

Original Research Article

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Evaluating Poplar Leaf Extract Concentration on Potential Plant Pathogens

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ABSTRACT

Today, Poplar clones, being raised as monocultures, are prone to attack of a large number of fungal pathogens. These pathogens are *in vitro* cultured for varied studies. It is believed that natural media (vegetable and plant extract host decoction), stimulates growth and sporulation of a fungus. Therefore, the present study was conducted to evaluate the influence of different concentrations of host (*P. deltoides*) leaf extract on its potential pathogens. Nine isolates three of each *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii* were taken for experimentation. All these pathogenic fungi had better growth in leaf extract medium than without it but fungal growth was inhibited at higher concentrations of leaf extract.

Keywords

Poplar, Extract, Pathogen, Growth.

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Introduction

Poplar belongs to the family Salicaceae, order Salicales and group Amentiflorae (Dickmann and Stuart, 1983; Eckenwalder, 1996). There are 35 species of poplar currently recognized in the world; out of those, 6 *Populus* species are indigenous to India— *P. alba*, *P. ciliata*, *P. euphratica*, *P. gamblei*, *P. glauca* and *P. laurifolia*, and during past many clones of exotic species of poplar (*P. deltoides* and *P. xuramericana*) have been introduced in India in area between 28° to 31°N. Poplar have very specialized climatic and edaphic requirements, this is the reason which restrict their cultivation in India (Tewari, 1993).

In India, poplars (*P. deltoides*) are eminently suited for agroforestry because of its fast

growth, straight- growing stem, short rotation, quality wood production and less adverse effect on agriculture crops (Bangarwa, 2008) and are being grown in Uttar Pradesh, Uttarakhand, Punjab, Haryana and Jammu and Kashmir (Singh *et al.*, 1999). *P. deltoides* enters in dormancy during winter and become leafless, and let the sunlight to pass through it, for the companion crops, therefore, suited well to companion crops. In the interspace of poplar plantations, several seasonal crops are cultivated for enhancement of overall productivity of the land and generation of supplementary income (Jaiswal *et al.*, 1993; Singh *et al.*, 1993; Jain and Singh, 1999).

The ease with which most materials can be vegetatively propagated is one of poplar's

premier assets. So that in India, poplar is grown under forestry programmes as clonal plantations i.e. from a single plant infinite number of plants can be developed to ensure the genetic superiority for better growth and superior wood quality (Pande and Dhiman, 2010). Meanwhile, favoured by their simple clonal propagation, relatively rapid growth rate and considerably small genome size among woody species, they are one of the major model plants used in both scientific and industrial research (Bradshaw *et al.*, 2000; Polle and Douglas, 2010). By taking cuttings from a single tree, infinite number of genetically identical trees can be produced - better known as clones (Charlton *et al.*, 2007). Several promising clones of *P. deltoides* namely; G, D, Sc, I, IC, PL, St, L, WSL, Wimco series, etc., have been identified and, subsequently, planted in farm/agro forestry systems throughout Punjab, Haryana, Tarai region of Uttarakhand, western part of Uttar Pradesh, some parts of Bihar, West Bengal and Assam (Mishra *et al.*, 2010; Dhiman and Gandhi, 2012).

About 90 percent of the poplar plantations in India are of the clones G-48, D-121, S7C15 and G-3 (Kumar *et al.*, 1999). The clonal forestry in India represents a very narrow genetic base of the planting material. Clones, being raised as monocultures, are thus prone to a large number of fungal pathogens and disease outbreaks (Singh *et al.*, 2012). Disease in poplar may occur in any stage from the time of planting of cutting in beds till they are extracted as entire transplant (ETPs) for field planting as well as in plantation. Large numbers of foliage diseases were recorded in poplar nurseries. Fungi causing leaf spots and blights are *Alternaria alternata*, *Bipolaris* sp., *Cercospora populina*, *Cladosporium humile*, *Corticium salmonicolor*, *Curvularia* sp., *Drechslera maydis*, *Fusarium solani*, *Pollaccia* sp., *Phoma* sp., *Phyllosticta adjuncta*, *Rhizoctonia*

microsclerotia, *Sclerotium rolfsii* and *Septoria* sp. (Pandey and Khan, 1992; Singh *et al.*, 2012). Poplar also suffers from root rot namely; white root rot, *Ganoderma* root rot, *Rhizoctonia* root rot and *Fusarium* root rot (Singh *et al.*, 1983). *Fusarium* is one of the important pathogens that cause mortality of planted cutting.

Sub culturing in forest pathology is to grow and sustain a pathogen's sample suitable for experimentation and tests. Sub culturing prolongs the lifespan of pathogens, allowing for long-term maintenance and observation of the culture. On the other hand, repeated sub culturing in axenic media cause the loss of sporulation in pathogen which is associated with the loss of virulence. Zhao and Shamoun (2006) suggested that nature of a culture medium has great role to play in the growth, sporulation and conidial discharge of *Valdensinia heterodoxa*. It is believed that natural media (vegetable and plant extract host decoction) stimulates the growth of fungus (Patil and Rao, 2003). Therefore, periodic mixing of host extract in the medium during sub culturing is one of the strategies to revive the fungal characteristics. Probably, the complex of constituents of the host extract offers some nutrients that, otherwise, are lacking in the synthetic medium. Therefore, the present study was conducted to evaluate the influence of different concentration of host (*P. deltoides*) leaf extract media on mycelial growth of *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii*.

Materials and Methods

Collection of Fungal Isolates

Nine isolates three of each *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii* were taken from Forest Pathology Division, Forest Research Institute, Dehradun for experimentation (Table 1 to 3).

Preparation of Leaf Extract Medium

Leaves were collected from *P. deltoides*, weighted 5, 10, 15, 20, 25 g for making 5, 10, 15, 20, 25 % leaf extract media, respectively. After weighing, leaves of poplar were washed thoroughly in tap water and sterilized distilled water and boiled in 100 ml distilled water and then filtered to separate the leaf extract from the leaves. Finally make up the leaf extract to 100 ml by adding distilled water because during the process of making leaf extract, some water evaporates. Then, 1.2 gm. PDB was added to 50ml leaf extract of different concentrations (5%; 10%; 15%; 20% and 25%), stirred vigorously and autoclave it to obtain natural leaf extract medium.

PDB (Potato Dextrose Broth) is liquid medium used as control medium for the study of fungi. Ready-made media of PDB was used 24g/1000ml of distilled water.

Inoculation

Five mm disc of 7 days old mother culture of different fungal isolates were transferred into the conical flasks of 100 ml volume containing 50 ml media. Then, it was incubated at $22^{\circ}\pm 1^{\circ}\text{C}$ in BOD incubator for 21 days.

Growth Measurement

Growth parameters Dry weight, Sclerotia Initiation, Maturation and Sclerotia Count (in case of sclerotia forming fungus *Sclerotium rolfsii*) were used for the measurement of the growth.

Statistical Analysis

Data was analyzed with the GENSTAT 5 Release 3.22. Two way analyses were used for growth. Treatments means were compared at 5% level of significance.

Results and Discussion

Present study deal with effect of poplar leaf extract on the growth of three fungi namely, *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii*. Following trends were found:

Table 4 depicted the pattern of growth of *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii* at different concentrations (5-25 %) of poplar leaf extract. It was found that all fungi exhibited growth only at lower concentration of leaf extract tested, i.e., 5 and 10 %. While no growth was recorded at higher concentrations of leaf extract (15-25%). Further, it was noticed that isolates of *Bipolaris* and *Curvularia* exhibited growth till 10 % leaf extract concentration (Fig.1, 2). In contrasting, *Sclerotium rolfsii* isolates showed no growth at even 10 % poplar leaf extract concentration (Fig.3).

Dry Mycelium Weight

The growth of *Bipolaris* sp. isolates showed differential pattern on different poplar leaf extract concentrations. Irrespective of leaf extract concentrations, isolate no. B-59 showed significant and maximum dry mycelium weight (0.27g) while, minimum growth (0.12g) was recorded for the isolate no. B-22. Isolates showed significantly more and maximum growth at 10 % leaf extract concentrations. Further, the growth was significantly minimum on control (Table 5.).

When interactions between fungal isolate and concentrations (I*C) were studied, it was observed that all isolates attained growth only at lower concentrations (5 and 10 %). Isolate no. B-59 showed maximum and significantly more growth at 10 % concentration (0.60g). Minimum dry mycelium weight (0.31g) was recorded by isolate no. B-22 on control which was at par with isolate no. B- 25 on same medium (0.31g). Barring isolate no. B-59

(0.50g) at 5 % concentration, all the isolates had significantly more dry mycelium weight than control (Table 5). While, dry mycelium weight of isolate no. B-59 was significantly higher on control than 5 % concentration (0.53 and 0.50g, respectively).

Irrespective of different poplar leaf extract concentrations tested, it was observed that isolate no. C-40 (0.29g) had maximum dry

mycelium weight (Table 6). While, minimum dry mycelium weight was recorded for the isolate no. C-29 (0.23g). Highest dry mycelium weight of the *Curvularia* sp. isolates were recorded at 10 % leaf extract concentration. No growth was recorded at 15 concentrations, i.e., none of the isolate could grow at 15- 25 % leaf extract concentration. Second to it, significantly low dry mycelium weight was recorded at control (0.35g).

Table.1 Details of the *Bipolaris* sp. isolates

Isolate No.	Place	Clone
B25	Baghwala,Rudrapur	G-3
B58	Baghwala,Rudrapur	G-3
B59	Wimco nursery, Saharanpur	W-39

Table.2 Details of the *Curvularia* sp. isolates

Isolate No.	Place	Clone
C29	Jawahar Nagar, Rudrapur	WSL-39
C35	Bagwala,Rudrapur	G-48
C40	Bagwala,Rudrapur	G-48

Table.3 Details of the *Sclerotium rolfsii* isolates

Isolate No.	Place	Clone
S21	Baghwala,Rudrapur	Udai
S97	Baghwala,Rudrapur	W-108
S104	Baghwala,Rudrapur	W -108

Table.4 Effect of different concentrations of poplar leaf extract on the growth of *Bipolaris* sp., *Curvularia* sp. and *Sclerotium rolfsii*

Isolate No.	Plant extract (%)/ Growth					
	0 (Control)	5	10	15	20	25
B-22	+	+	+	-	-	-
B-25	+	+	+	-	-	-
B-59	+	+	+	-	-	-
C-29	+	+	+	-	-	-
C-35	+	+	+	-	-	-
C-40	+	+	+	-	-	-
S-21	+	+	-	-	-	-
S-97	+	+	-	-	-	-
S-104	+	+	-	-	-	-

+ = Normal Growth, - = No Growth

Table.5 Effect of different concentrations of poplar leaf extract on the growth of *Bipolaris* sp.

Isolate No.	Plant extract (%) / Dry mycelium weight (g)						Mean
	Control (0)	5	10	15	20	25	
B-22	0.31	0.35	0.42	0.00	0.00	0.00	0.12
B-25	0.31	0.35	0.44	0.00	0.00	0.00	0.18
B-59	0.53	0.50	0.60	0.00	0.00	0.00	0.27
Mean	0.38	0.40	0.49	0.00	0.00	0.00	
	Isolate		Concentration		Interaction (I*C)		
SEM	0.003		0.004		0.006		
CD (5%)	0.01		0.01		0.02		

Table.6 Effect of different concentrations of poplar leaf extract on the growth of *Curvularia* sp.

Isolate No.	Plant extract (%) / Dry mycelium weight (g)						Mean
	Control (0)	5	10	15	20	25	
C-29	0.31	0.51	0.57	0.00	0.00	0.00	0.23
C-35	0.33	0.51	0.69	0.00	0.00	0.00	0.25
C-40	0.42	0.58	0.76	0.00	0.00	0.00	0.29
Mean	0.35	0.53	0.67	0.00	0.00	0.00	
	Isolate		Concentration		Interaction (I*C)		
SEM	0.004		0.005		0.009		
CD (5%)	0.01		0.01		0.02		

Table.7 Effect of different concentrations of poplar leaf extract on the growth of *Sclerotium rolfsii*

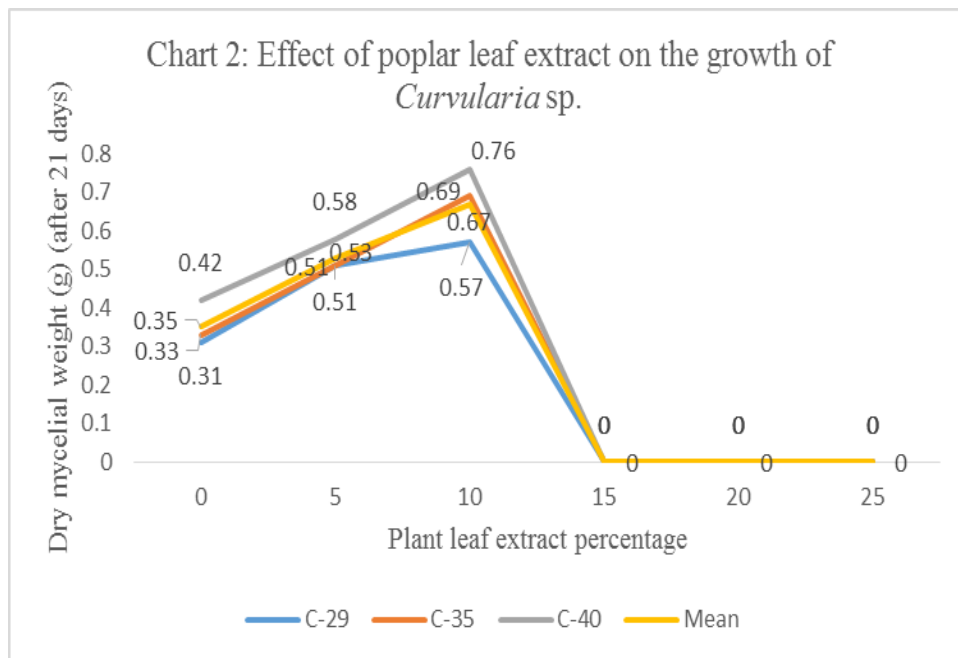
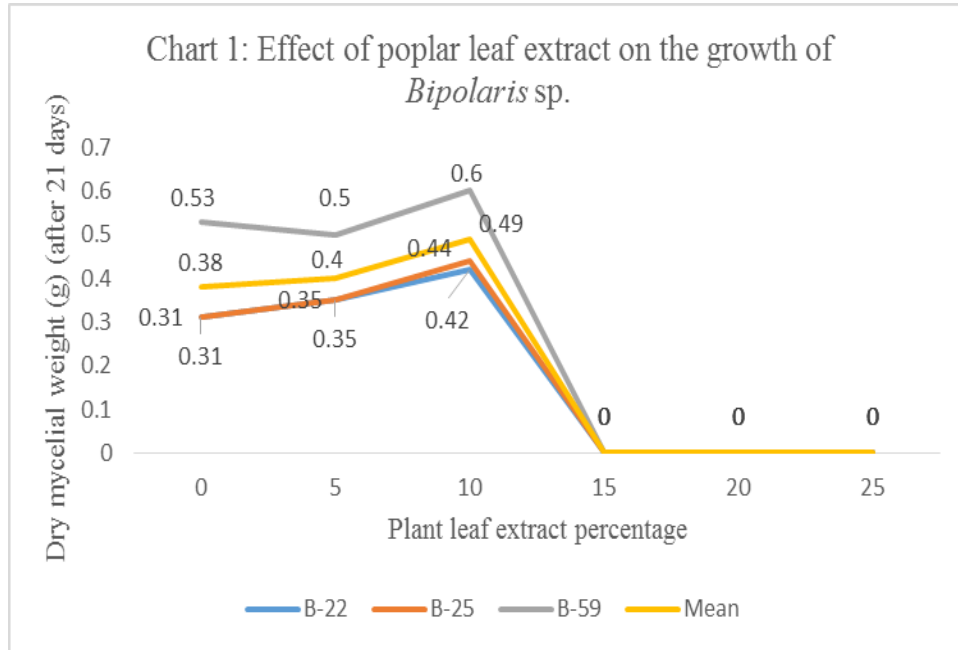
Isolate No.	Plant extract (%) / Dry mycelium weight (g)						Mean
	Control (0)	5	10	15	20	25	
S-21	0.70	0.79	0.00	0.00	0.00	0.00	0.25
S-97	0.69	0.76	0.00	0.00	0.00	0.00	0.24
S-104	0.53	0.63	0.00	0.00	0.00	0.00	0.19
Mean	0.64	0.72	0.00	0.00	0.00	0.00	
	Isolate		Concentration		Interaction (I*C)		
SEM	0.002		0.003		0.005		
CD (5%)	0.01		0.01		0.02		

Table.8 Time of Initiation and maturation of the sclerotia of *Sclerotium rolfsii* in different concentrations of poplar leaf extract

Isolate No.	Plant extract (%) / Initiation and maturation of sclerotia (Days)			
	0 Control		5	
	Initiation	Maturation	Initiation	Maturation
S-21	7	10	10	15
S-97	8	12	10	16
S-104	7	11	9	15

Table.9 Number of sclerotia of *Sclerotium rolfsii* in different concentrations of Poplar leaf extract

Isolate No.	Plant extract (%)/No. of sclerotia formed	
	0 Control	5
S-21	257	213
S-97	293	252
S-104	236	215



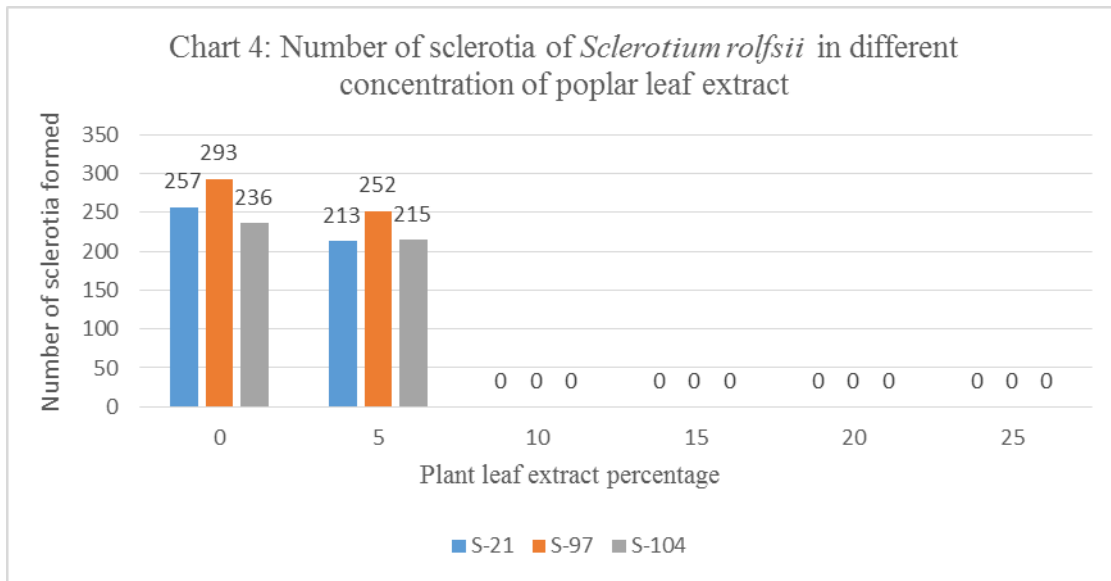
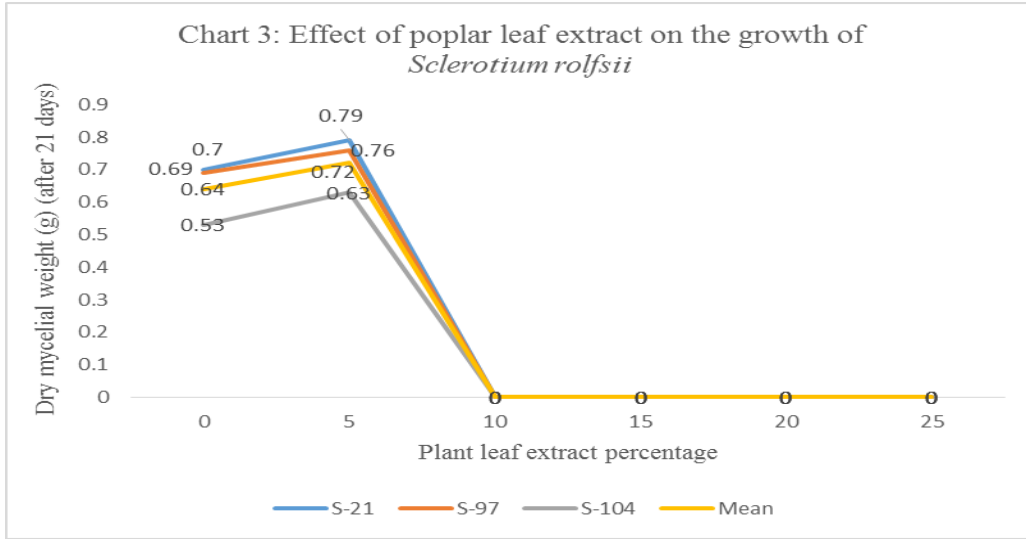


Fig.1 Growth pattern of *Bipolaris* sp. in different leaf extract concentration

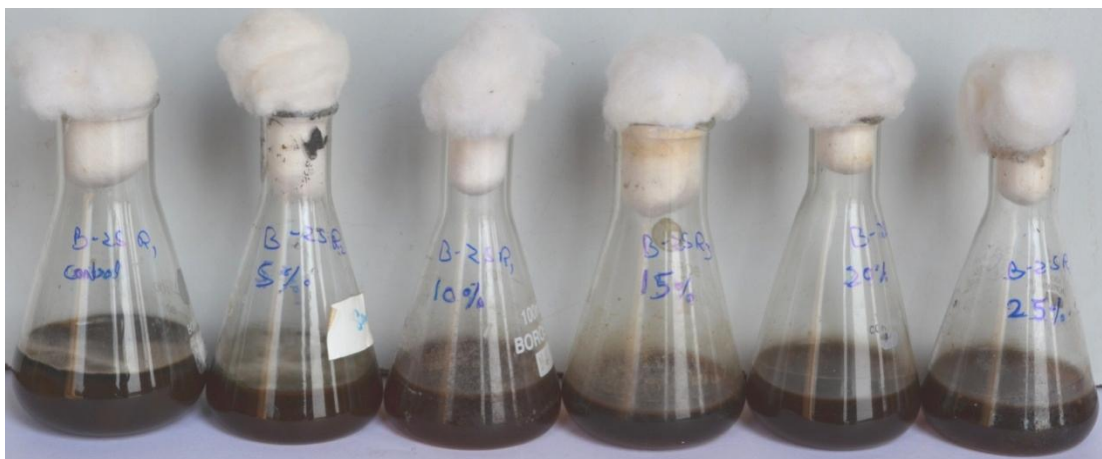


Fig.2 Growth pattern of *Curvularia* sp. in different leaf extract concentration

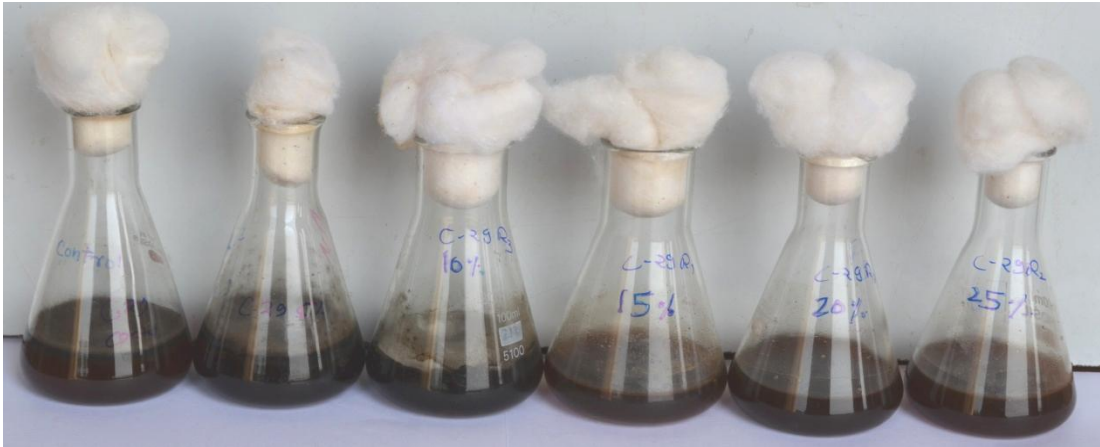
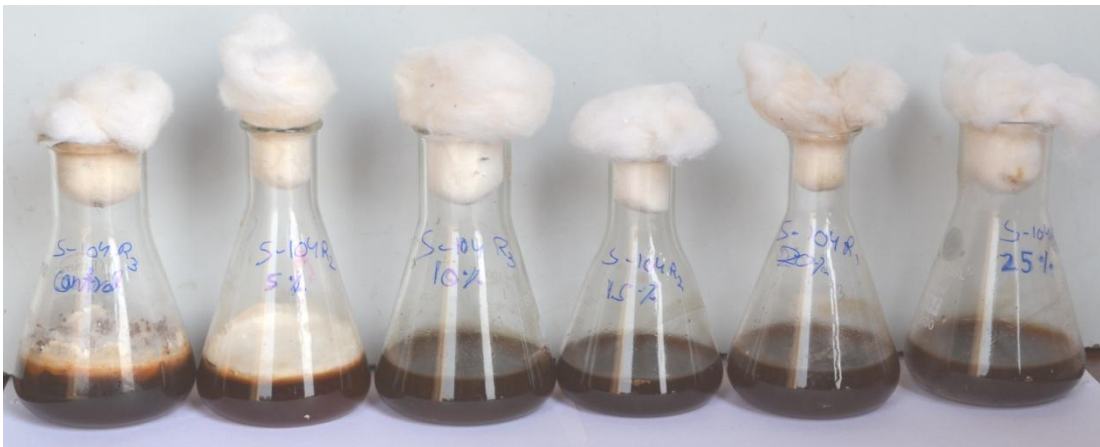


Fig.3 Growth pattern of *Sclerotium rolfsii* in different leaf extract concentration



When interactions between isolate and concentrations (I*C) were studied, it was found that isolate no. C-40 (0.76g) had maximum and significantly more dry mycelium weight. Minimum and significantly low dry mycelium weight was recorded by the isolate no. C-29 (0.31g) on control which was at par with isolate no. C-35 on the same medium. All isolates of *Curvularia* sp. exhibited significantly higher growth at 5 and 10 % leaf extract concentrations as comparative to control (Table 6).

Irrespective of different concentrations of poplar leaf extract tested, it was found that isolate no. S-21 (0.25g) had maximum dry

mycelium weight which was at par with dry mycelium weight of isolate no. S-97 (0.24g; Table 7). Maximum and statistically higher dry mycelium weight was recorded at 5 % leaf extract concentration when isolates were ignored while, minimum and significantly low dry mycelium weight were recorded at the control.

All the isolates of *Sclerotium rolfsii* showed significant and maximum dry mycelium weight at 5 % leaf extract concentration when interactions between isolates and different concentration of leaf extract were (I*C) studied (Table 7). Isolate no. S-21 had significant and maximum dry mycelium

weight (0.79g) at 5 % leaf extract concentration. While, minimum dry weight was recorded by the isolate no. S-104 (0.53g) on control. All the isolates at 5 % leaf extract concentration had significantly higher dry mycelium weight than control.

Initiation and maturation of sclerotia of *Sclerotium rolfsii*

The Sclerotia of *Sclerotium rolfsii* isolates showed differential pattern of initiation and maturation on different poplar leaf extract concentrations (Table 8). The average initiation period of sclerotia was 7-8 days in case of control medium while in 5% leaf extract medium it was 9-10 days. And the maturation period was 10-11 days in control medium and 15-16 days in 5% leaf extract medium. Isolate no. S-27 and isolate no. S-104 showed the minimum initiation period (i.e. 7 days) in control medium and Isolate no. S-97 showed the maximum maturation period (i.e. 16 days) in 5% leaf extract medium.

Number of sclerotia formed

In case of sclerotia formation, the maximum no. of sclerotia was formed in isolate no. S-97 in control medium (i.e. 293) and minimum no. of sclerotia were formed in isolate no. S-21 in 5% leaf extract medium (i.e. 213). It was also seen that sclerotia formation was more in every isolates of *Sclerotium rolfsii* in control medium in comparison to the 5% leaf extract medium (Table 10).

Present study reveals the effect of the host leaf extract on the growth of its pathogens. Experiment was done with three isolates of *Curvularia* spp., *Bipolaris* spp. and *Sclerotium rolfsii* and came to the conclusion that although host extract medium is good for the growth of the pathogens but it is good up to a certain level after that when we used the high concentration of the leaf extract, it

become poisonous to the isolates. It may be due to the fact that Plant have their own feature of resistance which is due to the biochemical present inside the plant. At higher concentration of the leaf extract this resistant chemical may become inhibitory as its concentration is also increased. This fact get support from the research evidence that some clone are resistant to the same fungus and some are less prone to the infection which is due to the quality or quantity of the resistant chemical compound present inside the host plant. Further study require in this, what chemical constituent is this? Which can control the growth of the potential pathogen and if there is any way to increase the amount of production of such compound inside the plant so that we can indirectly cure the disease by providing it resistance by itself. Because disease can only be cured by two ways. Either to give the plant, resistance from outside or let the plant too healthy that it can develop its own resistance.

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