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Research Paper

SEARCH OF A NATURAL REMEDY FOR CONTROL OF FUSARIAL WILTING OF SISHAM (*DALBERGIA SISSOO ROXB*)

Narendra Kumar[1*]

*Corresponding Author: **Narendra Kumar** ✉ narendra.microbiology@rediffmail.com

In present investigation frequent survey was conducted in order to collect wilted samples from 20 places of Eastern UP districts. Survey of these places revealed that wilting occurs in all age groups, even in nursery plants, in age group of 10-15 year, 15-20 year, 30-40 year, during humid months from July to September. In Basti district it was found highly alarming causing 50% wilting in July months followed by Siddharth Nagar, Santkabir Nagar and Gorakhpur district. The symptoms observed in all wilted plants were yellowing and death of leaves in acropetal succession and lastly entire plant in yellow appearance. So root samples were collected from wilted plants from these places for study of rhizoplane mycoflora. The root free from rhizosphere soil was cut into small pieces (5 mm-7 mm range) and plated on the medium at the rate of 5 pieces per petriplates and incubated at 28±2C for 6 days to observe the appearance of fungi. In all age group *Fusarium solani* and *Fusarium oxysporum* were found to be present in both sterilized and unsterilized root pieces. During pathogenicity testing of both *Fusarium solani, Fusarium oxysporum* it was found that *Fusarium solani* is highly powerful causing 80% wilting in plants. In nature plants are available which have volatile activity that can destroy fungal pathogens. So far 32 essential oils were extracted from higher plants and tested against *Fusarium solani* and *Fusarium oxysporum.* The oil of *Putranjiva roxburghii* showed complete inhibition of mycelia growth of both test fungi at 500 ppm. The oil was thermostable at its mic of 500 ppm and exhibited wide range of activity at 1000 ppm. Moreover, the fungitoxicity was not destroyed by autoclaving and storage upto 120 days.. In this way this plant have anti action against *Fusarium*. So a little mixed field trial at nursery bed level was conducted with *Putranjiva* which showed no wilting symptoms compared with separately grown control sets of sissoo

Keywords: Fusarium, Natural remedy, Putranjiva

INTRODUCTION

Shisham (*Dalbergia sissoo* Roxb) is a deciduous tree of family *Papillionaceae* and have great economic value. The species found in India, Nepal, Bhutan, Bangladesh, Pakistan and Afghanistan. It is also found in tropical to

[1] Bacteriology and Natural Pesticide Laboratory, Department of Botany, DDU Gorakhpur University, Gorakhpur-273009.

subtropical Africa and Asia, viz., Java, Nigeria, Mauritius, Sri Lanka, Kenya, Northern Zimbabwe, Palestine and South Africa (Tewari, 1994). *D. sissoo* is an important fuel wood, shade, shelter and fodder tree. It usually cultivated in forest plantations and along the canals, roadsides, railway lines, water channels and borders of the agricultural fields. Plants of Sisham are at the verge of extinction no remedy is available for control. In North Eastern Uttar Pradesh a lot of sisham plants have been found wilted. Review of literature reveals that disease is caused by *Fusarium solani* (Mart) Wr. F dalbergiae (Bagchee, 1945; Bakshi, 1954; Bakshi and Singh, 1954,1959). Serious wilt of sissoo seedlings in nurseries caused by *Fusarium oxysporum* Fr. are reported from Pakistan (Browne, 1968)

The number of diseases like powdery mildew, leaf rust, leaf blight, collar rot, wilt, die-back and Ganoderma root rot are reported by various research workers in Indo-Pakistan (Bakshi, 1954, Khan, 1989, Zakaullah, 1999, Khan and Khan, 2000). *Polyporus* and *Fusarium oxysporum* cause root rot and wilt respectively (Khan, 1989). *Fusarium solani* was isolated as a facultative parasite associated with wounds and on hosts weekends by unfavorable conditions. Baral (1995) traced the first report of sissoo die back in the plantations of Nepal. A new form of disease die back has been established in sissoo. This disease has been reached epidemic proportion in Bangladesh and other countries of South Asia. The heart wood of plant is strong and durable and have high timber quality so it needs protection. Elimination of pathogen from soil is not possible either chemically or by crop rotation (Bakshi, 1954). Chemicals are pollutive in nature. Biocontrol agents are available which have their own limitations. In nature plants are available which have volatile activity that can destroy fungal pathogens. Now a days, essential oil and their components are gaining increasing interest because of relatively safe status, their wide acceptance by consumers and their exploitation for potential multipurpose functional use (Ormancey *et al*., 2001; Sawamura, 2000; Kumar and Tripathi, 2007).

Synthetic pesticides pose a range of risks to human health and safety. Some present few dangers while others are highly toxic. Synthetic pesticides can affect the nervous system or cause skin and eye irritation. Long-term effects include hormone imbalances and increased risk of cancer. Organic insecticides are comparatively safe. Insecticidal soap can cause toxicity and reproductive effects if ingested in high doses, and soaps containing ammonium can cause permanent eye damage. Bt can cause allergic reactions in some people.

Since no systematic work has been done in sisham wilting in north Eastern UP so wilted samples were collected. Fungal organisms were isolated and pathogenicity tests were conducted and 32 essentials isolated from higher plants were evaluated. The fungitoxic properties of oil were studied and a little mixed field trial at nursery level was conducted in order to recommend most potent plant for protection of sisham after large scale field trials.

MATERIALS AND METHODS

Disease Sample Collection

A thorough survey of sisham growing area of North Eastern UP regions of districts, viz., Basti, Sant kabir nagar, Siddharthnagar and Gorakhpur district places were carried out in humid months (July to September) from 1997 to 2008 to find the

symptoms and severity of wilted plants of sisham. The natural diseased plants showing the typical symptoms of disease were selected from the field and samples of root portion of diseased sisham tree were collected for the isolation of associated pathogens.

Places Visited in Districts of North Eastern UP

Basti-Jamadi road, Mahso, Sonupar area, Munderwa, Orwara; in Santkabir nagar-Samariwa, Nandaur, Lohrauli, Mangua road, Khalilabad; in Siddhartha nagar-Dumariaganj, Hallore, Itwa tahsil, Bansi, Naugarh; in Gorakhpur-Bhiti road, Sahjanwa, Ghagsara, Khajani, Pipraich.

These places were visited for collection of root samples for isolation of mycoflora and recording of %wilting and age group of plants by discussion with local people. The symptoms of wilted plants were studied. The root samples were kept in presterilized polyethylene bags after labeling the name of district and place.

Isolation of Pathogens from Roots of Infected Sissoo Plants

Isolation of pathogen from these samples were carried out on Potato Dextrose Agar (PDA) medium . The pathogens were examined under microscope and maintained the culture on PDA plates at room temperature (25 ± 2 °C). The associated pathogens of sisham decline were isolated from the infected root pieces following Pathak, 1987. The infected root was cut into small pieces 5-7 mm in size with the help of sterilized knife. The pieces were surface sterilized in 2% sodium hypochlorite solution for 3 min followed by five washings with sterile distilled water. The surface sterilized and unsterilized root pieces were then placed in Petri plates having PDA medium seperately following Saleem and Nasir (1991). All Petri plates were incubated at 25±2^{o}C for seven days for isolation of associated fungi.

Percent occurence of each fungal species associated with root sample was calculated as per formula-

Percent occurrence = Number of colonies of a particular fungus/Total number of colonies of all the fungi X 100

PATHOGENICITY

Ten healthy plants of sissoo were selected from nursery. A cut was made in the stem of 9 healthy plants with the help of a sterilized knife and inoculated with 1×2 cm block of the isolated culture of *Fusarium oxysporum* and *Fusarium solani* isolated from naturally infected diseased plants in the field. Following this method a cut was made in the remaining one healthy plant to serve as a control and inoculated with 1×2 cm block of only PDA and wrapped with parafilm. Plants were monitored for the development of disease symptoms and pathogens were reisolated from stem of the test plants after seven days to confirm the pathogenicity according to Koch's Postulates following Saleem and Nasir (1991).

Isolation of Essential Oils from Higher Plants

The leaves of 32 higher plants collected separately from Gorakhpur locality were surface sterilized by dipping in 70% ethanol and then washed repeatedly with sterilized double distilled water. The surface sterilized leaves were macerated and hydrodistilled for isolation of volatile constituents separately for 6 h in clevengers apparatus. After hydrodistillation immiscible oil was separated and

dehydrated over anhydrous sodium sulphate separately to remove traces of moisture.

Fungitoxicity Testing

The toxicity of oil was assessed by using the inverted petri plate technique of Bocher (1938). The fungitoxicity of essential oils was measured following Dixit *et al.* (1978) and recorded in terms of per cent inhibition of mycelial growth.

Determination of mic of Most Active Oil

The mic of most active oil was determined by poisoned food technique of Grover and Moore (1962). Different concentration of the oil ranging from 200 to 600 ppm were prepared by dissolving requsite amount of oil in 0.5 ml acetone and then mixing with 9.5 ml czapeks dox agar medium seperately. In control sets the petriplates having acetone and medium without oil were used. Fungal discs (5 mm diam) obtained from periphery of seven d old culture of each of test fungi were aseptically inoculated in each of the treatment and control sets. All these sets were incubated at 28$\pm$2C for 6 days. Diameters of fungal colony of treatment/control sets were measured in mutually perpendicular directions on the 7th day and the average was used to calculate the percent inhibition of mycelia growth of test fungi separately. The oil treated discs of the fungi showing complete inhibition of their mycelia growth upto 7th day were washed with sterile water and placed again on fresh solidified medium to observe the revival of mycelia growth. The fungitoxic spectrum of the oil was studied against various fungi isolated from root samples. In addition effect of temperature, autoclaving and storage on the fungitoxicity of oil was determined following Pandey *et al.* (1982). Each experiment was repeated twice and contained 5 replicates.

Fungitoxic Studies in Root and Bark and Seed Extract of *Putranjiva*

This was determined by poisioned food technique of Grover and Moore (1962) against both species of Fusarium.

Mixed Field Trial with *Prautnjiva* Plant

For mixed field trial nursery bed of 1 m x 1 m area (5 such bed) were prepared at 6 inches above from the ground level. For mixed trial 100 seed of sissoo and 100 seed of Putranjiva were taken and kept in soil at each bed one after the other for growing and properly watering was done. For control set another bed of 1 m x 1 m area was prepared at 6 inches above ground level and 200 seeds were sown. Both alone and treatment sets were kept under observation for 2 years.

RESULTS AND DISCUSSION

Botany of Sisham

D. sissoo is a medium to large deciduous tree with a light crown which reproduces by seeds and suckers. It can grow up to a maximum of 25 m (82 ft) in height and 2 to 3 m (6 ft 7 in to 9 ft 10 in) in diameter, but is usually smaller. Trunks are often crooked when grown in the open. Leaves are leathery, alternate, pinnately compound and about 15 cm (5.9 in) long. Flowers are whitish to pink, fragrant, nearly sessile, up to 1.5 cm (0.59 in) long and in dense clusters 5-10 cm (2.0-3.9 in) in length. Pods are oblong, flat, thin, strap-like 4-8 cm (1.6-3.1 in) long, 1 cm (0.39 in) wide and light brown. They contain 1-5 flat bean-shaped seeds 8-10 mm (0.31-0.39 in) long. They have a long taproot and numerous surface roots which produce suckers. Young shoots are downy and drooping; established stems with light brown to dark gray bark to 2.5 cm (0.98 in) thick, shed in

narrow strips; large upper branches support a spreading crown.

During survey more than 30% plants has been found wilted at place of eastern UP districts. It is evident from Table 1, that wilting is highly alarming in Basti district causing 50% wilting in the month of July followed by Siddharthanagar, Santkabirnagar and Gorakhpur district.

As evident from Table 2, wilted plants of sissoo were observed in all age groups, in nursery plants, in age group 5-10 year, 10-15 year, 15-20 year, 30-40 year which showed yellowing and death of leaves in acropetal sucession and lastly death of entire plant in humid months from July to September.

It is evident from Table 3, that 16 fungal species were found to be associated with sterilized and unsterilized root pieces. The percent occurrence of *Fusarium oxysporum* in Basti, Santkabirnagar, Siddharthanagar, Gorakhpur district was found to be 80.4, 80.3, 78.6, 53.6%, respectively and that of *Fusarium solani* was 79.4,79.3,79.0 and 54.8%, respectively. Other fungi showed lower or moderate percentage of occurrence.

It is evident from Table 4, that during pathogenicity *Fusarium oxysporum* showed 70% wilting while *Fusarium solan*i showed 80% wilting while control set showed no wilting symptoms. On account of wide occurrence and their pathogenicity these were selected as test organisms.

The essential oil of *Putranjiva roxburghii* Wall exhibited absolute toxicity at 500 ppm inhibiting mycelial growth of both test fungi completely, while other oils at these concentrations showed lower level of fungitoxicity (Table 5).

Table 1: Percent Wilting of Sissoo at Different Places of Eastern UP in Months of July

Name of place	Percent Wilting
Basti D	
Jamdi Road	51
Mahsoo Road	55
Sonupar area	60
Munderava	50
Orwara	54
Santkabirnagar	
Sameriwa	30
Nandor	39
Lohrauli	30
Mangua Road	39
Khalilabad	38
Siddharthanagar	
Dumariaganj	42
Hallore	40
Itwa tahsil	44
Bansi	40
Naugrah	49
Gorakhpur	
Bhiti Road	30
Sahjanwa	31
Ghagsara	32
Khajani	33
Pipraich	29

Table 2: Symptoms Observed in Wilted Plants of Sissoo in Different Age Groups

Age group	Symptoms
Nursery plants 1-2 years	Complete yellow
5-10 year	Light yellow
10-15 year	Upper half yellow in acropetal order
15-20 year	yellow
30-40 year	Complete yellow

Table 3: Percent Occurrence of Isolated Fungi From Rhizoplane Of Sissoo In Different Districts

Fungal Species	Average Percentage of Isolated Fungi			
	Basti	Santkabirnagar	Siddharthanagar	Gorakhpur
Alternaria alternata	1.4	1.3	2.1	2.3
Aspergillus aculeatus	2.4	2.3	2.4	2.5
A.flavus	3.0	3.2	3.3	3.4
A.fumigatus	1.5	0.5	2.4	0.9
A.niger	3.0	2.0	2.5	15.0
A.ochraceous	3.9	4.0	0.5	0.5
A.sulphureous	2.5	1.2	4.2	0.6
A.terreus	2.4	2.5	2.5	2.6
A.versicolar	1.5	1.6	1.2	1.8
Curvularia lunata	3.4	4.5	4.0	4.6
Fusarium oxysporum	80.4	80.3	78.6	53.6
F.solani	79.4	79.3	79.0	54.8
Helminthosporium oryzae	3.5	4.9	4.3	3.1
Penicillium oxalicum	3.8	4.9	4.5	4.6
Rhizopus stolonifer	10.5	15.4	16.4	14.4
Trichoderma viride	3.5	2.5	1.5	2.0

Table 4: Fungal Species vis-à-vis Wilting of Sissoo

Fungal Species	Control Set	Treatment Set
Fusarium oxysporum	nil	70%wilting
Fusarium solani	nil	80% wilting

BOTANY OF ACTIVE PLANT

Other Recipients

Putranjeevak-*Puntrajiva roxburghii* Family-Euphorbiaceae (Eranda kula) Sanskrit name - *Putranjiva*, *Pavitra, Garbhad, Sutajeevak, Kutajeeva, Apatyajeeva, Arthasadhak, Garbhakar*. Description: Common name: Putranjiva, Lucky Bean Tree • Hindi: *Put*

Putranjeevak-*Puntrajiva roxburghii*

Family-Euphorbiacea

Sanskrit name - *Putranjiva, Pavitra, Garbhad, Sutajeevak, Kutajeeva, Apatyajeeva, Arthasadhak, Garbhakar.*

Description: Common name: Putranjiva, Lucky Bean Tree • Hindi: *Putijia*

Putranjiva is a famous, moderate-sized, evergreen tree, growing up to 12 m in height. It has pendant branches and dark grey bark having horizontal lenticels. Leaves are simple, alternately arranged, dark green, shiny, elliptic-oblong, distantly serrated. Male flowers, with short stalks,

Table 5: Evaluation of Essential oils of Higher Plants Against *Fusarium oxysporum and F. solani*

Plant species	Percent inhibition of mycelia growth of test fungi at 500ppm		
	Family	*Fusarium oxysporum*	*Fusarium solani*
Aegle marmelos(L.)Corea	Rutaceae	37.3	62.1
Ageratum conyzoides L.	Asteraceae	70.5	64.2
A. houstonianum	-	80.5	80.5
Anetum graveolens L.	Umbelliferae	31.0	33.0
Anisomeles ovate R.Br.	Lamiaceae	60.3	60.3
Artabotrys hexpetalous(Lamm)Merr.	Annonaceae	54.2	46.7
Azadirachta indica A. Juss.	Meliaceae	44.1	38.7
Caesulia oxillaris Roxb.	Asteraceae	47.1	47.1
Callestemon lanceolatus DC	Myrtaceae	33.3	48.2
Cannabis sativa L.	Cannabinaceae	10.0	9.5
Cinnamomum tamla Nees and Bbrem	Lauraceae	30.0	23.0
Citrus aurantifolia Christm	Rutaceae	32.2	29.3
*C.medica var limonia(*L.)	-	47.9	59.3
Eucalyptus citriodora Hook	Myrtaceae	29.1	35.8
E.globulus(l.) Herit	-	30.0	34.9
Eupatorium capillifolum (L.)	Asteraceae	30.0	30.9
Feronia elephantum Correa	Rutaceae	59.7	60.3
*F.limonia(*L.) Swingle	-	60.8	65.4
*Hyptis suaveolens(***L.) Poit**	Lamiaceae	37.2	27.4
Lantana camera L.	Verbenaceae	57.3	39.1
L.indica Roxb.	-	51.7	40.0
Mentha arvensis L.	Lamiaceae	43.9	38.6
M.piperata L.	-	60.3	50.3
M.spicata L.	-	63.3	48.2
Murraya koenighii(L.)Spreng	Rutaceae	20.8	40.1
Ocimum adscendens Willd	Lamiaceae	50.0	52.4
O.basilicum L.	-	45.1	50.1
O.canum Sims	-	55.1	75.0
O.sanctum L.	-	47.1	52.3
Putranjiva roxburghii Wall	Euphorbiaceae	100	100
Tagetes erecta L.	Asteraceae	40.0	30.7
Thuja occidentalis L.	Cuppressaceae	23.0	46.3

in rounded axillary clusters, female flowers 1-3 in leaf axil. Fruits ellipsoid or rounded drupes, white velvety seed normally one, one pointed, rugose, very hard.

The MIC of the oil was found to be 500 ppm against both the test fungi. The oil exhibited fungicidal nature at hyper MIC against both the test fungi (Table 6).

Table 6: MIC of the Essential oil of *Putranjiva roxburghii*

Dose of oil in ppm	*Fusarium oxysporum*	*Fusarium solani*
200	30	40
300	40	40
400	70	80
500	100	100
600	100*	100*

Note: *Fungicidal

Table 7: Fungitoxic Spectrum of oil of *Putranjiva roxburghii* at Sub Lethal, Lethal and Hyperlethal Doses

Fungal Species	Per cent inhibition of mycelial growth of isolated fungi			
	Subletha 300ppm	Lethal 500ppm	Hyperlethal 700ppm	Hyperlethal 1000ppm
Alternaria alternata	45.6	80.0	100.0	100.0
Aspergillus aculeatus	49.6	89.0	100.0	100.0
A.flavus	50.0	100.0	100.0	100.0
A.fumigatus	49.0	100.0	100.0	100.0
A.niger	60.0	100.0	100.0	100.0
A.ochraceous	68.0	100.0	100.0	100.0
A.sulphureous	79.0	100.0	100.0	100.0
A.terreus	65.6	100.0	100.0	100.0
A.versicolar	68.6	100.0	100.0	100.0
Curvularia lunata	68.6	79.6	100.0	100.0
Fusarium oxysporum	80.0	100.0	100.0	100.0
F.solani	79.0	100.0	100.0	100.0
Helminthosporium oryzae	74.0	100.0	100.0	100.0
Penicillium oxalicum	75.0	80.0	90.0	100.0
Rhizopus stolonifer	75.9	95.0	100.0	100.0
Trichoderma viride	74.6	95.6	98.6	100.0

The broad range of fungitoxicity against different fungi was also found in the present study, confirming a broad fungitoxic spectrum. The sensitivity of different fungi also varies with the variation in concentration of oils (Table 7).

According to Wellman (1967) a fungicide must retain its fungitoxicity at the extreme of temperatures. The fungitoxicity of the oil of Putranjiva was found to be thermostable upto 100C like *Ageratum conyzoides* (Dixit *et al., 1995)* and *Nardostachys jatamansi* (Mishra *et al*.,1995). The oil retained its fungitoxicity on autoclaving (15 lbs/square inch pressure). This quality of oil will facilitate the isolation of their constituents in active state.

A fungicide should be able to retain its activity during long period of its storage (Wellman, 1967). The fungitoxic factor in the oil of *Adenocalyma allicea* was lost within 21 d of storage (Chaturvedi, 1979) while persisted for long period in the oil of *Ageratum conyzoides* (Dixit *et al.*, 1995), *Trachyspermum ammi* (Singh and Tripathi, 1999). The essential oil of *Putranjiva* retained fungitoxic factor upto 120 d, the maximum period taken into consideration (Table 8).

During present investigation the oil of *Putranjiva* was found to be fungistatic in nature at its MIC of 500 ppm while it exhibited fungicidal nature of toxicity at higher concentration against both the test fungi, viz*., Fusarium oxysporum* and *Fusarium solani*. The oil was thermostable,

Table 8: Effect of Physical Factors on the Fungitoxicity of *Putranjiva roxburghii* Wall

Physical factors	Percent inhibition of mycelial Growth at its MIC
Temperature(°C)	
Time of treatment-60min	
40°C	100
60°C	100
80°C	100
100°C	100
Autoclaving (15lbs/sq inch pressure at 12°C) For 15 min	100
Storage in days	
15	100
30	100
45	100
60	100
75	100
90	100
105	100
120	100

Table 9: Evaluation of Extracts of *Putranjiva* at 1;10 Dilution Against Test Fungi		
	Percent inhibition of mycelial growth	
Name of extract	***Fusarium oxysporum***	***Fusarium solani***
Root extract	100	100
Bark extract	100	100
Seed extract	100	100

Table 10: Observation of Mixed Trial with *Putranjiva* Plant and Sissoo Plant upto Two Year	
Control set(only sissoo plant)	**20% wilting Treatment set(mixed nursery preparation with *Putranjiva* plant) No wilting**
Yellowing and deathof leaves and ultimatelydeath of entire plants	Plants found luxuriantly growing

retained absolute toxicity on autoclaving and storage upto 120 d. Further the oil showed broad range of activity at 1000 ppm .

It is evident from Table 9, the root, bark and seed extract of *Putranjiva* showed 100% mycelial inhibition of both test fungi at 1;10 dilution. This feature does not indicate the ineffectiveness in control of *Fusarium* because it has antiaction against both species and will reduce the population of *Fusarium*.

It is evident from Table 10, that a little mixed field trial at nursery level upto two year showed 20% wilting in control set when only sissoo plants were grown while in treatment set a mixed nursery preparation of sissoo and *Putranjiva* one after other showed complete protection of wilting symptoms. The separately grown set of sissoo showed yellowing and death of leaves and ultimately death of entire plants. So in order to protect sissoo *Putranjiva* may be used against *Fusarium* after successful large scale mixed planation field trials. The monoculture concept for sissoo should be removed in order to conserve this plant because this at the stage of extinction.

CONCLUSION

The work done tells that root cause of wilting of sisham is species of *Fusarium* and for their management *putranjiva* which is also a tree have capacity for their protection so that there is a need of large scale mixed plantation trials. It is requested from forest department and farmers please do mixed plantation for protection of sisham.

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