collection soil and sterilization

The soil was collected from botanical garden, (pH 5.6; N 0.33%; P 1.4 ppm and K 0.25 mg/g) North-Eastern Hill University, Shillong (Megahalaya). The roots and litter were removed and the soil was sterilized chemically (Sharma 1981). The sterilized soil was filled in plastic pots (5 liter capacity).

Collection of seeds, sterilization, sowing and inoculation

The pine seeds were collected in the months of December and January, 1982 from Shillong (Meghalaya) and stored at 10°C. Sterilization of seeds was done by keeping them in 1% mercuric chloride for 10 minutes. Later on several washings were given with sterilized water. Germination of seeds was carried out in moist humid chambers at 30°C. The seedlings (2 cm. long radicle) were transplanted in sterilized soil. After one week of transplantation, treatment was given to the seedlings. The pine seedlings were inoculated with the *Scleroderma aurenteum*, mycorrhizal fungi (Sharma 1981).

Aplication of fungicides

The fungicides used were copper sulphate and formalin, with three concentrations i.e., 50 ppm, 100 ppm and 500 ppm. Each concentration of fungicides was applied at an interval of 20 days to the upper surface of soil avoiding the aerial parts of the seedlings.

Harvesting and measurement of growth of seedlings

Seedlings along with their root system were harvested after 20 days of fungicides application and brought to the laboratory for the growth, measurement and mycorrhizal estimation.

A. Growth measurement of seedlings

Measurement of growth parameters like shoot height (cm), leaf production (number) and leaf size (cm) was carried out before harvesting. The dry weight of seedlings was determined by drying washed pine seedlings in an oven at 80°C for about 24 hours (M i s h r a 1969).

B. Mycorrhizal establishment and its development

After each harvesting, the seedlings were washed under tap water for several times. Counting of bifurcated rootlets and the total number of roots for the estimation of mycorrhizal establishment was done under binocular microscope (S h a r m a 1981). Mycorrhizal roots were preserved in F.A.A. and were passed through the alcohol series (J o h a n s o n 1949). After filtration bloks were prepared and the serial transverse sections were out (10 - 15 μm), spread, stained in cotton blue mounted in canada balsam. The external and internal morphological characters were observed under a compound microscope.

Measurement of phosphate contents in seedlings

Dried seedlings (shoot, leaves and roots) were ground using a mortar and pestle to fine powder and passed through sieves (0.05 mm). 300 mg powdered material was taken in crucible and 3 ml of 0.1 N Mg NO_3 was added. It was evaporated to dryness and kept in Muffle furnace at 500°C for 3 - 4 hours. After it cooled down, 10 ml of 10 N H_2SO_4 was added. It was washed gently, digested in a hot water bath for about 20 minutes and filtered. Total volume was made up to 100 ml with double glass distilled water. Further analyses were carried out following the procedures given by A l l e n (1974).

RESULTS

Growth of pine seedlings

An increased shoot growth was observed with the increase of the copper concentration in comparison to untreated seedlings (Table 1). However, in the seedlings treated with formalin, a toxic effect was noticed at higher concentration i.e. 100 and 500 ppm. But at low concentration (50 ppm) the seedlings showed a slight increase in shoot height as compared with than untreated ones (Table 1).

The leaf production was induced with the increase of copper application up to 100 ppm (Table 1). In case of seedlings treated with formalin, at low concentration slight stimulation was recorded (Table 2). Higher

Table 1
Effect of different doses of copper and formalin on the shoot growth of mycorrhizal pine seedlings

Treatments		Shoot height /Cm/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	4.25	4.52	5.20	5.40
	100 ppm	4.43	4.72	5.40	6.30
	500 ppm	4.50	4.75	5.72	6.50
Formalin	50 ppm	4.38	4.43	5.8	6.0
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		4.30	4.40	5.2	5.40

concentration caused the death of leaves. Variation in leaf size of treated and untreated seedlings was not significant (Table 4).

The roots were also found to be longer in seedlings treated with 100 ppm dose of copper (Table 3). However, in formalin treated seedlings, low concentrations induced the root lenght, whereas higher doses caused seedling mortality (Table 3).

Maximum biomass was produced by the seedlings treated with 100 ppm copper (Table 6), while formalin could not initiate biomass

Table 2
Effect of different doses of copper and formalin on the leaf production of mycorrhizal pine seedlings

Treatments		Leaf production /No./			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	19	20	24	33
	100 ppm	23	25	28	40
	500 ppm	19	20	25	38
Formalin	50 ppm	22	24	28	34
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		20	20	23	33

Table 3
Effect of different doses of copper and formalin on the root production of mycorrhizal pine seedlings

Treatments		Root length /Cm/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	11.40	12.60	12.20	13.80
	100 ppm	13.70	15.80	16.90	18.20
	500 ppm	13.20	14.10	14.30	15.10
Formalin	50 ppm	11.20	13.80	14.90	15.5
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		10.20	12.20	13.60	14.10

Table 4

Effect of different doses of copper and formalin on the leaf size of mycorrhizal pine seedlings

Treatments		Leaf size /Cm/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	2.60	2.68	2.70	2.76
	100 ppm	2.70	2.76	2.79	2.85
	500 ppm	2.75	2.79	2.82	2.94
Formalin	50 ppm	2.69	2.72	2.85	2.84
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		2.34	2.56	2.65	2.71

accumulation. In this set untreated seedlings produced more dry weight than formaline treated seedlings (Table 6). Mycorrhizal seedlings treated with higher doses (500 ppm) of copper showed an adverse effect on dry matter production (Table 6).

Mycorrhizal establishment and its development

Copper application induced the mycorrhizal establishment and its development upto 100 ppm concentration then it declined slowly. The highest mycorrhizal association was observed at IVth harvesting in 100 ppm copper treated seedlings (Table 5). The seedlings treated with low concentration of formalin did not exhibit a significant difference with untreated ones. However, no mycorrhizal association could be observed at higher concentrations on application of formaline (Table 5).

Table 5

Effect of different doses of copper and formalin on the mycorrhizal establishment /%/ of pine seedlings

Treatments		Mycorrhizal establishment /%/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	52.3	54.1	62.8	68.3
	100 ppm	59.4	62.5	70.4	78.8
	500 ppm	56.4	58.2	66.3	70.5
Formalin	50 ppm	56.9	58.6	69.8	65.0
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		51.2	55.6	62.2	65.8

Table 6

Effect of different doses of copper and formalin on the dry weight of mycorrhizal pine seedlings

Treatments		Dry weight /g/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	0.400	0.460	0.540	0.560
	100 ppm	0.470	0.500	0.570	0.620
	500 ppm	0.430	0.480	0.560	0.580
Formalin	50 ppm	0.420	0.480	0.610	0.62
	100 ppm	Dead	Dead	Dead	Dead
	500 ppm	Dead	Dead	Dead	Dead
Control		0.243	0.263	0.420	0.560

Microscopic observations on Harting net development and fungal sheath formation showed very interesting results. Thickness of mantle increased in 50 and 100 ppm copper applied seedlings. Maximum thickness of mantle i.e., 52 μm was observed in last harvesting in the roots of 100 ppm applied copper, formaline produced thin and irregular mantle. Harting net was more poorly developed in the root formalin treated roots than in untreated seedlings.

Phosphate uptake

Phosphate concentration in seedlings increased in relation to the harvesting interval. No significant difference in phosphate uptake was observed in formaline and untreated seedlings, however, marked difference was observed in 100 ppm copper treated seedlings (Table 7). The higher concentration of copper reduced the phosphate concentration in pine seedlings.

DISCUSSION

Application of copper in laterite soil of low nutrient status may induce the efficiency of mycorrhizal functioning. Container grown mycorrhizal seedlings showed increase in shoot and leaf size production in relation to increased application of copper (Table 1 and 4). Mycorrhizal development, phosphate uptake and dry matter accumulation in pine seedlings was markedly enhanced by 100 ppm copper application, however, lower and higher concentrations of copper had little effect (Table 2, 3, 5, 6 and 7). Lower doses of fungicides are reported to be stimulatory (Iloba, 1979; William et al. 1980) suggesting that it may reduce the rhizospheric microbial population which may ultimately provide better competitive ability to the mycorrhizal fungus. The results also indicate that efficiency of mycorrhizal fungus may also be stimulated by adding copper up to certain concentration, which may function as nutrient in zinc deficient soil (Hayman 1980).

In contrast higher doses of formalin induced mortality in mycorrhizal seedlings within 20 days of its application (Table 1 - 6). The higher doses which are toxic to fungi and plant system (Bruck et al. 1981; Reilly, Lamourax 1981; Pataky, Lim 1981; Talboys et al. 1976; Englander 1980) may not be adopted in the field application. However, the control of certain soil borne diseases by fungicides is reported (Bakshi, Dabriyal 1970), this may be correlated in the present study with the application of low doses of formaline which showed slight improvement on mycorrhizal development and its efficiency in inducing seedling growth and phosphate uptake (Table 7).

Table 7
Effect of different doses of copper and formalin on the phosphate uptake of mycorrhizal pine seedlings

Treatments		Phosphate concentration /mg/gm/			
		Harvestings			
		I	II	III	IV
Copper	50 ppm	0.600	0.725	0.900	1.00
	100 ppm	0.775	0.925	1.075	1.20
	500 ppm	0.700	0.900	1.00	1.10
Formalin	50 ppm	0.575	0.700	0.725	-
Control		0.500	0.600	0.675	0.750

However, formaline application under field conditions may decrease the microbial population for a short duration (Nauman 1970). But the highest rate of formalin application may eliminate the soil organic matter, which may not be restored even after several years of cropping (Jenkinson, Powlson 1970).

The increase in mantle and Hartig not development in copper sets could be related to its stimulatory effect on mycorrhiza in acidic and zinc deficient soils (Hayman 1980), therefore, its role may be related to the increase in nutrient sink in copper treated roots (Table 7) but the higher dose of copper may reduce the phosphate uptake in acidic soil (Singh, Swarup 1982). The tribasic salt of copper, applied in field soil may decrease the rate of nitrification and mineralization. Mycorrhiza are proven to enhance the phosphate uptake (Heap, Newman 1980; Sharma 1981) and root area (Daft, Elgiahmi 1978; Marx 1979). This inherent property of mycorrhiza may very under various environmental conditions (Hayman 1980; Graw 1979). Therefore, increased phosphate uptake by mycorrhiza in 100 ppm copper may be attributed to the production of more mycorrhizal roots induced at a particular copper level (Table 5 and 7). Whereas, sparse and irregular development of mycorrhiza in other copper treated sets and in formalin treated seedling may elp'am lower phosphate uptake. Henceforth, further detail studies on morphology of ectomycorrhiza may reveal better understanding of inbuilt nutrient sink mechanism alterd due to various treatments.

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