Journal of Tropical Forest Science 13(1):19–30 (2001)

EFFECT OF FUMIGANT AND PESTICIDES ON THE MYCORRHIZATION AND NODULATION OF TREE LEGUME SEEDLINGS

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Received June 1998

UDAIYAN, K., MUTHUKUMAR, T., VASANTHA, K., GREEP, S. & NARMATHA BAI, V. 2001. Effect of fumigant and pesticides on the mycorrhization and nodulation of tree legume seedlings. The influence of formaldehyde fumigation and pesticide drenching with Bavistin, Cuman, Copperthom, Sulfex, Furadan, and Termix on the dynamics of root colonisation by arbuscular mycorrhizal (AM) fungi, spore number and rhizobial nodule number was investigated. Plant growth and nutrient accumulation were assessed regularly for a period of 90 days in the forest tree legumes, viz. *Acacia mellifera, A. nilotica* and *Leucaena leucocephala,* under open nursery conditions. Pesticide drenching initially enhanced AM fungal colonisation and spore count gradually increased with age of the seedlings. Sulfex and Termix treatments significantly increased the spore number 90 days after emergence in all three tree legumes. Termix treatment enhanced the nodule numbers of the seedlings at 30 day after emergence in all three tree legumes. Sulfex responded at later stages when compared with the control, though the nodulation positively correlated with the age of the seedlings. Pesticides reduced plant growth of the legume seedlings at all age levels. Nitrogen and potassium contents were higher in seedlings grown in formaldehyde fumigated beds than in the control, but the accumulation of phosphorus was lower in all pesticide treatments.

Key words: AM - formaldehyde fumigation - pesticide drench - root colonisation - forest tree legume - seedlings

UDAIYAN, K., MUTHUKUMAR, T., VASANTHA, K., GREEP, S. & NARMATHA BAI, V. 2001. Kesan bahan pewasap dan racun perosak terhadap pemikorizaan dan penodulan anak benih pokok kekacang. Pengaruh pewasapan formaldehid dan penyemburan racun perosak dengan Bavistin, Cuman, Copperthom, Sulfex, Furadan dan Termix terhadap dinamik pengkolonian akar oleh kulat mikoriza arbuskular (AM), bilangan spora dan bilangan nodul rizobia diselidik. Pertumbuhan pokok dan penumpukan nutrien ditaksirkan secara tetap bagi tempoh 90 hari di dalam hutan pokok kekacang, iaitu *Acacia mellifera, A. nilotica* dan *Leucaena leucocephala,* di bawah tapak semaian terbuka. Penyemburan racun perosak pada mulanya menambahkan pengkolonian kulat AM dan bilangan spora bertambah secara beransur-ansur dengan umur anak benih. Rawatan Sulfex dan Termix menambahkan dengan bererti bilangan spora 90 hari selepas kemunculannya di dalam ketiga-tiga pokok kekacang. Rawatan Termix menambahkan bilangan nodul anak benih 30 hari selepas kemunculannya di dalam ketiga-tiga pokok kekacang. Sulfex bertindak balas pada peringkat yang kemudian berbanding dengan kawalan, walaupun penodulan bersaling tindakan secara positif dengan umur anak benih. Racun perosak mengurangkan pertumbuhan

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pokok anak benih kekacang pada semua peringkat umur. Kandungan nitrogen dan kalium adalah lebih tinggi di dalam anak benih yang ditanam di atas batas yang diwasapkan dengan formaldehid berbanding dengan kawalan yang tidak dirawat, tetapi penumpukan fosforus adalah lebih rendah di dalam kesemua rawatan racun perosak.

Introduction

A variety of nursery cultural practices may affect mycorrhizal fungi and their host species. The use of fumigation and pesticides for the production of healthy seedlings is common practice in most tropical forest tree nurseries. Although pesticides are designed against target pathogens, a good number of them have a deleterious effect on non-target organisms including arbuscular mycorrhizal (AM) fungi (Vyas 1988). In contrast, soil fumigants are non-selective biocides as they kill a wide range of soil organisms including nematodes, weed seeds, pathogenic, saprophytic and mycorrhizal fungi. Although the effects of fumigation and pesticides on ectomycorrhizal tree species are well documented (Kurle & Pfleger 1994), very little is known about the effect of these chemicals on endomycorrhizal tree species.

Previous studies on pesticides show that fungicides have varied effects on endomycorrhizal fungi. Most fungicides reduce the development of AM in plant roots while others are known to enhance AM formation (De Bertoldi *et al.* 1977) or even induce mycorrhizae in non-host species (Schwab *et al.* 1982). Some fungicides like dicarbamate, Captan, are known to produce a range of effects on AM formation depending on the AM fungal species and the host species with which they are associated (De Bertoldi *et al.* 1977). However, the cause of reduction in AM formation and subsequent low phosphorus (P) uptake due to pesticides has not been studied extensively. Some decline has been associated with the decrease in spore germination, hyphal growth or fungal activity (Knough *et al.* 1987).

Limited studies on the effect of nematicides and insecticides indicate that these chemicals do not reduce or stimulate AM formation and sporulation. The practical use of AM fungi in forest tree seedling production in nurseries necessitates the use of pesticides that do not have deleterious effect on AM symbiosis. The objective of this study was to determine the effect of formaldehyde fumigation and six pesticides on AM formation and functioning in three forest tree legume seedlings under nursery conditions.

Materials and methods

Study site

The experiment was conducted in an experimental field at the Department of Botany, Bharathiar University (11°1' N, 76° 93' E; altitude 410 m above sea-level), Coimbatore. The sandy loam soil (Alfisol) had the following properties: pH (H_2O) of 8.5 and electrical conductivity of 0.4 mS cm^{-1}. The nutrients determined according to Jackson (1973) were 10.4 mg of total nitrogen (N) kg^{-1}, 1.7 mg of P kg^{-1} and 23 mg of potassium (K) kg^{-1}. The soil was ploughed to a depth of *c.* 50 cm, levelled and 2 × 2 m beds were prepared 2 m apart. Total AM fungal spore density in the soil prior to treatments was 23 ± 2.5 spores g^{-1} dry soil, comprising *Glomus fasciculatum* (20%), *G. geosporum* (45%), *G. deserticola* (13%) and *Sclerocystis sinuosa* (22%).

Plant source

Seeds of *Acacia mellifera, A. nilotica* ssp. *indica* and *Leucaena leucocephala* were procured from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore. *Leucaena* seeds were scarified with concentrated H_2SO_4 for 20 min, and washed several times in distilled water; the seeds of acacias were soaked in boiling water for 3 min and soaked in distilled water overnight prior to sowing.

Treatments and cultural practices

Seven pesticides used in Indian forest nurseries were tested at realistic doses (Table 1). Fumigation was done with formaldehyde (37%) applied at the rate of 2L m^{-2}. The soil was covered for 48 h with transparent plastic sheets and then opened for aeration. For pesticidal drench, the pesticides were applied at the rate of 4L m^{-2} at recommended concentrations (Bavistin®, Cuman® 0.02%, Copperthom® 0.25%, Sulfex® 0.20%, Furadan®, Termix® 0.05%). The treatments were arranged in completely randomised block design with five replicate plots per treatment. Untreated control plots were subjected to the same operations except for fumigation and pesticide drench.

Table 1. Pesticides used and their modes of action

Commercial name	Active component	Formula	Characteristic	Mode of action
Formalin®	Formaldehyde	-	Fumigant	Inhibits respiration
Bavistin ®	Carbendiazim	Methyl 1-2-3 imidazole carbamate	Systemic fungicide	Inhibits fungal mitosis and microtubule formation
Cuman ®	Ziride	Zinc dimethyl dithiocarbamate	Non-systemic fungicide	Like formaldehyde
Copperthom ®	Copper oxychloride	-	Non-systemic fungicide	Interferes with enzyme system through denature of protein within fungal cells
Sulfex ®	Sulphur	-	Fungicide/ agaricide	Like formaldehyde.
Furadan ®	Carbofuran	2-3-Dihydro-2, 2-dimethyl 7-benzofuranyl methyl carbamate	Systemic insecticide/ nematicide	Interferes with enzyme systems of insects & nematodes
Termix ®	Chlordane	Hexa chlorocyclo pentadiene	Non-systemic insecticide	Acts on nervous system of insects

Seven days after pesticidal drench, seeds were sown in rows 30 cm apart. After emergence, the seedlings were thinned to allow *c.* 10 cm between adjacent seedlings within a row. Irrigation was initially done with a sprinkler and later with plastic tubes to minimise soil movements between plots.

Measurements

As the seedlings of different species emerged at different days, the harvests were staggered accordingly. The seedlings were harvested at 30, 60 and 90 days after emergence (DAE) with almost the entire root system intact. The roots were washed free of soil and nodule numbers were estimated visually. The mass of shoots and roots were measured after drying at 65 °C for 96 h. For the estimation of AM fungal colonisation, the roots were washed, cut into 1-cm long segments, cleared in 2.5% KOH at 90 °C, neutralised in 5N HCl and stained with 0.05% trypan blue in lactophenol. The percentage of root length colonised by AM fungi was determined using the root slide technique (Read *et al.* 1976). At harvests, 10 soil samples (*c.* 100 g) were collected at a depth of 0–11 cm and composited for assessment of AM fungal spore number. A 100-g composite soil sample was subjected to wet sieving and decanting (Gerdemann & Nicolson 1963). The sieve washings were collected over a filter paper with grid lines, spread on a Petri dish and counted under a dissection microscope. Only intact spores were counted and expressed as individuals per g of dry soil.

Plant analysis

Finely ground plant materials were wet digested in a triple acid mixture, and plant tissue P was determined by the molybdenum blue method (Jackson 1973). Tissue N was estimated after micro-Kjeldahl digestion (Humphries 1956) and K was estimated by flame photometry (David 1962).

Data analysis

All data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT). Linear regression analysis was used to determine the relationships between plant and mycorrhizal parameters.

Results

seedling growth. Root lengths colonised by AM fungi were significantly higher in all species grown in Sulfex treated soils at 30 DAE. Bavistin and Termix significantly enhanced root colonisation in *L. leucocephala* at 30 DAE. Termix enhanced root colonisation in *A. mellifera* and Furadan induced a similar effect at 30 and 90 DAE. The intensity of mycorrhizae was significantly low for pesticide treatments in *A. nilotica* at 90 DAE whilst such an effect in the same period was evident in *Leucaena* only in Sulfex treatment. In general, fumigation and pesticidal drench either significantly reduced or had no effect on spore numbers (Table 2). The spore number in Copperthom drenched soils in *Leuceana* at 30 DAE was significantly higher compared to the control; the other pesticides able to induce such an effect on mycorrhizal spore number were Sulfex, Furadan (both at 60 DAE) and Termix (at 90 DAE) in *A. mellifera*; and Bavistin and Cuman (both at 30 DAE) in *L. leucocephala*.

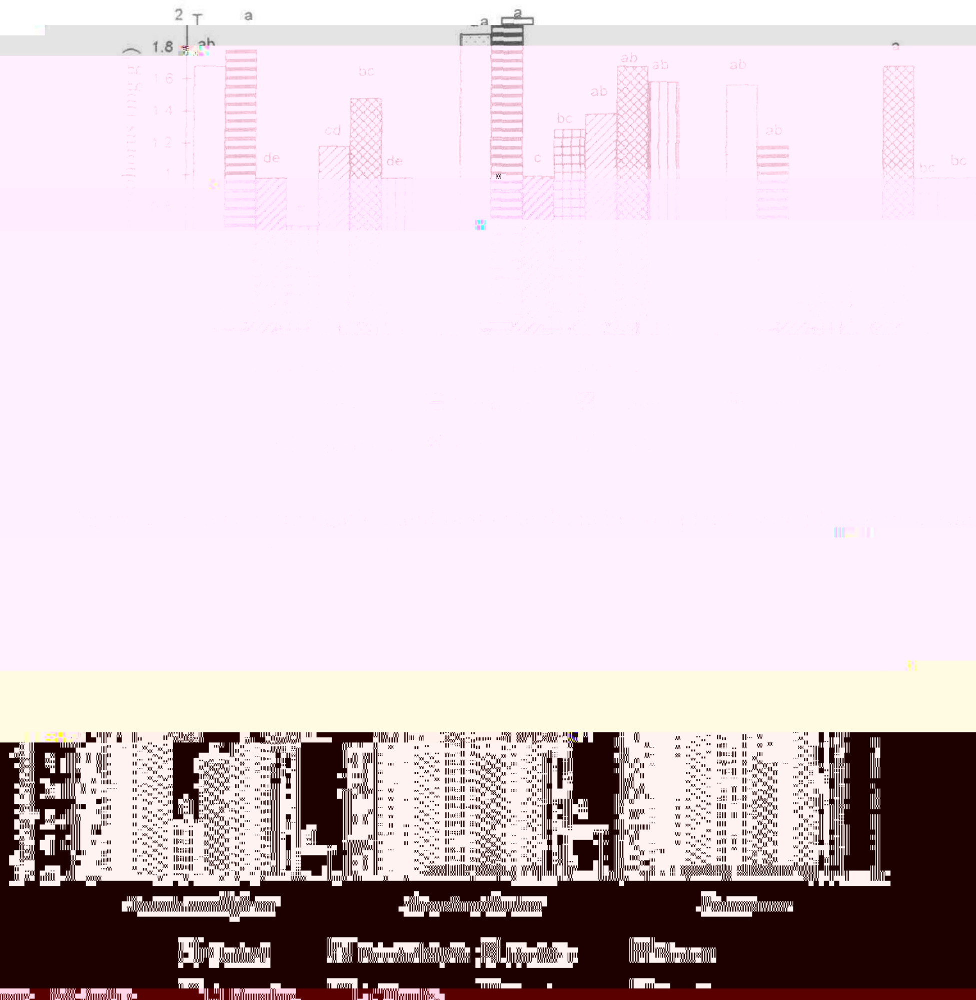
Root colonisation spore number and treatments	*Acacia mellifera* 30 DAE	60 DAE	90 DAE	*Acacia nilotica* 30 DAE	60 DAE	90 DAE	*Leucaena leucocephala* 30 DAE	60 DAE	90 DAE
Root colonisation									
Control	40cd	70c	67c	60bc	72bc	96a	50d	100a	100a
Formaldehyde	20e	100a	100a	40de	89a	98a	100a	100a	100a
Bavistin	50bc	68cd	72bc	70ab	26d	81b	70bc	79b	100a
Cuman	03f	59de	79bc	50cd	39d	50c	60cd	89ab	100a
Copperthom	30de	47e	73bc	30e	81ab	47c	30c	100a	100a

leucocephala seedlings in Sulfex and Bavistin drenched soil at 60 DAE and *A. millifera* seedlings in all treatments at 30 DAE had increased shoot dry weights (Table 3). Fumigation and pesticidal drench either reduced or had no significant effect on root dry weight in all the legumes, except for fumigation at 90 DAE

Table 4. Effect of fumigation and pesticide drenching on number of nodules per plant

	Acacia mellifera			*Acacia nilotica*			*Leucaena leucocephala*		
	30 DAE	60 DAE	90 DAE	30 DAE	60 DAE	90 DAE	30 DAE	60 DAE	90 DAE
Number of nodules plant[1]									
Control	18a	30a	38ab	15bc	40ab	43bc	29a	39ab	45b
Formaldehyde	20b	26bc	40ab	20a	43a	45bc	19cd	41a	49b
Bavistin	15cd	24c	37b	12de	38e	39bc	22bc	33c	50ab
Cuman	14d	25c	43ab	12de	43a	48b	18cd	38e	43b
Copperthom	15cd	24c	37b	11e	45c	55a	17d	39de	46b
Sulfex	13d	29ab	39ab	12de	49d	55a	16d	37b	56a
Furadan	14d	27abc	44a	14ed	41ab	48b	18d	32cd	49b
Termix	18a	30a	40ab	17b	42ab	46b	25ab	[illegible]	50ab

See footnote to Table 2.



et al. (1995). Formaldehyde has a poor penetrability (Hartley & West 1969). So AM fungal propagules within root bits or organic matter may remain unaffected and could serve as inocula in initiating mycorrhizal formation. Cuman (ziride) and Sulfex act similarly on fungi. The former reduced AM formation in the acacias. Indeed, Cuman and Sulfex application has already been reported to reduce ectomycorrhizal formation in *Pinus* (Bakshi & Dobriyal 1970) and endomycorrhizal formation in citrus (Singh *et al.* 1990) species. These fungicides, although non-systemic, interfere with the respiratory cycle of fungi, thereby affecting fungal metabolism. Copperthom either had no significant effect or reduced AM colonisation or spore numbers, which is in accord with Udaiyan *et al.* (1995). Copper oxychloride interferes with the fungal enzyme system by precipitating proteins, thereby influencing fungal metabolism. The low AM colonisation levels in *A. nilotica* and *Leucaena* in Bavistin (carbendiazim) treated soils support the results of Singh *et al.* (1990) that carbendiazim inhibits AM formation, but contradict those of Anusuya (1995) that carbendiazim either does not affect AM fungi or enhances AM colonisation and sporulation at the recommended level. Carbendiazim interferes with fungal growth, cell division and development through inhibition of mitosis (Morgan 1989).

The insecticides Furadan (carbofuran) and Termix (chlordane) had varied

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