Research Journal of

Forestry

ISSN 1819-3439

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Forestry

ISSN 1819-3439
DOI: 10.3923/rjf.2017.13.18

Research Article

Fusarium semitectum as a Dominant Seed-borne Pathogen in *Albezzia lebbeck* (Linn.) Benth., its Effect on Location and Transmission Studies

[1]Sarika Gupta, [2]Ashish Dubey and [3]Tribhuwan Singh

[1]Department of Bioscience and Biotechnology, Banasthali University, Banasthali, 304022 Tonk, Rajasthan, India
[2]Department of Botany, Agarwal P.G. College, Jaipur, India
[3]Department of Botany, UGC, BSR Faculty Fellow, University of Rajasthan, Jaipur, India

Abstract

Background and Objective: *Albezia lebbeck* (Linn.) Benth. (Siris) is a significant legume tree chiefly planted throughout the world. *Fusarium semitectum* was both externally and internally seed-borne affecting seed germination, seedling vigour causing pre and post emergence mortality. Since, only few studies were available on the seed borne nature of *F. semitectum*. Therefore, the present study aims to elucidate the location of pathogen along with its transmission from seed to seedling. **Materials and Methods:** Fifty seven seed samples were collected from various locations of Jaipur and are subjected to dry seed examination. For evaluating the location of the pathogen seed were subjected to cleared whole mount preparation, handcut and microtome sectioning. During transmission studies the seeds of all category were tested through Standard Blotter Method (SBM), water agar seedling symptom test and pot experiments. **Results:** *Albezzia lebbeck* (Linn.) Benth. (Siris) seeds naturally infected with *Fusarium semitectum* showed either white mycelial crust (2.5-14.25%) and insect damaged (5.75-38.5%) causing wilt disease. These seeds on incubation yielded pure growth of the pathogen. Seeds were characterized as asymptomatic and symptomatic (weakly and heavily infected) on the basis of severity of infection. Cleared wholemount preparation and their section of seed revealed mycelial fragments in cells of seed coat of asymptomatic seeds and in symptomatic seeds pathogen invaded the deeper tissues of seed till the embryonal axis through the hilar tissue into the trachidial bars from where it densely spreads to embryonal axis, cotyledons and the space between two cotyledons. The pathogen is both externally and internally seed-borne. The internal innoculum affect seed germination, viability and caused high total (pre and post emergence) losses 35-85%. **Conclusion:** The pathogen is transmitted from seed to seedling causing heavy losses to the tree plantation. The pathogen was externally as well as internally seed-borne and internal inoculum affects seed germination, viability and elevated pre and post emergence losses.

Key words: *Albezzia lebbeck*, *Fusarium semitectum*, seedling mortality, histopathological effect

Received: July 08, 2016 Accepted: October 17, 2016 Published: December 15, 2016

Citation: Sarika Gupta, Ashish Dubey and Tribhuwan Singh, 2017. *Fusarium semitectum* as a dominant seed-borne pathogen in *Albezzia lebbeck* (Linn.) benth., its effect on location and transmission studies. Res. J. For., 11: 13-18.

Corresponding Author: Sarika Gupta, Department of Bioscience and Biotechnology, Banasthali University, Banasthali, 304022 Tonk, Rajasthan, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Albezia lebbeck (Linn.) Benth. (Siris) is an important legume tree belonging to family Fabaceae, subfamily Caesalpiniaceae. It is native of India and largely planted throughout the country discussed by Troup[1] and has also found to be suitable species for planting in dry areas of Jodhpur, Rajasthan by Bhimaya *et al.*[2] as an avenue tree. Siris suffers from many fungal diseases reported by Bakshi[3]. Major diseases are caused by species of *Fusarium* as Fusarium wilt, damping off and grayish-black canker explained by Richardson[4]. *Fusarium* sp. is externally and internally seed-borne affects its germination and seedling vigour causing pre and post emergence mortality. Parrotta[5] reported occurrence of *Fusarium* sp. in plantation of *A. lebbeck* in Puerto Rico. *Fusarium solani* causes die-back of *D. sissoo* described by Bakshi[3]. *Fusarium oxysporum* and *Phytopthora* causes die-back of *A. lebbeck* Mehrotra *et al.*[6]. However, its pathogenicity was confirmed by artificial inoculation in healthy host tissue resulted in disease. Ashour and El-Kadi[7] stated that cultural studies demonstrated its contribution in the damping off of tomato seedling.

There is not much information available on seed-borne nature of *F. semitectum* in *A. lebbeck.* Therefore, the present study is carried out for determining the histopathological, phytopathological and transmission of pathogen from seed to seedling.

MATERIALS AND METHODS

Collection of seed samples: Fifty seven seed samples were collected from 12 locations of Jaipur in year 2000-2002 were subjected to dry seed examination and incubation tests by Standard Blotter Method (SBM), Potato Dextrose Agar (PDA) plate test as recommended by ISTA[8]. In dry seed examination besides normal, bold, oval-oblong, light brown asymptomatic seeds with compressed testa, various kinds of discolourations (white crusted, dark brown and green) and deformities (insect damaged and shriveled) symptomatic seeds were observed.

Dry seed examination: Seeds were incubated on moistened blotters both untreated and 3% chlorine pretreated for 5 min. In PDA test, pretreated seeds were spaced (10 seeds per plate) on petriplates containing PDA medium. Two samples were selected from Seed market and Bani park locations of sample No. 6 and 27 having high incidence of *F. semitectum*, respectively. These samples were used for histopathological and transmission studies. The seeds were categorized as asymptomatic and symptomatic (weakly and heavily infected) seeds on the basis of severity of infection.

Histopathological and phytopathological studies: Location of the pathogen in different seed components was studied using component plating (10 seeds/category/sample) handcut and microtome sectioning (5 seeds/category/sample) suggested by Singh *et al.*[9]. Transmission of seed-borne innoculum from seed to seedling per plant was studied using petriplate method and growing on tests ISTA[8]. In petriplate method four replicates of 100 pretreated seeds were sown on moistened blotters (10 seeds per plate) and on growing in water agar test tube seedling symptom test (1 seed per test tube), respectively. Observations were made to record percent germination, incidence of pathogen, seedling symptom and seedling mortality.

RESULTS

All the 57 seed samples collected from 12 locations of Jaipur were either covered with white mycelial crust (2.5-14.25%) were insect damaged (5.75-38.5%) carrying infection of *Fusarium semitectum* (Fig. 1a). Such seeds on incubation yielded pure growth of the pathogen. The fungus was detected in 54, 49 and 24 samples with Relative Percent Occurrence (RPO) incidence in the range 94.73, 10-88, 94.73, 3-48 and 42.10%, 10-40% in untreated and pretreated seeds in SBM and PDA test, respectively. Incidence was high in the samples from Seed market, Bani Park and Jhalana natural garden (Table 1).

For the location of the pathogen in various parts of seeds component plating, cleared wholemount preparation and sectioning (handcut and microtome) were performed. In component plating, pathogen was detected in seed coat (35, 30%), cotyledons (35, 30%) and embryonal axis (5, 10%) of asymptomatic seeds. Whereas, it varied from 45-75% on seed coat, 30-65% on cotyledons and 20-65% on embryonal axis of symptomatic (weakly and heavily infected) seeds of the samples with sample No. 6 and 27, respectively (Table 2).

In cleared whole mount preparation, all the parts of asymptomatic and symptomatic seeds revealed mycelial infection. It was 40, 40% in seed coat, 20, 40% in cotyledons and 15, 20% in embryonal axis of asymptomatic seeds and it varied from 80-85, 60-90 and 45-80% in seed coat, cotyledons and embryonal axis of weakly and heavily infected seeds of the two samples, respectively (Table 2). In symptomatic seeds, all the parts were densely colonized by the fungal infection

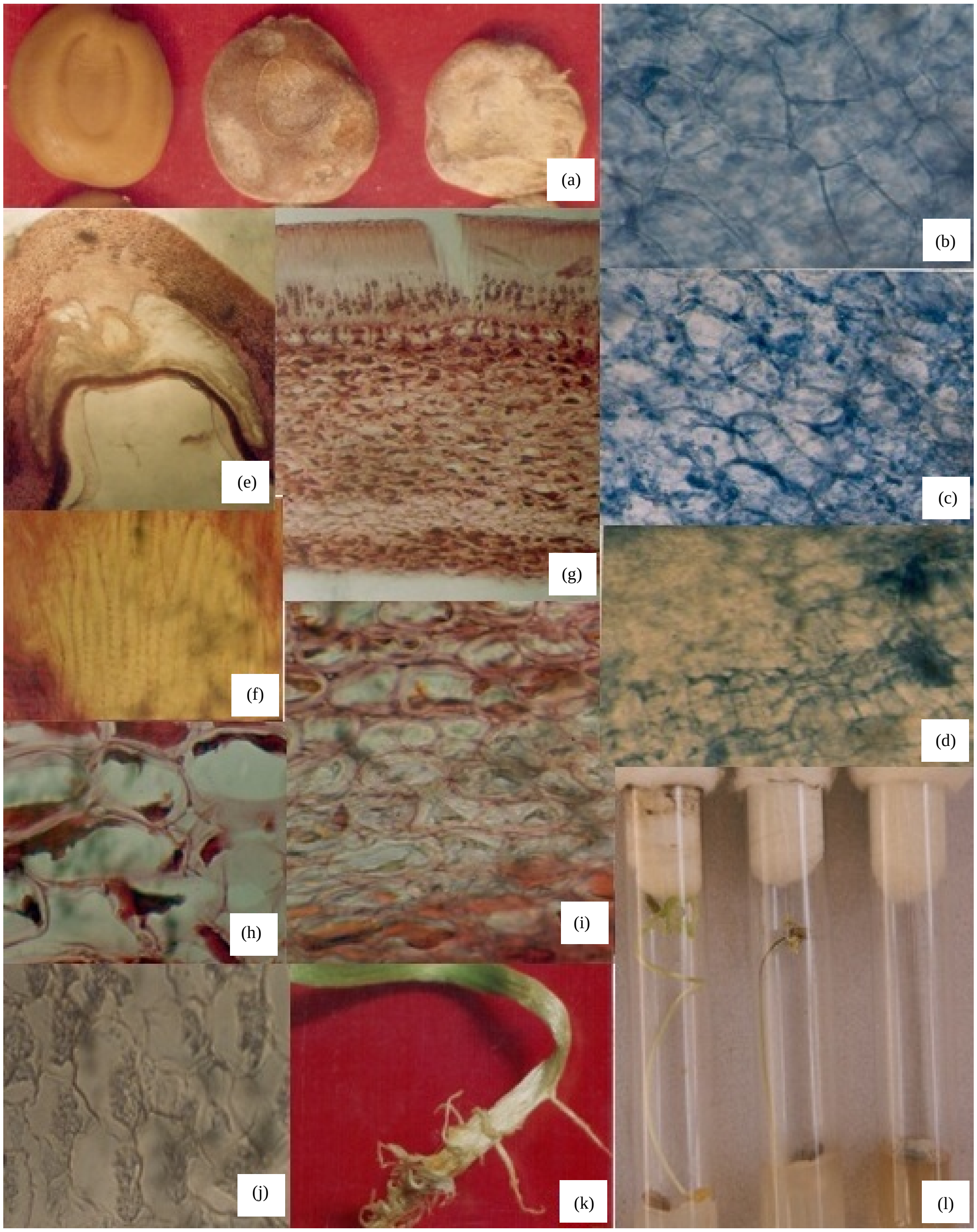

Fig. 1(a-l): Histopathological and cytopathological localization of *Fusarium semitectum* in *Albezzia lebbeck* (Linn.) Benth, (a) During dry seed examination, asymptomatic seeds, weakly and heavily symptomatic seeds covered with white mycelial crust caused by *F. semitectum* X18, (b-d) Cleared wholemount preparation of symptomatic seeds revealed mycelial infection in cells of seed coat, (b) Cotyledons, (c) Embryonal axis (d) X700, (e-i) Microtome sect of seeds infected with *F. semitectum*, (e) The T.S. part of seed showing mycelial bits and cellular disaggregation in the seed coat, hilar region in space between seed coat and cotyledons X350, (f) The T.S. part of weakly infected seeds showing hilar region of seed coat carried aggregation of hypae in their trachiedal elements X700, (g) Inter and intracellular mycelium in cells of all the layers of seed coat X350, (h) Disintegrated cells of cotyledons of heavily infected seed X700, (i) X700, (j) Mycelial ramification in cells of embryonal axis X700, (k) Characteristic wilting of roots due to the infection of *F. semitectum* and (l) Phytopathological studies in water agar seedling symptom test showing various degrees of infection normal seedling and infected seedling

Table 1: Seed samples of *Albizzia lebbeck* infected by *Fusarium semitectum* in Jaipur

		Dry seed examination		In standard blotter method		
Location	No. of samples	White crusted	Insect damaged	Untreated	Pretreated*	PDA test
Amer road	2	2	2	2	2	-
Bani park	6	6	6	6	4	-
Forest training centre	3	3	3	3	3	2
JDA circle	7	7	7	7	7	4
Jhalana natural garden	3	3	3	3	3	2
Police academy	6	6	6	6	6	4
Ram niwas bagh	3	3	3	3	2	2
Sanganer	3	3	3	2	2	-
Seed market	9	9	4	9	7	4
University campus	9	9	9	9	8	4
Vidhan sabha bhawan	3	3	2	1	-	-
World forestry arboratum	3	3	3	3	3	2
Total	57	57	51	54	49	24

*3% Chlorine pretreatment for 5 min

Table 2: Incidence of *Fusarium semitectum* in various components of seed and percent disease transmission

		Ac. No. 6			Ac. No. 27	
		Sym			Sym	
Categorys	Asy	W. sym	H. sym	Asy	W. sym	H. sym
Component plating						
Seed coat	35	45	75	30	65	70
Cotyledon	35	30	55	30	50	65
Embryonal axis	5	20	65	10	45	60
Cleared components						
Seed coat	40	80	80	40	80	85
Cotyledon	20	60	85	40	80	90
Embryonal axis	15	45	60	20	60	80
Disease transmission (%)						
Germination	15	10	5	25	20	5
Seedling symptoms	5	-	-	5	5	-
Seedling mortality	5	10	5	-	10	5
Disease transmission	66.6	100	100	40	75	100

Asy: Asymptomatic, Sym: Symptomatic, W.sym: Weakly symptomatic and H.Sym: Heavily symptomatic

showing mycelial bits and fragments in seed coat (Fig. 1b) cotyledonary cells, inter and intracellular mycelium was detected in cells of embryonal axis (Fig. 1d) showed dense mycelial ramification.

Handcut and microtome sections revealed infection with pathogen, 1 and 2 seed out of 5 seeds of the two samples, respectively of asymptomatic seeds showed presence of mycelial fragments and few hyphal bits were observed in the cells of seed coat. Tissues of cotyledons and embryonal axis did not show any infection. Whereas, samples of symptomatic seeds (with weak and heavy infection) revealed presence of pathogen in all the parts of seed. Inter and intracellular mycelium was detected in seed coat and cotyledon of seed with weak infection. But pathogen invaded the deeper tissues of the seed till embryonal axis (Fig. 1e). The cells of seed coat ere densely colonized showed depleted cell contents (Fig. 1h). Due to heavy infection sub-epidermal cells of seed coat were deformed and irregularly elongated (Fig. 1g, i). The hilar region of seed coat carried aggregation of hypae in their trachiedal elements. The fungal hypae invaded the seed through the hilar tissue into the trachiedal bar from where it further spreads to seed coat, embryonal cells and the space between two cotyledons (Fig. 1e, f). Hypae readily invaded and spread in embryonal axis (Fig. 1j).

During transmission studies in petriplate method, radical emergence began on 6th day of sowing which increased on 8th day. Maximum germination was 15-25% in asymptomatic seeds, 10-20% in weakly symptomatic and 5, 5% in heavily symptomatic seeds of sample No. 6 and 27, respectively. Dense growth of fungus leads to high preemergence loss. On growing on test the germination reached maximum on 10th day. The total pre and post emergence loss and disease transmission in the two samples were 60-35% and 66.6, 60.0% in asymptomatic seeds and it varied 70, 55, 100, 100 and 85, 75%, 100, 100% in symptomatic (both weakly and heavily infected) seeds of the two samples in both the test, respectively. On growing in test the germination was 80 and 100% in asymptomatic seeds whereas, it varied from 20-95% in symptomatic seeds (with weak and heavy infection) of both the samples on 8th day of incubation (Table 2).

Initial signs of disease symptoms appeared on 6th day of sowing as browning of radical and collar region, later the symptoms progressed upward causing browning of hypocotyls. The infected seedling either failed to form primary leaves and if formed were brown in colour and rudimentary. The seedlings were stunted, pale looking and finally collapsed leaves and hypocotyls become brittle due to heavy infection (Fig. 1k, l).

The present study showed that infected seeds were asymptomatic or symptomatic (weakly and heavily infected). The pathogen was mostly found to be associated with seeds with white mycelial crust and dark brown discolorations (Fig. 1a). The pathogen was both externally and internally seed-borne. The pathogen ramified the seed inter and intracellularly and was correlated with severity of disease symptoms. The pathogen invades the seed either through hilar region or through the pores on the seed coat surface.

During transmission studies the pathogen moved from seed to seedling per plant caused high pre and post emergence losses and produced brown necrotic spots on cotyledonary leaves, browning of radical and hypocotyls. Later the seedlings wilted and succumb to death (Fig. 1k, l).

DISCUSSION

Based on the review less study has been done on evaluating the seed-borne nature of *Fusarium semitectum* on *Albizzia lebbeck*. But to discuss the study it is correlated with the seed-borne nature of the pathogen in other plants and trees and their impact on seedling. A study on demonstrated the evidence of 6 seed-borne fungi were found to be dominant adversely affecting to seed health namely, seed germination, seedling emergence of the green gram described by Sadhu[10]. In another study, *Dalbergia sissoo* Roxb. seeds naturally infected with *Fusarium semitectum* showed either dark brown discoloration with compressed hilar region or covered with white mycelial crust causing wilt disease reported by Gupta *et al.*[11]. A similar study suggests the effect of 15 *Fusarium* species on seed germination and early plant development was examined in a laboratory study, together with the potential toxigenicity of selected isolates and the possibility of T-2 toxin production on soybean and pea grain reported by Ivic[12] and Sultana and Ghaffar[13] described *Lasiodiplodia theobromae, Fusarium semitectum Macrophomina phaseolina* and *Fusarium oxysporu*m were most frequently isolated from 33, 91, 50 and 66% seed samples of bottle gourd, respectively causing seed-borne infection. Seven different species of *Fusarium* were isolated from the seed lots assayed, these fungal species were present on seeds were found to be associated with damping off and root diseases in loblolly and slash pine seedlings. Seed of *Pinus elliottii* and *P. taeda* were collected from Northeastern Argentina and analyzed for seed-borne *Fusarium* Lori and Salerno[14]. This is the 1st study on economic loss along with seed-borne fungal pathogens having reduced seedling emergence due to *Cladosporium cladosporioides, Colletotrichum crassipes, Fusarium semitectum, Phomopsis dalbergiae* and *Pestalotiopsis* sp. on *Dalbergia nigra* seedlings in Brazil. Their colonisation of both, pericarp and seed by these fungi starts soon after pod formation and continues to increase to pod maturity evaluated by Dhingra *et al.*[15]. Internal and external seed-borne fungal pathogens of *Anadenanthera macrocarpa* reduced seedling emergence and the quality of seedlings grown from contaminated seeds with *Colletotrichum gloeosporioides, Fusarium lateritium, F. semitectum, Pestalotiopsis* sp. and *Phomopsis dalbergiae* were the only fungi consistently isolated from seed samples collected from afforestation described by Dhingra *et al.*[16] and Paik and Do[17] reported 7% infection of *F. moniliforme* in seed coat and 2% in the embryo of green gram seed. Similar results were studies by Wu and Li[18] on wilt disease of *A. julibrissin* caused by species of *Fusarium* in China. Shakir *et al.*[19] isolated *F. solani* from roots of *Dalbergia sissoo*. Browning of leaves in the tip region and discoloration (yellowing) of first few basal leaves was reported by Singh *et al.*[9]. Similar symptoms on radicle and hypocotyls due to infection of *F. moniliforme* and *F. semitectum* have also been reported by Saxena and Sinha[20]. Dwivedi and Dwivedi[21] observed blackish spot on collar region due to infection of *F. solani*. The severely infected seedlings were finally wilted. The pathogenicity was confirmed by artificial inoculation of healthy plant parts resulted in the appearance of disease such studies were also supported by Ashour and El-Kadi[7]. Paik and Do[17] confirmed the pathogenicity of *F. monoliforme, F. pallidoroseum* and *F. equseti* in seedling in water agar test tube method. Whereas, Davidson and Byther[22] detected symptoms and control of die-back of *A. lebbeck* by cultural means. Kochler[23] also reported seed as source of seedling infection in corn.

CONCLUSION

The pathogen *Fusarium semitectum* is both externally and internally seed-borne. The internal innoculum affects seed germination, viability and caused high total (pre and post emergence) losses. The pathogen is transmitted from seed to seedling causing heavy losses to the tree plantation.

SIGNIFICANCE STATEMENT

Seed-borne nature of *F. semitectum* associated with *Albezzia lebbeck* was not reported so far, the present study provides the comprehensive information regarding the location and transmission of the pathogen and proves it to be both internally and externally seed-borne.

REFERENCES

1. Troup, R.S., 1921. The Silviculture of Indian Trees. Oxford at the Clarendon Press, Oxford, UK., Pages: 413.
2. Bhimya, C.P., R.N. Kaul, B.N. Ganguli, I.S. Tyagi and M.D.S. Choudhari, 1964. Species suitable for afforestation of different habitats of Rajasthan. Ann. Arid Zone, 2: 162-168.
3. Bakshi, B.K., 1957. Wilt disease of *Shisham* (*Dalbergia sissoo* Roxb.). Indian For., 83: 316-322.
4. Richardson, M.J., 1990. An Annotated List of Seed Borne Diseases. 4th Edn., The International Seed Testing Association, Switzerland, Pages: 338.
5. Parotta, J.A., 1987. *Albizzia lebbeck* (L.) Benth. USDA Forest Service, Southern Forest Research Reports No. 8, Siris Research Note SO-ITF-SM-7, New Orleans, pp: 118-119.
6. Mehrotra, M.D., S.N. Khan, R.K. Tivari and B.M. Misra, 1992. A study on the periodicity of foliage diseases of *Albizia lebbek* and control of *Camptomeris* leaf spot in nursery. Indian For., 118: 594-598.
7. Ashour, W.E. and M.M. El-Kadi, 1959. Cultural studies on *Fusarium semitectum*, *Altermaria tenius* and *Rhizoctonia solani* which cause damping-off of tomato seedlings. A'in Shams Sci. Bull., 3: 57-68.
8. ISTA., 2016. International rules for seed testing. http://www.seedtest.org/en/international-rules-_content---1--1083.html.
9. Singh, D., S.B. Mathur and P. Neergaard, 1977. Histopathology of sunflower seeds infected by *Alternaria tenuis*. Seed Sci. Technol., 5: 579-586.
10. Sadhu, K.A., 2014. Seed-borne fungi and their effect on seed health of green gram. Biosci. Dis., 5: 251-255.
11. Gupta, S., A. Dubey and T. Singh, 2011. *Fusarium semitectum* as a dominant seed-borne pathogen in *Dalbergia sissoo* Roxb., its location in seed and its phytopathological effects. Indian J. Fundament. Applied Life Sci., 1: 5-10.
12. Ivic, D., 2014. Pathogenicity and potential toxigenicity of seed-borne *Fusarium* species on soybean and pea. J. Plant Pathol., 96: 541-551.
13. Sultana, N. and A. Ghaffar, 2009. Seed borne fungi associated with Bottle gourd (*Lagenaria siceraria* Mol.) Standl. Pak. J. Bot., 41: 435-442.
14. Lori, G.A. and M.I. Salerno, 2003. Fusarium species on seeds of *Pinus taeda* L. and *Pinus elliottii* Engelm. in Argentina. J. Plant Dis. Prot., 110: 437-443.
15. Dhingra, O.D., D.C. Lustosa, C.B. Maia and J.B. Mesquita, 2003. Seedborne fungal pathogens of jacaranda (*Dalbergia nigra*) tree. Seed Sci. Technol., 31: 341-349.
16. Dhingra, O.D., C.B. Maia, D.C. Lustosa and J.B. Mesquita, 2002. Seedborne pathogenic fungi that affect seedling quality of red angico (*Anadenanthera macrocarpa*) trees in Brazil. J. Phytopathol., 150: 451-455.
17. Paik, S.B. and E.S. Do, 1987. *Fusarium* spp. Isolated from seed, root and cultivated soil of *Phaseolus dissimus* and their pathogenicity. Korean J. Plant Pathol., 3: 8-12.
18. Wu, L. and S.K. Li, 1990. A preliminary study on wilt disease of *Albizia julibrissin*. For. Pest Dis., 4: 13-14.
19. Shakir, A.S., S.M. Khan and R. Ahmad, 1999. First report on shisham (*Dalbergia sissoo* Roxb.) decline in Pakistan. Pak. J. Phytopathl., 11: 106-106.
20. Saxena, R.M. and S. Sinha, 1979. Field mycoflora in *Vigna radiate* (L.) *Wilczek* var. radiate and *Vigna mungo* in relation to pre-emergence and post-emergence mortalities. Seed Res., 7: 159-164.
21. Dwivedi, S.K. and P. Dwivedi, 1999. Wilt disease of Guava: A national problem. J. Applied Horticult., 1: 151-154.
22. Davidson, Jr.R.M. and R.S. Byther, 1992. Tubercularia (Nectaria) canker and die-back of broadleaf trees. Extension Bulletin-Co-Operating Extension College of Agriculture and Home Economics, Washington State University, pp: 2.
23. Kochler, B., 1960. Corn stalk rot of Illinosis. Agricultural Experiment Station Bulletin No. 658, April 1960, Illinois, USA., pp: 90.