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PATHOLOGICAL PROBLEM AND ITS REMEDY OF SOME FAST GROWING FUEL WOOD TREE LEGUMES

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Seed and leaf infecting fungi were isolated from four fast growing fuel wood yielding tree legumes. Koch postulation studies confirms the presence of *Penicillium sp.* and *Rhizopus sp.* in seed infection, and in leaf infection a mixed population of fungi were observed. Effect of fungicides on isolated fungal pathogens and free phenol contents of investigated plant under control and inoculated conditions also observed.

Keyword : Fuel wood; Fungicides; Phenol; Plant pathogen.

Introduction

In nature, plants growing in fields are exposed to several pathogenic organisms that cause diseases. Fungi are an important group of fungi that are pathogenic to plants. A number of mycoflora like *Rhizopus*, *Erysiphae*, *Verticillum*, *Penicillium* and *Thermomyces* were associated with tree seeds such as *Acacia*, *Albizia* and *Cassia* causing germination failure of such species[1]. Seedlings are also particularly vulnerable to pathogens. Dargan and Dulat[2,3], Dargan *et al.*[4] and Mamatha *et al.*[5] studied the pathological problems and associated mycoflora of certain important multipurpose trees. Diseases of seeds and seedlings of *Acacia* species[6,7], *Albizia* species[8-10] and *Leucaena* species[11,12] have been reported from different geographical localities. Effect of fungicides like Bavistain, Blitox and Dithane M-45 against the isolated fungal pathogens of *Acacia*, *Albizia*, *Bauhinia*, *Dalbergia* have been reported[13-15].

Plants produce a large, diverse array of organic compounds, like phenolics, alkaloids and other secondary metabolites that appear to have no direct function in growth and development, but serve as defense compounds against herbivores and pathogen[16]. It is an established fact that many secondary metabolites of plants, particularly phenolics impart a great role in conferring resistance in plants against different pathogens[17,18].

In the present investigation with fuel wood yielding trees, plants were first screened, under field condition, for occurrence of pathological diseases and the responsible organisms. Disease occurrence and causal organism was confirmed through Koch's postulation experiment. Next, pathogenic fungi were tested against fungicides for their sensitivity. Finally, seedlings of the selected plants were analyzed for accumulation of phenolic substances as defense compound in response to pathogenic infection to specific fungi. This will give an insight of relative resistance of these fuel wood plants against fungal diseases, which help in afforestation programme.

Materials and Methods

(a) *Identification of plant pathogens responsible for diseases:* Seeds and leaves with infections were collected from in and around Santiniketan, surface sterilized with 0.1% $HgCl_2$ for 2-3 minutes[19], infected portions were cut into small pieces and were inoculated on the malt extract (ME) plate. The inoculated petridishes were incubated at 28 ± 2°C in BOD incubator for 3-5 days. The colonies appeared on these plates were counted and characterized. Identification of the fungal cultures was done by microscopic observation of the stained cultures following the standard literature [20, 21].

(b) Confirmation of causal agent for diseases in seeds or seedlings by reinoculation (Koch's postulation): For pathological study healthy seeds of fast growing species viz., *Acacia auriculiformis*, *A. holosrricea*, *Albizia lebbeck*, and *Leucaena leucocephala* were taken as plant material. Twenty days old seedlings were taken as plant samples. Koch postulation experiments were performed using cultures isolated from the infected plant parts. This was done using conidia of freshly grown isolates against seedlings of their specific hosts (the plant species from which they were originally isolated).

To infect, seedlings were first rubbed mildly with

Table 1. Fungal Pathogens Causing Diseases of Four Fast Growing Fuel Wood Species.

Plant Species	Infected Plant Parts	Name of the Pathogens
Acacia auriculiformis	Seed Leaf curl	*Penicillium sp., Erysiphae sp., Rhizopus sp., Curvularia sp.*
Acacia holosrricea	Seed Leaf curl Leaf spot	*Penicillium sp., Erysiphae sp.* *Curvularia sp.* *Phytophthora sp.*
Albizia lebbeck	Seed Leaf curl	*Rhizopus sp.* *Botrytis sp., Phytophthora sp.*
Leucaena leucocephala	Seed Leaf spot	*Penicillium sp.* *Phytophthora sp.*

Table 2. Organisms isolated after Koch's Postulation Experiments.

Plant Species	Seed infection	Leaf infection
Acacia auriculiformis	*Penicillium sp.*	*Curvularia sp.*
Acacia holosrricea	*Penicillium sp.*	*Curvularia sp.* *Phytophthora sp.*
Albizia lebbeck	*Rhizopus sp.*	*Botrytis sp.*
Leucaena leucocephala	*Penicillium sp.*	*Phytophthora sp.*

sterilized sand and then applied with conidia at different inoculum density. One control set was maintained. Five seedlings were inoculated for each treatment. After 15 days of treatment seedlings were observed carefully for the development of any symptoms comparable to their mother host. Next, infected seedlings (leaves and stem) were cut into pieces, thoroughly surface sterilized with 0.1% $HgCl_2$ for 2-3 minutes, washed three times with sterile water and dried with sterilized blotting paper before plating on to malt-agar medium (with same composition). The inoculated petridishes were incubated at 28 ± 2° C in BOD incubator for 3-5 days. Seeds were also mixed with isolated pathogens similarly under aseptic condition. After 15 days only monocultures were obtained in most cases.

(c) Effect of fungicides on isolated fungal pathogens: The fungi, which were isolated, were then tested for their growth responses in presence of two common fungicides viz., Blitox and Dithane M 45. Different concentrations (50-1000 μg ml^{-1}) of these fungicides were mixed with malt-agar medium and each fungus was tested for their ability for growth in presence of fungicides. The inoculated petridishes were incubated at 28 ± 2°C in BOD incubator for 3-5 days.

(d) Assessment of defense against pathogens: Assessment of the plant defense against pathogen was done by analyzing the accumulation of free phenols by the seedlings infected with specific pathogens. The extraction and estimation of the total free phenol content was done following standard method[22]. Content of phenols was calculated by comparing the absorbance with a standard curve prepared from catechol (Sigma Chemical Co., USA). The content of free phenol was expressed as mg g^{-1} fresh weight.

Results and Discussion

Infected seeds and leaves of the selected plants produced different fungal colonies, in ME plates, which are enlisted in Table 1. In infected seeds of *A. auriculiformis*, fungal species like *Penicillium*, *Erysiphae* and *Rhizopus* were found. For leaf curl diseases, infected leaf part produced colonies of *Curvularia* sp. In case of *A. holosericea*, seeds

Table 3. Fungicide tolerance level of the isolated pathogens (After koch's postulation against Blitox and Dithane M-45).

	Blitox (μg ml^{-1})						Dithane M 45 (μg ml^{-1})					
	0	50	100	200	500	1000	0	50	100	200	500	1000
Seed												
Penicillium sp.	+	+	+	+	+	-	+	+	+	+	+	-
Rhizopus sp	+	+	+	+	+	-	+	+	+	+	+	-
Leaf												
Curvularia sp.	+	+	+	-	-	-	+	+	+	-	-	-
Phytophthora sp.	+	+	+	-	-	-	+	+	+	-	-	-
Botrytis sp.	+	+	+	-	-	-	+	+	+	-	-	-

were infected with *Penicillium* sp. and *Erysiphae* sp. while *Curvularia* sp. and *Phytophthora* sp. were found to be associated with leaf curl and leaf spot respectively in this plant. In all cases single colonies of fungi were observed in malt-agar medium. In case of *A. lebbeck*, seed infection was due to *Rhizopus* sp. and leaf curl diseases was due to *Botrytis* sp., *Phytophthora* sp. and one other unknown fungal species. *L. leucocephala* seeds were infected by *Penicillium* sp. and in case of leaf spot disease the fungal colony produced on culture medium was very much similar to *Phytophthora* species.

After reinoculation of the pathogens (fresh conidia) in seeds or young seedlings (20 days old) of the respective plants followed by inoculation into malt agar medium, fungal colonies produced in ME- plates were found to be less in number compared to earlier (Table 2) for each plant species. Thus, only *Penicillium* species was observed in case of seed infection of *A. auriculiformis* after Koch's postulation. Two fungal species viz., *Erysiphae* sp. and *Rhizopus* sp., which appeared in the earlier study, were totally absent after reisolation experiment. While for leaf infection of young seedlings of this species single colony of *Curvularia* sp. could be isolated. After Koch's postulation experiment, infected seeds of *A. holosericea* showed only *Penicillium* species thus confirming this fungus as the casual organism of seed infection. Similarly, *Curvularia* sp. and *Phytophthora* sp. caused leaf curl and leaf spot diseases, respectively. In case of seed of *A. lebbeck* monoculture of *Rhizopus* sp. was isolated, while infected leaves produced colonies of *Botrytis* sp. only. Here also *Phytophthora* and some other unknown fungi, which were found earlier, were totally absent in reisolated plates. Newly infected seeds of *L. leucocephala* produced colonies of *Penicillium* sp. and infected leaf part showed the presence of *Phytophthora* sp. in ME medium.

The fungi, which were reisolated, were tested for their sensitivity to two common fungicides (Blitox and Dithane M-45). It was observed that (Table 3) the growth of the fungi responsible for seed infection (viz. species of *Penicillium* and *Rhizopus*) was not affected by lower concentration of both the fungicides (50-500 μg ml^{-1}), but at higher concentration (1000 μg ml^{-1}) colonies of those organisms were not formed at all in ME culture medium. In case of fungal species causing leaf diseases in all the fast growing species, 200μg ml^{-1} concentration and above inhibited growth as no colony appeared in these concentrations.

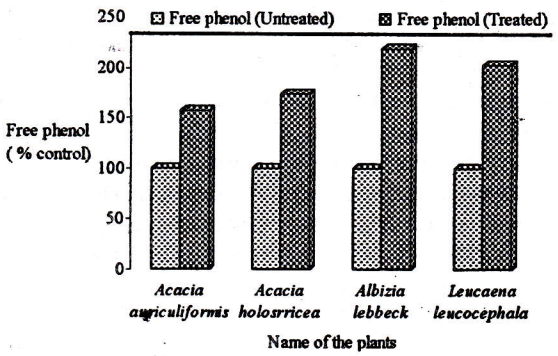

Fig 1. Free phenol contents of investigated plant under inoculated condition (by pathogen responsible for leaf infection).

When seedlings (20 days old) of the fast growing fuel wood yielding plants were inoculated, with pathogens responsible for leaf diseases, phenol content increased in leaves of all species (Fig. 1). However, such increase was highest in *A. lebbeck* seedlings followed by *L. leucocephala* while, *A. auriculiformis* seedlings showed lowest amount of phenol among all species.

In afforestation programme, large quantities of seeds of forest trees are being collected and used for raising seedlings in nurseries. Depending on moisture content seed can carry a number of pathogenic organisms and unhealthy seeds have the potential of introducing dangerous diseases to new plantation areas. Mostly fungi as compared to bacteria, viruses or nematodes cause large

number of plant diseases that occur more commonly in seeds.

In the present study both the species of *Acacia*, *A. auriculiformis* and *A. holosericea*, severe seed infection was found to occur due to infestation by *Penicillium* sp., while *Erysiphae* sp. and sometimes *Rhizopus* species were loosely associated with *Penicillium* as these species were totally eliminated through Koch's postulation experiment. For leaf curl disease, the causal organism was confirmed to be *Curvularia* species for both *A. auriculiformis* and *A. holosericea*. While *Phytophthora* species was confirmed to be the causal organism for the leaf spot diseases of *A. holosericea*. Diseases like, different types of seed infection, seedling blight, leaf spot, and defoliation are very common in *Acacia* spp. Several workers[8, 23-25] reported about different types of fungi responsible for seed and seedling diseases.

In the present investigation, causal organism of seed infection of *A. lebbeck* was *Rhizopus* species and the casual organism of leaf curl disease was *Botrytis* species. Earlier, there were reports on destructive damping-off of seedlings caused by *Rhizoctonia solani* in nurseries of *A. lebbeck* in India [9] while leaf spots of *A. lebbeck* caused by *Cercospora* species[26]. On the other hand, Khan and Mishra[10] described *Alternaria* sp. in leaf spot disease of *A. lebbeck*. Sharma and Bhardwaj[8] and Dargan and Dulat[2] reported about several other fungi responsible for diseases in *A. lebbeck*. But in no case *Botrytis* was reported so far as pathogenic organism for *A. lebbeck*. So, present report on the occurrence of *Botrytis* in causing disease in *A. lebbeck* is new.

Leucaena leucocephala is the most intensively researched, widely used and best known forage tree. Camptomeris leaf spot, caused by *Camptomeris leucaenae*, reduced forage production and quality of *L. leucocephala* throughout Central and South America, the Caribbean, India, Taiwan and Philippines[25]. Here in local environment *L. leucocephala* seeds were found to be infected by *Penicillium* species and in case of leaf spot disease the casual organism was *Phytophthora* species. Moreno *et al.*[11] reported seed infection by *Pseudomonas* species. But in our study no bacterial member was isolated from the seeds of *L. leucocephala* even on nutrient agar plate containing griseofalvin (200 µg ml^{-1}).

Chemicals are commonly, successfully and economically used to control seed and seedling diseases of tree legumes [5, 12, 15]. The fungi, which were isolated from the tree species used during our investigation, were tested for their growth responses in presence of two common systemic fungicides (Blitox and Dithane M-45). It was observed that the fungi responsible for seed infection of these trees viz., *Penicillium* and *Rhizopus* species were found to be less sensitive since their growth was inhibited only at higher concentration (1000 µg ml^{-1}) in ME medium. On the other hand, fungi responsible for leaf diseases viz., *Phytophthora*, *Curvularia* and *Botrytis* species were found to be far more sensitive to fungicides as they can be controlled at 200 µg ml^{-1} concentrations for both fungicides. There are variable reports on the effectiveness of the fungicides to control fungal diseases. In the present study, Blitox affected the growth of the test fungi (responsible for leaf diseases) remarkably at 200µg ml^{-1} concentration. However, some recent studies [13, 14] indicate the effectiveness of fungicides like Bavistin, Blitox and Dithane M-45 for the control of tree and forest mycopathogens. It may be assumed, therefore, that a particular fungicide may show differential effects on different species of pathogen under the same genus. Limited work has been done on control of foliage diseases of tree legumes. Tree pathologists have generally minimized the importance of foliar diseases as these rarely affect timber production, usually the most valuable forest product. However, if the tree legume is grown as a source of forage and green manure, effects of foliage diseases on forage production and quality become more significant.

When seedlings were inoculated with the pathogens responsible for specific leaf diseases, phenol content increased in leaves of all species. It is an established fact that many secondary metabolites of plants particularly phenolics impart a great role in conferring inducible resistance to the plant against different pathogens. However, such increase was highest in *A. lebbeck*. Seedlings of *A. auriculiformis* showed lowest amount of phenol accumulation in response to infection among all the species. This intrinsic character of the plant may be of constitutive or inducible in nature, which helps plants to overcome the unfavorable condition [17, 18]. If such intrinsic characters of plants are detected at seedlings or earlier stage of plant life, this could be utilized for mass cultivation without a significant pathogenic incidence.

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