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IN-VITRO EVALUATION OF PLANT EXTRACTS AND FUNGICIDES AGAINST MYCELIAL GROWTH OF *SCLEROTIUM ROLFSII* CAUSING FOOT AND ROOT ROT OF BETELVINE

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Abstract

Betelvine (*Piper betle* L.) is an important cash crop of Bangladesh. Foot and root rot caused by *Sclerotium rolfsii* is the most important disease of the crop. It decreases the production of betel leaf to a great extent. Management of the disease, the experiment was conducted to determine the effect of botanical extracts, chemical fungicides and bio-agents on *in-vitro* mycelium growth of *S. rolfsii*. Ten fungicides, 11 plant extracts and 2 bio-agents were evaluated. Among the fungicides, 100% inhibition of mycelium growth of *S. rolfsii* was achieved with the fungicide Provax 200. It was proved to be the best in inhibiting the radial mycelial growth of *S. rolfsii*. The highest growth inhibition of *S. rolfsii* was obtained with Garlic clove extract (96.67%) followed by Allamonda (51.12%), and bio-fungicide, *Trichoderma harzianum* reduced the colony diameter by 56.39% over untreated control.

Keywords: Betelvine, bio-fungicides, foot and root rot, plant extracts, *in-vitro* growth inhibition

Introduction

Betelvine (*Piper betle* L.) in Bengali called 'Pan' under the family *Piperaceae* is a perennial climber cultivated largely for its shiny, green heart-shaped leaves. It is an important cash crop of Bangladesh and used as a masticatory. Total cultivated area under the crop in Bangladesh in 2016-17 was about 23,803.20 hectares and the total annual production was about 2,14,252 metric tons. The average yield per hectare is 9.0 metric tons (BBS, 2018). Betelvine is usually plucked throughout the year but maximum production obtained in the months of July to October. At present, betelvine has a worldwide market. But in competition with India and other betelvine producing countries, Bangladesh has a very small share of the world betelvine market for low production of quality betelvine due to various diseases and insect pests. Foot and root rot caused by *S. rolfsii* is the most important disease of betelvine. It decreases the production of betelvine to a great extent (Islam, 2005). To increase its production management of the diseases is necessary.

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Sclerotium rolfsii Sacc is a serious soil borne fungus and harmful to many valuable crops in most of the tropical and subtropical regions of the world (Aycock, 1966).

Botanical extracts and bio-control agents are biodegradable and free from environmental contamination and health hazards (Grange and Ahmed, 1988). Many species of fungi and bacteria are reported to be effective bio-control agents against soil borne plant pathogens (Papavizas, 1985; Mukhopadhyay, 1994).

Hence, efforts have to be made to retain pathogen activity below economic threshold level by choosing methods alternative to chemicals only. So, the present experiment was undertaken to evaluate the effect of some chemical fungicides, bio-agents and botanical extracts on *in-vitro* growth of *S. rolfsii* the causal fungus of foot and root rot disease of betelvine.

Materials and Methods

The experiment was conducted in the Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka during May 2016 to June 2017.

Evaluation of botanical extract

A total of 11 plant species were collected from different areas of Bangladesh (Table 1). Their inhibitory effect on *in-vitro* growth of *S. rolfsii* was evaluated following Poison food technique using potato dextrose agar (PDA) as basic medium (Islam, 2005). Water extracts of suitable parts of botanicals were prepared according to Islam (2005). The plant parts were collected, washed with tap water, cut into small pieces, weighed on an electric balance and again washed with sterilized water. After soaking with blotting paper, weighed plant parts were blended in an electric blender adding equal amount of sterile water for preparing 1:1 botanical extract (100 ml water for 100 g plant parts). The blend was filtered through sterile cheesecloth. For getting 1:2 suspensions, another 100 ml sterilized water was added to the filtrate. PDA medium was prepared and poured into conical flasks and autoclaved. The requisite quantity of botanical extracts was added to the medium before solidification at 2 ml per 20 ml PDA and mixed thoroughly by stirring, and poured into sterilized Petriplate at 20 ml per plate.

Mycelial discs of 5 mm diameter were cut from 5 days old PDA culture of the pathogen with a sterile cork borer. The discs were transfered aseptically in the centre of the Petriplate containing amended PDA medium. The Petriplate of PDA without botanical extract was maintained for control. The inoculated Petriplates were incubated at 25±2^{o}C in an incubator. Control plates received non ammended PDA. Each treatment was replicated 4 times. The radial growth of mycelium of the causal pathogen was recorded at 24 hr interval until the colony in control plates reached the rim of Petriplates (Islam *et al.*, 2001).

Evaluation of chemical fungicides

Ten fungicides were evaluated *in-vitro* against *S. rolfsii* following Poison food technique in grove (Islam *et al*., 2001). Fungicide suspensions were prepared in requisite quantity of water. The suspensions were poured into 250 ml Erlenmeyer flasks. Flasks were labeled appropriately and shaken thoroughly before use.

Petriplates were prepared with 20 ml PDA medium. After solidification, 5 mm discs of the medium were scooped from PDA plate in three places maintaining an equal distance from the centre using a sterilized disc cutter. One milliliter of suspension (Table 2) was put into each hole and the plates were stored overnight in refrigerator for diffusion of fungicide into the medium around the hole. The next day, one 5 mm blocks of 5 days old PDA culture of *S. rolfsii* was placed at the centre of the plate. The plates were placed in an incubation chamber at 25±2^{o}C. The radial mycelial growth of *S. rolfsii* was recorded at 24 hr interval until the colony reached the rim of Petriplate in control plates (Islam *et al*., 2001; Islam, 2005).

***In-vitro* evaluation of bio-agents**

Two bio-agents, *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated against mycelium growth of *S. rolfsii* following Growth inhibition techniques in Dual culture method (Islam, 2005). The bio-agents were collected from Plant Pathology Division, Bangladesh Agriculture Research Institute (BARI), Gazipur. The fungal antagonists *Trichoderma harzianum* was cultured on PDA and the bacteria *Pseudomonas fluorescens* was cultured on Nutrient Agar (NA) medium.

PDA media was prepared and sterilized in an autoclave at 121^{o}Cunder 1.1 kg/cm^{2} for 15 minutes. The medium was poured into sterilized Petriplates (90 mm) at 20 ml per dish. Five millimeter diameter blocks of 5 days old PDA culture of both bio-agents and pathogen were cut separately with the help of sterilized cork bores (5 mm). The culture discs of pathogen and bio-agent were transferred aseptically and placed at periphery of Petriplate at opposite to each other. The inoculated Petriplates were incubated into an incubator at 25±2^{o}C. The inoculated plate with pathogen culture without antagonists was maintained for control. The growth of the pathogen was observed periodically and measured the colony diameter in each Petriplate.

Measurement of radial mycelia growth and computation percent inhibition

The radial growth of mycelium in each plate was measured by taking average of the two diameters taken at right angles for each colony. The linear growth of mycelium of the causal pathogen was recorded at 24 hr interval until the colony reached the rim of control plates (Islam *et al*., 2001; Nene and Thaplial, 1979).

Inhibition percentage of radial growth was computed based on colony diameter at 4 DAI in control plate using the following formula:

% growth inhibition of pathogen over control, $I = \frac{C - T}{C} \times 100$ (Vincent, 1947)

Where C = Colony diameter in control (T_{10}) and T= Colony diameter in treatment ($T_{n,n=1-9}$)

Table 1. List of plant species tested in bioassay against *Sclerotium rolfsii*

Local name	English name	Scientific name	Plant parts
Neem	Margosa	*Azadiracta indica*	Leaf
Biskatali	Knotweed	*Polygonum hydropiper*	Leaf
Allamanda	Allamanda	*Allamanda cathartica*	Leaf
Lemon grass	Lemon grass	*Cymbopogon citratus*	Leaf
Korobi	Yellow oleander	*Cascabela thevetia*	Leaf
Tamak	Tobacco	*Nicotiana tabacum*	Leaf
Durba	Burmuda grass	*Cynodon dactylon*	Leaf
Roshun	Garlic	*Allium sativum*	Clove
Ada	Ginger	*Gingiber officinales*	Rhizome
Peaz	Onion	*Allium cepa*	Bulb
Mahogany	Mahogany	*Swietenia mahagoni*	Seed

Table 2. List of fungicides tested in the bioassay *in vitro* against *Sclerotium rolfsii*

Trade name	Chemical name	Active ingredient	Conc. used
Tilt 250 EC	1-[2- (2,4-Dicholorophenyl4-propyle- 1 ,3-diooxalane-2 EI-Methyl]-IH, 1,2,4-Triyazole	25% Propiconazole	0.1 %
Score 250 EC	Difenconazol	25% Difenconazole	0.05 %
Rovral 50 WP	3-(3,5 dichlorophenyl)-N-(1methyl ethyl)-2,4 dioxuimidazolidene Carboxamide $(C_{13}H_{13})_3N_3C_{12}$	50 % Iprodione	0.2 %
Bavistin 50 WP	Mythyl-2-Benzimidazole Carbamate	50 % Carbendazim	0.2 %
Provax 200	$C_{12}H_{13}NO_2S + C_6H_{12}N_2S_4$	Carboxin 17.5%+ Thiram 17.5%	0.25%
Topgan	Copper-oxychloride	50% Copper-oxychloride	0.2 %
Ridomil Gold MZ-68 WP	N- (2,6 dimethyl phenyl)-N-(methoxyacetyl)-alanine methyl ester (C14H2N04)	Metalaxil- 4% Mancozeb- 64%	0.5 %
Pencozeb 80 WP	Mancozeb	80 % Mancozeb	0.45 %
Cuprofix 30 D	Copper + Mancozeb	30% Mancozeb+ 12% Copper	0.45%
Bordeaux mixture	Copper sulphate + Calcium Hydro-oxide + Water	$CuSO_4$ - 5 lbs $Ca(OH)_2$ - 5 lbs H_2O - 50 gallons	100.00%

Experimental design and analysis of data:

Completely Randomized Design (CRD) was followed for the experiments. The data were statistically analyzed by using computer package program (Statistix 10). The significant differences of the treatment means were compared by Duncan's Multiple Range Test (DMRT).

Results and discussion

Effect of botanicals on *in-vitro* mycelial growth of *Sclerotium rolfsii*

At 1, 2 and 3 days after inoculation, *in-vitro* radial mycelium diameter was 22.75, 49.75 and 74.25 mm, respectively under control. The growth ranged 0.00-21.50 mm at 1 DAI, 0.00-47.50 mm at 2 DAI and 0.00-66.75 mm at 3 DAI, due to amendment of PDA with different botanicals. During first three days after inoculation, the fungus failed to grow on PDA amendment with Garlic clove extract. The lowest colony diameter was achieved with Allamanda leaf extract and the highest under tobacco leaf extract. At 4 DAI, the *in-vitro* colony diameter under different treatments including control ranged 3.00-89.50 mm (Table 3). The maximum growth was observed under control and the minimum under Garlic clove extract. The highest growth inhibition was obtained with Garlic clove extract followed by Allamanda and Bishkatali leaf extract. Garlic clove extract and Allamanda leaf extract were noted as most effective botanicals to inhibit colony growth of *S. rolfsii*. Colony growth increased gradually with the progress of incubation period under every botanicals and control (Plate 3).

The findings of the present investigation are in agreement with many other investigators. Masuduzzaman *et al*. (2008) found that at higher concentrations of 1:1 and 1:2 completely inhibited *in-vitro* growth of *S. rolfsii* whereas at lower concentrations of 1:3 and 1:4 its growth was suspended to some extent.

Sahana *et al*. (2017) conducted an experiment to evaluate the efficacy of leaf extracts of Neem, Eucalyoptus, Jathropa, Tulsi and Marigold, extracts of Garlic clove and Onion bulb at 0, 5, 10 and 15% concentrations to inhibit *in-vitro* colony growth of *S. rolfsii*. Among the botanicals tested in the present investigation Garlic clove extract showing 97.77, 98.88 and 100% inhibition at 5, 10 and 15% concentrations, respectively. The least inhibition of mycelium diameter was 22.55, 24.44 and 44.07% at 5, 10 and 15% concentrations, respectively in Jathropa leaf extract.

Effect of fungicides on *in-vitro* colony growth of *S. rolfsii*

At 1, 2, 3 and 4 DAI, the colony diameter under control was 24.25, 54.75, 81.25 and 89.25 mm, respectively. The fungus failed to grow when PDA was amended with Provax-200. Other nine fungicides reduced the colony growth of the pathogen within the ranges of 10.25-21.25, 12.75-49.25, 17.50-78.75 and 9.50-87.75 mm at 1, 2, 3 and 4 DAI, respectively (Table 4). At 4 DAI, Provax-200, Score 250 EC, Tilt 250 EC, Pencozeb 80 WP and Rovral 50 WP gave 100.00,

78.15, 75.64, 59.10 and 44.53% growth inhibition, respectively. These five fungicides were noted as highly effective (Plate 4).

Similar findings were also reported by many other investigators. Suryawanshi *et al*. (2015) conducted an *in-vitro* experiment and found that Vitavax, Tebuconazole and Penconazole gave 100.00, 99.25 and 99.03% mycelial growth inhibition, respectively of *S. rolfsii*.

In vitro evaluation of bio-agents against *S. rolfsii*

The *in-vitro* radial colony diameter was 23.75, 62.00, 81.00 and 90.00 mm at 1, 2, 3 and 4 DAI, respectively under control. The colony diameter was reduced to 12.50, 20.75, 31.00 and 39.25 mm due to use of *Trichoderma harzianum* and 19.00, 32.25, 45.25 and 60.50 due to *P. fluorescens* at 1, 2, 3 and 4 DAI, respectively (Table 5).

The findings of the present investigation are in agreement with many other investigators. Almeida and Landim (1981) reported that an isolate of *Trichoderma* sp. was hyper parasite of *S. rolfsii* on PDA culture and found to be most effective in controlling *S. rolfsii* on cowpea in green house. Parvin *et al*. (2016) conducted an experiment *in-vitro* to observe inhibition of mycelial growth of *S. rolfsii* in dual culture method. The performance of *T. harzianum* in reduction of radial mycelial growth was the best followed by *P. fluorescens*.

Table 3. Efficacy of plant extracts in inhibition of *in-vitro* mycelial growth of *Sclerotium rolfsii*

Treatments	Radial colony diameter (mm) at different days after inoculation (DAI)			
	1	2	3	4
Neem leaf extract	16.50 d[α](4.12)*	36.25 c(6.06)	53.00 de(7.31)	66.50 d(8.19)
Biskatali leaf extract	16.25 d(4.09)	28.25 e(5.36)	36.50 g(6.08)	50.00 e(7.11)
Allamanda leaf extract	14.75 e(3.90)	26.00 e(5.15)	31.75 h(5.68)	43.75 e(6.65)
Garlic clove extract	0.00 f(0.71)	0.00 f(0.71)	0.00 I (0.71)	3.00 f(1.41)
Zinger rhizome extract	16.75 d(4.15)	27.25 e(5.27)	39.50 f(6.32)	50.75 e(7.16)
Lemon grass leaf extract	21.50 b(4.69)	37.25 c(6.14)	52.00 de(7.25)	74.25 bcd(8.65)
Onion bulb extract	16.25 d(4.09)	35.50 c(6.00)	54.50 d(7.42)	70.50 cd(8.43)
Korobi leaf extract	16.25 d(4.09)	31.00 d(5.60)	51.00 e(7.18)	72.25 bcd(8.53)
Tobacco leaf extract	20.50 b(4.58)	43.50 b(6.63)	66.75 b(8.20)	81.25 ab(9.04)
Bermuda grass leaf extract	21.25 b(4.66)	47.50 a(6.93)	62.75 c(7.95)	82.50 ab(9.11)
Mehagony seed extract	18.75 c(4.38)	31.25 d(5.63)	54.50 d(7.41)	79.50 abc(8.94)
Control	22.75 a(4.82)	49.75 a(7.09)	74.25 a(8.64)	89.50 a(9.49)

[α]Values within the same column with a common letter(s) do not differed significantly (P=0.01).

*Data within parenthesis are square root or arc-sine transformed values

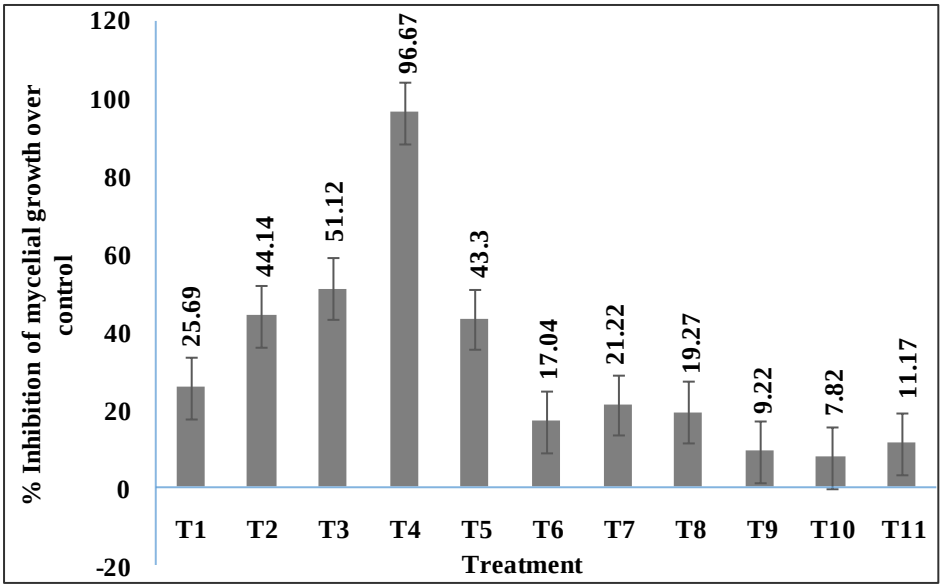

Fig. 1. Percent inhibition of mycelial growth of *S. rolfsii* over control at 4 days after inoculation [T_1= Neem leaf extract, T_2= Biskatali leaf extract, T_3 = Allamanda leaf extract, T_4= Garlic clove extract, T_5= Zinger rhizome extract, T_6= Lemon grass leaf extract, T_7= Onion bulb extract, T_8= Korobi leaf extract, T_9= Tobacco leaf extract, T_{10}= Bermuda grass leaf extract and T_{11}= Mehagony seed extract]

Table 4. Efficacy of fungicides to inhibit *in-vitro* mycelial growth of *Sclerotium rolfsii*on PDA Growth Inhibition Technique (Cup method)

Fungicides	Radial colony growth (mm) of *S. rolfsii* at different days after inoculation (DAI)				% Inhibition of mycelial growth over control at 4 DAI
	1	2	3	4	
Tilt-250 EC	10.50 ef$^{\alpha}$ (3.31)*	14.75 g (3.90)	18.25 h (4.32)	21.75 f (4.72)	75.64 b (58.29)
Score 250 EC	10.25 f (3.28)	12.75 h (3.64)	17.50 h (4.24)	19.50 g (4.47)	78.15 b (59.95)
Rovral 50 WP	11.00 ef (3.39)	22.50 e (4.79)	36.00 f (6.04)	49.50 d (7.07)	44.53 d (40.38)
Bavistin 50 WP	19.50 c (4.47)	45.50 c (6.78)	57.00 e (7.58)	65.00 c (8.09)	27.16 e (30.29)
Provax 200	0.000 g (0.71)	0.00 i (0.71)	0.000 i (0.71)	0.00 h 0(.71)	100.00 a (86.82)

Fungicides	Radial colony growth (mm) of *S. rolfsii* at different days after inoculation (DAI)				% Inhibition of mycelial growth over control at 4 DAI
	1	2	3	4	
Topgan	19.00 c (4.42)	40.00 d (6.36)	63.00 d (7.97)	79.00 b (8.92)	11.48 f (19.08)
Ridomil Gold MZ-68 WP	21.25 b (4.66)	48.25 b (6.9)	72.25 c (8.53)	87.75 a (9.40)	1.67 g (6.48)
Pencozeb 80 WP	11.50 e (3.46)	16.75 f (4.15)	26.25 g (5.17)	36.50 e (6.08)	59.10 c (48.47)
Cuprafix 30 D	16.25 d (4.09)	40.75 d (6.42)	63.00 d (7.97)	79.25 b (8.93)	11.19 f (18.80)
Bordeaux mixture	17.50 d (4.24)	49.25 b (7.05)	78.75 b (8.90)	87.75 a (9.39)	1.69 g (5.84)
Control	24.75 a (5.02)	54.75 a (7.43)	81.25 a (9.04)	89.25 a (9.47)	0.00 h (0.062)

[α]Values within the same column with a common letter(s) do not differed significantly (P=0.01).

*Data within parenthesis are transformed (square root and arc-sine for percent inhibition) values

Table 5. Efficacy of bio-agents to inhibit *in-vitro* radial mycelial growth of *Sclerotium rolfsii* in dual culture method

Treatments	Radial mycelial growth (mm) at different days after inoculation (DAI)				% Inhibition of mycelial growth over control at 4 DAI
	1	2	3	4	
Trichoderma harzianum	12.50 c[α]	20.75 c	31.00 c	39.25 c	56.39 a(46.96)*
Pseudomonas fluorescens	19.00 b	32.25 b	45.25 b	60.50 b	32.78 b(33.69)
Control	23.75 a	62.00 a	81.00 a	90.00 a	00.00 c(0.062)

[α]Values within the same column with a common letter(s) do not differed significantly (P=0.01).

*Data given in parenthesis are transformed values

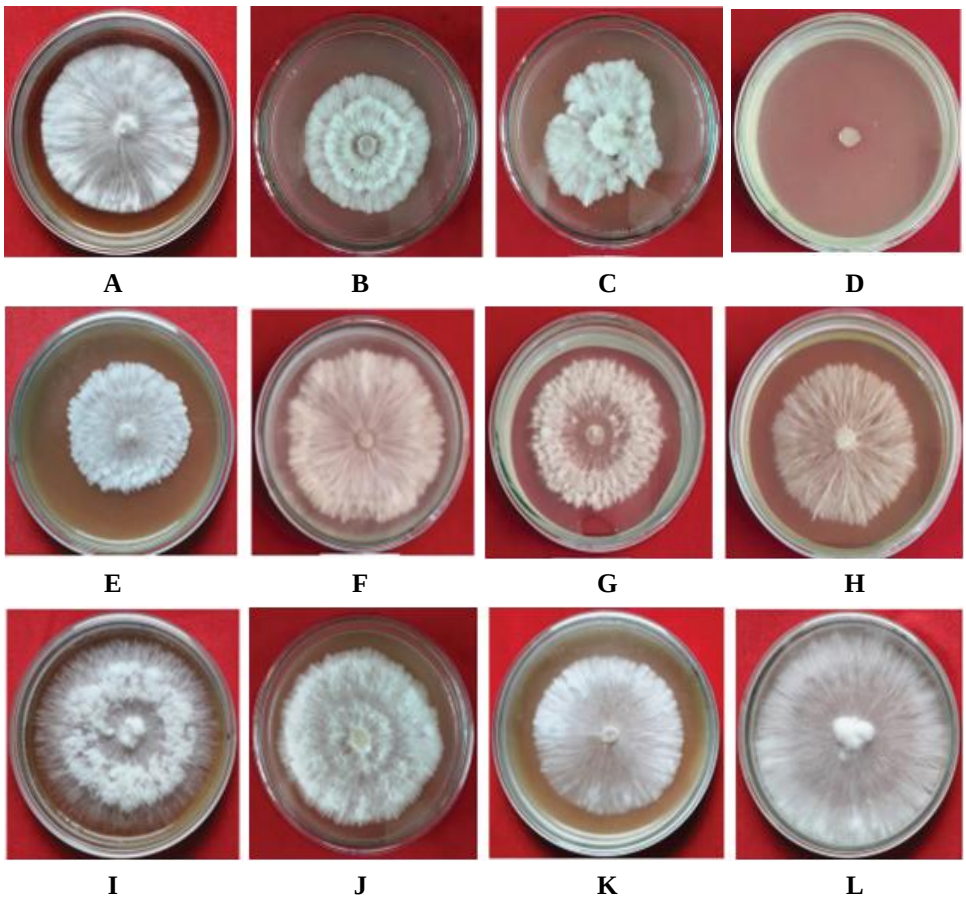

Fig. 1. Pictorial view of radial mycelial growth of *Sclerotium rolfsii* in Petriplates containing PDA amended with botanicals [(A) Neem leaf extract, (B) Biskatali leaf extract, (C) Allamanda leaf extract, (D) Garlic clove extract, (E) Zinger rhizome extract, (F) Lemon grass leaf extract, (G) Onion bulb extract, (H) Korobi leaf extract, (I) Tobacco leaf extract, (J) Bermuda grass leaf extract, (K) Mehagony seed extract and (L) Control at 4 DAI of pathogen]

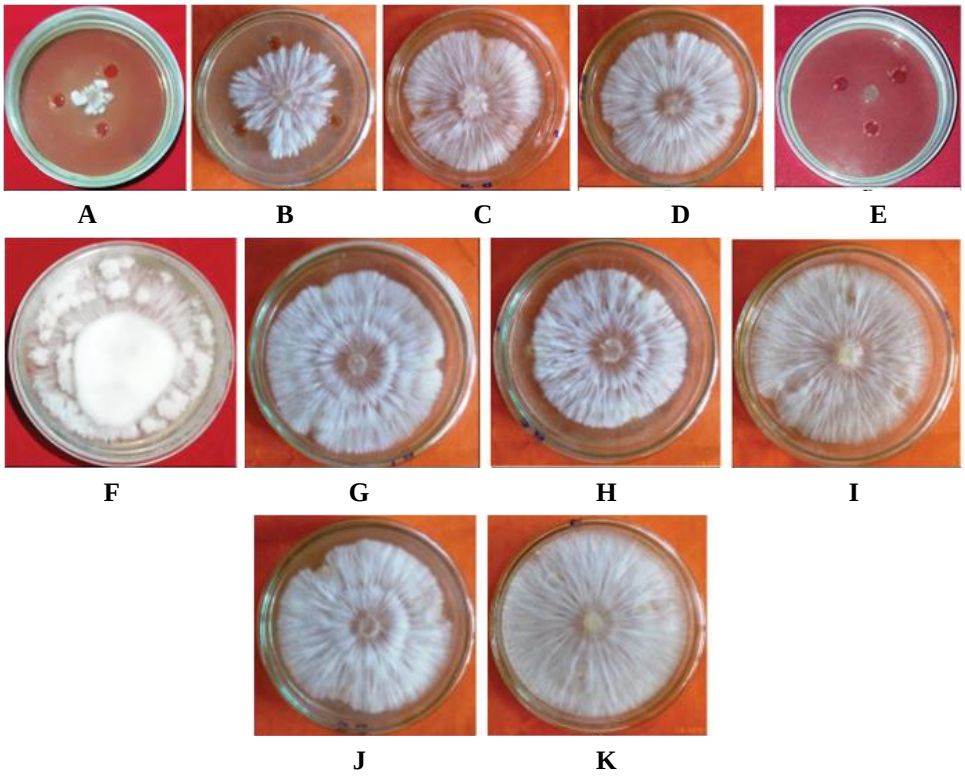

Fig. 2. Pictorial view of radial mycelial mycelial growth of *Sclerotium rolfsii* against (A) Tilt-250 EC, (B) Score 250 EC, (C) Rovral 50 WP, (D) Bavistin 50 WP, (E) Provax 200, (F) Topgan,(G) Ridomil Gold MZ- 68 WP, (H) Pencozeb 80 WP, (I) Cuprafix 30 D, (J) Bordeaux mixture and (K) Control at 4 days after inoculation

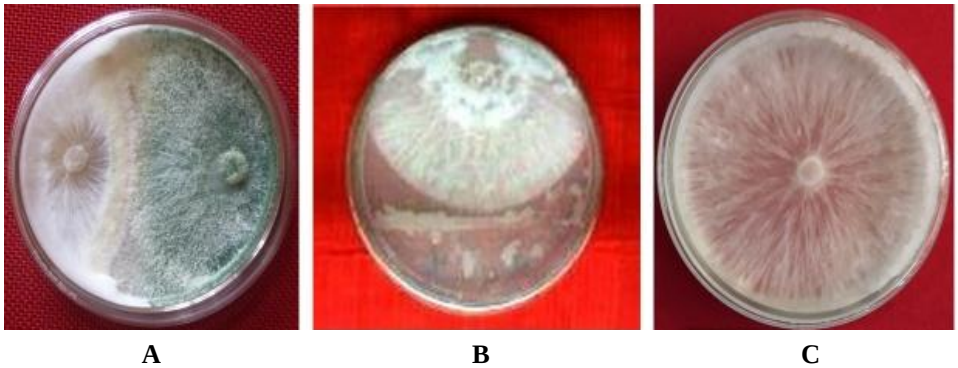

Fig. 3. Pictorial view of radial mycelial growth of *S. rolfsii* against (A) *Trichoderma harzianum*, (B) *Pseudomonas fluorescens* and (C) Control at 4 days after inoculation of pathogen.

Conclusion

Based on findings of three *in-vitro* tests, two botanicals viz., Garlic clove extract and Allamanda leaves extract, five fungicides, *viz*., Provax 200, Tilt 250 EC, Score 250 EC, Rovral 50 WP and Pencozeb 80 WP and a bio-agent *T. harzianum* are most effective against *in-vitro* growth of *S. rolfsii*. The materials may be tested under pot and field condition to find out the efficacy to control betelvine foot and root rot disease.

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