Research Paper Botany section

Diversity and biocontrol aspects of rhizospheric fungal flora of healthy and diseased plants of *Dalbergia sissoo in* Central East India

Ravi Shanker Mishra #	P.G Dept. of Biotechnology, TM.Bhagalpur University, Bihar#
Neeraj Shrivastava#	Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, UP.#
Manoj Kumar#	Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, UP.
Awadh Kishore Roy	P.G Dept. of Biotechnology, TM.Bhagalpur University, Bihar
Shankar Kumar Pandey	Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, UP.
Ajit Varma	Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, Noida, UP
Vivek Kumar*	Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, UP. *Corresponding author

ABSTRACT

In eastern part of India, two major diseases viz. wilt and Dieback are responsible for the mortality of *Delbergia* sissoo (Shisham). The Dieback disease cause severe loss to the plants rapidly and is mainly caused by pathogenic rhizospheric fungus. It is quite difficult to get rid of pathogenic mycoflora in the Shisham growing lands; therefore, disease management helps in the protection of D. sissoo. Better methods for disease management are integrated disease management and it includes judiciousapplication of chemical and biological approaches. The pathogenic fungus infectsthe plant through roots. Thus, management of pathogenic *fungi* by natural biocontrol agents (Trichoderma viride isolated from rhizospheric soils) could be a sustainable approach.Under in vitro conditions, Trichodrma viride was found to inhibit the growth of Fusarium oxysporum up to 46.4% inhibition (zone of inhibition on Petri plate was 29.5 mm). T. harizanum inhibitedthe F.oxysporum up to 25.2mm (58% inhibition). T.harizanum showed better antagonistic potential than T. veride. This could be due to rapid growth of T. harizanum and excretion of potent antifungal substances.The antagonistic substancesviz. Gliotoxin, volatile and non-volatile substances inhibited the growth and proliferation of pathogenic fungus F. oxysporum.

KEYWORDS Biocontrol agents, *Dalbergia sissoo*, Dieback, Pathogenic *Fungi*.

Introduction

Dalbergia sissoo (Shisham) is a most planted timber trees species in north central India owing to its high economical values and wide industrial and rural applicability.In Bihar state Sisham plantation is commonly noticed from West Champaran to Purnia district in Bihar.It is a multipurpose tree species and due to its durability, elasticity and strength it is priced as a valuable timber used in furniture, door, and window and in gun butt(Bhattacharya et. al. 2014, Chandra et.al. 2014,Sharma et. al. 2000).

Two major diseases wilt and Dieback are responsible for the mortality of *D. sissoo*. The Dieback disease cause severe loss of the plants very rapidly. This disease is mainly concerned with pathogenic rhizospheric fungus. It is quite difficult to remove mycoflora from the Shisham growing lands, thus the disease management could help in safeguarding of Shisham plants. In a study, Rajput et. al. (2011) showed the positive efficacy of different Neem (*Azadirachta indica* A. Juss) products namely Neem oil, Neem seed decoction, Neem seed without coat, Neem seed coat and Neem leaf extract were tested for *in vitro* growth of Shisham seedlings inoculated with *Fusarium solani* isolated from dieback infected Shisham plant (Rajput et. al. 2012, Chandra et. al. 2014, Ojha et. al. 2010, Bhandari, 2014)

The disease has also been reported from Pakistan (Rehman et.al. 2012, Arif et.al. 2013, Poussio et. al. 2010), Nepal (Baksha and Basak 2000, 2010), Bangladesh (Basak, 2006) and Iran (Abdollahzadeh et. al. 2010). Valdez et.al. (2013) also established the possible role of bacteria as pathogens in dieback affected *D. sissoo* Roxb. trees in Bangladesh. The growth of the pathogens;*Fusarium, Aspergillus* and *Rhizoctonia* are more in the rainfall ranges between 500 to 1500mm and the rainy season also favor the growth of *D. sissoo*. Wilting of affected tree is the common symptom;therefore,*Fusarium solani* (Mart) has been consistently isolated from dying roots (Bakshi, 1974; Bakshi and Singh 1959).

Biological control of plant pathogen using antagonistic *fungi* such as *Trichoderma veride* and *Trichoderma harizanum* which have shown effective and promising results in controlling Shisham diseases. The antagonists produce antifungal biochemicals which causes the pathogenic fungal hyphae tobecome vacuolated and finally collapse(Singh, 2002, Kundu and Chatterjee 2003). The use of *Trichoderma* in agriculture provides advantages by colonizing the rhizosphere rapidly by controlling pathogenic and competitive microflora, thereby improving plant health (Harmanet.al.2004). Numerous species of *Trichoderma* can also protect plants by producing biologically active compounds such as cell wall degrading enzymes, which mitigate the negative effects of plant pathogens and promote plant growth. For these reasons, these beneficial

biocontrol *fungi* have been commercially employedas biopesticides, biofertilizers and soil amendment agents (Vinalea et. al. 2008, Basak and Basak 2011, Chandra et. al. 2014).Therefore, keeping the above in view, an attempt was made to study the diversity of rhizosphere *fungi* associated with drying or wilting of *D.sissoo* plants and biocontrol aspects of pathogenic *fungi*.

Materials andmethods

Selection of study area and tree species

The *D. sissoo* growing areas of North Bihar was conducted during 2012-2013 and 2013-2014 and were divided into eight sectors (Fig. 1) based on its wide range of habitat and microbial infestation. The targeted area was surveyed and documented the incidence of wilt disease of host plants at different age groups i.e. 5 years and more than 10 years.

Isolation of fungal species

Rhizospheric soil samples were collected in separate polyethylene bags and brought to the laboratory for fungal profiling associated with healthy and diseased sissoo plants. For microbial isolation, methods as described by Kumar (2011) were adopted. The identification of microbes was carried out as per Manual of Soil *Fungi*(Gilman, 1998).

Isolation of biocontrol agent *T. harizanum*

Trichoderma harizanum was isolated from rhizospheric soil by using selective Agar medium (TSM) for quantitative isolation prescribed by Elad et al, (1981). For biocontrol study in fields, the *T. harizanum* was grown in liquid broth to achieve spore count of 10^7 ml^{-1}.

Soil amendment with Bio-agents:

The soil amendment for growth study of D. sissoo seedling was carried outwith biocontrol agent (*T. harizanum)* having following experimental set up in triplicate.

T1 = Control (Sterilized soil only)
T2 = Sterilized soil + *F. oxysporum* (Pathogen)
T3 = Sterilized soil + *F. oxysporum* (Pathogen) + *T. harizanum* (Biocontrol)
T4 = Garden/Natural soil
T5 = Garden soil + *F. oxysporum* (Pathogen)
T6 = Garden soil + *F. oxysporum* (Pathogen) + *T. harizanum* (Bio-control)

Results and Discussion

During survey of Gangetic and Kosi river belts of Central East India, Distt. Khagaria, Bihar (Fig. 1) it was observed that *D. sissoo* plants of different age groups (Fig. 2)were infested with wilt disease. Data collected from this district revealed that out of 357 (05-10 years age group plants) and 259 plants (above 10 years age group), 28 and 73 plants were found partially and 42 and 111 plants completely wilted, respectively. The percentage disease incidence was maximum (71.05) in 10 years and above age group plants whereas, the lowest disease incidence (38.45) was recorded among the plants planted in Katihar district (Fig. 2). Survey report of all these eight districts showed that highest disease incidence was found more than 10 years old plants. Earlier, Rehmanet. al. (2012), Sharma et. al. (2000), Rajput et.al. (2012) and Singh et. al. (2009) reported the incidence of disease affecting more than 40% plant part in more than 10yrs age group of plants which lead to considerable loss of *D. sissoo*.

Diversity and occurrence of *fungi* are documented in Table-2. Result indicated that four fungal species viz., *A.niger, F.solani*, *F. oxysporium, P. citrinum* were isolated from all the plant sites but the *Fusarium*sps. were invariably present in association with diseased plants (Table 2, 3). Some *fungi* viz., *Gliocladium sp. C. lunata, A.clavatus* and*Alternaria* sps.were distributed unevenly in all sites. The results showed that the presence of *P. citrinum, F. solani, F. oxysporum* in diseased samples might be correlated with the incidence of wilt disease of *D.sissoo*. The present findings are in conformity with the reports of Rajput et. al.(2008) and Singhet. al. (2009) related to wilting of *D. sissoo* plants in the different agro climatic conditions.

The pathogenic fungus grows rapidly than the non-pathogenic fungus during the month of October to July and the dominant *fungi Aspergillus* species, *Fusarium* species, *Rhizoctonia* species and *Penicillium* species wereobservedat Site-1, while at Site-2, *Aspergillus* species, *Fusarium* species, *Penicillium* species, *Rhizoctonia* species and *Helminthosporium* species were observed. Similarly at Site-3, *Aspergillus* species, *Fusarium* species and *Penicillium* species were found to be dominant, similarly same fungal species were also observed from Site 4-8 (Table 3).Their association with host plants is obvious and therefore, reported in rhizospheric soil of many perennial trees (Ahmad et. al. 2012). The experiment conducted with sterilized and garden soil (Table 1) showed that sterilized soil supported healthy plant growth and less sign of wilting was observed,inoculated with *T. harizanum*.Sterilized soil and garden soil without biocontrol agent exhibited 100% wilting effect on plants. Likewise, in garden soil, biocontrol effect of *T. harizanum* also supported more growth of healthy plants (54%) compared to wilted plants (46%). This could be due to antifungal metabolites excreted by *T. harizanum*, which inhibited the growth of fungal pathogen in rhizosphere (Pathan et. al. 2007, Chandra et.al. 2014, Basak and Basak 2011).

From the present study, it can be concluded that employment of potential biocontrol agent/s could be an option but to completely eradicate an inclusive survey of the dieback disease in different ecological zones is necessary to make an evaluation of the damage caused by the parasite in the natural and planted areas. Since the tree is an important tree crop in the Central East India,scientists from these regions can collaborate on the research, exchange new findings, and develop a program for exchange of resistant genetic material and testing them for performance and disease resistance.

Table 1: Percentage wilting of *Dalbergia sissoo* Treatment

Treatments	Composition	% Healthy plants	% wilted plants
T1	Sterilized Soil (Control)	100	00
T2	Sterlized Soill + *Fusarium oxysporum*	00	100
T3	Sterlized Soil + *F. oxysporum* + *Trichoderma harizanum*	58	42
T4	Natural/Garden soil	89	11
T5	Natural/Garden soil + *Fusarium oxysporum*	00	100
T6	Natural/Garden Soil + *F. oxysporum* + *Trichodermaharizanum*	54	46

Table-2:Disease incidence of *D. sissoo* in 8 districtsof North Bihar

S. No.	District	Age of Plants	No of Plants Studied	Healthy plants (H)	Partially diseased plan s(PD)	Dead (D) Plants	% of H Plants	% of PD Plants	% of D Plants	Total % of D Plants
	Bhagalpur	5-10yrs	197	139	18	40	70.56	09.14	20.30	29.44
		Above 10 yrs	149	59	43	47	39.60	28.86	31.54	60.4
	Khagaria	5-10yrs	357	287	28	42	80.40	07.85	11.77	19.62
		Above 10yrs	259	75	73	111	28.96	28.19	42.86	71.05
	Begusarai	5-10yrs	56	37	07	12	66.07	12.5	21.43	33.48
		Above 10yrs	43	18	14	11	41.86	32.56	25.58	58.14
	Saharasa	5-10 yrs	75	46	14	15	61.33	18.67	20	38.67
		Above 10 yrs	41	13	18	10	31.71	43.90	24.39	68.29
	Madhepura	5-10yrs	165	98	26	41	59.39	15.76	24.85	40.61
		Above 10 yrs	89	28	23	38	31.46	25.84	42.70	68.54
	Darbhanga	5-10yrs	63	41	09	13	65.08	14.29	20.63	34.92
		Above 10yrs	49	22	17	10	44.90	34.69	20.41	55.1
	Katihar	5-10yrs	32	25	04	03	78.13	12.5	09.38	21.88
		Above 10yrs	52	33	08	12	63.46	15.38	23.07	38.45
	Purnia	5-10yrs	83	57	11	15	68.67	13.25	18.07	31.32
		Above 10yrs	92	38	33	21	41.40	35.87	22.83	58.07

'H' – Healthy 'P.D.' – Partially Diseased 'D' – Dead

Table-3:Distribution of *Fungi* in association with *D.sissoo* Roxb.in various sites of North Bihar

S.N	Name of *fungi*	Site-1		Site-2		Site-3		Site-4		Site-5		Site-6		Site-7		Site-8	
		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
1	***Alternaria spp***	_	_	_	_	_	03	_	_	_	_	_	_	_	_	02	_
2	***Aspergillus clavatus***	_	_	_	_	04	_	_	_	_	_	03	_	_	_	_	_
3	***Aspergillus niger***	08	11	07	05	05	04	04	05	04	08	05	06	05	06	05	09
4	***Aspergillus flavus***	04	_	04	_	_	_	03	06	04	08	06	05	05	06	05	11
5	***Chaetomium globosum***	_	_	_	_	03	_	05	-_	05	_	05	_	_	_	_	_
6	***Cladosporium spp***	03	04	04	_	_	_	_	03	_	02	_	_	_	_	_	05
7	***Curvularia lunata***	02	_	_	_	04	_	_	_	_	_	_	_	_	04	_	_
8	***Fusarium moniliforme***	_	02	_	03	_	04	03	_	_	_	_	02	_	04	_	_
9	***Fusarium oxysporum***	02	05	03	06	02	05	03	05	01	04	03	05	_	07	02	08
10	***Fusarium solani***	01	04	_	03	03	04	_	05	04	04	04	05	_	06	02	05
11	***Gliocladium spp***	_	_	_	02	_	_	04	_	_	_	_	_	_	_	_	_
12	***Helminthosporium sp***	03	_	04	04	04	03	01	03	01	_	04	_	_	_	03	04
13	***Mucor flavus***	02	_	_	_	_	_	_	_	_	_	_	_	03	_	_	_
14	***Penicillium citrinum***	03	03	_	03	05	06	04	05	04	04	05	08	_	02	03	07
15	***Penicillium spp.***	_	_	05	_	_	_	05	_	05	_	_	03	06	_	04	_
16	***Rhizopus oryzae***	_	_	_	_	02	_	_	_	_	_	_	__	_	02	_	_
17	***Trichoderma album***	_	_	02	_	_	_	_	_	05	_	_	_	-	_	_	_
18	***Trichoderma flavus***	_	_	_	_	_	_	_	_	_	_	_	03	_	_	_	_
19	***Verticillium glaucum***	04	03	_	_	_	_	_	_	_	_	_	_	_	04	_	_
20	Fungal diversity	10/32	7/32	7/32	7/32	9/32	7/32	9/32	7/32	9/32	6/32	8/32	8/32	5/32	9/32	8/32	7/32

H- Healthy, D- Diseased.

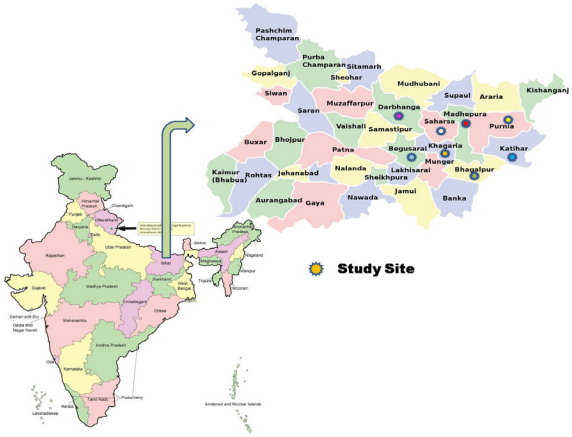

Fig.1 The study area

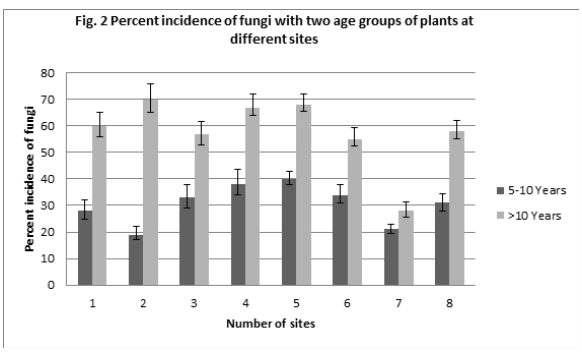

Fig. 2 Percent incidence of *fungi* with two age groups of plants at different sites

REFERENCES

1. Abdollahzadeh, J., Javadi, A., Mohammadi Goltapeh, Zare, R. and Phillips, A.J.L. (2010). Phylogeny and morphology of four new species of Lasiodiplodia from Iran. Persoonia, 25:1-10. | 2. Ahmad I., Khan, R.A., Siddiqui, M.T. 2012. Incidence of dieback disease following fungal inoculations of sexually and asexually propagated shisham (*Dalbergia* sissoo). Forest Pathology. 43:77-82. | 3. Arif, M., Zaidi, N. W., Haq Q. M. R., Singh, Y. P., Khan S. and Singh U. S. 2013 Molecular phylogeny and pathotyping of Fusarium solani: a causal agent of *Dalbergia* sissoo decline, For. Path. 43: 478–487 | 4. Baksha, M. W. and Bask A. C. 2010. Mortality of sissoo in Bangladesh. Proc. of 3rd Natl. Conf. of Pl. Pathol. 2001, NARC, Islamabad. pp. 33-37. | 5. Baksha, M.W. and Basak, A.C. 2000. Mortality of sissoo (*Dalbergia* sissoo Roxb.) in Bangladesh. In: Proceedings of International Seminar on Dieback of Sissoo. Appanah, S., Allard, G. and Amatya, S.M. (Eds) 25–28 April 2000, Kathmandu, pp. 1–4. | 6. Bakshi, B. K. 1974. Control of root disease in plantation in reforested stands. Indian Forester, 100: 77-78. | 7. Basak A. C. 2006. Mortality of sissoo (*Dalbergia* sissoo Roxb.) in Bangladesh and its management. PhD thesis, Jahangirnagar University, Savar. | 8. Basak A.C. and Basak, S.R. 2011. Biological control of Fusarium solani sp. *dalbergiae*, the wilt pathogen of *Dalbergia* sissoo, by Trichoderma viride and T. harzianum, Journal of tropical forest science 23(4): 460-466 | 9. Bhandari M. S., Kant R, Ahmed, N., Dobhal, S., Luna, L. K., Nautiyal, S., Kumar, V., Kumar, A. 2014. Shisham Mortality in Hoshiarpur, Punjab: Causes and Remedy, The Indian Forester, 140(2): published online | 10. Bhattacharya, M., Singh, A. and Ramrakhyan, C. 2014. *Dalbergia* sissoo - An Important Medical Plant, Journal of Medicinal Plants Studies, 2 (2): 76-82 | 11. Chandra, S., Prasad, R., Harsh, N. S. K., Ahuja, R. and khatri, S. 2014. Bark canker and die-back of *Dalbergia* sissoo in haryana and Punjab caused by lasiodiplodia theobromae, Indian Forester, 140 (1) : 76-79, | 12. Elad, Y., Chet, I. and Henis, Y. 1981. A selective medium for improving quantitative isolation of Trichoderma spp. From soil, Phytoparasitica 9(1): 59-67 | 13. Gilman, C.J. 1998. A manual of soil *fungi*. Biotech books. New Delhi | 14. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. 2004. Trichoderma species-Opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2: 43-56 | 15. Kumar, N. 2014. Search of a natural remedy for control of Fusarial wilting of Sisham (*Dalbergia* Sissoo Roxb), Int. J. Engg. Res. & Sci. & Tech. 3(1): published online. | 16. Kumar, V. 2011. Laboratory Manual of Microbiology, Scientific Publishers, Jodhpur, pp 100-101. | 17. Kundu, A. and Chatterjee, N.C. 2003. Antagonism of Trichoderma species to Polyporus sanguineus-an incitant of bamboo decays. Indian Forester 129: 1281–1288. | 18. Ojha, S., Khatun, S., Chakraborty, M.R. and Chatterjee, N. C. 2010. Occurrence of die-back of *Dalbergia* sissoo in West Bengal and evaluation of *fungi*cidal control of its pathogen. International Journal of Plant Protection, 3:17-19. | 19. Pathan, M. A., Rajput, N. A., Jiskani, M.M., Wagan, K. H. 2007. Studies on intensity of shisham dieback in Sindh and impact of seed borne *fungi* on seed germination. Pak. J. Agric. Agril. Engg. Vet. Sci., 23: 12 - 17. | 20. Poussio, G. B., Kazmi, M. R., Akem, C., Fateh, F. S. 2010: First record of Ceratocystis fimbriata associated with shisham (*Dalbergia* sissoo) decline in Pakistan. Australian Plant Dis. Notes 5: 63–65. | 21. Rajput, N. A., Pathan, M. M., Jiskani, A.Q., Rajput, R. and Arain, R. 2008. Pathogenicity and Host rang of Fusarium solani (MART.) SACC. Causing Die-back of Shisham (*Dalbergia* sissoo Roxb.) Pak. J. Bot., 40 (6); 2631-2639, | 22. Rajput, N. A.; Pathan, M. A.; Lodhi, A. M.; Dou, D.; Liu, T.; Arain, M. S.; Rajer, F. U., 2012: In vitro evaluation of various *fungi*cides against Fusarium solani isolated from *Dalbergia* sissoo dieback. Afr. J. Microbiol. Res. 6, 5691–5699. | 23. Rajput, N.A., Pathan M.A., Lodhi, A.M., Dou, D. and Rajput, S. 2011. Effect of neem (Azadirachta indica) products on seedling growth of shisham dieback. African Journal of Microbiology Research Vol. 5(27): 4937-4945 | 24. Rehman, A., Sahi, S.T., Khan, M.A. and Mehboob, S. 2012. *Fungi* associated with bark, twigs and roots of declined shisham (*Dalbergia* sissoo roxb.) trees in Punjab Pakistan, Pak. J. Phytopathol Vol. 24(2):152-158. | 25. Sharma, M. K., Singhal, R. M., and Pokhriyal, T. C. 2000. *Dalbergia* sissoo in India. Field Document No 18. In: Proc. Sub Regional seminar on Die-back of Sissoo (*Dalbergia* sissoo), Ed. by Appanah, S.; Allard, G.; Amatya, S. M. Bangkok: Kathmandu, Nepal, April 25–28, 2000, Forestry Research Support Programme for Asia and Pacific (FORSPA), 65 pp. 5–16 | 26. Singh K. P., Kumari, P. and Bhadauria, S. 2009. A survey of the Indian rose wood tree *Dalbergia* sissoo Roxb. under threat of fungal pathogens. Proceedings of National Academy of Sciences India, Sect. B, Pt. II, 79: 86-95 | 27. Singh Y. 2002. Biological control of Sclerotium leaf blight of Gmelinas arborea. Indian Forester 128: 41–44. | 28. Valdez, N., Karlovsky, P., Dobrindt, L., Hoque, M.I., Sarker, R.H., Tantau, H. and Mühlbach, H-P.2013. Role of bacteria in dieback disease of *Dalbergia* sissoo roxb Bangladesh J. Bot. 42(1): 1-16 | 29. Vinalea, F., Sivasithamparam, K. and Ghisalberti, E.L. 2008. Trichoderma plant pathogen interactions. Soil Biology and Biochemistry, 40(1):1-10. |