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SEED BORNE DISEASES OF WOODY TREES IN EGYPT

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ABSTRACT

Routine and specific techniques were carried out to detect seed-borne microflora of five trees grown in Egypt i.e., Acacia mangium, Araucaria angustifolia, Casuarina equisetifolia, Eucalyptus camaldulensis and Ficus bengalensis. Seeds were collected from a number of trees grown in commercial nurseries. The blotter test was the most acceptable method for the detection of seed-borne fungi. Eight fungal genera i.e., Botryodiplodia, Botrytis, Cephalosporium, Colletotrichum, Curvularia, Fusarium, Macrophomina, and Phoma along with two genera of bacteria viz., Pseudomonas and Xanthomonas were detected. Botryodiplodia theobromae, Fusarium moniliforme and F. oxysporum were the most prevalent fungi isolated. They caused seed rot, seedling diseases, seedling mortalities, wilt, growth suppression, and stem deformation. Pseudomonas sp. and Xanthomonas sp. were also common causing various seedling diseases including dieback. Colletotrichum gloeosporioides, Fusarium moniliforme, F. oxysporum and Phoma spp might be seed transmitted as they were isolated from embryos, on the other hand, Botrytis spp, Cephalosporium spp, Curvularia tuberculata, Macrophomina phaseolina were found in seed coat. Trichoderma spp when mixed with fertilizers improved seedling growth and minimizing the incidence of seed-borne diseases.

Keywords: Woody trees , seed borne microflora , blotter test , fertilizers.

INTRODUCTION

Although natural forests are uncommon in Egypt, wood trees and shrubs are often common in orchards, gardens, streets and agricultural roads. In nurseries, seedlings of such trees are facing many diseases . Because orchards contain many trees of the same species, they are more susceptible than individual trees scattered across a farm or community. Seeds of softwood and hardwood trees are known to be attacked by fungi and other micro organisms Ivory, (1987). Seed-borne pathogens of wood trees affect nursery seedling and reduce seed germination and seedling vigor. They also decrease the longevity of the stored seeds. Several species of fungi which are generally considered as saprophytes , behave as pathogens under certain circumstances, such as injury to seed or seed coat, conducive moisture and temperature conditions which favor the fungal growth and increase physiological and physical vulnerability of tree cone,fruit, seed, or seedling to infection Mittal et al.,(1990). Studies on the mycoflora of eucalyptus seeds have shown that a large number of fungal species were associated with seeds. Although most of these fungi are saprophytes, a number of them are pathogens or potentially pathogenic. (Mittal et al., (1990); Mohanan and Sharma (1991); Pongpanich (1990); Sharma and Mohanan (1991); Yuan et al. (1990). Colletotrichum and Curvularia are associated with damping-off of Eucalyptus camaldulensis in Malaysia Ahmad (1987). Gibson (1978) described Botryodiplodia theobromae as important

fungus causing stem canker and die-back which killed young Pinus seedling. This fungus was currently causing severe die-back of Acacia mangium Wiley (1993). Several fungi are known to cause deformation, decay, and reduction in germination or may even cause complete destruction of seeds Gravatt (1931). Seeds of agricultural crops may carry fungal spores on their coat or inside . Fungi that found inside the seed, most often occur as mycelium. The seed-borne fungi, in general, may be classified as externally associated or internally found . Number of methods are available to detect fungi in seeds ISTA(1981) and Singh and Mathur(1993). In certain methods, the actual presence of the fungus is detected. In others, the effects they cause in seeds, or by the symptoms they produce on seedlings and young plants. Therefore, the present investigation was carried out with the aim to survey seed borne diseases of some woody trees grown in Egypt and to find out the possibilities to minimize their harmful effect.

MATERIAL AND METHODS

Seed samples of Araucaria angustifolia (Bertol) Kuntze, Acacia mangium R. Br., Eucalyptus camaldulensis Dehnh, Casuarina equisetifolia Forster et G. Forester and Ficus bengalensis. L. were collected from 20 commercial nurseries where seedlings are often damaged. Seeds of each specie was subjected for health test by different standard methods ISTA(1993).

Seeds, were incubated on a substrate, such as water soaked standard blotter, deep freezing blotter and PD agar medium and the developing fungal genera were recorded .

Standard Blotter method: 200 seeds were plated on water-soaked filter papers, five seeds per 15 cm diameter Petri dish. The seeds were incubated at 22 ± 2 °C under 12 hours of alternating cycles of darkness and fluorescent light.

Deep-freezing blotter method: The preparation and procedures were the same as for standard blotter, except that the dishes were transferred to -20 oC in the second day of incubation, at 20 oC . Then they were given five days incubation at 20 oC.

Agar-plate method: 200 seeds were surface sterilized in 1% sodium hypochlorite solution for 1 minute, then plated five seeds per 15 cm diameter Petri dish. Incubation was as for the blotter test.

Isolation and identification of fungi:

Fungi which developed from the five tested seeds were transferred to agar media, then identified based on colonies formed on the culture media after 7 days incubation at 25°C using stereo-or compound microscope, according to Neergaard(1973) .

Isolation and identification of bacteria:

Extraction of the bacterium was carried out using the liquid plating assay (Bolkan et al. (1997). 400 of the seed sample were put in a doubled

plastic bag (20 cm x 25 cm and 0.15 mm thick) containing 150 ml sterile phosphate-Tween buffer (7.75 g/l of Na2HPO4 + 1.65 g/l KH2PO4 + 0.2 ml/l Tween 20), pH 7.4. The plastic bag with its contents was incubated in a refrigerator at 4°C for 15 min. After the refrigeration, the plastic bag with its contents was placed in a shaker and shaking for 15 min. Pipette 0.1 ml of 0, 1:10, 1:100 dilutions (prepared using phosphate buffer without Tween) of each sample was incorporated onto each of the three plates containing semi-selective medium (2 g K2HPO4; 0.5 g KH2PO4; 0.25 g MgSO4.7H2O; 1.5 g boric acid; 10 g sucrose, 0.1 g yeast extract and 15 g of agar in 980 ml distilled water after autoclaving, cool to 45-50°C in a water bath and add 100 mg nicotinic acid (dissolved in 20 ml sterile distilled water); 30 mg nalidixic acid (sodium salt, dissolved in 1 ml of 0.1 M NaOH); 10 mg potassium tellurite (1 ml of 1% Chapman tellurite solution from Difco); and 200 mg cycloheximide (dissolved in 1 ml absolute methanol). Spread with an L-shaped glass rod and incubated at 26°C Fatmi and Schaad(1988). The bacteria were identified based on Gram reaction Suslow et al.(1982), tobacco hypersensitivity Klement et al. (1964), spore formation Bradbury (1986), ability to cause soft rot of potato, onion and cucumber slices Friedman (1951) and levan production Lelliot and Stead (1987).

Pathoginicity test:

Pathogenicity test was carried out to acertain the disease causal pathogens. Four pots (20 cm diameter) for each treatment were filled with autoclaved aerated sandy clay soil. The pots were inoculated with the isolated fungi and bacteria, and sown with sodium hypochlorite surface sterilized seeds of each specie (5 seeds/pot). Check treatment comprised also four pots for each specie but without inoculation . The experiment lasted for 60 days.

Location of seed-borne fungi of *Araucaria angustifolia* and Eucalyptus camaldulensis:

To determine the location of fungi in seed tissues of Eucalyptus camaldulensis and Araucaria angustifolia, 100 seeds were first soaked in tap water for 12 hours in order to facilitate the separation of seed parts viz., seed coat, and embryo. Seed parts were surface sterilized and plated on PDA. Petri dishes were incubated at as mentioned before. The developing fungal colonies were examined and percentages were recorded.

Control of seed-borne fungi and bacteria:

The commercial biopreparation of Promote based on Trichoderma harzianum 2×107/g and Trichoderma koningii 3×107/g was mixed with mineral fertilizers. These applied fertilizers were new plant care (20, 20, 20) NPK (30Kg) + LAV (ammonium nitrate with limestone, 30Kg) + DASA (ammonium nitrate, ammonium sulphate, 30Kg). The biopreparation Promote was mixed together with the fertilizers at 2 different rates (o.5 g and 1 g per 1 Kg of fertilizers). The health status of seedlings was recorded .

RESULTS AND DISCUSSION

Detection and isolation of fungi and bacteria associated with the five tested tree seeds:

Fungi detected from seeds of Araucaria angustifolia, Acacia mangium, Eucalyptus camaldulensis, Casuarina equisetifolia and Ficus bengalensis using the standard blotter, deep freezing blotter, agar plate media and liquid assay techniques are shown in Tables 1 & 2. 8 genera and 9 species of fungi were detected in seeds of the tested 5 trees (Table1).

Botryodiplodia theobromae was developed on blotter, deep freezing blotter and PD agar media at high rate in average of 11.3%, 3.5% and 5.9% respectively,followed by Botrytis cinerea 5.6%, 2.0% and 1.0%, Curvularia tuberculata 18.7%,2.8% and 9.4%, Cephalosporium spp. 6.7%, 5.0% and 2.3%, ,Colletotrichum gloeosporioides in 6.8%, 1.7%, 2.0%, Fusarium moniliform in 14.6%, 2.3% and 5.2%), F. oxysporum in 6.3%, 1.4% and 1.5%, Macrophomina phaseolina in 5.5%, 1.4% and 2.0% and Phoma spp in 5.1%, 2.2% and 0.4%. The liquid assay cropped up 2 bacteria i.e., Pseudomonas syringae pv. syringae and Xanthomonas campestris pv. campestris from seeds of the 5 tested tree seeds with averages means of 15.9% and 9.4% respectively. These results were in harmony with those of Mittal et al. (1990), who showed that large number of fungal species associated with Eucalyptus. Some of them infected seedlings after germination, for example,Macrophomina phaseolina and Verticillium albo-atrum and mentioned that seed health testing was primarily concerned with the evaluation of the presence or absence of disease causing organisms viz., fungi, bacteria, virus and nematodes.He added that fungi considered the most important group of microorganisms causing loss of seed viability. Some of soil borne pathogens were found associated with seeds of E. grandis in India Mohanan and Sharma (1991). In the present investigation, the 3 tests i.e. standard blotter, deep freezing blotter and PD agar displayed most of the fungi associated with the seeds of the 5 tested trees. Among all methods tested, standard blotter manifested the maximum percentages of fungi that were associated with the 5 tree species seeds with total frequencies of 80.6% against 22.3% and 29.7% in the deep freezing blotter and agar plate tests . The standard blotter technique demonstrated 52.4% of total frequency of the 9 species of fungi for Araucaria angustifolia against 28.4% and 16.4% when deep freezing blotter and agar plate methods were used. It showed 35% for Acacia mangium against 10.3% and 11.4% in case of both the other techniques. For Eucalyptus camaldulensis it was 147% compared with 2% and 53.5%. For Casuarina equisetifolia, the standard method exhibited 62.1% against 31.1% and 17.1%. It also illustrated 110.8% for Ficus bengalensis comaperd with 25.6% and 48.1%. These results were in agreement with those reported by Nath et al.(1970); Neergaard(1973) ; Bilgrami et al.(1979) and Abdelmonem and Rasmy(1996), who stated that

Table 1: Percentage of Seed borne fungi which were detected in seeds of 5 tested tree species using the three standard methods of isolation .

Fungi	% of seed-borne fungi																	
	Araucara Angustifolia			Acacia *Mangium*			*Eucalyptus camaldulensis*			*Casuarina equisetifolia*			*Ficus bengalensis*			Mean		
	SB	DF	PDA	SB	DF	PDA	SB	DF	PDA	SB	DF	PDA	SB	DF	PDA	SB	DF	PDA
Botryodiplodia theobromae	11.5	5.0	2.0	3.3	1.5	0.0	14	0.0	10.0	5.8	2.8	1.3	22	8.0	16	11.3	3.5	5.9
Botrytis cinerea	3.5	3.0	1.8	1.8	1.0a	0.5	10	0.0	0.0	3.5	3.0	2.5	12	3.0	0.0	5.6	2.0	1.0
Curvularia tuberculata	4.8	1.5	1.0	5.3	1.5	0.0	48	0.0	32.0	11.5	4.8	2.0	24	6.0	12	18.7	2.8	9.4
Cephalosporium spp.	8.8	5.0	1.0	1.5	0.8	0.5	7.0	2.0	3.5	6.0	3.5	1.5	10	0.0	3.0	6.7	5.0	2.3
Colletotrichum gloeosporioides	1.0	0.8	2.8	5.3	1.5	0.0	10	0.0	0.0	3.5	3.0	1.8	14	3.0	5.0	6.8	1.7	2.0
Fusarium moniliforme	5.5	5.5	3.0	8.0	2.0	5.8	38	0.0	8.0	11.5	4.0	3.0	10	0.0	6.1	14.6	2.3	5.2
F. oxysporum	5.8	2.8	1.3	5.0	0.0	3.8	10	0.0	0.0	4.8	1.5	1.0	5.8	2.8	1.3	6.3	1.4	1.5
Macrophomina phaseolina	5.0	1.3	3.5	2.8	2.0	0.8	8	0.0	0.0	5.5	3.0	2.0	6.0	0.8	4.8	5.5	1.4	2.0
Phoma spp.	6.5	3.5	0.0	2.0	0.0	0.0	2	0.0	0.0	10	5.5	2.0	7.0	2.0	0.0	5.1	2.2	0.4
Total frequency	52.4	28.4	16.4	35	10.3	11.4	147	2.0	53.5	62.1	31.1	17.1	110.8	25.6	48.	80.6	22.3	29.7
Mean	5.8	3.2	1.8	3.9	1.1	1.3	16.3	0.2	5.9	6.9	3.5	1.9	12.3	2.8	5.3	9.0	2.5	3.3

SB = Standard blotter test
DF = Deep freezing blotter test
PDA = PD agar plating test
200 seeds were used in each test .

Table 2: Percentage of Seed borne bacteria which were detected on seeds of 5 tested tree species using liquid plating assay.

Isolated bacteria / Host	% of seed-borne bacteria.					
	Araucaria angustifolia	Acacia mangium	Eucalyptus camaldulensis	Casuarina equisetifolia	Ficus bengalensis	Mean
Pseudomonas syringae pv. syringae	16	9.5	14	20	20	15.9
Xanthomonas campestris pv. campestris	10.5	6.5	10	10	10	9.4
Total frequency	26.5	16	24	30	30	25.3
Mean	13.3	8.0	12	15	15	12.7

the blotter test might detect the most microorganisms associated with some shrub seeds. They mentioned that the standard blotter, deep freezing blotter and PDA plate methods were favorable for the growth of various species of fungi when seeds were sized for health test. They also recorded that the standard blotter and agar methods were the best for detecting F. moniliforme and F. solani from seeds of some crops.

Data in Table 2 showed the significance of the liquid plating assay in detecting of 2 bacteria with total frequency of 25.3%. Pseudomonas syringae pv. syringae and Xanthomonas campestris pv. campestris were found associated with the seeds of the 5 tested trees at the average of 15.9% and 9.4% respectively.

Pathogenicity of seed-borne fungi and bacteria:

Pathogenicity tests were carried out to recognize the main causal pathogen(s). The obtained results ascertained the seed infection with all isolated species causing typical seed and seedling disease symptoms. Table 3 showed different symptoms created by the isolated seed borne fungi and bacteria . Botryodiplodia theobromae caused root rot, stem canker, die-back and seed decay. Botrytis cinerea caused seed decay. Curvularia tuberculata produced damping-off and seedling wilt. Cephalosporium spp expressed growth suppression and stem deformation.Colletotrichum gloeosporioidescausing damping-off, leaf spots, anthracnose and blight. Fusarium moniliforme and F. oxysporum induced seed rot, root rot, growth suppression, stem deformation, seedling mortality and wilt.

Table 3: disease symptoms induced by fungi or bacteria borne with the seeds of the five tested trees.

Seed borne fungi or bacteria	Disease symptom
Botryodiplodia theobromae	Root rot, Stem canker, die-back and seed damage
Botrytis cinerea	Seed decay
Curvularia tuberculata	Damping-off and seedling wilt
Cephalosporium spp.	Growth suppression and stem deformation
Colletotrichum gloeosporioides	Damping-off, leaf soots, anthracnose and blight
Fusarium moniliforme.	Seed rot, root rot, growth suppression, stem deformation , seedling mortality, and wilt
F. oxysporum	Seed rot, root rot, growth suppression, stem deformation , seedling mortality, and wilt
Macrophomina phaseolina	Seed rot, seedling mortality and yellowing of foliage
Phoma spp.	Seed rot, and growth suppression
Pseudomonas syringae pv. Syringae	Seed decay, watery root, blight and dieback
Xanthomonas campestris pv. campestris	root rot, water soaked and dieback

Macrophomina phaseolina, brought out seed rot, seedling mortality and clorosis .Phoma spp induced seed rot, and growth retardaton.

Pathogenicity tests with the isolated bacteria produced irregular-shaped spots (1 to 5 mm diameter), black or grey, slightly sunken lesions developed on cotyledons and leaves and to a lesser extent on the growing apical tip of shoot. Pseudomonas syringae pv. syringae, gave rise to seed decay, watery root, blight and dieback. Xanthomonas campestris pv. campestris induced root rot, water soaked and dieback symptoms .

Location of fungi and bacteria in Eucalyptus camaldulensis and Araucaria angustifolia seeds:

Table 4 presents of the location of fungi in seed coat and embryo of Eucalyptus camaldulensis and Araucaria angustifolia seeds. The majority of the isolated fungi were found located on seed coat. Colletotrichum gloeosporioides, Fusarium moniliforme, F. oxysporum and Phoma spp were also detected in seed embryos of both tested trees.

Table 4: Location of seed-borne fungi in seed tissues of E. camaldulensis and A. angustifolia .

Fungi / Host	Eucalyptus camaldulensis		Araucaria angustifolia	
	Seed coat	Embryo	Seed coat	Embryo
Botryodiplodia theobromae	++	-	+	-
Botrytis cinerea	+	-	++	-
Curvularia tuberculata	++	-	++	-
Cephalosporium spp.	+	-	+	-
C. gloeosporioides	+	+	+	+
Fusarium moniliforme	+	++	++	+
F. oxysporum	-	+	-	+
Macrophomina phaseolina	++	-	+	-
Phoma spp.	+	+	+	+

Improvement of seedling growth:

Infestation of Acacia mangium, Araucaria angustifolia, Casuarina equisetifolia, Eucalyptus camaldulensis and Ficus bengalensis by the isolated pathogenic fungi and bacteria and the influence of Trichoderma harzianum and Trichoderma koningii in minimizing their harmful effect was studied and data in table 5 showed that developed seedlings were healthy and the mixture of fertilizers with the antagonistic fungi i.e. Promote when applied at the rate of 1kg fertilizer to 1g Promote (Trichoderma harzianum and T. koningii) or 1 kg fertilizer to 0.5g of Promote significantly increased the number of healthy developed seedlings in the 5 tested trees species. Soil treated by the concentration 1.0 g Promote per 1Kg of the fertilizer proved to have the optimal.

Effect of the antagonistic fungi (Trichoderma harzianum and Trichoderma koningii) against the seed borne pathogenic fungi used in soil infestation as they increase the mean number of the healthy developed seedling in the 5 tested trees to 77, 79, 80, 78 and 81 for A. angustifolia, A. mangium, E. camaldulensis, C. equisetifolias and F. bengalensis

respectively. These results coincide with those of Chet and Baker, 1981; Duskova, 1995; Lo et al., 1996 and De Meyer et al.,1998 who reported that Trichoderma harzianum suppressed many pathogenic fungi among which Botrytis cinerea and Colletotrichum lindemuthianum. In the present investigation, other tested fungi like, Botryodiplodia theobromae, Curvularia tuberculata, Cephalosporium spp, Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina and Phoma spp were supressed by application of the biological agents and the suppression level was very similar.

CONCLUSION

Results obtained during this study showed that the blotter test was the most acceptable method for detection of seed borne fungi and the liquid plating assay detected most of the bacteria.Trichoderma harzianum and T. koningii when mixed with fertilizers was found to improve seedling growth and minimizing seed borne diseases to a minimum level.

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حصر لامراض قاطنات البذرة في الأشجار الخشبية بمصر

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مركز البحوث الزراعية- الجيزة – مصر.

أجريت التقنيات الخاصة والعادية بتحديد الميكروفلورا الهامة و المحمولة بالبذرة لخمسة أجناس من الاشجار الخشبية المنزرعة فى مصر وهى : الأكاسيا مانجيم و أروكاريا انجوستيفوليا و كازوارينا اكوستيفوليا و يوكاليبتس كاملايولنسيز و فيكس بنجالنسيز وتم جمع البذور من عدد من حضانات الاشجار التجارية.

أثبتت التجارب الكفاءة الأعلى لاختبار البلوتر في الكشف عن قاطنات البذور الفطرية .سجلت التجارب مصاحبة ثمانية أجناس فطرية لبذور الخمسة أشجار تحت الدراسة وهى :البتريودبلوديا و البوتريتس و السيفالوسبوريم و الكوليتوتريكم و الكرفيولاريا و الفيوزاريم و المكروفومينا و الفوما . علاوة على مصاحبة جنسان من البكتيرات الممرضة هما البسودوموناس والزانثوموناس لتلك البذور .جرى عزل كل من الفطر بوتريودبلوديا ثيوبروما و فيوزاريم مونيليفورم و فيوزاريم أوكسيسبورم حيث كانت الاكثرتواجدا بالبذور المصابة مسببة لأعفان البذور وامراض البادرات وموتها وامراض الذبول و تناقص النمو و تحورات لنمو السيقان . وكانت البكتيرات بسودوموناس وزانثوموناس هي الأكثر مصاحبة للبذور وتسبب أمراض مختلفة للبادرات منها مثل موت الأطراف وأثبت البحث إمكان نقل وحمل الفطريات كوليتوتريكم جليوسبورويدس و فيوزاريم مونيليفورم و فيوزاريم أوكسيسبورم و جنس الفوما بالبذور حيث تم عزلها من أجنة البذور المصابة . بينما تواجدت الفطريات البوتريتس و سيفالوسبوريم و كرفيولاريا تيوبركيولاتا و ماكروفومينا فاصولينا فقط مصاحبة لأغلفة البذور . وظهر من النتائج المتحصل عليها تحسن نمو البادرات عند خلط المخصبات بالمبيد الحيوي بر يموت بالاضافة آلي تقليل الأمراض المتسببة عن الفطريات والبكتيرات المحمولة ببذوالخمسة أشجار المستخدمة في الدراسة آلي الحد الأدنى .