

Inhibition of *Fusarium* causing death before life in *sissoo* (*Dalbergia sissoo* Roxb.) by a botanical-*Putranjiva*

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Abstract: *Sissoo* or *sisham*(*Dalbergia sissoo* Roxb.) is a medium to large-sized deciduous tree. The species occurs throughout the plains in north India, from Punjab to Assam ascending upto 100m in the Himalayas. Due to the value of timber, *sissoo* is raised in plantations. During survey at 20 different places of four districts of Eastern U.P it has been found that in a one kilometer area if 100 plants of *sisham* are growing more than 30 plants has been found wilted. So frequent survey was conducted in order to collect wilted samples from 20 places of Eastern U.P 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 rhizosphere mycoflora. The root free from rhizosphere soil was cut into small pieces with the help of sterilized scalpel and forceps and plated on the medium and incubated at 20± 2C for 6 days to observe the appearance of fungi. Both sterilized and unsterilized root pieces were observed. In all age group *Fusarium solani* and *Fusarium oxysporum* were found to be present in both sterilized and unsterilized root pieces. Seven species of *Fusarium* isolated from roots were tested for their pathogenicity on *sissoo*. During pathogenicity testing of *Fusarium solani*, *Fusarium oxysporum* it was found that *Fusarium solani* is highly powerful causing 100% 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 500ppm. The oil was thermostable at its MIC of 500ppm and exhibited wide range of activity at 1000ppm. Moreover, the fungitoxicity was not destroyed by autoclaving and storage upto 120 days. The oil obtained from *Feronia limonia* also showed inhibitory activity at 1000ppm. The root and bark extract of *Putranjiva* was also found to be effective against both species of *Fusarium*. In this way this plant has overall 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*. So *Putranjiva* may be used against *Fusarium* to protect *sissoo* after successful large scale mixed plantation field trials.

I. Introduction

Sissoo or *Shisham* (*Dalbergia sissoo* Roxb) is a deciduous tree of family *Papilionaceae* and has great economic value. The species is found in India, Nepal, Bhutan, Bangladesh, Pakistan and Afghanistan. It is also found in tropical to 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 is 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. 25-30% mortality was observed in Kasur, Pakistan (Khan *et al.*, 2004). The heart wood of plant is strong and durable and has high timber quality so it needs protection. Elimination of pathogen from soil is not possible either chemically or by crop

rotation(Bakshi,1955).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).

Since no systematic work has been done in sisham wilting in north Eastern U.P 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.

II. Materials and Methods

Disease sample collection

A thorough survey of sisham growing area of North Eastern U.P regions of districts viz.,Basti,Sant kabir nagar,Siddharthnagar , Gorakhpur and Maharajganj 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 U.P

Basti-Ganeshpur,Kalwari,Makhauda,Chhawni Bazar,Walterganj in Santkabir nagar-Baghnagar,Mehdaul,Matiuli,Alinagar,Gagargarh, in Siddhartha nagar-Bansi,Itwa,Chandapar,Chilia,Birdpur inGorakhpur-Brhalganj,Golabazar,Kauriram,Kusmi,Pali in Maharajganj-Nautanwa,Sanduriya,Khucha,Paniyara,Nichlaul

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

Wilted sissoo plants collected during survey were brought to the laboratory and washed to remove adhering soil from roots.These were then sterilized by dipping in 0.5 per cent NaOCl(sodium hypochlorite) solution for 3-5 minutes.The root were then dried on paper towels and cut into pieces of 1cm size.A vertical slit was made in each piece which were placed sperately in petridishes containing solidified potato dextrose agar(PDA) medium and incubated at 20 ± 2 °C for six days to observe the appearance of fungi.The fungi thus appeared were purified by single hyphal tip culture technique and maintained on PDA slants.The fungi were identified by comparing their morphological and cultural characteristics with authentic culture maintained in the Mycology laboratory of my department as well as with help of literature(Gillman,1967;Booth,1971;Ellis,1971,1976).

Per cent occurance 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

The pathogenicity of all species of *Fusarium* on the host plant was evaluated.The isolates of *Fusarium* were grown on PDA in petriplates.Fungal discs of each species was taken from 7-day old culture and mixed with maizemeal and sterilized soil(1;1;8w/w/w respectively).The soil thus inoculated was placed in earthen pots.Sterilized soil upto 2cm height was again filled in the pots which were given light irrigation.The sick pots thus prepared were covered with parchment paper and incubated for 7 days in greenhouse before sowing.Control sets were prepared by using only sterilized soil mixed with maize meal(9;1 w/w) without fungal inoculam.Seeds of sissoo were surface sreilized by dipping in 5 per cent NaOCl solution for 5 minutes.Five such seeds were sown in the top layer of sterilized soil of each pots.The pots were irrigated at regular intervals.Experiment was repeated twice,each containing 10 replicates and observation were recorded on 40th day.

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 hour 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 600ppm were prepared by dissolving requisite amount of oil in 0.5ml acetone and then mixing with 9.5ml czapeks dox agar medium separately. In control sets the petriplates having acetone and medium without oil were used. Fungal discs(5mm 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±2C for 6 days. Diameters of fungal colony of treatment/control sets were measured in mutually perpendicular directions on the 7th d 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 thir mycelia growth upto 7d 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 poisoned 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 1mx1m area(5such 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 1mx1m area was prepared at 6 inches above ground level and 200 seeds were sown. Both alone and treatment sets were kept under observation for 3 years.

III. Results

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 20 places of eastern U.P districts. It is evident from Table1, 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 Table2, 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 succession and lastly death of entire plant in humid months from July to September.

It is evident from Table3, that 16 fungal species were found to be associated with sterilized and unsterilized root pieces. The per cent occurrence of *Fusarium oxysporum* in Basti, Santkabirnagar, Siddharthanagar, Gorakhpur district was found to be 80.3, 80.1, 78.5, 53.2 per cent respectively and that of *Fusarium solani* was 79.2, 79.2, 79.3 and 54.4 per cent respectively. Other fungi showed lower or moderate percentage of occurrence.

It is evident from Table4, that during pathogenicity *Fusarium oxysporum* showed 90% wilting while *Fusarium solani* showed 100% wilting while control set showed no wilting symptoms. Except *Fusarium oxysporum* and *Fusarium solani*, none else exhibited wilting in more than 50% plants. Amongst *Fusarium*

species, *F. avenaceum* exhibited different levels of pathogenicity on sissoo plants. 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 500ppm inhibiting mycelial growth of both test fungi completely, while other oils at these concentrations showed lower level of fungitoxicity (Table 5).

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

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).

The essential oil of *Putranjiva* retained fungitoxic factor upto 120d, 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 500ppm 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, retained absolute toxicity on autoclaving and storage upto 120d. Further the oil showed broad range of activity at 1000ppm.

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 anti-action 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 three year showed 25% 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.

IV. Discussion

Bakshi (1954) reported that wilt disease of sisham in Dehradun and Saharanpur district of western Uttar Pradesh is due to *Fusarium solani*. In present investigation both *Fusarium oxysporum* and *Fusarium solani* are responsible for wilting. Bakshi (1954) mentioned that only trees of 15-25 year old suffer from this disease, but in present investigation even nursery plants have found suffering from wilting disease. Browne (1968) recorded serious wilt of sissoo seedlings in nurseries due to *Fusarium oxysporum* and *Rosellina aquila* from Pakistan.

The leaflets gradually turn yellow and eventually turn yellow and eventually drop off. The entire tree becomes thin and light coloured as observed by Bakshi (1954) have been also observed in present investigation.

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 100°C like *Ageratum conyzoides* (Dixit *et al.*, 1995) and *Nardostachys jatamansi* (Mishra *et al.*, 1995). The oil retained its fungitoxicity on autoclaving (15lbs/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 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 plantation field trials. The monoculture concept for sissoo should be removed in order to conserve this plant because this at the stage of extinction.

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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Table1.Per cent wilting of sissoo at different places of eastern U.P in months of July

Name of place	Per cent wilting
Basti District	
Ganeshpur	50
Kalwari	54
Makhauda	59
Chhawni Bazar	50
Walterganj	54
Santkabirnagar District	
Baghnagar	30
Mehndawl	38
Matiuli	30
Alinagar	39
Gagargarh	37
Siddharthanagar	
Bansi	41
Itwa	40
Chandapur	43
Chilia	40
Birdpur	48
Gorakhpur	
Barhalganj	30
Golabazar	30
Kauriram	31
Kusmi	32
Pali	28

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. Per cent occurrence of isolated fungi from rhizoplane of sissoo in different district

Fungal species	Average per centage of isolated fungi			
	Basti	Santkabirnagar	Siddharthanagar	Gorakhpur
<i>Absidia spinosa</i> Lender	1.0	1.0	2.0	2.1
<i>Acremonium album</i> Cattaneo	2.3	2.1	2.3	2.4
<i>Alternaria solani</i> Keissler	3.1	3.1	3.0	3.2
<i>A.niger</i> Van Teighmer	1.4	0.7	2.1	0.8
<i>A.tamari</i> Kita	3.2	2.2	2.4	15.0
<i>F.avenaceum</i> Schl	3.7	4.1	0.6	0.6
<i>F.chlamydosporum</i> Wallen Welber and Reinking	2.2	1.0	4.0	0.4
<i>F.equseti</i> (Corda) Saccardo	2.1	2.2	2.2	2.5
<i>F.moniliforme</i> Schl	1.0	1.2	1.1	1.2
<i>F.nivale</i> Schl	3.2	4.2	4.1	4.6
<i>Fusarium oxysporum</i> Schl	80.3	80.1	78.5	53.2
<i>F.solani</i> (Mart)Sacc	79.2	79.2	79.3	54.4
<i>Humicola grises</i> Omvik	3.4	4.7	4.0	3.0
<i>Penicillium chrysogenum</i> Thom	3.7	4.5	4.1	4.4
<i>P.funiculosum</i> Thom	10.0	15.0	16.3	14.3
<i>P.oxalicum</i> Currie and Thom	3.0	2.0	1.0	2.2

Table 4. Fungal species vis-à-vis wilting of sissoo

Fungal species	Per cent wilting
<i>F.avenaceum</i>	20.0
<i>F.chlamydosporum</i>	2.0
<i>F.equseti</i>	2.0
<i>F.moniliforme</i>	8.0
<i>F.nivale</i>	5.0
<i>F.oxysporum</i>	90.0
<i>F.solani</i>	100.0

Table 5. Evaluation of essential oils of higher plants against *Fusarium oxysporum* and *F.solani*

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

<i>Putranjiva roxburghii</i> Wall	Euphorbiaceae	100	100
<i>Tagetes erecta</i> L.	Asteraceae	40.0	30.7
<i>Thuja occidentalis</i> L.	Cupressaceae	23.0	46.3

Table 6. MIC of the essential oil of *Putranjiva roxburghii*

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

*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			
	Sublethal 300ppm	Lethal 500ppm	Hyperlethal 700ppm	Hyperlethal 1000ppm
<i>Absidia spinosa</i> Lender	45.6	80.0	100.0	100.0
<i>Acremonium album</i> Cattaneo	49.6	89.0	100.0	100.0
<i>Alternaria solani</i> Keissler	50.0	100.0	100.0	100.0
<i>A.niger</i> Van Teighmer	49.0	100.0	100.0	100.0
<i>A.tamari</i> Kita	60.0	100.0	100.0	100.0
<i>F.avenaceum</i> Schl	68.0	100.0	100.0	100.0
<i>F.chlamyosporum</i> Wallen Welber and Reinking	79.0	100.0	100.0	100.0
<i>F.equesii</i> (Corda) Saccardo	65.6	100.0	100.0	100.0
<i>F.moniliforme</i> Schl	68.6	100.0	100.0	100.0
<i>F.nivale</i> Schl	68.6	79.6	100.0	100.0
<i>Fusarium oxysporum</i> Schl	80.0	100.0	100.0	100.0
<i>F.solani</i> (Mart)Sacc	79.0	100.0	100.0	100.0
<i>Humicola grises</i> Omvik	74.0	100.0	100.0	100.0
<i>Penicillium chrysogenum</i> Thom	75.0	80.0	90.0	100.0
<i>P.funiculosum</i> Thom	75.9	95.0	100.0	100.0
<i>P.oxalicum</i> Currie and Thom	74.6	95.6	98.6	100.0

Table 8. Effect of physical factors on the fungitoxicity of *Putranjiva roxburghii* Wall

Physical factors	Per cent 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 120C) 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 Per cent inhibition of mycelial growth

Name of extract	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>
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 three year

Control set (only sissoo plant) 25% wilting	Treatment set (mixed nursery preparation with <i>Putranjiva</i> plant) No wilting
Yellowing and death of leaves and ultimately death of entire plants	Plants found luxuriantly growing