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Effect of pesticides on AM colonization and spore production in some endemic trees of Western Ghats

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The frequently observed arbuscular mycorrhizal fungi in the rhizosphere of the endemic trees, *Gluta travancorica*, *Bentinckia condapanna* and *Myristica malabarica* were isolated. Attempts were made to identify the rapidly infecting fungi on these hosts and *Glomus fasciculatum* was found highly colonized with both *G. travancorica* and *M. malabarica*, while *Glomus intraradices* was observed as the rapidly infecting fungi on *B. condapanna*. Effect of phosphorus addition on AM root colonization and spore production on these endemic trees were determined. In order to find out the effect of pesticides on AM fungal development, a few fungicides and insecticides at two concentration levels (RL & HRL) with and without application. Effect of pesticides and phosphorus on colonized root length and spore number to colonized root length were also determined.

Key Words : Arbuscular mycorrhizal fungi, *Gluta travancorica*, *Myristica malabarica*, *Bentinckia condapanna*, pesticides

INTRODUCTION

The potentials of mycorrhizal symbiosis for increased productivity of agronomic and forest tree species have been demonstrated (Miyasaka *et al.*, 1993; Rahangdale and Gupta, 1998). Mycorrhizal fungi may be important for the establishment and early growth of the trees in regions where growth and nutrient uptake are seasonal. Eventhough most studies in forest trees are concentrated on the use of ectomycorrhizal fungi, arbuscular mycorrhizal (AM) fungi were also used successfully for the early growth of many trees (Miyasaka *et al.*,1993; Michelsen, 1993). The most important feature of AM is the restricted spreading of fungus within the root and its extensive spreading to the root zone for nutrient absorption. Root colonization and proliferation of external hyphae are complex and dynamic processes that interact with host environment, and they appears to be related directly to the onset and duration of the benefits achieved (Jarstfer and Sylvia, 1992). The spread of infection and nature of host fungus interaction were determined by the type of host tissue colonized (Gianinazzi–Pearson and Gianinazzi, 1987), internal P concentration (Jasper *et al.*, 1979) and the pesticide application to the soil (Nemec, 1980; Wilson and Hartnett, 1998).

A wide variety of pathogens and insects adversely affect the growth and establishment of tree species in forest nurseries and natural habitat. The pathogens, especially, seed-borne and soil-borne mycoflora cause considerable damage to the plants during their germination and early establishment. Large number of insects also cause considerable damage to the forest nurseries and plantations. Earlier studies have proposed different pesticides against the pathogens and insects causing damage to the plants in natural habitats (Pandey *et al.*, 1996; Gupta and Borse, 1997). However, application of these plant protecting chemicals may have certain deleterious effects on the associated non-target beneficial mycoflora (Parvati *et al.*, 1985; Sugavanam *et al.*, 1994; Udaiyan *et al.*, 1995; Wilson and Hartnet, 1998), which in turn negatively affect the growth and establishment of host trees. In the present study, a few representatives of the endemic trees of the Western Ghats were selected for the study of mycorrhizal colonization with two indegenous AM fungi. The effect of certain commonly used biocides like dithane, bavistin, endosulfan and malathion were

also studied on AM root colonization and spore production.

MATERIALS AND METHODS

The plants selected for this study were *Gluta travancorica* Bedd., *Bentinckia condapanna* Berry ex Roxb. and *Myristica malabarica* Lam. growing in the Agasthyamala hills of Western Ghats. *B. condapanna* is a beautiful palm confined to the streep slopes. *G. travancorica* and *M. malabarica* are hard wood trees and the wood of *G. travancorica* is extensively used in carving industry.

Seeds of *B. condapanna* were cleared by removing the pulpy mesocarp and washed in tap water. Seeds of *M. malabarica* after removing the aril, were washed well in tap water. Seeds of both these plants were then surface sterilized with 10% sodium hypochlorite followed by successive rinses with sterilized deionized water. Seeds of *G. travancorica* were washed well in tap water and scarified with 10% H_2SO_4 for 20 minutes, followed by successive rinses with sterilized deionized water. Seeds of these plants were then placed in small plastic pots containing sterilized sand and germinated in dark at 25°C. On germination seedlings were thinned to one plant per pot.

The most frequently observed AM fungal spores viz. *Glomus fasciculatum* and *G. intraradices* isolated from the rhizosphere of these selected plants used as inoculant fungi. Based on the preliminary colonization experiments, *G. fasciculatum* was used to inoculate both *G. travancorica* and *M. malabarica* and *G. intraradices* is used for inoculating *B. condapanna*. Inoculum of these fungi was prepared on sorghum plants and it was air dried kept at 4°C.

The pesticides used in this study include two fungicides and two insecticides at recommended level (RL) and half the recommended level (HRL) of field application. The fungicides used are Dithane M45 – Mancozeb 75% (Manganese ethylene bis dithiocarbamte+ Zinc) and Bavistin – Carbendazim 50% (Methyl-2-benzimidazole carbamate). The insecticides used are Endosulfan 35EC (C, C- [1, 4, 5, 6, 7-hexachloro-8, 9, 10-trinoborn-5-en -2,.3-yelenedimethyl sulphite) and Malathion–50EC (S-1, 2-bis (ethoxy carbonyl) ethyl - O, O - dimethyl phosphorodithiorate). In the phosphorus treatment, 15 mg KH_2PO_4 kg^{-1} soil was supplied as 50 ml aqueous solution. The substrate used in this experiment was double autoclaved sand-soil mixture in 2 :1 proportion. Thus the experiment consisted of mycorrhizal plants with and without P and RL and HRL of pesticides supplied mycorrhizal plants with and without P.

The experiment was conducted in a randomized complete block design with four replicates to each treatment. Ten gram of inoculum of AM fungi containing 2500 infective propagules per gram substrate was placed as a layer 5 cm below the germinated seeds before transplanting into 15 cm pots. Both RL and HRL of pesticides were thoroughly mixed with the substrate separately. Phosphorus was supplied as aqueous solution to the substrate. The pots were watered daily with sterilized deionized water, and Hoagland nutrient solution without P was added twice in a week.

Terminal feeder roots of the seedlings after 90 and 180 days of treatment were stripped, cleaned and stained followed by the methods of Phillips and Hayman (1970). Percentage of root colonization was determined by Gridline intersect method (Giovannetti and Moss, 1980). Soil samples from the pots were collected after 90 and 180 days of treatment, wet-sieved and spore count was estimated (Gerdemann and Nicolson, 1983).

RESULTS AND DISCUSSION

Marked differences were observed in colonization and spore production between the fungi and among the host plants. Percentage of root colonization was highest in *B. condapanna* inoculated with *G. intraradices*. Variations in colonization and spore production were observed among *G. travancorica* and *M. malabarica* when inoculated with *G.fasciculatum*. Colonization of 74% and 68% was observed in *M. malabarica* and *G. travancorica* respectively at 180 days of inoculation (Table 1). Variations in colonization may be due to the preference of the fungi for host plants. Differences in colonization potential of AM fungi was attributed to the ability of the AM fungi to colonize the host root quickly and extensively (Abbott and Robson, 1982) and the high level of colonization may be the prime determinant of the efficacy of symbiosis (Menge, 1983). Differences in the response of AM fungi within hosts suggest that under certain conditions selection should occur to favour certain host-fungus combinations (Sanders and Fitter, 1992).

Spore production by AM fungi in pot culture was highly variable among the host plants. The plant *B. condapanna*, with highest root colonization yielded significantly higher number of spores than the other two plants. The spore count was 238 for *G. travancorica* and 284 for *M. malabarica* per 50-g soil after 180 days of inoculation (Table 2). The differential ability to sporulate on different host plants may be

Table 1. Effect of pesticides on AM root colonization of the selected plants

Treatments	Percent Colonization					
	Gluta travancorica		*Myristica malabarica*		*Bentinckia condapanna*	
	90 days	180 days	90 days	180 days	90 days	180 days
AMF	71^{a}	68^{ab}	77^{a}	74^{a}	84^{a}	86^{a}
AMF+P	67^{bc}	62^{cd}	69^{bc}	70^{bc}	71^{cd}	68de
Dithane-RL	50^{ef}	61^{cd}	57^{e}	64de	63de	70de
Dithane-HRL	64^{bc}	69^{a}	68^{bc}	73^{ab}	76^{bc}	81^{b}
Dithane-Rl+P	50^{ef}	56^{ef}	47^{gh}	54^{gh}	58^{ef}	64^{ef}
Dithane-HRL+P	56de	63^{c}	51^{fg}	66^{cd}	66^{cd}	76^{c}
Bavistin-RL	36^{hi}	55^{fg}	41^{hi}	56^{g}	38^{hi}	49^{hi}
Bavistin-HRL	53de	62^{cd}	60de	67^{cd}	51^{fg}	60^{fg}
Bavistin-RL+P	41^{gh}	53^{fg}	44^{gh}	53^{h}	41^{gh}	46^{i}
Bavistin-HRL+P	49^{ef}	59de	52^{fg}	66^{cd}	49^{fg}	58^{fg}
Endosulphan-RL	34^{i}	47^{i}	44^{gh}	53^{h}	34^{i}	51^{h}
Endosulphan-HRL	54de	58^{e}	58de	64de	52^{fg}	63^{ef}
Endosulphan-RL+P	37^{hi}	52^{gh}	38^{i}	50^{i}	40^{h}	49^{hi}
Endosulphan-HRL+P	49^{ef}	56^{ef}	51^{fg}	59^{ef}	49^{fg}	60^{fg}
Malathion-RL	49^{ef}	61^{cd}	52^{fg}	64de	52^{fg}	63^{ef}
Malathion-HRL	57^{d}	68^{ab}	71^{bc}	73^{ab}	72^{bc}	79^{bc}
Malathion-RL+P	45^{fg}	53^{fg}	56^{ef}	60^{ef}	49^{fg}	66^{c}
Malathion-HRL+P	58^{cd}	64^{bc}	68^{bc}	69^{bc}	64de	76^{c}

Columns followed by same letter (s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

Table 2. Effect of pesticides on AM spore count of the selected plants

Treatments	Number of spores (50 g soil)					
	Gluta travancorica		*Myristica malabarica*		*Bentinckia condapanna*	
	90 days	180 days	90 days	180 days	90 days	180 days
AMF	158^{bc}	238^{bc}	193^{bc}	284^{bc}	266^{bc}	386^{bc}
AMF+P	176^{a}	264^{a}	224^{a}	306^{a}	286^{a}	426de
Dithane-RL	106^{fg}	156^{gh}	116^{gh}	168^{gh}	186^{fg}	284^{fg}
Dithane-HRL	168^{ab}	213de	178^{cd}	264^{cd}	286^{a}	376^{bc}
Dithane-Rl+P	108^{fg}	1326^{i}	108^{h}	176^{gh}	198^{ef}	232^{hi}
Dithane-HRL+P	126de	184^{cf}	119^{gh}	188^{fg}	264^{bc}	344^{cd}
Bavistin-RL	93^{gh}	138^{hi}	118^{gh}	142^{i}	158^{gh}	214^{hi}
Bavistin-HRL	114^{ef}	145^{hi}	146^{cf}	212^{ef}	216de	262^{g}
Bavistin-RL+P	107^{fg}	137^{hi}	128^{fg}	166^{gh}	148^{hi}	208^{i}
Bavistin-HRL+P	96^{gh}	144^{hi}	101^{hi}	153^{hi}	188^{fg}	284^{fg}
Endosulphan-RL	86^{gh}	146^{hi}	92^{i}	174^{gh}	184^{fg}	272^{fg}
Endosulphan-HRL	114^{ef}	164^{gh}	126^{fg}	198^{fg}	216de	336de
Endosulphan-RL+P	73^{i}	132^{i}	112^{gh}	162^{h}	138^{i}	226^{hi}
Endosulphan-HRL+P	123^{ef}	148^{h}	116^{gh}	176^{gh}	202^{ef}	258^{gh}
Malathion-RL	121^{ef}	198^{e}	138^{fg}	208^{ef}	186^{fg}	312^{ef}
Malathion-HRL	145^{cd}	224^{cd}	172de	262^{cd}	274^{ab}	408^{ab}
Malathion-RL+P	118^{ef}	178^{fg}	122^{gh}	196^{fg}	168^{gh}	296^{ef}
Malathion-HRL+P	167^{ab}	244^{bc}	144^{cf}	214^{ef}	244^{cd}	382^{bc}

Columns followed by same letter (s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

related to several factors. There are reports that percentage of colonization is directly related to the spore production (Hayman, 1970) But a few others are on the opinion that there is no correlation between these two factors (Simpson and Daft, 1990). Schenck and Kilnoch (1990) compared the spore production of six AM fungi in identical conditions and observed that differences in sporulation were due to the host species. Since the growth conditions were similar in this experiment, the differences in spore production shown by the endophyte might be due to host factors.

Response of both the AM fungi to addition of P was similar. A reduction in root colonization was observed among the host plants by the addition of P. The spore number showed an initial decrease, but increased after 90 days of inoculation. But the total spore count did not show any correlation with their root colonization.

The high percentage of infection among the host plants indicates that mycorrhizal infection in plants grown in added P may suggest the negative effect of P in mycorrhizal development as indicated by Miller and Jackson (1998). However, there are also reports of high AM infection as well as enhanced P uptake at high concentration of added P (Amijee *et al.*, 1989).

Differences have been observed among the AM fungal apecies in response to fungicide application. Fungicides at RL have reduces AM root colonization compared to the HRL (Table 1) as reported by Sreenivasa and Bagyaraj (1989). The inhibitory effect on colonization was higher during the initial stage of AM development. Increases in both colonization and spore count have been observed after 90 days of inoculation. Udaiyan *et al.* (1985) also have observed

Table 3. Effect of fungicides on root length (A) and spore number to colonized root length (B) after 180 days of treatment

Treatments	*Gluta travancorica*		*Myristica malabarica*		*Bentinckia condapanna*	
	A	B	A	B	A	B
AMF	2.07^{ab}	115.00^{bc}	11.23^{ab}	25.29^{ab}	28.27^{a}	13.65^{de}
AMF+P	1.98^{b}	133.33^{a}	10.95^{bc}	27.94^{a}	22.71^{bc}	18.76^{a}
Dithane-RL	1.69^{cd}	92.31^{cd}	8.93^{cd}	18.81^{cd}	19.66^{cd}	14.45^{cd}
Dithane-HRL	2.18^{a}	97.7^{cd}	12.88^{a}	20.50^{cd}	25.49^{ab}	14.75^{cd}
Dithane-Rl+P	1.67^{cd}	79.04^{de}	7.62^{de}	23.10^{bc}	19.14^{cd}	12.12^{e}
Dithane-HRL+P	2.00^{ab}	92.00^{cd}	10.03^{bc}	18.74^{cd}	24.06^{ab}	14.30^{cd}
Bavistin-RL	1.55^{de}	89.03^{d}	6.81^{e}	20.85^{cd}	13.29^{de}	16.10^{bc}
Bavistin-HRL	1.91^{bc}	75.91^{e}	10.53^{bc}	20.13^{cd}	19.08^{cd}	13.73^{de}
Bavistin-RL+P	1.40^{e}	97.86^{cd}	7.23^{de}	22.96^{bc}	13.27^{e}	15.67^{bc}
Bavistin-HRL+P	1.63^{cd}	88.34^{de}	9.81^{bc}	15.60^{e}	16.88^{de}	16.82^{bc}

Columns followed by same letter (s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

Table 4. Effect of insecticides on root length (A) and spore number to coloniazed root length (B) after 180 days of treatment

Treatments	*Gluta travancorica*		*Myristica malabarica*		*Bentinckia condapanna*	
	A	B	A	B	A	B
AMF	2.07^{ab}	115.00^{bc}	11.23^{a}	25.29^{bc}	28.27^{a}	13.65^{e}
AMF+P	1.98^{ab}	133.33^{a}	10.95^{ab}	27.94^{a}	22.71^{bc}	18.76^{ab}
Endosulphan-RL	1.18^{e}	123.73^{ab}	6.35^{de}	27.40^{ab}	13.47^{e}	20.19^{a}
Endosulphan-HRL	1.70^{bc}	96.47^{de}	8.42^{cd}	23.52^{cd}	17.91^{cd}	18.76^{ab}
Endosulphan-RL+P	1.44^{d}	91.67^{e}	6.11^{e}	26.51^{ab}	13.71^{de}	16.48^{cd}
Endosulphan-HRL+P	1.58^{cd}	93.67^{de}	8.22^{cd}	21.41^{de}	17.16^{d}	15.03^{de}
Malathion-RL	1.76^{bc}	112.50^{b}	8.17^{cd}	25.46^{bc}	16.64^{de}	18.75^{ab}
Malathion-HRL	2.10^{a}	106.67^{cd}	11.07^{ab}	23.67^{cd}	23.00^{bc}	17.74^{bc}
Malathion-RL+P	1.43^{d}	124.48^{ab}	8.44^{cd}	23.22^{cd}	18.77^{cd}	15.77^{cd}
Malathion-HRL+P	1.90^{ab}	128.40^{ab}	10.11^{ab}	21.17^{e}	23.47^{bc}	16.27^{cd}

Columns followed by same letter (s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

an inhibition of colonization and spore count by the application of pesticides. However, both the fungicides at HRL did not show much inhibitory effect on AM fungi at 90 days of inoculation. Fungicides are expected to affect mycorrhizal fungi most profoundly and its formation on hosts. (Trappe *et al.*, 1984). However, most of them selectively affect some fungi or some fungi can differ in their response to particular chemicals (Edington, 1971). Among the fungicides used, inhibition of colonization was minimum with dithane compared to bavistin. It was reported that the effect of bavistin persists for a long period while the toxicity of dithane gets neutralized within a short period (Sugavanam *et al.*, 1994). Udaiyan *et al.* (1995) have observed that bavistin application exhibited measurable inhibition in root colonozation. Addition of P to the fungicide applied plants did not show any consistent result.

Among the insecticides applied, endosulfan was found to reduce colonization and spore count compared to malathion. The inhibitory effect of endosulfan on AM colonization was reported earlier by Parvati *et al.* (1985). The inhibitory effect of endosulfan was more pronounced initially and had affected root colonization after 90 days of treatment. Reduction in the number of mycorrhizal structures might be associated with the inhibition of infection process. The toxicity of endosulfan was found persisting for longer period in this experiment compared to malathion. However, Sreenivasa and Bagyaraj (1984) have reported that, both these insecticides are deleterious to AM colonization when applied at RL of field application. There are many reports on the inhibitory effect of pesticides on AM colonization (Sukarno *et al.*, 1993; Miller and Jackson, 1998). However, there are also reports on the stimulatory effect of pesticides on AM colonization (Jabaji-Hare and Kendrick, 1985). Since the root exudate is an important factor governing mycorrhizal colonization (Graham *et al.*, 1981), any alteration in the quantity or quality of root exudation by pesticide application may increase or decrease mycorrhizal colonization. Like fungicides the addition of P to the insecticide applied plants did not show any consistent result either on colonization among the AM fungi or different hosts.

The observed reduction in spore number by pesticide application is probably due to the reduction of infected root length or may be due to the exhaustion of extramatrical hyphae by the toxic chemicals. Though bavistin application generally reduces AM development, *G. intraradices* produced higher number of spores on *B. condapanna* compared to *G. fasciculatum* on the other two hosts. Dodd and Jeffries (1989) have reported differential response of three *Glomus* species to fungicides. This differential response appears to be related to the tolerance of AM fungi to toxic chemicals.

Total root length varied greatly among the host plants

and hence infected root length also differed considerably. The largest root length was recorded for *B. condapanna*, while the shortest for *G. travancorica*. An increase in root length was noticed by P addition. Addition of pesticides at RL considerably reduced the root length infection and it was found to be proportional to the percentage of infection (Table 3 & 4). These toxicants usually decrease mycorrhizal infection and spore germination, which affect all aspects of fungal development in plant roots. But addition of dithane did not affect the length of colonized root. The ratio of spore number to infected root length also showed variation on pesticide application. This ratio was largest in case of *G. travancorica* and lowest for *B. condapanna*. Sukarno *et al.* (1983) observed reduction in percentage of root infection and infected root length by the application of biocides. In the present study, it was found that pesticide application at RL resulted in the reduction of mycorrhizal development. The higher mycorrhizal colonization at the HRL suggests that, application of these pesticides at HRL or below that level will not affect the mycorrhiza formation and the symbiosis.

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REFERENCES

Abbott, L.K. and Robson, A.D. (1982). The role of vesicular-arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Aust J. Agric* Res. **33** : 389-408.

Amijee, F., Tinker, P. B. and Stribley, D. P. (1989). The development of endomycorrhizal root systems. 7. A detailled study of effects of soil phosphorus on colonization. *New Phytol.* **111** : 435-446.

Dodd, J. C. and Jeffries, P. (1989). Effect of fungicides on three vesicular-arbuscular mycorrhizal fungi associated with winter wheat (*Triticum aestivum L.*) *Biol. Fertil Solis.* **7** : 120-128.

Edington, L. V., Khew, K. L. and Barron, G. L. (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopathol.* **61** : 42-44.

Gerdemann, J.W. and Nicolson., T.H. (1983). Spores of endomycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46** : 235-244.

Gianinazzi-Pearson, V. and Gianinazzi, S. (1987). Morphological integration and functional compatibility between symbionts in vesicular-arbuscular endomycorhizal associations. In : Cell to signals in plant, animal and microbial symbiosis. S. Scannerini, DC. Smith, P. Bonfantae-Fasolo and Gianinazzi-Pearson (eds). NATO ASI series.

Giovannetti, M. and Moss, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84** : 489-500.

Graham, J. H., Leonard, R. T. and Menge, J. A. (1981). Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiol* **68** : 548-552.

Gupta, B. K. and Borse, S. S. (1997). Relative toxicity of some insecticides as contact poison against third instar larvae of *Hyblea purea* (Lepidoptera : Hyblaeidae). *Indian For.* **123** : 427-429.

Hayman, D. S. (1970). Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans Br. Mycol. Soc.* **54** : 53-63.

Jabaji-Hare, S. H. and Kendrick, W.B. (1985). Effects of fosetyl-Al on root exudation and composition of extracts mycorrhizal and non-mycorrhizal leek roots. *Can. J. Plant Pathol.* 7 : 118-126.

Jarstfer, A.G. and Sylvia, D.M. (1992). Inoculum production and inoculation strategies for vesicular-arbuscular mycorrhizal fungi. In : Soil microbial ecology. FB Metting (ed) Marcel Decker Inc. New York. pp 349-377.

Jasper, D.A., Robson, A.D. and Abbott, J.K. (1979). Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biol. Biochem.* **11** : 501.

Menge, J.A. (1983). Utilization of vesicular-arbuscular mycorrhizal fungi in Agriculture. *Can. J. Bot.* **61** : 1015-1024.

Michelson, A. (1993). The mycorrhizal status of vascular epiphytes in bale Mountains National Park. Ethiopia. *Mycorrhiza* **4** : 11-15.

Miller, R.L. and Jackson, L.E. (1998). Survey of arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *J. Agic Sci.* **130** : 173-182.

Miyasaka, S.C., Habtae, M. and Matsuyama, D.T. (1993). Mycorrhizal dependency of two Hawaiian endemic tree species : Koa and Mamane. *J. Plant Nutr.* **16** : 1339-1356.

Nemec, S. (1980). Effects of 11 fungicides on endomycorrhizal development in sour orange. *Can. J. Bot.* **58** : 522-526.

Pandey, P.C., Singh, A.,Karnatak, D.C. and Bhartari, B.K. (1996). *Melamspora Larci-populina* on Poplar in India and its control in nursery. *Indian For* **122** : 1062-1067.

Parvati, K., Venkateswarlu, K. and Rao, A. S. (1985). Toxicity of soil-applied fungicides and gypsum to the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* in groundnut. *Can J. Bot.* **63** : 1673-1675.

Philips, J.M. and Hayman, D.S. (1970). Inproved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55** : 158-160.

Rahangdale, R. and Gupta, R. (1998). Selection of VAM inoculants for some forest tree species. *Indian For.* **124** : 331-341.

Sanders, I.R. and Fitter, A.H. (1992). Evidence for differential responses between host-fungus combinations of vesicular-arbuscular mycorrhizas from grassland. *Mycol. Res.* **96** : 415-419.

Schenck, N.C. and Kinloch, R. A. (1980). Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. *Mycologia* **72** : 445-456.

Simpson, D. and Daft, M.J. (1990). Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. *Plant Soil* **121** : 171-178.

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

Sugavanam, V., Udaiyan, K. and Manian, S. (1994). Effect of fungicides on vesicular-mycorrhizal infection and nodulation in groundnut (*Arachis hypogea* L.) *Agric. Ecosyst. Environ.* **48** : 285-293.

Sukarno, N, Smith., S.E. and Scott, E.S. (1993). The effect of fungicides on vesicular-arbuscular mycorrhizal symbiosis. 1. The effects on vesicular-arbuscular mycorrhizal fungi and plant growth. *New Phytol.* **25** : 139-147.

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

Udaiyan, K., Manian, S., Muthukumar, T. and Greep, S. (1995). Biostatic effect of fumigation and pesticide drenches on an endomycorrhizal-Rhizobium-legume tripartite association under field conditions. *Biol. Fertil. Soils* **20** : 275-283.

Wilson, G.W.T. and Hartnett, D.C. (1998). Effects of mycorrhizae on plant growth and dynamics in experimental tallgrass prairie microcosms. *Amer. J.Bot.* **84** : 478-482.

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