

## Effects of Formaldehyde Fumigation and Fytolan Drench on VAM Fungi and Nodulation in Some Leguminous Forest Tree Seedlings in India

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**Keywords:** formaldehyde fumigation, Fytolan drench, VAM colonization, nodulation, field survival

### ABSTRAK

Anak benih 12 spesies pokok kekacang (*Acacia caesia*, *A. farnesiana*, *A. holosericea*, *A. leucocephala*, *A. nilotica*, *Albizia lebbeck*, *Dichrostachys cinerea*, *Leucaena latisiliqua*, *Prosopis cineraria*, *Dalbergia latifolia* dan *Pterocarpus marsupium*) dibesarkan dalam formaldehid yang dipewasap/batas Fytolan basah ditapak semaian. Anak benih dalam batas formaldehid yang di pewasap terbantut membesar dan klorotik; mempunyai perkolonian akar VAM yang lemah (18-25.3%) dan densiti spora ( $3.1 - 0.16 \text{ g tanah}^{-1}$ ), jumlah bintil yang rendah (3 – 8 tanaman<sup>-1</sup>) dan biojisim berbintil (100 – 870 mg plant<sup>-1</sup>); jumlah biojisim (15.5 – 72 g plant<sup>-1</sup>) dan kadar terus hidup lapangan 31.2 – 40.4%) anak benih adalah sangat rendah. Spesies mikoriza yang diasingkan adalah *Acaulospora bireticulata*, *G. fasciculatum* dan *G. geosporum*. Sebagai perbandingan, anak benih dari batas Fytolan yang basah menunjukkan pertumbuhan biasa, biojisim yang bertambah (18 – 82.2 f g plant<sup>-1</sup>) dan kadar terus hidup lapangan lebih tinggi (71 – 86%); pengkolonian akar VAM yang sangat menggalakkan (53.4 – 100%) dan jumlah bintil densiti spora lebih tinggi ( $36 - 82.8 \text{ g. tanah}^{-1}$ ) dan jumlah bintil yang lebih tinggi (7.4 – 17.6 plant<sup>-1</sup>) dan biojisim berbintil (195 – 950 mg plant<sup>-1</sup>) dibandingkan dengan anak benih terkawal. Akar untuk tanaman-tanaman ini mempamerkan perkembangan struktur arbuskular dan vesikular yang sangat menggalakkan. Daripada tujuh spesies VAMF yang direkodkan dari tanah-tanah rizosfera terkawal dan batas basah Fytolan, *A. bireticulata*, *G. fasciculatum* dan *G. geosporum* merupakan spesies dominan. Secara statistik, perbezaan antara rawatan adalah ( $p < 0.05$ ).

### ABSTRACT

Seedlings of 12 legume tree species (*Acacia caesia*, *A. catechu*, *A. farnesiana*, *A. holosericea*, *A. leucocephala*, *A. nilotica*, *Albizia lebbeck*, *Dichrostachys cinerea*, *Leucaena latisiliqua*, *Prosopis cineraria*, *Dalbergia latifolia* and *Pterocarpus marsupium*) were raised in formaldehyde-fumigated/Fytolan-drenched beds in a nursery. Seedlings in the formaldehyde fumigated beds had stunted growth and were chlorotic; had poor VAM root colonization (18-25.3%) and spore density ( $3.1 - 10.6 \text{ g. soil}^{-1}$ ) and lower nodule number (3 – 8 plant<sup>-1</sup>) and nodular biomass (100 – 870 mg plant<sup>-1</sup>); the total biomass (15.5 – 72 g plant<sup>-1</sup>) and field survival rate (31.2 – 40.4%) of the seedlings were very low. The mycorrhizal species isolated were *Acaulospora bireticulata*, *Glomus fasciculatum* and *G. geosporum*. In contrast, seedlings from Fytolan-drenched beds showed normal growth, enhanced biomass (18 – 83.2 f g plant<sup>-1</sup>) and higher field survival rate (71 – 86%); intense VAM root colonization (53.4-100%) and higher spore density ( $36 - 82.8 \text{ g soil}^{-1}$ ) and higher nodule number (7.4 – 17.6 plant<sup>-1</sup>) and nodular biomass (195 – 950 mg plant<sup>-1</sup>) compared with the control seedlings. Roots of these plants exhibited extensively developed arbuscular and vesicular structures. Of the seven VAMF species recorded from the rhizosphere soils of control and Fytolan-drenched beds, *A. bireticulata*, *G. fasciculatum* and *G. geosporum* were the dominant species. The differences between treatments were statistically significant ( $P < 0.05$ ).

## INTRODUCTION

In India, 68% of the population is rural based and depend on forests for fuelwood, timber, cattle fodder and other minor products. Over-exploitation of forest lands has led to the dwindling of forest cover, causing severe ecological imbalance and environmental disaster. As part of the national effort to open new forests, barren substandard soils and low productivity agricultural lands have been used to raise multi-utility trees through plantation programmes. The seedlings needed for these programmes are usually raised in forest tree nurseries.

Legumes often have double symbiotic associations with *Rhizobium* spp. and mycorrhizal fungi (VAM and/or ECM). Such associations often benefit the leguminous plants through improved P and N supplies and also from N-P interactions (Munns and Mosse 1980). Nitrogen fixation by members of Papilionoideae and Mimosoideae needs high phosphate levels, which could probably be satisfied through mycorrhiza symbiosis. Mycorrhizal fungi not only help the plant itself, but also aid the bacterial system to fix N in the nodular tissue. Hence, legumes with dual symbioses are preferred for forestation programmes in marginal environments.

The use of fumigants and fungicides is an important management practice in nurseries to control root pathogenic fungi, soil-inhabiting insects, nematodes and weed seeds. Properly applied fumigation also usually eliminates the beneficial mycorrhizal population (Kormanik *et al.* 1977; Riffle 1980; Maronek *et al.* 1981; Udaiyan *et al.* 1995) as well as nitrogen-fixing and symbiotic bacteria (Trappe and Strand 1969; Abrahamson 1980; Molina and Trappe 1984; Kishinevsky *et al.* 1992). Since most fungicides are not pathogen-specific, they affect a wide range of non-pathogenic fungi (Bollen 1979; Vyas 1988),

including those which are beneficial to plant growth, such as VAMF. VAMF are also affected by fungicide application (Manjunath and Bagyaraj 1984; Kough *et al.* 1987; Fitter and Nichols 1988; Plenchette and Perrin 1992; Sukarno *et al.* 1993; Udaiyan *et al.* 1995).

Studies on the effect of different fungicides, both systemic and non-systemic, on the development of VAM infection do not, however, show consistent overall trends (Trappe *et al.* 1984). Non-systemic fungicides have been reported to be ineffective on VAM (Jalali and Domsch 1975; Sutton and Sheppard 1976; Nemeč 1980) to reduce VAM development (Nesheim and Linn, 1969; El-Giahmi *et al.* 1976; Plenchette and Perrin 1992) or, surprisingly, to stimulate it (Sugavanam *et al.* 1994).

The purpose of the present study is therefore to assess the effects of formaldehyde fumigation and Fytolan drench on the VA mycorrhizal and nodular endophytes and their subsequent effects on seedling quality and field survival of some leguminous forest tree species.

## MATERIALS AND METHODS

The study was carried out in the nursery in the experimental plot of Bharathiar University, Coimbatore, Tamil Nadu, India. The red alfisol - calcareous soil had a pH of 8.1 and electric conductivity of  $0.2 \text{ mScm}^{-1}$ . Soil nitrogen (N), phosphorus (P) and potassium (K) concentrations were 104, 4 and  $380 \text{ kg ha}^{-1}$  respectively. Total N and available P were respectively determined by the micro Kjeldahl and molybdenum blue method (Jackson 1973). Exchangeable K was measured using a digital flame photometer (Jackson 1973). The soil was ploughed to a depth of 50 cm, levelled and  $1.5 \times 1.5 \text{ m}$  nursery beds prepared with 2-m intervals between them.



The treatments of the beds were: a) fumigated with 0.4% formaldehyde applied at the rate of 2 l/m<sup>2</sup> covered with polyethylene sheets for 48 h and then exposed to air for 15 days prior to sowing; b) drenched with 0.2% Fytolan, a non-systemic fungicide with protectant properties containing 88% (w/w) copper oxychloride, applied at the rate of 75ml/m<sup>2</sup> for six hours prior to sowing and; c) untreated control. Each treatment was replicated five times. The plots were arranged in a completely randomised block design.

Fully mature, uniform size, viable seeds of 12 forest tree species (*Acacia caesia* (L.) Willd., *A. catechu* (L.F) Willd., *A. farnesiana* (L.) Willd., *A. holosericea* A. Cunn., *A. leucocephala* (Roxb.) Willd., *A. nilotica* (L.) Willd. ex. Del. subsp. *indica* (Benth.) Brenan, *Albizia lebbek* (L.) Willd., *Dichrostachys cinerea* (L.) Wight & Arn., *Leucaena latisiliqua* (L.) Gills, *Prosopis cineraria* (L.) Douce, *Dalbergia latifolia* Roxb. and *Pterocarpus marsupium* Roxb.) from the Institute of Forest Genetics and Tree Breeding (IFG &TB), Coimbatore, were sown in nursery beds in June 1992 and watered by irrigation at weekly intervals. Uniform 60-day-old seedlings were transferred to 30 × 12 cm polyethylene bags, each filled with ca. 3 kg soil from the respective seedling beds. Holes were punched in bags for drainage. The bags were arranged closely for sprinkle-irrigation. Samples of feeder root and rhizosphere soil were collected randomly from 10 seedlings for each species and each treatment 60 days after planting.

#### Root Colonization

Randomly selected root segments were cleaned and stained for assessment of mycorrhizal colonization. The cleared root segments were washed in distilled water, acidified with 5N HCl and stained in trypan blue (0.05% in lactophenol) adopt-

ing the technique of Phillips and Hayman (1970). Stained root segments were then examined for the presence of VAM structures, and the percentage of mycorrhizal infection determined by the root slide technique of Read *et al.* (1976).

#### Spore Population

Total spore count in soil samples was estimated by a modified wet sieving and decanting technique of Gerdemann and Nicolson (1963). Spore population was expressed as the number of individuals per gram of dry soil.

#### Field Survival Rate

The respective 150-day-old seedlings (100 seedlings for each species from the different treatments) were subsequently transplanted to degraded, barren land at the foot of the Maruthamalai hills, Western Ghats in the Bharathiar University campus in the monsoon month of October 1992. A 4 × 4 spacing was maintained for all seedlings. Data on the field survival of these seedlings were collected in February 1993.

#### Statistical Analyses

The data were analysed by analysis of variance (ANOVA) and the means were separated by Duncan's new multiple range test ( $P < 0.05$ ). Pearson's coefficient correlations were performed for plant dry weight with nodule number, nodule dry weight, root colonization, spore number and field survival rate.

## RESULTS

#### Soil

The soil at the study area was sandy loam, and low in available nutrients especially phosphorus (4 kg ha<sup>-1</sup>). However, supplementary fertilizers were not added.

#### Formaldehyde Fumigation

Seedlings in the fumigated beds were found to be stunted and chlorotic. They had very

poor biomass and stunted growth. Maximum reduction was found in *Prosopis cineraria* followed by *Acacia caesia*, *A. catechu* and *Pterocarpus marsupium*. After 60 days in polythene bags, VAM root colonization and spore density, the number, size and biomass of nodules and field survival rate of these seedlings decreased significantly ( $P < 0.05$ ) compared with control seedlings. This effect was maximum in *Acacia nilotica* and *Dichrostachys cinerea*. Spores of *Acaulospora bireticulata*, *Glomus fasciculatum*, *G. geosporum* and *G. macrocarpum* were isolated from the respective rhizosphere soils.

#### *Fytolan Drench*

Seedlings from the Fytolan-drenched soils showed significantly ( $P < 0.05$ ) higher biomass and field survival compared to the control beds. *Acacia caesia*, *A. holosericea*, *Leucaena latisiliqua* and *Prosopis cinerea* had increased biomass. Field survival rate increased by 21.7, 14.5 and 12.8% in *Acacia leucocephala*, *Pterocarpus marsupium* and *Dalbergia latifolia* respectively. VAM root colonization, spore density, legume nodule number and biomass were significantly greater ( $P < 0.05$ ) in *Dichrostachys cinerea*, *Acacia catechu*, *A. farnesiana* and *A. holosericea* respectively, than in control seedlings (Table 1). Well-developed VAM structures were observed in treated and control seedlings. Of the seven VAMF species isolated (*Acaulospora bireticulata*, *A. sporocarpia*, *Gigaspora margarita*, *Glomus australe*, *G. fasciculatum*, *G. geosporum* and *G. macrocarpum*), *A. bireticulata*, *G. fasciculatum* and *G. geosporum* were the dominant species, contributing 30, 25 and 20%, respectively, to the total spore count. The field survival rate of the transplanted seedlings from formaldehyde-fumigated, Fytolan-drenched and control soils were 31-40, 71-86 and 64-80%, respectively (Table 1).

A significant positive correlation was established (Table 2) between plant dry

weight and nodule dry weight in *Acacia caesia* ( $P < 0.001$ ), *A. catechu* ( $P < 0.05$ ), *A. holosericea* ( $P < 0.001$ ) and *A. nilotica* ( $P < 0.05$ ), but not in the others. The field survival rate of *A. nilotica* and *Pterocarpus marsupium* significantly and positively correlated ( $P < 0.05$ ) with plant dry weight.

#### DISCUSSION

The results showed that seedlings raised in formaldehyde-fumigated soil were chlorotic and had stunted growth. Their field survival rate was about half that of seedlings from control as well as Fytolan-treated beds. These adverse effects on the quality and performance of seedlings are correlated with a reduction in nodular biomass and poor root colonization by VAMF. Similar results on formaldehyde fumigation in leguminous crops have been reported by Udaiyan *et al.* (1995).

Poor root colonization by VAM was probably due to reduced spore density in the fumigated nursery soil. A similar reduction in VAMF spore density was reported in the rhizosphere of wheat (Hayman 1970) and citrus (Nemec 1980) as a consequence of formaldehyde fumigation. The reduction in nodule number may be due to destruction of the rhizobial population by fumigation (Kishinevsky *et al.* 1992); and the non-availability of sufficient P supply for nodulation (Mosse *et al.* 1976).

It has been suggested that VAMF may play a role in satisfying the high P demand for good nodulation and nitrogen fixation in the control beds (Asimi *et al.* 1980). The synergistic interactions between the bacterium *Rhizobium* and the mycorrhizal endophytes not only enhanced nutrient content in the above-ground plant material, but also seemed to provide well-balanced nutrients to the plants. This subsequently resulted in an improvement in biomass production. Furthermore, mycorrhizal in-

TABLE 1  
Effects of formaldehyde fumigation and Fytolan drenching on growth, root colonization, sporulation and field survival of forest tree seedlings

Parameters	Treatments	<i>Acacia caesia</i>	<i>Acacia catechu</i>	<i>Acacia farnesiana</i>	<i>Acacia holosericea</i>	<i>Acacia leucoccephala</i>	<i>Acacia nilotica</i>	<i>Albizia lebbek</i>	<i>Dichrostachys cinerea</i>	<i>Leucaena latisiliqua</i>	<i>Prosopis cineraria</i>	<i>Dalbergia latifolia</i>	<i>Pterocarpus marsupium</i>
Plant d. wt. (g plant) <sup>-1</sup>	Control	58.0 ab	43.0 a	30.0 a	42.5 b	67.0 b	72.8 a	43.2 a	28.0 a	34.1 b	80.0 a	16.2 b	34.0b
	Formaldehyde	55.0 b	40.0 ab	28.0 b	41.0 b	66.5 b	71.0 b	41.3 b	26.3 b	32.4 c	72.0 b	15.5 b	31.0 b
	Fytolan	61.0 a	45.0 a	31.0 a	45.0 a	68.0 a	73.0 a	44.0 a	29.1 a	36.3 a	83.2 a	18.0 a	35.6 a
No. nodules (plant) <sup>-1</sup>	Control	14.6 a	18.0 a	12.0 b	16.1 b	20.3 a	21.4 a	8.2 a	20.3 a	19.4 a	15.4 a	7.5 a	9.4 a
	Formaldehyde	4.8 b	6.0 b	8.0 c	4.4 b	7.5 c	6.0 b	3.0 c	6.0 b	7.1 c	4.1 b	5.2 b	4.2 c
	Fytolan	12.0 a	17.6 a	14.3 a	15.0 a	13.0 a	16.4 a	7.4 b	12.2 a	11.0 b	16.3 a	8.1 a	11.3 a
Nodule d. wt (mg plant) <sup>-1</sup>	Control	400 ab	380 b	475 b	900 b	193 a	250 a	200 a	620 a	700 a	195 a	285 b	270 a
	Formaldehyde	370 b	320 c	460 c	870 c	180 b	100 b	120 b	400 b	520 b	170 b	150 c	250 b
	Fytolan	430 a	427 a	510 a	950 a	195 a	280 a	210 a	630 a	725 a	200 a	300 a	300 a
Root colonization (%)	Control	58.3 a	98.4 a	70.0 a	40.7 b	90.6 a	100.0 a	87.4 b	37.4 b	57.5 a	85.2 a	77.3 a	81.2 a
	Formaldehyde	21.0 b	18.0 c	21.5 b	22.3 c	25.3 b	23.1 b	19.0 c	18.3 c	16.6 b	25.0 b	22.0 b	16.0 b
	Fytolan	53.4 a	89.6 b	77.2 a	56.2 a	95.1 a	100.0 a	94.3 a	56.0 a	56.0 a	91.3 a	85.2 a	83.3 a
No. of spores (g soil) <sup>-1</sup>	Control	70.2 a	41.1 a	50.6 a	82.8 a	48.0 a	27.5 a	42.2 a	94.2 a	55.3 a	40.7 a	38.6 b	37.7 b
	Formaldehyde	5.1 b	4.5 b	8.3 b	3.1 c	6.2 b	8.3 c	8.4 b	10.6 c	5.7 b	9.1 b	7.3 c	6.3 c
	Fytolan	62.6 a	58.2 a	60.6 a	70.4 b	58.5 a	36.0 a	48.1 a	82.8 b	50.6 a	44.5 a	43.4 a	49.4 a
Field Survival rate (%)	Control	68.3 a	76.8 b	80.3 a	69.0 a	64.3 b	80.6 a	69.0 a	73.0 a	80.0 a	78.3 a	65.2 b	68.6 b
	Formaldehyde	38.2 b	32.3 c	40.4 b	34.5 b	37.2 c	31.2 b	38.6 c	39.2 b	38.3 b	37.6 b	32.1 c	37.2 c
	Fytolan	71.0 a	82.0 a	84.2 a	72.4 a	86.0 a	73.5 a	82.8 a	78.5 a	82.2 a	79.2 a	78.0 a	83.1 a

Means within a parameter followed by the same superscript are not significantly different according to Duncan's new multiple range test ( $P < 0.05$ )



TABLE 2

Pearson's correlation coefficient (r) for plant dry weight (PDW) with nodule number (NN), nodule dry weight (NDW), root colonization (RC), spore number (SN) and field survival rate (FSR)

Sl. Tree Species	PDW × NN	PDW × NDW	PDW × RC	PEW × SN	PDW × FSR
1. <i>Acacia caesia</i>	+0.6053	+1.0000**	+0.7992	+0.8076	+0.9007
2. <i>A.catechu</i>	+0.9056	+0.9989*	+0.8734	+0.9957	+0.7513
3. <i>A. farnesiana</i> *	+0.9993f	+0.9142	+0.9771	+0.9869	+0.9682
4. <i>A. holosericae</i>	+0.7302	+1.0000**	+0.9814	+0.6880	+0.8332
5. <i>A. leucocephala</i>	+0.2497	+0.8305	+0.7925	+0.8664	+0.9679
6. <i>A. nilotica</i>	+0.9152	+0.9978*	+0.9958	+0.9773	+0.9985*
7. <i>Albizia lebbek</i>	+0.9064	+0.9818	+0.9718	+0.9880	+0.9849
8. <i>Dichrostachys cinerea</i>	+0.5397	+0.9351	+0.9933	+0.8645	+0.9635
9. <i>Leucaena latisiliqua</i>	+0.2392	+0.8844	+0.8881	+0.7754	+0.8422
10. <i>Prosopis cineraria</i>	+0.9770	+0.9922	+0.9805	+0.9832	+0.9658
11. <i>Dalbergia latifolia</i>	+0.8391	+0.7766	+0.7915	+0.7963	+0.8781
12. <i>Pterocarpus marsupium</i>	+0.9961	+0.9585	+0.9485	+0.9964	+0.9993*

\*,\*\*Correlations are significant at P=0.05 and 0.001 respectively.

fection has also been reported to increase shoot nitrogen content in nodulated plants (Ross and Harper 1970; Ross 1971). This probably explains the normal growth of the control seedlings.

In general, fungicides are less damaging/deleterious to mycorrhiza population than are fumigants. Nesheim and Linn (1969) suggested that stunting of seedlings can be avoided by using fungicides that eliminate root pathogens but are harmful to mycorrhizal fungi. Sugavanam *et al.* (1994) have reported that Fytolan promoted VAM root colonization, rhizosphere spore population and nodulation in *Arachis hypogaea*. In the present study, drenching of the nursery beds with Fytolan was found to increase endomycorrhizal colonization and *Rhizobium* nodulation in the legume seedlings. But the extent of increase varied among the host species. The differential response to treatments probably reflects the difference in the genetic constitution as well

as the microfloral composition in the rhizosphere of the host species. The higher root colonization and spore density in the Fytolan-treated beds is probably due to the following reasons: i) increased survival of VAM fungal propagules at seedling emergence stage, made possible by the suppression of microbes antagonistic to VAMF (Groth and Martinson 1983; Afek *et al.* 1990; Hetrick and Wilson 1991); ii) The insensitivity of certain *Rhizobium* strains to fungicides (Chiranjeevi 1982; Kataria *et al.* 1985; Radhakrishnan and Chatrath 1989; Singh and Agarwal 1990); iii) The synergistic interaction between *Rhizobium* and the mycorrhizal endophytes, where the mycorrhiza fungi enhance P availability for greater nodule formation (Kucey and Paul 1982).

Results from the present study also showed that seedlings with high biomass with roots extensively colonized by the VA mycorrhiza fungi also have higher field

survival rate. Seedlings from Fytolan-drenched beds in particular showed significantly higher field performance than the control. The effective synergistic interactions between the symbionts could probably have provided the necessary prerequisites for the high performance in the field environment. Presence of VAM probably helps to alleviate drought stress during transplanting (Michelsen and Rosendahl 1990) and enhances seedling growth, vigour and survival after transplanting (Brandeau 1970; Biermann and Lindermann 1983). Fytolan drench favours the establishment of the VAM and nodular endophytes of legumes in nutrient-deficient soils and should therefore be employed in nursery management along with other cultural practices for the production of high quality seedlings. The very hazardous chemical, formaldehyde, is not recommended for use in the nursery management practices, if it can be avoided.

#### ACKNOWLEDGEMENT

One of us (V.S.) thanks the Council of Scientific and Industrial Research, New Delhi, India for the award of Senior Research Fellowship, that supported this research.

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(Received 29 July 1995)

(Accepted 28 November 1995)