J. Soil Biol. Ecol. 16(1) : 40-45 March 1996

Influence of Fungicides on Mycorrhizal Association, Seedlings Growth and Enzyme Activities in Root Region of *pinus kesiya* seedlings

A.K. SHUKLA, G.D. SHARMA, RAJ KUMAR AND R.R. MISHRA

Department of Botany, School of Life Sciences,
North-Eastern Hill University, Shillong - 793 022, India.

Abstract

Effect of fungicides carbendazim and mancozeb on the development of mycorrhiza, seedlings growth and enzymes activities in the root region of pine was studied. Both fungicides suppressed mycorrhizal formation as well as seedlings growth, however, a stimulation of percentage infection in mycorrhizal fungi was found with mancozeb treated seedlings at 500 ppm concentration. Phosphatase activity in roots, and dehydrogenase, urease and phosphatase activity in soil was also inhibited by the application of fungicides. Inhibition of enzyme activities increased with increasing concentration of fungicides.

Introduction

An integration of the activities by the microorganisms from the soil may be achieved by determining the activities of different enzymes in the soils. The biological activity in soil also provides better insight in understanding the transformation of organic matter (Trevors, 1984). Mycorrhizal association results in increased growth of plant, nutrient uptake, production of growth promoting substances, tolerance to drought, transplant shock and synergetic interaction with other beneficial soil microorganisms (Marks and Kozlowski, 1973). Various seed dressing and soil drenching fungicides are used in nurseries to raise seedlings free from soil borne plant pathogenic fungi (Marx *et al.*, 1986; Sreenivasa and Bagyaraj, 1989). These fungicides beside controlling pathogens affect non-target microorgaanisms and mycorrhizal fungi. Our aim was to investigate the effect of fungicides on the mycorrhizospheric region and seedling growth.

Material and Methods

The study was carried out near University campus (altitude 1500 m; latitude 25° 34" N, longitude 91° 54" E). The soil was sandy loam (Sand 65%, silt 13% and clay 22%). Various physico-chemical parameters of soil during the study period were : pH 5.8, organic carbon 1.2%, total nitrogen 0.045%, available phosphorus 0.011% and exchangeable potassium 0.031%.

The experimental plots were sub divided into seven micro plots, one square meter each and 50 cm distance was maintained from plot to plot. Sterilized pine seeds were grown in moist chamber at 30°C under continuous light condition. Seedlings (2-3 cm long radicle) were transferred to microplots and eighty seedlings were maintained for

each microplot. Sporocarps of *Scleroderma aurantium* were collected from pine forest in sterilised polythene bags and brought to the laboratory. Thereafter, inoculum of mycorrhizal fungus was prepared from the sporocarps (4.17×10^4 spores/ml) as suggested by Riffle and Maronek (1982). Mycorrhizal formation was established by adding to each microplot 500 ml of spore suspension after five days of seedlings transplantation.

Fungicides were sprayed to the upper surface of soil after one month of transplantation of the seedlings. Control was maintained without application of fungicides. The foliar spray of fungicides were continued at monthly interval upto six months of seedlings growth. The common names, chemical names, commercial names and manufacturers of the fungicides were as follows : Carbendazim, 2-) methoxy-carbamoyl) - benzimidazol, Bavistin, BASF India Limited; Mancozeb, Zinc ethylene bisdithiocarbamate, Dithane M-45, Indofil Chemicals Limited.

Seedlings along with their root system were harvested after 180 days of transplantation and brought to the laboratory for the measurement of different parameters. The root system of seedlings was gently washed under tap water for several times. The mycorrhizal and nonmycorrhizal rootlets were counted and observed for the development of mycorrhiza under binocular sterio-microscope. To determine dry weight of shoot and root seedlings were kept in hot air oven at 80°C for 48 hours. Acid phosphatase activity of mycorrhizal roots and soil attached to it was measured by the method of Dodd *et al.* (1987). Dehydrogenase activity of soil was determined by the method of Casida (1977). Urease activity was estimated as suggested by McGarity and Myers (1967). Statistical analysis of data was done by performing lattice square design (LSD).

Results and Discussion

Application of fungicides significantly reduced mycorrhizal infection, however, an enhancement of mycorrhizal infection was found at 500 ppm in case of mancozeb treated seedlings. All concentrations of carbendazim reduced the mycorrhizal formation. Inhibition of the mycorrhizal formation increased with increase in the concentration of the fungicides. In fungicides treated plots, shoot height and root length of seedlings always remained low compared to control. Dry weight of shoot and root was also adversely affected except in case of seedlings treated with 500 ppm concentration of mancozeb. Phosphorus content in shoot and root were slightly more compared to control with mancozeb (500 ppm) treated seedlings. Otherwise P content was found low compared to control with different concentrations of fungicides. Cudlin *et al.* (1983) observed that highest rate of fungicides had no phytotoxic effect although the growth of treated seedlings was reduced due to complete or partial inhibition of mycorrhizal formation. Stimulation of mycorrhizal development at low dose of pesticides was also recorded by Trappe *et al.* (1984) and Kumar *et al.* (1991). Root exudation is an important

Table I

Growth response and percentage of ectomycorrhizae of pine seedlings treated with fungicides

Treatment		MI (%)	Shoot			Root		
			Height (cm)	Weight (g)	P (%)	Length (cm)	Weight (g)	P (%)
Control		70.20	10.60	0.61	0.23	13.50	0.32	0.20
Mancozeb	500 ppm	86.00	8.74	0.57	0.25	11.30	0.33	0.21
	1000 ppm	70.00	8.24	0.53	0.22	10.20	0.30	0.20
	2000 ppm	61.00	7.00	0.48	0.21	08.20	0.29	0.19
Carbendazim	500 ppm	59.00	7.86	0.54	0.22	08.98	0.31	0.20
	1000 ppm	36.20	7.24	0.43	0.20	06.32	0.30	0.19
	2000 ppm	22.40	4.64	0.36	0.18	04.82	0.26	0.17
L.S.D. (p=0.05)		5.23	2.78	NS	NS	6.17	NS	NS

Table II

Enzymatic activity of soils and phosphatase activity of pine roots

Treatments			Dehydrogenase (μg TPF g^{-1} soil $24h^{-1}$)	Urease (μg $NH_4^+ g^{-1}$ soil $3h^{-1}$)	Phosphatase $^{-1}$ (μg p - nitrophenol g^{-1} h^{-1})	
					soil	root
Control			159	97	55.0	152.0
Mancozeb	500	ppm	143	75	52.8	148.8
	1000	ppm	124	61	45.6	131.8
	2000	ppm	63	51	41.8	121.2
Carbendazim	500	ppm	123	65	50.2	111.0
	1000	ppm	85	48	43.6	100.0
	2000	ppm	34	29	37.4	72.4
L.S.D. (p=0.05)			10.44	6.08	3.20	6.7

factor governing mycorrhizal colonization (Graham *et al.*, 1986) and pesticides can alter the quality and quantity of root exudation (Schwab *et al.*, 1982). Perhaps both fungicides at high rate altered the exudates which consequently inhibited the infection of mycorrhizal fungi.

Phosphatase activity in roots of fungicides treated plants was always lower than control. Dehydrogenase, urease and phosphatase activity of soil were also adversely affected with application of fungicides and always minimum values were recorded compared to control (Table II). Enzymes are indicator of microbial population and its status in the soil (Perucci and Scarponi, 1994). Perhaps due to alteration of root exudates and direct effect of fungicides on the microorganisms surrounding the symbiotic system of pine roots were also suppressed, which resulted in reduction of enzymatic activity of soils. Fungicides detrimental to mycorrhizal formation were also inhibitory to phosphatase activity in root surface and soil. Dodd *et al.* (1987) have reported that phosphatase activity in mycorrhizal root surfaces was directly correlated with mycorrhizal infection. Comparatively carbendazim was more toxic than mancozeb for inhibition of mycorrhizal formation, plant growth and enzymatic activity. It can be stated that low rates of fungicides are not much toxic but increase in concentration may be more harmful. Since ectomycorrhizal fungi are instrumental in root health and nutrient uptake of the plants, the influence of fungicides on ectomycorrhizae need to be identified and distinguished for any effect on the plant.

References

Casida, L.E., 1977. Microbial metabolic activity in soil as measured by dehydrogenase determination. *Appl. Environ. Microbiol.* **34:** 630-636.

Cudlin, P. Mejstrik, V. and Skoupy, J., 1983. Effect of pesticides on ectomycorrhizae of *Pinus sylvestris* seedlings. *Plant Soil* **71:** 353-361.

Dodd, J.C., Burton, C.C., Burns, R.G. and Jefferies, P., 1987. Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi. *New Phytoll.* **107:** 163-172.

Graham, J.H., Timmer, L.W. and Fardelmann, D., 1986. Toxicity of fungicidal copper in soil to citrus seedlings and vesicular-arbuscular mycorrhizal fungi. *Phytopathol.* **76:** 66-70.

Kumar, R., Shukla, A.K., Sharma, G.D. and Mishra, R.R., 1991. Response of mycorrhizal pine seedlings (*Pinus kesiya*) to fungicides. *Acta Botanica Indica* **19:** 157-161.

Marks, G.C. and Kozlowski, T.T., 1973. *Ectomycorrhizae - their ecology and physiology*. Academic Press New York and London, pp 444.

Marx, D.H., Cordell, C.E. and France, R.C., 1986. Effect of triadimefon on growth and ectomycorrhizal development of loblolly and slash pines in nurseries. *Phytopathol.* **76:** 824-831.

McGarity, J.W. and Myers, M.G., 1967. A survey of urease activity in soils of northern New South Wales. *Plant Soil* **27:** 217-238.

Perucci, P. and Scarponi, L., 1994. Effect of herbicide imazethapyr on soil microbial biomass and various soil enzyme activities. *Biol. Fert. Soils* 17: 237-242.

Riffle, J.W. and Maronek, D.M., 1982. Ectomycorrhizal inoculation procedures for greenhouse and nursery studies. In: *Methods and Principles of Mycorrhizal Research* (N.C. Schenck Ed.). American Phytopathological Society, USA. 147-155.

Schwab, S.M., Johnson, E.L.V. and Menge, J.A., 1982. Influence of simazine on formation of vesicular-arbuscular mycorrhizae in *Chaenopodium quinona* Wild. *Plant Soil.* 64: 283-287.

Sreenivasa, M.N. and Bagyaraj, D.J., 1989. Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. *Plant Soil* 119: 127-132.

Trappe, J.M., Molina, R. and Castellano, M., 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. *Annu. Rev. Phytopathol.* 22: 331-359.

Trevors, J.T., 1984. Effect of substrate concentration, inorganic nitrogen, O_2 concentration, temperature and pH on dehydrogenase activity in soil. *Plant Soil* 77: 285-293.