FUNGI ASSOCIATED WITH IPIL-IPIL (*LEUCAENA LEUCOCEPHALA*) (LAM) DE WITH SEEDS AND THEIR CONTROL^{a/}

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ABSTRACT

Thirteen fungi were isolated from *Leucaena* seeds from different places in the Philippines. These were; *Fusarium moniliforme*, *F. solani*, *F. semitectum*, *Botryodiplodia theobromae*, *Cephalosporium* sp., *Phoma* sp., *Cladosporium* sp., *Chaetomium* sp., *Penicillium* sp. and *Aspergillus flavus*. *C. graminicola* completely inhibits the germination of seeds once it has colonized the seeds. Ten fungicides were tested for seed treatment and only Benlate, Daconil, Delsene MX and AgroCaptan were capable of protecting and/or eradicating the growth of *Colletotrichum* spp., *Fusarium* spp. and *Botryodiplodia theobromae* in seeds in storage.

INTRODUCTION

The involvement of seed borne fungi in forest tree seeds in the Philippines is not well documented. In preliminary observations of these investigators some fungi were found attacking the dipterocarp seeds while they are still in the trees. Consequently, if these infected seeds fall to the ground, few will ever germinate.

Tamesis (1949) in reviewing the Philippine forest and forest products researches observed that damping-off got a toll of 40% in nurseries in Mindanao. In a preliminary survey made by Agmata (1979) of the fungi associated with 16 species of forest trees, she found a high percentage infection of *Fusarium semitectum* in all samples collected from Ilocos Norte and Zambales. Eusebio and Quimio (1979) also found damping-off as a serious disease of Benguet pines during summer months with infection reaching to 40%.

The extensive use of giant ipil-ipil as reforestation species has caused some problems in the availability of "good" seeds. It was observed that seeds in the market are infested with insects and fungi. Feedbacks from farmers who planted them get very low germination.

REVIEW OF LITERATURE

Studies on the association of different fungi on tree species had been conducted by some workers. Agmata (1979) in a preliminary study of Philippine forest tree seeds, reported high percentage of infection by *Cephalosporium* sp. in Mindoro pine, *Fusarium solani* in malapapaya, *Phomopsis* sp. in narra and in large mahogany seed. In Malaysia, Lee and Abdul Manab (1981) reported 33 species of fungi isolated from seven species of Dipterocarp the most common of which are *Botryodiplodia theobromae* and *Colletotrichum gloeosporoides*.

In agricultural corps, Jamil Khan (1974) found different species of *Aspergillus* on seeds of different crops in Pakistan. In the Philippines *Aspergillus* spp. and *Penicillium* spp. were also found in soybean and peanut seeds causing poor germination (Dalmacio and Daya 1977). *Aspergillus flavus* was also high in pre-harvested, dried and stored corn (Ilag 1973).

Thirumalachar (1977) observed that when *Macrophomina phaseolina* was inoculated to beans it would cause damping-off, browning, and rotting of the entire cotyledon of seedlings.

Different fungi either seed borne, field and storage can be controlled by several methods. In the study of Vir et al (1970) they found that Dithane M-45 and Ceresan gave a complete control of *Dreschlera* spp. on barley and *Trichoconis padwickii* on stockburn disease of rice. Besides chemical control, use of hot water treatment was found to control the fungus. Neergaard (1977) stated that loose smut of wheat can be controlled by pre-soaking the wheat to ordinary water for 5 hours and then soaking the seeds at 54°C for 10 min.

OBJECTIVES:

- a. To identify the fungi associated with ipil-ipil seeds from the field and in storage.
- b. To determine the most effective fungicide(s) that could protect giant ipil-ipil seeds in storage.

MATERIALS AND METHODS

Isolation and identification of fungi

Seventy four seed samples were collected from different areas in the islands of Luzon, Visayas, Mindanao for the study.

The standard blotter and agar plate tests recommended by International Seed Testing Association (ISTA 1966) were followed. For blotter test, 200 seeds of each samples were used at 25 seeds per plate. The petri dishes were lined with 3 layers of moistened filter paper to provide proper conditions for fungal growth. For agar plate test, 20 ml of sterile potato dextrose agar (PDA) was plated in each sterile petri dish and allowed to congeal. Seeds were surface sterilized with 5% NaOCl for 30 min, washed on several changes of sterile distilled water and plated at 10 seeds/plate. Plated seeds were incubated at room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) 12 hr cycle under ultra violet light and then under daylight fluorescent lamps for 7 days. On the 7th day, seeds were examined with stereo binocular microscope (6X – 40X) for the presence of fungi. Where fungal growths were present slide mounts were made and examined with an Olympus stereo microscope.

1. MORPHOLOGICAL STUDIES

In order to get proper morphological growth for identification a slide culture of each fungus isolated was made. These were examined under the microscope daily to observe for the appearance of the fruit bodies. These cultures were made into semi-permanent mounts. The shape and color of conidia, conidiophores, spore measurements and mode of attachment were noted.

2. PHYSIOLOGICAL STUDIES

To determine the species of fungi within the same genus, special media were used.

These were potato dextrose agar (PDA), malt agar (MA), Czapeck's agar (CZA), yeast extract agar (YEA) and Malt extract agar (MEA). These sterilized media were plated in sterile petri dishes with about 20 ml in each plate. A bit of mycelial growth of each pure culture was placed at the middle of each plate and the plates were incubated at different temperatures (15°C, 25°C, 28°C and 33°C). At least 5 plates/treatment were used. One hundred thirty five plates were used for the 9 fungi replicated 3 times using 5 different media. The kind of mycelial growth, and the sizes of fruiting structures that are used for identification like spores, conidiophores and setae were noted.

PATHOGENICITY TEST ON MATURE PODS.

Apparently healthy mature pods of local and giant ipil-ipil were collected for inoculation with pure culture of the different fungi previously isolated and identified. The pods were disinfected with 10% NaOCl for 5 min, washed with sterile distilled water for 3 times and blotted with sterile filter paper. Injuries were made on the pods with pins to simulate insect damage before the fungal spore suspension were inoculated. The inoculated pods were incubated in moistened plastic bags for 1 week. Control pods with similar treatment but without fungi were provided. Disease reaction using following rating was noted after 1 week.

Disease rating	Infection rate
0	no infection
1	1-3 mm infection
2	3.1-5 mm infection
3	5.1-7 mm infection
4	7.1-9 mm infection
5	whole inoculated area infected

PATHOGENICITY TEST ON SEEDS

Apparently healthy seeds of local and giant ipil-ipil were secured from around Los Banos. The seeds were sacrificed with sulfuric acid for 30min, washed in tap water for 3 changes and then soaked overnight. On the next day the seeds were disinfected with 10% NaOCl for 3 min washed with sterile distilled water three times and blotted dried. The seeds were dipped in fungal suspension before they were sown in sterile soil, 100 seeds/treatment, replicated 2 times. The number of seeds that germinated were recorded everyday until no more seeds germinated. From these data the percentages of germination were computed.

BIOASSAY OF DIFFERENT FUNGICIDES IN THE LABORATORY

To determine the effect of 10 different fungicides in protecting the seeds and/or in eradicating the growth of seedborne fungi in storage, 7 test fungi namely: *Botryodiplodia theobromae*, *Colletotrichum gloeosporoides*, *C. graminicola*, *C. truncatum*, *Eusarium solani*, *F. semitectum* and *F. moniliforme* were used. The 10 fungicides were Agro-captan (50% ai), Benlate (50% ai), Manzata (50% ai), Daconil (75% ai), Plantineb (80% ai), Thiovi (80% ai) and Vitigran blue (58% ai). Four concentrations were used; 0 ppm, 50 ppm, 100 ppm and 200 ppm. Required amounts of each chemical were mixed with the medium (PDA) suitable for this test and poured on sterile petri dishes.

A bit of mycelial growth of each fungi was placed in the middle of each plate before they were incubated at room temperature. The percentage inhibition of each fungicides on radial growth of fungus was expressed by:

$$\text{Percentage inhibition} = \frac{\text{average radial growth of control} - \text{average radial growth of treated}}{\text{average radial growth of control}} \times 100$$

Effect of seed treatment on the germination of ipil-ipil seeds stored at 10°C

Three fungicides; Benlate, Delsene MX and Daconil that were found effective in the laboratory assay against the fungi associated with the seeds were used for seed treatment to determine their ability to protect the seeds from fungus contamination and/or to eradicate the fungi that are already present inside the seed. Ten replications were made at 200 seeds/replicate. Each replicate was weighed and amount of fungicide was computed according to this formula:

$$\frac{\text{Amount of chemical used/ kg of seed}}{\text{kg of seed}} = \frac{\text{Recommended rate}}{\% \text{ active ing. of chemical}}$$

The seeds in a flask were sprinkled with sterile distilled water before the fungicides were added. The flask was shaken for sometime until the seeds were coated with the fungicides. The treated seeds were stored in plastic bags kept in a refrigerator at 10°C. Samples were taken every 15 days and sown in washed river sand to get the percentage germination. The sampling was done for a period of 6 mon.

RESULTS

Using the blotted and agar palte methods of isolation (Fig. 1a and 1b) 9 genera of fungi and 13 species were identified. The frequency of distribution of these fungi by collection sites is as follows:

Fusarium spp.	74
F. semitectum	47
F. solani	11
F. moniliforme	16
Colletotrichum spp	46
C. graminicola	43
C. garminicola	2
C. truncatum	1
Botryodisporium sp.	40
Cephalosporium sp.	29
Aspergillus spp.	24
Penicillium spp.	16
Phoma sp.	12
Cladosporium sp..	2
Chaetomium sp.	1

1. *Fusarium semitectum* Berk. and Rav.

On seeds: Mycelial growth are whitish, shiny bearing macroconidia at the tips. Conidiophores arise singly or in groups. On severely infected seeds mycelial growths are dull yellow to yellow.

Fig. 1a. Agar method: Giant ipil-ipil seeds showing colony characteristics of *Fusarium* spp. and *Colletotrichum gloesporoides* (X 1/2).

Fig. 1b. Blotter test: Giant ipil-ipil seeds showing colony characteristics of *Botryodiplodia theobromae* (X 1/3)

On PDA: The presence of branched conidiophores with macroconidia is marked diagnostic characteristics. Sporechium is absent. Macroconidia 3-5 septe, curved, wedge shaped, hyaline, pedicellate foot cell absent. Microconidia absent. (Fig. 2). Conidial measurements vary on different media. On YEA, $30.4 \times 5.0 \mu$; PDA, $27.9 \times 4.9 \mu$; CZA, $25.0 \times 6.0 \mu$ and on MEA, $22 \times 4.6 \mu$. Maximum growth on PDA incubated at 28°C – 30°C was observed.

Fig. 2. Photomicrograph of *Fusarium semitectum* Berk and Rav. from a slide culture, 4 days old (X 600)

2. *Fusarium solani* (Mart.) Sacc.

On seeds: Mycelial growth dull white and very loose. Milky white droplets are seen on the mycelium with naked eye but when examined under the microscope, these are the microconidia. On slide culture, microconidia are broader and oval shape. Macroconidia fusoid, with rounded foot cell (Fig. 3). Apical cell is pointed and somewhat beaked.

Fig. 3. Photomicrograph of *Fusarium solani* (Mart) Sacc. from a slide culture, 4 days old (X 600)

Macro and microconidia develop abundantly at 28°C in PDA. At 25 °C, only macroconidia are produced. At 33°C macroconidia are sparse but microconidia are plenty. On PDA micro and macroconidia measures 13.2 x 2.9 μ and 32.5 μ ; YEA, 10.9 μ and 37.2 x 4.8 μ ; MEA 12.9 x 3.3. μ and 32.5 x 5.3 μ and CZA, 9.7 x 3.6 μ and 36.5 x 5.2 μ , respectively.

3. *Fusarium moniliforme* Sheldon

On seeds: Abundant mycelial growth which are whitish to purplish white or very light orange mycelial growth are seed under the stereo binocular microscope (40X).

On PDA: Mycelium scanty, chain of microconidia can be observed under binocular microscope (40X) but with naked eye they appear powdery. Microconidia are hyaline, fusiform to elevate (Fig. 4a). Measurements of spores vary on different media; PDA, 6.8 x 3.1 μ ; YEA, 8.0 x 2.7 μ and on MEA, 5.1 x 2.7 μ . Macroconidia are inequilaterally fusoid, thin walled with elongated sharply curved pedicellate basal cell, 3-7 septate (Fig. 4b). Average measurements in different media; 27.5 x 3.0 μ (PDA), 19.6 x 3.3 μ (YEA), 34.2 x 3.2 μ (MEA) and 12.0 x 3.9 μ on (CZA.) Maximum temperature for growth in 28°C to 30°C in PDA.

Fig. 4a & b. Photomicrograph of *Fusarium moniliforme* Sheldon from a slide culture showing microconidia (4a) macroconidia (4b) (X 1500)

4. *Collectotrichum gloesporoides* (Penz) Sacc.

On seeds: Pale orange to salmon orange mycelial growth when in mass but scanty; sometimes with brownish coloration.

On PDA: Greyish white at first then turning orange to dark orange with diurnal zonation. Conidia oblong or ellipsoidal, uninucleate, hyaline and aseptate (Fig. 5). Spores vary in measurements. On PDA, average sizes, $15 \times 5.0 \mu$; MEA $12.0 \times 4.0 \mu$ and $14 \times 5.0 \mu$ on MA. If setae is formed, it is 1-3 septate, swollen at the base, darker than the tip. Setae measurements on PDA, $63.6 \times 4.3 \mu$.

5. *Colletotrichum graminicola* (Ces.) Wilson

On seeds: Spore masses grey or dirty pink to salmon orange. Mycelium scanty.

On PDA: Grey compact growth; zonation absent. Conidia falcate, hyaline and aseptate (Fig. 6). Average measurements vary; on PDA, $22 \times 3 \mu$, $28.0 \times 3.0 \mu$ (MEA) and $28 \times 4.0 \mu$ on MA. Setae brown, slightly swollen at the base. Setae measurements also vary; on PDA, $134.9 \times 5.8 \mu$, MEA, $143.2 \times 6.4 \mu$ and $170.1 \times 6.9 \mu$ on MA.

Fig. 5. Photomicrograph of *Collectotrichum gloesporoides* from a slide culture, 6 days old. No setae is produced yet (X 600)

Fig. 6. Photomicrograph of *Colletotrichum graminicola* (Ces.) Wilson from a slide culture 6 days old (X 600)

6. *Colletotrichum truncatum* (Schw.) Andrus et Meore

On seeds: Spore masses grey to salmon orange. Mycelian scanty.

On PDA: Grey to dark mycelial growth, zonation not so distinct. Conidia falcate, uninucleate, hyaline and aseptate (Fig. 7). Average measurements on different media; on PDA, 25 x 3.0 μ , MEA 24 x 3.0 μ and on MA, 14 x 5.0 μ . Setae brown, 1-7 septate, base not swollen, tip tapered measurements vary on different media; on PDA 132.5 x 6.6 μ , MA, 93 x 6.0 μ and on MEA, 143.1 x 6.6 μ .

Fig. 7. Photomicrograph of *Colletotrichum truncatum* (Schw.)
Andrus et Moore from a slide culture 6 days old (X 600)

7. *Botryodiplodia theobromae* Sacc.

On seeds: Pycnidia black and ostiolate. Mycelial growth fluffy. In slide culture pycnidia develop within 10 days under ordinary light. Conidiophores simple and short. Conidia aseptate, hyaline, double cell wall when young, round to oblong in shape (Fig. 8). Matured conidia are 2 celled, dark ovoid with apical portion somewhat pointed, basal portion rounded to oblong. Spore measurements vary on different media, on PDA, 25.3 x 16.2 μ , MA, 25.2 x 18.0 μ and on MA 24.4 x 14.8 μ .

8. *Cephalosporium* sp.

On seeds: Conidial masses attached to the conidiophores in somewhat slime drops, light in color.

On PDA: Mycelium not so very distinct, sparse conidiophores slender and simple. Conidia produced successively at the tip and collection in a slime drops, oblong in shape, similar to microconidia of *Fusarium moniliforme* (Fig. 9).

Fig. 8. Cross section of a pycnidium of *Botryodiplodia theobromae* showing immature conidia (X 600)

Fig. 9. Photomicrograph of *Cephalosporium* sp. from a slide culture, 4 days old (X 600)

9. *Aspergillus flavus* Link

On seeds: Yellowish to dark green especially on seeds stored for several months. Conidiophores simple, uncolored. Conidial heads radiate, splitting into several columns, vesicle globose to subglobose (Fig. 10). Cultures when old produced sclerotial bodies; white at first, then turning brown to dark brown with age. Conidia measure $3.6 \times 4.5 \mu$.

Pathogenicity test on matured local and giant
ipil-ipil pods

Fig. 10. Photomicrograph of *Aspergillus flavus* Link from a slide culture, 4 days old (X 600)

Table 1 shows that all 13 isolates of fungi were pathogenic to local ipil-ipil pods while *C. gloesporoides*, *C. graminicola*, *C. truncatum*, *F. solani*, *Cephalosporium* sp. and *Botryodiplodia theobromae* were pathogenic to giant ipil-ipil pods (Fig. 11). The rest of the isolates were less pathogenic with infection ranges from 1.2 – 3.6.

Fig. 11. Disease reaction initiated by different fungi: Fm, *Fusarium moniliforme*; Fs, *F. semitectum*; Fso, *F. solani*; Con, Control; Cepha, *Cephalosporium* sp.; Clad, *Cladosporium* sp.; Cogr, *Colletotrichum graminicola* and Bd, *Botryodiplodia theobromae* on mature giant ipil-ipil pods, 1 week after inoculation.

Pathogenecity test on local and giant ipil-ipil seeds.

Table 2 shows that of the 13 isolates tested *C. graminicola* completely infested and inhibited the germination of local and giant ipil-ipil seed. *F. moniliforme* also affected the germination of local ipil-ipil seed (4%) as compared with 54.5% germination of giant ipil-ipil. The rest of the isolates were not so effective in arresting germination of local and giant ipil-ipil seeds.

Table 1 : Disease reaction of 13 fungal isolates on mature local giant ipil-ipil pods

Isolates	Disease rating ^a	
	Local	Giant
<i>Fusarium moniliforme</i>	5.0	3.6
<i>F. solani</i>	5.0	4.8
<i>F. semitectum</i>	5.0	4.2
<i>Botryodiplodia theobromae</i>	5.0	4.5
<i>Collectotricum graminicola</i>	5.0	5.0
<i>C. gloesporoides</i>	5.0	4.8
<i>C. truncatum</i>	5.0	5.0
<i>Cephalosporium</i> sp.	5.0	4.8
<i>Cladosporium</i> sp.	5.0	2.2
<i>Chaetomium</i> sp.	5.0	1.3
<i>Phoma</i> sp.	5.0	1.6
<i>Aspergillus flavus</i>	5.0	1.2
<i>Penicillium</i> sp.	5.0	1.2
Control	—	—

Disease rating	Infection rate
0	No infection
1	1-3 mm infection
2	3. 1-5.0 mm infection
3	5. 1-7.0 mm infection
4	7. 1-9 mm infection
5	Total inoculated area infected

Table 2: Percentage germination of local and giant ipil-ipil seeds inoculated with different fungal isolates.^a

Isolates	Percentage germination ^b	
	Local	Giant
<i>Fusarium moniliforme</i>	4.0	54.5
<i>F. solani</i>	80.0	48.5
<i>F. semitectum</i>	65.0	44.5
<i>Colletotrichum graminicola</i>	0.0	0.0
<i>C. gloeosporoides</i>	42.0	35.0
<i>C. truncatum</i>	67.0	40.0
<i>Cephalosporium</i> sp.	60.0	43.5
<i>Botryodiplodia theobromae</i>	53.0	60.5
<i>Cladosporium</i> sp.	68.0	60.5
<i>Chaetomium</i> sp.	65.0	77.0
<i>Phoma</i> sp.	59.0	39.0
<i>Aspergillus</i> sp.	69.0	30.0
<i>Penicillium</i> sp.	64.0	30.0
Control	—	—

^areadings done after 1 week

^b100 seed/treatment, 50 sees/replicate

Assay of different fungicides against *Botryodiplodia*
Fusarium spp. and *Colletotrichum* spp. on PDA

Table 3 shows the percentage inhibition of *B. theobromae* by the different fungicides tested. The 3 fungicides that were effective in inhibiting the growth of the fungus were Daconil 200 ppm with 90.1% inhibition, Benlate at 50 ppm with 95.9 and Delsene MX at 50 ppm with 97.6% inhibition.

Table 3: Percentage inhibition of growth of *Botryodiplodia theobroame* on potato dextrose agar treated with different concentrations of fungicides^a

Fungicides ^b	Treatment (ppm)		
	50	100	200
Agro-Captan	51.0	63.7	77.3
Belate	95.7	95.7	95.9
Daconil	52.4	64.0	90.1
Agrofungex	3.8	64.1	70.6
Manzate	65.0	68.0	73.7
Bra			
Brassicol	89.0	89.0	89.0
Delsene MX	97.6	97.6	97.6
Plantineb	51.9	63.3	70.8
Thiovit	4.1	8.1	18.7
Vitigan blue	39.0	52.0	84.3

^aAverage of 4 readings/3 replications/treatment

^bAgro-Captan (50% AI) N-trichloromethyl thio 4-cyclohexene 1-2 dicarboximide.

Daccoil (75% AI) tetrachloroisophthalonitrile
 Benlate (50% AI) Benomyl methyl 1 (butylcarbomyl) 2 benzimidazole carbamate
 Agrofungex (50% AI) N (trichloromethylthio) cyclohexene-4-ene 1-2 dicarboximide
 Manzate (50%AI) Maneb-zinc ion; ethylene bis (dithiocarbamate) iron
 Brassicol (75% AI) Pentachloronitrobenzene
 Delsene MX (72% AI) Mancozeb- 6 methyl 1-2 benzimidazole carbamate
 Plantineb (80% AI) Manganese ethylene bis (dithiocarbamate)
 Thiovit (80% AI) elemental sulfur
 Vitigran blue (58% AI) copperoxycchloride

Fusarium semitectum was also inhibited by Benlate 100 ppm with 94.0% inhibition. Daconil at 200 ppm 80% inhibition and Delsene MX at 200 ppm with 94% inhibition. Delsene MX (77%) and Daconil (76%) showed much lower inhibition. Delsene MX (77%) and Daconil (76%) showed much lower inhibition compared with Benlate. At low concentration (50 ppm), Benlate completely inhibited the growth of *F. moniliforme*, Daconil (77%) and Delsene MX (90%) at 200 ppm respectively.

Table 4: Percentage inhibition of the growth of different *Fusarium* spp. on potato dextrose agar with different concentrations of fungicides^a

Fungicides ^b	Treatment (ppm)								
	F. semitectum			F. solani			F. moniliforme		
	50	100	200	50	100	200	50	100	200
Agro-Captan	40.0	58.0	60.0	67.0	73.0	74.0	31.5	69.0	69.4
Benlate	91.0	92.0	94.0	78.0	93.0	94.4	100-	100	100
Daconil	53.0	63.0	80.0	74.0	75.0	76.0	64.0	65.0	77.0
Agrofungex	9.0	20.0	24.0	7.0	58.0	58.3	63.0	66.0	69.0
Manzate	13.0	32.0	34.0	4.0	64.0	67.0	31.2	65.0	74.0
Brassiccol	46.0	49.0	53.0	6.0	69.0	70.3	53.0	63.0	69.0
Delsene MX	79.0	90.0	94.0	6.2	69.0	77.0	73.6	76.0	90.0
Plantineb	20.0	25.0	49.0	18.0	30.3	34.3	24.5	37.7	57.0
Thiovit	31.0	34.0	53.0	3.4	22.0	29.0	26.1	30.8	63.0
Vitigran blue	2.7	16.0	18.0	2.5	5.9	18.0	11.0	26.0	50.0

^a Average of 7 readings, 3 replications/treatment

^b Agro Captan (50% AI) N-trichloromethylthio 4-cyclohexene, 1-2 dicaboximide
 Benlate (50% AI) Benomyl methyl 1 (butylcarbomyl) 2 benzimidazole carbamate
 Daconil (75% AI) tetrachloroisophthalonitrile
 Agrofungex (50% AI) N-trichloromethylthio cyclohex-4-ene; 1-2 dicarboximide
 Manzate (50% AI) Maneb-zinc ion; ethylene bis (dithiocarbamate) iron
 Delsene MX (72% AI) Mancozeb; 6 methyl, 1-2 benzimidazole carbamate
 Plantineb (80% AI) elemental sulfur
 Vitigran blue (58% AI) copper oxychloride

Table 5 shows the effect of 10 fungicides on 3 species of *Collectotrichum* in culture. *C. gloesporoides* was completely inhibited at 100 ppm using Benlate, Delsene MX at 200 ppm with 94.3% inhibition. Agro-Captan showed 78% inhibition at 200 ppm. For *C. graminicola* inhibition was higher at 200 ppm, Benlate with 85%, Agro-Captan (87%) and Delsene MX, 80% inhibition. Benlate completely inhibited the growth of *C. truncatum* at 50 ppm. Agro-Captan at 100 ppm while Delsene MX at 200 ppm.

Table 5: Percentage inhibition of growth of *Colletotrichum* sp. on potato dextrose agar treated with different concentrations of fungicides^a

Fungicides ^b	Treatment (ppm)								
	<i>C. gloesporoides</i>			<i>C. graminicola</i>			<i>C. truncatum</i>		
	50	100	200	50	100	200	50	100	200
Agro Captan	40.5	73.2	78.0	58.0	81.7	87.0	53.2	100	100
Benlate	95.0	100	100	82.0	82.0	85.0	100	100	100
Daconil	40.3	43.0	47.1	32.0	39.0	44.0	20.0	49.0	75.0
Agrofungex	7.1	13.0	18.0	3.1	20.0	22.0	8.2	12.0	17.0
Manzate	30.0	32.0	41.0	15.2	37.0	49.0	4.2	5.6	12.0
Brassicol	35.0	48.0	54.0	62.0	67.1	67.1	21.0	26.0	40.3
Delsene MX	80.5	92.5	94.3	74.0	80.1	80.1	88.0	92.3	100
Platineb	10.9	11.3	40.3	20.7	31.7	47.0	2.3	16.0	17.7
Thiovit	12.0	36.0	37.6	24.3	30.2	0	11.2	15.0	
Vitigran blue	16.8	18.0	39.0	10.3	10.7	29.0	15.0	14.4	25.3

^a Average of 7 readings, 3 replications/treatment

^b Agro Captan N-trichloromethyl-thio 4 cyclohexene, 1, 2 di-carboximide

Benlate (50% AI) Benomyl methyl 1 (butycabomyl) 2 benzimidazole carbamate

Daconil (75% AI) tetrachloroisophthalonitrile

Agrofungex (50% AI) N-tricholormethyl thio cyclohex-4-ene 1-2 dicarboximide

Manzate (50% AI) Maneb-zinc ion; ethylene bis (dithiocarbamate) iron

Brassicol (75% AI) Pentachloronitrobenzene

Delsene MX (72% AI) Manconzeb; 6 methyl 1-2 benzimidazole carbamate

Plantineb (80% AI) Manganese ethylene bis (dithiocarbamate)

Thiovit (80% AI) elemental sulfur

Vitigran blue (58% AI) copper oxychloride

Effect of seed treatment of the germination of ipil-ipil seeds stored at 10°C

Benlate, Daconil and Delsene MX were used for seed treatment to determine their ability to protect the seeds from fungus contamination and to eradicate the fungus that is already in seeds. Table 6 shows that seeds treated with fungicides had higher germination compared with the control. Seeds treated with Delsene MX had much higher germination (44.2%), as compared with Benlate, 32.0% and Daconil (40.1%). However, for all treatment there was a reduction in seed germination after 2½ months os storage, Benlate (16.0%) Daconil (16.5%), Delsene MX (11.0%) and Control (6.0%).

Table 6: Effect of seed treatment on the germination of giant ipil-ipil seeds in storage

Sampling ^a	Treatment			
	Control	Benlate	Daconil	Delsene MX
15	22.5 ^b	30.0	37.0	45.0
30	30.0	31.0	37.0	49.0
45	21.5	37.0	34.0	43.0
60	12.5	40.0	43.0	42.0
75	17.0	32.0	40.1	44.2
90	6.0	16.0	16.5	11.0
105	6.5	18.5	18.0	13.0
120	9.5	20.5	11.0	12.5
135	4.0	1.0	7.0	5.5
150	3.5	1.0	5.0	5.0

^aSampling was done at 15 days interval

^bAverage of 10 replications; 200 seeds/treatment

DISCUSSIONS

Results of this study showed that local and ipil-ipil seeds are affected by a wide variety of fungi. Six of 13 fungal species are known potential pathogens, *F. moniliforme*, *F. solani*, *F. semitectum*, *C. gloeosporoides*, *C. truncatum* and *C. graminicola* (Mathur et. al. 1975, CMI 1971 and Ram Nath et al 1970).

The occurrence of *F. semitectum* in all samples supported the observation of Agmata (1979) when she found high percentage of infection of the forest tree seed samples collected from Ilocos Norte and Zambales. In related studies Mathur et al (1975) and Dayan (1980) also found the presence of *F. moniliforme* and *F. semitectum* in sorghum seeds collected in India and Philippines. *F. solani* was also found associated with wilt diseases of ipil-ipil (Quiniones 1978) besides causing seed rotting in this report.

Of the 3 *Collectotrichum* species isolated, *C. graminicola* is the most pathogenic. Infected ipil-ipil seeds never germinate once this species has colonized the seeds. The same fungus was also isolated on ipil-ipil seedlings from Mabuhay Vinyl Corporation causing seedling rot (Quiniones 1981).

Botryodiplodia theobromae and *Cephalosporium* sp. also occurred in most samples collected. Agmata (1979) also found these fungi on seeds of Benguet pine and large mahogany. Although *B. theobromae* is known to be a weak parasite. Quiniones (1980) found it to be causing seedling dry rot (*Sweitenia macrophylla*) in the Bureau of Forest Development (BFD) nursery in Peddig, Ilocos Norte. In Jekutong, Malaysia (*Dyera costulata*), Griffith (1966) and Hong (1976) found this organism causing stains on the wood.

The occurrence of *Penicillium* spp. and *Aspergillus* spp. in most samples was also noted. Seeds heavily infected with these fungi normally have low germination. Christensen (1973) found these fungi caused deterioration of soybean seeds in United States of America (USA) by reducing the quality of stored seeds.

Pathogenicity tests conducted on pods and seeds showed that the different isolates can caused infection and reduced seed viability in various proportions. Microscopic examina-

tion showed that the fungi invaded both the cotyledon and the embryo. In the case of *F. moniliforme*, Mathur et al. (1975) reported the fungus initially colonizes the scutellar region and such proximity to the embryo could easily explain the reduced germination of the infected seeds. This was also true to ipil-ipil seed inoculated with *C. graminicola*.

The higher percentage germination as a result of seed treatment may probably be due to reduction in seed infection during storage. This observation collaborated with the works of Hansing (1970) and Dayan (1980) who found that seeds treated with Captan had protected sorghum seeds from infection with smut, *F. moniliforme* and *Curvularia lunata*. Benlate was effective in protecting and eradicating fungi attacking ipil-ipil seeds. Lapis et al (1980) also found Benlate effective for the control of *F. moniliforme*, causal pathogen of Bakanae disease of rice.

RECOMMENDATIONS

1. To safeguard the infection of seeds with fungi in seed orchard, the trees must be sprayed with a combination of insecticides and fungicides. Azodrin and Benlate are recommended for at least 3 to 4 application during seed production.

2. Newly harvested seed should be sun-dried for 4 days to reduce moisture content of the seed to 10%. Benlate, Daconil and Delsene MX may be used for seed treatment but treated seeds should be planted at once or not go beyond 2 months of storage to ensure high germination.

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