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Synergistic interaction between *Fusarium solani* and *Ganoderma lucidum*, two root pathogens of *Dalbergia sissoo*

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Abstract

In nature different pathogens cause diseases on a common host plant which may develop simultaneously and can infect the same host at a time. Co-occurring pathogens may affect each other, through antagonism and/or synergism. In *Dalbergia sissoo*, *Ganoderma lucidum* and *Fusarium solani* are two serious pathogens which cause root rot and vascular wilt diseases, respectively and are responsible for large scale mortality of this tree species. These two pathogens were tested to find out whether they live in a synergistic way or inhibit one another. Results showed that these two pathogens can co-exist on the same host plant at the same time and cause disease in *Dalbergia sissoo* independently. None of these fungi affect adversely the growth of other fungus; instead they favour the growth of each other.

Key words – Antagonism – disease – mortality – root rot – vascular wilt

Introduction

Dalbergia sissoo (shisham, sissoo, tali), a perennial deciduous tree, is native to Indian subcontinent and found in tropical to subtropical climates. Besides an excellent timber, the wood has high caloric value and ability to increase soil fertility through nitrogen fixation. This tree species is subjected to various man-made and natural threats. Diseases and insect pests being natural deterrents adversely affect its growth and multiplication. Its large scale mortality in recent years throughout the entire north Indian plains is an alarming phenomenon which has disrupted the economic targets of state forest departments and farmers who grow it along their field boundaries.

Ganoderma lucidum and *Fusarium solani* are two serious pathogens which cause root rot and vascular wilt diseases, respectively. *Ganoderma lucidum* infects roots through intact as well as injured surfaces and kills the bark and causes white fibrous rot in the sapwood. The fungus spreads from tree-to-tree through root contact. Symptoms of the disease start appearing from third year in plantations. In affected trees sporophores of *Ganoderma lucidum* appear which are usually stalked, corky and later become woody. In older trees the branches in the crown show die-back exhibiting 'stag-headedness'. Finally the entire tree becomes leafless with the death of branches and dies in few years (Bakshi et al. 1972).

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Fusarium solani f. sp. *dalbergiae* has been reported by Baghchee (1945), Bakshi (1954), and Bakshi & Singh (1959). It affects the vascular system causing moisture stress that leads to wilting and killing of the plants. Symptoms are yellowing and death of leaves in accropetal succession up the tree. In advanced stages of the disease, the leaves are shed rendering the branches bare and finally the tree dies within a few months. Outer sapwood just below the bark exhibits a characteristic pink to reddish stain. The fungal hyphae and jelly like substance plug the xylem vessels resulting in wilt disease symptoms.

Both *Ganoderma lucidum* and *Fusarium solani* share the common host, *Dalbergia sissoo* (Fig. 1). The present study was planned to find the interaction between these two pathogens *in vitro* and to confirm the interaction in simulated environment.

Materials & Methods

Isolation of fungal cultures

Ganoderma lucidum was isolated on Potato Dextrose Agar (PDA) medium from sporophores attached to the roots of diseased *Dalbergia sissoo* trees. *Fusarium solani* was isolated from diseased root samples following blotter technique (de Temp 1963). Fungal cultures were maintained on PDA slants in a refrigerator at 4°C. Isolated fungi were identified using the mycological literature (Booth 1971, Sen 1973) and expertise at Forest Pathology Division, Forest Research Institute, Dehra Dun. For the interaction study following experiments were conducted.

1. Dual culture method: Mycelial discs (5 mm dia.) were cut from actively growing margin of the colony of *Ganoderma lucidum* (5 days old) and placed on one side of a Petri plate containing sterilized PDA. After two days of incubation, a mycelial disc of *Fusarium solani* (5 days old, 5 mm dia.) was placed 2 cm juxtaposed to that of *Ganoderma lucidum*. Control was set for each fungus separately. All the inoculated plates were incubated at 27±1°C for 15 days. After completion of incubation period each fungus was re-isolated on PDA plates to confirm its status. The number of spores of each fungus was calculated from inoculation to end point of the growth using a Haemocytometer slide.

2. Mycelial dry weight: Potato Dextrose Broth (PDB) was prepared and 50 mL PDB was taken in each 150 mL conical flask and sterilized in a vertical autoclave at 121ºC and 15 lbs psi pressure for 15 min. When cooled to room temperature, the flasks were inoculated with *Ganoderma lucidum* and *Fusarium solani* cultures (5 days old, 5 mm dia.) separately and incubated at 27±1°C. After 15 days when proper growth had occurred in flasks, culture filtrates of both the fungi were obtained separately by filtering first by Whatman No. 1 filter paper and then with a bacterial syringe filter (0.45 µm, Schuell). For estimation of mycelial yield in 'cell-free' culture filtrate (autoclaved and unautoclaved), each fungus was inoculated with 5 agar discs (5 mm dia.) cut from actively growing margin in culture filtrate of other fungus. After incubation at 27±1°C for 15 days each culture was filtered through pre-weighed Whatman filter paper No. 1. The mycelial mat of each fungus was dried at 80±2°C in a Hot Air Oven for 24 h to determine the mycelial yield.

3. Colony growth in culture filtrate: Sterilized PDA plates were prepared and wells of 5 mm dia. were cut at 2 cm distance from the centre with sterilized cork borer. An agar disc (5 mm dia.) cut from actively growing margin of *Ganoderma lucidum* and *Fusarium solani* was separately inoculated in the centre of PDA plates. 'Cell-free' and 'with-cell' culture filtrate (0.5 mL) of *Fusarium solani* and *Ganoderma lucidum* was poured in wells of PDA plates inoculated with a disc of opposite fungus in the center. Sterilized distilled water was poured in PDA plates for comparison as control in the same way. All the plates were incubated at 27±1°C for 7 days and colony growth was measured.

4. *In vitro* spore germination: Chlamydospores of *Ganoderma lucidum* and conidia of *Fusarium solani* were harvested from 7 days old cultures grown on PDA. Germination and hyphal development of both fungi was studied in cavity slides containing 'cell-free' and 'with-cell' culture filtrate of *Ganoderma lucidum* and *Fusarium solani*. Glucose solution (1%) was used as control. Petri plates containing the cavity slides were incubated at room temperature in dark and in light and chlamydospores and conidia germinating in each slide were counted after 6 h of incubation till 48 h. Germination was defined when the hypha produced by a chlamydospore and conidium to about half of its diameter.

5. Interaction in wood chips in soil: Wood chips of *Bombex ceiba* Linn. (1.0 × 0.5 × 0.3 cm) were taken in culture tubes, moistened and sterilized. The tubes were then inoculated with 5 agar discs of *Ganoderma lucidum* (5 mm dia.) and incubated at 27±1^{o}C for 15 days till white mycelial mat covered the wood chips. Carboxymethylcellulose medium was prepared in flasks (250 mL) and inoculated with culture of *Fusarium solani* and incubated at 27±1^{o}C for 15 days. Conidial suspension of *Fusarium solani* (10^5/mL) was prepared with the help of a magnetic stirrer and mixed in flasks (50 mL) containing pre-sterilized soil (20 gm) with wood chips colonized with *Ganoderma lucidum*. Flasks of *Ganoderma lucidum* colonized wood chips and with *Fusarium solani* conidia in sterilized soil were kept as control separately. Flasks were incubated at 27±1^{o}C. Status of both fungi was observed after 21 days of incubation and wood chips from the flasks of control and interaction sets were taken out and re-isolation of *Ganoderma lucidum* was done on PDA. Colony forming units (CFU) of *Fusarium solani* were recorded on Fusarium specific medium (FSM) from soil in control and interaction sets and counted up to 10^{-3} dilution.

Statistical analysis

Data were analysed statistically using a two-way analysis of variance (ANOVA). Standard error of the means and ANOVA were calculated using STATISTICA 7 Statistical Software (Stat Soft. Inc.,Tulsa, USA).

Results

Dual culture method

Inter-fungal interaction *in vitro* showed that the taxa did not inhibit the growth of one another, but displayed a synergistic interaction. *Ganoderma lucidum* and *Fusarium solani* grew rapidly and covered the whole Petri plates overlapping each other. Both fungi could be isolated on PDA plates from different sides of the interaction plates.

Spore counts of both fungi decreased with the increase in distance from inoculation point but increased significantly under the influence of another test fungus (Table 1). Both fungi were able to sporulate in their area of growth as well as in the growth area of each other, while maximum sporulation was found at the point of inoculation of both fungi. As *Fusarium solani* showed more sporulating tendency than *Ganoderma lucidum*, mean spore count of *Fusarium solani* was greater than *Ganoderma lucidum*. No abnormalities in hyphal morphology and structure were observed from zone of interaction in dual cultures of *Ganoderma lucidum* and *Fusarium solani* (Fig. 2).

Effect of 'cell-free' culture filtrate on dry mycelial yield: After 15 days of incubation white mycelial mat of *Ganoderma lucidum* was observed in culture filtrate of *Fusarium solani* and cream to pink coloured mycelial mat of *Fusarium solani* in cultural filtrate of *Ganoderma lucidum*. Mycelial weight of each fungus showed approximately similar growth in culture filtrate as well as in control. Mycelial weight of *Fusarium solani* was 0.09±0.00 gm in control, 0.10±0.01 gm in sterilized culture filtrate and 0.11±0.02 gm in unsterilized culture filtrate which were nearly same. Similarly, mycelial weight of *Ganoderma lucidum* was recorded as 0.08±0.01 gm, 0.09±0.01 gm and 0.08±0.00 gm in control, sterilized culture filtrate and unsterilized culture filtrate, respectively (Fig. 3).

Fig. 1 – Disease symptoms in *Dalbergia sissoo* tree; (a) wilting due to *Fusarium solani;* (b) fruiting bodies of *Ganoderma lucidum* causing root rot.

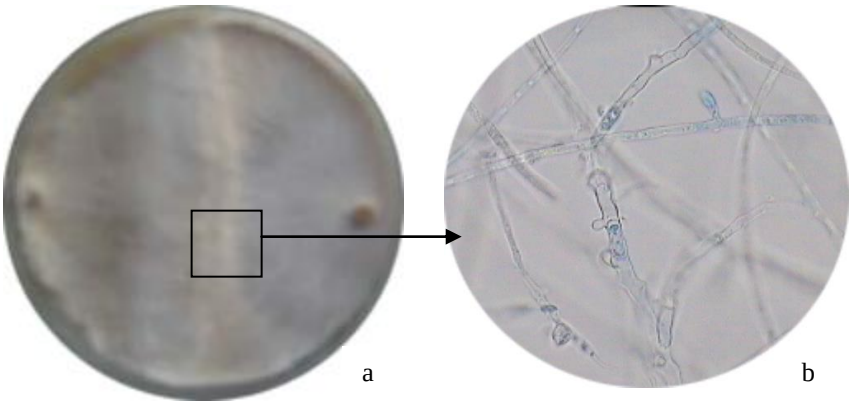

Fig. 2 – Inter-fungal interaction; (a) dual culture of *Ganoderma lucidum* and *Fusarium solani*; (b) microscopic view from zone of interaction.

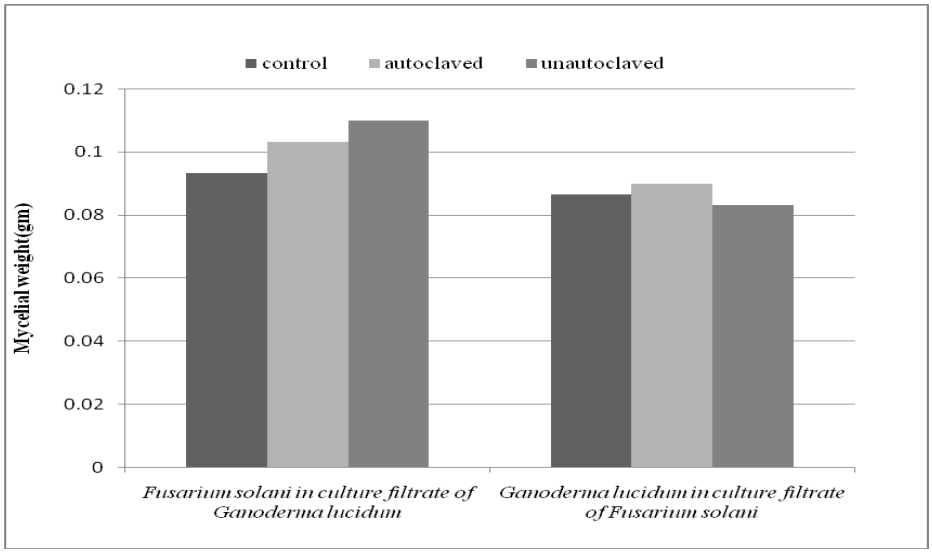

Fig. 3 – Comparison between mycelial dry weight yield in culture filtrates.

Colony growth by culture filtrate

In 'cell-free' culture filtrate (CFCF): After 15 days of incubation, mycelium of both taxa covered the entire Petri plate including the wells in which 'cell-free' culture filtrate of opposite fungus was taken. No growth inhibition was observed in both taxa and similar growth was also observed in control. 'Cell-free' culture filtrates of both *Fusarium solani* and *Ganoderma lucidum* did not impart any adverse effect on the growth of each other (Fig. 4 a-c).

In 'with-cell' culture filtrate (WCCF): After 15 days of incubation, mycelium of *Ganoderma lucidum* and *Fusarium solani* grew in all the 4 wells containing culture filtrate. Clear zones of growth of both taxa were noticed in the Petri plate but no zone of inhibition was recorded. Colony of both fungi overlapped each other. Similar results were found with *Ganoderma lucidum* culture filtrate against *Fusarium solani* as in 'cell-free' culture filtrate against *Fusarium solani* (Fig. 4 d-e).

Spore germination: Table 2 shows germination of spores of *Ganoderma lucidum* and *Fusarium solani* at 48h. Germination of spores of *Ganoderma lucidum* and *Fusarium solani* was favoured more in dark than under light. Culture filtrate of *Fusarium solani* favoured spore germination of *Ganoderma lucidum* and vice versa. Maximum spore germination in *Ganoderma lucidum* was recorded in 'with-cell' culture filtrate of *Fusarium solani* and minimum in control (1% glucose), in both the conditions. Spore germination of *Ganoderma lucidum* was found more in *Fusarium solani* culture filtrate than in *Ganoderma lucidum* culture filtrate. In light and dark conditions spore germination of *Fusarium solani* was found more in 'cell-free' culture filtrate of *Ganoderma lucidum* followed by *Fusarium solani* 'cell-free' culture filtrate. Minimum spore germination was observed in control (1% glucose) in comparison to 'with-cell' culture filtrate of *Ganoderma lucidum* and *Fusarium solani* (Table 2; Fig. 5).

Interaction in wood chips in soil: Table 3 shows the count of *Fusarium solani* in both sets. CFUs of *Fusarium solani* were significantly higher in soil containing *Ganoderma lucidum*-colonized wood chips as compared to control where they were not placed. When isolations were made from the wood chips placed in soil, *Ganoderma lucidum* was successfully re-isolated from control and interaction sets.

Discussion

Fusarium solani f. sp. *dalbergiae* and *Ganoderma lucidum* have been reported as serious pathogens that attack and kill *Dalbergia sissoo* (Bakshi 1976). Generally synergism has been focused to enhance the productivity of the crop by using plant growth promoting and antagonistic microorganisms. If the pathogens are synergistic, they may lead to severe disease symptoms. Synergistic interactions between the plant pathogens are relatively rare (Johnson 1990). Some examples of synergistic interaction in fungal pathogens are *Tilletia tritici* increasing susceptibility of wheat foliage to infection by *Puccinia glumarum* (Dillon-Weston 1927), powdery mildew increasing susceptibility of wheat to leaf rust (Simkim & Wheeler 1974), rust predisposing bean plants to foliar infection of *Colletotrichum lindemuthianum* in anthracnose-resistant bean varieties (Yarwood 1977), barley leaves infected with rust being more severely infected with *Septoria avenae* f. sp. *triticea* (Shearer et al. 1978) and in mature fruits in citrus between *Geotrichum candidum* and *Penicillium digitatum* (Morris 1982).

Antagonistic/synergistic effect of pathogens has also been described by Le May et al. (2009) who observed interaction between *Didymella pinodes* and *Phoma medicaginis* var. *pinodella*, the causal agents of blight disease in *Pisum sativum*. The presence of the two pathogens on the same host plant organ limited the disease development and their reproduction. Damages caused by the two pathogens were increased when previously inoculated plants were inoculated with other species. Davidson et al. (2011) also observed similar effect in *Didymella pinodes*, *Phoma koolunga* and *Phoma medicaginis* var. *pinodella* in pea.

On the basis of the results it maybe concluded that both *Ganoderma lucidum* and *Fusarium solani* can co-exist with each other in synergistic manner at the same time in the soil as well as in the host *Dalbergia sissoo*. Nutrient competition, mycoparasitism and antibiosis were not observed among both the fungi when interaction was studied on solid culture medium. Mycelial biomass was observed higher or similar in culture filtrates of one another as compared to control which clearly indicates that the culture filtrates were not suppressing the growth of other fungus. It was found that there was no harmful effect on colony growth by 'cell-free' and 'with-cell' culture filtrate. *Ganoderma lucidum* and *Fusarium solani* interaction showed synergism and compatibility between them. Similarity such as their common host, mode of nutrition and co-existence may be the reason for their compatibility. The synergistic effect may lead in severe symptoms expression individually and jointly in the host. Interaction between these two pathogens of a common host sharing same niche has not been reported earlier.

Table 1 Spore count of *Fusarium solani* and *Ganoderma lucidum* in dual culture method ($\times 10^3$/mL).

Distance	*Fusarium solani*	*Ganoderma lucidum*
At inoculation point	9 ± 0.18	5 ± 0.14
0.5 cm away from inoculation	7 ± 0.18	3 ± 0.22
1.5 cm away from inoculation	7 ± 0.11	2 ± 0.34
2.5 cm away from inoculation	6± 0.29	1± 0.05
3.5 cm away from inoculation	6 ± 0.13	2 ± 0.07
4.5 cm away from inoculation	7± 0.07	2 ± 0.02
5.5 cm away from inoculation	7 ± 0.10	3 ± 0.12
6.5 cm away from inoculation	8 ± 0.08	4 ± 0.02
7.0 cm away from inoculation	8±0.11	4 ±0.11
Mean ± SEM	7±0.17	3±0.25
CD	0.45	

Table 2 Spore germination (%) in *Ganoderma lucidum* and *Fusarium solani.*

Germination medium	*Ganoderma lucidum*		*Fusarium solani*	
	In light	In dark	In light	In dark
Control (1% Glucose solution)	20.33±0.88	30.00±2.88	37.00±5.50	45.67±2.33
'With-cell' culture filtrate of *Ganoderma lucidum*	63.33±3.71	72.67±1.45	64.33±2.33	73.00±2.51
'Cell-free' culture filtrate of *Ganoderma lucidum*	53.33±0.33	62.67±1.45	79.67±2.02	80.67±0.67
'With-cell' culture filtrate of *Fusarium solani*	69.00±1.20	73.00±1.73	66.33±7.21	76.33±0.88
'Cell-free' culture filtrate of *Fusarium solani*	67.66±3.71	72.00±2.00	76.33±1.20	77.33±1.66
Mean ± SEM	54.80±4.92	62.06±4.46	64.73±4.33	70.60±3.46

Table 3 Colony forming units of *Fusarium solani* on *Fusarium* specific medium

Dilution	Control(10^3/mL)	Interaction (10^3/mL)
10^{-1}	34.90±0.88	54.00±0.57
10^{-2}	7.19±0.18	9.03±0.18
10^{-3}	1.20±0.12	1.95±0.04
Mean ± SEM	14.60±5.24	22.00±8.31
CD	2.60	

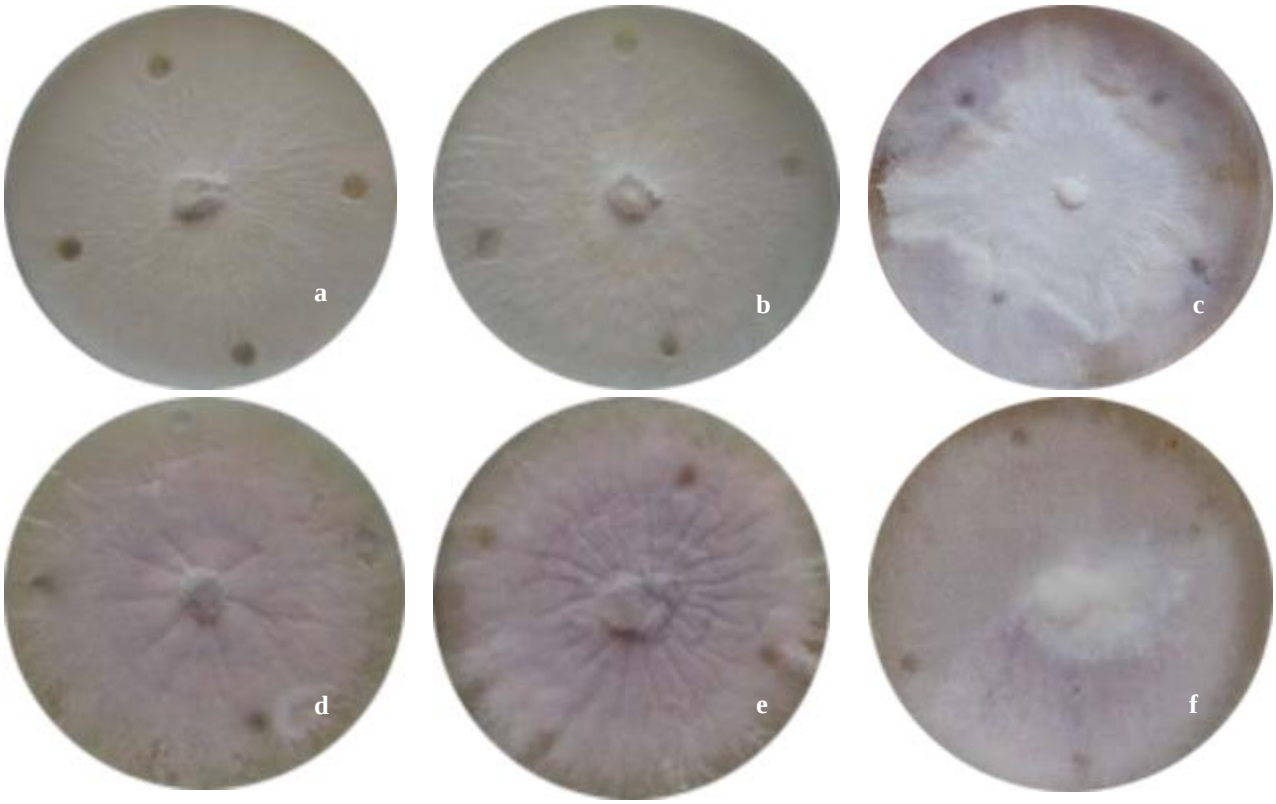

Fig. 4 – Colony growth in culture filtrates: *Ganoderma lucidum* colony- control (a) in CFCF (b) in WCCF (c); *Fusarium solani* colony- control (d) in CFCF (e) in WCCF (f) *CFCF: 'Cell-free' Culture Filtrate, WCCF: 'With-cell' Culture Filtrate

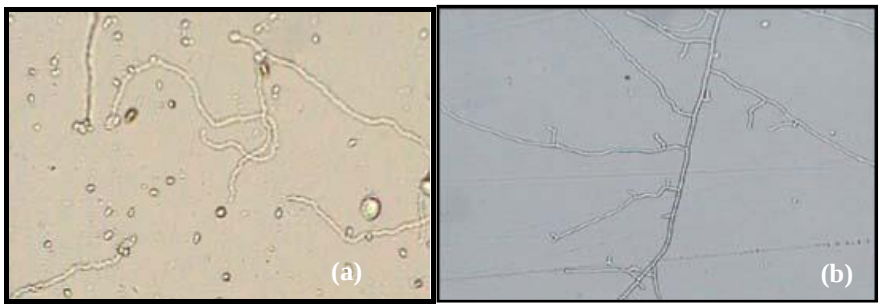

Fig. 5 – Spore germination in (a) *Ganoderma lucidum* and (b) *Fusarium solani*

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References

Bagchee KD. 1945 – Wilt and die-back of shisham, babul and khair in the artificial regeneration under agriculture-cum-forestry management. Indian Forester 71, 20–24.

Bakshi BK. 1954 – Wilt disease of shisham (*Dalbergia sissoo* Roxb.) due to *Fusarium solani* Sensu Snyder and Hansen. Nature 174, 278.

Bakshi BK, Singh S. 1959 - Root disease of Shisham (*Dalbergia sissoo* Roxb.) Inoculation studies on wilt. Indian Forester 85, 415–421.

Bakshi BK, Reddy MAR, Puri YN, Singh S. 1972 – Forest disease survey (Final Technical Report). Forest Pathology Branch, F.R.I., Dehradun, 117 pp.

Bakshi BK. 1976 – Forest Principles and Practice in Forestry, Controller of Publications, F.R.I. & Colleges, Dehradun, 400 pp.

Booth C. 1971 – The genus Fusarium. Kew, England: Commonwealth Mycological Institute.

de Temp J. 1963 – The blotter method for seed health testing. Proc. Int. Seed Test. Assoc. 28, 1933.

Dillon-Weston WAR. 1927 – The Incidense and Intensity of *Puccinia glumarum* Eriks on Wheat Infected and Non-infected with *Tilleteia tritici*. Annals of Applied Biology 14, 105–112.

Davidson JA, Krysinska-Kaczmarek M, McKay HA. 2011 – Comparison of cultural growth and in planta quantification of *Didymella pinodes*, *Phoma koolunga* and *Phoma medicaginis* var. pinodella, causal agents of ascochyta blight on field pea (*Pisum sativum*). Mycologia 104, 93–101.

Johnson KB. 1990 – Assessing multiple pest populations and their effects on crop yield. In: IRRI Crop Loss Assessment in Rice. Los Banos The Philippines. IRRI. 203–214 pp.

Le May C, Potage G, Andrivon D, Tivoli B, Outreman Y. 2009 – Plant Disease Complex: Antagonism and Synergism between Pathogens of the Ascochyta Blight Complex on Pea. Journal of Phytopathology 157, 715–721.

Morris SC. 1982 - Synergism of *Geotrichum candidum* and *Penicillium digitatum* in infected citru fruit. Phytopathology 72, 1336–1339.

Sen M. 1973 – Cultural diagnosis of Indian polyporaceae. Indian Forest Records, F.R.I. press, Forest Research Institute, Dehradun, 283–284 pp.

Shearer BL, Wilcoxson RD, Skovmand B, Anderson WH. 1978 – Infection of barley by *Septoria avenae* f. sp. triticea enhanced by *Puccinia hordei*. Journal of Plant Diseases and Protection. 85, 461–470.

Simkin MB, Wheeler BEJ. 1974 – Overwintering of *Puccinia hordei* in England. Cereal Rusts. 2, 2–4.

Yarwood CE. 1977 – *Pseudoperonospora cubensis* in rust-infected bean. Phytopathology 67, 1021–1022.