FUNGI ASSOCIATED WITH LEUCAENA SEEDS AND THEIR INFLUENCE ON GERMINATION

M. Satish Chandra Prabhu, P. Venkatasubbaiah, K.M. Safeeulla and H.S. Shetty

Downy Mildew Research Laboratory, Manasagangotri, Mysore, India.

ABSTRACT

Seed health analysis was conducted on 5 different cultivars of *Leucaena leucocephala*. Twenty-seven different fungal species of both field pathogenic and storage importance were recorded. *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Verticillium* sp., *Trichoderma viridae* and *T. harzianum* were among the most important field fungi while *Aspergillus flavus*, *A. niger*, *A. candidus*, *A. fumigatus*, *A. ruber*, *A. versicolor* and *Penicillium* sp. were some of those with seed storage significance. Of the 3 standard methods employed for the seed health test, standard blotter method was found superior to deep freeze and agar plate system. Hot water treatment at 85°C for 5 min was shown to ward off most of the seed-borne fungi and retain the viability of the seeds.

Ann. Trop. Res. 4: 151-155.

KEY WORDS: *Leucaena leucocephala.* Fungal incidence. Seed health analysis. Standard blotter method. Deep freeze method. Agar plating.

INTRODUCTION

Leucaena *(Leucaena leucocephala* (Lam.) de Witt.) legume has become popular among tree farmers for its multiple use as fuel and charcoal, fodder, organic fertilizer, timber and in soil conservation. Out of 3 known varieties which include the Hawaiian type, Salvador type (K-8 and K-28) and Peru type, the Hawaiian type has been introduced with success in countries like India, Hawaii, Thailand, Indonesia, Philippines and Taiwan (Kushalappa, 1981).

Leucaena is generally propagated through seeds. However, seeds sown to raise seedlings often decay in the germination bed or get infection of hypocotyl leading to severe loss of seedlings (Quiniones, 1978). So far, no systematic study regarding the seed mycoflora of

Leucaena has been reported. The present investigation deals with the identification of mycoflora, their per-cent incidence and their effect on seed germination.

MATERIALS AND METHODS

Seeds of Hawaiian Giant, K-8, K-28 and 2 local varieties of Leucaena used in this study were collected and stored in paper packets at 25-28°C. To investigate the seed mycoflora and per cent seed germination, both hot water-treated (85°C for 5 min) and untreated seeds were tested using the following methods:

1. **Standard-blotter method:** Three layers of moistened circular blotting papers were placed in petri dishes of 9 cm dia. Twenty-five seeds were placed in each plate (ISTA, 1966).
2. Deep-freeze technique: Seeds were first incubated at 20 ± 2°C for 24 hr then deep-frozen at -20°C for the same number of hours. These were then placed in petri dishes as in the standard-blotter method (Limonard, 1968).
3. Agar-plating method: Seeds were surface-sterilized for 2 min using sodium hypochloride solution containing 1% chlorine and 10 seeds were placed into each petri plate with agar.

Four hundred seeds were used for each method. All the plates were incubated at 20 ± 2°C, alternating 12 hr of ultraviolet light kept at a height of 40 cm above the plates and 12 hr of darkness for 7 days. After the incubation period, the seeds were examined under stereo-binocular microscope at 40x magnification for the development and growth, color and fructification of fungal colonies. Slides were prepared to aid in the identification of fungi whenever necessary.

To study the percentage of germination in both hot water-treated and untreated seeds, 400 seeds from each variety were subjected to Ragdol paper towel method (ISTA, 1966). After 10 days, the percentage of germination was determined and recorded.

RESULTS AND DISCUSSION

As many as 27 fungal species were found on the untreated seeds and 10 in the hot water treated seeds (Tables 1 and 2). The field fungi which occurred in high per-centage were *Cladosporium herbarum, Alternaria tenuis, Fusarium solani, F. oxysporum, F. moniliforme, F. semitectum, Verticillium* sp., *Trichoderma viridae, T. harzianum* and *Trichothecium roseum;* and *Aspergillus flavus, A. niger, A. candidus, A. fumigatus, A. ruber, A. versicolor* and *Penicillium* spp. for the storage fungi. Among the cultivars used; Hawaiian Giant, K-8 and K-28 seed samples showed high percentage of field fungi whereas the 2 local varieties had the maximum incidence of storage fungi. The field fungi observed in the hot

Table 1. Incidence of different fungi commonly recorded on untreated seeds of 5 Leucaena varieties using blotter, deep-freeze and agar methods.

Fungi Recorded		Percentage of Fungi Detected H.G.	K-8	K-28	Local variety 1	Local variety 2	Ave. Incidence (%)
Alternaria tenius	B	82.5	5.0	2.0	30.5	0.5	25
	D	73.5	4.0	5.0	20.0	4.0	21.3
	A	2.5	4.0	1.0	0.5	0.5	1.7
A. tenuissima	B	0.5	—	0.5	—	1.0	0.4
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
Aspergillus flavus	B	3.0	6.0	11.5	8.5	39.5	14.2
	D	—	0.5	6.0	52.0	62.0	24.5
	A	0.5	—	1.0	2.5	4.0	1.6
A. niger	B	—	3.0	1.0	0.5	47.0	10.3
	D	—	—	—	22.5	81.0	20.7
	A	—	—	5.5	7.0	40.0	10.5
A. candidus	B	—	—	2.5	—	39.5	8.4
	D	—	—	3.5	4.0	46.0	11.1
	A	—	—	—	—	—	—
A. flavus	B	—	1.5	—	—	—	0.3
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
A. niber	B	—	—	—	—	—	—
	D	—	—	—	—	12.0	2.4
	A	—	—	—	—	—	—
A. fumigatus	B	—	—	—	0.5	—	0.1
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
A. nidulans	B	—	—	1.0	—	—	0.2
	D	—	—	5.0	—	—	1.0
	A	—	—	—	—	—	—
A. versicolor	B	—	1.5	1.0	26.0	—	5.7
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
A. ochraceous	B	—	—	—	6.0	—	1.2
	D	—	—	—	—	—	—
	A	—	—	—	2.0	—	0.4
Cephalosporium acremonium	B	—	—	—	—	—	—
	D	—	0.5	—	—	—	0.1
	A	—	1.0	2.5	1.0	—	0.9
Chaetomium spp.	B	0.5	1.0	—	—	2.5	0.8
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
Curvularia lunata	B	—	0.5	—	0.5	—	0.2
	D	—	1.5	—	—	—	0.3
	A	—	—	—	—	—	—
Cladosporium herbetum	B	11.5	—	—	5.5	0.5	3.5
	D	6.5	1.0	—	—	—	1.5
	A	—	—	—	—	—	—
Fusarium oxysporum	B	0.5	—	0.5	—	—	0.2
	D	—	—	—	4.0	—	0.8
	A	2.0	0.5	0.5	1.5	2.5	1.4
F. solani	B	0.5	2.5	—	2.5	—	1.1
	D	—	4.5	1.0	—	1.0	1.3
	A	1.0	1.0	6.5	4.0	0.5	3.0
F. semitectum	B	—	—	—	0.5	1.0	0.2
	D	—	—	—	4.0	—	0.8
	A	—	—	0.5	—	—	0.1
F. moniliforme	B	—	—	—	—	4.0	0.8
	D	—	—	—	—	—	—
	A	—	—	0.5	—	0.5	0.2
Mycotypha spp.	B	—	1.0	2.5	—	—	0.7
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
Penicillium spp.	B	2.0	1.0	2.5	10.5	1.0	3.4
	D	10.0	1.0	6.0	6.0	2.0	5.0
	A	0.5	0.5	2.5	1.0	1.0	1.1
Phoma spp.	B	1.0	0.5	2.0	—	1.5	1
	D	—	—	—	—	—	—
	A	—	—	4.0	—	2.5	1.3
Rhizopus spp.	B	—	1.0	0.5	—	—	0.3
	D	—	—	0.5	—	—	0.1
	A	—	—	—	—	—	—
Trichoderma viridae	B	2.0	5.5	20.0	5.5	42.5	15.1
	D	—	—	—	—	—	—
	A	3.5	4.0	25.5	6.0	50.5	17.9
Trichothecium roseum	B	—	3.5	—	—	5.5	1.8
	D	—	—	—	—	—	—
	A	—	7.5	—	—	6.5	2.8
Trichoderma harzianum	B	1.0	2.5	—	0.5	14.5	3.7
	D	—	—	—	—	—	—
	A	0.5	1.0	0.5	0.5	8.5	2.2
Verticillium spp.	B	—	3.5	3.5	—	—	1.4
	D	—	7.5	1.0	—	—	1.7
	A	—	—	—	—	2.0	0.4

B: Blotter method
D: Deep freeze method
A: PDA method.

water treated seeds were *Cladosporium herbarum, Fusarium solani, F. moniliforme, Trichoderma harzianum* and *Verticillium* sp. Storage fungi observed were *Aspergillus flavus, A. niger,* and *A. fumigatus.* However, the percentage of each fungus was considerably less compared to untreated seeds.

Higher number of fungal species was noticed on the blotter when compared to deep-freeze and agar-plate methods. Few fungal species, **one of them a *Mycotypha* spp., were observed in K-8 and K-28 samples using the blotter method.**

In all the samples made, the hot water-treated seeds showed high percentage of germination compared to the untreated seeds (Fig. 1). The highest percentage of

Table 2. Incidence of different fungi commonly recorded on hot-water treated seeds of 5 Leucaena varieties using blotter, deep-freeze and agar methods.

Fungi Recorded		Percentage of Fungi Detected H.G.	K-8	K-28	Local variety 1	Local variety 2	Ave. Incidence (%)
Aspergillus flavus	B	0.5	2.5	2.5	2.5	2.5	2.40
	D	—	—	5.5	20.0	6.5	6.40
	A	—	—	—	—	0.5	0.10
A. niger	B	—	—	0.5	0.5	17.0	3.60
	D	—	—	—	10.0	30.0	8.00
	A	—	—	—	2.5	2.0	0.90
A. fumigatus	B	—	0.5	—	1.0	0.5	0.40
	D	—	—	—	—	—	—
	A	—	—	—	—	—	—
Cladosporium herberum	B	24.5	0.5	0.5	7.5	—	6.50
	D	22.5	2.0	2.5	15.0	2.5	8.90
	A	0.5	—	—	—	—	0.10
Fusarium solani	B	—	—	—	0.5	—	0.10
	D	—	—	1.0	2.0	0.5	0.70
	A	0.5	0.5	1.5	0.5	0.5	0.70
F. moniliforme	B	—	—	—	—	0.5	0.10
	D	—	—	—	—	1.0	0.20
	A	—	—	—	—	1.0	0.20
Penicillium spp.	B	—	0.5	—	2.5	—	0.60
	D	4.0	—	2.0	4.0	2.0	2.40
	A	0.5	—	—	—	—	0.10
Rhizopus spp.	B	—	—	—	—	—	—
	D	—	—	—	—	—	—
	A	0.5	—	—	—	—	0.10
Trichoderma harzianum	B	0.5	—	5.0	2.0	2.5	2.00
	D	—	—	—	—	—	—
	A	1.0	—	6.5	1.0	5.5	2.60
Verticillium spp.	B	—	0.5	—	—	—	0.30
	D	—	2.0	—	—	0.5	0.50
	A	—	—	—	—	—	—

B: Blotter method
D: Deep freeze method
A: PDA method.

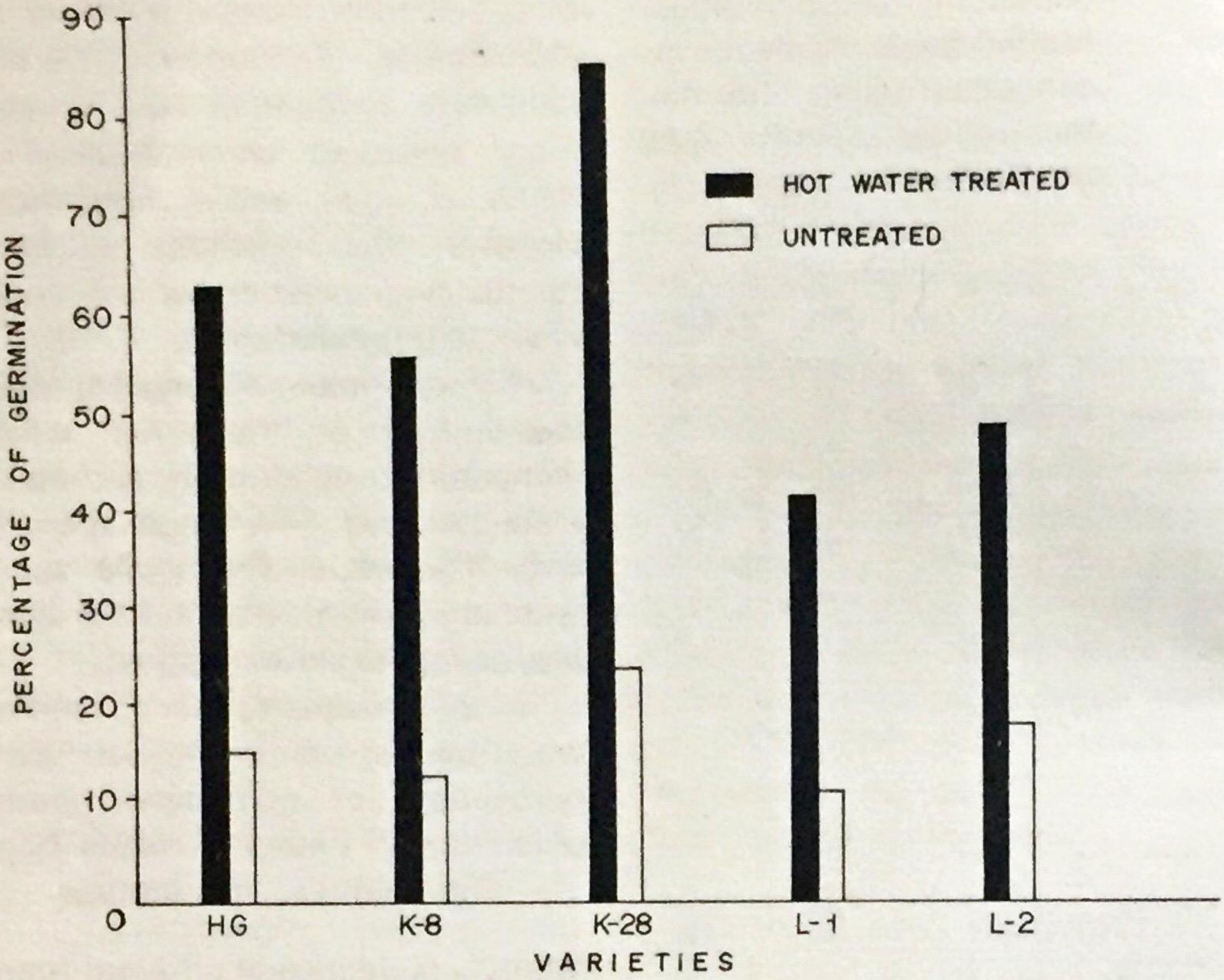

Fig. 1. Percentage of germination in hot-water treated and untreated Leucaena seeds.

germination observed was in K-28 (84%). The local varieties showed comparatively low percentage of germination. In some cases the germinated seedlings became stunted in growth and died prematurely as evidenced by the presence of *Fusarium* sp., *Trichoderma* sp., and *Verticillium* sp. which infected the seeds. Seeds infected by *Aspergillus flavus, A. niger* and *A. fumigatus* also showed a decline in germination.

Out of the 3 methods of testing percentage germination, the blotter method was found to be most suitable since most of the fungi got the same conditions of growth. In deep-freeze method, growth depended upon the degree of saprophytism of the fungi; and storage fungi, like *Aspergillus flavus* and *A. niger* could overlap the growth of other fungi. Likewise, in the agar-plate method, the added nutrients could support the growth of a batch of fungi which dominate the growth of slow growing ones.

Hot water treatment (Chaturvedi, 1981) was found to be a novel practice to preserve the viability of the seeds. In this study the hot water treatment preserved the viability of the seeds as well as reduced fungal contamination. The 17 fungi observed on the untreated seeds such as *Fusarium semitectum, F. oxysporum, Trichoderma viridae,*

Trichothecium roseum and *Cephalosporium acremonium* were absent in the hot water treated seeds. This might be due to their sensitivity to heat in the hot water treatment. The low percent incidence of other fungi such as *Fusarium solani, F. moniliforme, Cladosporium* sp., *Trichoderma harzianum* and *Verticillium* sp. in the hot water treated seeds might be due to their deep-seated nature as well as their inherent resistance to temperature (Neergaard, 1977). The deep-seated nature of *Fusarium* sp. was stressed by Mathur et al. (1975) in sorghum seeds. Neergaard (1977) reported the embryo-borne *Fusarium, Cladosporium* and *Trichoderma* fungal species. He added that the presence of *Fusarium* sp., *Verticillium* spp., *Trichoderma* spp. and *Cephalosporium* spp. in Leucaena seeds needs further investigation, since most of the fungal species are the causal agents of wilt diseases.

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