J. Agrofor. Environ. 2(2): 193-196, 2008 ISSN 1995-6983

Study on seed borne fungi of ipil-ipil (*Leucaena leucocephala*) and their control by plant extracts

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Abstract: Investigations have been done to detect the seed borne infections of Ipil-ipil (*Leucaena leucocephala*) and its control with plant extract. Seed borne fungi viz. *Aspergillus flavus, Aspergillus niger, Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Botryodiplodia theobromae* and *Curvularia lunata* were detected on two types of ipil-ipil seeds (newly harvested seeds and market seeds) following blotter method of seed health testing. The germination percentage was higher in newly harvested seeds than market seeds. Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Neem (*Azadirachta indica*) showed highest germination either in freshly or newly harvested seeds. Garlic (*Allium sativum*) was most effective in controlling seed borne infection of all the recorded fungi. The fungi *Fusarium* spp., *Botryodiplodia theobromae* and *Curvularia lunata* were effectively inhibited by dipping seeds in the extracts of clove of garlic for 15 minutes. The extracts of ginger (*Zingiber officinale*) and neem (*Azadirachta indica*) were also effective against *Fusarium* spp., *Botryodiplodia theobromae* and *Curvularia lunata*. In all cases comparatively lower effect was recorded in case of onion and arjun extracts. However, vitavax-200 as check fungicide was found best as seed treating agent for reducing fungal population. The extract of garlic clove was the best in controlling all the recorded fungi among the five plant extracts.

Key words: Seed borne fungi, control by plant extracts.

Introduction

The ipil-ipil is a native of Mexico and is widely distributed throughout the tropics. Probably this forage tree introduced into Philippine in the 16th century as a feed for ruminant livestock and subsequently spread throughout Asia-Pacific region. Previously it was used in only agroforestry but no longer it is also commonly planted in Bangladesh as forage crop. It is used as ruminant forage and as a fuel wood by subsistence and semi-commercial farmers of Bangladesh. Ipil-ipil foliage is noted for its very high nutritive value for ruminant production. Typical values for the edible fraction are 55-70%, digestibility 3-4.5% N, 6% ether extract, 6-10% ash, 30-50% N- free extract, 0.8-1.4% Ca and 0.23-0.27% P (Lenne, 1991). Healthy and Quality seeds are one of the most important requirement for yielding quality tree production which in turn give quality wood and wood products. But quality of forest tree seed in the country is poor. One of the major reason for poor seed quality or health of forest tree seeds is that often the seeds are infected by pathogens specially by pathogenic fungi (Srivastava, 1956; Salehuddin, 1988; Agmata, 1979; Mamata *et al.*, 1996; and Mehorotra *et al.*, 1998). Such infected seeds fail to germinate, resulting germination failure or seed rot. Infected seeds may also cause pre-emergence damping off and seedling disease complex in the nurseries. Apparently, no research works on the efficacy of botanical extracts on seed borne fungi of ipil-ipil has yet been taken in Bangladesh. Chemicals are being used extensively for controlling different diseases of agro-social forest trees. But reducing disease incidences with the chemicals create some unwanted effects on economic, ecological and sociological aspects. So to produce disease free ipil-ipil tree, disease free seeds are very much needed. However to avoid the harmful side effect of chemicals, seed treatment with botanicals might be considered as one of the important alternatives. There are some plant extracts which have antimicrobial qualities and antagonistic to some pathogens (Hossain *et al.*, 1993; Suratuzzaman *et al.*, 1994). In the view of the above facts, the present study has been undertaken to record the seed borne fungal pathogens associated with the seeds of ipil- ipil tree and to determine the effect of five plant extracts (Onion, Garlic, Neem, Ginger, Arjun) in controlling seed borne pathogens of ipil- ipil.

Materials and Methods

The experiment was carried out in the Seed Pathology Center (SPC), Bangladesh Agricultural University (BAU), Mymensingh during the period from October, 2007 to January, 2008. Seeds of ipil-ipil trees were collected from the Botanical garden of BAU, Mymensingh and Momenshahi Seed Store, Mymensingh. The seed samples of Botanical garden were collected immediately after ripen of pod in October, 2007. The seed samples were tested by blotter method for the presence of seed borne fungi following the International Rules for Seed Health Testing Association (ISTA, 2003). For each treatment 200 seeds with four replications were placed on 20 petridishes. The petridishes with seeds were then incubated at room temperature (24 ± 2^{0}C) under 12/12 hours alternating cycles of NUV and darkness in the incubation room of the seed pathology center, BAU, Mymensingh. Time to time watering was made to keep the filter paper moist. On the 12th days of incubation the germination and the pathogen associated with the seeds were recorded. Here incubation for 12 days were maintained due to hard and thick of seed coat. Seed-borne infections of fungi observed under the sterio microscope at 20X were identified by observing their growth characters on the incubated seeds. The fungi were identified to species level, wherever possible, following the keys of Barnett (1965), Ramnath *et al.* (1970) and Booth (1971). Temporary slides were prepared from each fungal colony, in the cases where they could not be identified under steriobinocular microscope. Identification of the fungi confirmed by preparing temporary slides and examining them under compound microscope. In fewer cases the fungi from the incubated seeds were transferred to PDA medium in petridishes asceptically and incubated under

controlled temperature (28^0C ± 1) for 3-10 days and then examined under compound microscope for their identification. The extracts of bulb of onion, clove of garlic, rhizome of ginger, bark of arjun and leaf of neem were used for the experiment (Table 1).

Table 1. Particulars of plants used

SL. No.	Local Name	Scientific Name	Plant parts used (25gm/100ml, H_2O)
1	Onion	*Allium cepa*	Bulb
2	Garlic	*Allium sativum*	Clove
3	Neem	*Azadirachta indica*	Leaf
4	Ginger	*Zingiber officinale*	Rhizome
5	Arjun	*Terminalia arjuna*	Bark

The plant extracts were prepared by chopping after cleaning in running water. From each plant sample, 25 gms were taken and macerated in a mortal and pestale and suspended separately in 100 ml distilled water. After 24 hours of soaking, samples were filtered through 3 folds of fine cloth. These filtrates were arbitrarily termed as standard and the filtrate was used as extract. The extracts were stored in a refrigerator at 4^0C ± 1 until used. The seeds were dipped in plant extracts 1:2 concentration and duration for 15 minutes in previously prepared Onion, Garlic, Ginger, Neem and Arjun extracts. In case of control, seeds were treated only with sterile water. The treated seeds were then placed in moist blotters in petridishes, following standard blotter method (ISTA, 2003). In case of Vitavax-200, 200 seeds were taken in a small conical flask. Fungicide of requisite amount (0.25% of seed wt) was taken into the flask and shaken to mix the chemicals thoroughly. The experiment was laid out in Completely Randomized Design (CRD) with four replication. Five petridish were considered as one replication. Two hundreds seeds were taken at random as per ISTA (2003) rules. Out of 200 seeds 10 seeds per petridish were placed in each petridish. The data collected from the experiment were analyzed for finding out test of significance and compared the treatment means following Completely Randomized Design (CRD) by using DMRT test at 5% level of probability.

Results and Discussion

Germination test

The result of germination test carried out by blotter in incubation test of ipil-ipil seed of two locations were shown in Table 2. The germination percentage was higher (68.75%) in BAU (newly harvested seeds) than Notun Bazar (61.25%) (Market seeds) for untreated (control) were recorded.

Effects of different plant extracts on the germination of ipil-ipil seed

Effect of different plant extracts on the germination of ipil-ipil seeds for BAU location, and Notun Bazar location was determined and presented in Tables 2 and 3. Different plant extracts showed significant variation on the germination of ipil-ipil seed of two locations. In case of freshly harvested seed, comparatively highest germination (83.75%) was recorded in Vitavax 200 than garilic, ginger, neem (82.50%). The lowest (68.75%) was found in control. Vitavax- 200 increased seed germination by 21.82% over control. Extract from garlic, ginger and neem increased seed germination by 20.00% over control (Table 2).

Table 2. Effect of five different plant extracts on the germination of ipil-ipil seeds

Treatments	*SEED GERMINATION (%)*		Germination increased over control (%)	
	NEWLY HARVESTED SEEDS	*MARKET SEEDS*	*NEWLY HARVESTED SEEDS*	*MARKET SEEDS*
Control	68.75b	61.25c	-	-
Vitavax-200 (Check fungicides)	83.75a	78.75a	21.82	28.57
Garlic	82.50b	77.50ab	20.00	26.53
Ginger	82.50b	75.00ab	20.00	22.45
Neem	82.50b	67.50abc	20.00	10.20
Arjun	72.50bc	65.00bc	5.45	6.12
Onion	80.00b	72.50abc	16.36	18.37

Two hundred seeds were tested for each sample
Figure with common letters did not differ significantly at 5%level of DMRT

In case of market seeds, collected from Notun bazaar location, the highest germination (78.75%) was recorded in Vitavax-200 followed by garlic extract (77.50%). The lowest germination (61.25%) was found in control treatment. There was no significant differences among garlic, ginger, neem, arjun and onion. Vitavax-200 increased, seed germination by 28.57% over control (Table 2).

Effect of different plant extracts in controlling of seed borne fungi of ipil-ipil

Effect of different plant extracts on the prevalence of different seed borne fungi of ipil-ipil seed (BAU location) was determined and presented in Table 3. Different plant extracts showed significant variation on the prevalence of *Aspergillus flavus, Aspergillus niger, Penicillium* spp., *Fusarium* spp., *Rhizopus* sp., *Botryodiplodia theobromae*, *Curvularia lunata*.

Garlic and Vitavax-200 were found most effective for controlling seed borne fungus, *Aspergillus flavus, Aspergillus niger, Penicillium* spp. Here, the fungus was completely controlled (0.00%) by vitavax-200.In case of seed treated with garilic, the prevalence of fungus was 1.50%. Seed treated with distilled water (Control) were more affected by this fungus with 18.50% prevalence, which was statistically significant and different from other treatments. The extract from ginger was found more effective than neem, arjun and onion. Percentage of fungi prevalence in ginger, neem, arjun and onion were 4.50%, 5.50%, 6.00% and 8.50% respectively. Plant extracts garlic, ginger, neem, arjun and onion were statistically identical.

The associations of the above mentioned seed-borne fungi were also detected by other researcher on the seeds of different forest tree species (Salehuddin, 1974; Rahman, 1977; Rahman and Choudhury, 1978; Haque and Fakir, 2002; Vhumic, 2006). The seed treated with the extract of garlic, giner, neem and vitavax-200 showed highest percentage of germination. Similar findings were noticed by of Suratuzzaman *et al.* (1994); Khan and Kumar, (1992) and Islam (2001). The extract of bulb of onion and bark of arjun also found to increase the germinations percentage over the control (Table 2). In Pakistan one research work had been carried out for the occurrence of mycoflora on the seeds of ipil-ipil and their effect on seed germination, khalid *et al.* (2002). In our country one similar research work had been done by Vhumic (2006), where she worked on the occurence of seed borne fungi of agro-social forest tree. Many research works had been done with plant extracts against seed borne fungi of many other crops but no research on this aspect about forest trees had been recorded. Considering the findings of the present studies, it was found that Vitavax-200 as check fungicides was the best for germination and reduction of seed borne fungal pathogens but considering all aspects performance of selected plant extracts clove of garlic, rhizome of ginger and neem leaves were satisfactory. As Vitavax-200 is hazardous and serious threat to our ecology as well as our terrestrial and aquatic species of animal, use of plant extract is essential to keep our environmental pollution free.

Table 3. Effect of five different plant extracts on the prevalence of different seed borne fungi of newly harvested seeds of ipil-ipil

Treatments	% prevalence of seed borne fungi						
	Aspergillus flavus	*Aspergillus niger*	*Penicillium spp.*	*Fusurium spp.*	*Rhizopus spp.*	*Botryodiplodia theobromae*	*Curvularia lunata*
Control	18.50a	14.50a	15.00a	16.00a	17.50a	7.50a	7.00a
Check fungicide (vitavax-200)	0.00f	0.00e	0.00d	3.00c	1.50d	2.00c	1.00d
Garlic	1.50e	2.00d	1.00d	0.50e	1.00d	1.00d	1.50d
Ginger	4.50d	3.50c	2.00cd	1.50cde	3.00cd	1.50d	2.00cd
Neem	5.50c	4.00c	5.00b	1.00d	4.00cd	1.00d	2.50bcd
Arjun	6.00c	3.50c	4.50b	2.50cd	3.50cd	4.00b	3.50bc
Onion	8.50b	7.50b	3.50bc	6.50b	5.00b	3.50bc	4.00b

Two hundred seeds were tested for each sample; Figure with common letters did not differ significantly at 5%level of DMRT
Before analysis data were transformed following Arcsin transformation

Table 4. Effect of five different plant extracts on the prevalence of different seed borne fungi of market seeds of ipil-ipil

Treatments	% prevalence of seed borne fungi						
	Aspergillus flavus	*Aspergillus niger*	*Penicillium spp.*	*Fusurium spp.*	*Rhizopus spp.*	*Botryodiplodia theobromae*	*Curvularia lunata*
Control	17.00a	14.00a	15.00a	15.50a	17.50a	7.50a	6.50a
Check fungicides (Vitavax-200)	0.00e	7.00b	2.00cd	2.50c	1.50d	1.00c	1.50b
Garlic	2.00d	2.00c	1.50d	0.50c	2.50cd	1.00c	2.00b
Ginger	4.00c	3.50c	2.50bcd	1.00c	2.50cd	2.00c	2.50b
Neem	5.00c	3.00c	4.50b	1.50c	4.00c	1.50c	3.50b
Arjun	5.50c	3.50c	3.50bcd	2.50c	4.00c	4.00b	3.50b
Onion	8.00b	2.50c	4.00bc	6.00b	5.00b	4.50b	3.50b

Two hundred seeds were tested for each sample; Figure with common letters did not differ significantly at 5%level of DMRT
Before analysis data were transformed following Arcsin transformation

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