STUDIES ON SOILBORNE DISEASES OF STEVIA AND THEIR MANAGEMENT WITH SPECIAL REFERENCE TO SCLEROTIUM WILT

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INTRODUCTION

Stevia is a genus of about 240 species of herbs and shrubs in the family Asteraceae, native to subtropical and tropical South America and Central America, but the history of culture of stevia mainly stems from Paraguay and Brazil. *Stevia rebaudiana* Bertoni is a small, short day and perennial plant. In wild state, it grows up to 65-80 cm, but when cultivated it grows to a height of one m with sessile, oppositely arranged leaves contain several potential sweetening compounds. Its leaves are about five cm long and two cm wide.

Stevia rebaudiana is commonly known as "Sweetest plant of the world", "sweet leaf", "honey leaf" and "sugar leaf" in English, whereas in Sanskrit it is referred as "Madhupatra". It is widely grown for its sweet leaves, as a sweetener and sugar substitute. Diterpene glycoside is the group of natural sweeteners that have been extracted from stevia. The leaves contain 9.1 per cent stevioside, 3.8 per cent rebaudioside A, 0.6 per cent rebaudioside C and 0.3 per cent dulcoside and they are 20 times sweeter than cane sugar and pure extract is 300 times sweeter than sugar. Stevia has garnered attention with the rise in demand for low carbohydrate and low sugar food alternatives. Medical research has also shown possible benefits of stevia in treating obesity and high blood pressure, because stevia has a negligible effect on blood glucose. It is attractive as a sweetener to people on carbohydrate controlled diets.

The documented properties of stevia are antibacterial, antifungal, anti-inflammatory, antimicrobial, antiviral, anti-yeast, cardio tonic, diuretic, hypoglycaemic and hence a boon to diabetic people. Stevia is widely used as a sweetener in Japan and it is now available in Canada as a dietary supplement. Reports of useful effects in human body (Konoshima and Takasaki, 2002, Jayaraman *et al*., 2008) favour its commercialization in several countries including Latin America, Canada, China, Japan, Indonesia and USA (Andolfi *et al*., 2006).

Possible treatment of osteoporosis has been suggested by the patent application claim that egg shell breakage can be reduced by 75 per cent by adding a small quantity of stevia leaf powder to chicken feed. In addition, it has suggested that pigs fed with stevia extract had twice calcium content in their meat.

Rebiana is a trade name of zero-calorie sweetener containing mainly the steviol glycoside rebaudioside A (Reb-A) which is extracted from stevia. Truvia is the consumer brand for a sweetener made of erythritol, rebiana and natural flavour marketed by Cargill and developed jointly with the Coca-Cola Company. In December 2008, the US food and drug administration permitted Reb-A based sweeteners as food additives. Pure via is the Pepsico and Merisant brand of sweetener made of erythritol, isomaltulose, Reb-A, cellulose powder and natural flavours (Brandle, 1998).

Stevia can be grown on relatively poor soil. The plants can be used for commercial production for six years, during which five times a year harvest of above ground parts take place. The roots remain in place, so that plant regenerate again. Plants, which are one meter height, have a dry weight of 70g on an average. The dry weight of the leaves can vary from 15-35g/plant.

Stevia suffers cold, usually does not tolerate temperatures below 9℃ but occasionally can tolerate temperatures near to zero, and 20-24℃ are necessary for a rapid growth (Singh and Rao, 2005). On the other side, Stevia has remarkable water needs, leaves and stems can wilt rapidly and also recover rapidly if the stress is not prolonged, this is a limitation to the area suitable for its cultivation. It grows fast and can be grown as an annual herb during late spring and summer. Consequently, Stevia could become an interesting and profitable new crop for the tropics, for warm areas including temperate areas with hot and rainy summer (as an annual summer crop). Productivity ranges according to climate parameters, soil fertility and management. Researchers have found a range from 4.4 t ha^{-1} leaves dry matter in Brazil (Fronza and Folegatti, 2003), 13.3 t ha^{-1}at the first year (Ruta *et al*., 1999). In Italy it ranged from 3.1 t ha^{-1} at the first year to about 6.3 t ha^{-1}at the 5^{th} year (Andolfi *et al*., 2006; Tedone *et al*., 2009) and it was 16.76 t ha^{-1} in annual cash crop irrigated in Central Italy.

In the early 1970's, Japan began cultivating stevia as an alternative to artificial sweeteners such as cyclamate and saccharin, which were suspected to be carcinogens. Steviol glycosides were first commercialised as a sweetener in 1971 by the Japanese firm Morita Kagaku Kogyo Co. Ltd. a leading stevia extract producer in Japan. Stevia has been grown on experimental basis in Ontario, Canada since 1987 for the purpose of determining the feasibility of growing the crop commercially. In the US, Rebiana is generally recognised as a dietary supplement.

Stevia has also been approved as a dietary supplement in Australia, New Zealand and Canada. Since June, 2008 it is approved as a sweetener for food and beverages in Australia and New Zealand. In Japan and South American countries, stevia is also used as a food additive.

In India too, stevia is being successfully cultivated in the recent years at many areas of Rajasthan, Kerala, Maharashtra and Karnataka. Demands for natural sweeteners have driven the farmers in India for large scale cultivation of stevia. As several other plants, stevia is also attacked by several fungal and bacterial diseases. Among fungal diseases, wilt complex caused by *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia solani* is a major threat, which has posed a serious problem in establishing the crop on a commercial basis..

Although, the soil borne diseases causing wilt of stevia have assumed economic importance in India and Karnataka, not much information pertaining to symptomatology, etiology, variability, epidemiology and integrated management strategies of the disease is available. Keeping these points in view, objectives were set as follows:

1. Survey for incidence of soil borne fungal diseases of Stevia in Karnataka.
2. Cultural, nutritional and physiological studies on soil borne fungal pathogens
3. Studies on cultural, mophological and molecular variability among isolates of *Sclerotium rolfsii*.
4. Estimation of losses due to wilt caused by *Sclerotium rolfsii* and biochemical changes due to soil borne fungal pathogens.
5. Studies on epidemiology of *Sclerotium rolfsii*
6. Integrated management of Sclerotium wilt of stevia by using bioagents, organic amendments and chemicals.

MATERIAL AND METHODS

The present investigation was carried out from 2007-2012 in the Department of Plant Pathology and the field experiments were conducted at the Medicinal and Aromatic crops, Saidapur Farm, Department of Horticulture, University of Agricultural Sciences, Dharwad, Karnataka. The materials used and methods followed in conducting the experiments are described in this chapter.

General Laboratory Procedures

Glassware cleaning

For all the laboratory experimental studies, Borosil and Corning glassware were used. Before using, the glassware were kept for 24 h in the cleaning solution containing 60 g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water and then washed with detergent solution followed by tap water and finally rinsed with distilled water.

Sterilization

All the glassware were sterilized in an autoclave at 1.1 kg/cm^2 pressure for 20 minutes. All the media were sterilized for 15 minutes at 1.1 kg/cm^2 pressure and soil used for experiment was sterilized at 1.33 kg/cm^2 pressure for two hours.

Surface sterilization of plant parts

Plant materials were surface sterilized using 0.1 per cent mercuric chloride solution for 20-30 seconds and then washing in sterile water thrice.

3.1 Survey for incidence of diseases of stevia in Karnataka

An intensive survey was conducted to monitor the incidence of diseases of stevia in stevia growing districts like Belgaum, Gadag, Dharwad, Bangalore, Mysore, Mandya, Koppal, Shimoga and North Kannada districts during 2008-09. Survey was conducted in Gangenahalli, Hirethimmanahalli, Agoli, Nidsoshi and Gangavati (Koppal district). Thirtahalli and Ripponpete (Shimoga district), Kappatagudda (Gadag district), Kalenahalli (Mandya district), Sirsi (North Kannada) Saidapur (Dharwad district) and Herbal garden of Gandhi Krishi Vigyna Kendra (G.K.V.K.), University of Agricultural Sciences (UAS), Bangalore.

Samples of soils and roots were collected from infected fields from the rhizosphere of stevia crop. In the similar manner totally about 10 spots were selected randomly for taking soil and root samples representing the whole field. Later from this, a composite sample of 200g of soil and 5g of root were formed. Hundred plants were selected in each field and number of plants wilted was counted and the per cent wilt incidence was calculated. Wilted plants were also collected for isolation and other studies. Per cent disease incidence (PDI) was calculated by using the following formula.

$$PDI = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Each sample was collected in polythene bag separately and labelled immediately. The roots were analyzed on the day of collection or after keeping for a few days under refrigerated conditions. Root samples were used for detection of the fungi associated with wilted plants.

3.1.1 Isolation of pathogens

Isolation of fungal pathogens

Stevia plant showing the typical wilt symptoms were collected. The infected portion of plant parts were taken for isolation. The isolation of fungus was made by following standard tissue isolation procedure.

The infected specimens were cut into small bits and washed in running water. These bits were surface sterilised in 1% sodium hypochlorite solution for one minute. Afterwards, these bits were washed thoroughly in sterilized distilled water three times to remove traces of sodium hypochlorite solution if any and then aseptically transferred to sterile Petri plates containing potato dextrose agar. The plates were incubated at room temperature (27±1^{0}C) for three days for *Sclerotium rolfsii* and seven days to obtain good growth of *F. solani* and *R. bataticola.*

3.1.2 Identification of the pathogens

The pathogens were identified upto species level based on their morphological and cultural characters.

3.1.3 Purification of fungal pathogenic cultures

a) Single spore isolation

This method was followed for maintaining pure culture of *Fusarium solani*. Ten ml of two per cent clear filtered water agar was poured into sterile Petri plate and allowed to solidify. Dilute suspensions of spores (8-10 spores/ml) were prepared in sterile distilled water of which one ml was spread uniformly on water agar plates and the suspension was drained off aseptically from the plates. Such plates were incubated at 27±1^{0}C for few hours. They were examined under the microscope, well isolated spores of *Fusarium solani* were marked with ink on the glass surface of the plate. These marked agar areas were cut and transferred aseptically to potato dextrose agar slants and incubated at 27±1^{0}C. Such culture was used for further studies.

b) Hyphal tip isolation

This method was done for maintaining pure cultures of *Sclerotium rolfsii* and *Rhizoctonia bataticola*. Hyphal tip isolation was done on water agar plates. Dilute mycelial suspension was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Single mycelial bit was then marked under the microscopic field with ink on the glass surface of the plate and it was allowed to grow. Such plates were incubated at 27±1^{0}C and hyphae coming from each end cell of the mycelial bit was traced and marked with the ink. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 27±1^{0}C for 10 days. Later, mycelial bits of the fungus were placed in the center of Petri plates containing potato dextrose agar medium and incubated at 27±1°C for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. Such culture was used for further studies.

3.1.4 Maintenance of fungal pathogenic cultures

The fungus was sub-cultured on potato dextrose agar slants and allowed to grow at 27±1^{0}C for one week. Such slants were preserved in a refrigerator at 5^{0}C and renewed once in two months.

3.1.5 Mass multiplication

By using sand-corn meal

Sand corn meal medium was prepared in the proportion of 90:10 in order to get maximum inoculum of fungus. Four hundred gram of sand corn meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.01 kg/sq cm pressure for one hour. The pure cultures of *Sclerotium rolfsii, Fusarium solani and Rhizoctonia spp.* were inoculated separately to different flasks under aseptic condition and incubated at 27±1^{0}C for 20 days. The flasks were shaken on alternate days to get uniform growth. The giant cultures thus obtained were used for further studies.

3.1.6 Proving the pathogenicity of wilt causing pathogens

The rooted stevia cuttings were grown in polythene bags filled with sterile soil. 20-30 days old rooted cuttings were used for inoculation with one month old giant culture. Control treatment was maintained in which no inoculum was added. Treatments are as follows.

1. *Sclerotium rolfsii*
2. *Fusarium solani*
3. *Rhizoctonia bataticola*
4. Control

Giant cultures of *S. rolfsii, Fusarium solani and Rhizoctonia* sp. were inoculated to separate sets of pots. The pots were maintained at 25 per cent moisture holding capacity for *F.solani*, 30 per cent for *R. bataticola* and 30 to 35 percent moisture holding capacity for *S.rolfsii*. Observations were made every day on the development of wilt symptoms. When the plants showed wilt symptoms, such

plants were carefully up rooted and the pathogens were reisolated by standard tissue isolation method. The pathogens reisolated were compared with original cultures.

3.2 Cultural, nutritional and physiological studies of fungal pathogens

3.2.1 Growth characters on different solid media

The growth characters of *Sclerotium rolfsii, Fusarium solani and Rhizoctonia* spp. were studied on different solid media, *viz.,* Czapek's Dox agar, Richards's agar, Sabouraud's agar, carrot agar, corn meal agar, host leaf extract agar, basal agar, malt extract agar, oat meal agar, potato dextrose agar, V8 juice agar, Tochinai's agar, Yeast extract agar, Brown's medium, Rose Bengal agar and Elliott's agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1^{0}C. Each treatment was replicated thrice. Observations were taken when the fungus covered complete petriplate in any one of the medium. The colony diameter was recorded. The fungal colony colour, margin, sclerotial/microsclerotial production and sporulation were also recorded. The data on radial growth was analyzed statistically.

The composition of each medium used is furnished below.

a) Potato dextrose agar (Tuite, 1969)

1. Peeled and sliced potatoes : 200 g
2. Dextrose ($C_6H_{12}O_6$) : 20 g
3. Agar-agar : 20 g
4. Distilled water (to make up) : 1000 ml

The peeled and sliced potatoes were boiled in 400 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was added to the mixture. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

b) Malt extract agar (Tuite, 1969)

1. Malt extract : 25 g
2. Agar-agar : 20 g
3. Distilled water (to make up) : 1000 ml

Malt extract was dissolved in 400 ml of distilled water. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly and volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

c) Oat meal agar (Tuite, 1969)

1. Oat flakes : 30 g
2. Agar-agar : 20 g
3. Distilled water (to make up) : 1000 ml

Oat flakes were boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

d) Corn meal agar

1. Corn meal : 30 g
2. Agar-agar : 20 g
3. Distilled water (to make up) : 1000 ml

Corn meal was boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

e) Sabouraud's agar

1. Dextrose ($C_6H_{12}O_6$) : 20 g
2. Peptone : 10 g
3. Agar-agar : 20 g
4. Distilled water (to make up) : 1000 ml

Agar-agar was melted in 400 ml of distilled water. All other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. This was sterilized at 1.1 kg/cm^2 pressure for 15 min.

f) Richards's agar (Ainsworth, 1971)

1. Sucrose ($C_{12}H_{22}O_{11}$) : 50 g
2. Potassium dihydrogen phosphate (KH_2PO_4) : 5 g
3. Potassium nitrate (KNO_3) : 10 g
4. Magnesium sulphate ($MgSO_4$. $7H_2O$) : 2.5 g
5. Ferric chloride ($FeCl_3$. $6H_2O$) : 0.02 g
6. Agar-agar : 20 g
7. Distilled water (to make up) : 1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at 1.1 kg/cm^2 pressure for 15 min and subsequently mixed together.

g) Czapek's dox agar (Tuite, 1969)

1. Sucrose ($C_{12}H_{22}O_{11}$) : 30 g
2. Sodium nitrate ($NaNO_3$) : 2 g
3. Potassium dihydrogen phosphate (KH_2PO_4) : 1 g
4. Magnesium sulphate ($MgSO_4$. $7H_2O$) : 0.5g
5. Potassium chloride (KCl) : 0.5g
6. Ferrous sulphate ($FeSO_4$. $7H_2O$) : 0.01g
7. Agar-agar : 20 g
8. Distilled water (to make up) : 1000 ml

Agar-agar was melted in 500 ml of distilled water. All the other ingredients were mixed in 400 ml of distilled water. The two solutions were mixed thoroughly. The volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

h) V8 juice agar

1. V8 juice (Hi-media) : 44.3 g
2. Distilled water (to make up) : 1000 ml

44.3 g of V-8 juice agar powder obtained from high media was suspended in 1000 ml distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

i) Host leaf extract agar

1. stevia leaves : 200g
2. Sucrose ($C_{12}H_{22}O_{11}$) : 20 g
3. Agar agar : 20g
4. Distilled water (to make up) : 1000ml

Stevia leaves were boiled in 400 ml of water for one hour and then the extract was filtered through muslin cloth and mixed with the sucrose. Agar agar was melted in 400 ml of water. Both the solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water.

j) Brown's medium

1. Glucose ($C_6H_{12}O_6$) : 2.0 g
2. L-asparagine : 0 2 g
3. Dipotassium hydrogen phosphate (K_2HPO_4) : 1.25 g
4. Magnesium sulphate ($MgSO_4$. $7H_2O$) : 0.75 g
5. Trace element mixture : 1 ml
6. Agar-agar : 15.0 g
7. Distilled water (to make up) :1000 ml

Agar-agar was melted in 400 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm2 pressure for 15 min.

k) Tochinai's agar

1. Potassium dihydrogen phosphate (KH_2PO_4) : 0.5 g
2. Potassium nitrate (KNO_3) : 2.0 g
3. Magnesium sulphate ($MgSO_4$. $7H_2O$) : 1.0 g
4. Ferric chloride ($FeCl_3$. $6H_2O$) : Trace
5. Sucrose ($C1_2H_2O_{11}$) : 30 g
6. Agar-agar : 15 g
7. Distilled water (to make up) : 1000 ml

Agar-agar was melted in 400 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm^2 pressure for15 min.

3.2.2 Growth phase

The growth phase study was conducted on potato dextrose broth. Thirty ml of broth was added in each of the 100 ml conical flasks and sterilized at 1.1 kg/cm^2 pressure for 15 min. These flasks were allowed to cool and 5 mm discs of *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola* was inoculated to each of the conical flasks. They were incubated at 27 ± 1^0C. Each treatment was replicated thrice. Culture was filtered through Whatman No. 42 filter paper disc of 12.15 cm diameter, which was dried to a constant weight at 60^0C in an electrical oven, prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated. One set of flasks were harvested on second day. Subsequent harvesting was done at regular interval of one day for *Sclerotium rolfsii* upto 14^{th} day and at an interval of two days upto 24^{th} day *for F.solani* and *Rhizoctonia bataticola*. The filter papers along with mycelial mat were dried to a constant weight in an electrical oven at 60^0C, cooled in a dessicator and weighed immediately in an analytical electric balance and dry mycelial weight was calculated. Results were analyzed statistically.

3.2.3 Growth studies in different liquid media

The liquid media used were same as that of solid media except that agar agar was not added to the liquid media. All the liquid media were sterilized at 1.1 kg/cm^2 for 15 minutes. Cultures of *S. rolfsii, F. solani* and *R. bataticola*. inoculated separately and incubated at 27±1^{0}C for the growth. Each treatment was replicated thrice. The mycelial mat was filtered through whatman number 42 filter paper of 12.5 cm diameter. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with the mycelium and dried to a constant weight in an electrical oven at 60^{0}C, cooled in desiccators and weighed immediately on an analytical electric balance. The weight of dry mycelium was recorded and the data were statistically analyzed. The best synthetic medium was found out and used for further studies as basal medium.

3.2.4 Nutritional studies

3.2.4.1 Carbon utilization

The carbon requirements of the fungi *i.e.*, *S. rolfsii, F.solani* and *Rhizoctonia sp.* was studied by replacing sucrose in Richards`s solution with different carbon compounds. The carbon sources used in present study were dextrose, fructose, glucose, mannitol, starch, maltose, sucrose, lactose, citric acid and galactose. The quantity of each carbon compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of carbon as that of sucrose present in the basal medium. Each treatment was replicated thrice. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. Separate sets of flasks were maintained for each pathogen. Cultures of *S. rolfsii, F.solani, Rhizoctonia sp.* were inoculated separately to the respective sets of flasks and incubated at 27±1°C for ten, twelve, fourteen and twelve days respectively. Dry mycelial weight of the fungus was recorded and results were analyzed statistically.

3.2.4.2 Nitrogen utilization

The nitrogen requirements of the fungi was studied by replacing potassium nitrate of Richards`s medium. With different nitrogen sources *viz.*, ammonium chloride, ammonium nitrate, asparagine, potassium nitrate, sodium nitrate, calcium nitrate, peptone, glycine, methionine and ammonium sulphate. The quantity of each nitrogen compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of nitrogen as that of potassium nitrate present in the basal medium. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. The flasks were inoculated and incubated as described earlier. The dry mycelial weight of the fungus was recorded and results were analyzed statistically.

3.2.5 Physiological studies

3.2.5.1 Temperature requirement

Richards's broth was used in this experiment. Conical flasks of 100 ml capacity containing 30 ml of liquid medium were inoculated with 5 mm mycelial disc and incubated at different temperature levels *viz.,* 5, 10, 15, 20, 25, 30, 35 and 40°C. In each case, three replications were maintained. The dry mycelial weight at each temperature level was recorded after incubating as described earlier and the results were analyzed statistically. The same study was conducted on potato dextrose agar medium to know the mycelia growth.

3.2.5.2 Hydrogen ion concentration

Richards's liquid medium was used as a basal medium. pH of the liquid media was adjusted by using 0.1N alkali (NaOH) or 0.1N acid (HCl). The reaction of the medium was adjusted to the desired pH by using di-hydrogen phosphate citric acid buffer. The pH of the medium used were 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0,7.5 and 8.0. After sterilization there was slight change in pH, which was negligible. The culture was inoculated to each of 100 ml flask containing 30 ml of basal medium and incubated as described earlier. Three replications were maintained in each treatment.. The pH of the culture filtrate was determined by using Precision pH meter. Dry mycelial weight was obtained as described earlier and results were analyzed statistically.

3.3 Studies on cultural, morphological and molecular variability among isolates of *Sclerotium rolfsii*

3.3.1 Collection of isolates of *Sclerotium rolfsii*

Wilt affected coleus plants were collected from different parts of the Karnataka constituting 14 localities. Details of location and designation given for each isolate are furnished in Table 1.

Details of isolates of *Sclerotium rolfsii* used for variability study

Sl. No.	Place	District	Designation
1	Nidshoshi	Koppal	SrNID
2	Hosagudda	Koppal	SrHOS
3	UAS., Bengaluru	Bengaluru	SrBEN
4	Thindlu	Bengaluru	SrTHI
5	Kalenahalli	Mandya	SrKAL
6	Saidapur	Dharwad	SrSAI
7	Sirsi	North Canara	SrSIR
8	Thirthalli	Shimoga	SrTHIR
9	Rippenpete	Shimoga	SrRIP
10	Gangavathi	Gangavathi	SrGAN
11	Belgaum	Belgaum	SrBGM
12	Gadag	Gadag	SrGDG
13	Raichur	Raichur	SrRCR
14	Mysore	Mysore	SrMYS

3.3.2 Studies on variability among the isolates of *Sclerotium rolfsii*

The possibility of existence of variability among 14 isolates of *Sclerotium rolfsii* collected from different locations of Karnataka were attempted through a series of experiments.

3.3.3 Cultural and morphological variability among isolates of *Sclerotium rolsii*

About 15 ml of PDA was poured into each of the 90 mm diameter Petri dishes. One ml of streptomycin sulphate solution of 100 ppm strength was added to the medium just before pouring into the plates to avoid bacterial contamination. Five mm diameter circular discs taken from the margin of an actively growing (3-4 days old) colony was punched out and placed onto the center of the plate with the mycelial side facing downwards, under aseptic conditions. The plates were incubated at 28^{o}C for 7 days.

Morphological characteristics such as growth rate, radial colony diameter, colony colour and type, number of sclerotia, shape and colour and location of sclerotia, test weight of 100 sclerotial bodies and days for sclerotium formation for each isolates were noted. The observations were recorded at 12 hours interval up to 3 days and then at 24 hours intervals for 7 days.

3.3.4 Mycelial compatibility/Vegetative compatible groups of isolates of *S. rolfsii*

Fourteen isolates of *S. rolfsii* were subjected to mycelial compatibility reaction on PDA plates in order to identify the morphological similarities/differences among various strains as described by Punja & Sun (2001). Fourteen isolates in combination of three at a time were tested. All combinations *viz.* (1,2,3) (1,4,5) (1,6,7) (1,8,9) (1,10,11) (1,12,13) (2,4,6) (2,5,7) (2,8,10) (2,9,14) (2,13,11) (2,12,1) (3,4,7) (3,5,8) (3,6,9) (3,10,12) (3,11,14) (3,13,4) (4,8,11) (4,9,10) (4,12,14) (5,6,14) (5,10,12) (5,11,9) (5,10,13) (6,11,12) (6,10,14) (6,13,8) (7,8,14) (7,9,12) (7,10,11) (7,13,1) (8,12,14) (9,12,13) were inoculated in dublicate.

The PDA plates were marked into three portions. Five mm diameter mycelial disc of the respective isolate was inoculated on each portion of PDA plate. After inoculation, the plates were incubated at 28°C for 15 to 20 days. The pairings were macroscopically examined daily for the development of clearing zone and sclerotial formation in the region of mycelial contact.

3.4.5 Oxalic acid production by isolates of *S. rolfsii*

All the 14 isolates were tested for oxalic acid production in culture filtrates. For this purpose each isolate was grown separately in PDB. Fifty ml of the media was poured in to each 250 ml conical flask and were sterilised at 1.1 kg/cm^2 pressure for 15 minutes. Each of the flasks was inoculated aseptically with five mm culture disc obtained from the growing periphery of four days old culture of the individual isolate grown on PDA in Petriplate and incubated at 27±1°C for 10 days. The mycelial mat was filtered through Whatman No. 42 filter paper disc of 12.50 cm diameter and the aliquot was centrifuged at 5000 rpm for 10 min to remove the mycelial fragments. Then, 10 ml cell free culture filtrate, 8 ml of calcium chloride – acetate buffer (pH – 4.5) was added and mixed thoroughly. The mixture was allowed to stand overnight and then centrifuged at 5000 rpm for 10 min. Supernatant was discarded and the residue was washed with 10ml of 50 per cent acetic acid saturated with calcium oxalate and centrifuged. The residue was dissolved in 10 ml on 4N H_2SO_4. The solution was transferred to 100 ml flask and heated at 80^0 C on a water bath. While hot, it was titrated with 0.02 N potassium permanganate until a faint pink colour persisted. The amount of oxalic acid present in the culture filtrate was calculated as 1 ml of 0.02 N potassium permanganate react with 1.2 mg of oxalic acid (Mahadevan and Sridhar, 1986). Three replications were made for each isolate and data were analysed statistically.

3.3.6 Virulence index of isolates of *Sclerotium rolsii*

A pot experiment was conducted in the glass house of Department of Plant Pathology, University of Agricultural Sciences, Dharwad to find out virulence index of different isolates. Four seedlings were planted in each pot. The giant culture of 14 isolates was inoculated to each pot @ 20 g/pot, each treatment was replicated thrice. Observations were noted on PDI and number of days taken for wilting (latent period).

The numerical values of Percent disease incidence and latent period were used to calculate the Virulence index using the following formula (Thakur and Rao, 1997).

Virulence index (VI) = Per cent disease incidence (PDI) X Latent period^{-1}

3.4 Molecular variability among isolates of *Sclerotium rolfsii*

The molecular variability among the isolates of *S. rolfsii* was studied by using ITS region of rDNA and Random Amplified Polymorphic DNA (RAPD).

Requirements/stock solutions

Extraction buffer	: 1M Tris HCl
	5M NaCl
	0.5M EDTA
	0.1% mercaptoethanol
	2% C-TAB
	1% PVP
	10% SDS
10% SDS	: Dissolved 10 g of sodium dodecyl sulphate in 100 ml of distilled water
5M NaCl	: Dissolved 292 g of NaCl in 1 litre of distilled water
Chloroform	: Isoamyl alcohol – 24:1
70% Ethanol	: Dilute 70 ml of alcohol in 30 ml of distilled water
$T_{10}E_1$	: 10 mM Tris
	1 mM EDTA

pH 8.0

100 □□ random primers

25 ng/□l template DNA

3 U/□l taq DNA polymerase

Rnase 10 g/ml

3.4.1 DNA extraction

Disc of 5mm diameter *S. rolfsii* isolates were cut from periphery of an actively growing 4 to 5 days old culture on PDA and inoculated into 250 ml conical flask containing 100 ml of sterile potato dextrose broth and incubated at 28 ± 2°C. The resultant growth of mycelial mat was harvested and excess moisture was completely removed through sterile blotting paper and used for DNA extraction. The total genomic DNA of *S. rolfsii* isolates was extracted from vegetative mycelium using the procedure of Murray and Thompson (1980) and Saddala *et al*. (2010) with slight modifications. Fungal mat of 0.5 g grounded to fine powder in liquid nitrogen and transferred to sterile eppendorf tube. To this, 1 to 2 ml of extraction buffer (1 M Tris-5.0 mL; 5 M Nacl-14.0 mL; 0.5 M $EDTA^2$.0 mL; 0.1% Mercaptoethanol-50.0 µL; 2% CTAB-1.0 g; 1% PVP-0.5 g) was added and incubated for 1 h in water bath at 65℃. The samples were allowed to cool at room temperature Then the tubes were centrifuged at 13,000 rpm for 8 min at room temperature. The supernatant was transferred into other tubes. To the supernatant equal ml of chloroform and isoamyl alcohol (24:1) was added and the tubes were rocked gently to mix the content for five minutes. RNase (1 µL/100 µL) was added and incubated at room temperature for 10-20 min. The tubes were centrifuged at 13,000 rpm for 8 min, separated the supernatant and added 0.6 vol of ice cold isopropanol + 0.1 vol. of sodium acetate and incubated at 20℃ for overnight. Next day, the tubes were centrifuged at 13,000 rpm for 20 min at 4℃, the supernatant was discarded and the pellet washed with 70 per cent ethanol and centrifuged at 13,000 rpm for 20 min at 4℃. Again the supernatant discarded, the pellet air dried and dissolved in 100 µl of sterile distilled water or $T_{10}E_1$. The DNA samples were stored at -20℃ for further studies.

3.4.2 RAPD profiles through Polymerase Chain Reaction

Primers for RAPD

Totally twenty random primers (5pM) were used for molecular characterization of 14 isolates of *Sclerotium rolfsii.*. Commercial kit OPA, OPB and OPF of decamer DNA primers were obtained from M/s Integrated DNA technologies supplied by Sigma Industrial and Laboratory Equipments Inc., Bangalore, India.

dNTP's

The four individual dNTP's such as dATP, dGTP dCTP and dTTP were obtained from M/s Bangalore Genei, Pvt. Ltd., Bangalore.

Taq DNA polymerase

Taq DNA polymerase and 10 x buffers were obtained from M/s Bangalore Genei, Pvt. Ltd., Bangalore.

Thermo cycler

Corbett research gradient PCR supplied by M/s JH Bio Innovation Pvt. Ltd., R. T. Nagar, Bangalore was used for cyclic amplification of DNA.

Master mix for PCR

Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing following components. The total volume of each reaction mixture was 20 µ1. The following reaction mixture was found to be optimum for PCR amplification.

10x assay buffer with 15 mM $Mgcl_2$	: 2.00 µ1
dNTPs mix (2.5 mM each)	: 1.00 µ1
Primer (5PM/ µ1)	: 1.00 µ1
Template DNA (25 mg/ µ1)	: 1.00 µ1

Sterile distilled water : 14.83 µ1

Taq DNA polymerase (6.0 $\mu 1^{-1}$) : 0.17 µ1

Except template the master mix was distributed to PCR tubes (18 µ1 /tube) and later 2 µ1 template DNA from the respective isolates was added making the final volume of 20 µ1.

The thermo profile for PCR

The PCR amplification for RAPD analysis was performed according to Williams *et al.* (1990) with certain modifications. The optimum conditions for DNA amplification used were as follows.

Steps	Temperature (^oC)	Duration(min.)	Number of cycles
Denaturation	94	4	1
Denaturation	94	1	40
Annealing	36	1	
Extension	72	2	
Final extension	72	5	1
Hold temperature	4	--	--

After the completion of the PCR, products were stored at 4^{o}C until the gel electrophoresis was done. A total of 20 random primers with the following sequence were used in the study.

The Oligonucliotide primer sequences of random primers used in RAPD analysis for different isolates of Sclerotium rolfsii are given below

Primer	Sequence 5^1-3^1	Primer	Sequence 5^1-3^1
OPA-01	CAGGCCCTTC	OPB-01	GTTTCGCTCC
OPA-02	TGCCGAGCTG	OPB-02	TGATCCCTGG
OPA-03	AGTCAGCCAC	OPB-03	CATCCCCCTG
OPA-04	AATCGGGCTG	OPB-04	GGACTGGAGT
OPA-05	AGGGGTCTTG	OPB-05	TGCGCCCTTC
OPA-06	GGTCCCTGAC	OPB-09	TGGGGGACTC
OPA-07	GAAACGGGTG	OPB-10	CTGCTGGGAC
OPA-08	GTGACGTAGG	OPF-01	ACGGATCCTG
OPA-09	GGGTAACGCC	OPF-02	GAGGATCCCT
OPA-10	GTGATCGCAG	OPF-03	CCTGATCACC
OPA-11	CAATCGCCGT	OPF-05	CCGAATTCCC
OPA-17	GACCGCTTGT	OPF-06	GGGAATTCGG

Separation of amplified products by agarose gel electrophoresis

Requirements

1. Electrophoretic unit: Gel trough, gel combs, power-pack, UV- Transilluminator
2. Agarose (1.2%)
3. Bromophenol blue
4. Ethidium bromide (0.5 ml μg^{-1})
5. 50 x TAE (stock): Tris-base-60.5g
   Glacial acetic acid-14.25 ml
   0.5 M EDTA 25 ml
   Make up the volume to 250 ml, pH 8.0
6. Working solution (1 x TAE): 20 ml of 50 x TAE was made upto 1000 ml by using distilled water.

Procedure

- Three grams of agarose was weighed and added to a conical flask containing 250 ml of 1 x TAE buffer.
- The agarose was melted by heating the solution in microwave oven and the solution was stirred to ensure even mixing and complete dissolution of agarose.
- The solution was then cooled to about 40-45^{o}C.
- Two to three drops of ethidium bromide (0.5 µg ml^{-1}) was added.

- The solution was mixed and poured into the gel casting platform after inserting the comb in the trough. While pouring sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1x TAE) so as to cover the wells completely.
- The amplified products (20 µ1) were carefully loaded into the sample wells, after adding bromophenol blue with the help of micropipette.
- Electrophoresis was carried out at 60 volts, until the tracking dye migrated to the end of the gel.
- Ethidium bromide stained DNA band were observed under UV-transilluminator and photographed for documentation.

Scoring the amplified fragments

The amplified products along with 2.0 µ1 of loading dye (Bromophenol blue) were separated on 1.2 per cent agarose gel at 80 volts (45 volts per cm of gel) using 1 X TAE buffer of pH 8.0 containing ethidium bromide (0.5 µ1 /10ml of gel). The gels were photographed using gel documentation system. The amplified profiles for all the primers were compared with each other and the bands of DNA fragment were scored as '1' for the presence and '0' for the absence of a band generating the '0' and '1' matrices and per cent polymorphism was calculated by using the formula.

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Statistical analysis

DNA bands that could be scored unequivocally for presence or absence were included in the analysis. Faint bands were not scored. Binary matrices were analysed by NTSYS-PC (version 2.0; Exeter Biological Software, Setauket, NY). Jacard's coeffients were clusterd to generate dendrogram using the SHAN clustering programme, selecting the unweighted pair group method with arithmetic average (UPGMA) algorithm in NTSYS-PC (Rohlf 1998).

3.4.3 Characterisation of *S.rolfsii* isolates by ITS-PCR

PCR amplification of ITS region

PCR amplification of Internal Transcribed Spacers (ITS) region of rDNA was performed using universal primers ITS-1 (5' - TCC GTA GGT GGA CCT GCG G - 3') as forward primer and ITS-4 (5' - TCC TCC GCT TAT TGA TAT GC - 3') as reverse primer (White *et al*., 1990) in eppendorf PCR master cycler.

The thermo profile for PCR

The PCR amplification for RAPD analysis was performed according to Williams *et al.* (1990) with certain modifications. The optimum conditions for DNA amplification used were as follows.

Steps	Temperature (^{o}C)	Duration(min.)	Number of cycles
Denaturation	94	4	1
Denaturation	94	1	35
Annealing	56	1	
Extension	72	1.5	
Final extension	72	6	1
Hold temperature	4	--	--

After the completion of the PCR, products were stored at 4^{o}C until the gel electrophoresis was done

Agarose gel electrophoresis

Amplified PCR products were observed in 1.0 per cent agarose gel in 1X TBE buffer and visualized under UV transilluminator with ethidium bromide staining as mentioned in the earlier procedure. The size of the PCR product was estimated by comparison with known DNA marker of 1 kb DNA ladder. The banding profiles of ITS-PCR products were documented in gel documentation system.

ITS rDNA Sequencing

The PCR products (50 µl) and 10 µl each of both the primers were given to CHROMOUS BIO-TECH COMPANY PVT.LTD for direct sequencing.

Sequence analysis

DNA sequence for the internal transcribed spacer region (ITS) of the 18S rDNA was obtained. After collecting the sequences from Chromous Biotech Pvt, Ltd., all sequences obtained using forward and reverse primers were assembled using Vector NTI software. Further the sequences were submitted to the NCBI, BLAST (National Center for Bioinformatics) BLAST GenBank to check the identity of isolates based on previously published sequences and were used for phylogenetic analyses.

Also DNA sequences were aligned using MEGA 4.0.2.

Phylogenetic analysis

Dendrogram was constructed using neighbour-joining tree method available in MEGA 4.0.2 software for the nucleotide sequences of 18S rDNA region of all isolates. Published sequence *Athelia rolfsii* Bean isolate (KC293992.1) was downloaded from NCBI and used in the phylogenetic analysis as out group in the construction of dendrogram.

3.5 Estimation of loss

3.5.1 Growth and Yield parameters

Field experiments were conducted during *Kharif* 2012 at Medicinal and Aromatic plants Unit, Saidapur Farm, Department of Horticulture, University of Agricultural Sciences, Dharwad, Karnataka to estimate the losses in growth parameters *viz.,* plant height, number of branches and yield due to wilt in stevia in treated and control.

Treatment details:

1. Hexaconazole @ 0.1%
2. Control

Observations on growth parameters *viz.,* plant height and number of branches at 60 and 120 days after planting, per cent disease incidence and yield were taken (1 harvest per season).

3.5.2 Biochemical parameters

3.5.2.1 Biochemical changes in healthy and infected stevia leaves

Fungal pathogens (*R. bataticola* (R), *F. solani* (F) and *S. rolfsii* (S) were grown on sand corn meal medium. The pathogens were inoculated to plants individually. At the same time uninoculated plants served as control.

Sampling

Sampling was done at 20 days after inoculation (DAI). The infected leaves were collected randomly from all treatments including uninoculated plants. The leaves collected were used for further biochemical analysis in the laboratory.

Extraction of plant tissues in alcohol

Estimation of metabolites requires their complete extraction from the tissues. The activities of the enzymes which synthesize and utilize them need to be stopped at once to get reliable values. Plant constituents possess different solvents. Though water is the universal solvent, it does not penetrate tissues quickly enough to stop enzymatic activity. In this context hot alcohol, was used as suitable solvent for the extraction.

Reagent

Ethanol (80%)

Procedure

One g of tissue was weighed and made into small pieces and plunged immediately in boiling alcohol. Then it was cooled and passed through double layered muslin cloth. The pieces of the tissue were ground thoroughly in a pestle & mortar with little hot alcohol. Again it was passed through muslin cloth. The above procedure was repeated once again. The filtrates were pooled and filtered through Whatman No. 41 filter paper and made upto ten ml. volume with alcohol. Then the extract was stored in a refrigerator at 4^0C. This alcoholic extract of the tissue contains reducing sugars, non-reducing sugars, phenols, O.D. phenols and amino acids which were used for further analysis.

Clarification of alcoholic extracts

Dark coloured alcohol extracts of the tissue create a great problem in analytical procedure. The interference due to coloured plant pigments like chlorophyll, carotenes and xanthophylls is enormous which need to be eliminated prior to analysis. Heavy metal salts were used for clarification of alcoholic extracts.

Reagent

Saturated solution of neutral lead acetate and saturated solution of disodium hydrogen phosphate were used.

Procedure

Two ml. of saturated lead acetate solution was added dropwise to ten ml. of the coloured alcoholic extract with three ml. of saturated solution of disodium hydrogen phosphate till the precipitation completed. The above solutions were mixed thoroughly and kept for over night. Further, it was filtered through whatman No. 41 filter paper and made upto 15 ml volume with 80 per cent alcohol and stored in a refrigerator at 4^0C.

Estimation of Reducing Sugar

The reducing sugar was estimated following Nelson's modification of Somogyi's method (Nelson, 1944).

Solution A

Twenty-five g of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of sodium sulphate was dissolved in about 800 ml. of distilled water and diluted to one liter.

Solution B

Fifteen g of copper sulphate was dissolved in distilled water to which one or two drops of conc. Sulphuric acid was added and volume was made up to 100 ml. with distilled water. Solutions A & B was mixed in 24:1 (v/v) proportion just before use.

Arsenomolybate Reagent

1. Twenty-five g of ammonium molybdate was dissolved in 450 ml. of distilled water. Twenty one ml. of conc. Sulphuric acid was added and mixed with above solution.
2. Three g of sodium orthoarsenate was dissolved in 25 ml. distilled water. Two solutions were mixed stirring and placed in a incubator at 37^0C for 24-48 hr. The reagent was stored in brown bottle.

One ml. of each sample (alcohol extract) was pipetted to a test tube. To each one ml. of extract one ml. of mixture of solution of A and B was added. The test tubes were heated in a hot water bath for 20 min. the tubes were then cooled under a running tap water. After cooling one ml. of arsenomolybdate reagent was added. The colour developed after 15 min. and then the above solution was diluted to 15 ml. The absorbance of the solution was measured in spectrophotometer at 500 nm. The amount of reducing sugars was determined by using standard curve prepared with glucose.

Acid hydrolysis of non-reducing sugar and its estimation as reducing sugar

Non-reducing sugar was first hydrolyzed with the help of diluted mineral acid like hydrochloric acid. Then the hydrolysate was neutralized and the reducing sugar was estimated by Nelson Somogyi's method.

Reagents

- 0.1 and 1 N hydrocholoric acid and 1 N sodium hydroxide.
- Phenolphthalein indicator solution in alcohol.

Procedure

One ml of each alcohol extract was taken in a test tube and to it 1- N HC 1 was added and placed in hot water bath at 50^0C for 20 min. After cooling one drop of indicator was added and mixed well. To the solution 1-N sodium hydroxide was added drop wise till the colour turns pink due to excess alkali. The excess alkali was reneutralized with 0.1 N hydrocholoric acid till the solution became colorless. Then the volume was made upto five ml.

From above five ml solution one ml was taken and reducing sugar present in the hydrolysate was estimated by Nelson Somogyi's method. The reducing sugar in the hydrolysate was a measure of total sugar. To get the quantity of non-reducing sugar, the quantity of reducing sugar was subtracted from this value and it was multiplied by a conversion factor of 0.95.

Estimation of Total phenol

Estimation of total phenols present in plant samples was done following Folin-Ciocalteau Reagent Methods.

Reagents

1. Folin-Ciocalteau reagent (FCR)
2. Sodium carbonate (2%)

Procedure

One ml of each alcohol extract was taken in a test tube to which one ml of Folin- Ciocalteau reagent was added followed by two ml of sodium carbonate solution (2%). The tubes were shaken well and heated in a hot water bath for exactly one min. and then cooled under running tap water. The blue colour developed was diluted to 25 ml with water and its absorbance was read at 650 nm in spectrophotometer. The amount of phenols present in sample was calculated from a standard curve prepared from catechol.

3.6 Epidemiological studies

Local (UASD) isolate was used for all epidemiological studies.

3.6.1 Standardization of inoculum density

Soil, sand and farmyard manure were sieved by passing through (2mm mesh sieve) and sterilized separately and then mixed in 3:1:1 ratio. After mixing, it was weighed and filled in surface sterilized earthern pots. The fungal cultures (*F. solani, Rhizoctonia* sp.and *S. rolfsii*) grown on sand corn meal media were mixed separately to each pot to obtain different inoculum levels *viz.*, 0, 2 ,4, 6, 8, 10 and 12 percent. Each treatment was replicated three times. The pot filled with sterilized soil without any inoculum served as control (uninoculated). Apparently healthy rooted cuttings of stevia (four cuttings per pot) were planted in pots. Water was added to the pots at regular intervals to maintain 30 per cent soil moisture. The observations were recorded on the initiation and development of wilt symptoms from the 7^{th} day after inoculation.

3.6.2 Effect of soil temperature on *Sclerotium rolfsii*

The experiment was conducted to access the optimum temperature required for growth and development of S. *rolfsii.*

Finely powdered, sterilized soil sieved through 2 mm sieve was mixed with the inoculum at 4 per cent level. Each petriplate was filled with 100g of soil infected with the culture. The soil in the study was moistened to 25 per cent moisture holding capacity (MHC) by adding required quantity of

water every day. Each treatment was replicated thrice. The plates were incubated at temperature levels *viz.,* 10^0C, 20^0C, 30^0C, 40^0C and 50^0C for 7 days.

After 7 days of incubation, the survival of the fungus was determined by baiting technique and the percent colonization of the fungus on sorghum seeds was calculated. After 7 days of incubation, sclerotial bodies were plated on PDA by following standard procedure and percent germination of sclerotial bodies were recorded at each temperature.

3.6.3 Effect of soil moisture on *Sclerotium rolfsii*

This experiment was conducted to know the favourable moisture conditions for growth and development of *S. rolfsii*. Autocalved soil was mixed with giant culture at four per cent in Petri plates of 20 cm diameter. The soil moisture levels were adjusted to 10, 20, 30, 40, 50, 60 and 70 percent of moisture holding capacity and the moisture level in each treatment was maintained throughout experiment by adding water to nullify the evaporation loss. Each treatment was replicated three times. After 7 days of incubation, the survival of the fungus was determined by baiting technique and the percent colonization of the fungus on sorghum seeds was calculated. One hundred slcerotia of *S. rolfsii* were kept in each treatment to determine the viability of sclerotia. After 7 days of incubation sclerotial bodies were plated in PDA by following standard procedure and percent germination of sclerotial bodies were recorded.

3.6.4 Effect of soil pH on *Sclerotium rolfsii*

This experiment was conducted to determine the exact pH range for growth and development of the *S. rolfsii* in the soil. Twenty gram of soil was taken and suspended in 40 ml sterile distilled water in 250 ml conical flask and stirred for 30 min. to get the soil to water ratio of 1:2, pH of this soil was 7.5. The soil pH was adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0. and 9.5 by using Na_2Co_3 and Oxalic acid. Hundred grams of soil of each pH level was taken and 4g of giant culture was mixed to each treatment and placed in 20 cm diameter Petriplates. Each treatment was replicated thrice. The soil in the study was maintained at 25 per cent moisture holding capacity (MHC) by adding required quantity of water every day. The plates were incubated at 30^0C for seven days. After seven days healthy sorghum seeds, previously boiled in water, were placed equidistantly on it at the rate of 10 seeds per plate. Per cent colonization on sorghum seeds was recorded. A separate 100 sclerotia of *S. rolfsii* were kept in each treatment to know the viability. After seven days of incubation, sclerotial bodies were plated on PDA by following standard procedure and per cent germination of sclerotial bodies were recorded

3 .6.5 Viability of sclerota of *Sclerotium rolfsii* at different depths and duration in Soil

This study was conducted to get the information on the survival of *S. rolfsii* at different depths of soil at different durations of time. Sclerotia of the *S. rolfsii* was obtained from giant culture. Approximately 100 sclerotia were wrapped with small quantity of soil in nylon net (mesh size 1mm) and 3 such bundles were buried at different depths ranging from 1 to 20 cm with difference of one cm, for 12 months in surface sterilized earthen pots (30 cm diameter) containing field soil after the exposure of 12 months, all the nylon bundles of sclerotia were taken out and the Sclerotial viability was tested (Mishra *et al.* 1995 and Gurjar *et al.,* 2004). The bundles were also buried for different durations, in each of the 20 surface sterilized earthen pots (15 cm diameter) at depth of two cm. The pots were kept in the glass house. To test the duration of viability of sclerotia, one bundle each from 20 pots were taken out at monthly interval by dipping in 0.1 per cent mercuric chloride solution for one minute followed by three washings with sterilized distilled water and inoculated aseptically to Petri plates containing PDA by following the -standard procedure. The per cent germination of sclerotial bodies was recorded.

3.6.6 Survival of fungal pathogens on infected debris

Survival of pathogens was carried out to know the period of viability of the pathogens on the infected debris. Stevia plants infected with *F. solani* were collected and stored in various environmental conditions *viz*., refrigeration, laboratory (room temperature), glasshouse (Department of Plant Pathology) and field conditions (Saidapur). Presence of pathogens in plant debris was confirmed by periodic isolations *i.e*., at 15 days interval from infected plant debris stored under different conditions by using standard tissue isolation technique.

3.6.7 Identification of susceptible stage of stevia for *Sclerotium rolfsii*

To know the susceptible stage of the crop, an experiment was conduced under glass house condition. Five stage of the crop were taken for their susceptible reaction with *Sclerotium rolfsii*. These stages of the crop were maintained in the earthern pots filled with sterilized soil. After planting, all the stages of the crop (4 cuttings per pot), inoculum (6%) was added at a time to all the treatments. After 30 days, number of plants showing wilting symptoms due to *S. rolfsii* was recorded.

Treatment details

Sl. No.	Age of the crop (days)
T_1	15
T_2	30
T_3	60
T_4	90
T_5	120

3.6.8 Interaction among pathogens

Pot culture studies were conducted to investigate the interaction among the wilt causing pathogens like *S. rolfsii, F. solani and Rhizoctonia bataticola*. The experimentation details are presented hereunder.

Treatment details

Tr. No.	Treatments
T_1	*Sclerotium rolfsii* alone (S)
T_2	*Fusarium solani* alone (F)
T_3	*Rhizoctonia* spp. alone (R)
T_4	*Sclerotium rolfsii* + *Fusarium solani*
T_5	*Fusarium solani* + *Rhizoctonia bataticola*
T_6	*Sclerotium rolfsii* + *Fusarium solani* + *Rhizoctonia bataticola*

Six per cent of fungal giant culture was applied individually to 30 days old rooted cuttings of stevia grown in 3:1 sterile soil sand mixture in earthen pots. Inoculums of all the said pathogens were applied at the base of the plant, singly or in combinations in sequence as per the treatments. Stevia rooted cuttings in sterilized soil without any inoculum served as control. Three replications were maintained for each treatment

Disease incidence was recorded from the first day of symptoms appearance and continued until fresh infections ceased to appear. An observation on per cent disease incidence was recorded. Later pathogens were detected for each treatment.

3.6.9 Host range of *Sclerotium rolfsii*

Host range experiment was undertaken with an objective of knowing whether the pathogen can infect any host other than stevia. Totally 18 different hosts were selected and details of plants are given below. The pathogen was inoculated to the following medicinal plants at the rate 4 per cent to know the host range.

Common name	Botanical name	Family
Indian Borej	*Coleus amboinicus*	Lamiaceae
Makandiberu	*Coleus forskohlii*	Lamiaceae
Mint	*Mentha spicata*	Lamiaceae
Aloe	*Aloe vera*	Liliaceae
Ashwagandha	*Withania somnifera*	Solanaceae
Tulsi	*Ocimum sanctum*	Lamiaceae
Shatavari	*Asparagus racemosus*	Liliaceae
Brahmi	*Centella asiatica*	Apiaceae
Patchouli	*Pogostemon patchouli*	Lamiaceae
Rosemary	*Rosmarinus officinalis*	Lamiaceae
Lemon grass	*Cymbopogan flexuosus*	Poaceae
Pandanus	*Pandanus amaryllifolius*	Pandanaceae
Vetiver	*Vetiveria zizanioides*	Poaceae
Kachora	*Kaempferia galanga*	Zingiberaceae
Clocimum	*Ocimum gratissimum*	Lamiaceae
Vajravalli	*Cissus quadrangularis*	Vitaceae
Insulin plant	*Costus pictus*	Costaceae
Chakramani	*Sauropus androgynous*	Euphorbiaceae

Above plants were raised in earthern pots of 12"size. Gaint culture of *Sclerotium rolfsii* multiplied on sorghum grains was added to the pots when the plants were established. These were then incubated in glass house at 27±1^{o}C and watered regularly. The early stage of symptoms development and type of symptoms developed on different hosts were recorded

3.7 Management studies

3.7.1 *In vitro* evaluation of bioagents against *S. rolfsii, F. solani and R. bataticola*

The efficacy of seven bioagents was tested against *S. rolfsii, F. solani* and *Rhizoctonia* spp., for radial growth inhibition on the potato dextrose agar media using dual culture technique (Vincent, 1947)

Bioagents used against *S. rolfsii*

1. *Trichoderma viride* Pers.
2. *Trichoderma harzianum* Rifai
3. *Trichoderma harzianum* (Dharwad isolate),
4. *Trichoderma koningii* Oudern
5. *Trichoderma virens* Miller
6. *Pseudomonas fluorescens* Migula
7. *Bacillus subtilis* Cohn.

3.7.1.2 Dual culture test

Bioagents were evaluated for their efficacy through dual culture technique. Both biocontrol agents and test pathogen were cultured on potato dextrose agar in order to get fresh and active growth of fungus. The cultures of antagonistic micro organisms used in the present study were obtained from the project directorate of biological control (PDBC) Bengaluru and Department of Plant Pathology, UAS, Dharwad and IABT Dharwad, Karnataka state.

Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile Petriplate and allowed to solidify. For evaluation of fungal bio control agents, mycelial disc of test fungus was inoculated at one end of the Petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at the middle of the pertiplates and mycelial disc of the test fungus was placed on either side at the centre of each half of the plate. The plates were incubated at 27±1^{o}C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism.

The colony diameter of the pathogen in control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where, I = Percent inhibition

C = Growth in control

T = Growth in treatment

3.7.2 *In vitro* evaluation of botanicals against *S. rolfsii, F. solani and R. bataticola*

In the present study, the leaf and bulb extracts of the following plants were selected for testing their efficacy against various pathogens causing wilt in stevia.

Botanical name	Common name	Family	Plant part used
Adhathoda vasica Nees	Adathoda	Acanthaceae	Leaf
Allium cepa L.	Onion	Alliaceae	Bulb
Allium sativum L.	Garlic	Liliaceae	Bulb
Azadirachta indica A. *Juss.*	Neem	Meliaceae	Leaf
Bougainvillea spectabilis L.	Bougainvillea	Nyctaginaceae	Leaf
Calotropis gigantea L.	Calotropis	Apocyanaceae	Leaf
Cassia fistula L.	Negro coffee	Caesalpiniaceae	Leaf
Clerodendron inermae Gaerth	Kashmir bouquet	Verbenaceae	Leaf
Chrysanthemum indicum	Crysanthemum	Asteraceae	leaf
Murraya koenigii	Currey leaf	Rutaceae	leaf
Moringa oleifera	Drumstick	Moringaceae	leaf
Durantha repens L.	Durantha	Verbenaceae	Leaf
Eucalyptus globulus Labill	Eucalyptus	Myrtaceae	Leaf
Eupatorium odoratum L.	Communist weed	Asteraceae	Leaf
Glyricidia maculata L.	Glyricidia	Leguminaceae	Leaf
Jatropha curcas L.	Jatropha	Euphorbiaceae	Leaf
Lantana camara L.	Lantana	Verbenaceae	Leaf
Tagetes erecta	Marigold	Asteraceae	Leaf
Ocimum sanctum L.	Tulsi	Labiateae	Leaf
Parthenium hysterophorus L.	Congress weed	Asteraceae	Leaf
Nodilittorina unifasciata	Periwinkle	-	leaf
Pongamia glabra L.	Honge	Simarubaceae	Leaf
Prosopis juliflora L.	Bellary jali	Mimosaceae	Leaf
Tridax procumbens L.	Tridax/Deer foot	Asteraceae	Leaf
Multineem	Multineem	-	Liquid suspension

Preparation of cold aqueous extract

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts poisoned food technique was used (Nene and Thapliyal, 1973). Five and ten ml of stock solution was mixed with 95 and 90 ml of sterilized molten PDA medium, respectively so as to get 5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of medium was poured into sterile petriplates, mycelium of five mm size discs form periphery of actively growing culture of test pathogens were cut out by sterile cork borer and one such disc was placed at the center of each agar plate. Totally 19 plant extracts were evaluated at two concentrations. Suitable control plates were maintained by growing the test pathogens on PDA plates.

Then such plates were incubated at 27±1^{0}C temperature and radial growth was taken when maximum growth occurred in the control plates.

The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the following formula (Vincent, 1947). The data were analysed statistically.

3.7.3 *In vitro* evaluation of fungicides

In vitro evaluation of non-systemic fungicides

The efficacy of eight non-systemic fungicides at of 0.1, 0.2 and 0.3 per cent was assayed by following poisoned food technique. The fungicides used are given here under

Common name	Chemical name	Trade name
Captan	N (Trichloromethyl thio) cyclohelix 4-ere-1, 2 dicarboximide	Captaf 50%wp
Chlorothalonil	Tetrachloro isopthalonitrile	Kavach 75 % WP
Copper oxy chloride	Copper oxychloride	Blitox-50% WP
Methoxyethyl mercury chloride	2-methoxyethyl mercury chloride	Emissan-6
Mancozeb	Manganese + Zinc ethylene bis dithiocarbamate	Indofil M-45 75%WP
Propineb	Zincpropylene bisdithiocarbamate	Antracol 70WP
Thiram	Tetra methyl thiuram disulphide (TMTD)	Thiram 75% WP
Zineb	Zinc ethylene bis dithiocarbomate	Dithane Z-78

In vitro evaluation of systemic fungicides

The efficacy of six systemic fungicides were assayed at 0.025, 0.05 and 0.1 per cent by following poisoned food technique. The fungicides used are given here under.

Common name	Chemical name	Trade name
Carbendazim	2-(Methoxy-Carboxyl)-benzimidazole	Bavistin 50%WP
Benomyl	Benzimidazole carbomate	Benomyl 50%s WP
Carboxin+ thiram	3-(3-5-dichlorophenyl)-N-(1-methylethyl)^2-4-dioxo-1-lemadazolidine carboximide + Tetramethyl thiuram disulphide	Vitavax Power
Propiconazole	1-(2 (2,4 dichlorophenyl)-4-propyl-1, 3-dioxolanyl methyl)-1H-1-4-triazole.	Tilt 25% EC
Hexaconazole	(RS^2-C2, 4-dichlorophenyl)-1-(14-1, 2, 4-triazole-1-41)hexan^2-Ol	Contaf 5% EC
(Carbendazim 12% + Mancozeb 63% WP)	Manganese zinc ethylenebisdithiocarbamate +2-(Methoxy carboryl) –benzimidazole	Saaf

Required quantity of individual fungicides was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later 20 ml of the poisoned medium was poured into sterile petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the center of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated four times. Then such plates were incubated at room temperature till growth of the fungus touched the pereiphery in control plate. The diameter of the colony was measured in two directions and average was worked out. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947). The data were analysed statistically.

3.7.4 *In vivo studies*

3.7.4.1 Management by organic amendments

A pot experiment was conducted in glasshouse of University of Agricultural Sciences, Dharwad, Department of Plant Pathology, College of Agriculture, Dharwad. This experiment was conducted to know the efficacy of various organic amendments. Sieved soil was filled in earthen pots and one month old culture of *S. rolfsii,* grown on sand corn meal was mixed at four per cent w/w basis

to have sick soil in pots. Moisture was maintained at 25 per cent water holding capacity (W.H.C.). After one month, organic amendments (listed below) were added to the pots and left for two weeks at 25 per cent W.H.C. one month old rooted stevia cuttings were planted and cuttings in sterilized soil served as control. Three replications were maintained for each treatment. Observations like per cent death of plants and plant height were recorded. The organic ammandments used are given hereunder.

Treatment No.	Treatment details
T_1	Enriched Farm Yard Manure (25g/kg of soil) (FYM + *Trcihoderma harzianum* + *Pleurotus* sp. and *Aspergillus nizer*
T_2	Neem cake (20g/kg of soil)
T_3	Safflower oil cake (20g/kg of soil)
T_4	Cotton oil cake (20g/kg of soil)
T_5	Vermicompost (20g/kg of soil)
T_6	Groundnut cake (20g/kg of soil)
T_7	Control

3.7.4.2 Integrated disease management (IDM) for Sclerotium wilt of stevia in the pot experiment

A pot culture experiment was conducted in the glasshouse, Department of Plant Pathology, University of Agricultural Sciences, Dharwad to find out the best treatment for control of wilt disease of stevia caused by *S.rolfsii.* The rooted stevia cuttings were grown in pots containing sick soil. The effective fungicides, botanicals, and bioagents evaluated in *in vitro* studies were further evaluated under pot culture. About 2 kg of sterile soil was filled in each pots. Rooted Stevia cuttings were planted separately at the rate of three cuttings per pot. Each treatment was replicated thrice. Inoculum of *S. rolfsii* was added after establishment of seedlings to prepare the sick soil. After proper establishment of the seedlings, fungicides, plant extracts were drenched individually to respective sets of pots. stevia plants planted in the sick soil without treatment served as control.

Treatment No.	Treatment details
T_1	Untreated control
T_2	Eucalyptus leaf extract @ 10%
T_3	Hexaconazole @ 0.1 %
T_4	FYM @ 10 t /ha + *Trichoderma harzianum* @ 25 kg/ha
T_5	Carboxin + thiram @ 0.1%
T_6	*Azadiracta indica* leaf extract @ 10%
T_7	Duranta leaf extract @ 10%
T_8	Carbendazim + mancozeb @ 0.1%

3.7.4.3 Integrated disease management (IDM) for Sclerotium wilt of stevia in the field

A field trial was undertaken in Saidapur Farm, Department of Horticulture, University of Agricultural Sciences, Dharwad, for one year i.e. during *kharif* 2012. Eight treatments were laid out in randomized complete block design (RCBD) with plot size of 6 sq m (gross size). The transplanting of the stevia rooted cuttings was done during August 2012.Three replications were maintained for each treatment. In each treatment, planting of stevia rooted cuttings was done at a spacing of 45 x 40 cm. Rooted stevia cuttings of 15-20 cms with 5-6 pairs of leaves were taken for planting. Fertilizers were applied @ 60: 30: 45 of N: P: K kg/ha. In the experimental wilt sick plot, established rooted cuttings were planted. Treatments were imposed by drenching the rooted cutting with the plant extracts, biocontrol agents, organic amendments and fungicide solution soon after the appearance of symptoms (wilting) in control and the second treatment was applied at 30 days after planting. FYM was applied to the soil only once before transplanting. The above mentioned treatments were imposed in randomized block design (RCBD). Observations were recorded for plant growth parameters like plant height, number of branches (at 30, 60 and 120 days after transplanting), no of leaves and per cent disease incidence. Yield parameters like fresh and dry herbage yield out of one harvest was recorded. Results were analysed statistically.

3.8 Statistical analysis

The data obtained in the present investigations for various parameters were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized block design for *in vivo* studies by using M-STATC programme.

REVIEW OF LITERATURE

Stevia is one of the important medicinal plants. A scanning of literature on diseases of stevia showed that wilt is the major constraint in the production of stevia. Wilt caused by *Sclerotium rolfsii* is a major disease distributed wherever stevia cultivation is pursued intensively. Wilt caused by *Fusarium solani* is a very serious disease in nurseries. *Rhizoctonia bataticola* also causes wilt in some pockets of stevia growing areas of Karnataka. However, research work on stevia wilt is scanty and very little information is available on stevia wilt in the literature. The work pertaining to the various aspects of pathogen(s) and disease(s) have been initiated. Review was made on the diseases and pathogens of stevia and other hosts are quoted hereunder.

2.1 Survey and distribution

Sclerotium rolfsii

Sclerotium rolfsii is predominantly distributed in tropical and subtropical countries. It is common where high temperatures exist during the rainy season. Weber (1931) and Garret (1956) reported that, the fungus survived in the soil for many years by producing sclerotial bodies and causing the disease either in the form of stem rot or foot rot or root rot on several hosts. Nargund (1981) carried out survey on foot rot of wheat during 1978-79 and 1979-80 in Malaprabha Project area and reported maximum disease incidence of 10.20 per cent and 5.20 per cent in rainfed and irrigated fields respectively.

Kumar (2008) reported the prevalence of wilt complex of *Coleus forskohlii* in northern Karnataka and reported the association of *S. rolfsii* along with other soil borne pathogens of wilt complex disease. Mallesh (2009) and Ammajamma (2010) conducted survey for the incidence of wilt complex of *C. forskohlii* in Karnataka and revealed the association of *S. rolfsii* in the complex disease.

Hegde *et al. (*2010b) conducted survey in six districts during 2008 and 2009, and reported maximum incidence of Sclerotium wilt of stevia in Gangenalli village (13.75%) and least in Nidshoshi village of Koppal district (6.25%).

Fusarium solani

Singh and Angadi (1990) reported maximum incidence of wilt of patchouli caused by *Fusarium solani* during April-May 1990 at the Farms of IIHR, Bangalore. Incidence of the disease gained momentum from June and continued throughout the year causing heavy plant mortality.

Sreedevi (2007) observed higher incidence of Patchouli wilt in Dharwad district (48.26%) followed by Belgaum district (40.25%) and Haveri (38.63%), while least incidence was noticed in Uttar Kannada district.

Rhizoctonia bataticola

Ramprasad (2005) conducted survey on root rot complex of *Coleus forskohlii* in northern districts of Karnataka and revealed the association of *F. chlamydosporum, R. bataticola, S. rolfsii* and *M. incognita* by following standard tissue isolation technique from infected plant parts.

Kumar (2008) conducted survey for the incidence of wilt complex of *C. forskohlii* in Karnataka and revealed the association of *R. bataticola* in the complex disease. Mallesh (2009) reported the prevalence of wilt complex of *C. forskohlii* in northern Karnataka and reported the association of *S. rolfsii.*

2.1.1 Symptomatology

Sclerotium rolfsii

Many researchers have described the symptoms of Sclerotium rot of potato as dark brown lesions appearing on the stem just below the soil surface followed by wilting of lower leaves and gradually drying of the entire plant. Such wilted plants showed white cover of fungal threads, girdling the basal part of the stem, which moved above and below to the stem and roots. Sclerotia resembling mustard seeds, developed on infected plant parts and also on soil (Bisht, 1982; Kulkarni *et al.,* 1995; Somani and Chauhan, 1996 and Anahosur, 2001).

Symptoms of *Sclerotium* infection in coleus, stevia, patchouli and aloe has been described by many researchers. Initially symptoms appear as water soaked lesions near soil surface. Leaves become flaccid and dropp off. White, fan shaped mycelial strands crept over the stem portion, developing small light to dark brown sclerotia on the infected portion. The sclerotial bodies were white at first, later turned brown with age finally the plants wilted and dried (Ramprasad, 2005; Kamalakannan *et al.,* 2006; Sreedevi, 2007; Rashmi and Pandey, 2009; Ammajamma, 2010 and Hegde *et al*., 2010b, Shwetha, 2011).

Fusarium solani

Singh and Angadi (1990) reported that *Fusarium solani* wilted plants of patchouli were characterized by blackening of roots at collar regions of fully grown plants. Severe disintegration of secondary roots surface was also observed.

Rathnamma (1994) described the symptoms of wilt of Garden rue (*Ruta graveolens* L.), a medicinal plant caused by *F. solani* as yellowing of the lower leaves which, later advanced to middle and terminal leaves. The tap root of the plants showed dark brown to red discoloration, which covered entire tap root with longitudinal fissures along the main root.

Raju (1997) recognized *F. solani,* the cause of wilt of crossandra and the disease was characterized by loss of turgidity, drooping and wilting of leaves followed by brown to black discoloration of roots.

Rhizoctonia bataticola

Prashanthi (1994) reported that safflower plant infected by *R. bataticola* was characterized by gradual yellowing and drying of leaves followed by loss of vigour and pre-mature death. The bark of such plants could be easily peeled off. There was extensive sloughing off and shredding of affected bark.

In coleus, the infection started at the collar region of plants as water soaked areas and tissues soon turned into a soft, watery mass. Later spread to the roots of the plant and caused decay, which ultimately toppled and collapsed. These infected plants can be easily pulled off from the soil and exhibited brown discoloration of roots followed by rotting or roots. In addition, the extensive sloughing off of affected bark and shredding of roots was also observed. In advanced stage, the aerial portion of the plants decayed completely. The causal organism was isolated and identified as *R. bataticola* (Sachidananda (2005), Ramprasad (2005) and Ammajamma (2010).

2.1.2 Pathogen

Sclerotium rolfsii

Sclerotium rolfsii is a well known polyphagous pathogen. The fungus was first reported by Rolfs (1892) as a cause of tomato blight in Florida. Saccardo (1911) named the fungus as *S. rolfsii* sp. nov. His Latin description of the fungus was based on the material collected in Florida by Rolfs and submitted by Stevens. In India, Shaw and Ajrekar (1915) isolated an organism from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. This name was also used in numerous papers. But, later studies revealed that the fungus involved was actually *S. rolfsii* (Ramakrishnan, 1930).

Higgins (1927) worked in detail on the physiology and parasitism of *S. rolfsii*. However, its perfect state was first studied by Curzi (1931) and proposed generic name as *Corticium*. Mundkur (1934) successfully isolated the perfect state of *S. rolfsii*. McClintock (1917) and Butler and Bisby (1931) reported the disease for the first time from USA and India, respectively.

Fusarium solani

The genus *Fusarium* was identified by Link in 1809 and was accepted in an amended form by Fries (1849) for species with fusiform, septate spores described and clearly illustrated by Martius and Von (1842).

Rhizoctonia bataticola

The fungus *Rhizoctonia bataticola* is known to infect a wide range of host plants such as both monocots and dicots with a high aggressiveness and these attributes have made this fungus economically important pathogen. The genus *Rhizoctonia* was erected by de Candole (1815) for the violet root rot pathogen, *Rhizoctonia erocorum* D.C. ex. Fr.+

Rhizoctonia is predominantly subterranean in habit living both as a parasite and saprophyte. According to ecological grouping of soil fungi (Garrett, 1956), it is placed under soil inhabitants and characterized as unspecified parasite generally distributed worldwide.

2.2 Cultural studies

2.2.1 Growth charaters on different media

Sclerotium rolfsii

Lingaraju (1977) reported that the isolates of *S. rolfsii* from sunflower showed maximum growth in potato dextrose broth on tenth day. Palaiah (2002) observed significant variation in the mycelial growth of different isolates on different solid media and isolates also differed with regard to time taken for sclerotial initiation on solid media. Out of six media, maximum radial growth of *S. rolfsii* was recorded on PDA at 4 and 7 days after inoculation, followed by chickpea meal agar, rice meal agar and Richard's agar (Tripathi and Khare, 2006)

Ramprasad (2005) and Ammajamma (2010) reported that maximum dry mycelial weight of *S. rolfsii* was recorded on Richard's medium. Kamalakannan *et al*. (2006) found that the sclerotia produced on PDA were round to oblong, initially white and later turned brown, with an average diameter of 0.5-2.0mm in stevia. Out of 14 solid media tested, the best mycelial growth of *S.rolfsii* was noticed on oat meal agar, PDA and Sabouraud's agar (Basamma, 2008). Maximum radial growth of *S. rolfsii* was recorded in oat meal agar, Richard's medium, PDA and Sabouraud's agar (Hegde *et al.,* 2010b)

Fusarium solani

Venkataraman (1955) showed that culture of pathogen causing *Fusarium* wilt of muskmelon produced fluffy mycelium with sparse number of conidia differing with 'wild type' strain having abundant sporulation.

Monoconidial lines of the linseed wilt pathogen *Fusarium oxysporum* f.sp*. lini* isolated from different linseed growing regions differed in their cultural and morphological characters with marked diversity in virulence according to Sharma and Mathur (1971).

Jhamaria (1972) reported that, potato dextrose agar, Richards's agar and Czapek's agar provided maximum growth and sporulation of *F. oxysporum.* f .sp*. niveum.*

Sowmya (1993) studied the growth of *Fusarium oxysporum* f. sp. *cubense* on different nutrient media and observed maximum growth and sporulation of the pathogen on Potato sucrose agar and Richards's agar, respectively.

Rhizoctonia bataticola

Radial growth of *Rhizoctonia bataticola* was found maximum after 24 hours of incubation on PDA (40.3 mm), followed by potato sucrose agar (39.0 mm), Czapeck's dox agar (36.0 mm) and Oatmeal agar (35.5 mm) in the studies conducted by Chowdary and Govindaiah (2007).

Kulkarni *et al*. (1992) reported that, *R. bataticola* grew best on cotton root extract agar, potato dextrose agar, Richard's agar and Sabouraud's agar, while sclerotial production was excellent on the dextrose agar, Richard's agar and Sabouraud's agar and on Czapek's agar.

Sahi *et al.* (1992) observed that, potato dextrose agar supported the maximum colony diameter (9 cm) of *Macrophomina phaseolina* while Richard's agar supported maximum sclerotial production. Four isolates of *Macrophomina phaseolina* on pigeon pea were compared for cultural variability. Potato dextrose agar and Czapek's agar were found best for the growth of all the isolates (Lokesha, 2002).

2.2.2 Growth phase of the pathogens

Lilly and Barnett (1951) discussed the growth pattern of fungi and outlined the following growth phase a) Stationary phase, b) Phase of accelerated growth of maximum c) stationary phase and d) Phase of decline or autolysis. They attributed these phases of fungus to the environmental and nutritional conditions in which it grows.

Sclerotium rolfsii

Lingaraju (1977) reported that *S. rolfsii* reached maximum growth after 10 days of incubation on potato dextrose broth. Ramprasad (2005) reported that *S. rolfsii* isolated from coleus plants, reached maximum growth after 10 days of incubation on potato dextrose broth. *Sclerotium rolfsii* isolated from coleus plant, reached maximum growth after 10 days of incubation on PDB (Ramprasad, 2005 and Ammajamma, 2010)

Hegde *et al.* (2010b) and Shwetha (2011) reported that maximum dry mycelial weight *Sclerotium rolfsii* isolated from stevia plant was observed on 10^{th} day.

Fusarium solani

Shyla (1998) used potato dextrose broth to study the growth phase of *F. chlamydosporum* and observed maximum growth at either 18^{th} or 20^{th} day after inoculation. The autolysis stage of the fungus was recorded after 20 days of inoculation.

Sreedevi (2007) reported maximum growth of the fungus on 10^{th} day of incubation.

Rhizoctonia bataticola

Rhizoctonia follows the curve in a liquid medium having an initial period of accelerating growth followed by a phase of very rapid growth and then decreased in weight due to autolysis (Israel and Ali, 1964) reported that the fungus *Macrophomina phaseolina* attained maximum growth after 11 days of inoculation in Richards's liquid medium.

Ramprasad (2005), Ammajamma (2010) and Hegde *et al.* (2012) reported maximum growth of the fungus on 12^{th} day of incubation in *C. forskholi* and jatropha respectively.

2.2.3 Growth on liquid media

Sclerotium rolfsii

Ramprasad (2005) Ammajamma (2010) reported that maximum dry mycelial weight of *S. rolfsii* was observed in Richard's medium and potato dextrose broth and minimum in host leaf extract.

Fusarium solani

Among the media tested for growth and sporulation of *F. chlamydosporum*, maximum growth was obtained on potato dextrose agar and Rose Bengal agar while the least growth was observed on host extract agar (Shyla, 1998).

Desai (1982) reported that *F. moniliforme* was able to grow very well in Czapek's-Dox broth while, the least growth was recorded in Elliot's broth. Richard's medium was found to be best suited medium for the growth of *F. udum* the causal agent of *Fusarium* wilt on pigeonpea (Sataraddi *et al*., 2003).

Rhizoctonia bataticola

Rhizoctonia followed the classical growth curve in liquid medium, having an initial period of accelerating growth followed by a phase of very rapid growth and then a decrease in weight due to autolysis (Ikeno, 1933; Townsend, 1957; Israel and Ali, 1964).

Shanmugam and Govindswamy (1973) and Desai (1982) reported that Richards's medium gave the maximum growth of groundnut isolate of *Macrophomina phaseolina*. Among the liquid media tried, Richards's broth and potato dextrose broth gave the maximum growth and sclerotial production Chowdary and Govindaiah (2007) reported that maximum dry mycelial weight of *R. batoticola* in potato dextrose broth (998.3 mg) and minimum in Oatmeal broth (439.3 mg).

2.2.4 Nutritional studies

2.2.4.1 Effect of carbon sources

Sclerotium rolfsii

Hussain *et al.* (2003) reported that, among all carbon sources starch was the best for growth of the *S. rolfsii* whereas other carbon sources did not showed good results. The fungus may utilize certain simple form of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al*., 1970).

Ramprasad (2005), Basamma (2008) and Ammajamma (2010) reported that sucrose supported maximum mean dry mycelial weight (183.67mg) of *S. rolfsii* followed by glucose (153.33mg), while least dry mycelial weight (62.33mg) was recorded in citric acid.

Fusarium solani

Kesavan and Prasad (1975) reported that among the carbon sources, glucose and sucrose were best for the production of fusaric acid and sporulation by the muskmelon wilt pathogen while starch provided maximum growth.

Brayford and Bridge (1989) reported growth and pigmentation of 99 strains of *Fusarium* mainly *F. oxysporum* and *F. solani* on ammonium salts agar containing either manitol, sorbitol or xylitol as sole source of carbon. Patel (1991) demonstrated that, among carbon sources, maltose and mannitol were best utilized by *F. solani*.

Rhizoctonia bataticola

Patil and Kulkarni (1977) reported that arabinose, dextrin, glucose, lactose and sucrose supported good growth of *Macrophomina phaseolina* isolated from cotton, sesame, groundnut and castor.

Singh *et al. (*1974) noticed that abundant growth and sclerotial production in media with carbon sources as sucrose, glucose and fructose while, glactose, sorbose, lactose and mannitol permitted poor growth of *R. bataticola*. Similarly, among the carbon sources, sucrose was utilized most efficiently followed by maltose. Maximum mean mycelial dry weight of the fungus (205.67 mg) was obtained when sucrose was used as a carbon source. It was followed by fructose (Ramprasad, 2005 and Ammajamma, 2010).

2.2.4.2 Effect of nitrogen sources

Sclerotium rolfsii

Hussain *et al.* (2003) reported that, among all nitrogen sources peptone (9.0 cm) was found to be the best source of nitrogen for *S. rolfsii*. It was followed by potassium nitrate.

Ramprasad (2005) and Ammajamma (2010) reported that *S. rolfsii* fungus grew well utilizing potassium nitrate (323.67 mg dry mycelial weight) followed by ammonium nitrate (219.33 mg dry mycelial weight).

Fusarium solanii

Kushwaha *et al.* (1974) working with eight strains of *F. oxysporum* f .sp*. lentis* for nutritional requirement noticed variation with regard to utilization of nutrients. Kesavan and Prasad (1975) found that sodium nitrate and ammonium nitrate were the best nitrogen sources for the growth of *F. oxysporum* f .sp*. melonis.*

Mahendrapal and Grewal (1975) showed that ammonium salts in general supported growth of *F. Oxysporu m* f .sp*. ciceri*. Sreedevi (2007) found that potassium nitrate and sodium nitrate nitrate were the best nitrogen sources for the growth of *F. Solani.*

Rhizoctonia bataticola

Shanmugam and Govindaswamy (1973) obtained significantly higher growth of *M. phaseolina* in asparagine followed by glutamine, peptone and potassium nitrate. *R. bataticola* metabolized a number of nitrogen compounds for the growth and that the amount of growth varied with the type of nitrogen source. Similarly, among the nitrogen sources, glutamic acid supported the maximum growth and sclerotial production of the fungus *R. bataticola* while least growth was supported by ammonium chloride (Ramamurthy, 1982).

2.2.5 Physiological studies

2.2.5.1 Temperature

Sclerotium rolfsii

Sulladmath *et al.* (1977) studied variation in requirement of temperature by different isolates and found that all isolates grew well between 25-35℃. The optimum temperature for groundnut isolate was 25℃ and 30℃ for tobacco and potato, but 35℃ for rest of the isolates. Prasad *et al.*

(1986) found that, mycelial growth was best at 30°C, while most sclerotial bodies were formed at 25°C.

Harlapur (1998) reported that in the optimum soil temperature for growth and activity of *Sclerotium rolfsii* was found to be 25^0C and 30^0C and the growth was completely ceased at 45^0C in wheat.

Basamma (2008), Baswaraj (2005) and Kulkarni (2007) found that maximum growth *of Sclerotium rolfsii* was observed at 30^0C and least was recorded at 10^0C. Hegde *et al.* (2010b) observed maximum growth *of Sclerotium rolfsii* at 30^0C and 35^0C.

Fusarium solani

Optimum temperature for growth of *F. oxysporum* f .sp*. cubense* isolates was found to be 25 – 30°C (Chuang, 1988). Sowmya (1993) noticed maximum growth of four isolates of *F. oxysporum* f .sp. *cubense* at 35°C. Desai *et al.* (1994) reported that maximum growth of all the four races of *F. oxysporum* f .sp*. ciceri* was recorded at 25°C.

The fungus *F. solani* showed good growth and sporulation in Duggar's modified basal medium at 28-30^0C and very poor growth at 36°C (Madan, 1983). Sreedevi (2007) reported maximum growth of *F. Solani* at 30^oC.

Rhizoctonia bataticola

Bainade *et al.* (2006) while studying various levels of temperature for culturing *M. phaseolina* causing leaf spot of mungbean, observed that the temperature of 38^0C favoured maximum growth and sclerotial production. Singh and Mehrotra (1980) observed that the incubation temperature of 35-^{0}C stimulated the mycelial growth of *R. bataticola.*

Sandhu *et al.* (1999) reported that the temperature of 30^0C favored maximum disease development. Kulkarni (2000) noticed variation in growth of *M. phaseolina* due to different temperatures. Significant growth of all the isolates was observed at temperature of 35^0C and 40^0C indicating their preferential range to be between 35 and 40^0C.

2.2.5.2 Hydrogen ion concentration

Scerotium rolfsii

Maximum growth of *S. rolfsii* was observed at pH of 5 and 6 (Hari *et al.,*1988; Tripathi and Khare, 2006). Basamma (2008) reported that the maximum growth of *S. rolfsii* was obtained at pH range of 4-5. Ramprasad (2005) and Ammajamma (2010) reported that pH of 3.5 supported the maximum growth of *S. rolfsii.*

Fusarium solani

Jhamaria (1972) reported that *F. oxysporum* f .sp*. niveum* could grow well on wide range of pH varying from 3.2 to 8.3 and the optimum was between pH 5.5 to 6.5. Marras *et al.* (1981) reported that the optimum pH for *F. roseum* var *avanaceum* was 7.0.

Desai *et al.*(1994) reported that all the four races of *F. oxysporum* f .sp. *ciceri* recorded maximum growth at pH 6.0.Profuse growth and sporulation of *F. oxysporum* f. sp *sesamum* was recorded at pH 6.6 to 7.5 (Raghuvanshi, 1995). Jadhav *et al*. (2000) reported that, *F.chlamydosporum* recorded maximum growth at pH 6.5 which was followed by pH 6.0 and 5.5.

Rhizoctonia bataticola

Kulkarni (2000) reported that variation due to change in pH level was evident in *M. phaseolina* isolates. Highest growth was observed at pH 7.0 closely followed by pH 6.5 indicating preferential range to be between pH 6.5 and 7.0.

Maximum growth of *M. Phaseolina* was observed at pH 7.0 in mulberry (Chowdary and Govindaiah (2007). Bainade *et al.* (2006) studied various pH levels for the growth of *M. phaseolina* of mungbean. They observed that the pH levels of 7.0 was recorded (89.66) mm growth followed by pH 8.0.

2.3 Studies on cultural, morphological, biochemial and molecular variability among isolates of *Sclerotium rolfsii*

2.3.1 Cultural and morphological variability of isolates of *Sclerotium rolsii*

Ansari and Agnihotri (2000) studied the variation existing among 40 isolates of *S. rolfsii* collected from different soybean growing areas in India and categorized into groups based on morphological characters of sclerotia like sclerotial arrangement, size and colour on potato dextrose agar medium.

Prabhu (2003), studied the variation existed among isolates *S. rolfsii* isolates collected from soybean growing areas and reported that, isolates showed marked differences in their growth rate, sclerotial initiation, colour, size, number and test weight of sclerotia.

Jyothi (2006) studied variation among the isolates of *S. rolfsii* on different crops and observed considerable variation among the isolates. The colony diameter varied from 52.00 to 89.83 mm at 72 hours of incubation. The colour of sclerotia was light to dark brown, size of sclerotia varied from 1.3 to 3.40 mm and they were spherical to round in shape. The test weight of 100 sclerotial bodies was recorded between 73.00 to 383.30 mg and number of sclerotia per cm^2 ranged between 1.14 to 6.55. Groundnut isolate recorded the highest dry mycelial weight (280.70 mg) while wheat isolate recorded lowest (132.70 mg) in potato dextrose broth.

Morphological characteristics of eight fungal strains grown on PDA plates revealed that mycelial growth rate of different strains varied considerably upto three days. The formation of sclerotia initiated after 72 hours of incubation and continued till 168 hours. Initially, white colored sclerotia were formed. Then their color changed from white to off-white, light brown and dark brown as they attained maturity (Darakhshanda *et al*., 2007)

Abida *et al.* (2008) reported that isolates of *S. rolfsii* varied in colony morphology, mycelial growth rate, colony colour, sclerotial production, number and sclerotial size of sclerotia. Out of twelve isolates, colonies of seven isolates were fluffy, while five isolates were compact. The growth rate of the isolates varied substantially with isolates SRC-1, SRC-18, SRC-19 and SRC-112 were fast growing (76.7-90 mm diam.). The average size of sclerotia for most of the isolates were >40 µm in diameter. The color of sclerotia was generally dark to reddish brown at maturity

Morphological characters studied on PDA at room temperature revealed a considerable variation among ten isolates of *S. rolfsii*. With respect to various attributes studied, growth rate ranged from 0.63 to 1.25 mm per hour. Highly virulent isolates (SrNID, SrKAL and SrSAI) exhibited faster growth and sclerotial initiation as compared to less virulent isolates (Shwehta, 2010).

2.3.2 Mycelial compatibility/Vegetatively compatability groups of *S. rolfsii*

Punja and Sun (1997) observed that when mycelia of different isolates belonging to the same species confront with one another, either on agar media or a suitable growth substrate, a distinct zone of demarcation (barrage or aversion zone) was developed between the colonies. Recognition of non-self from self is the underlying basis of the incompatible reaction

Sarma *et al*. (2002) reported 13 vegetative incompatibility groups (VCG) among the isolates. In all the antagonistic reactions, sclerotia were not formed at the interaction zone. Sclerotia were formed only in the border of the lytic zone of the two isolates in only 23.6% combinations. However, a few sclerotia produced later on such lytic zone in some combinations failed to develop to the full size as those produced on the border of such barrages. Mycelial compatibility reaction was also used by Darakhshanda *et.al.* (2007) to study variability and relatedness among fungal species belonging to different geographical regions.

Abida *et al.* (2008) also studied mycelial compatibility/incompatibility among the isolates of *S. rolfsii*. Out of 66 combinations, only 26 combinations (39%) showed compatible reactions (VCG). For combinations which showed antagonistic reactions with each other, a thin band of living or dead mycelia was formed

Shanthalakshmi *et al. (*2012) reported 84 compatible reactions out of 231 parings of 22 isolates. 9 vegetatively compatibility groups (VCGs) were observed. In compatible reactions, mycelia of the two isolates intermingled at the zone of interaction without a visible border between them. A clear barriage zone of dead myelia was formed in combinations which showed antagonistic reactions with each other.

2.3.3 Oxalic acid production

Bateman and Beer (1965) observed synergistic action between polygalacturonase and oxalic acid as significant factor for destruction of plant tissue. Oxalic acid creating acid medium which is favourable for the polygalacturonase activity, by tying up of calcium in the pectage of cell wall, thereby permitting rapid destruction of pectic substance.

Punja and Damiani (1996) reported the production of oxalic acid by all the three isolate of *S. rolfsii* collected from diverse geographical areas.

Ansari and Agnihotri (2000) and Palaiah (2002) noticed oxalic acid production in culture filtrate of isolates of *S. rolfsii* from soybean and found positive correlation between oxalic acid production and virulence of the isolates.

Prabhu (2003) noticed variation in production of oxalic acid by isolates. Based on the quantity of oxalic acid produced he grouped the isolates in four groups.

Shwetha (2011) noticed the isolates differed with regard to production of oxalic acid in culture filtrate. Maximum oxalic acid production (5.50 mg/ml) was observed in highly virulent isolates like SrKAL, SrNID and SrSAI. However, less virulent isolate viz., SrBEN produced least oxalic acid (1.30 mg/ml).

2.4 Genetic variability

Information on molecular polymorphism is reviewed here with special emphasis on Random amplified polymorphic DNA (RAPD) and ITS.

2.4.1 Random Amplified polymorphic DNA (RAPD)

RAPD technique has quickly gained wide spread acceptance and application because it has provided a relatively simple tool of genetic variability studies in biological systems. However, RAPD is the best assay when the nucleotide sequence is not known. Unlike other PCR protocols, which utilize, two primers of defined sequence, RAPD detects nucleotide polymorphism using only one primer of an arbitrary nucleotide sequence.

RAPD involves the use of single short random oligonucleotide sequence (called random primers) defined cyclic amplification of DNA, which exposes the polymorphism, distributed throughout the genome. The amplified fragments are called random amplified polymorphic DNA (Williams *et al.,* 1990).

Punja and Sun (2001) studied the genetic relationships among 132 isolates of *S. rolfsii* collected during 1967-97 from 36 different host species over a wide geographical range representing 13 countries. They investigated variability using mycelial compatibility grouping and RAPD analysis. A smaller group of 15 *S. delphinii* Welch. isolates from five host species and a limited geographical distribution was also studied. Some mycelia compatibility group in *S. rolfsii* and *S. delphinii,* that had identical RAPD patterns were considered to be clonally derived. The extent of genetic diversity among the isolates of *S.delphinii* was lower than that observed in *S. rolfsii.*

Francisco and Patrica (2001) reported genetic variability in *Sclerotium cepivorum* Berk. which causes white rot of garlic. The PCR amplification of ribosomal 18S gene generated a DNA fragment of a size close to 22 kb in all isolates and reference strains, as compared to that of 1.8 kb amplified in control fungi. Variability was analysed by RAPD technique. Some of the bands identified here can be useful as molecular markers in identification studies of this plant pathogen. Dendrogram analysis of data revealed a tendency of isolates to group according to the geographic precedence.

Tyson *et al.* (2002) studied a subset of 51 *S. cepivorum* Berk. isolates and investigated for genetic diversity using universally primed PCR and Random Amplified Polymorphic DNA analysis. Results revealed that, from two primers (L15 and OPA X 15) were combined into six genetic groups. Molecular methods demonstrated the presence of genetic diversity among New Zealand field populations of *S. cepivorum*.

Saddala *et al*. (2010) reported genetic variability among the virulent isolates of *Sclerotium rolfsii* using molecular techniques like RAPD. The RAPD banding pattern reflected the genetic diversity among the isolates by formation of two clusters. A total of 221 reproducible and scorable polymorphic bands ranging approximately as low as 100 bp to as high as 2500 bp were generated with five RAPD primers.

2.4.2 Genetic variability by Internal Transcribed Spacers (ITS) region of rDNA

Harlton *et al*. (1995) screened a worldwide collection of *S. rolfsii* which revealed variation in ITS regions of 12 sub-groups of *S. rolfsii.* Almeida *et al.* (2001) studied variability among thirty isolates of *S. rolfsii* from different hosts and regions of Brazil by RAPD and were differentiated into distinct groups by ITS-PCR. The 'ITS types' within isolates were almost phylogenetically distinct. There was no clear correlation between ITS based phylogeny and isolate origin.

Saddala *et al*. (2010) reported that the primers *viz*., ITS-1 and ITS-4 were used for PCR amplification of ITS region of rDNA cluster which includes ITS-1, 5.8S and ITS-2 regions of all eight isolates. ITS region of rDNA amplification with specific ITS1 and ITS4 universal primers produced approximately 650 to 700 bp in all the isolates confirmed that all the isolates obtained are *Sclerotium rolfsii.*

2.5 Estimation of loss

2.5.1 Growth and yield parameters

Estimates of loss are a pre-requisite to the rational development of any agricultural research programme with an important component of plant protection (Campbell and Madden, 1990; Gaunt, 1987; Teng and Johnson, 1988). Reliable estimates of loss facilitate the objective of identification of the relative importance of biotic pests. Consequently, limited resources can be assigned on a priority basis and optimize returns from a given effort. Accurate information concerning losses is also needed by growers and plant protection specialists to develop decision thresholds for determining, when cost effective control measures should be deployed. The need for reliable crop loss assessment assumes added importance, given the current worldwide concern about improving or maintaining environmental quality by reducing the use of pesticides (Stern *et al*., 1959).

Results of the loss estimation with respect to growth and yield parameters obtained were almost similar during both the years (2007-08 and 2008-09). Maximum suppression of the disease was observed in carboxin+thiram treatment which recorded significantly superior growth parameters, *viz*. plant height and number of branches. The same could reduce the disease incidence to the extent of 68.85 -69.99 per cent and recorded the increase in yield up of 218.86 per cent and 147.60 per cent during 2007-08 and 2008-09 respectively (Ammajamma, 2010).

2.5.2 Biochemical parameters

Phenols

It has been widely recognized that, the aromatic compounds such as mono and dihydric phenols, phenolic glucosides, flavonoids, anthocyanidins, aromatic aminoacids and coumarin derivatives are increased in host tissues invaded by a parasite. One of the major biological properties of phenolic compounds is their antimicrobial activity and it is often assumed that their main role in plants is to act as protective compounds against disease agents such as fungi, bacteria and viruses. Involvement of phenolic compounds in many aspects of plant-parasite relationship other than plant protection has been reported by Friend in the year 1979. The role of phenolics in the mechanism of disease resistance in plants has been reviewed by several workers (Walker and Stahmann, 1955; Farkas and Kiraly, 1962; Tomiyanma, 1963; Kuc, 1966; Klement and Goodman, 1967 and Rohringer and Samborsk, 1967; Arora and Wagle, 1985 and Saini *et al*., 1988) and found that the concentration of phenolic compounds is usually higher in resistant than in susceptible genotypes of different crop plants. Studies have also shown that, qualitative and quantitative changes in these compounds occur after infection (Arora and Wagle, 1985 and Luthra *et al*., 1988).

Sharma *et al*. (1992) studied the biochemical relationship in resistant and susceptible cultivars of maize with *Turcicum* leaf blight. Biochemical analysis were done at 15 days interval after 30 days of sowing. Total sugars and free aminoacids were reduced in all the cultivars, but the rate of reduction was more in susceptible than in resistant cultivars.

The total phenolic content in healthy and diseased leaves was found non-significant. Due to infection with *S. rolfsii*, the total phenol content increased in potato (Basamma, 2008).

Sugars

In general, infection by some pathogens bring about lot of changes in the photosynthetic and respiratory pathways, which are very vital processes occurring in the plants. This leads to a wide fluctuations in sugars in the plants (Klement and Goodman, 1967).

Kuprevica (1947) studied the physiology of the diseased plants in relation to the general question of parasitism and reported that, the sugar content of infected plant cell sap from leaves and stems was correspondingly less in diseased plants.

Horsfall and Diamond (1957) classified the diseases of plants based on sugar content and the incidence of disease as “low sugar” and high sugar” diseases. Basamma (2008) reported that, there was significant increase in the reducing sugars in healthy potato tubers while reducing, non-reducing and total sugars decreased in infected tubers.

2.6 Epidemiological studies

2.6.1 Inoculum level and infection

Garrett (1965) defined inoculum potential as the energy for growth of a parasite available for infection of a host, at the surface of the organ to be infected. For development of root rot diseases, certain number of fungal propogules should always survive in soil.

Rattink (1986) reported the influence of inoculum level of *F. oxysporum* f.sp. *dianthi* on wilt disease development, viability and pathogenicity. Shalini (2006) reported that, the increase in per cent inoculum increases the disease incidence in rhizome rot of ginger and turmeric caused by *F. solani*. However, considerable amount of infection was recorded in two per cent inoculum and 100 per cent disease in six per cent and above inoculum levels (Nargund, 1981).

Palakshappa *et al.* (1987) observed considerable foot rot infection, when betelvine were inoculated with two and three per cent inoculum. They also recorded 100 per cent infection at four per cent and above inoculum levels. Harlapur (1998) reported that, two per cent inoculum was essential for infection. But, maximum infection (100%) was noticed in inoculum level of more than four per cent in foot rot disease of wheat.

Singh and Thapliyal (1998) reported inoculum density levels of 2.5 to 10 g kg^{-1} soil significantly increased the pre emergence rot which ranged from 36.7 to 90 per cent seed and seedling rot of soybean caused by *S. rolfsii* respectively. Hanumanthegowda (1999) observed 92.50 per cent pre-emergence collar rot of groundnut at two per cent inoculum level and above two per cent lead to 100 per cent seedling mortality.

Prabhu (2003) studied the effect of inoculum density on foot rot of soybean which indicated cent per cent pre-emergence disease incidence in four per cent and above inoculum levels and maximum post emergence disease incidence was noticed in three per cent inoculum level. The disease intensity ranged from 36.70 to 90 per cent in seed and seedling rot of soybean caused by *S. rolfsii*.

2.6.2 Effect of soil temperature on *Sclerotium rolfsii*

Rolfs as early as in 1892 observed that moist warm weather was necessary for serious disease outbreaks. Higgins (1927) emphasized that, temperature was the limiting factor in the geographical distribution of the fungus.

Gondo (1962) reported the optimum soil temperature of 30^0C for mycelia and 25^0C for sclerotia. Weerapat and Schroeder (1966) observed that the optimum soil temperature for the growth of rice seedlings and the two strains of *S. rolfsii* were 30-35^0C. Infection occurred at all temperatures and severe disease development occurred between 25 to 35^0C. However, the severity of the disease was greatest at 30^0C.

Manjappa (1979) reported that the sunflower isolate of *S. rolfsii*, made maximum growth at 30^0C which was significantly superior to the growth at all the temperature levels followed by 25^0C. They concluded that the optimum temperature for the growth of *S. rolfsii* was between 25-30^0C. However, there was no significant difference between the temperatures of 20^0C and 35^0C and 15^0C. Least growth was noticed at 40^0C. Harlapur (1998) reported that in foot rot of wheat caused by *S. rolfsii*, the optimum soil temperature for growth and activity of the fungus was 25 and 30^0C and the growth was ceased at 45^0C.

2.6.3 Effect of soil moisture on *Sclerotium rolfsii*

Epps *et al.* (1951) observed no growth of fungus through sand from infected wheat seeds when the moisture content was 0.93 per cent or less, but good growth occurred at 1.02 per cent. They further reported that, *S. rolfsii* is capable of growing from inoculum through soil at a moisture level much below that is required for seed germination of rice and soybean. Flados (1958) found reduction in growth of the fungus with the increase in soil moisture.

Lingaraju (1977) reported that the saprophytic activity of the fungus was more at 10 per cent soil moisture and the fungus did not survive well at 50 and 70 per cent moisture levels. Khati *et al.* (1983) reported that the survival of *S. rolfsii* was highest at soil moisture levels between 30 and 50 per cent of water holding capacity. Nargund *et al.* (1983) observed that, the wheat seedlings survival and yields were considerably higher in *S. rolfsii* sick soil plots receiving 4-6 irrigations than those with 1-3 irrigations or none.

Palakshappa (1986) studied the effect of seven soil moisture levels on foot rot of betelvine caused by *S. rolfsii* and reported that, the fungus *S. rolfsii* survived better at low soil moisture levels than at high soil moisture levels. The survival range was highest between 20 and 40 per cent soil moisture levels. However, highest saprophytic activity of the fungus was observed at 40 per cent moisture level (86.66%). Least was found at 60 and 70 per cent soil moisture levels where the saprophytic activity of the fungus was found to be very less (43.33 and 30.00% respectively).

Harlapur (1998) studied the effect of soil moisture on foot rot of wheat caused by *S. rolfsii* and reported that the fungus survived better at low soil moisture levels as compared to high level. Thirty per cent of soil moisture was found to be optimum for maximum saprophytic activity of the fungus.

2.6.4 Effect of soil pH on *S. rolfsii*

The fungus is reported to make fairly good growth over a wide range of pH (Higgins, 1927; Abeygunawardena and Wood, 1957). Chowdhury (1946), while testing the effect of soil pH on the disease incidence, reported that there was no correlation between soil pH and disease incidence. The percentage of death of betelvines (*Piper betle* L.) was almost similar at all levels of pH tested *viz.,* 4.1, 5.4, 5.7, 6.0, 6.9, 7.6 and 8.5.

Mathur and Sinha (1968) observed that the infection in guar was maximum at pH 6.6 (54.2%) and in gram at 5.7 (89.6%). Alkaline condition reduced the disease in both the crops. Chattopadhyay and Mustafee (1977) reported that *S. rolfsii* from jute, showed considerable increase in population at all pH levels tested, however, growth was good at pH 5 and 6. Lingaraju (1977) found that the saprophytic activity of *S. rolfsii* from sunflower was more at pH 5.5 and 8.5.

Harlapur (1998) found that the fungus *S. rolfsii* which cause foot rot of wheat survived better in soil at wide range of pH levels. Maximum level was observed at soil pH level of 6.0

2.6.5 Viability of sclerotia at different depths and duration

Bateman and Beer (1965) reported that, *S. rolfsii* survives from year to year in sclerotial stage in soil and this might be influenced by soil environment. Coley-smith and Cooke (1971) reported that sclerotium of *S. rolfsii* can survive up to five years.

Gurjar *et al.* (2004) noticed that per cent viability of sclerotia of *S. rolfsii* was significantly reduced when buried in soil beyond 4 cm depth and the sclerotia lost their viability completely beyond 14 cm depth. With increasing duration of burial there was a gradual reduction in the viability of sclerotia, which was completely lost after 19 months of burial (Kulkarni, 2007).

2.6.6 Survival of inoculum

Fusarium solani

Mustafee and Chattopadhyay (1983) revealed that survivability of fungi increased with host tissues being most pronounced. Shyla (1998) reported that *F. chlamydosporum* survived more than 210 days in host debris under room temperature. Liddell and Burgess (1985) demonstrated that *F. moniliforme* microconidia could survive upto 900 days under various humidity and temperature conditions in the Laboratory. *F. moniliforme* conidia and hyphae survived for two Kansas winters in sorghum stalks without any loss of viability or pathogenicity (Manzo and Claflin, 1984). Nyvall and Kommedhal (1970) studied the survival of *F. moniliforme* for eight months in maize stalk residue in

Iowa field. Shalini (2006) reported the survival of *F. solani* in ginger and turmeric infected debris upto 32 weeks.

2.6.7 Identification of susceptible stage of the plant

Singh and Dwivedi (1988) recorded that, barley seedlings were found most susceptible to *Sclerotium rolfsii* during first fifteen days of growth and the per cent infection of the plant reduced with age. Similarly, Mishra and Bais (1985) also reported that the barly seedling of first 15 days were found most susceptible to *S. rolfsii* and the per cent of infection of the plant reduced with age.

Kulkarni *et al*. (1994) while studying the most susceptible growth stage of groundnut to *S. rolfsii* reported maximum infection, colonization, disease development and mortality in 15 days plants and least, mortality in 105 days old plants.

2.6.8 Interaction among the pathogens

In nature, plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognized that “nature does not work with pure cultures” and that many plant diseases were influenced by associated organisms.

There is no doubt about pathogenic potential of *R. bataticola, F. chlamydosporum, S. rolfsi* and *R. solanacearum* on coleus. Whether these pathogens alone or are part of disease complexes is not certain. This has been an area of research in recent years. Such studies would help us to target control measures against appropriate pathogen. Dake and Edison (1989) stated that, *F. oxysporum* can form complex with *Pythium spp.* They also reported association of *Pythium spp, Fusarium spp*. or *R. solanacearum* in rhizome rot complex of ginger in Kerala.

Shalini (2006) reported complexity of disease due to *Pythium aphanidermatum* and *F. solani*. Disease complex between *Pythium spp.* and *Fusarium spp.* was known with other pathosystems (Hendrix and Campbell, 1973). Association of *Pythium spp*. and *Fusarium* especially *F. solani* and *F. equiseti* have been demonstrated by Bhardwaj *et al*. (1988).

Ammajamma (2010) reported that when all pathogens were simultaneously (*Rhizoctonia + Fusarium + Sclerotium + Ralstonia*) inoculated, the plants exhibited the symptoms within 15 days and recorded more disease incidence when compared to independent inoculations.

2.6.9 Host range studies

Epps *et al.* (1951) demonstrated pathogenicity of four isolates of *S. rolfsii* on several hosts and observed the variations in the level of infection and number of plants killed.

Sengupta and Das (1970) studied the cross inoculation of isolates of *S. rolfsii* from groundnut, wheat, potato, guava and bengalgram. They found that, bengalgram was the most susceptible host. Although isolates were most virulent to their appropriate hosts and specialization was not demonstrated conclusively.

Hall (1991) reported that *S. roflsii* infects more that 500 species of monocotyledonous and dicotyledous plants, but especially severe on legumes, soalanceous crops, cucurbits and other vegetables grown in ratation with beans.

Hegde *et al.* (2010b) reported that *S. roflsii* infects many of medicinal and aromatic plants like patchouli, aloe, coleus, rose merry, brahami, shatavari and tulsi.

Sclerotium rolfsii is a polyphagous pathogen which infects many of the cultivated crop plants *viz*., potato, chilli, groundnut, sunflower, onion, tomato, betel vine *etc*. (Agrios, 2005).

2.7 Management studies

2.7.1 *In vitro* evaluation of botanicals

Plant derivatives possessing pesticidal properties are gaining worldwide importance as alternative or supplement for the existing pesticides because of low cost, less environmental hazards and no risk of development of resistance by the pathogens. These chemicals are also known to leave harmful residues on plants and have deleterious effect on the existing eco system (Toriyama, 1972)

Sclerotium rolfsii

Mycelial growth and sclerotial production were completely inhibited by different concentrations of *Ageratum conyzoides, Eupatorium cannabinum* and *Crotalaria medicaginea* (Kumar and Tripathi, 1991).

Dayaram and Tewari (1994) observed that soil application of green leaves of *Azadiracta indica* and *A. vasaka* were very effective for controlling collar rot of chickpea caused by *S. rolfsii*.

Seshakiran (2002) tested the role of plant extracts in the management of stem rot of groundnut caused by *Sclerotium rolfsii* and reported that among 30 plant extracts evaluated *in vitro* by cold aqueous method, leaf extract of *Agave americana* L. exhibited maximum inhibition of mycelial growth and sclerotial formation at 10 per cent. Out of 29 plant extracts evaluated *in vitro* by hot aqueous method, leaf extracts of *Prosopis juliflora* (Swartz) Dc. exhibited maximum inhibition of mycelial growth and sclerotia formation at 10 per cent concentration.

Shwetha (2011) also reported the efficacy of Eupatorium and *A. indica* against *S. rolfsii* causing wilt of stevia.

Fusarium solani

Spore germination of all the fungi tested was completely inhibited by 100% aqueous extract of *Azadiracta indica* while only 20% of germination of spores was observed with treatment of 100% aqueous extract of *Alium sativum* (Tripathi *et al.,* 1999).

Leaf extracts of *Lantan camara* followed by *A. indica,* and *Acalypha indica* were found to be equally effective in inhibiting the growth of *F. solani in vitro*. Leaf extracts of *L. camara* has been reported to exhibit maximum toxicity against spore germination of *F. solani* (Mamatha and Ravishankar Rai, 2004)

Among eleven plant extracts tested against *F. solani,* neem seed kernel extract at 10 per cent was significantly superior to other plant extracts followed by Eucalyptus, garlic and parthenium (Sreedevi, 2007)

Rhizoctonia bataticola

Sindhan *et al. (*1999) reported the efficacy of leaf extracts of *Azadiracta indica, Mentha arvensis* L., *Eucalyptus globules* L., *Ocimum sanctum* L., *Duranta alba* L., *Bougainvillea spectabilis* L., rhizome extract of *Zingiber officinalis*, bulb extract of *Allium sativum* and *Allium cepa* against the mycelial growth of *R. solani* and *R. bataticola* in vitro at 5, 10 and 20 per cent concentrations. Results showed that all the plant extracts were inhibitory to *R. solani* and *R. botaticola* even at five per cent.

Magar *et al*. (2011) reported that bulb extract of garlic was most effective against *M. Phasiolina* causing leaf spot in green gram followed by ginger and onion at 10 per cent.

2.7.2 *In vitro* evaluation of bioagents

Biological control of soil borne diseases is a popular and challenging goal and has been a focus for research since many years. Biological control is attractive in an environmental and economic sense because it offers safe and cost effective alternative to fungicides.

Sclerotium rolfsii

Bagwat (1997) tested the antagonistic organisms against *S. rolfsii*. Among them, *Trichoderma harzianum* was found to be superior which produced an inhibition zone of 3.27 mm. Maximum reduction of sclerotial bodies was observed in *T. harzianum* followed by *T. viride.*

Baswaraj (2005), Kulkarni (2007) and Basamma (2008) reported that among the bioagents *T. harzianum* and *T. viride* were found to be highly effective in reducing wilt in potato wilt by *Sclerotium rolfsii*. Hegde *et al*. (2008) and Shwetha (2011) reported that *T. harzianum* causes a maximum *in vitro* inhibition of *S. rolfsii* causing wilt of stevia followed by *T. Viride.* Bacillus isolates were least effective.

Fusarium solani

Ram *et al. (*1997) reported that, biocontrol agents, *T. harzianum* and *Pseudomonas fluorescens* when introduced to soil for control of rhizome rot of ginger caused by *F. solani* and *P. myridylum* effectively inhibited the growth of pathogen.

The fungal antagonist *T.harzianum* inhibited the growth of *F. moniliformae* Similarly, the bacterial antagonist, *B. subtilis* showed maximum inhibition compared to *P. fluorescens* in controlling *F. moniliforme* (Karunakaran *et al*., 2003).

Verma and Sharma (2007) revealed that *T. harzianum* causes a maximum (72%) *in vitro* inhibition of *F. Solani* causing wilt of mango seedlings followed by *T. viride, T. virens, and T. hamatum*. Bacillus isolates were least effective.*Rhizoctonia bataticola*

The fungal antagonists like *Trichoderma viride T. harzianum, T. hamatum, T. koningii, T. pseudokoningii, T. longibrachirum, and Gliocladium virens* were evaluated against blackgram root rot fungus *Macrophomina phaseolina*, *T harzianum* and *T. longiorachiarum* were on par in controlling *M. phaseolina* (Indra *et al*., 2003).

Kamalakannan *et al.* (2003) studied the effects of volatile and diffusible compound of two *Trichoderma. spp* (*T. harzianum* and *T. viride*), two *Pseudomonas fluorescens* (Pf 6 and Pf1) isolates and *Bacillus subtilis* (BSC 7 and BSC 8) isolates against coleus root rot pathogens like *R. solani* and *M. phaseolina*. Volatile compounds of *T. harzianum* and Pf1 effectively reduced the Sclerotial production of *R. solani*. Similarly, volatile compound of Pf 1 and *T. harzianum* effectively inhibited the mycelial growth and sclerotial production of *M. phaseolina.*

2.7.3 *In vitro* evaluation of fungicides

Sclerotium rolfsii

Prabhu (2003) studied the *in vitro* evaluation of different systemic and non systemic fungicides against collar rot of soybean caused by *Sclerotium rolfsii* and reported cent percent mycelial inhibition by carboxin, carbendazim (63%)+ mancozeb (12%) and propiconazole. Thiram was most effective while zineb showed least effectiveness.

Kulkarni (2007) reported that systemic fungicides *viz.,* carboxin, carbendazim +mancozeb, tridemefon and propiconazole were effective against *Sclerotium rolfsii*. Among non systemic fungicides, emissan, zineb and mancozeb recorded cent percent inhibition followed by captan (88.51%) at 0.3% concentration.

Hegde *et al.* (2010a) reported that among the six systemic fungicides evaluated, carbendazim + mancozeb, carboxin + thiram, propiconazole, hexaconazole and benomyl completely (100 %) inhibited the growth of the fungus at all concentrations tested. Least inhibition of mycelial growth of pathogen was observed in carbendazim (57.74 %).

Shwetha (2011) reported that among systemic fungicides tested, propiconazole, difenconazole and hexaconazole were highly effective at all the concentrations tested. And among the non-systemic fungicides captan, zineb, chlorothalonil and mancozeb and among combi products carboxin + thiram, tricyclazole + mancozeb, carbendazim + mancozeb and zineb + hexaconazole were found to be highly effective in inhibiting mycelial growth at all the concentrations tested

Fusarium solani

Kapoor and Kumar (1991) reported that carbendazim and benomyl (500 mg a.i. /ml) were highly toxic to *Fusarium solani*. Etebarian (1992) found that iprodione and carbendazim totally inhibited fungal growth of *F. solani* at 10 ppm and 100 ppm. Wahid *et al.* (1995) concluded that Derosol (carbendazim) and benlate (benomyl) at 10 ppm completely inhibited the growth of *F. solani* which was isolated from soybean seed. Topsin-M and vitavax gave 100 percent inhibition at 50 ppm.

Growth and sporulation of *Fusarium solani* causing root rot of garden rue was completely inhibited by methoxy ethyl mercury chloride (1000 ppm), thiram (1000 ppm) and carbendazim (50 ppm) (Rathnamma, 1994). Sreedevi (2007) repoted that among systemic fungicides, carbendazim and carbendazim + mancozeb were superior followed by propiconazole and vitavax. Among non systemic fungicides, mancozeb at 0.2 and 0.3 per cent and propineb at 0.3 per cent completely inhibited the growth of *F. solani*

Rhizoctonia bataticola

Peshney *et al.* (1992) reported that growth and sclerotial germination of *R. bataticola* were effectively controlled by thiram (0.2%), captan (0.2%), mancozeb (0.2%), iprodione (0.2%), carbendazim (0.2%) and tridemorph (0.7%).

Kulkarni (2000) screened various chemicals against safflower root rot caused by *M. phaseolina* and concluded that carbendazim and propiconazole were the most effective fungicides.

Four systemic and three non-systemic fungicides were evaluated against the *M. phaseolina in vitro*. Benomyl significantly inhibited the growth of the fungus at 250 ppm followed by carbendazim among systemic fungicides. Among non-systemic fungicides, mancozeb and thiram at 500 ppm inhibited significantly the fungal growth followed by captan (Lokesha, 2003).

Magar *et al. (*2011) reported that among various fungicides tested *against M. phaseolina* causing leaf spot in green gram, carbendazim (0.1%), propiconazole (0.05%) and mancozeb (0.25) were very effective. Carbendazim (500 ppm), chlorothalonil (500 ppm) and hexaconazole (500 ppm) were very effective against *M. Phaseolina* causing root rot of safflower (Khalikar *et al*., 2011)

2.7.4 *In vivo* disease management

2.7.4.1 Greenhouse evaluation of organic amendments

Gurjar *et al.* (2004) studied the effect of organic amendments like farm yard manure, vermicompost, cotton oil cake, mustard oil cake, castor oil cake, neem oil cake and groundnut oil cake against collar rot of chilli caused by *S. rolfsii*. All amendments were found significantly superior compared to control. Neem cake was found most effective with the least disease incidence of 18.50 per cent.

Baswaraj (2005), worked on management of wilt of potato caused by *S. rolfsi* and reported that organic amendments *viz.,* neem cake with oil and neem cake without oil were found effective in reducing the disease incidence.

Vinod Dange (2006) studied the effect of organic amendments *viz.,* farm yard manure, vermicompost, neem cake, paddy hull, wheat bran and groundnut oil cake against root rot of chilli caused by *S. rolfsii*. Farm yard manure was found most effective with the least disease incidence of 19.34 per cent.

Kulkarni (2007) tried five organic amendments (farm yard manure, sunhemp green manure, vermicompost, neem cake and safflower cake) against potato wilt caused by *S. rolfsii* in green house conditions. He noticed least disease incidence (40%) in farm yard manure amended soil, followed by vermicompost (41.25%) and neem cake (42.50%).

2.7.4.2 Integrated disease management

Literature related to management of wilt of stevia involving soil borne fungal pathogens under field conditions is very limited. Hence, efforts have been made to review the work done on closely related aspects.

Though the use of bio control agents offers an environmental friendly, economic and safer alternative for fungicides. They often give better results than a good fungicide (Chet, 1987). However, biocontrol agents have not attained efficiencies matching those of currently available fungicides under all environmental conditions. Therefore, it is necessary to develop methods to enhance the efficiency of biocontrol agents and to attain consistency in their performance over a wide range of agro climatic conditions.

Mukhopadhaya *et al. (*1992) found that seed treatment with *G. virens* (10^7 conidia/ml) and 0.1 per cent carboxin was very effective in controlling *S. rolfsii* in lentil, chickpea and in groundnut. Seed treatment with potential *G. virens* (1×20 spores/ml) reduced 30-40 per cent of stem rot of groundnut (Sreenivasaprasad and Rao, 1993).

Singh and Thapliyal (1998) studied the effect of seed treatment with fungicide and bioagents on seed and seedling rot of soybean caused by *S. rolfsii*. Both pre and post emergence seedling rot were effectively managed by seed treatment with carboxin and *T. harzianum* or *G. virens*. Mukharjee *et al. (*2000) noticed integration of captan (0.25%) and *G. virens* (0.1%) as the best combination to manage wilt complex of French bean, caused by *S. rolfsii* and *R. solani* under field condition.

Patibanda *et al.* (2002) reported the efficiency of *T. harzianum* alone and in combination with fungicides against sclerotium wilt of groundnut. Synergistic and positive effects on disease control was observed when *T. harzianum* was applied to soil in integration.

Kulkarni (2007) evaluated fungicides and bioagents alone or in combination as seed dressers along with soil amendments as components for integrated management of potato wilt caused by *S. rolfsii* under glasshouse conditions. He noticed least disease incidence (13.33%) in treatment consisting carboxin + *T. harzianum* + farm yard manure.

EXPERIMENTAL RESULTS

In the present investigation, both laboratory and pot experiments were conducted in the department of Plant Pathology while field experiments were conducted at Medicinal and Aromatic plant unit, Saidapur Farm, Department of Horticulture, University of Agricultural Sciences (UAS), Dharwad. The results including *in vitro* studies conducted during the period 2008 to 2012 and field experiments conducted during 2011-2012 are presented hereunder.

4.1 Survey for diseases of *Stevia rebaudiana*

A fixed plot survey was carried out in different parts of Karnataka *viz*., Gangavati, Nidshoshi, Gangenahalli, Hirethimmanahalli and Agoli (Koppal district), Gandhi Krishi Vignyana Kendra (Bengaluru), Kalenahalli (Mandya), Saidapur (Dharwad), Thirthalli, and Ripponpete (Shimoga), Kappadagudda (Gadag), Sirsi (North Kannada), Mysore, Raichur and Belgaum districts. During survey *Sclerotium* wilt, *Fusarium* wilt and root rot was noticed (Table 1, Fig. 1 and Plate 1).

Survey data indicated that the *Sclerotium* wilt was present in all the areas surveyed followed by *Fusairum* wilt and very low incidence of *Rhizoctonia* root rot was recorded.

Sclerotium wilt incidence ranged from 6.25 to 35.80 per cent. In Koppal district, maximum disease incidence was observed in Gangavati (35.80 %) followed by Nidshoshi (16.50%), Gangenalli (13.75%), Hirethimmanahalli (8.75%), Hosagudda (7.25%) and lowest was in Agoli (6.25%). In Shimoga district, maximum disease incidence was noticed in Rippenpete (16.25%) and Thirtahalli (8.75%). In Bengaluru district maximum disease incidence of 39.65 per cent was recorded in Thindlu followed by 18.65 per cent disease incidence in GKVK, UAS, Bengaluru In Kappadagudda (24.50 %), Kalenahalli (25.25%), Sirsi (12.50%), Mysore (19.75%), Dharwad (Saidapur) (20.00%), Raichur (24.50%) and in Belgaum (14.00%) sclerotium wilt was noticed.

Fusarium wilt incidence ranged from 5.25 to 42.75 per cent. In Koppal district, maximum disease incidence was observed in Agoli (42.75%), followed by Hirethimmanahalli (12.75%), Nidshoshi (12.28%), Gangenalli (11.25%) and Gangavati (7.50%). Maximum Fusarium wilt of 35.00 per cent was noticed in Sirsi followed by Saidapur with 25.80 per cent and Gandhi Krishi Vignan Kendra with 24.50 percent in nursaries. Least incidence of 10.00 % was recorded in Belgaum followed by Thirthalli (6.25 %) and Mysore (5.25 %). Disease was very severe in nursery bed at Saidapur, Sirsi and GKVK with 80-100% mortality.

Root rot incidence was observed in Gangavati (6.25 %) followed by Saidapur (8.75 %)

4.1.1 Pathogens causing wilt disease in *Stevia rebaudiana*

From the survey, it was found that the wilt caused by *Sclerotium rolfsii* was more severe in all the districts surveyed followed by *Fusarium solani* and *Rhizoctonia bataticola.* During the course of survey, collection and analysis of the infected root samples showed that *Sclerotium rolfsii, Fusarium solani,* and *Rhizoctonia bataticola* were found to be associated with wilted plants.

4.1.2 Symptomatology

Sclerotium wilt

Initial symptoms appeared as yellowing and drooping of leaves, later resulting in wilting of plants and with white cottony mycelial growth at the collar region Brown sclerotial bodies were observed on the diseased areas in severe cases. Fungus produced white colonies. The mycelium of the fungus was hyaline and septate. The sclerotia were abundant in number, round to oblong, initially white and later turned to brown colour (Plate 2a).

Fusarium wilt

Symptoms could be observed either in main field or in nursery.

a) Nursery: Symptoms started as yellowing followed by drying, defoliation and wilting of entire seedlings (Plate 2b).
b) Main field: Symptoms can be observed at any stage of the crop. Initially symptoms appear as yellowing of lower leaves and later resulted in drying of branches or entire plant in severe cases. Infection was observed at collar region of stem in the form of brown/ black discoloration.

Table 1: Survey for diseases of stevia in Karnataka

District	Location	Percent disease incidence (%)		
		Sclerotium wilt	*Fusarium* wilt	Root rot
Koppal	Gangenahalli	13.75	11.25	0
	Hirethimmanalli	8.75	12.75	0
	Agoli	6.25	42.75	0
	Nidshoshi	16.50	12.28	0
	Gangavati	35.80	7.50	6.25
	Hosagudda	7.25	0	0
District Mean		16.21	17.30	1.04
Bengaluru	Gandhi Krishi Vignyana Kendra	18.65	24.50	0
	Thindlu	39.65	0	0
District Mean		29.15	12.25	0
Shimoga	Thirtahalli	8.75	6.25	0
	Rippenpete	16.25	0	0
District Mean		12.50	3.12	0
Gadag	Kappatgudda	24.50	0	0
Mandya	Kalenahalli	25.25	0	0
North Kananda	Sirsi	12.50	35.00*	0
Mysore	Mysore	19.75	5.25	0
Dharwad	Saidapur	20.00	25.80*	8.75
Raichur	Raichur	24.50	0	0
Belgaum	Belgaum	14.75	10.00	0

*Disease incidence in nursery

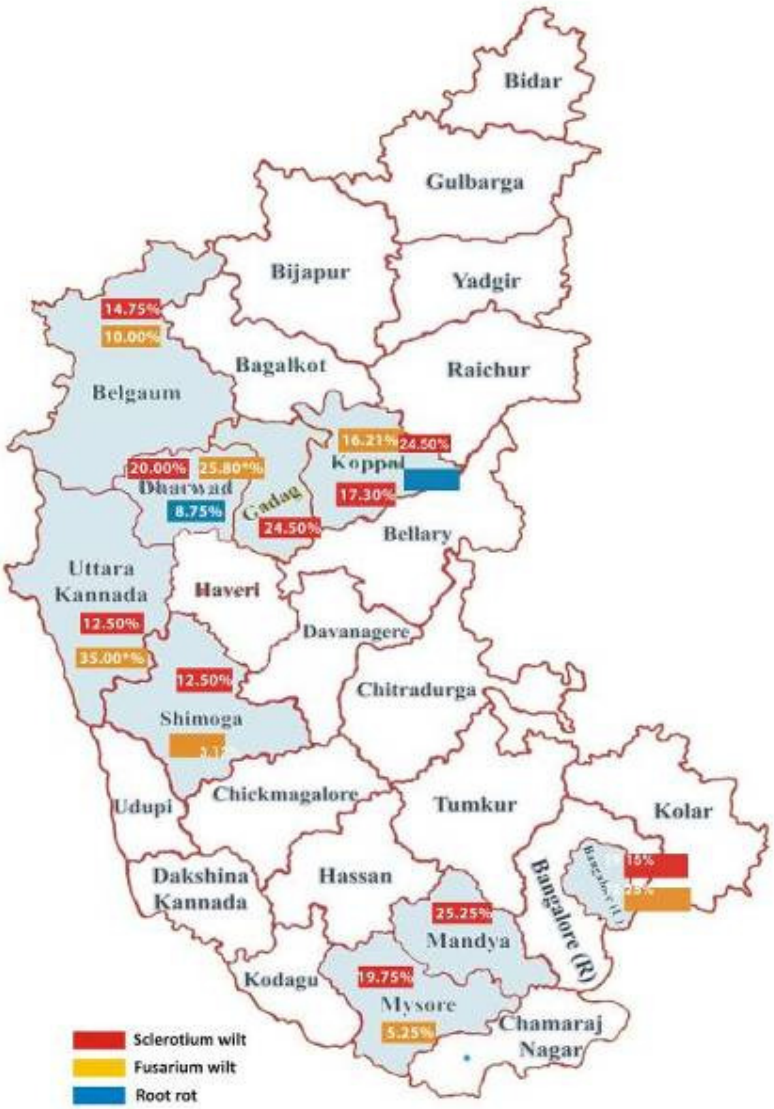

Fig 1: Karnataka map showing district wise average incidence of soilborne fungal pathogens of stevia

Healthy field

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Infected field

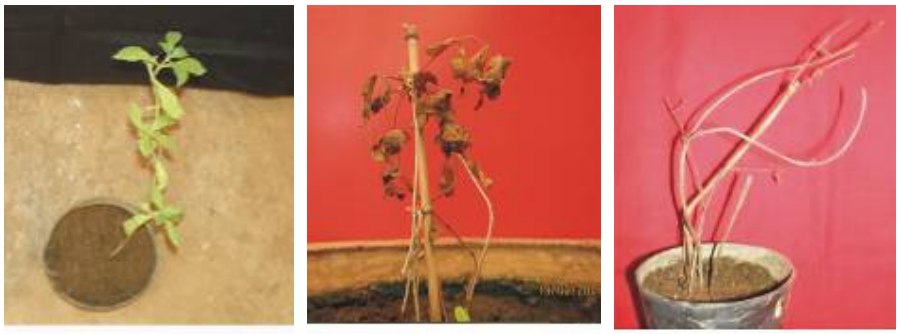
Plate 1: Wilt on farmers field in Gangavati during survey

Wilting of plants

White mycelial growth
on collar and root portion.

Discoloration

Plate 2a: Symptoms of *Sclerotium rolfsii*



Yellowing Wilting Defoliation

In nursery

Plate 2b: Symptoms of *Fusarium solani*

Wilting Discoloration Shredding

Plate 2C: Symptoms of *Rhizoctonia bataticola*

Rhizoctonia bataticola

Symptoms started as drooping of leaves, followed by drying of leaves and resulted in wilting of entire plants. Brown to black discoloration of stem, resulting in necrosis of the tissue and shredding of infected part. Symptoms were observed in nursery as well as in main field. Fungus produced white colonies in the beginning later changed to dark colour (Plate 2c).

4.1.3 Isolation of pathogens

The wilt samples collected during survey were used for isolation of pathogens. Standard tissue isolation techniques were followed to get culture of causal organisms from diseased parts of stevia as detailed in 'Material and Methods'. Pathogens isolated from infected plants were *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola. Sclerotium rolfsii* was isolated from all the locations and proved to be most predominant pathogen among the wilt causing pathogens. Next predominant pathogen was *F. solani* followed by *R. bataticola*. However, *R. bataticola* was detected only from Saidapur and Gangavati.

4.1.4 Identification of pathogens

On the basis of morphological and cultural characteristics, the pathogens were identified as *Sclerotium rolfsii* Sacc., *Fusarium solani* (Mart.) Sacc., and *Rhizoctonia bataticola*. Further, the identity of *Fusarium solani* was confirmed from Maharashtra Association for cultivation of sciences, Agharkar Research Institute, Pune.

Sclerotium rolfsii

The fungus produced white, dense radiating mycelial growth on PDA. In early stages of growth, the mycelium was silky white, it gradually lost its lustre and became dull in appearance and colony grew radially (Plate 3(1a)). Sclerotial initials were observed from 6th day onwards. At the initial stage, the bodies were white in colour later they turned chocolate brown and spherical then to dark brown at maturity. Matured sclerotia were spherical to ellipsoidal (Plate 3(1A)). The fungus isolated from the affected plant tissue was compared with the type species originally described and was found resembling with *Sclerotium rolfsii* in all the morphological character (Saccardo, 1911).

Fusarium solani

Repeated isolations from the infected plants yielded *Fusarium solani*. The culture was identified based on morphology, mycelial character, spore production and pigmentation on PDA. *Fusarium solani* on potato dextrose agar put forth moderately rapid growth covering the Petriplate in 7-10 days (Plate 3(2)). The mycelium was sparse to dense, greyish white to light pinkish in colour. Hyphae are septate and hyaline and produced both macro- and microconidia (Plate 3(2A)). Micro conidia were abundant, hyaline, continuous or one-septate, ovoid and ovate and measured 6.6 – 19.8 x 3.3 – 6.6 □m (average 13.5 x 4.3 □m) and macro conidia were 3 – 4 septate measuring 29.7 – 47.8 x 4.9 – 6.6 □m (average 9.78 x 8.11 □m).

Rhizoctonia bataticola

The morphological and cultural characters of the fungus studied showed that the growth of the fungus was fast on potato dextrose agar, mycelium was fluffy and white at initial stages of growth but turned dark brown with white periphery as the culture grew old and ultimately turned into dark brown colour (Plate 3(3)). In mycelium, right-angled branching was noticed with a small contraction at the basal part of branched hyphe (Plate 3(3A)). Excellent production of sclerotia was also observed. The sclerotia were spherical, ellipsoidal and dark brown, measuring 70 µm in diameter (Plate 3(3B)).

4.1.5 Mass multiplication of pathogens

By using sand-corn meal

Sand corn meal medium was prepared in the proportion of 90:10 to get maximum inoculum of fungus. Four hundred gram of sand corn meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.04 kg/sq cm pressure for one hour. The pure cultures of *S. rolsii, F. solani* and *Rhizoctonia bataticola* were inoculated separately to different flasks under aseptic condition and incubated at 27±1^{0}C for 20 days. The flasks were shaken on alternate days to get uniform growth. The giant cultures thus obtained were used for further studies (Plate 4).

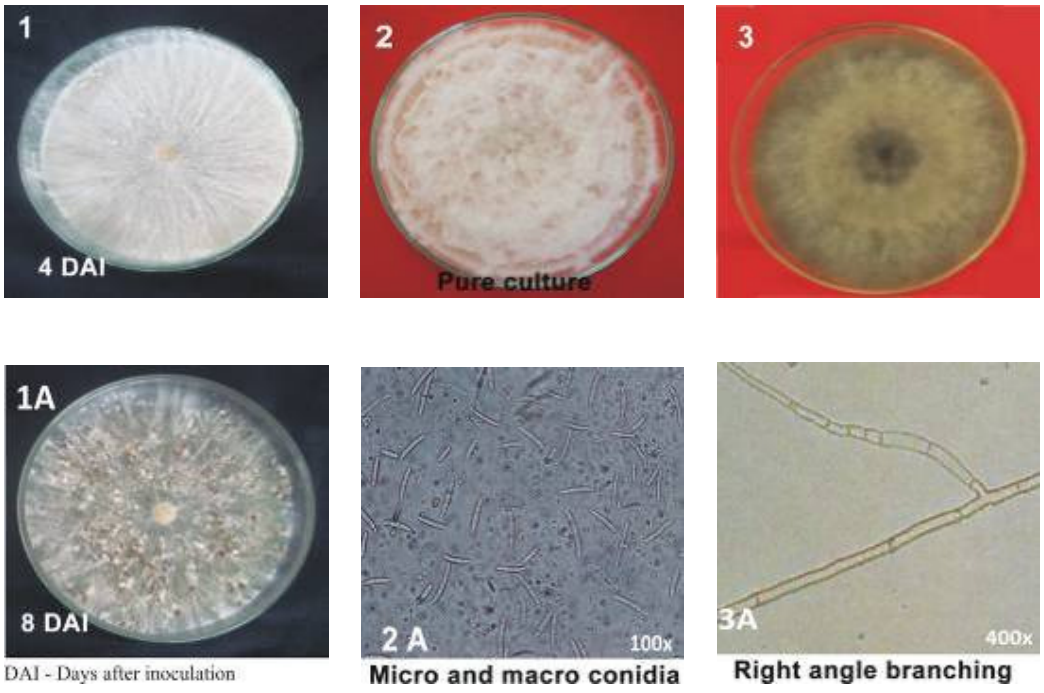
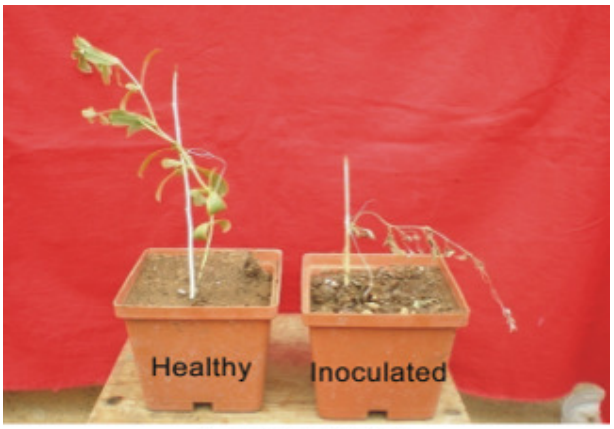


Plate 3: Cultural and morphological characters of soilborne fungal pathogens

Plate 4: Mass multiplication of soilborne fungal pathogens

A. *Sclerotium rolfsii*



B. *Fusarium solani*

C. *Rhizoctonia bataticola*

Plate 5: Pathogenicity of soilborne fungal pathogens

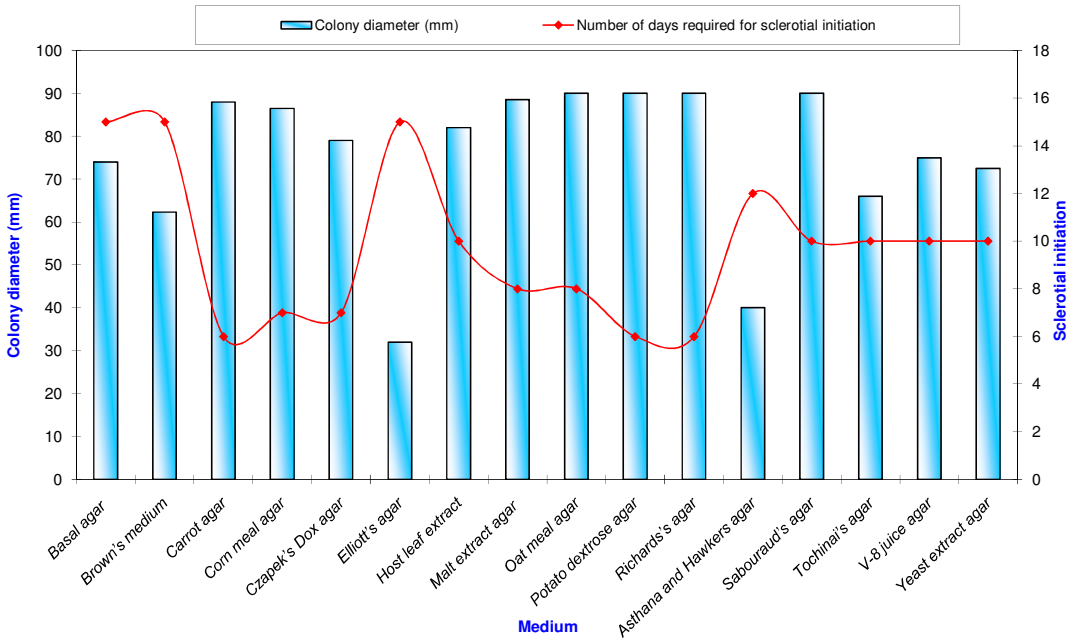


Fig. 2: Effect of solid media on mycelial growth of *Sclerotium rolfsii*

Fig 2: Effect of solid media on mycelial growth of *Sclerotium rolfsii*

Table 3: Cultural characteristics of *Fusarium solani* on different solid media

Medium	Colony diameter (mm)	Growth characters	Sporulation
Asthana and Hawker's agar	73.00	White cottony growth	++
Brown's medium	42.33	Pink cottony growth with smooth margin	+
Carrot agar	84.33	White sparse growth	++
Corn meal agar	88.16	Pink cottony growth	++
Czapek's agar	89.23	White cottony growth with smooth margin	++
Host extract agar	72.33	Pink cottony mycelium with smooth margin	++
Malt extract agar	69.00	Pinkish white cottony growth	++
Oat meal agar	90.00	White cottony growth	+++
Potato dextrose agar	90.00	Pink dense and pluffy growth, smooth margin	+++
Richards's agar	90.00	White cottony and pluffy growth, smooth margin	+++
Basal agar	48.20	Pink sparse growth	-
Sabouraud's dextrose agar	89.00	Pink cottony growth	++
Tochinai's agar	78.33	White sparse growth	++
V-8 juice agar	75.00	White cottony and pluffy growth	++
Yeast extract agar	53.77	Pink sparse growth	-
Elliots agar	52.16	White sparse growth	+
S.Em. ±	1.07		
CD at 1%	3.25		

* +++ Excellent (> 50 conidia / microscopic field)
++ Moderate (30-50 conidia / microscopic field)
+ Scanty (< 30 conidia / microscopic field)
- No sporulation

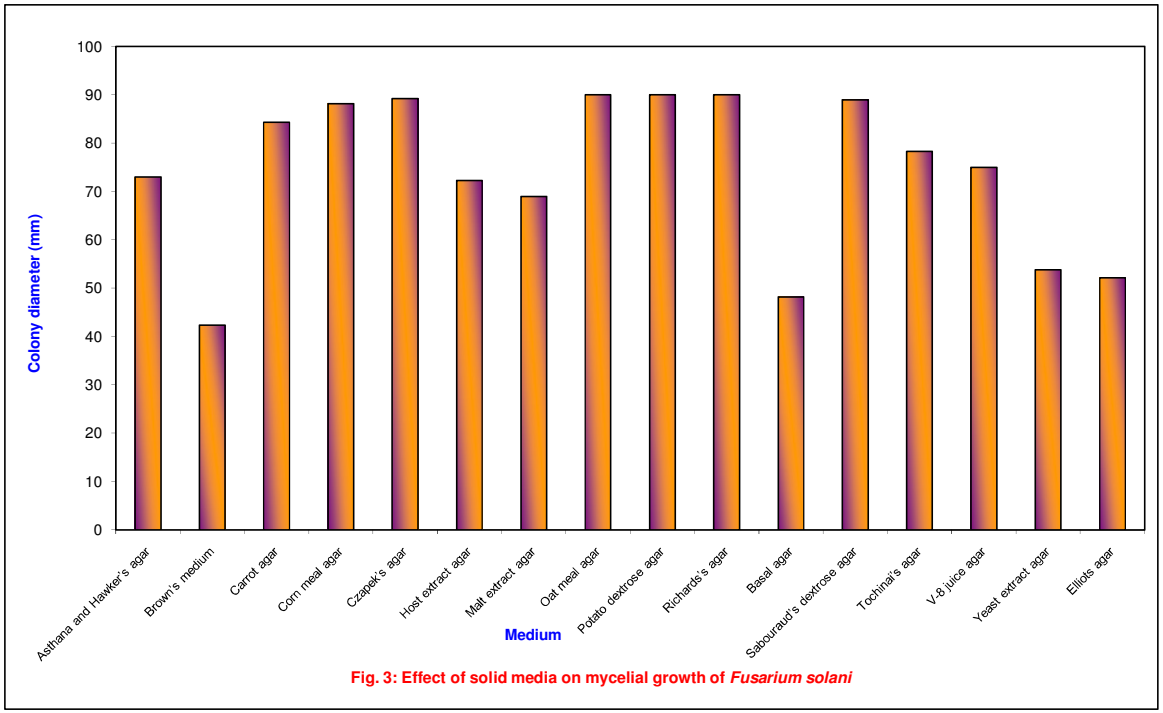


Fig. 3: Effect of solid media on mycelial growth of *Fusarium solani*

Fig 3: Effect of solid media on mycelial growth of *Fusarium solani*

Table 4: Cultural characters of *Rhizoctonia bataticola* on different solid media

Medium	Colony diameter (mm)	Growth characters	Sclerotial production *
Asthana and Hawker's agar	70.67	Good growth irregular margin, sparse mycelium, slightly raised colony, sclerotial the third day.	+
Brown's medium	71.00	Good growth, irregular margin, raised colony, sclerotial initiation on the third day.	+
Carrot agar	87.67	Good growth, smooth margin, uniformly dense mycelium initiation on the third day.	++
Corn meal agar	65.33	Moderate growth, flat colony, sclerotial initiation on the fourth day.	+
Czapek's agar	73.23	Good growth, smooth margin, colony raised, sclerotial initiation on the third day and mycelium black colour.	++
Host extract agar	61.33	Moderate growth, mycelium sporse, colony flat and sclerotia not produced.	+
Malt extract agar	81.67	Good growth, smooth margin, colony raised, sclerotial initiation on the third day.	-
Oat meal agar	32.67	Slow growth, indistinct margin, sparse mycelium, raised and black coloured colony, no microsclerotial production.	++
Potato dextrose agar	90.00	Good growth, dense mycelium, smooth margin, mycelium black colour and sclerotial initiation on the third day.	+++
Richards's agar	90.00	Good growth uniformly dense mycelium, colony raised, sclerotial initiation on the third day and mycelium balck coloured.	+++
Sabouraud's dextrose agar	49.00	Slow growth, sparse growth, colony slightly raised, sclerotial initiation on the third day	++
Tochinai's agar	74.33	Good growth, irregular margin, colony raised, sclerotial initiation on the third day.	+
V-8 juice agar	73.00	Good growth, margin smooth, colony flat, sclerotial initiation on fourth day	++
S.Em. ±	1.13		
CD at 1%	3.41		

* +++ : Excellent (> 20 microsclerotia / microscopic field)
++ : Good (> 5-20 microsclerotia /microscopic field)
+ : Poor (< 5 microsclerotia /microscopic field)
– : No microsclerotial production

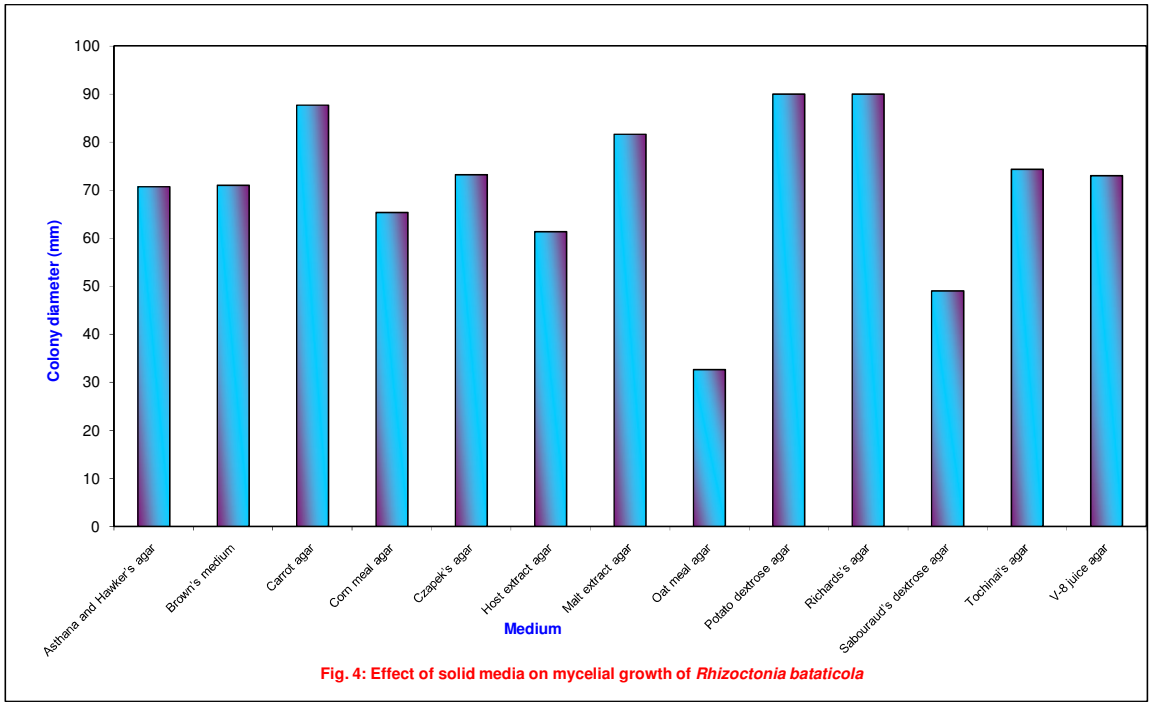
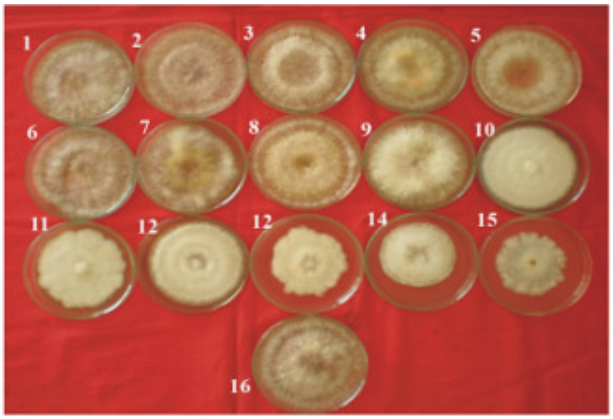


Fig 4: Effect of solid media on mycelial growth of *Rhizoctonia bataticola*

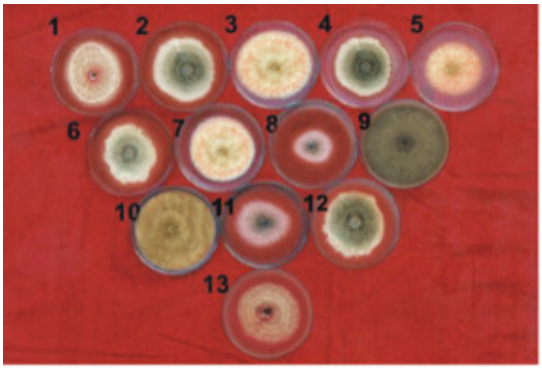
1. Richards's agar
2. Potato dextrose agar
3. Oat meal agar
4. Sabouraud's agar
5. Czapek's Dox agar
6. Corn meal agar
7. Host leaf extract agar
8. Asthana and Hawker's agar

A. ***Sclerotium rolfsii***

9. Malt extract agar
10. Carrot agar
11. V-8 juice agar
12. Tochinai's agar
13. Yeast extract agar
14. Basal agar
15. Elliott's agar
16. Brown's medium



B. *Fusarium solani*



1. Asthana and Hawker's agar
2. Brown's medium
3. Carrot agar
4. Corn meal agar
5. Czapek's agar
6. Host extract agar
7. Malt extract agar
8. Oat meal agar
9. Potato dextrose agar
10. Richards's agar
11. Sabouraud's dextrose agar
12. Tochinai's agar
13. V-8 juice agar

C. *Rhizoctonia bataticola*

Plate 6: Growth phase *of Fusarium solani* and *Rhizoctonia bataticola* in potato dextrose broth at different incubation period

4.1.6 Pathogenicity

The pathogenicity test of *S. rolfsii, F. solani and R. bataticola* was proven by soil inoculation with giant culture carried out under glasshouse conditions as per the proceedure described in 'Material and Methods'. Control was maintained without adding any inoculums (Plate 5).

Sclerotium rolfsii

Pathogen infects first at collar region. Leaves of such infected plants became pale green followed by yellowing. Later, leaves became flaccid and droop off. White, fan shaped mycelial strands crept over the stem portion, developing small light to dark brown sclerotia on the infected portion. The sclerotial initials were white at first later turned brown with age. Finally the plant wilted and dried. The fungus was reisolated from affected plant tissue and compared with the original culture, thus proving the pathogenicity following Koch's postulates (Plate 5A).

Fusarium solani

Artificial inoculation of stevia rooted cuttings were carried out as explained in "Material and Methods". The symptoms developed were recorded. The characteristic symptoms started as yellowing of lower leaves extended upwards and whole leaves gradually turned brown coloured. The infected plants showed discoloration of roots and complete destruction of root system. The bark of such plants was easily peeled off. The affected plants were killed finally due to severe rot (Plate 5B).

Rhizoctonia bataticola

Pathogenicity test for *Rhizoctonia bataticola*. was carried out as described in "Material and Methods". The disease was initially expressed as water soaked areas and the affected tissues soon turned into a soft black, watery mass at the collar region of the plant. The infection was also found on roots and caused decay, which ultimately resulted in collapse of the plant. The infected plant roots showed discoloration, followed by rotting of root hairs. Extensive sloughing and girdling off of affected bark was also observed (Plate 5C).

4.2 Cultural Studies

4.2.1 Growth characters on different solid media

Sclerotium rolfsii

Cultural characters were studied on sixteen different solid media. The radial growth of *S. rolfsii* was measured when the maximum growth was attained in any of the media tested. Observations on various cultural characters were recorded as described in "Material and Methods''. The results are presented in Table 2, Fig. 2 and Plate 6A.

The results of the cultural studies on solid media indicated that the radial growth of *S. rolsii* was maximum on Richards's agar (90 mm), Potato dextrose agar (90 mm), Sabouraud's agar (90 mm) and Oat meal agar (90 mm). These were on par with malt extract agar (88.50 mm), carrot agar (88 mm), corn meal agar (86.50 mm), host leaf extract agar (82 mm), Czapeck's Dox agar (79 mm), V-8 juice agar (75 mm), Basal agar (74 mm), Yeast extract agar (72.50 mm), Tochinai's agar (66 mm) and Brown's medium (62.33 mm). Minimum radial growth was observed in Elliott's agar (32 mm) followed by Asthana and Hawker's agar (40.10 mm).

Excellent sclerotial production was observed in potato dextrose agar, Oat meal agar, Sabouraud's agar and Richard's agar. In carrot agar, corn meal agar, host leaf extract, Czapeck's Dox agar, V-8 juice agar, Basal agar and Yeast extract agar, moderate sclerotial production was observed. Poor sclerotial production was observed in Tochinai's agar, Brown's medium, Elliott's agar and Asthana and Hawkers agar.

Sclerotial initiation was observed on sixth day in potato dextrose agar and Richards's agar, whereas in Oat meal agar, malt extract agar and Sabouraud's agar sclerotial initiation was observed on eighth day of incubation. However, in host leaf extract agar, Tochinai's agar, yeast extract agar and V-8 juice agar, sclerotial initiation was recorded on 10^{th} day. In Basal agar, Elliots agar and Brown's medium sclerotial initiation was on fifteenth day after incubation.

Fusarium solani

The effect of sixteen different media on the growth of the fungus was significant (Table 3, Fig. 3 and Plate 6B).

. The maximum radial growth of the mycelium was observed on Richards's agar (90 mm), Potato dextrose agar (90 mm) and Oat meal agar (90.00 mm). These were followed by Czapek's Dox agar (89.23mm), Sabouraud's agar (89.00mm), corn meal agar (88.16 mm), carrot agar (84.33 mm), Tochinai's agar (78.33mm), V-8 juice agar (75.00mm), Asthana and Hawker's agar (73.00mm), host leaf extract (72.33 mm) and Brown's medium (42.33mm), Minimum radial growth was observed in Basal agar (48.20mm), followed by Yeast extract agar (53.77 mm).

Sporulation was excellent in Richards's agar, oat meal agar and potato dextrose agar media. It was moderate in Sabouraud's agar, malt extract agar and Brown's medium, corn meal agar, carrot agar, host extract agar, malt extract agar, Sabouraud's agar, V-8 juice agar and Tochinai's agar and Asthana and Hawker's agar. Scanty sporulation was observed in Brown's agar. However, Yeast extract agar and Basal agar did not support sporulation.

Rhizoctonia bataticola

Among thirteen media tested, Potato dextrose agar and Richards's agar supported maximum radial growth (90 mm) of mycelium. However they were on par with carrot agar (87.67 mm), followed by malt extract agar (81.67 mm), Czapek's Dox agar (73.23mm), Tochinai's agar (74.33mm), V-8 juice agar (73 mm), Brown's medium (71 mm), corn meal agar (65.33 mm) and host extract agar (61.33 mm). Minimum radial growth was observed in oat meal agar (32.67 mm).

Excellent sclerotial production was observed in Richards's agar and potato dextrose agar. Good sclerotial production was observed in V8 juice agar, Sabouraud's agar, Czapek's agar, oat meal agar and carrot agar. Poor sclerotial production was observed in corn meal agar, Asthana and and Hawker's agar, Tochinai's agar and host root extract agar. However, malt extract agar did not support sclerotial production of the fungus (Table 4, Fig. 4 and Plate 6C).

4.2.2 Growth phase

The experiment was conducted as detailed in material and methods to ascertain the period when the maximum growth of the pathogen could occur.

Sclerotium rolfsii

The data depicted in Table 5 and Fig. 5 indicated that dry mycelial weight of *S. rolfsii* was minimum on fourth day after inoculation and on subsequent harvest, it significantly increased and finally reached maximum on 10^{th} day. Later, the growth started decreasing. Maximum mycelial growth (220.66 mg) was observed on tenth day of incubation and was significantly superior to all other treatments. This period was used as maximum growth period for further studies.

Fusarium solani

The results indicated that there was a significant difference in the growth of the fungus among the incubation period (Table 6). Maximum growth of the fungus (208.66 mg) was observed on 12^{th} day of incubation. Later the growth was decreased significantly. As the growth of the fungus was maximum on 12^{th} day of inoculation, this period was used as maximum growth period for further studies.

Rhizoctonia bataticola

Maximum growth of the fungus (234 mg) was observed on 14^{th} day of inoculation and later the growth decreased significantly. As the maximum growth was observed on 14^{th} day after inoculation, this period was used as a maximum growth period for further studies (Table 6).

4.2.3 Growth on different liquid media

Growth of the fungus was studied in the liquid media to select a medium that would support maximum growth as explained in material and methods.

Table 5: Growth phase of *Sclerotium rolfsii* in potato dextrose broth

Incubation period (days)	Mean dry mycelial weight (mg)
4	70.00
5	99.90
6	107.30
7	134.20
8	160.00
9	172.90
10	220.66
11	186.40
12	175.00
13	159.60
14	119.90
S.Em. ±	0.70
CD at 1 %	2.79

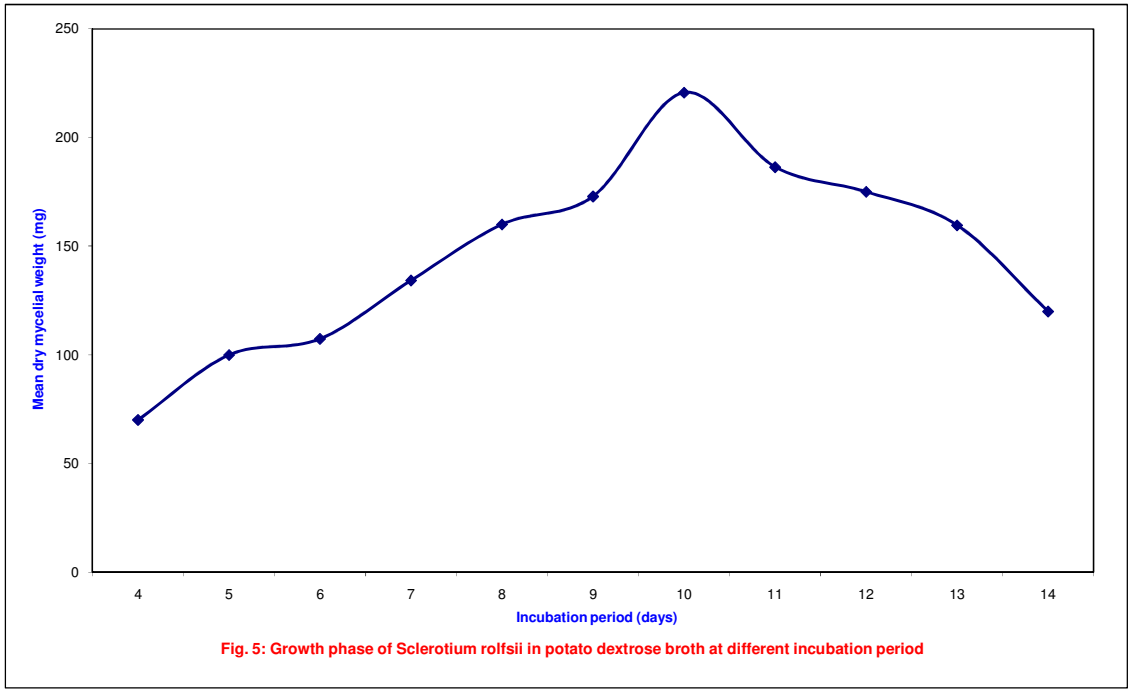


Fig 5: Growth phase of *Sclerotium rolfsii* in potato dextrose broth at different incubation period

Table 6: Growth phase *of Fusarium solani* and *Rhizoctonia bataticola* in potato dextrose broth

Incubation period (days)	Mean dry mycelial weight (mg.)	
	F. solani	*R. bataticola*
2	41.66	48.76
4	62.14	66.46
6	102.46	109.51
8	136.46	142.09
10	180.10	191.22
12	208.66	206.73
14	198.64	234.00
16	191.85	212.40
18	156.87	194.33
S.Em. ±	0.45	0.06
CD at 1 %	1.82	0.23

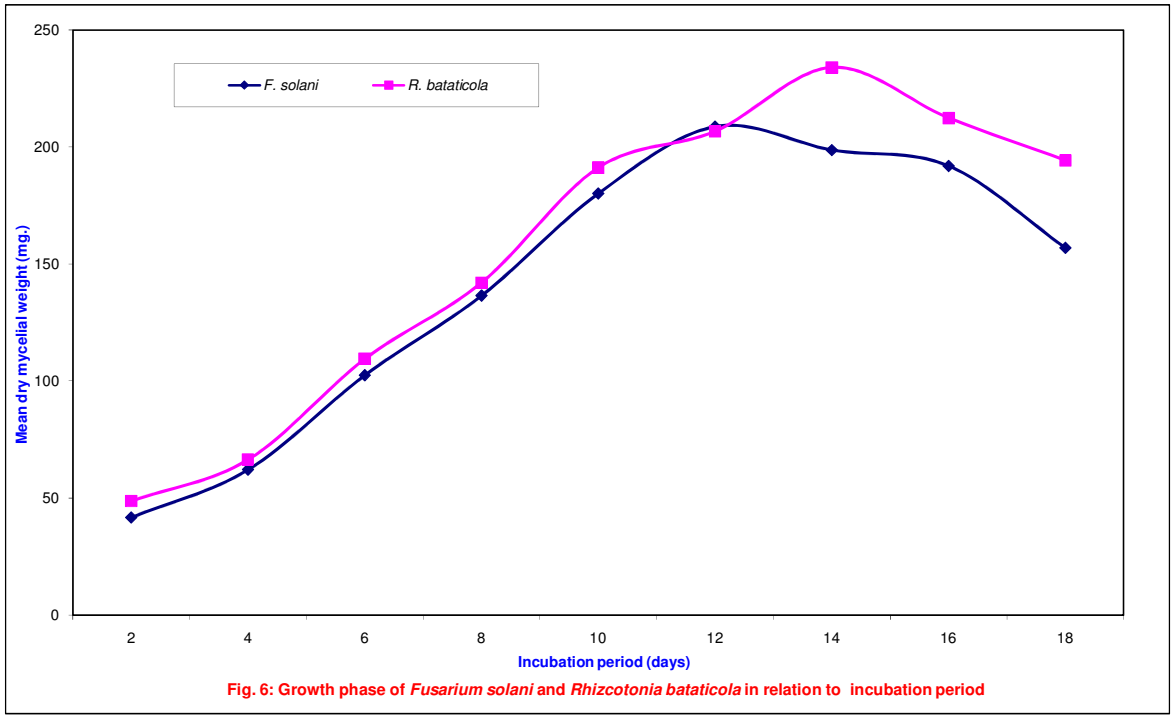


Fig. 6: Growth phase of *Fusarium solani* and *Rhizcotonia bataticola* in relation to incubation period

Fig 6: Growth phase of *Sclerotium rolfsii* in potato dextrose broth at different incubation period

Sclerotium rolfsii

Among the liquid media tested maximum dry mycelial weight of fungus was obtained in Richards's broth (248.33 mg) which was significantly superior to other media tested (Table 7a and Fig. 7a and Plate 7). This was followed by potato dextrose broth (238.67 mg), Sabouraud's broth (235.33) and Oat meal broth (250.17 mg). Least mycelial weight was obtained in Brown's medium (57.23 mg) and V8 juice broth (122.33 mg).

Fusarium solani

Results indicated that maximum dry mycelial weight of fungus was obtained in Richards's broth (362.33 mg) which was significantly superior to other media (Table 7a and Fig. 7a and Plate 7). This was followed by potato dextrose broth (352.33 mg) and Oat meal broth (315.66 mg) and host extract broth (259 mg). Least mycelial weight was obtained in Brown's medium (66.33 mg) and malt extract broth (66.66 mg).

Rhizoctonia bataticola

Among the liquid media tested, fungus growth was maximum in Richards's broth (317.70 mg) which was on par with potato dextrose broth (305 mg) (Table 7b and Fig. 7b and Plate 7). This was followed by malt extract broth (245.33 mg) and Czapeck's broth. Least growth (225 mg) was observed in Brown's broth (62.67 mg) followed by Asthana and Hawker's broth (65.33).

4.2.4 Nutritional studies

4.2.4.1 Carbon utilization

Utilization of eight carbon sources by the fungi was tested as described in material and methods. The results of the experiments are presented in Table 8 and Fig. 8.

Sclerotium rolfsii

The Effect of different carbon sources on the mycelial growth was significant. Maximum dry mycelial weight of 358.67 mg was obtained when sucrose was used as a carbon source. This was significantly superior to rest of the sources tested. This was followed by fructose (302.33 mg), glucose (296.67 mg), dextrose (265.57 mg), starch (237.35mg), and mannitol (179.67) which were on par with each other. Least dry mycelial weight was observed in maltose (149.33 mg) and lactose (111.00 mg).

Fusarium solani

Effect of different carbon sources on the mycelial growth of the *F.solani* was also significant. Maximum dry mycelial weight of 365.30 mg was obtained when sucrose was used as a carbon source. This was significantly superior to rest of the sources tested and was followed by fructose (345.33 mg), dextrose (268.50 mg), glucose (247.66 mg), maltose (184.35mg), and mannitol (156.66 mg) which were at par with each other. Least dry mycelial weight of the fungus was observed in starch (122.73 mg) (Table 8).

Rhizoctonia bataticola

Effect of different carbon sources on the growth of the fungus was significant. Maximum growth of the fungus (328.67 mg) was obtained when sucrose was used as a carbon source. It was found significantly superior over rest of the sources tested. This was followed by fructose (286.33 mg), glucose (250.17mg), dextrose (229.21 mg) and maltose (154.33 mg). Least growth of the fungus (87.13mg) was observed in case of mannitol. Since, sucrose was found to be the best carbon source, for subsequent studies, sucrose as the carbon source was used.

4.2.4.2 Nitrogen utilization

In the present investigation, utilization of eight nitrogen sources by the fungus was tested as detailed in the 'Material and Methods'. The results of the experiment are presented in Table 9 and Fig. 9.

Sclerotium rolfsii

Effect of different nitrogen sources on the growth of the fungus was significant (Table 9). Maximum growth of 302.33 mg was recorded when potassium nitrate was used as a source of nitrogen. Growth supported by ammonium nitrate (250.33 mg), asparagine (203.00 mg), ammonium sulphate (120.66 mg), and sodium nitrate (138.33 mg) were significantly different amongst each other. Minimum growth was observed in ammonium orthophosphate (84.33 mg), urea (94.26 mg) and ammonium chloride (111.80 mg).

Table 7a: Effect of liquid media on mycelial growth of *Sclerotium rolfsii* and *Fusarium solani*

Medium	*S. rolfsii*	*F. solani*
Asthana and Hawker's broth	202.50	189.66
Brown's medium	57.23	66.33
Carrot broth	176.33	288.33
Corn meal broth	188.00	211.33
Czepeck's broth	203.67	242.66
Host extract broth	212.67	259.00
Elliot's broth	87.23	63.47
Tochinai's broth	142.66	154.52
Malt extract broth	146.67	66.66
Oat meal broth	230.17	315.66
Potato dextrose broth	238.67	352.33
Richards's broth	248.33	362.33
V8 juice broth	122.33	199.00
Basal medium broth	154.77	119.33
Yeast extract broth	217.17	121.23
Sabouraud's dextrose broth	235.33	215
S.Em. $\pm$	1.57	3.85
CD at 1%	4.48	11.96

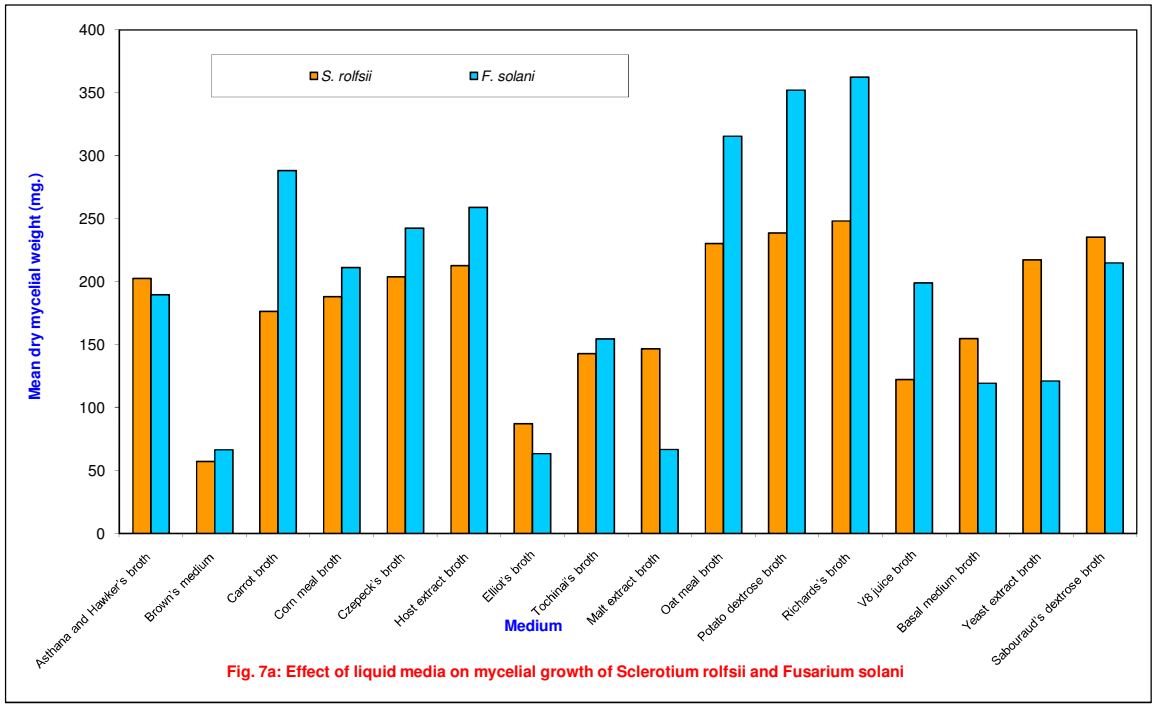


Fig 7a: Effect of liquid media on mycelial growth of *Sclerotium rolfsii* and *Fusarium solani*

Table 7b: Effect of liquid media on mycelial growth of *Rhizoctonia bataticola*

Medium	*R. bataticola*
Asthana and Hawker's broth	65.33
Brown's medium	62.67
Carrot broth	255.50
Corn meal broth	96.52
Czepeck's broth	225.00
Host extract broth	72.47
Malt extract broth	245.33
Oat meal broth	120.77
Potato dextrose broth	305.00
Richards's broth	317.70
V8 juice broth	174.17
Sabouraud's broth	206.55
Tochinai's broth	230.337
S.Em. $\pm$	4.37
CD at 1%	12.44

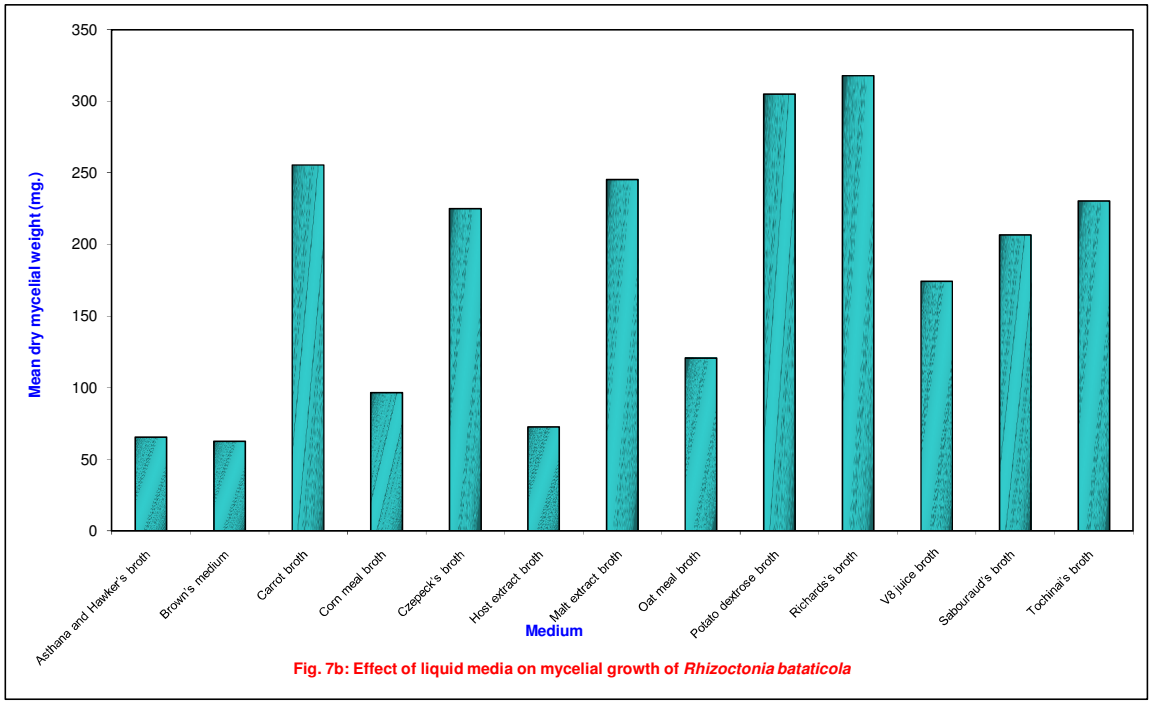
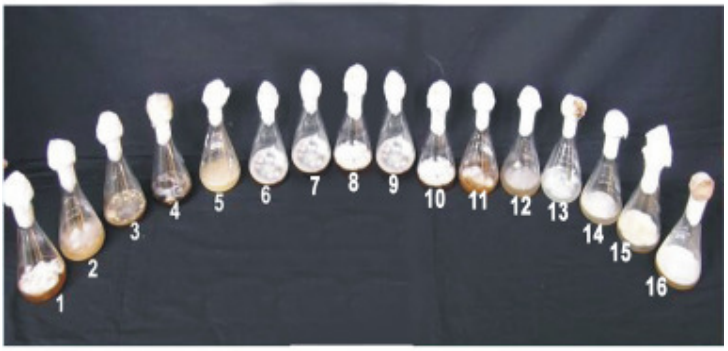


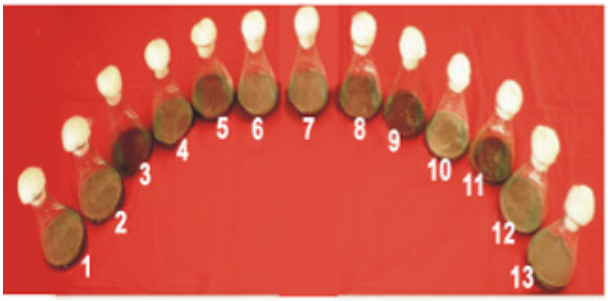
Fig 7b: Effect of liquid media on mycelial growth of *Rhizoctonia bataticola*

Sclerotium rolfsii

1. Richards's broth
2. Potato dextrose broth
3. Oat meal broth
4. Sabouraud's broth
5. Czapek's broth
6. Corn meal broth
7. Host leaf extract broth
8. Asthana and Hawker's broth
9. Malt extract broth
10. Carrot broth
11. V-8 juice broth
12. Tochinai's broth
13. Yeast extract broth
14. Basal broth
15. Elliott's broth
16. Brown's medium



Fusarium solani



Rhizoctonia bataticola

1. Asthana and Hawker's broth
2. Brown's broth
3. Carrot broth
4. Corn meal broth
5. Czapek's broth
6. Host leaf extract broth
7. Malt extract broth
8. Oat meal broth
9. Potato dextrose broth
10. Richards's broth
11. Sabouraud's broth
12. Tochinai's broth
13. V-8 juice broth

Plate 7: Cultural characters of soilborne fungal pathogens on different liquid media

Table 8: Effect of carbon sources on the growth of *Sclerotium rolfsii, Fusarium solani,* and *Rhizoctonia bataticola*

Carbon sources	Mean dry mycelial weight (mg)		
	S. rolfsii	*F. solani*	*R. bataticola*
Dextrose	265.67	268.50	229.21
Fructose	302.33	345.33	286.33
Glucose	296.97	247.66	250.17
Lactose	111.00	265.33	97.13
Maltose	149.33	184.00	154.33
Mannitol	179.67	156.66	87.13
Starch	237.35	122.73	106.13
Sucrose	358.67	365.30	328.67
S.Em. ±	0.82	1.26	0.59
CD at 1 %	2.50	3.87	1.92

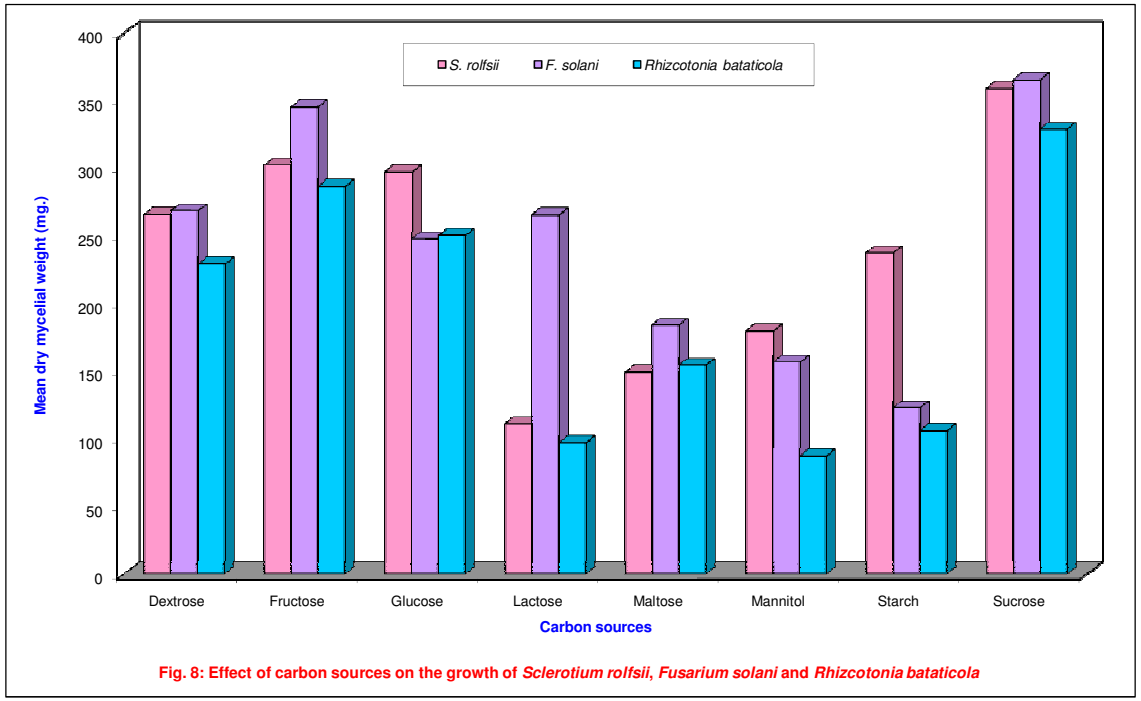


Fig. 8: Effect of carbon sources on the growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizcotonia bataticola*

Fig 8: **Effect of carbon sources on the growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoconia bataticola***

Table 9: Effect of nitrogen sources on the growth of *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola*

Nitrogen sources	Mean dry mycelial weight (mg)		
	S. rolfsii	*F. solani*	*R. bataticola*
Ammonium chloride	111.80	91.26	174.00
Ammonium nitrate	250.33	250.71	203.00
Ammonium orthophosphate	84.33	81.33	127.46
Ammonium sulphate	120.66	214.96	179.50
Aspargine	203.00	282.33	206.60
Potassium nitrate	302.33	374.30	290.53
Sodium nitrate	138.33	341.30	118.13
Urea	94.26	210.33	105.46
S.Em. ±	2.44	1.80	1.57
CD at 1 %	7.52	5.70	4.88

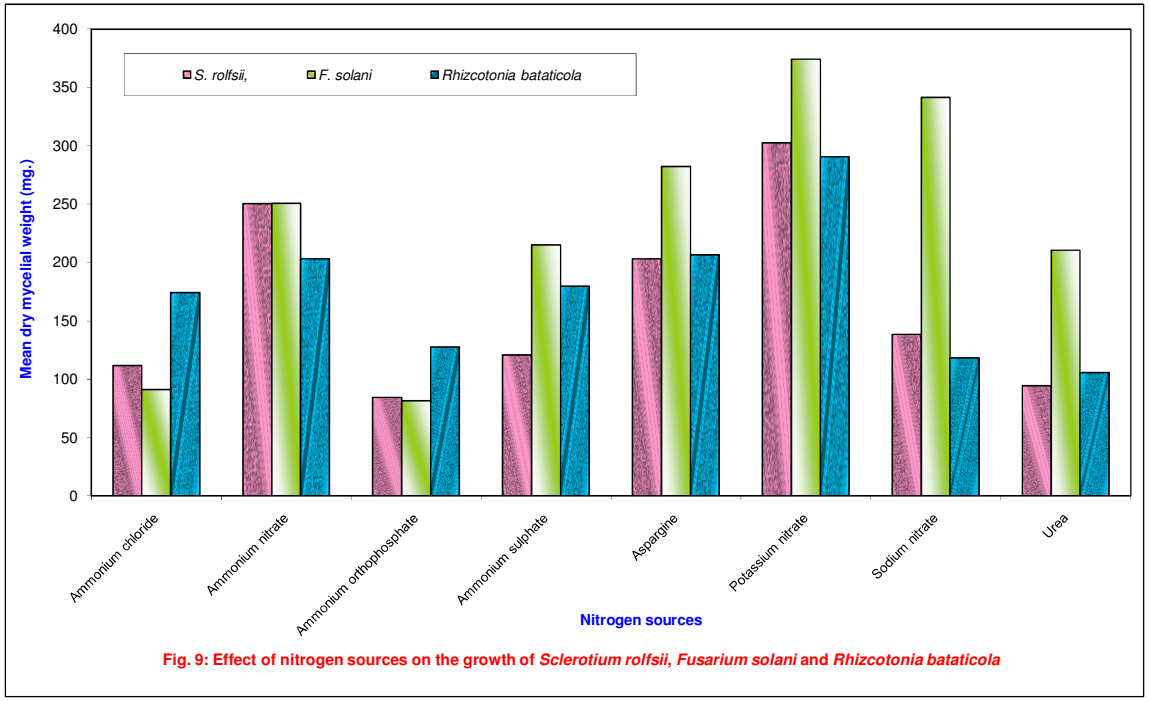


Fig. 9: Effect of nitrogen sources on the growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizcotonia bataticola*

Fig 9: Effect of nitrogen sources on the growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoconia bataticola*

Fusarium solani

Significant difference was noticed among nitrogen sources tested (Table 9). Maximum growth of 374.30 mg was recorded when potassium nitrate was used as nitrogen source. The next best sources were sodium nitrate (341.30 mg), aspergine (282.33 mg), ammonium nitrate (250.71 mg) and ammonium sulphate (214.96 mg). Least dry mycelial weight was recorded by ammonium orthophosphate (81.33 mg).

Rhizoctonia bataticola

The effect of different nitrogen sources on the growth of the fungus was significant (Table 9). Maximum growth of 290.53 mg was recorded when potassium nitrate was used as a source of nitrogen. Growth supported by asparagine (206.60 mg), ammonium nitrate (203.00 mg), ammonium sulphate (179.50 mg) and ammonium chloride (174.00 mg) were significantly different amongst each other. Minimum growth was observed in urea (105.46 mg) and ammonium orthophosphate (127.46 mg).

4.2.5 Physiological studies

4.2.5.1 Effect of temperature

The effect of temperature on the growth of the fungi was studied as explained in material and methods and the results are presented in Table 10 and Fig. 10.

Sclerotium rolfsii

The growth of the fungus was significantly influenced by different temperature levels tested (Table 10). Significantly higher growth of the fungus (255.90 mg) was observed at temperature of 30^0C which was on par with temperature level of 35^0C (225.66mg) and was followed by 25^0C (190.80 mg) and 40^0C (112.33 mg). The least growth was recorded at 5^0C (38.00 mg).

Good sclerotial production was obtained at 30 and 35^0C, moderate sclerotia were produced at 25 and 40^0C, less at 20^0C and no sclerotia were produced at 5, 10 and 15^0C temperature.

Fusarium solani

The effect of different temperature levels on the growth of the fungus was significant (Table 10). The maximum growth of the fungus (365.33 mg) was observed at a temperature of 25^0C which was significant and superior to the rest of the temperature levels tested. The next best temperature was 30^0C (325.62 mg) and 35^0C (282.66 mg). Least growth was supported by 5^0C temperature (52.44 mg).

Excellent conidia were produced at 25 and 30^0C, moderate conidia were produced at 20 and 35^0C, less at 20^0C and no conidia were produced at 5, 10 and 15^0C temperature.

Rhizoctonia bataticola

Different temperature levels significantly influenced the growth of the fungus (Table 10). The maximum growth of the fungus (225.40 mg) was observed at temperature of 30^0C. The next best temperature was 35^0C (198.66 mg). Least growth was recorded at 5^0C (42.50 mg). Excellent sclerotial production was noticed at 30^0C and good at 25 and 35^0C respectively. Poor sclerotia were produced at 20 and 40^0C. No sclerotia were produced at low temperatures.

4.2.5.2 Effect of hydrogen ion concentration

The experiment was carried out to know the effect of pH on the growth of fungi. The growth of the fungi was studied in various pH levels as detailed in “Material and Methods” and the results are presented in Table 11 and Fug. 11.

Sclerotium rolfsii

The growth of the fungus was significantly influenced by different pH levels tested (Table 11). The maximum growth of the fungus was noticed at a pH level of 4.0 (365.67 mg). It was found significantly superior to rest of the pH levels tested. This was followed by pH 4.5 (298.33 mg), 5.0 (242 mg) and 5.5 (196 mg). The least mean dry mycelial weight of the fungus was observed at pH level of 8.0 (46.13 mg). Good sclerotial production was obtained at 4.0 pH, moderate sclerotia were produced at pH 4.5 and sclerotial production was absent from pH 5.5 onwards.

Table 10: Effect of temperature on dry mycelial weight of *Sclerotium rolfsii, Fusarium solani and Rhizoctonia bataticola*

Temperature (^{0}C)	*S. rolfsii*		*F. solani*		*R. bataticola*	
	Colony diameter (mm)	Sclerotial formation*	Colony diameter (mm)	Sporulation**	Colony diameter (mm)	Sclerotia production**
5	38.00	-	52.44	-	42.50	-
10	52.10	-	96.81	-	62.10	-
15	96.52	-	218.33	+	96.80	-
20	106.53	-	282.66	++	140.90	+
25	190.80	++	325.62	+++	192.90	++
30	255.90	+++	365.33	+++	225.40	+++
35	225.66	+++	282.66	++	198.66	++
40	112.33	++	142.33	+	117.96	+
S.Em. ±	0.21		0.03		0.05	
CD at 1 %	0.87		0.09		0.18	

**+ + + :	Good > 100 sclerotia / Petri plate	*+ + + :	Good sporulation > 50 conidia / microscopic field	**+ + + :	Excellent (> 20 microsclerotia / microscopic field)
+ + :	Moderate 50-100 sclerotia / Petri plate	+ + :	Moderate sporulation 30-50 conidia / microscopic field	+ + :	Good (> 5-20 microsclerotia /microscopic field)
+ :	Scanty - <50 sclerotia / Petri plate	+ :	Scanty sporulation <30 conidia / microscopic field	+ :	Poor (< 5 microsclerotia /microscopic field)
- :	No sporulation	- :	No sclerotia	- :	No microsclerotial production

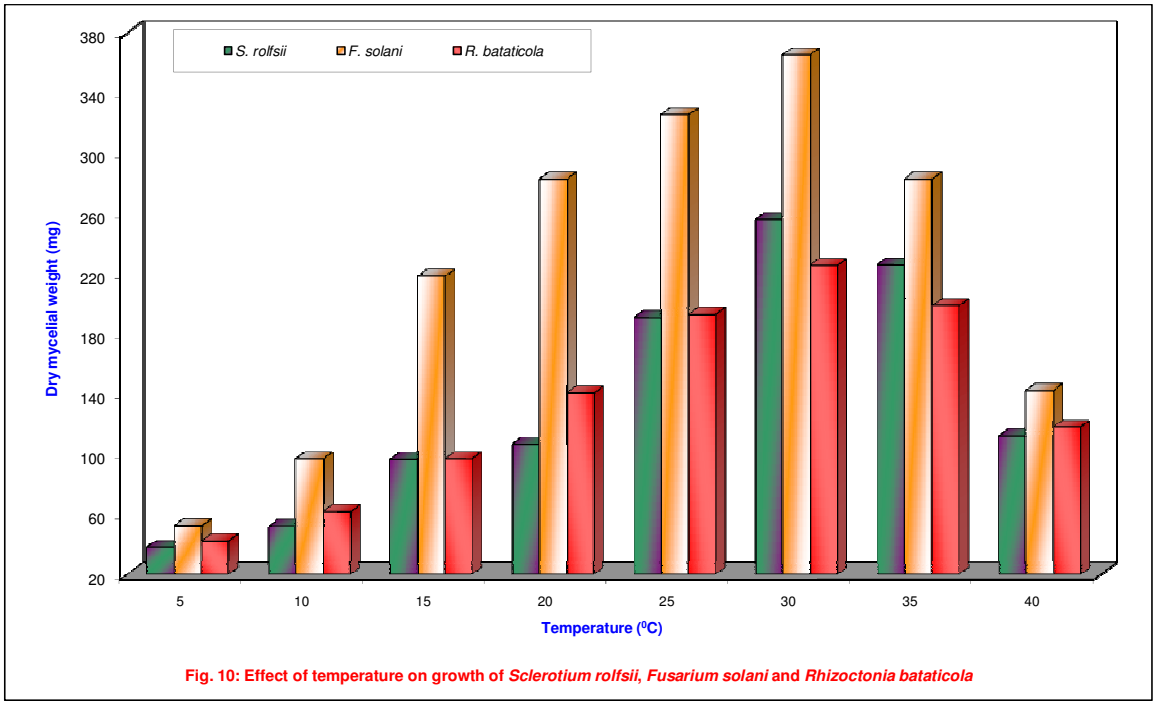


Fig. 10: Effect of temperature on growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoctonia bataticola*

Fig 10: Effect of temperature on growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoctonia bataticola*

Table 11: Effect of pH on mycelial growth of *Sclerotium rolfsii, Fusarium solani and Rhizoctonia bataticola*

pH	*S. rolfsii*		*F. solani*		*R. bataticola*	
	dry mycelial weight (mg)	Sclerotial* formation	Dry mycelial weight (mg)	Sporulation**	dry mycelial weight (mg)	Sclerotial*** production
4.0	365.67	+ ++	54.70	-	79.80	-
4.5	298.33	++	94.33	-	110.13	-
5.0	242.80	+	148.67	-	158.44	-
5.5	196.300	-	212.62	+	225.33	+
6.0	174.01	-	314.67	+ + +	308.70	+ + +
6.5	120.80	-	352.70	+ + +	364.77	+ + +
7.0	98.46	-	240.67	+ +	325.62	+ +
7.5	62.10	-	148.92	+	142.92	-
8.0	46.13	-	91.70		89.62	-
S.Em. ±	1.30		0.28		0.32	
CD at 1 %	3.97		0.92		0.94	

**+ + + :	Good > 100 sclerotia / 100 ml flask	*+ + + :	Good sporulation > 50 conidia / microscopic field	**+ + + :	Excellent (> 20 microsclerotia / microscopic field)
+ + :	Moderate 50-100 sclerotia / 100 ml flask	+ + :	Moderate sporulation 30-50 conidia / microscopic field	+ + :	Good (> 5 microsclerotia /microscopic field)
+ :	Scanty - <50 sclerotia / 100 ml flask	+ :	Scanty sporulation <30 conidia / microscopic field	+ :	Poor (< 5 microsclerotia /microscopic field)
- :	No sclerotial production	- :	No sporulation	- :	No microsclerotial production

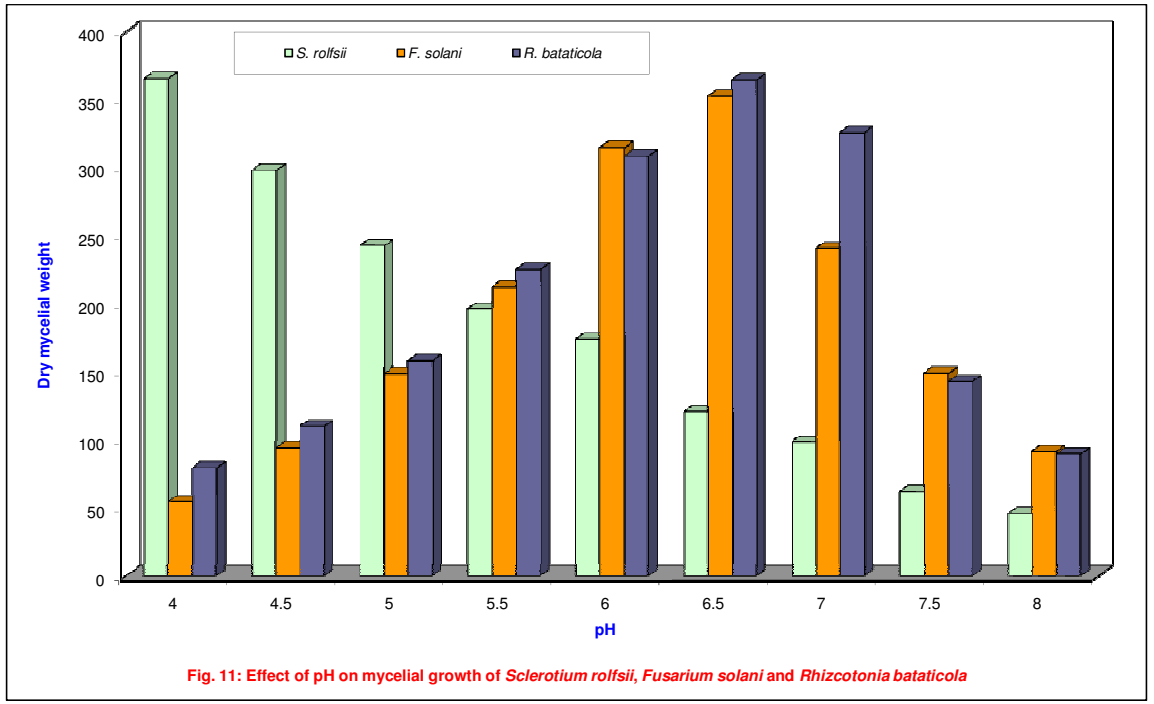


Fig. 11: Effect of pH on mycelial growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizcotonia bataticola*

Fig 11: Effect of pH on mycelial growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoconia bataticola*

Fusarium solani

Significant difference was noticed among various pH levels tested. The maximum growth of the fungus was noticed at a pH level of 6.5 (352.70 mg). It was found significantly superior to rest of the pH levels tested. This was followed by pH 6.0 (314.67mg), 7.0 (240.67 mg) and 5.5 (212.62 mg). The least mean dry mycelial weight of the fungus was observed at pH level of 4.0 (54.70 mg).

Excellent conidia were produced at 6.0 and 6.5 pH, moderate conidia at 7.0 pH, scanty at 5.5 and 7.0 pH whereas no conidia were produced at other pH levels tested.

Rhizoctonia bataticola

The results indicated significant difference among the different treatments on the growth of the fungus (Table 11). The maximum growth of the fungus was noticed at a pH level of 6.5 (364.77 mg) which was significantly superior over the rest of the pH levels tested. The next best pH level for the growth of the fungus was 7.0 (325.62 mg) which is also significant over rest of the pH level tested followed by 6.0 (308.70mg). The least mycelial growth of the fungus was at pH levels of 7.5 (142.92.33 mg) and 8.0 (89.62 mg).

Excellent sclerotia were produced at 6.0 to 6.5 pH, good sclerotia were produced at 7.0 pH. No sclerotia were produced at other pH levels tested.

4.3 Studies on cultural, morphological and molecular variability among isolates of *Sclerotium rolfsii*

4.3.1 Collection of isolates of *Sclerotium rolfsii*

During the survey wilted plant samples were collected from different places and isolated. *Sclerotium rolfsii* was isolated from all the places collected and proved to be most predominant pathogen among the wilt causing pathogens recorded. These isolates were designated as SrNID (Nidshoshi), SrHOS (Hosagudda), SrGKVK (UAS, Bengaluru)), SrTHI (Thindlu), SrKAL (Kalenahalli), SrSAI (Saidapur), SrSIR (Sirsi), SrTHIR (Thirthalli), SrRIP (Rippenpete), SrGAN (Gangavati), SrBGM (Belgaum), SrGDG (Gadag), SrRCR (Raichur), SrMYS (Mysore). These 14 isolates of *S.rolfsii* were used for further variability studies.

4.3.2 Cultural and morphological variability of isolates of *Sclerotium rolfsii*

All the fourteen isolates showed marked differences in their test parameters as described in the “Materials and Methods” (Table 12, 13 and Plate 8)

4.3.2.1 Growth rate

Wide range of variation was noticed among 14 isolates of *S. rolfsii* with respect to various attributes studied. Growth rate ranged from 0.63 to 1.25 mm per hour in different isolates. Based on growth rate, the isolates were grouped into three groups. Group I consisted of isolates with faster growth *viz*., SrGKVK, SrKAL, SrSAI, RCR and SrBGM (1.25 mm h^{-1}). Group II consisted four isolates with medium growth rate (0.94 mm h^{-1}) *viz*., SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS. Group III had isolates like SrRIP, SrTHIR and SrGAN with slow growth rate (0.63 mmh^{-1}) (Table 12).

4.3.2.2 Time taken for sclerotial initiation

The isolates varied with respect to time taken for sclerotial formation. Isolates were grouped into three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) which took just four days for sclerotial initiation. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS. Group) in which sclerotial initiation was noticed on 5^{th} day. Group III consisted of three isolates (SrRIP, SrTHIR and SrGAN) which took maximum number of 7 days for sclerotial initiation. In all isolates, sclerotial bodies were initially white and later turned dark brown (Table 12).

4.3.2.3 Colony colour and colony type

All the isolates showed light white colonies except the isolates SrGAN, SrTHIR and SrRIP showed extra whtie colony. Isolates *viz*., SrKAL, SrNID, SrTHI, SrGKVK, SrSIR, SrSAI, SrHOS, SrMYS, SrRCR, SrGDG and SrBGM showed compact mycelium while the isolates SrGAN, SrTHIR and SrRIP showed fluffy mycelium (Table 13).

Table 12: Mycelial growth and sclerotial formation in isolates of *Sclerotium rolfsii*

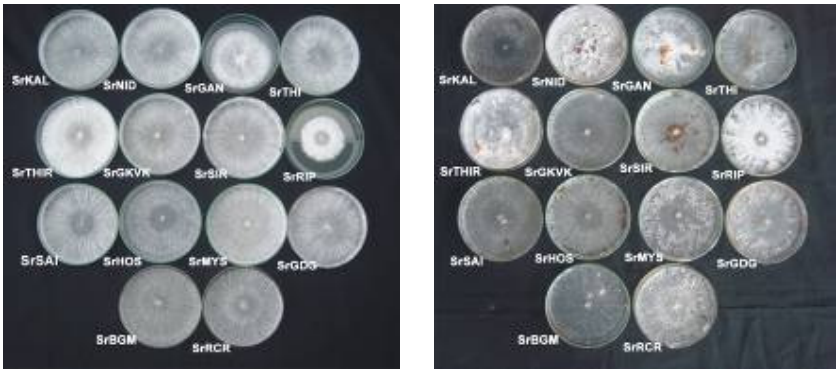
Isolate		Colony growth (mm)							Growth rate mm h^{-1}	Sclerotial formation and colour				
	12 h	24 h	36 h	48 h	72 h	96 h	120 h	144 h		96 h	120 h	144 h	168h	192h
Sr KAL	9	24	41	78	90				1.25	+	W	LB	B	DB
Sr NID	-	16	39	78	90				1.25	+	W	LB	B	DB
Sr GAN	-	20	41	64	90				1.25	+	W	LB	B	DB
Sr THI	-	15	40	56	90				1.25	-	W	W	LB	DB
Sr THIR	9	18	35	56	90				1.25	-	+	W	LB	LB
Sr GKVK	-	14	22	33	83	90			1.25	-	+	W	LB	DB
Sr SIR	-	14	22	36	75	90			0.94	-	+	W	LB	RB
Sr RIP	-	15	32	59	83	90			0.94	-	-	W	LB	RB
Sr SAI	-	14	22	35	81	90			0.94	-	-	+	W	DB
Sr HOS	-	18	24	32	83	90			0.94	-	-	-	+	DB
Sr MYS	-	17	22	35	83	90			0.94	-	+	-	B	RB
Sr GDG	-	16	28	33	49	68	81	90	0.63	-	-	-	+	W
Sr BGM	-	14	18	34	50	70	82	90	0.63	-	-	-	+	W
Sr RCR	-	14	20	40	52	74	85	90	0.63	-	-	-	+	W

+ = sclerotial initiation, - = nil, W = white, LB = light brown, B = brown, RB = reddish brown

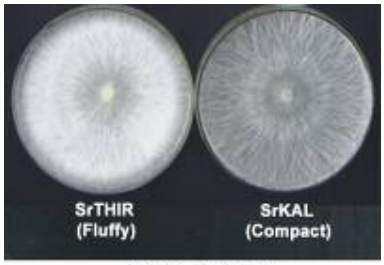
Table 12: Mycelial growth and sclerotial formation in isolates of *Sclerotium rolfsii*

Isolate		Colony growth (mm)							Growth rate mm h^{-1}	Sclerotial formation and colour				
	12 h	24 h	36 h	48 h	72 h	96 h	120 h	144 h		96 h	120 h	144 h	168h	192h
Sr KAL	9	24	41	78	90				1.25	+	W	LB	B	DB
Sr NID	-	16	39	78	90				1.25	+	W	LB	B	DB
Sr GAN	-	20	41	64	90				1.25	+	W	LB	B	DB
Sr THI	-	15	40	56	90				1.25	-	W	W	LB	DB
Sr THIR	9	18	35	56	90				1.25	-	+	W	LB	LB
Sr GKVK	-	14	22	33	83	90			1.25	-	+	W	LB	DB
Sr SIR	-	14	22	36	75	90			0.94	-	+	W	LB	RB
Sr RIP	-	15	32	59	83	90			0.94	-	-	W	LB	RB
Sr SAI	-	14	22	35	81	90			0.94	-	-	+	W	DB
Sr HOS	-	18	24	32	83	90			0.94	-	-	-	+	DB
Sr MYS	-	17	22	35	83	90			0.94	-	+	-	B	RB
Sr GDG	-	16	28	33	49	68	81	90	0.63	-	-	-	+	W
Sr BGM	-	14	18	34	50	70	82	90	0.63	-	-	-	+	W
Sr RCR	-	14	20	40	52	74	85	90	0.63	-	-	-	+	W

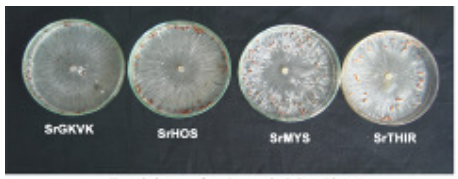
+ = sclerotial initiation, - = nil, W = white, LB = light brown, B = brown, RB = reddish brown



Colony colour

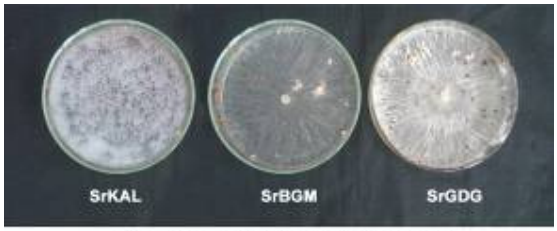


Colony type



Position of sclerotial bodies

SrKAL (Kalenahalli)
SrNID (Nidshoshi)
SrGAN (Gangavati)
SrTHI (Thindlu)
SrTHIR (Thirahalli)
SrGKVK (UAS, Bangalore)
SrSIR (Sirsi)
SrRIP (Rippenpete)
SrSAI (Saidapur)
SrHOS (Hosagudda)
SrMYS (Mysore)
SrGDG (Gadag)
SrBGM (Belgaum)
SrRCR (Raichur)



Number of sclerotial bodies

Fig 8: Cultural and morphological variability among 14 isolates of *Sclerotium rolfsii*

4.3.2.4 Number of sclerotia per plate and shape

With regard to number of sclerotia produced per plate, isolates, SrKAL, SrNID, SrTHI, SrGKVK, SrSIR produced more sclerotia (241 to 324/plate). Medium number of sclerotia were produced by the isolates SrGAN, SrTHIR,SrSAI, SrHOS, SrMYS, SrGDG, SrRCR and SrBGM (310to 346/plate) and very less number of sclerotia were produced in SrRIP isolate (142/plate). Isolates SrNID, SrTHIR, SrMYS and SrRIP showed oval shaped sclerotia whereas, others were round (Table 13).

4.3.2.5 Position of sclerotia in Petri plate

With respect to position of sclerotia, in maximum isolates (SrSIR, SrTHI, SrGKVK, SrRIP, SrSAI, SrHOS, SrBGM and SrGDG) sclerotia were found near the edges of Petri plates. SrGAN and SrSIR isolates produced sclerotia at centre in concentrated form. Where as isolates like SrKAL, SrNID, SrTHIR, SrMYS and SrRCR) produced sclerotia irregularly all over Petri plate (Table 13).

4.3.2.6 Size of sclerotial bodies

The variation in size of sclerotial bodies of isolates under study was found significant. SrRIP, SrTHIR and SrGAN isolates produced biggest sclerotia with mean diameter of 2.00 mm, 1.67mm and 1.43mm respectively. In isolates SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS medium sized sclerotia ranging from 1.00mm to 1.25 mm were produced. Where as in SrGKVK, SrKAL, SrRCR, SrSAI and SrBGM isolates produced very small sized sclerotia *viz.,* 0.85, 0.82, 0.95, 0.83 and 0.82 mm respectively (Table 13).

4.3.2.7 Hundred sclerotial weight

The test weight of sclerotial bodies revealed that it varied significantly (Table 13). Maximum test weight was recorded in SrTHIR isolate (436mg) and minimum in SrSAI isolate (272 mg). Based on test weight the isolates were grouped into three categories. Group I (SrGKVK, SrKAL, SrRCR, SrBGM and SrSAI) with test weight ranging from 270 – 304 mg. Group II consisted of six isolates (SrTHI, SrNID. SrSIR, SrMYS, SrGDG and SrHOS) with test weight ranging from 210-342 mg and group III (SrRIP, SrGAN, SrTHIR) test weight ranged from 426 to 436 mg. Results clearly indicated the variability among isolates with respect to sclerotial weight.

4.3.2.8 Mycelial compatibility/Vegetative compatible groups

In the present investigation, The PDA plates were marked into three portions and mycelial discs of 5 mm diameter from the edge of an actively growing colony (3 to 4 day old) of the respective isolate was inoculated on each portion of PDA plate. After inoculation, the plates were incubated at 28^{o}C for 5 to 15 days. The pairings were macroscopically examined daily for the development of clearing zone and sclerotial formation in the region of mycelial contact as described in the “Materials and Methods”.

Based on the mycelial compatibility seven vegetatively compatible groups (VCG) were found among the fourteen isolates (Table 14 and Plate 9).

Group I : Compatible with all (13) isolates (SrKAL).

Group II : Compatible with 12 isolates (SrRIP).

Group III: Compatible with 11 isolates (SrNID).

Group IV: Compatible with 10 isolates (SrBGM, SrHOS, SrMYS, SrTHI and SrSIR)

Group V : Compatible with 9 isolates (SrSAI, SrGDG and SrTHIR)

Group VI: Compatible with 8 isolates (SrRCR)

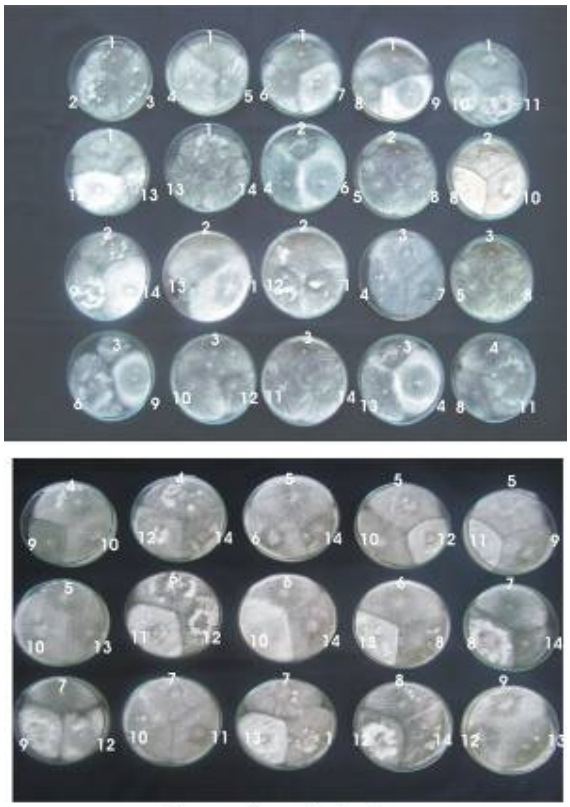
Group VII: Compatible with 7 isolates (SrGAN, SrGKVK)

Among these, SrKAL was highly compatible with other isolates. In the compatible reaction mycelia of the two isolates intermingled at the zone of interaction (Plate 9b). Whereas, in the incompatible reactions, a clear barriage zone of dead mycelia was formed (Plate 9a). In most of the antagonistic reactions, sclerotia were formed all along the border of the lytic zone of the two isolates (Plate 9c), while some were produced on the mycelium and along the edges (Plate 9c)

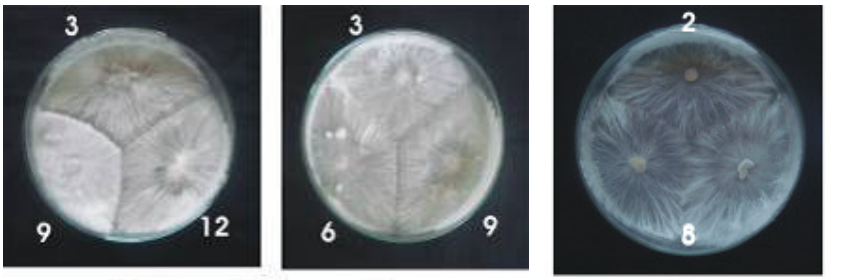
Table 14: Mycelial compatibility among fourteen isolates of *Sclerotium rolfsii*

Isolates	Isolates													
	SrBGM	SrGAN	SrGKVK	SRHOS	SrKAL	SrMYS	SrNID	SrTHI	SrRCR	SrRIP	SrSAI	SrTHIR	SrGDG	SrTHIR
SrBGM		C	C	C	C	C	IC	C	C	IC	C	C	C	IC
SrGAN			IC	IC	C	C	C	C	IC	C	IC	C	C	IC
SrGKVK				IC	C	IC	C	C	IC	C	C	C	C	IC
SRHOS					C	C	C	IC	C	C	C	C	C	C
SrKAL						C	C	C	C	C	C	C	C	C
SrMYS							IC	C	IC	C	C	C	C	C
SrNID								C	C	C	C	C	C	C
SrTHI									C	C	C	C	C	IC
SrRCR										C	C	IC	IC	C
SrRIP											C	C	C	C
SrSAI												C	IC	C
SrTHIR													IC	C
SrGDG														C
SrTHIR														

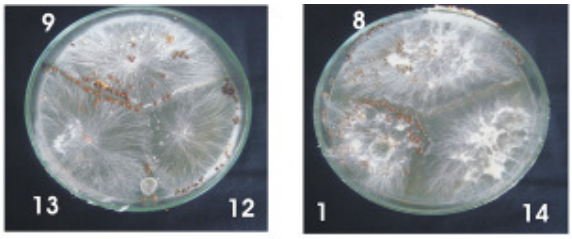
C: Compatible IC: Incompatible



Experimental view



a. Incompatible reaction b. Compatible reaction



c. Sclerotial development

1 - SrBGM	2 - SrGAN	3 - SrGKVK	4 - SrHOS	5 - SrKAL
6 - SrMYS	7 - SrNID	8 - SrTHI	9 - SrRCR	10 - SrRIP
11 - SrSAI	12 - SrSIR	13 - SrGDG	14 - SrTHIR	

Plate 9: Mycelial compatibility of fourteen isolates of *Sclerotium rolfsii*

4.3.2.9 Oxalic acid production by isolates of *S. rolfsii*

In the present investigation, each isolate of *S. rolfsii* was grown individually in potato dextrose broth and oxalic acid produced in medium was quantified separately as described in "Material and Methods" and results are presented in the Table 15 and Fig. 12.

The experimental results indicated that oxalic acid produced by different isolate was found to vary significantly. Maximum oxalic acid production (5.90 mg/ml) was observed in SrKAL isolate followed by SrGKVK (5.80 mg/ml), SrBGM (5.12mg/ml) and SrRCR (4.93 mg/ml) isolate. However, least oxalic acid production (1.33 mg/ml) was observed in SrRIP isolate followed by SrGAN (1.45 mg/ml) and SrTHIR (1.80 mg/ml) isolate. Other isolates (SrTHI, SrNID. SrSIR, SrMYS, SrGDG and SrHOS) remained intermediate in oxalic acid production (2.90 to 3.80 mg/ml).

4.3.2.10 Virulence index

All the fourteen isolates were inoculated to stevia to study the variation in pathogenicity. The inoculation procedures followed are described under "Material and Method". Observations on number of days taken for wilting and virulence index were recorded and presented in the Table 15, Fig. 12 and Plate 10. Maximum degree of virulence was recorded in SrKAL (10.00) which took less number of days (10) for complete wilting of plant followed by SrGKVK (8.33), SrBGM (7.64), SrSAI (6.41) and SrRCR (6.11) which required 12, 13 and 15 days for wilting. SrRIP was least virulent with minimum virulence index of 1.98 and required maximum period for wilting of plant. Isolates with maximum virulence index required less period for causing wilting of plants.

Based on virulence index the isolates have been classified into three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) with high virulence index ranging from 6 to 10. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS) with virulence index ranging from 3 – 5. Group III consisted of three isolates (SrGAN, SrTHIR and SrRIP) with low virulence index ranging from 1 –3.

4.3.2.11 Grouping of *S. rolfsii* isolates based on different characters

In the present study, virulence was related to the time taken for wilting, sclerotial maturity, sclerotial size, virulence index and oxalic acid production. The isolates which took less days for wilting, high growth rate, which took less time for sclerotial maturity, with small sized sclerotia, high virulence index and oxalic acid production were highly virulent. Whereas isolates with medium growth rate, medium time for sclerotial maturity, medium sized sclerotia, moderate virulence index, oxalic acid production and which took medium days for wilting were just virulent. While, isolates which took more days for wilting, slow growth rate, which took more days for sclerotial maturation, with big sized sclerotia, low virulence index and oxalic acid production were less virulent.

Based on number of days taken for wilting, growth rate, sclerotial maturity and size, virulence index, oxalic acid production, the isolates of *Sclerotium rolfsii* were classified in to three groups. Group I consisted of highly virulent isolates *viz.,* SrGKVK, SrKAL, SrSAI, RCR and SrBGM which took 10 to 15 days for wilting, high growth rate (1.25 mm h^{-1}), which took six to seven days for sclerotial maturity, with sclerotial size varied from 0.85 to 0.95 mm, virulence index of 6 to 10, oxalic acid production varied from 4 to 6 mg/ml. Group II consisted of six moderately virulent isolates *viz*., SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS which took medium days for wilting (18-22), medium growth rate 0.94 mm h^{-1}),which took medium time for sclerotial maturity, with sclerotial size varied from 1.00 to 1.50 mm, virulence index of 3 to 6 and oxalic acid production varied from 2.00 to 4.00 mg/ml. Group III consisted of three less virulent isolates viz., SrTHIR, SrGAN, SrRIP which took more days for wilting (21-38), less growth rate 0.63 mm h^{-1}), with sclerotial size varied from 1.00 to 3.00 mm, virulence index of 1 to 2.5 and oxalic acid production varied from 1.00 to 2.00 mg/ml (Table 16 and Fig. 13).

4.4 Molecular variability among isolates of *Sclerotium rolfsii*

4.4.1 Characterization of *S. rolfsii* by RAPD-PCR

It is difficult to distinguish these isolates using traditional morphological differences. The suitability of random amplified polymorphic DNA (RAPD) and ITS was used to detect the variations among the isolates of *S. rolfsii.* OPA, OPB and OPF series primers were used to determine genetic distance between isolates and to construct a dendrogram. Banding profile of different primers for fourteen isolates of *S. rolfsii* is given in Table 17.

Table 17: Scorable DNA bands generated with 24 RAPD primers in 14 isolates of *Sclerotium rolfsii*

Primer	Total number of bands amplified	Number of polymorphic bands produced	% polymorphism
OPA-01	6	2	33.33
OPA-02	10	10	100.00
OPA-03	13	12	92.31
OPA-04	10	10	100.00
OPA-05	8	4	50.00
OPA-06	8	8	100.00
OPA-07	5	4	80.00
OPA-08	8	2	25.00
OPA-09	8	8	100.00
OPA-10	12	13	92.31
OPA-11	6	3	50.00
OPA-17	12	7	58.30
OPB-01	13	12	92.31
OPB-02	6	6	100.00
OPB-03	8	6	75.00
OPB-04	4	4	100.00
OPB-05	10	8	80.00
OPB-09	3	2	66.67
OPB-10	5	4	80.00
OPF-01	6	4	66.67
OPF-02	8	8	100.00
OPF-03	7	6	85.71
OPF-05	5	4	80.00
OPF-06	8	2	25.00

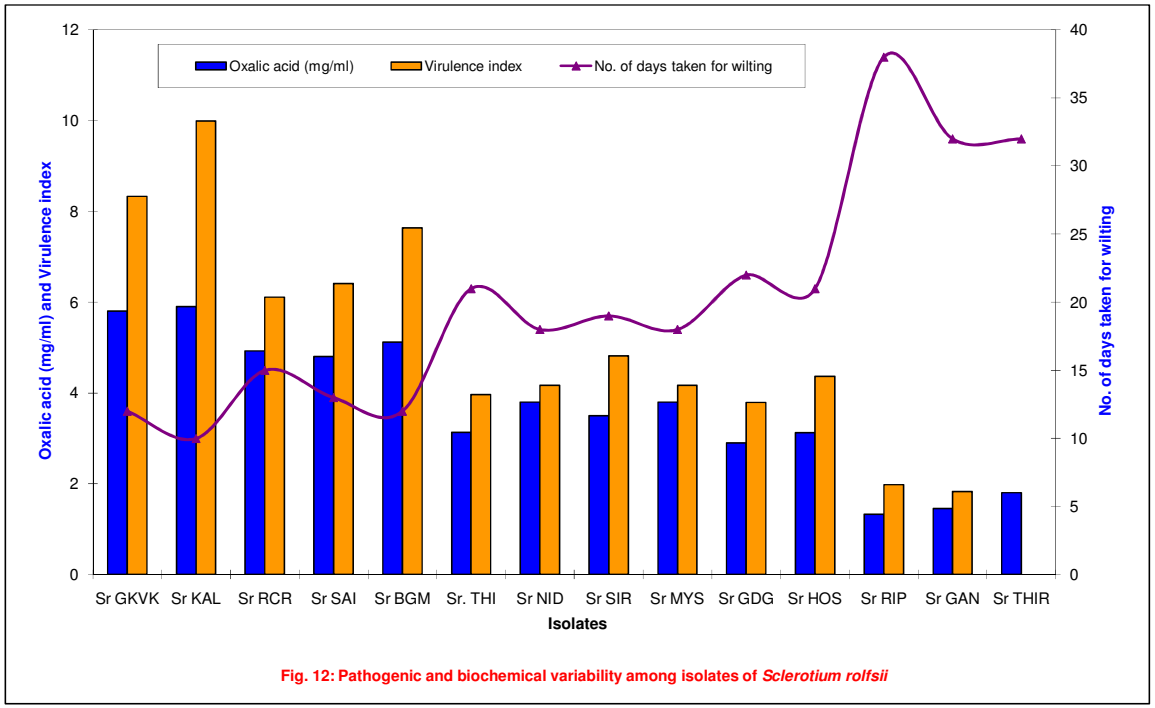


Fig. 12: Pathogenic and biochemical variability among isolates of *Sclerotium rolfsii*

Fig 12: Pathogenic and biochemical variability among isolates of *Sclerotium rolfsii*

15DAP

35DAP

60DAP

1. Sr GKVK (UAS, Bangalore
2. Sr KAL (Kalenahalli)
3. Sr THI (Thindlu)
4. Sr HOS (Hosagudda)
5. Sr MYS (Mysore)
6. Sr NID (Nidshoshi)
7. Sr SIR (Sirsi)
8. Sr RIP(Rippenpete)
9. Sr THIR (Thirtahalli)
10. Sr GAN (Gangavati)
11. Sr GDG (Gadag)
12. Sr BGM (Belgaum)
13. Sr SAI (Saidapur)
14. Sr RCR (Raichur)

Plate 10: Studies on virulence of isolates of Sclerotium rolfsii

Of the 24 primers used for amplification, OPA02, OPA 04, OPA06, 0PA09, OPB02, OPB4 and OPF 02 showed 100 per cent polymorphism (Plate 11a). A total of 175 amplicon levels resulted from 24 primers and were available for analysis. Information on banding pattern for all the primers was used to determine genetic distance between isolates and to construct a dendrogram.

Based on simple matching coefficient a genetic similarity matrix was constructed to assess the genetic relatedness among the isolates of *S.rolfsii.* Genetic similarity coefficient of fourteen isolates of *Sclerotium rolfsii* based on RAPD analysis is given in the Table 18. Similarity coefficient ranged from 0.63 to 0.93. The maximum genetic similarity of 93 was noticed between SrGDG (Gadag) and SrNID (Nidshoshi). Whereas, least genetic similarity was observed between SrRIP (Rippenpete) and SrMYS (Mysore). The dendrogram for pooled data showed three major clusters (Fig 13a). The isolates SrMYS, SrKAL, SrRCR, SrSAI,SrBGM, SrTHI and SrGKVK were found in one cluster, isolates SrTHIR and SrRIP were found in a separate cluster, SrGDG, SrNID, SrHOS, SrGAN and Sr SIR isolates were found in another cluster.

4.4.2 Characterization of *S.rolfsii* by ITS-PCR

ITS-1 and ITS-4 primers were used for PCR amplification of ITS region of rDNA of all fourteen isolates of *S. rolfsii*. Both the primers yielded a single band of approximately 650-700 bp in all the fourteen isolates (Plate 11b). These results confirmed that all the isolates belong to genus *Sclerotium.* Nucleotide sequences of 14 isolates of *S.rolfsii* are given in Table 19.

Nucleotide sequences of fourteen *S.rolfsii* isolates of 18S, 5.8S and 28S rDNA region were analyzed using NCBI (National Center for Biotechnology Information) BLAST programme. Based on sequence comparison, all the fragment of 18S, 5.8S and 28S rDNA gene sequences of fourteen isolates were confirmed as *Athelia rolfsii* (Table 20). Fragment of 18S, 5.8S and 28S rDNA gene sequences of SrHOS, SrKAL, SrMYS, and SrTHIR isolates showed 100.00 per cent similarity with NCBI published sequences *viz*., *Athelia rolfsii* strain HUTC1T1 (HQ895874.1), *Athelia rolfsii* isolate AS-1 (JN241563.1), *Athelia rolfsii* strain NAGC12T1 (HQ895931.1) and *Athelia rolfsii* 18S ribosomal RNA gene (HM222638.1) respectively.

The isolates SrGAN and SrGDG showed 99% similarity with the sequence of *Athelia rolfsii* strain orchid 18S ribosomal RNA gene (GQ358518.1), SrNID and SrTHI isolates showed 99% similarity with the isolates *Athelia rolfsii* isolate AS-1 (JN241563.1) and SrBGM, SrRCR and SrSIR showed 99% similarity with the isolates *Athelia rolfsii* strain srpo3 (KC460985.1), *Athelia rolfsii* isolate SR001 18S ribosomal RNA gene (HQ420816.1) and *Sclerotium* sp. LS341-1 (GQ358518.1). SrGKVK and SrRIP isolates showed 98% similarity with the isolates *Athelia rolfsii* 18S ribosomal RNA gene (HM222638.1) and *Sclerotium delphinii* strain Sd-1(JQ982485.1) respectively. Whereas the isolate SrSAI showed 98% similarity with *Athelia rolfsii* strain KACC42087 18S ribosomal RNA gene (HM355751.1).

4.4.3 Phylogenetic analysis

Dendrogram was constructed using neighbour-joining tree method for the nucleotide sequences of 18S rDNA region of all 14 *S.rolfsii* isolates. Published sequence of *Athelia rolfsii* isolate BeanScRs (Accession no:KC293992.1) was downloaded from NCBI and was used in the construction of dendrogram. Phylogram results of fourteen isolates of *S. rolfsii* revealed that, there are two major clusters (Fig 13b). Cluster A comprising all *Sclerotium rolfsii* (*Athelia rolfsii*) species of stevia, whereas cluster B comprises *Athelia rolfsii* isolate BeanScRs (Accession no:KC293992.1). A glance towards dendrogram reveals that, there is no much diversity among the 14 isolates of *Sclerotium rolfsii*. *Athelia rolfsii* isolate BeanScRs (Accession no:KC293992.1) with separate branch in dendrogram shows a wide divergence with the above 14 isolates.

4.5 Estimation of loss

4.5.1 Growth and yield parameters

The data from the field experiment revealed that the *Sclerotium* affected plot recorded 51.19 and 58.88 per cent decrease in plant height 60 DAP and 120 DAP, respectively. Further, 59.11 and 46.08 per cent decrease in number of branches was observed when compared to the plot treated with hexaconazole (Table 21). Soil drenching with hexaconozole @ 0.1% could reduce the disease to the extent of 80.02 per cent and yield loss of 65.12 per cent (fresh yield) and 64.84 per cent (dry yield) was estimated (Table 22).

Table 16: Grouping of *Sclerotium rolfsii* isolates based on different characters

Group	Isolate	Location	Growth rate mm h^{-1}	Sclerotial size (mm)	Sclerotial maturity in days	Virulence index	Oxalic acid (mg/ml)	No. of days taken for wilting	Pathogenic reaction
Group I	SrGKVK	UAS,Bangalore	1.25	0.85	06	8.33	5.80	12	Highly virulent
	SrKAL	Kalenahalli	1.25	0.82	06	10.00	5.90	10	
	SrRCR	Raichur	1.25	0.91	07	6.11	4.93	15	
	SrSAI	Saidapur	1.25	0.83	07	6.41	4.80	13	
	SrBGM	Belgaum	1.25	0.82	06	7.64	5.12	12	
Group II	Sr THI	Thindlu	1.25	1.00	08	3.97	3.13	21	Moderately virulent
	SrNID	Nidshoshi	0.94	1.23	10	4.17	3.80	18	
	SrSIR	Sirsi	0.94	1.00	09	4.82	3.50	19	
	SrMYS	Mysore	0.94	1.25	09	4.17	3.80	18	
	Sr GDG	Gadag	0.94	1.11	08	3.79	2.90	22	
	Sr HOS	Hosagudda	0.94	1.00	08	4.37	3.12	21	
Group III	Sr RIP	Rippenpete	0.63	2.00	15	1.98	1.33	38	Less Virulent
	Sr GAN	Gangavati	0.63	1.43	12	1.83	1.45	32	
	Sr THIR	Thirthalli	0.63	1.67	12	2.08	1.80	33	

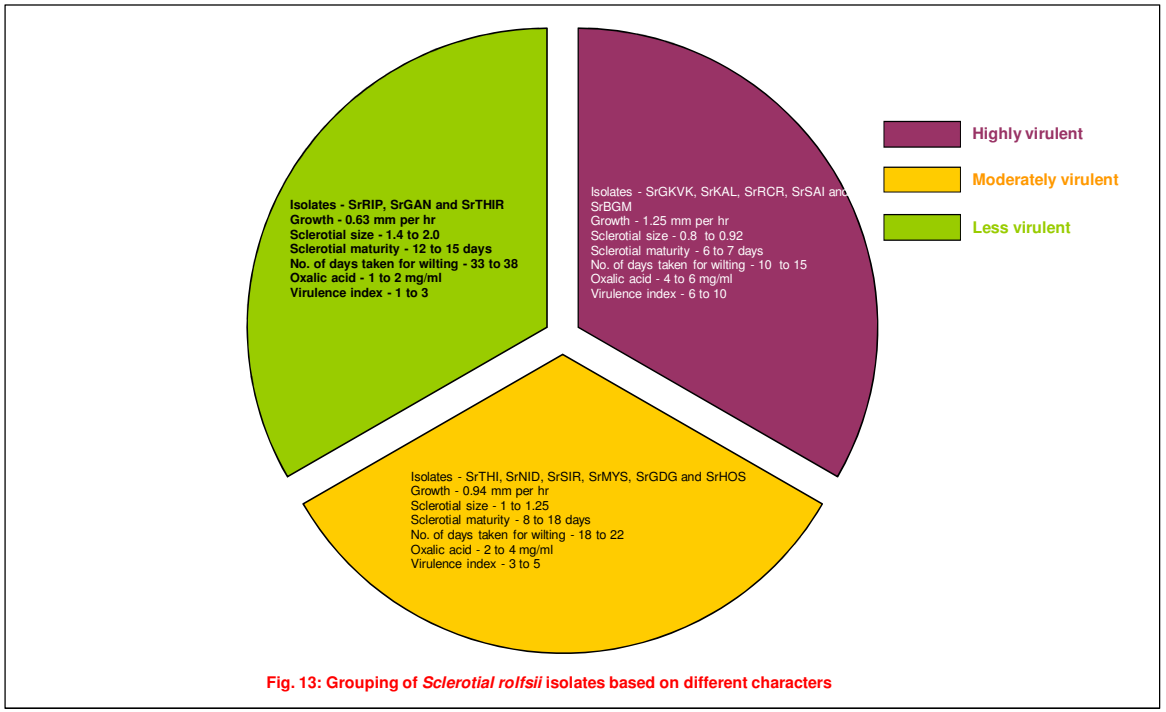


Fig. 13: Grouping of *Sclerotial rolfsii* isolates based on different characters

Fig 13: Grouping of *Sclerotial rolfsii* isolates based on different characters

Table 17: Scorable DNA bands generated with 24 RAPD primers in 14 isolates of *Sclerotium rolfsii*

Primer	Total number of bands amplified	Number of polymorphic bands produced	% polymorphism
OPA-01	6	2	33.33
OPA-02	10	10	100.00
OPA-03	13	12	92.31
OPA-04	10	10	100.00
OPA-05	8	4	50.00
OPA-06	8	8	100.00
OPA-07	5	4	80.00
OPA-08	8	2	25.00
OPA-09	8	8	100.00
OPA-10	12	13	92.31
OPA-11	6	3	50.00
OPA-17	12	7	58.30
OPB-01	13	12	92.31
OPB-02	6	6	100.00
OPB-03	8	6	75.00
OPB-04	4	4	100.00
OPB-05	10	8	80.00
OPB-09	3	2	66.67
OPB-10	5	4	80.00
OPF-01	6	4	66.67
OPF-02	8	8	100.00
OPF-03	7	6	85.71
OPF-05	5	4	80.00
OPF-06	8	2	25.00

Table 18: Similarity coefficient based on RAPD analysis using 24 primers in 14 isolates of *Sclerotium rolfsii*

Isolates	SrMYS	SrSrHOS	SRTHIR	SrKAL	SrSIR	SrRCR	SrSAI	SrBGM	SrTHI	SrGKVK	SrMYS	SrRIP	SrNID	SrGDG
SrMYS	1.000													
SrHOS	0.863	1.000												
SrTHIR	0.861	0.851	1.000											
SrKAL	0.898	0.871	0.896	1.000										
SrSIR	0.774	0.808	0.756	0.770	1.000									
SrRCR	0.804	0.823	0.800	0.814	0.783	1.000								
SrSAI	0.775	0.781	0.763	0.742	0.845	0.851	1.000							
SrBGM	0.759	0759	0.756	0.732	0.784	0.815	0.898	1.000						
SrTHI	0.674	0.696	0.689	0.724	0.781	0.778	0.820	0.817	1.000					
SrGKVK	0.674	0.703	0.689	0.723	0.780	0.806	0.849	0.815	0.910	1.000				
SrMYS	0.652	0.660	0.667	0.700	0.786	0.761	0.817	0.798	0.839	0.912	1.000			
SrRIP	0.636	0.689	0.899	0.710	0.753	0.867	0.811	0.800	0.853	0.887	0.857	1.000		
SrNID	0.828	0.848	0.851	0.814	0.761	0.812	0.768	0.793	0.722	0.722	0.725	0.878	1.000	
SrGDG	0.851	0.902	0.868	0.805	0.795	0.817	0.768	0.779	0.723	0.715	0.726	0.887	0.934	1.000

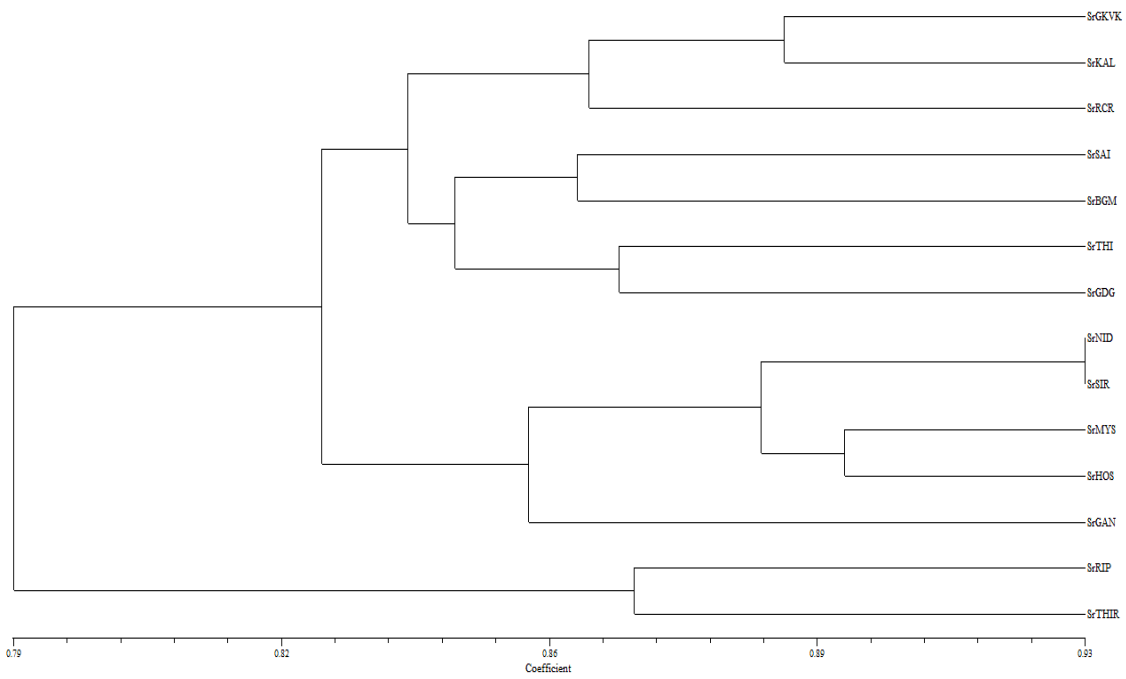


Fig. 13a: Polyogram of isolates of *Sclerotium rolfsii* of stevia based on ITS rDNA sequence data

Table 19: Samples of sequences of representative *Sclerotium rolfsii* isolates

>SrBGM (Belgaum)

GTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATAACCTGTGAACCAACT
GTAGTCAGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAACGATCT
CATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACAACGGATC
TCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGAAATGTGAATTGCAGAATCCA
GTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTGTTTGA
GAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGTTGCTGG
TTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCTACGGTTG
GTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTTCTTATAAAATC
ATGCGCATATATCTAGCATATAGAGTGCATATAT

>SrGAN (Gangavati)

AGTTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAAC
CAACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAA
CGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATTGAGGTCAAATGGTCA
ATATATGGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATACAAGCTAGAATC
CCCTTCCGGTATAGGCGTAGACATATTATCACACCAACCGTAGGCATTTCTACATGTCCTACTAAT
AGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCAACTCTCACATCCAAGCCTTGACAAAT
ACAAAAATTTGTAAGGTTGAGAATTTAATGACTCTCAAACAGGCATGCCCCTCGGAATACCAAAG
GGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCAT
TTCGCTGCGTTCTTCATCGATGCAAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATATTTTT

>SrHOS (Hosagudda)

TTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAACCA
ACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC
GATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACAAC
GGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTG
TTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGTT
GCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCTAC
GGTTGGTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTACTTAT
AAAATCATGCGCATATATCTAGCATATAAGTGCATATATTGA

> SrKAL (Kalenahalli)

TCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTCATATATGCAAAGAGTTGTGCTGGTAAT
AAATATTGCATGTGCACACTCTGAAGCTATATAATATATACACCTGTGAACCAACTGTAGTCTGGA
GAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAGAACGATCTCATATTGAA
GTGTGAACCAACTGTACAAGTTTCTCTTAATTGAAAAAACACAACTTTCAACAACGGATCTCTTGG
CTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAAT
CATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTGTTTGAGAGTCA
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ATATCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATATAGAAATGCCTGCGGTTGGTGTGATA
ATATGTCTACGCCTATACCAAAGGGGATTCTAGCTTGTATGCACTACTTATAAAATCATGCGCATA
TATCTAGCATATAAGTGCATATATTGACCATTTGACCTCAAATCAGGTAGGACTACCCGCTGAACT
TAAGCATA TCTGTGAACCAACTG

>SrMYS (Mysore)

AGTTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAAC
CAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGA
ACGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACAA
CGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCT
GTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGT
TGCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCTA
CGGTTGGTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTACTTA
TAAAATCATGCGCATATATCTAGCATATAAGTGCATATATTGACCATTTGAC

> SrNID (Nidshoshi)

TTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAACCA
ACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC
GATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACAAC
GGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTG
TTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGTT
GCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCTAC
GGTTGGTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTACTTAT
AAAATCATGCGCATATATCTAGCATATAAGTGCATATATTTGACCAATTGACCTCAAA

>SrTHI (Thindlu)

GGTCAAATGGTCAATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAGTACATA
CAAGCTAGAATCCCCCTTGTTGTGCTGGTAATGATATTGCATGTGCACACTCTGGAGCTATATAA
TATATACACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTT
ATTGTATGTTACATAGAACGATTTCATATTGAAACTTTGTTTTAACTTAAGCATA
TCTGTGAACCAACTG

>SrRCR (Raichur)

GAGTTGTGCTGGCAATAAATATTGCATGTGCACACTCTGAAGCTATATAACACATACACCTGTGA
ACCAACTGTAGTCTGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAG
AACGATCTCATATTGAAGCTTTGTTTTTTTTTACAAGTTTCTCTTAATTGAAAAATATACAACTTTCA
ACAACGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCAT
GCCTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGA
GAGTTGCTAGTTAAGAATATCTGACTGGCTCTCTTTAAAATTATTAGTAGGACATGTAGAAATGCC
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GACTACCCGCTGAACTTAAGCATATCAATAAATTTGAGGTCAAATGGTCAATGTATGCACTTATAT
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ATGCAAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATATTTTTCAATTAAGAGAAACTTGTAAAAA
AAAACAAAGCTTCAATATGAGATCGTTCTATGTAACATACAATAAGAGTTATATAGAGTAATCATA
GTCAGGATTTCTCCAGACTACAGTTGGTTCACAGGTGTATGTGTTATATAGCTTCAGAGTGTGCA
CATGCAATATTTATTACCAGCACAACTCCTTTGCATATATGAATTCAATAATGATCCTTCCGCAGG
TTCA

>SrRIP (Rippenpete)

GTTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAACC
AACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAGAAC
GATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAGAAAAAAACAATTTGAGGTCA
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ACTAATAGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCAACTCTCACATCCAAGCCTTG
ACAAATACAAAAATTTGTAAGGTTGAGAATTTAATGACTCTCAAACAGGCATGCCCCTCGGAATAC
CAAAGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGGATTCTGCAATTCACATTACTTAT
CGCATTTCGCTGCGTTCTTCATCGATGCAAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATATTTT
TAATTTAAAAAAACTTGGCCAAAAAACAAATTTCCATAATAAAACCTTCTTTGGA

>SrSAI (Saidapur)

GAGTTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAA
CCAACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACATAG
AACGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACA
ACGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGC
CTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGA
GTTGCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCC
TACGGTTGGTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTACT
TATAAAATCATGCGCATATATCTAGCATATAAGTGCATATATTGACCATTGACCTCAA

>SrSIR (Sirsi)

GAGTTGTGCTAACCTGCGGAAGGATCATTATTGAATTCATTATGCGAAGGAGTTGTGCTGGTAAT
GAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAACCAACTGTAGTCAGG
AGAAATCCTACTATGATCACCCTATATAACTCTTATTGATAACTCTTAGAACGATCTCATATTGAAA
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TTGATCGATGAAGAACGCAGCGAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCG
AATCTTAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTGTT
TGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGA
TGTGAGAGTTGCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAG
AAATGCCTACGGTTGGTGTGATAATTGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGT
ACTACTTATAAAATCATGCATATATCTAGCATATAAGTGCATATATT

>SrGDG (Gadag)

AGTTGTGCTGGTAATGAATATTGCATGTGCACACTCTGGAGCTATATAATATATACACCTGTGAAC
CAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGA
ACGATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCAACAA
CGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCT
GTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGT
TGCTGGTTAGAGTATATTCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATGTAGAAATGCCTA
CGGTTGGTGTGATAATATGTCTACGCCTATACCGGAAGGGGATTCTAGCTTGTATGTACTACTTA
TAAAATCATGCGCATATATCTAGCATATAAGTGCATAT

> SrTHIR (Thirthalli)

GAGTTGTGCTGGCAATAAATATTGCATGTGCACACTCTGAAGCTATATAACACATACACCTGTGA
ACCAACTGTAGTCTGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACATAG
AACGATCTCATATTGAAGCTTTGTTTTTTTTTACAAGTTTCTCTTAATTGAAAAATATACAACTTTCA
ACAACGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCAT
GCCTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGA
GAGTTGCTAGTTAAGAATATCTGACTGGCTCTCTTTAAAATTATTAGTAGGACATATAGAAATGCC
TGCGGTTGGTGTGATAATATGTCTACGCCTATACCAAAGGGGATTCTAGCTTGTATGTACTACTTA
TAAAACCATGCGCATATATCTAGCATATAAGTGCATACATTGACCATTTGACCTCAAATCAGGTAG
GACTACCCGCTGAACTTAAGCATATCAATAAGAGGTCAATGGTCAATGTATGCACTTATATGCTA
GATATATGCGCATGGTTTTATAAGTAGTGCATACAAGCTAGAATCCCCTTTGGTATAGGCGTAGA
CATATTATCACACCAACCGCAGGCATTTCTACATGTCCTACTAATAATTTTAAAGAGAGCCAGTCA
GATATTCTTAACTAGCAACTCTCACATCCAAGCCTTGACAAATACAAAAATTTGTAAGGTTGAGAA
TTTAATGACTCTCAAACAGGCATGCCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGATT
CGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCA
AGAGCCAAGAGATCCGTTGTTGAAAGTTGTATATTTTTCAATTAAGAGAAACTTGTAAAAAAAAAC
AAAGCTTCAATATGAGATCGTTCTATGTAACATACAATAAGAGTTATATAGAGTAATCATAGTCAG
GATTTCTCCAGACTACAGTTGGTTCACAGGTGTATGTATTATATAGCTTCAGAGTGTGCACATGCA
ATATTTATTACCAGCACAACTCCTTTGCATATATGAATTCAATAATGATCCTTCCGCAGGTTCAC

Table 20: Comparison and identify of fragment of the 18S rDNA gene of *Sclerotium rolfsii* of stevia with that of referred gene bank available in NCBI

Sl. No.	Location	Designation	Reference Accession no	Species	Maximum Identity
1	Belgaum	SrBGM	KC460985.1	*Athelia rolfsii*	99%
2	Gangavati	SrGAN	GQ358518.1	*Athelia rolfsii*	99%
3	UAS, Bengaluru	SrGKVK	HM222638.1	*Athelia rolfsii*	98%
4	Hosagudda	SrHOS	HQ895874.1	*Athelia rolfsii*	100%
5	Kalenahalli	SrKAL	JN241563.1	*Athelia rolfsii*	100%
6	Mysore	SrMYS	HQ895931.1	*Athelia rolfsii*	100%
7	Nidshoshi	SrNID	JN241563.1	*Athelia rolfsii*	99%
8	Thindlu	SrTHI	JN241563.1	*Athelia rolfsii*	99%
9	Raichur	SrRCR	HQ420816.1	*Athelia rolfsii*	99%
10	Rippenpete	SrRIP	JQ982485.1	*Athelia rolfsii*	98%
11	Saidapur	SrSAI	HM355751.1	*Athelia rolfsii*	97%
12	Sirsi	SrSIR	KC832506.1	*Athelia rolfsii*	99%
13	Gadag	SrGDG	GQ358518.1	*Athelia rolfsii*	99%
14	Thirthalli	SrTHIR	HM222638.1	*Athelia rolfsii*	100%

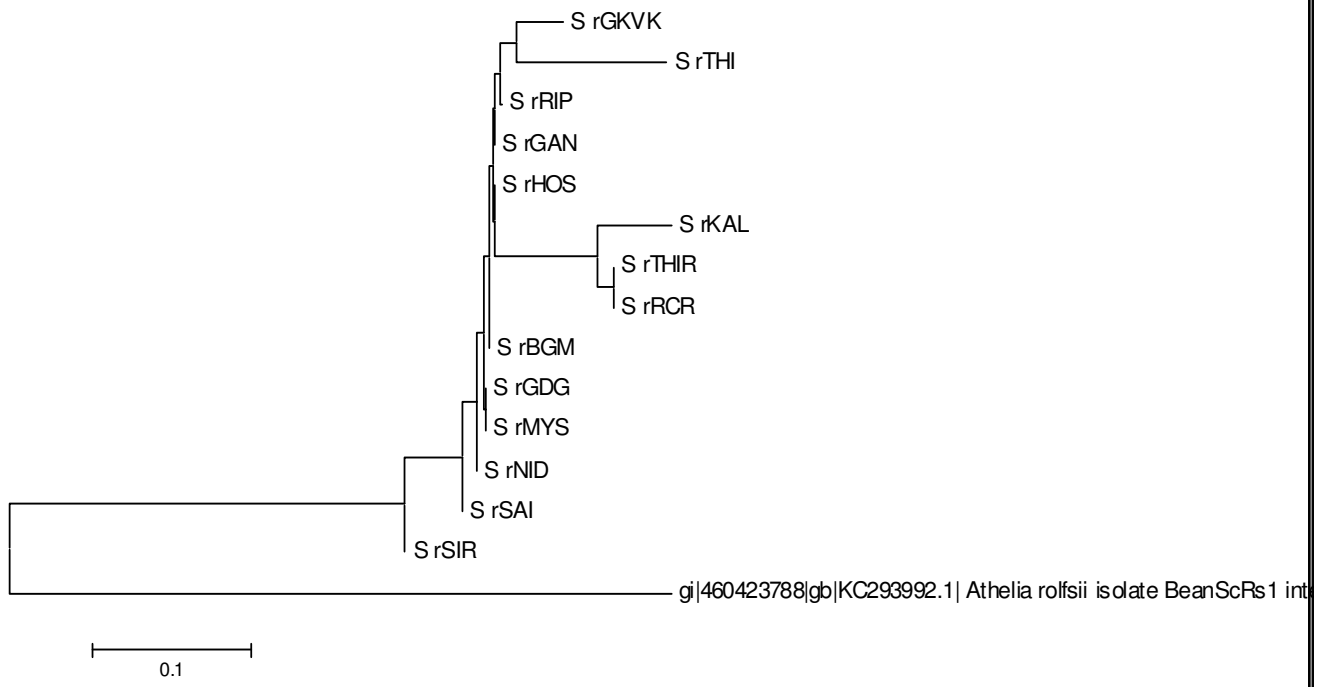
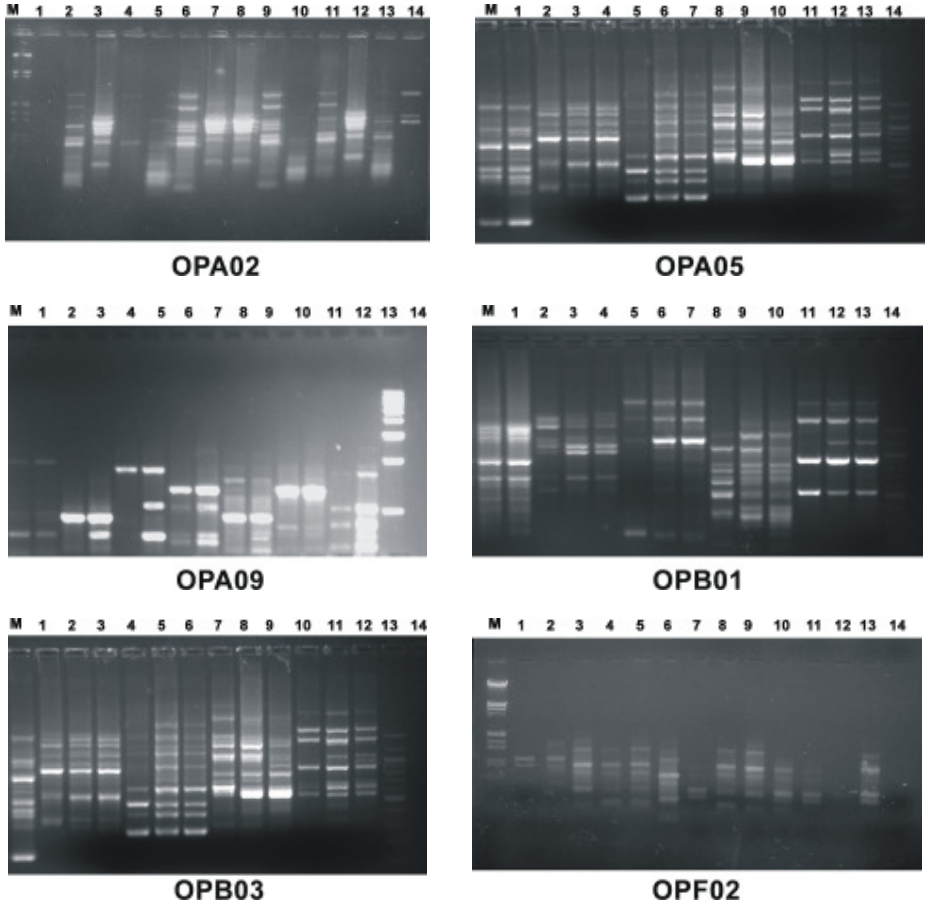
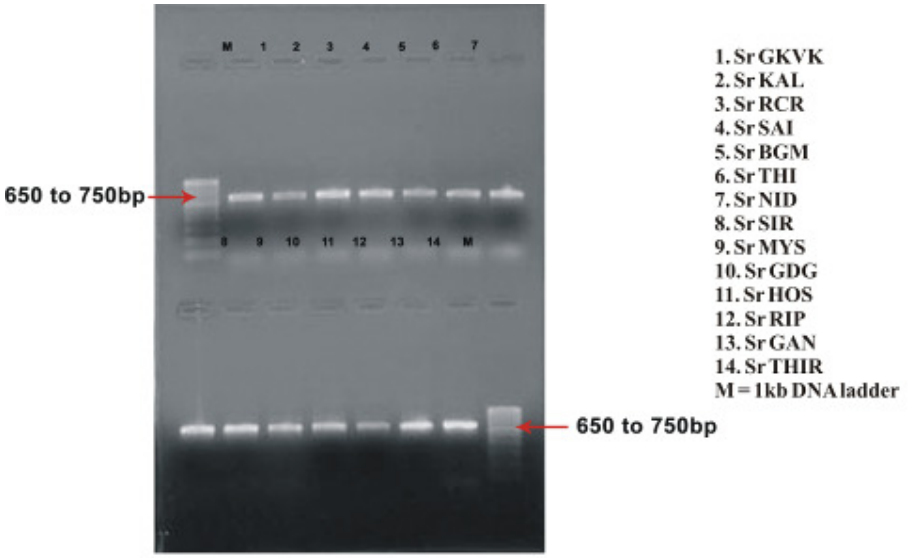


Fig. 13b: Polyogram of isolates of *Sclerotium rolfsii* of stevia based on ITS rDNA sequence data





Amplification product of Internal Transcribed Spacer (ITS) with ITS-1 and ITS-4 ribosomal DNA primers

Plate 11b: Genetic variability among 14 isolates of *Sclerotium rolfsii* by ITS

Table 21: Loss assessment with respect to growth parameters in treated and control plots at different intervals during 2011-12

Treatments	60 DAP		120 DAP		60 DAP		120 DAP	
	Plant height (cm)	% DOT	Plant height (cm)	% DOT	Number of branches	% DOT	Number of branches	% DOT
Hexaconazole	62.21	-	83.09	-	21.67	-	45.26	-
Untreated control	30.36	51.19	34.16	58.88	8.86	59.11	24.40	46.08

DAP= Days after planting
% DOT = Per cent decrease over treatment

Table 22: Loss assessment with respect to percent disease incidence and yield (fresh weight and dry weight) in treated and control plots

Treatments	2011-12					
	Percent disease incidence		Fresh herbage yield (t/ha)	% loss in yield	Dry herbage yield (t/ha)	% loss in yield
	120 DAP	%DOC				
Hexaconazole	13.33 (21.14)*	80.02	10.78	-	2.56	-
Untreated control	66.74 (35.25)	-	3.76	65.12	0.9	64.84

*Values in parenthesis are arc-sine transformed values
% DOC = Per cent decrease over control
DAP= Days after planting

Table 23: Biochemical changes in stevia due to infection *by Sclerotium rolfsii*, *Fusarium solani* and *Rhizoctonia bataticola*

Treatments	Healthy Stevia leaves	*Sclerotium* infected leaves	% increase/ decrease over healthy	*Furarium* infected leaves	% increase/ decrease over healthy	*Rhizoctonia* infected leaves	% increase/ decrease over healthy
Reducing sugars	35.5	20.50	- 42.25	19.89	- 43.97	24.16	-31.94
Non reducing sugars	18.5	15.11	- 18.32	13.19	- 28.70	15.50	- 16.21
Total Sugars	54	35.61	- 34.05	33.08	- 38.74	39.66	- 26.55
Total phenols	2.70	3.72	+ 37.77	3.80	+ 40.74	3.65	+ 35.18
OD phenols	0.380	0.460	+ 21.05	0.465	+ 22.36	0.463	+ 21.84

Table 24: Evaluation of minimum inoculum levels of *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola* for causing wilt of stevia

Inoculum level (%)	*S.rolfsii*		*F. solani*		*R. bataticola*	
	No. of days taken for wilting	PDI	No. of days taken for wilting	PDI	No. of days taken for wilting	PDI
0	-	-	-	-	-	-
2	31	25.00 (29.99)*	28	33.33 (35.26)	32	16.66 (24.09)
4	22	58.33 (49.80)	20	66.66 (54.76)	28	33.33 (35.26)
6	17	75.00 (60.63)	18	83.33 (65.94)	21	58.33 (49.80)
8	16	91.66 (73.41)	12	91.66 (73.41)	17	83.33 (65.94)
10	13	100.00 (90.00)	10	100.00 (90.00)	15	91.66 (73.41)
12	12	100.00 (90.00)	10	100.00 (90.00)	14	100.00 (90.00)
14	10	100.00 (90.00)	08	100.00 (90.00)	12	100.00 (90.00)
S.Em. ±		1.11		1.03		1.18
CD at 1 %		3.38		3.14		3.57

*Values in parenthesis are arc-sine transformed values

4.5.2 Biochemical changes in healthy and infected stevia leaves

The results of biochemical study of healthy and infected stevia are presented in the Table 23.

Sclerotium rolfsii

In the present study, the infected leaves recorded 34.05 per cent decrease of total sugars, 42.25 per cent decrease of reducing sugars and 18.32 per cent decrease of non reducing sugars when compared to healthy leaves. Where as 37.77 per cent increase in total phenol and 21.05 per cent increase in ortho dihydroxy (OD) phenols was recorded compared to healthy leaves

Fusarium solani

The infected leaves recorded 38.74 per cent decrease of total sugars, 43.97 per cent decrease of reducing sugars and 28.70 per cent decrease of non reducing sugars when compared to healthy leaves. Whereas 40.74 per cent increase in total phenol and 22.36 per cent increase in ortho dihydroxy (OD) phenols was recorded compared to healthy leaves (Table 23).

Rhizoctonia bataticola

In the infected leaves 26.55 per cent decrease of total sugars, 31.94 per cent decrease of reducing sugars and 16.21 per cent decrease of non reducing sugars was recorded when compared to healthy leaves. Whereas 35.18 per cent increase in total phenol and 21.84 per cent increase in ortho dihydroxy (OD) phenols was recorded compared to healthy leaves (Table 23).

4.6 Epidemiological Studies

4.6.1 Inoculum levels and disease incidence

The fungal culture of *S. rolfsii, F. solani and R. bataticola* grown on sand corn meal media were mixed separately to each pot to obtain different inoculum levels *viz*., 0, 2 ,4, 6, 8, 10, 12 and 14 percent as detailed in the 'Material and Methods'. The data on the effect of different inoculum levels on the per cent disease incidence is presented in Table 24 and Plate 12.

Sclerotium rolfsii

The results indicated that at zero per cent inoculum level, the percent disease incidence recorded was zero, but increase in per cent inoculum levels resulted in significant increase in the per cent disease incidence. At the concentration of ten per cent, there was cent percent disease incidence. Minimum disease incidence (25.00%) was noticed in two per cent inoculum level and with the increase in inoculum levels, maximum of 100 per cent disease incidence was recorded.

Fusarium solani

There was significant increase in the per cent disease incidence with increase in inoculums level. Cent per cent disease incidence was noticed when inoculum of 10 per cent was added, which was on par with inoculum at 8 per cent (91.66). Disease incidence of 33.33 per cent was recorded at minimum of two per cent inoculum level and increased with increase of inoculum levels.

Rhizoctonia bataticola

The results indicated that there was cent per cent disease incidence with the inoculum levels of 12 per cent and above. There was no disease incidence in uninoculated control. Minimum disease incidence (16.66) was noticed in two percent inoculum level and increased with increase inoculum level and reached 100 per cent at 10 per cent inoculum level.

4.6.2 Effect of soil temperature on *Sclerotium rolfsii*

Soil temperature is one of the important factors influencing the growth of the fungus. The soil temperatures tested were 15, 20, 25, 30, 35, 40, 45 and 50^{o} C as described in 'Material and Methods'. The per cent colonization of sorghum seed baits at different soil temperatures and the germination of sclerotia are given in Table 25, Fig 14 and Plate 13a.

S. rolfsii produced maximum growth at 30^{0}C (65.35 per cent colonization) which was significantly superior over all levels of soil temperatures tested. However there was no significant difference between the temperature regime of 20^{0}C and 40^{0}C. No growth was obtained at 50^{0}C.

Table 25: Influence of soil temperature on competitive saprophytic ability and viability of S*clerotium rolfsii*

Soil temperature (^{0}C)	Per cent colonization of sorghum seeds	Germination of sclerotia (%)
15	15.75(23.38)*	25.75(30.49)
20	28.33(32.17)	46.00(42.71)
25	53.67(47.10)	75.00(60.00)
30	65.35(52.54)	92.67(73.95)
35	36.33 (37.06)	65.43(53.99)
40	25.63(30.41)	34.30(35.87)
45	12.92(21.07)	14.33(22.24)
50	0.00(0.00)	0.00(0.00)
S.Em. ±	1.57	2.72
CD at 1 %	4.78	7.46

*Values in parenthesis are arc-sine transformed values

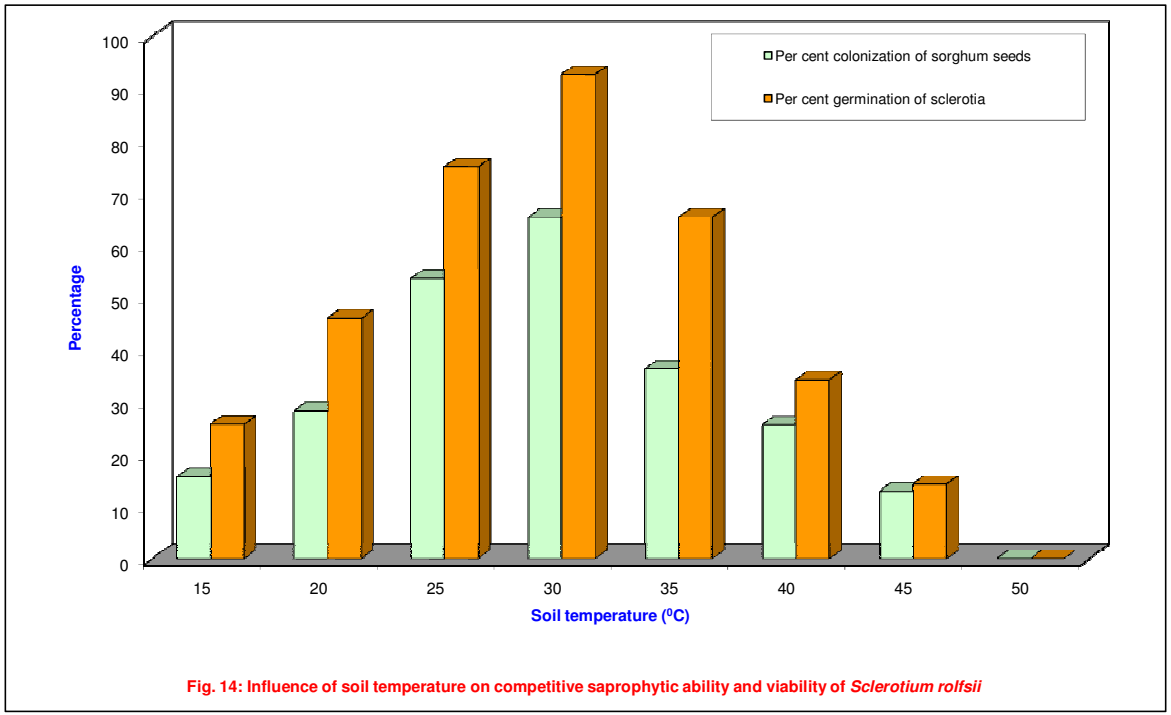


Fig. 14: Influence of soil temperature on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Plate 14: Influence of soil temperature on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Table 26: Influence of soil moisture on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Per cent soil moisture	Per cent colonization of sorghum seeds	Germination of sclerotia (%)
10	32.33 (34.67)*	71.43 (71.43)
20	56.87 (48.98)	80.13 (63.56)
30	84.13 (66.56)	100.00 (89.85)
40	63.67 (52.96)	73.67 (59.16)
50	52.00 (46.17)	62.90 (52.50)
60	26.90 (31.26)	55.33 (48.09)
70	12.67 (20.86)	25.69 (30.45)
S.Em. ±	3.68	2.25
CD at 1 %	11.10	6.79

*Values in parenthesis are arc-sine transformed values

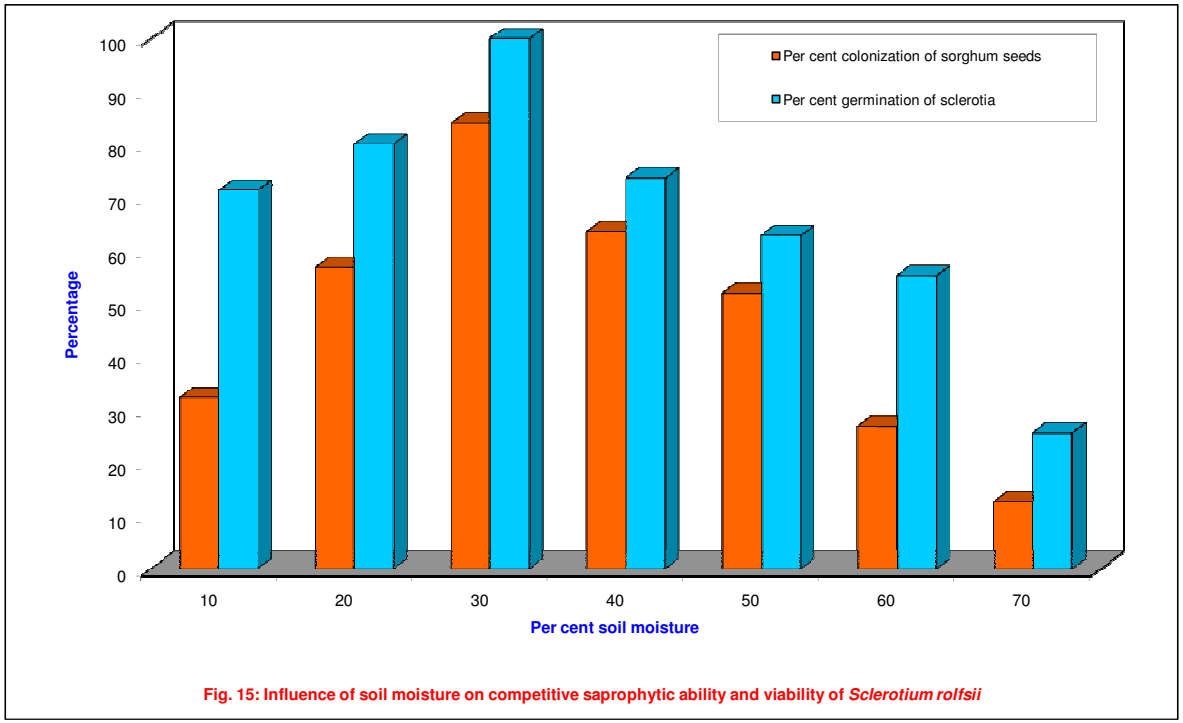
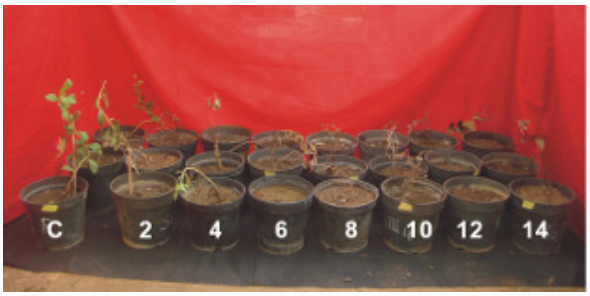


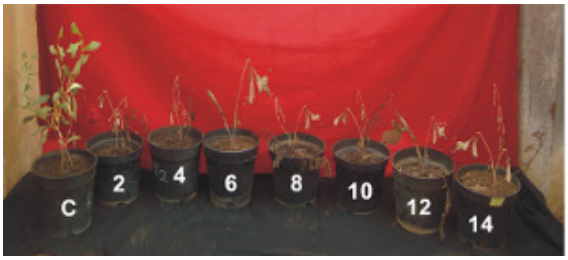
Fig. 15: Influence of soil moisture on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Fig 15: nfluence of soil moisture on competitive saprophytic ability and viability of *Sclerotium rolfsii*



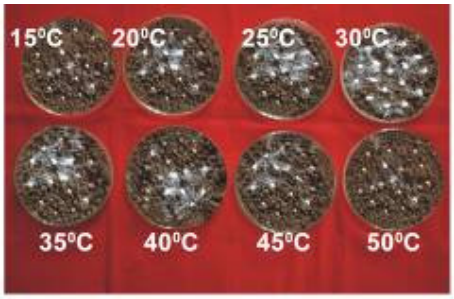
Sclerotium rolfsii

Fusarium solani



Rhizoctonia bataticola

Plate 12: Evaluation of inoculum levels of soilborne fungal pathogens



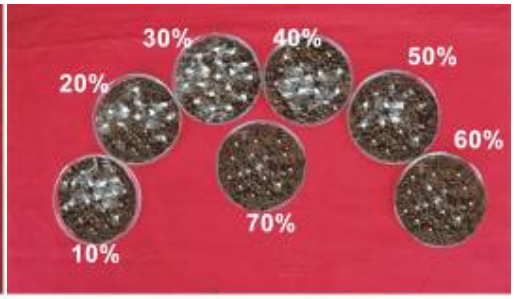


Plate 13a: Influence of soil temperature on competitive saprophytic ability of *S. rolfsii*

Plate 13b: Influence of soil moisture on competitive saprophytic ability of *S. rolfsii*

Plate 13: Epidemiological studies

Similar trend was observed in per cent germination of Sclerotia. Maximum germination of 92.67 per cent was observed at 30^0C which is significantly superior to all other treatments. Least germination (14.33 %) was noticed at 45^0C. No germination of sclerotia was obtained at 50^0C.

4.6.3 Effect of soil moisture on *Sclerotium rolfsii*

The effect of seven moisture levels was tested on saprophytic activity of fungus and germination of sclerotia and the data are presented in Table 26, Fig. 15 and Plate 13b.

It is clear from the data that, the fungus *S. rolfsii* survived better at low soil moisture levels than at high moisture levels. The saprophytic activity of the fungus was 32.33 per cent at 10 per cent soil moisture level and increased to 56 per cent at 20 per cent soil moisture level. The maximum saprophytic activity of the fungus was found at 30 per cent moisture level (84.13 %). Least was recorded at 70 per cent soil moisture level (12.67 %). With respect to per cent germination of sclerotia, maximum of 100 per cent germination was observed at 30 per cent soil moisture level which was significantly superior to all other treatments. This was followed by 20 per cent (80.13%) which was on par with 40 per cent (73.67 %). Least germination was noticed in 70 per cent soil moisture level (25.69 %).

4.6.4 Effect of soil pH levels

Nine pH levels were tested as described in 'Material and Methods' to understand the saprophytic activity of the fungus and germination of sclerotia. The data are presented in Table 27 and Fig. 16.

The fungus showed moderate to good growth over a pH range of 5.5 to 6.5. However, maximum fungal colonization of sorghum seeds was recorded at pH 6, where the saprophytic activity of the fungus was 79.10 per cent which was on par with 6.5, 7.0 and 7.5 pH (66.12, 53.33 and 52.97 per cent respectively). Lower saprophytic activity of 32.42 per cent was observed at pH of 9.5. Treatments differed significantly with respect to per cent germination of sclerotia also. Highest germination of sclerotia was observed at pH 6.0 (92.25%) which was on par with 6.5 (90 %) followed by 7.0 (85.00%) and 5.5 (83.33 %) pH levels. Significantly least germination of sclerotia was noticed at pH level of 9.5 (24.48 %) followed by 9.0 (38.33 %)

4.6.5 Viability of sclerotia of *S. rolfsii* at different depths and duration in soil.

An experiment was carried out in the glass house of Department of Plant Pathology, College of Agriculture, Dharwad to know the viability of sclerotia at different depths and duration as described in 'Material and Methods'. The per cent germination of sclerotia was recorded at different time intervals and specific depths. The data were analysed statistically and are presented in Table 28. The experimental results indicated that maximum per cent germination of sclerotia (87.57%) was noticed at one cm depth which was significantly superior over all other treatments. Per cent germination was reduced gradually as the depth increased. Significantly least germination of two per cent was noticed at 15 and 16 cm depths. Zero per cent germination was recorded at 17, 18, 19 and 20 cm depth. The germination of sclerotia was 100 per cent before storage and one month after the storage which was significantly superior than all the treatments and it gradually decreased with increase in storage duration. The germination was zero per cent at the end of 19th month after storage.

4.6.6 Survival

The survival ability of *Fusarium solani* was carried out as described in 'Material and Methods'

Fusarium solani

The results presented in Table 23 revealed that, *F. solani* was detected upto 36, 30, 24 and 22 weeks at refrigeration, laboratory, glass house and field conditions respectively (Table 29)

4.6.7 Susceptible stage of the crop

To find out the susceptible stage of the crop to sclerotium wilt, an experiment was laid out in glass house conditions as explained in "Material and Methods" (Table 30, Fig. 17 and Plate 14). The results revealed that, there was significant difference in wilting percentage among the different stages of the plants. Significantly highest per cent wilt of 91.66 per cent was recorded in 15 days old rooted stevia cuttings. This was followed by 83.33, 75.00 and 66.66 per cent wilting in 30, 60 and 90 days old stevia rooted cuttings respectively. 120 days old stevia plants showed 41.67 per cent wilt. However, it took a maximum of 54 days for complete wilting.

Table 27: Influence of soil pH on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Soil pH	Per cent colonization of sorghum seeds	Per cent germination of sclerotia
5.5	41.27(39.97)*	52.47(46.41)
6.0	79.10(62.80)	92.25(73.83)
6.5	66.12(83.60)	90.00(71.57)
7.0	53.33(46.92)	85.00(65.70)
7.5	52.97(63.77)	83.33(65.95)
8.0	51.97(60.70)	66.12(83.60)
8.5	42.19(45.00)	58.75(50.02)
9.0	37.53(37.10)	38.33(38.27)
9.5	32.42(28.77)	24.48(29.67)
S.Em. ±	1.80	2.89
CD at 1 %	5.47	8.76

*Values in parenthesis are arc-sine transformed values

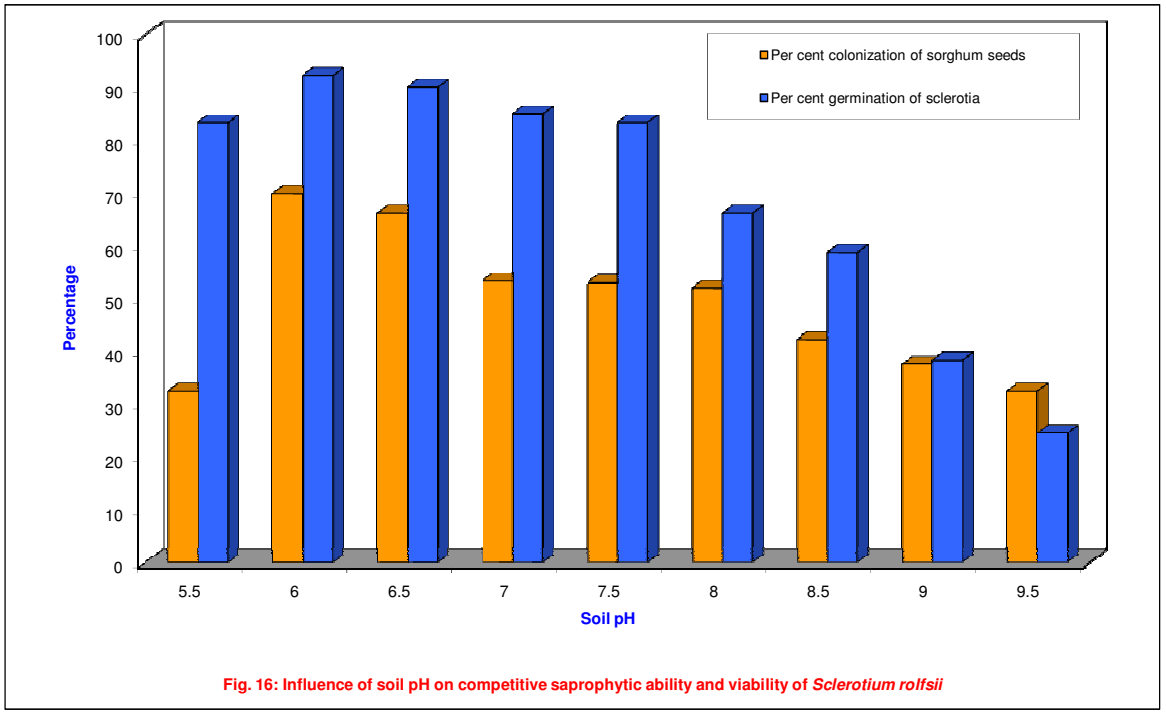


Fig. 16: Influence of soil pH on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Fig 16: Influence of soil pH on competitive saprophytic ability and viability of *Sclerotium rolfsii*

Table 28: Viability of Sclerotia of *Sclerotium rolfsii* at different depths and duration in the soil

Depth (cm)	Germination of sclerotia (%) (after 10 months)	Duration (Months)	Germination of sclerotia (%) (at monthly interval)
-	-	0	100.00 (89.85)*
1	87.57 (63.88)*	1	100.00 (89.85)
2	77.50 (61.72)	2	96.67 (79.52)
3	76.17 (60.81)	3	90.50 (72.52)
4	74.37 (59.62)	4	87.50 (62.33)
5	65.15 (53.85)	5	82.67 (65.43)
6	56.50 (48.76)	6	78.50 (62.41)
7	51.50 (45.88)	7	74.50 (59.70)
8	48.09 (43.93)	8	70.00 (56.82)
9	44.50 (41.86)	9	65.50 (54.06)
10	42.00 (40.52)	10	62.80 (52.45)
11	34.30 (35.87)	11	60.33 (50.99)
12	29.06 (32.64)	12	56.67 (48.86)
13	19.50 (26.22)	13	50.00 (45.02)
14	10.50 (18.91)	14	46.33 (42.92)
15	4.43 (12.16)	15	28.67 (32.39)
16	2.00 (8.14)	16	20.50 (26.94)
17	0.00 (0.71)	17	16.50 23.98)
18	0.00 (0.71)	18	6.00 (14.18)
19	0.00 (0.71)	19	0.00 (0.71)
20	0.00 (0.71)	20	0.00 (0.71)
S.Em. ±	1.51		2.56
CD at 1 %	4.45		7.72

Table 29: Survival of *Fusarium solani* in infected debris of Stevia at different environmental conditions

Weeks	Refrigeration	Laboratory (Room temperature)	Glasshouse	Field conditions
0	+	+	+	+
2	+	+	+	+
4	+	+	+	+
6	+	+	+	+
8	+	+	+	+
10	+	+	+	+
12	+	+	+	+
14	+	+	+	+
16	+	+	+	+
18	+	+	+	+
20	+	+	+	+
22	+	+	+	+
24	+	+	+	-
26	+	+	-	-
28	+	+	-	-
30	+	+	-	-
32	+	-	-	-
34	+	-	-	-
36	+	-	-	-
38	-	-	-	-

+ = Presence - = Absence

Table 30: Identification of susceptible stage of the crop

Age of the Plant	Percent disease incidence
15 days old	91.66
30 days old	83.33
60 days old	75.00
90 days old	66.66
120 days old	41.67
S.Em ±	3.68
CD at 5 %	11.04

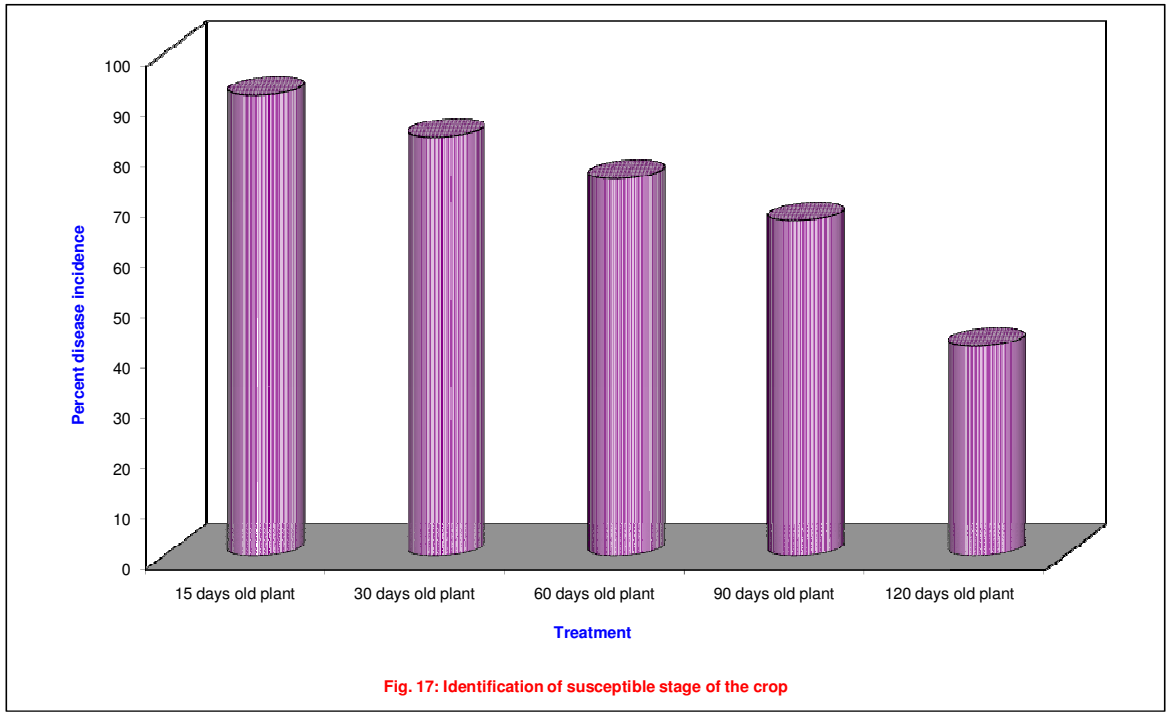


Fig 17: Identification of susceptible stage of the crop

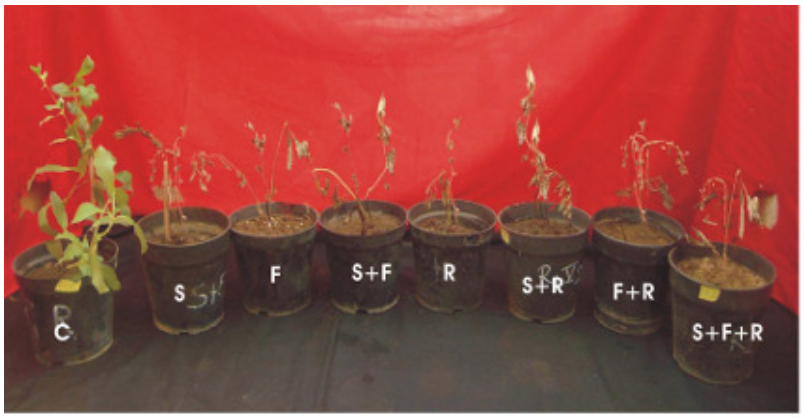
30 Days after inoculation

65 Days after inoculation

Plate 14: Susceptible stage of stevia for *Scierotium rolfsii*

Experimental view

10 days after inoculation



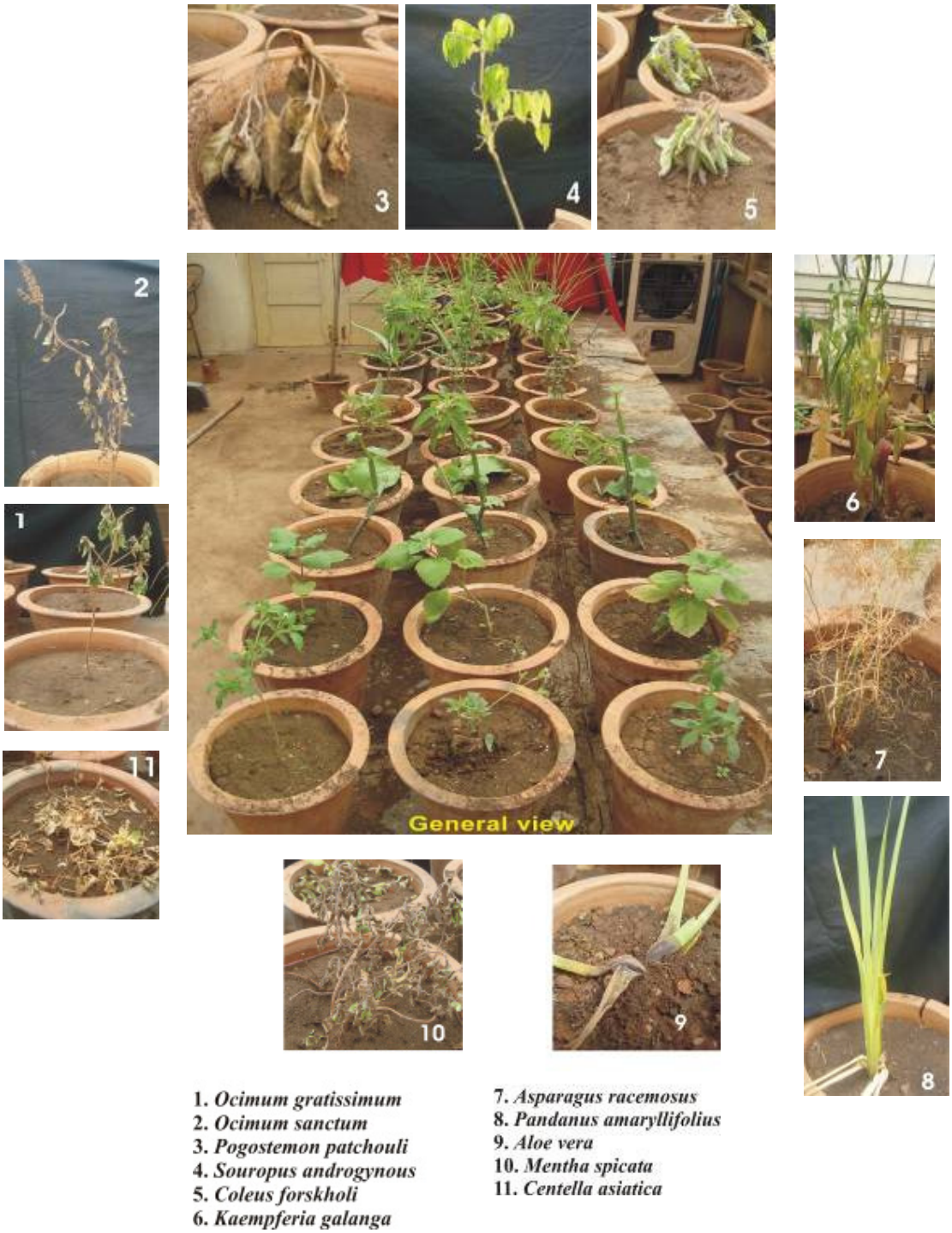
35 days after inoculation

C - Control
S - *Sclerotium rolfsii*
F - *Fusarium solani*
R - *Rhizoctonia bataticola*
S+F - *S. rolfsii* + *F. solani*
S+R - *S. rolfsii* + *R. bataticola*
F+R - F. solani + R. bataticola
S+F+R - S. rolfsii + F. solani + R. bataticola

Plate 15: Interaction among the soil born pathogens causing wilt of stevia

Table 32: Host range of *Sclerotium rolfsii*

Common Name	Botanical name	Days taken for symptom expression	Symptoms
Brahmi	*Centella asiatica*	5	Yellowing & drying of leaves
Mint	*Mentha spicata*	5	Yellowing and drying
Tulsi	*Ocimum sanctum*	7	Yellowing & drying of leaves
Coleus	*Coleus forskholli*	7	Yellowing, drooping wilting
Aloe.	*Aloe vera*	8	Yellowing & rotting at basal portion
Rosemary	*Rosmarinum officinalis*	8	Yellowing, drying
Shatavari	*Asparagus racemosus*	10	Yellowing, wilting
Patchouli	*Pogostemon patchouli*	10	Yellowing, wilting and drying
Pandanus	*Pandanus amaryllifolius*	40	Yellowing
Lemon grass	*Cymbopogon flexuosus*	-	-
Citronella	*Cymbopogon winterianus*	-	-
Clocimum	*Ocimum gratissimum*	-	-
Chakramani	*Souropus androgynous*	20	Yellowing
Vetiver	*Vetiveria zizanioides*	-	-
Kachora	*Kaempferia galanga*	12	Yellowing, drying
Vajravalli	*Cissus quadrangularis*	-	-
Insulin plant	*Costus pictus*	-	-



1. *Ocimum gratissimum*
2. *Ocimum sanctum*
3. *Pogostemon patchouli*
4. *Souropus androgynous*
5. *Coleus forskholi*
6. *Kaempferia galanga*
7. *Asparagus racemosus*
8. *Pandanus amaryllifolius*
9. *Aloe vera*
10. *Mentha spicata*
11. *Centella asiatica*

Plate 16: Host range of *Sclerotium rolfsii*

4.6.8 Interaction among pathogens

There is no doubt about pathogenic potential of *S. rolfsii*, *F. solani and R. bataticola.* Whether these pathogens act alone or in combination are part of disease complexes. This has been an area of research in recent years. Such studies would help us to design management practices against the appropriate disease/diseases. Pot culture tests were conducted to investigate the interaction among the wilt causing pathogens *viz*., *S. rolfsii, F. solani and R. bataticola* (Table 31, Fig. 18 and Plate 15).

Individual pathogens inoculated to the plants showed varying symptoms. Plants inoculated with *S. roflsii* showed early wilt symptoms within 15 days of inoculation. The leaves turned yellow in colour, became flaccid and dropped off. Finally the plant wilted and dried. *S. rolfsii* when inoculated alone to the plant recorded 65.56 per cent disease incidence.

F. solani alone inoculated plants exhibited symptoms within 22 days of inoculation and recorded 55.56 percent disease incidence. The symptoms developed were recorded. The characteristic symptoms started as yellowing of lower leaves extended upwards and whole leaves gradually turned brown coloured. The infected plants showed discoloration of roots and complete destruction of root system.

In case of *R. bataticola* alone inoculation, the plant exhibited symptoms after 35 days of inoculation and recorded 44.46 per cent disease incidence. Water soaked areas and the affected tissues soon turned into a soft black, watery mass at the collar region of the plant

Simultaneous inoculation of two pathogens i.e., *S.rolfsii + F.solani*, *F.solani + R. bataticola* and *S. rolfsii + R bataticola* exhibited the symptoms at 12, 17 and 15 days after inoculation with a per cent disease incidence of 88.89, 77.78 and 77.78 respectively.

When simultaneously three pathogens (*Sclerotium + Fusarium + Rhizoctonia*) were inoculated, the plants exhibited the earlier symptoms within 10 days and recorded cent per cent disease incidence. When pathogens were isolated later from this sample S, F, and R were recorded indicating these are dominant pathogen.

4.6.9 Host range of *Sclerotium rolfsii*

Eighteen different hosts were artificially inoculated with gaint culture of *S. rolfsii* in earthen pots under glass house condition as described in 'Material and Methods'. The results are furnished in Table 32 and Plate 16.

Eleven hosts *viz*., *Coleus amboinicus, Ocimum sanctum, Coleus forskohlii, Aloe vera, Mentha spicata, Asparagus racemosus*, *Rosmarinus officinalis, Centella asiatica, Pogostemon patchouli*, *Pandanus amaryllifolius* and *Sauropus androgynous* were infected by *S.rolfsii* isolated from stevia. The symptoms of the disease observed were similar to that of stevia. However, there were some differences in the symptoms expression among the hosts. *Withania somnifera, Cymbopogan flexuosus, Costus pictus, Kaempferia galanga, Ocimum gratissimum, Cissus quadrangularis and Vetiveria zizanioides* did not show any infection by *S. rolfsii.*

Symptoms were first expressed on *Mentha spicata, Centella asiatica* and *Coleus forskohli* in just five days followed by *Coleus amboinicus* and *Ocimum sanctum* showed drooping, wilting and yellowing, drying respectively in seven days. In Aloe rotting at basal portion was noticed in eight days. *Rosmarinus officinalis* wilted after eight days. *Asparagus racemosus* and *Pogostemon patchouli* wilted after ten days. Whereas, *Pandanus amaryllifolius* and *Sauropus androgynous* took maximum days to exhibit the symptoms i.e. 40 and 20 days respectively.

4.7 Management studies

4.7.1 *In vitro* evaluation of botanicals

As plant extracts are cost effective means of management, an effort was made to know the efficacy of different plant extracts against *S. rolfsii* and *F. solani and R. bataticola.* This was carried out by adopting the poison food technique as described in 'Material and Methods'. Observations on diameter of mycelial growth of fungus were recorded when untreated control plate showed full growth of the fungus. Results relating to the effects of plant extracts on per cent inhibition of *S. rolfsii, F. solani and R. bataticola* are presented here under.

Table 33: Inhibition of mycelial growth of *Sclerotium rolfsii* by different plant extracts

Plant extracts	Per cent inhibition of mycelial growth		Mean
	Concentration (%)		
	5	10	
Adathoda	16.03(23.62)	22.11 (28.06)	19.07(25.84)*
Bougainvillea spectabilis	27.78(31.82)	44.63(41.94)	36.20(36.88)
Calotropis	13.33 (21.43)	16.77(24.19)	15.05 (22.81)
Cassia fistula	10.37(18.79)	12.03(20.30)	11.70 (20.28)
Durantha repens	69.92 (56.77)	75.14 (60.13)	72.54(58.40)
Eucalyptus globes	33.77(35.55)	44.81(42.04)	39.29(38.80)
Euphorbium odoratum	19.00(25.85)	27.84(31.86)	23.42(28.85)
Allium sativum	24.74 (29.84)	31.77 (34.32)	28.25(32.08)
Glyrecidia maculata	68.51(55.95)	72.74 (58.55)	70.62(57.18)
Jatropa carcas	25.33 (30.23)	33.66(35.48)	29.50(32.85)
Prosopis juliflora	10.00 (18.44)	13.73(21.76)	11.01(19.37)
Lantana camera	36.66 (37.28)	45.81 (42.61)	41.24(39.95)
Azadirachta indica	38.66 (38.47)	47.62 (43.66)	43.14(41.06)
Allium cepa	13.66 (21.70)	17.88(25.02)	15.77(23.36)
Parthenium hysterophorus	46.00(42.72)	50.74(45.44)	48.37(44.08)
Pongamia glabra	30.66(33.64)	38.62(38.44)	34.64(36.04)
Tridax procumbens	29.33(32.81)	33.70(35.50)	31.51(34.15)
Ocimum sanctum	18.00(25.11)	23.70(29.14)	20.85(27.12)
Mean	29.16(31.99)	36.30(36.58)	-
	Plant extract (P)	Concentration (C)	P x C
S.Em.±	0.18	0.05	0.24
CD at 1%	0.57	0.16	0.78

*Figures in the parenthesis indicate arc sine transformed values

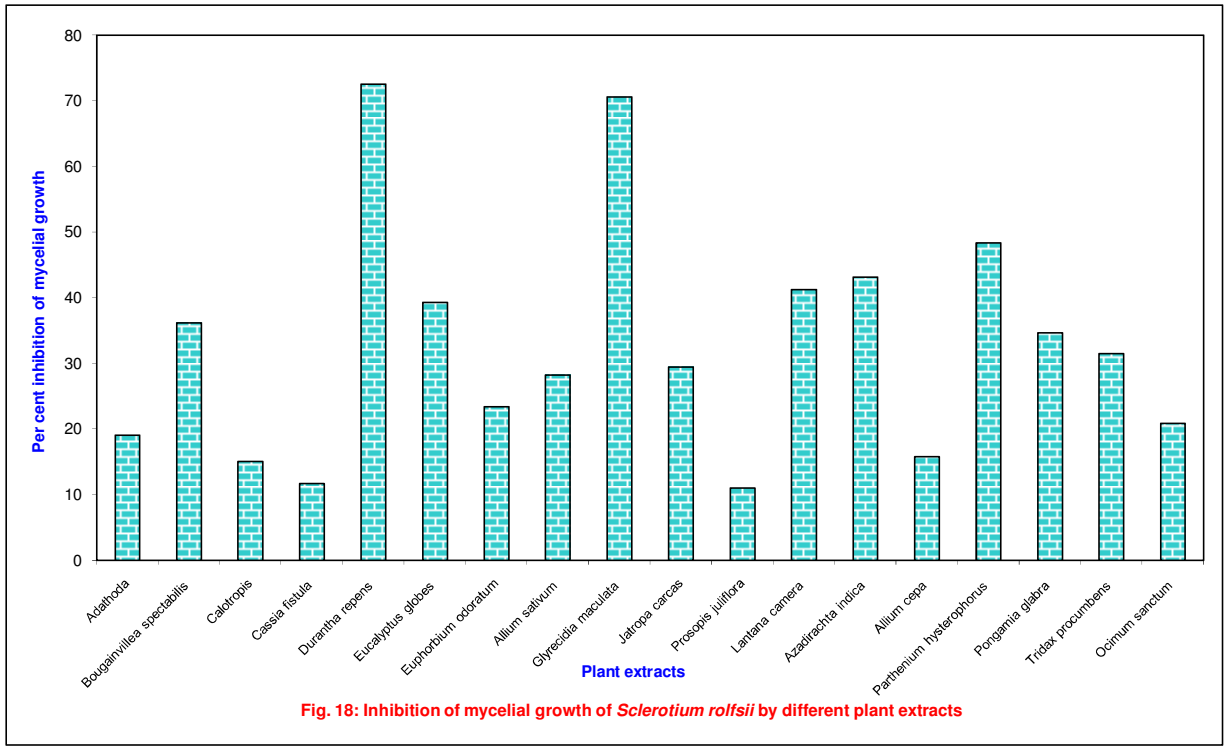


Fig. 18: Inhibition of mycelial growth of *Sclerotium rolfsii* by different plant extracts

Fig 18: *In vitro* evaluation of bioagents against soilborne fungal pathogens

Sclerotium rolfsii

Eighteen botanicals were evaluated against *S. rolsii.* The results revealed that the effect of plant extracts on the fungal growth was significant (Table 33, Fig. 18 and Plate 17A). Among 18 plant extracts evaluated against *S.rolfsii, Durantha repens* showed maximum inhibition of mycelial growth (72.54%) followed by *Glyrecidia maculata* (70.62%) and were significantly superior over all other plant extracts. These were followed by *Parthenium hysterophorus* (48.37%), *Azadirachta indica* (43.14%) and *Lantana camera* (41.24%). *Prosophis juliflora* showed least inhibition of mycelial growth (11.01%).

Among the two concentrations, the leaf extracts at 10 per cent were significantly superior to five per cent. In the interaction between plant extract and concentration *Duranta repens* showed significant increase in inhibition of mycelial growth at 10 per cent concentration (75.14%) compared to 5 per cent concentration (69.92%) and which was on par with *Glyrecidia maculata* at 10 per cent concentration (72.74%) followed by *Parthenium hysterophorus* (50.74%), *Azadirachta indica* (47.62%) and *Lantana camera* (45.81%). *Cassia fistula* showed least inhibition of mycelial growth 10 per cent concentration (12.03%) and *Prosophis juliflora* showed least inhibition of mycelial growth at 5 per cent concentration (10.00%).

Fusarium solani

Eighteen botanicals were evaluated against *F.solani.* The results revealed that the effect of plant extracts on the fungal growth was significant (Table 34, Fig. 19 and Plate 17B). Among 18 plant extracts evaluated against *F. solani, Bougainvillea spectabilis* showed maximum inhibition of mycelial growth (52.41%) followed by *Adathoda* (49.72%) and were significantly superior over all other plant extracts. The next best were *Duranta repens* (47.59%) followed by *Eucalyptus globes* (47.58%) and *Tridax procumbens* (47.44%). *Prosophis juliflora* showed least inhibition of mycelial growth (30.59%).

Among the two concentrations, the leaf extracts at 10 per cent were significantly superior to five per cent. In the interaction between plant extract and concentration *Bougainvillea spectabilis* showed significant increase in inhibition of mycelial growth at 10 per cent concentration (62.78%) compared to 5 per cent concentration (42.04%) followed by *Euphorbium odoratum* at 10 per cent concentration (55.63%) *Prosophis juliflora* showed least inhibition of mycelial growth at 10 per cent concentration (33.37%) and *Lantana camera* showed least inhibition of mycelial growth at 5 per cent concentration (24.48%).

Rhizoctonia bataticola

Fifteen botanicals were evaluated against *R.bataticola,* multineem showed cent per cent inhibition which was significantly superior over all other plant extracts (Table 35 and Plate 17C). This was followed by garlic bulb extract (87.24%), *Lantana camera* (63.92%) and calotropis (52.24%). Least inhibition was recorded in *Bougainvillea spectabilis* (1.57%) followed by *Ocimum sanctum* (4.26%) *and Tridax procumbans* (11.81%). Remaining eight plant extracts failed to inhibit the mycelial growth (Table 35)

Among the two concentrations, the plant extracts at 10 per cent were significantly superior to five per cent. In the interaction between plant extract and concentration, Multineem recorded maximum inhibition of mycelial growth (100.00%) which was significantly superior over all plant extracts both at 10 and 5 per cent followed by garlic bulb extract which recorded 100 per cent inhibition at 10 per cent concentration followed by 74.48 per cent inhibition at 5 per cent. The next best treatments were calotropis which showed (84.44%) inhibition of mycelial growth at 10 per cent concentration followed by *Lantana camera* (65.59%). Least inhibition was recorded in *Ocimum sanctum* (8.52%) and *Bougainvillea spectabilis* (3.15%)

4.7.2 *In vitro* evaluation of bioagents by dual culture technique

The antagonistic microorganisms *viz.*, *Trichoderma harzianum* Rifai., *T. viride* Pers., *T. koningii* Oudem., *T. virens* Miller Gidden., *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn along with native isolate of *T. harzianum* (Dharwad isolate) were evaluated for their antagonistic nature against *S. rolfsii, F. solani* and *R. bataticola* under *in vitro* conditions by dual culture technique as explained in 'Material and Methods'. Inhibition zone in mm was recorded and the per cent inhibition was calculated and the results thus obtained are presented in the Table 36, Fig. 20 and Plate 18.

Table 34: Inhibition of mycelial growth of *Fusarium solani* by different plant extracts

Plant extracts	Per cent inhibition of mycelial growth		Mean
	Concentration (%)		
	5	10	
Adathoda	47.06(43.22)	52.41(46.40)	49.72(44.84)*
Allium cepa	38.85(38.58)	44.11(41.64)	41.48(40.10)
Allium sativum	33.41(35.33)	42.04(40.44)	37.72(37.89)
Azadirachta indica	44.81(42.04)	53.70(47.15)	49.25(44.60)
Bougainvillea spectabilis	42.04(40.44)	62.78(52.43)	52.41(46.40)
Calotropis	30.93(33.80)	48.15(43.96)	39.54(38.93)
Cassia fistula	33.70(35.51)	49.26(44.60)	41.48(40.09)
Durantha repens	42.04(40.44)	53.15(46.83)	47.59(43.62)
Eucalyptus globes	44.52(41.87)	50.04(45.04)	47.58(43.61)
Euphorbium odoratum	35.22(36.25)	55.63(48.26)	45.42(42.37)
Glyrecidia maculata	34.48(35.98)	53.19(46.85)	43.83(41.45)
Jatropa carcas	44.48(41.85)	50.04(45.04)	47.26(43.43)
Lantana camera	24.48(29.67)	46.78(43.17)	35.63(36.65)
Ocimum sanctum	39.44(38.93)	51.44(45.83)	45.44(42.38)
Parthenium hysterophorus	38.93(38.62)	44.48(41.85)	41.70(40.22)
Pongamia glabra	30.74(33.67)	38.33(38.27)	34.53(35.99)
Prosopis juliflora	27.81(31.85)	33.37(35.30)	30.59(33.58)
Tridax procumbens	44.70(41.98)	50.19(45.13)	47.44(43.53)
Mean	37.64(37.84)	48.87(44.35)	
	Plant extract (P)	Concentration (C)	P x C
S.Em.±	0.74	0.28	1.03
CD at 1%	2.75	1.02	3.12

*Figures in the parenthesis indicate arc sine transformed values

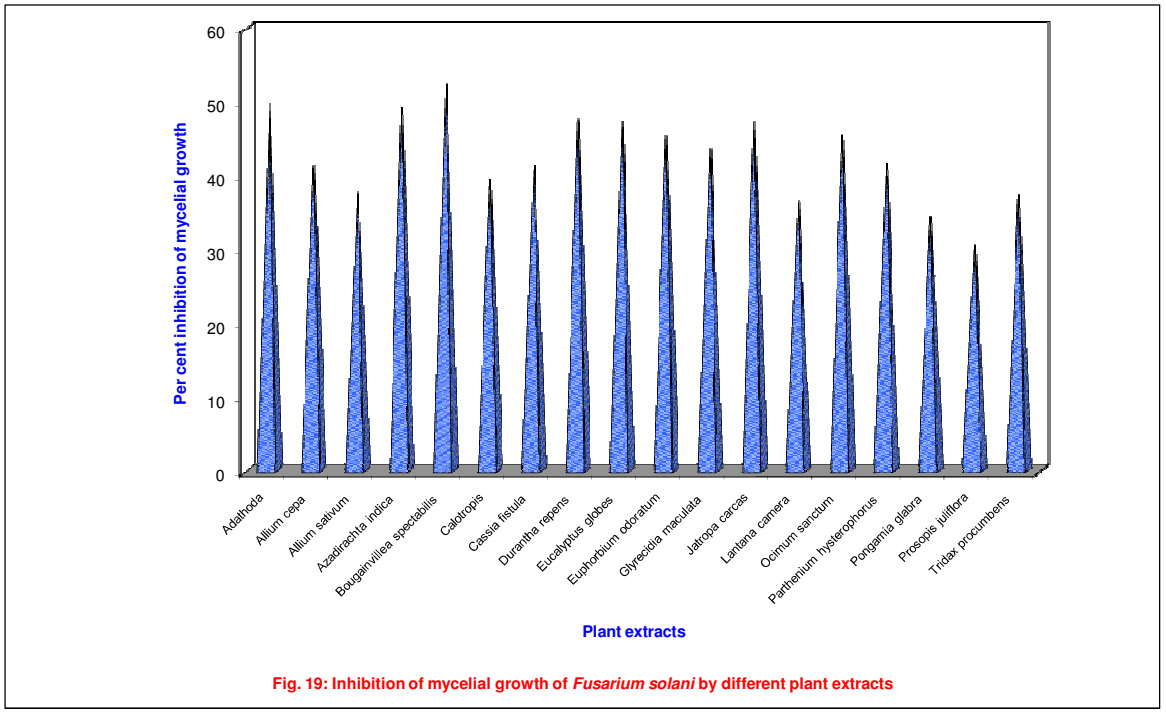


Fig 19: Inhibition of mycelial growth of *Fusarium solani* by different plant extracts

Table 35: Inhibition of mycelial growth *of Rhizoctonia bataticola* by different plant extracts

Plant extracts	Percent inhibition of mycelial growth		Mean
	Concentration (%)		
	5%	10%	
Allium satium	74.48 (59.69)	100.00 (90.05)	87.24 (69.07)
Azadirachta indica	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Bougainvillea	0.00 (0.00)	3.15 (10.22)	1.57 (7.20)
Calotropis	20.04 (26.61)	84.44 (66.80)	52.24 (46.28)
Crysanthemum	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Duranta repens	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Eucalyptus globes	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Lantana camera	62.26 (52.12)	65.59 (54.11)	63.92 (53.08)
Moringa oleifera	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Multineem	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)*
Murraya koenigii	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Oscimum sanctum	0.00 (0.00)	8.52 (16.97)	4.26 (11.91)
Periwinkle	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Tegetes erecta	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Tridax procumbens	0.00 (0.00)	23.63 (29.10)	11.81 (20.10)
Mean	17.12 (24.44)	25.69 (30.45)	-
	Plant extract (P)	Concentration (C)	P x C
S.Em. ±	0.07	0.03	0.10
CD at 1 %	0.28	0.10	0.36

*Values in parenthesis are arc-sine transformed values

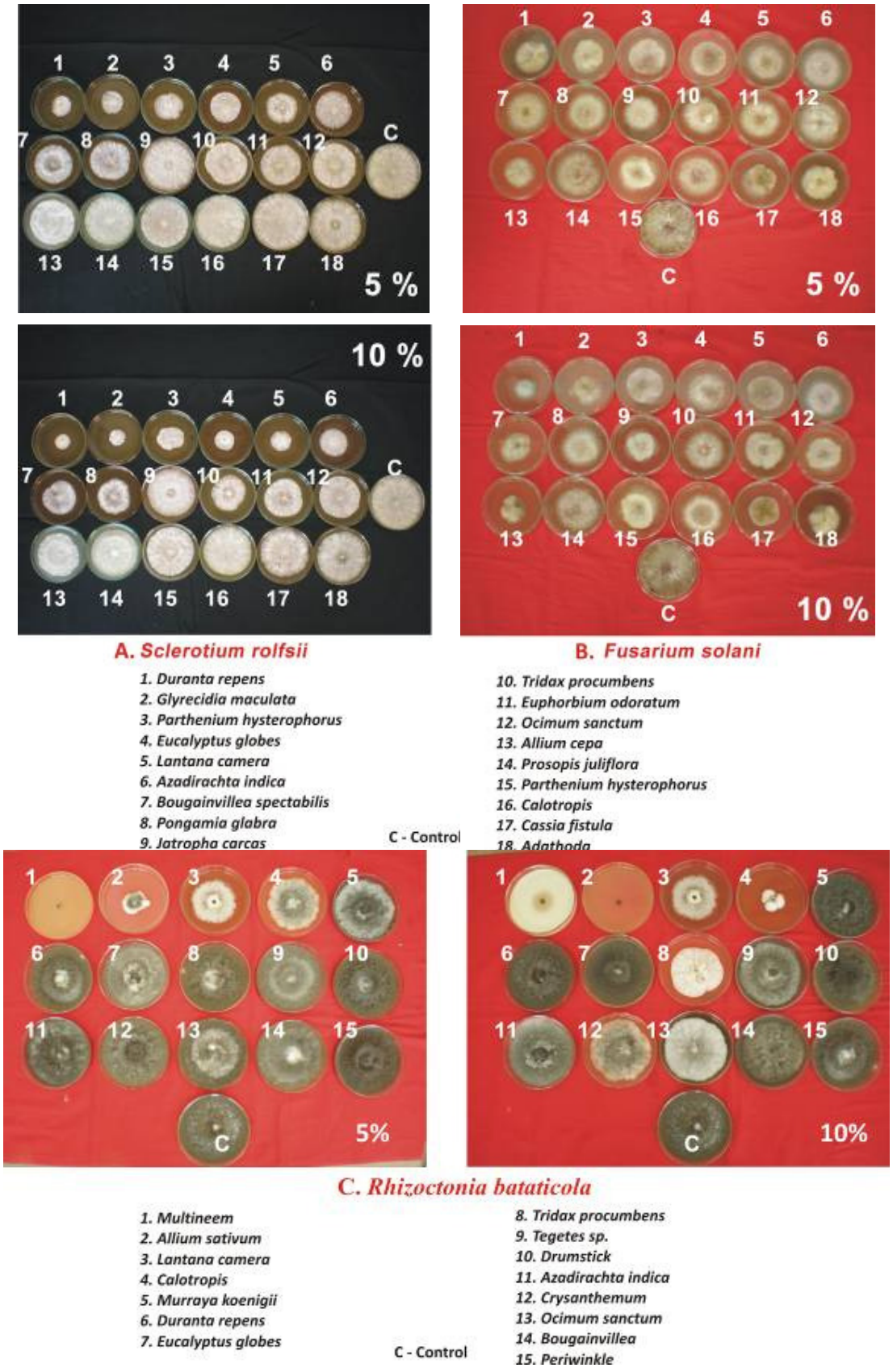


Plate 17: Inhibition of mycelial growth of soilborne fungal pathogens by plant extract

Table 36: Per cent inhibition of mycelial growth of *Sclerotium rolfsii and Fusarium solani*

Bioagents	Per cent inhibition of mycelial growth		
	S. rolfsii	*F. solani*	*R. bataticola*
Trichoderma harzianum (Dharwad isolate)	78.11 (62.13)	78.51 (62.23)*	69.11 (56.26)
Bacillus subtillis	57.02 (49.06)	40.99 (39.76)	30.50 (33.64)
Pseudomonas fluorescens	65.10 (53.82)	61.11 (51.41)	36.92 (38.99)
T. harzianum (PDBC Isolate)	75.63 (60.45)	77.03 (61.32)	66.23 (54.50)
Trichoderma koningii	62.04 (52.01)	73.54 (59.65)	55.54 (48.21)
Trichoderma virens	70.16 (56.92)	76.29 (60.83)	57.23 (49.18)
Trichoderma viridae	74.92 (59.98)	76.01 (60.05)	63.83 (53.06)
S.Em. ±	0.69	0.52	0.78
CD at 1 %	2.23	1.65	2.44

*Values in parenthesis are arc-sine transformed values

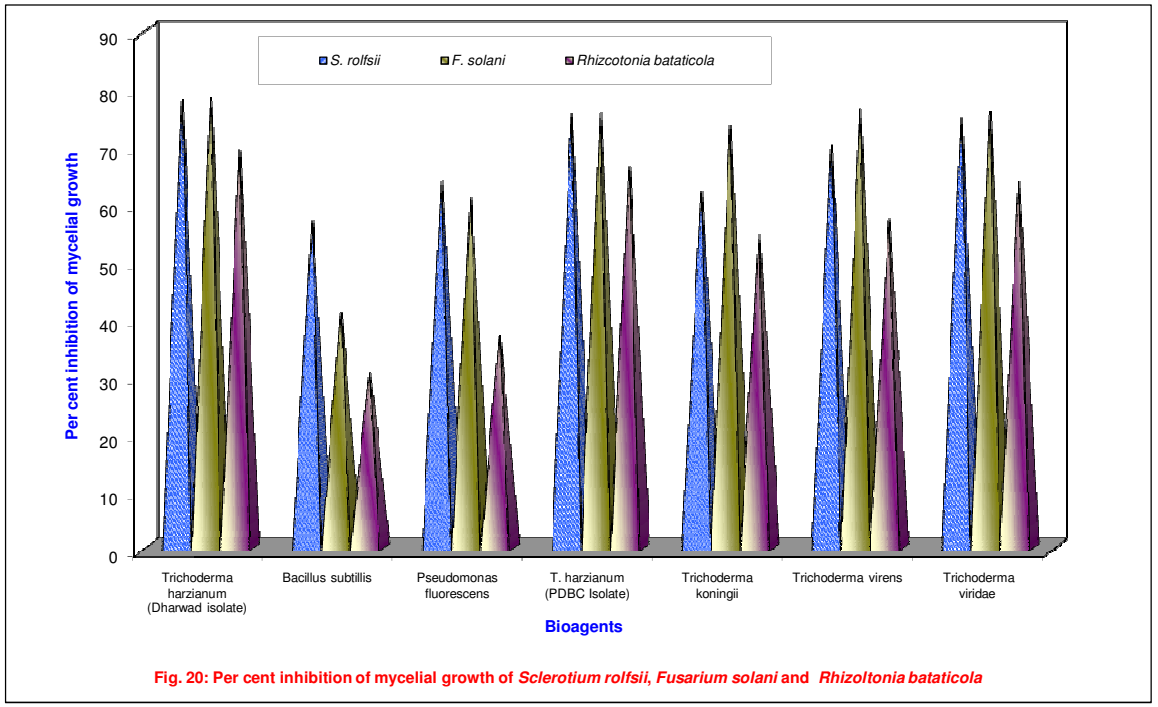


Fig. 20: Per cent inhibition of mycelial growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoltonia bataticola*

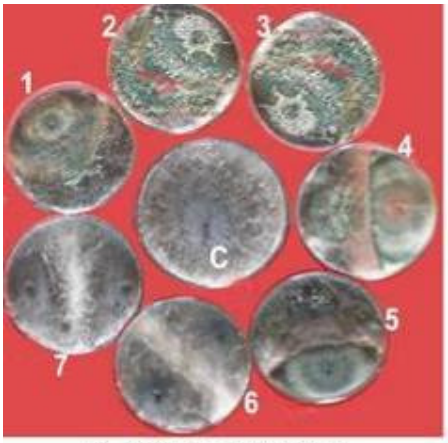
Fig 20: Per cent inhibition of mycelial growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoltonia bataticola*

1. *Trichoderma koningii*
2. *Trichoderma harzianum (Dharwad isolate)*
3 *T. harzianum (PDBC Isolate)*
4. *Trichoderma viride*
5. *Trichoderma virens*
6. *Bacillus subtillis*
7. *Pseudomonas fluorescens*
C. *Control*

A. Sclerotium rolfsii

1. *Trichoderma koningii*
2. *Trichoderma harzianum (Dharwad isolate)*
3 *T. harzianum (PDBC Isolate)*
4. *Trichoderma virens*
5. *Trichoderma viride*
6. *Bacillus subtillis*
7. *Pseudomonas fluorescens*
C. *Control*

B. Fusarium solani



1. *Trichoderma viride*
2. *Trichoderma harzianum (Dharwad isolate)*
3 *T. harzianum (PDBC Isolate)*
4. *Trichoderma koningi*
5. *Trichoderma virens*
6. *Bacillus subtillis*
7. *Pseudomonas fluorescens*
C. *Control*

C. Rhizoctonia bataticola

Plate 18: *In vitro* evaluation of bioagents against soilborne fungal *pathogens*

Sclerotium rolfsii

There was a significant difference between the bioagents evaluated with respect to per cent inhibition of mycelial growth of *S. rolfsii*. Maximum inhibition of mycelial growth was obtained when native isolate of *T. harzianum* (Dharwad isolate) (78.11%) was used as bioagent. It was found to be significantly superior over all other treatments, and was on par with *T. harzianum* (PDBC isolate) (75.63%) and *T. viride* (74.92%). Least inhibition was noticed in *B. subtilis* (57.02%) (Table 36, Fig. 20 and Plate 18A).

Fusarium solani

Maximum reduction in colony growth of *F. solani* was observed in *T. harzianum* (Dharwad isolate) (78.51%) which was significantly superior to all other bioagents tested and was on par with *T. harzianum* Rifai (77.03%). Next best were by *T. viride* (76.01%) and *T. virens* (76.29%). Least inhibition was noticed in *B. subtilis* (40.99%) followed by *Pseudomonas fluorescens* (61.11%) (Table 36, Fig. 20 and Plate 18B)

Rhizoctonia bataticola

There was significant difference between the bioagents tested with respect to per cent inhibition of mycelial growth of *R. bataticola.* Maximum per cent inhibition of mycelial growth was obtained when native isolate of *T. harzianum* (Dharwad isolate) was evaluated (69.11%). It was found to be significantly superior to the rest of the bioagents tested and was on par with *T. harzianum* (66.23%) and *T. viride* (63.83%). The least inhibition was observed in *B. subtilis* (30.50%) (Table 36, Fig. 20 and Plate 18C)

4.7.3 *In vitro* evaluation of fungicides

Efficacy of systemic and contact fungicides was tested at three concentrations in the laboratory against *S. rolfsii, F.solani* and *R. bataticola*. Poisoned food technique was followed as detailed in ''Material and Methods''. The per cent inhibition over control was worked out based on the fungal growth in control plate. The results thus obtained are presented hereunder.

4.7.3.1 *Sclerotium rolfsii*

In vitro evaluation of contact fungicides

The results indicated that there was a significant difference among the contact fungicides in inhibiting the growth of *S. rolfsii* Among six contact fungicides, emisan, mancozeb, thiram and captan completely inhibited the growth of *S. rolfsii* (100.00%) at all the concentrations (0.1, 0.2, 0.3%) tested followed by zineb (56.91%) while chlorothaloni was less effective (50.24%) (Table 37 and Plate 19A).

In vitro evaluation of systemic fungicides

The results indicated that there was a significant difference among the systemic fungicides in inhibiting the growth of *S.rolfsii*. Among the six systemic fungicides evaluated, carbendazim + mancozeb, carboxin + thiram, propiconazole, hexaconazole and benomyl were found to be most effective and significantly superior over control which inhibited cent per cent growth of *S.rolfsii* at all the three concentrations (0.025, 0.05 and 0.1%) tested. Carbendazim was least effective in inhibiting mycelial growth at all the concentration tested (Table 38 and Plate 20A).

4.7.3.2 *Fusarium solani*

In vitro evaluation of contact fungicides

Significant differences were recorded in the per cent inhibition of mycelial growth of *F. solani* with contact fungicides. Mancozeb (99.66%) gave maximum inhibition of mycelial growth, which was on par with the propineb (98.72%). Least inhibition of mycelial growth was observed in zineb (56.93%) (Table 39 and Plate 19B)

Among the contact fungicides tested, mancozeb at 0.2 and 0.3 per cent and propineb at 0.3 per cent completely (100%) inhibited the growth of *F. solani.* Least inhibition of mycelial growth was recorded in zineb at 0.1 per cent (49.33%).

Among the different concentrations tested, 0.3 per cent concentration (81.44%) of non-systemic fungicide was effective and least inhibition of mycelial growth was observed in 0.1 per cent concentration (69.66%).

Table 37: Effect of contact fungicides on inhibition of mycelial growth of *Sclerotium rolfsii*

Treatments	Percent Inhibition (PI) of mycelial growth			
	Concentration (%)			
	0.1	0.2	0.3	Mean
Emissan	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)*
Captan	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)
Chlorothalonil	37.03 (37.50)	47.77 (43.74)	65.92 (54.31)	50.24 (45.18)
Mancozeb	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)
Thiram	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)
Zineb	54.81 (47.78)	51.85 (46.08)	64.07 (53.20)	56.91 (49.02)
Mean	81.97 (74.24)	83.27 (75.00)	88.33 (77.95)	
	Fungicide (F)	Concentration (C)	FXC	
S.Em. ±	0.19	0.13	0.34	
CD at 1%	0.65	0.41	1.12	

*Values in parenthesis are arcsine-transformed values

Table 38: Effect of systemic fungicides on inhibition of mycelial growth of *Sclerotium rolfsii*

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.025	0.05	0.10	
Carbendazim	55.90 (48.41)	57.38 (49.26)	59.96 (50.76)	57.74 (49.48)*
Carbendazim + mancozeb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Carboxin + thiram	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Hexaconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Benomyl	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Fungicides (F)	Concentration (C)		F x C
S. Em.±	0.07	0.06		0.13
CD at 1%	0.24	0.19		0.41

*Figures in the parenthesis indicate angular transformed values

Table 39: Effect of contact fungicides on inhibition of mycelial growth of *Fusarium solani*

Treatments	Percent Inhibition (PI) of mycelial growth			
	Concentration (%)			
	0.1	0.2	0.3	Mean
Propineb	97.59(81.14)	98.59(83.29)	100 (90.04)	98.72(84.83)*
Mancozeb	99.00 (85.42)	100 (90.04)	100 (90.04)	99.66(88.68)
Thiram	55.62(48.25)	64.33(53.35)	77.24(61.54)	65.73(54.38)
Captan	66.96(54.94)	76.21(60.84)	78.07(62.11)	73.75(59.29)
Zineb	49.33(44.64)	55.36(48.10)	66.10(54.42)	56.93(49.05)
Copper oxy-chloride	49.50(44.73)	55.17(47.99)	67.28(55.13)	57.31(49.28)
Mean	69.66(59.85)	74.94(63.85)	81.44(68.88)	
	Fungicide (F)	Concentration (C)	FXC	
S.Em.±	0.35	0.25	0.60	
CD at 1%	1.35	0.96	2.30	

*Figures in the parenthesis indicate angular transformed values

Table 40: Effect of systemic fungicides on inhibition of mycelial growth of *Fusarium solani*

Fungicides	Per cent inhibition of mycelial growth			
	Concentration (%)			Mean
	0.025	0.05	0.10	
Carbendazim	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)*
Carbendazim + mancozeb	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Carboxin + thiram	98.86 (86.50)	99.23 (87.13)	99.60 (87.94)	99.23 (87.19)
Propiconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Benomyl	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
	Fungicides (F)	Concentration (C)		F x C
S. Em.±	0.06	0.05		0.13
CD at 1%	0.25	0.22		0.45

*Figures in the parenthesis indicate angular transformed values

Table 41: Inhibition of mycelial growth of *Rhizoctonia bataticola* by contact fungicides

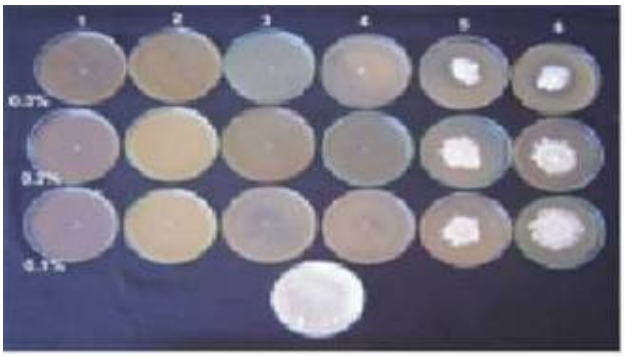
Treatment	Per cent inhibition (PI) at different concentrations			
	Concentration			
	0.1%	0.2%	0.3%	*Mean*
Thiram	98.52 (84.33)*	99.26 (87.19)	100.00 (90.05)	99.26 (87.19)
Mancozeb	40.74 (39.65)	62.22 (52.12)	69.26 (56.36)	57.41 (49.38)
Captan	54.82 (47.79)	56.67 (48.86)	77.41 (61.66)	63.00 (52.77)
Chlorothalonil	7.41 (12.99)	55.19 (48.00)	64.45 (53.44)	42.35 (38.14)
Copper oxychloride	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	40.30 (36.95)	54.67 (47.23)	62.22 (52.30)	52.40 (45.49)
	Fungicide (F)	Concentration (C)	F X C	
S.Em. ±	1.223	0.947	2.118	
CD at 1%	4.742	3.672	8.213	

* Values in parenthesis are arc-sine transformed values

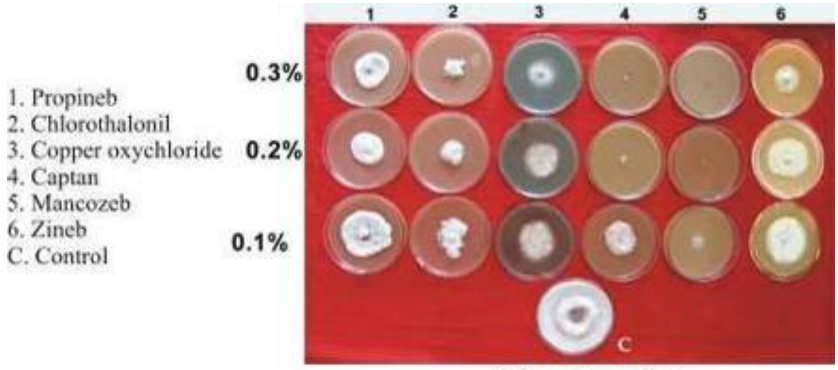
Table 42: Inhibition of mycelial growth of *Rhizoctonia bataticola* by systemic fungicides

Treatment	Per cent inhibition			
	Concentration			
	0.025%	0.05%	0.1%	Mean
Carboxin+Thiram	99.63 (88.03)*	100.00 (90.05)	100.00 (90.05)	99.88(89.37)
Carbendazim	0.00 (0.00)	29.63(29.18)	53.71(47.15)	27.78(25.44)
Hexaconazole	94.44 (76.40)	95.93(78.43)	100.00(90.05)	96.79(81.62)
Metalaxyl	1.85 (4.55)	29.63 (32.90)	100.00(90.05)	43.83(42.50)
Triademifon	76.67(61.45)	89.63(71.27)	100.00(90.05)	88.77(74.26)
Iprodione + carbendazim	34.00 (39.21)	91.85(73.48)	95.18(77.44)	75.68(63.38)
Mean	52.10 (11.94)	72.78(62.55)	91.48(80.80)	-
	Fungicide(F)	Concentration(C)	FXC	
S.Em. ±	0.26	0.19	0.46	
CD at 1%	1.00	0.73	1.77	

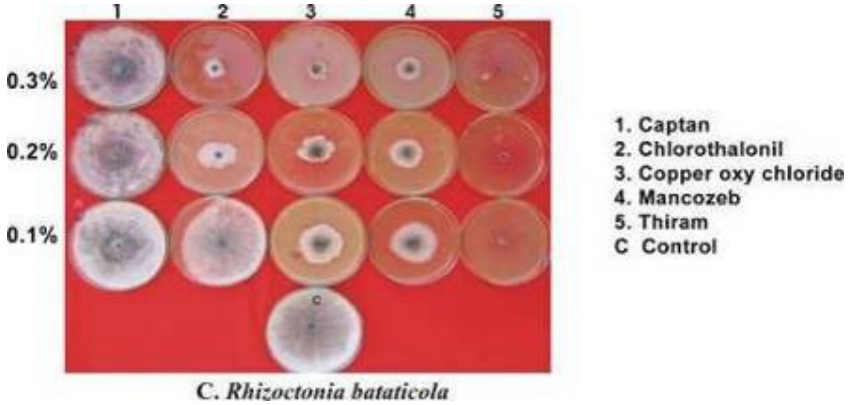
* Values in parenthesis are arc-sine transformed values



A. Sclerotium rolfsii

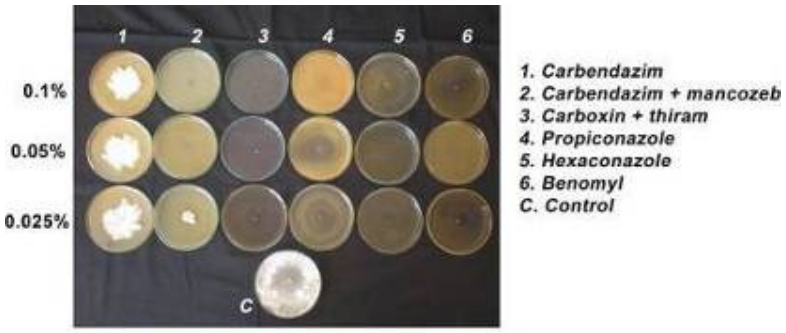


B. Fusarium solani

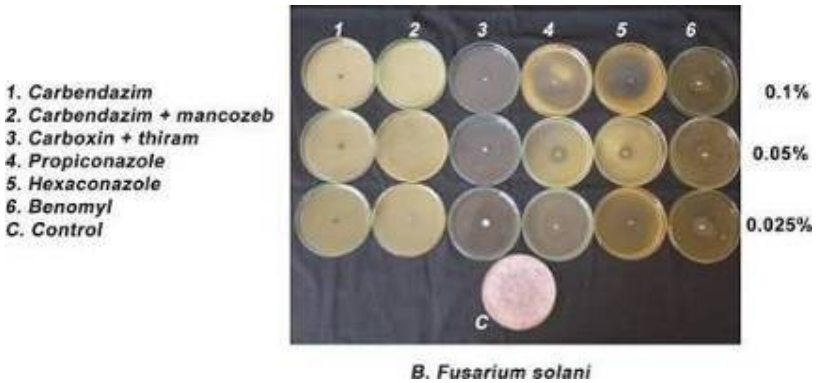


C. Rhizoctonia bataticola

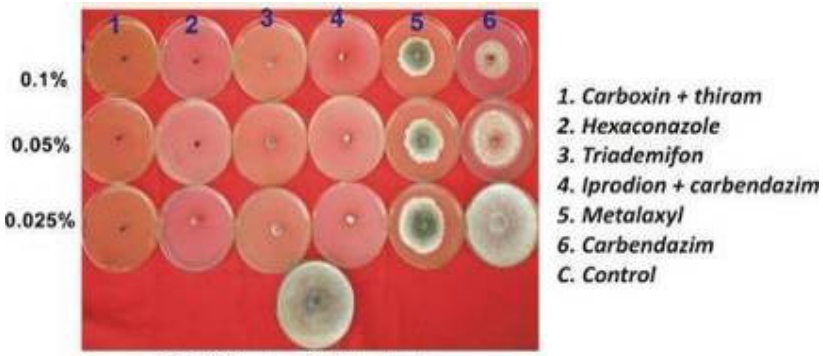
Plate 19: *In vitro* evaluation of contact fungicides against soilborne fungal pathogens



A. Sclerotium rolfsii



B. Fusarium solani



C. Rhizoctonia bataticola

Plate 20: In vitro evaluation of systemic fungicides against soilborne fungal Pathogens

Table 43: Effect of organic ammendments on incidence of Sclerotium wilt of stevia

Treatments	Percent disease incidence	Plant height (90 DAP)
Enriched Farm Yard Manure	33.33 (35.26)*	84.0
Neem cake	41.67 (40.26)	81.6
Safflower oil cake	58.33 (49.80)	74.6
Cotton oil cake	50.00 (45.00)	78.0
Vermicompost	41.67 (40.22)	80.6
Groundnut cake	58.33 (49.80)	78.3
Control	83.33 (65.91)	20.3
S.Em. ±	1.69	0.88
CD at 5 %	5.10	2.66

*Values in parenthesis are arc-sine transformed values

DAP: Days after planting

In vitro evaluation of systemic fungicides

Among the six systemic fungicides evaluated, carbendazim, carbendazim + mancozeb, propiconazole, hexaconazole and benomyl were found to be most effective and significantly superior over control which inhibited cent per cent growth of *F.solani* at all the concentrations (0.025, 0.05 and 0.1%) tested. Carboxin + thiram was least effective in inhibiting mycelial growth at all the concentration tested (Table 40 and Plate 20B).

4.7.3.3 *Rhizoctonia bataticola*

In vitro evaluation of contact fungicides

The results of the study presented in (Table 41 and Plate 19c) revealed that, there was a significant difference between the non-systemic fungicides in per cent inhibition of mycelial growth. Thiram was found to be most effective and significantly superior over other treatments which inhibited 99.26 per cent growth of the fungus. The next best treatment was captan which inhibited 63.00 per cent growth of the fungus. However, there was no inhibition by copper oxy chloride at all concentrations.

Among the three concentrations assessed, 0.3 per cent was found significantly superior over 0.2 per cent and 0.1 per cent concentration in inhibiting growth of the fungus. Thiram inhibited the mycelial growth completely at 0.3 per cent whereas at 0.2 and 0.3 per cent concentration, there was 99.26 and 98.52 per cent inhibition. Captan at 0.3 per cent showed 77.41 per cent of mycelial inhibition whereas 56.67 and 54.82 per cent inhibition was found at 0.2 and 0.1 per cent respectively.

In vitro evaluation of systemic fungicides

Combiproduct of carboxin + thiram at 0.05 and 0.1 per cent inhibited the mycelial growth completely and were found to be significant over other treatments. Next best treatment was hexaconazole (96.79%). Least inhibition of mycelial growth was observed in metalaxyl (43.83%) (Table 42 and Plate 20c).

Among the concentrations, systemic fungicides at 0.1 per cent was found significantly superior compared to 0.05 and 0.025 per cent. Carboxin + thiram, hexaconazole, metalaxyl and triademifon inhibited cent per cent of mycelial growth of *R. bataticola* at 0.1 per cent and carboxin + thiram at 0.05 per cent concentration. Mycelial inhibition at 0.05 per cent was significantly superior over 0.025 per cent concentration.

4.7.4 *In vivo* studies

4.7.4.1 Management by organic amendments

Six organic amendments *viz*., Enriched Farm Yard Manure, Neem cake, groundnut oil cake, safflower oil cake, cotton oil cake and vermicompost were tested against *S. rolfsii* as described in 'Material and Methods'. Observations on per cent disease incidence was recorded. The data are presented in Table 43 and Plate 21.

Among six organic amendments tested against *S. rolfsii*, enriched FYM was found to be superior, which recorded least disease incidence (33.33%), which was significantly superior other treatments. This was followed by neem cake and vermicompost (41.67 %), cotton oil cake (50.00%), safflower oil cake and groundnut cake showed 58.33 per cent disease incidence.

The plant height (90 DAP) was maximum in enriched FYM treatment (84.00cm) which was significantly superior to other treatments. This was followed by neem cake and vermicompost (81.6cm and 80.6cm) respectively.

4.7.4.2 Integrated Disease Management (IDM) for Sclerotium wilt of stevia in the pot experiment

A glass house experiment was conducted in department of plant pathology, i.e. during 2011-12 on the integrated management of *S. rolsiif* to evaluate the efficacy of organic amendments, bioagents and chemicals which performed well under *in vitro* studies as described in "Materials and Methods" Observations were recorded on plant growth parameters and per cent disease incidence and the results are presented in the Table 44.

Table 44: Integrated Disease Management (IDM) for Sclerotium wilt of stevia during 2011-12

Tr. No	Treatments	Plant height (cm)			Number of branches			Per cent wilt	
		15 DAP	30 DAP	60 DAP	15 DAP	30 DAP	60 DAP	30 DAP	60 DAP
T1	Untreated control	10.50	13.06	16.10	2.33	4.00	5.70	100.00 (90.00)	100.00 (90.00)*
T2	Eucalyptus leaf extract @ 10%	12.00	15.40	27.76	4.70	6.26	13.26	66.66 (54.73)	100.00 (90.00)
T3	Hexaconazole @ 0.1 %	24.70	36.30	62.66	7.33	12.70	18.40	0.00 (0.00)	0.00 (0.00)
T4	Carboxin+thiram @ 0.1%	22.50	31.74	58.70	7.00	12.00	16.70	0.00 (0.00)	0.00 (0.00)
T5	FYM @ 10 t /ha + *Trichoderma harzianum* @ 25 kg/ha	20.70	31.74	56.40	6.34	11.67	14.30	0.00 (0.00)	33.33 (35.26)
T6	*Azadiracta indica* leaf extract @ 10%	16.23	30.00	42.57	4.16	8.50	13.10	33.33 (35.26)	66.66 (54.73)
T7	Duranta leaf extract @ 10%	20.50	31.74	54.50	5.60	10.67	13.97	0.00 (0.00)	33.33 (35.26)
T8.	Carbendazim + mancozeb @ 0.1%	13.00	28.80	34.82	4.60	10.30	13.20	66.66 (54.73)	100.00 (90.00)
	S.Em. ±	0.48	0.40	0.26	0.14	0.09	0.63	3.34	1.20
	CD at 1 %	1.67	1.22	1.07	0.42	0.29	1.92	10.28	3.65

DAP – Days after Planting
* Figures in parentheses are arc-sine transformed values

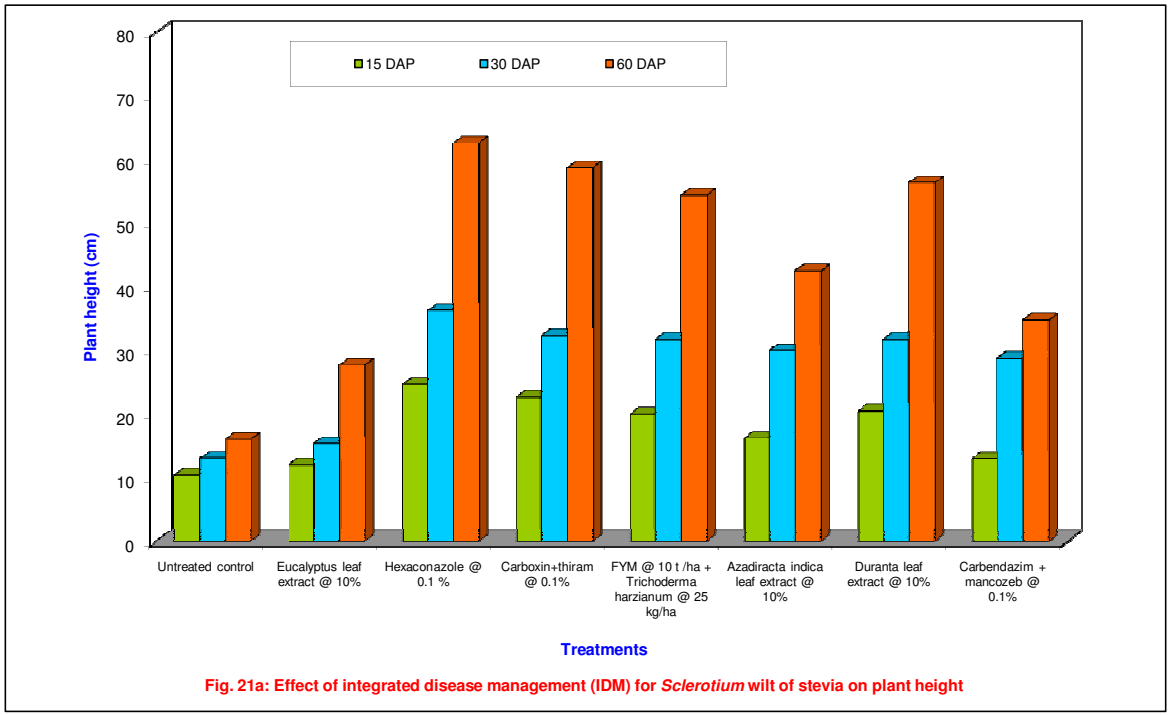


Fig. 21a: Effect of integrated disease management (IDM) for *Sclerotium* wilt of stevia on plant height

Fig 21a: Effect of integrated disease management (IDM) for Sclerotium wilt of stevia on plant height

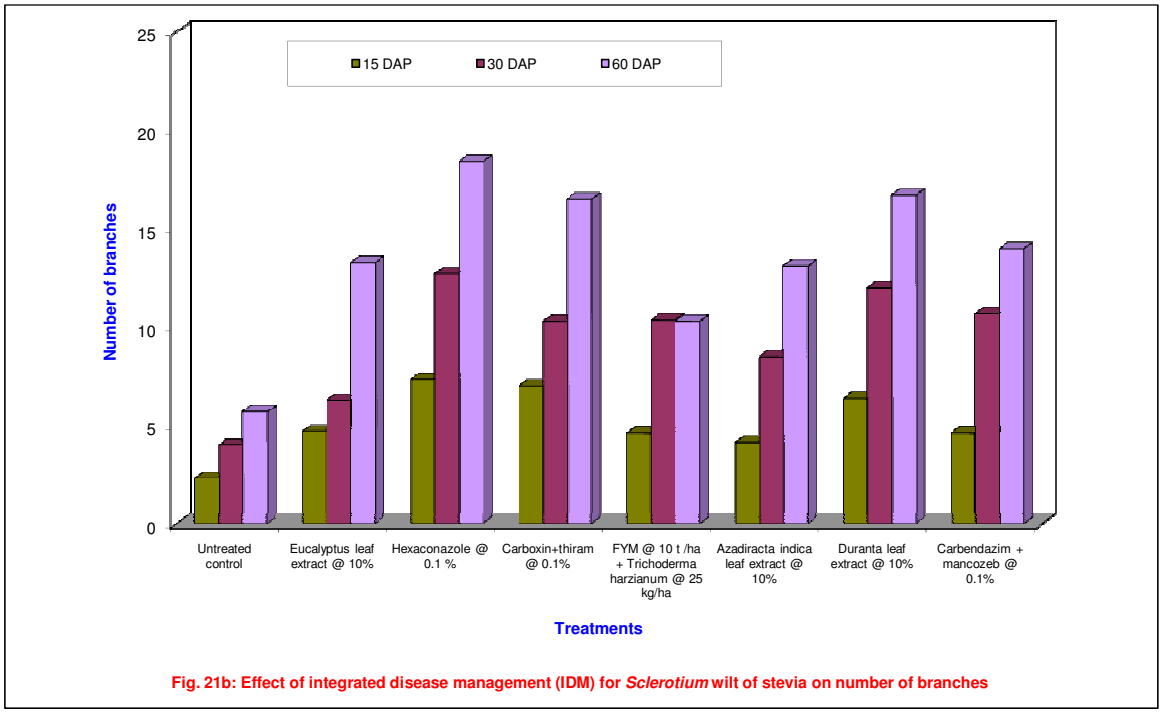


Fig. 21b: Effect of integrated disease management (IDM) for *Sclerotium* wilt of stevia on number of branches

Fig 21b: Effect of integrated disease management (IDM) for Sclerotium wilt of stevia on number of branches

1. Groundnut cake
2. Neem cake
3.Vermicompost
4. Enriched FYM
5. Cotton oil cake
6. Safflower oil cake
C. Control

Plate 21: Effect of organic amendments on incidence of Sclerotium wilt of stevia

60 days after inoculation

90 days after inoculation

1. *Azadiracta indica* @ 10%
2. Eucalyptus leaf extract @ 10%
3. FYM @ 10 t/ha + *T. harzianum* @ 25 kg/ha
4. Carboxin + thiram @ 0.1%
5. Hexaconozole @ 0.1%
6. Duranta leaf extract @ 10%
7. Carbandazim + mancozeb @ 0.1%
C. Control

Plate 22: Integrated disease management for Sclerotium wilt of stevia in the pot experiment

Table 45: Integrated Disease Management (IDM) for Sclerotium wilt of stevia during 2011-12

Tr.No	Treatments	Plant Height (cm)			Number of branches					No. of leaves / plant		Percent Disease Incidence			Yield	
					30 DAP		60 DAP		120 DAP							
		30 DAP	60 DAP	120 DAP	No. of main branches	Total No. of branches	No. of main branches	Total No. of branches	Total No. of branches	60 DAP	120 DAP	30 DAP	60 DAP	120 DAP	Fresh weight (t/ha)	Dry Weight (t/ha)
T_1	Untreated control	27.33	30.36	34.16	3.33	6.24	5.85	8.86	24.40	204.46	226.33	26.44 (30.94)	43.33 (41.16)	66.74 (54.78)*	3.76	0.9
T_2	Eucalyptus leaf extract @ 10%	35.60	45.56	48.21	4.80	7.33	6.86	14.42	34.53	542.00	601.00	15.48 (23.14)	28.89 (32.51)	58.72 (50.02)	5.55	1.16
T_3	Hexaconazole @ 0.1 %	45.44	62.21	83.09	8.91	12.03	16.33	21.67	45.26	712.33	850.01	3.57 (10.87)	8.89 (17.35)	13.33 (21.41)	10.78	2.56
T_4	Carboxin+thiram @0.1%	40.80	53.33	78.79	6.20	10.98	12.63	19.57	42.63	683.00	808.00	6.67 (14.90)	7.85 (16.26)	15.56 (23.23)	9.78	2.33
T_5	FYM@10t/ha+ *T.harzianum* @25kg/ha	40.21	50.49	75.68	5.41	9.80	12.26	19.26	40.86	634.03	792.33	9.00 (17.44)	13.33 (21.41)	17.78 (24.94)	9.16	2.00
T_6	*Azadiracta indica* leaf extract @ 10%	35.78	45.80	62.31	5.21	8.26	6.94	16.64	36.93	602.33	702.67	11.11 (19.47)	25.55 (30.36)	62.78 (52.40)	7.16	1.45
T_7	Duranta @ 10%	39.91	52.21	74.50	6.01	9.93	12.01	19.16	39.40	656.67	798.00	6.67 (14.96)	10.04 (18.47)	18.89 (25.76)	8.33	1.70
T_8	Carbendazim + mancozeb @ 0.1%	32.36	48.23	52.80	5.56	8.56	9.55	17.43	37.33	572.67	753.33	12.22 (20.46)	23.34 (28.90)	36.32 (37.06)	6.00	1.0
	S.Em. ±	0.61	0.81	1.16	0.32	0.26	0.39	0.46	1.04	2.37	3.53	0.53	0.74	1.04	0.69	0.20
	CD at 5%	1.80	2.36	3.40	0.95	1.00	1.19	1.28	2.86	7.19	10.71	1.60	2.29	2.86	2.10	0.82

*Values in parenthesis are arc-sine transformed values6

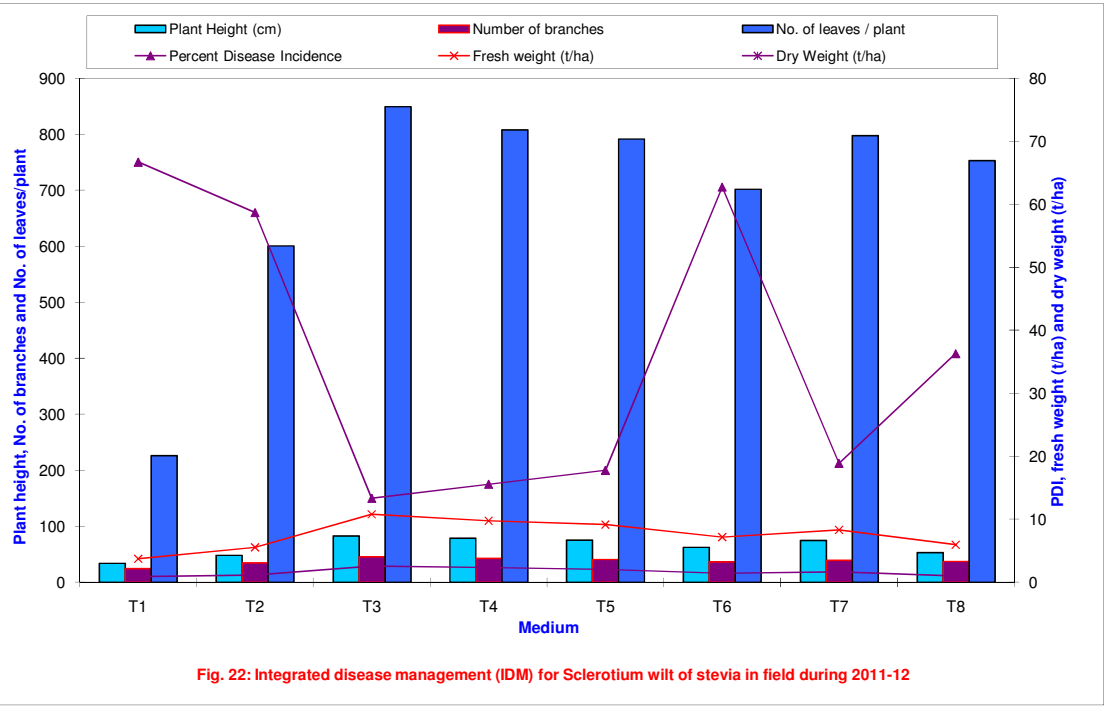


Fig. 22: Integrated disease management (IDM) for Sclerotium wilt of stevia in field during 2011-12

Fig 22: Integrated disease management (IDM) for Sclerotium wilt of stevia in field during 2011-12

Experimental field view

A. Hexaconazole

B. Carboxin + Thiram

C. Untreated control

Plate 23: Integrated disease management of Sclerotium wilt of stevia

7.4.3 Per cent disease incidence

Among the various treatments tested, fungicides have given the good results. Disease was absent in hexaconazole and carboxin + thiram treatment followed by FYM + *Trichoderma harzianum* and duranta leaf extract. The highest disease incidence was noticed in untreated control (100%). Plants did not show wilt even after 120 DAP in hexoconozole and corboxin treatment (Table 44, Fig. 21c and Plate 22).

4.7.4.4 Plant height and number of branches

Plant height was significantly superior in all treatments as compared to untreated control. The plant height (60 DAP) was maximum in hexaconazole (62.66 cm) with more number of branches (18.40) which was significantly superior to all other treatments. This was followed by carboxin + thiram (58.70 cm) which was on par with FYM + *Trichoderma harzianum* (56.40 cm) and duranta leaf extract (54.50 cm) (Table 44, Fig. 21a, 21b and Plate 21).

4.7.4.5 Integrated Disease Management (IDM) for Sclerotium wilt of stevia in field during 2011-12

A field experiment was conducted during *kharif* 2011-12 on the integrated management of sclerotium wilt of stevia. Treatments were allocated under Randomized Block Design as described in 'Material and Methods' and observations were recorded on plant height, number of branches, no of leaves per plant, per cent disease incidence and yield which are presented in the Table 45, Fig. 22 and Plate 23.

4.7.4.6 Plant height

The data from the field experiment revealed that there was a significant difference in plant height among the treatments. The treatment with soil drenching of hexaconazole recorded highest plant height of 45.44 cm at 30 DAP, 62.21cm at 60DATand 83.09cm at 120 days after planting (DAP) respectively. The next best treatment was carboxin + thiram which recorded plant height of 40.80 and 53.33 cm and 78.79 at 120 DAP respectively. Lowest plant height was recorded in eucalyptus leaf extract (35.60 cm) at 30 DAP (45.50 cm) at 60 DAP and (48.21 cm) at 120 DAP respectively. Untreated control, recorded 34.16cm of plant height at 120 DAP (Table 45).

4.7.4.7 Number of branches

There was significant increase in number of branches in all the treatments compared to control. Maximum number of branches were recorded in soil drenching with hexaconazole (45.26) at 120 DAT followed with carboxin + thiram showed maximum number of branches (42.63) and FYM + *T. harzianum* (40.86) which was on par with duranta leaf extract (36.40). Lowest number of branches was recorded in untreated control (24.40) (Table 45).

4.7.4.8 Per cent disease incidence

There was a significant difference in treatments with respect to disease incidence (Table 45). Among all treatments, hexaconazole recorded very less per cent disease incidence at all stages *viz.,* at 30 DAP (3.57), at 60 DAP (8.89) and 120 DAP (13.33) which was significantly superior over other treatments followed by carboxin + thiram treatment with 6.67, 7.85 and 15.56 per cent disease incidence at 30, 60 and 120 DAP, respectively. These two were on par with FYM + *T. harzianum* with disease incidence of 9.00, 13.33 and 17.78 per cent at 30, 60 and 90 DAP, respectively. Next best treatment was duranta leaf extract which showed minimum percent disease incidence of 6.67, 10.04 and 18.89 at 30, 60 and 120 DAP, respectively. Untreated plots recorded highest per cent disease incidence of 26.44, 43.33 and 66.74 at 30 DAP, 60 DAP and 120 DAP respectively.

4.7.4.9 Yield

The data revealed that there was a significant increase in yield in treated plots when compared to untreated control plot. Maximum fresh weight of 10.78 t/ha and dry weight of 2.56 t/ha was recorded in hexaconazole treatment followed by corboxin + thiram which recorded fresh weight of 9.78 t/ha and dry weight of 2.33 t/ha which is significantly superior over other treatments and they are statistically on par with each other. Fresh weight of 9.16 t/ha and dry weight of 2.00 t/ha was recorded in FYM + *T. harzianum* treatment followed by duranta leaf extract which yielded 8.33 t/ha of fresh weight and 1.70 t/ha of dry weight respectively. Untreated control recorded a very less yield 3.76 t/ha of fresh weight and 0.9 t/ha of dry herbage yield (Table 45).

DISCUSSION

Stevia (*Stevia rebaudiana* Bertoni.) is a herbaceous perennial plant of the Asteraceae, native to Paraguay where it grows in sandy soils near streams (Katayama *et al.,* 1976). The leaves of this plant are 30 times sweeter than sugar with zero calories where as pure extract is 300 times sweeter than sugar. The main active ingredient, stevioside, is 100 to 300 times as sweet as sucrose. The glycosides in its incredible sweetness, makes it unique among the nearly 300 species of stevia plants. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future.

Stevia is commonly propagated by stem cuttings. It is affected by fungal diseases, caused by *Sclerotium rolfsii, Fusarium solani,* and *Rhizoctonia bataticola*. Among all these diseases, *Sclerotium rolfsii* Sacc., which causes wilt is one of the limiting factors in commercial cultivation of stevia (Hegde *et al*., 2010b and Shwetha, 2011). This disease was first reported in India by Kamalakannan *et al.* (2006).

Sclerotium rolfsii, Fusarium solani, and *Rhizoctonia bataticola* are well known ubiquitous polyphagous and non-target pathogens. These are the most universally distributed soil borne pathogens of various crops. There are limited numbers of studies carried out on the disease as well as the pathogens in stevia. Therefore, in the present investigation, different aspects of wilt disease and the pathogens have been investigated, *viz*. survey for identification of pathogens. Morphological, cultural, physiological, nutritional studies, bio-chemical, morphological and genetic variability of the predominant fungus (by RAPD and ITS), epidemiology, host range, interaction and management were studied.

5.1 Survey for disease of stevia in Karnataka

Survey and surveillance work would help to identify the hot spots for the diseases which may become a major constraint in cultivation of a crop in future. The lack of information on etiology and management aspects of the diseases made an impact as to undertake the present investigation on a war footing. With these objective in view, an intensive survey was carried out in ten districts, *viz*., Dharwad, Belgaum Bangalore, North Canara, Shimoga, Koppal, Gadag, Mandya, Raichur and Mysore districts of Karnataka during 2007-08 and 2011-12 to monitor the incidence of diseases of stevia.

Results revealed that incidence of soil borne diseases of *Stevia rebaudiana* caused by *Sclerotium rolfsii, Fusarium solani and Rhizoctonia bataticola* were prevalent in all the locations surveyed. Sclerotium wilt incidence ranged from 8.75 to 35.80 per cent. Highest wilt incidence was recorded in Gangavati (35.80%) of Koppal district followed by Kalenahalli (24.50%) of Mandya district. These places can be considered as hot spots for sclerotium wilt. Least incidence of 8.75% was recorded in Thirthalli of Shimoga district and Hirethimanahalli of Koppal district.

In Koppal district, maximum disease incidence was observed in Gangavati (35.80 %) followed by Nidshoshi (16.50%), Gangenalli (13.75%), Hirethimmanahalli (8.75%) and lowest was in Agoli (6.25%). In Shimoga district, maximum disease incidence was noticed in Rippenpete (16.25%) and Thirthalli (8.75%). In Gandhi Krishi Vignan Kendra of Bangalore district the incidence was 18.65%. In Kappadagudda (24.50 %), Kalenahalli (25.25%), Sirsi (12.50%), Mysore (19.75%), Saidapur (20.00%) and in Belgaum (14.00%) incidence was recorded.

Fusarium wilt incidence ranged from 5.25 to 5.25 to 42.75 per cent in various regions. Maximum wilt incidence was observed in Sirsi (35.00) of North Canara, Saidapur (25.80%) followed by GKVK, UAS Bangalore (24.505). Least incidence was noticed in Thirthalli of Shimoga district. In these places Fusarium wilt was more in the nursaries where 80-100 per cent mortality of the cuttings were observed. Root rot incidence was observed only in Saidapur (8.75 per cent) and Gangavati (6.25 per cent).

Disease incidence varied from locality to locality, may be due to pattern, environmental conditions and buildup of inoculum. Further, monocropping system has also aggravated the disease situation.Similar type of survey was carried out by Hegde *et al.,* 2010b and Shwetha (2011) for diseases of stevia, Ammajamma (2010) and Ramprasad (2005) in coleus. Characteristic symptoms of wilt disease were noticed during survey and samples were collected for isolation of pathogens. Pathogens associated were *Sclerotium rolfsii* Sacc., *Fusarium solani* (Mart.) Sacc., *and Rhizoctonia bataticola*.. The pathogens alone or combination of pathogens aggrevate the wilt in stevia.

Sclerotium rolfsii was isolated from the samples collected from different localities and proved to be the most predominant pathogen among the wilt causing pathogens. Fourteen isolates of *Sclerotium rolfsii* were designated as SrNID (Nidshoshi), SrHOS (Hosagudda), SrBEN (UAS, Bengaluru), SrTHI (Thindlu), SrKAL(Kalenahalli), SrSAI (Saidapur), SrSIR (Sirsi), SrTHIR (Thirthalli), SrRIP (Rippenpete), SrGAN (Gangavathi), SrBGM (Belgaum), SrMYS (Mysore), SrGDG(Gadag) and SrRCR (Raichur). These isolates were used for further variability studies.

5.1.1 Isolation of different pathogens

Wilt causing pathogens *viz., S. rolfsii, F. solani and R. bataticola* were isolated from diseased plants of stevia by following standard tissue isolation method. Pure culture of all the fungal pathogens and isolates of *S. rolfsii* were maintained on slants containing potato dextrose agar and stored in refrigerator for use for further studies. Kamalakannan *et al.* (2006), Hegde *et al.* (2010b), Shwetha (2011) isolated *Sclerotium* from collar region of stevia following standard tissue isolation technique. Sachidananda (2005), Shyla (1998) Boby and Bhagyaraj (2003), Sumitra (2006) and Sreedevi (2007) isolated *Fusarium* by tissue isolation and purified by hyphal tip method and Kamalakannan *et al.* (2003) and Sachidananda (2005) isolated *R. bataticola* from coleus.

5.1.2 Identification of causal agents of wilt complex disease

The causal organisms of wilt were identified based on cultural and morphological characters of the pathogens and the pathogens were identified as *S. rolfsii, F. solani and R. bataticola.*

S. rolfsii was identified based on morphological characters described by Domsch *et al.* (1980). *S. rolfsii* showed white, dense, radiating mycelium. Sclerotial initial were noticed six days after incubation which were white in the beginning, later they turned to chocolate brown and dark brown at maturity. Fully matured sclerotia were spherical to ellipsoidal and measured 0.5 to 2.00 mm in diameter. Fungus produced characteristic white, abundant mycelial growth on the infected parts. Large number of sclerotial bodies were observed on mycelial mat. These were superficial on the infected plant parts. Morphological characters on PDA and infected tissue are in agreement with the results of the earlier workers (Saccardo, 1911; Ramprasad, 2005, Kulkarni, 2007 and Ammajamma, 2010).

The pathogen was identified as *Fusarium solani* based on their morphological characters as described by Booth (1971). It was also confirmed by Maharashtra Association for the Cultivation Sciences (MACS), Agharkar Research Institute, Pune (M.S.). The pathogen produced three kinds of spores *viz*., macroconidia, microconidia and chlamydospores. Microconidia were abundant, hyaline, cylindrical, single or two celled and measures 6.60-19.80 x 3.30 – 6.60 µm. Macroconidia were 3-4 septate and measured 29.70 –47.85 x 4.95-6.60 µm. Chlamydospores were hyaline, spherical and 1-celled and measured 8.25-11.5 x 6.60-9.90µm. They were produced singly or sometimes in chains. The description of the pathogen is in agreement with that of *F. solani* (Mart.) Sacc. Emend Snyder and Hansen, given by Commonwealth Mycological Institute, Kew, Surrey, England, Booth (1971) and Rathnamma (1994) and Sreedevi (2007).

Rhizoctonia bataticola grew rapidly and profusely on PDA covering the entire Petri plate within five days of incubation. Hyphae were hyaline at first and gradually turned grey to brownish black. As the culture grew old, abundant tiny black sclerotia were produced and the culture turned completely black. Mycelium was septate and branched at right angle having a septum at the point of origin. The microsclerotia were irregular, ellipsoidal and oblong in shape and were dark brown to black coloured. The description of the fungus agreed with the description given for *R. bataticola* (Taub.) Butler (Pycnidial stage of *Macrophomina phaseolina*) by Ashby (1927).

5.1.3 Pathogenicity test

Pathogenicity test for *S. rolfsii, F. solani and R. bataticola* was carried out under pot culture condition in glass house. Infected plants showed slight yellowing of leaves in four days. Pathogen infected the stem and produced the dark brown lesion at collar region causing wilt and ultimately plants dried in seven days. White mycelium was seen on roots also. Such infected plants when pulled out from the soil only shoot portion came out leaving the roots in soil. Superficial sclerotial bodies were found and were chocolate brown in colour. The symptoms produced were in agreement with description given by Hegde *et al.* (2010b) and Shwetha (2011) in stevia. Ramprasad (2005) and Ammajamma (2010) in coleus.

The symptoms produced by *F. solani* were similar to the reports of Hegde *et al.* (2010b*)* in stevia and Sreedevi (2007) in patchouli. Symptoms can be observed in the nurseries and in the main field. Initially started as yellowing of lower leaves and later resulted in drying of branches or entire plant in severe cases. Infection was observed at collar region of stem in the form of brown/ black discoloration.

The infection of *R. bataticola* was initially started at the collar region of the plants as water soaked areas and the affected tissues soon turned into a soft, black and watery mass. Later the infection spread to the roots and caused decay. This ultimately caused toppling and collapse of the infected plants. These infected plants were easily pulled off from the soil and showed brown discoloration of roots followed by rotting and the pathogen reisolated was similar to that of original culture. Similar finding was also observed by Sachidananda (2005) and Ammajamma (2010) in *Coleus forskohlii*.

These pathogens were reisolated from infected roots (Collar region) and the identity of the causal organisms was confirmed by comparing with the original cultures.

5.2 Cultural studies

5.2.1 Growth characters on different solid media

Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from the substrate upon which they grow. In order to culture the fungi artificially it is necessary to supplement in the medium, those essential nutrients needed for their growth, development and other metabolic processes. To find out the best sources of nutrients for the fungal growth, different synthetic and non-synthetic media were tested. The radial growth of the fungus was used to determine growth on solid media. While dry mycelial weight was used for liquid media.

S. rolfsii was grown on 16 different solid media. Results indicated that the best mycelial growth was recorded on Richards's agar, Potato dextrose agar, Sabouraud's agar and Oat meal agar. These four media were significantly superior over the rest of all other media tested. Minimum radial growth was observed in Elliott's agar (32.00mm) followed by Asthana and Hawkers agar (40.10 mm).Excellent sclerotial production was observed in potato dextrose agar, Oat meal agar, Sabouraud's agar and Richard's agar. Poor sclerotial production was observed in Tochinai's agar, Brown's medium, Elliott's agar and Asthana and Hawkers agar. *S. rolfsii* also differed with regard to time taken for sclerotial initiation on solid media. Similar observations were made by Sulladmath *et al.* (1977), Manjappa (1979) Hari *et al.* (1988), Basamma (2008), Hegde *et al.* (2010a*)* and Ammajamma (2010).

The fungus when grown on thirteen different solid media indicated that the maximum radial growth of *F. solani* was observed on potato dextrose agar (90 mm), Richards's agar (90 mm) and oat meal agar (90 mm). Minimum growth of fungus was observed in Browns agar (42.33 mm) and Asthana and Hawker's agar (73.00mm).The results are in confirmity with the findings of Sreedevi (2007) in *F. solani*, Ramprasad (2005) and Ammajamma (2010) in case of *Fusarum chlamydosporum*. Colony was of pink in colour in potato dextrose agar, and Sabouraud's agar and in all other cases colony was white. Margin was irregular in potato dextrose agar and Sabouraud's agar and smooth margin was observed in all other media. Sporulation of fungus was found to be abundant on Richards's agar, oat meal agar and potato dextrose agar. Good sporulation was observed in the same. Sporulation was sparse in Asthana and Hawker's agar and Brown's agar. Similar resulst were reported by Jhamaria (1972), Sherkar and Utikar (1982), Shyla (1998) and Sachidananda (2005), Ramprasad (2005) and Ammajamma (2010) in case of *Fusarium chlamydosporum* and Sreedevi (2007) in *F. solani.*

R. bataticola was grown on 14 different solid media. The results indicated that the best mycelial growth was made on potato dextrose agar (90.00) and Richard's agar (90.00 mm) followed by carrot agar (87.67 mm). Least mycelial growth was recorded in oat meal agar (32.67mm). Similar observations were made by Kulkarni *et al.* (1992); Sahi *et al*. (1992); Karunanithi *et al. (*2000), Lokesha (2002) and Sachidananda (2005), Ammajamma (2010) in *C. forskohlii* infected with *R. bataticola.*

R. bataticola produced abundant microsclerotia in Richard's agar and potato dextrose agar. Similar observations were made by Vasudeva (1937); Ghosh and Sen (1973); Kulkarni *et al*. (1992), Lokesha (2002), Sachidananda (2005) and Ramprasad (2005).

5.2.1 Growth phase

Every living organism has a definite growth pattern, in which it attains a maximum growth and declines thereafter. Barnett and Hunter (1951) have reported that cellular enzymes begin to digest the various cell constituents.

In the present study, the fungus *S. rolfsii* attained maximum vegetative growth on the 10^{th} day of incubation. This was indicative of optimum growth period beyond which, autolysis occurred. Bagyaraj and Sirisi (1965) while working with an isolate of *S. rolfsii* observed maximum growth eight to ten days of incubation. Similar obsesrvations were also made by by Lingaraju (1977), Ramprasad (2005), Kulkarni (2007), Ammajamma (2010) and Shwetha (2011).

F. solani attained maximum growth on the 12^{th} day of incubation in potato dextrose broth and there after a decline in dry mycelial weight was observed. This is in confirmity with the findings of Sataraddi (1998) in *Fusarium udum*, Sreedevi (2007) in *F. solani*.

R. bataticola also attained maximum growth after 12 days of incubation in potato dextrose broth. Ramamurthy (1982) and Sachidananda (2005) and Ammajamma (2010) also made similar observations.

5.2.2 Growth studies in different liquid media

Fungi posses an ability to utilize a wide range of nutrients as a source of energy. Among the liquid media Richard's broth and potato dextrose broth supported the maximum growth of of *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola.* Least mycelia weight was obtained in Brown's medium.

In the radial measurements, it is difficult to consider the amount of submerged mycelium. Hence, Cochrane (1958) has opined determination of dry mycelial weight as the best method for precise work. Good growth of *Sclerotium rolfsii, Fusarium solani, Rhizoctonia bataticola*. was obtained in Richard's medium. Hence, ability of fungus to grow in Richards's medium indicated the requirement of certain nutrients and vitamins which may be present in the medium. This is in conformity with the findings of Shanmugam and Govindswamy (1973), Kulkarni (2000) in *R. bataticola,* Sataraddi *et al.* (2003) in case of *F. udum,* Sreedevi (2007) in *Fusarium solani*, Sachidananda (2005) and Ramprasad (2005) and Ammajamma (2010) *F. chlamydosporum*; Lingaraju (1977) and Ramprasad (2005) in case of *S. rolfsii*.

5.2.3 Nutritional studies

5.2.3.1 Carbon utilization

Carbon is an important element required, by all the living organisms for metabolic activities. Almost half of the dry mycelial weight of the fungal cell consists of carbon which is the main structural element (Lilly and Barnett, 1951). In the present study, among the carbon sources tested, sucrose supported maximum mycelial growth of *Sclerotium rolfsii*, *Fusarium solani* and *Rhizoctonia bataticola*. Next best sources were fructose and dextrose. The utilization of various carbon compounds may depend on either on the activity of the fungus to utilize certain simpler forms or on its power to convert the complex carbon compounds into simpler forms, which may be easily utilized.

Sucrose was found to be the better carbon source than others for *S. rolfsii, F. solani* and *R. bataticola.* Sucrose being the major component of photosynthesis in plants is generally utilized as a good carbon source by most of the plant pathogenic fungi. Similar observations were made by Patel (1991) and Ramprasad (2005) and Sachidananda (2005), Sreedevi (2007) in case of Fusarium sp. Similar observations were made by Prasad *et al.* (1986) in *S. rolfsii* isolated from tomato fruit, Ramprasad (2005) and Ammajamma (2010) in *Rhizoctonia* and *Sclerotium* causing wilt complex of coleus.

5.2.3.2 Nitrogen utilization

Nitrogen is an important element for protein synthesis and like carbon it is used by fungi for functional as well as structural purpose. In the present study, among the nitrogen sources tested for *S. rolfsii, F. solani* and *R. bataticola*, potassium nitrate was found superior over others. Minimum growth of all these three fungi was obtained in ammonium orthophosphate. *S. rolfsii, F. solani* and *R. bataticola* utilize potassium nitrate more efficiently and it is a better nitrogen source than any other nitrogen source.

The nitrate compounds are excellent nitrogen sources for imperfect fungi and also ascomycetes. Similar findings were made by Moore (1924), Subramanian and Srinivas (1953) and Agarwal (1958). Ramprasad (2005), Sachidananda (2005) and Ammajamma (2010) reported the same in case of *Fusarium* sp. *Sclerotium* and *R. bataticola.* Similarly, Hussain *et al.* (2003) reported that, among nitrogen sources tested potassium nitrate was found to be best source of nitrogen for *S. rolfsii*. Khattabia *et al.* (2004) reported that, *Sclerotium rolfsii* did not utilize urea as source of nitrogen while ammonium sulphate and potassium nitrate allowed the growth of the fungus.

5.2.4 Physiological studies

5.2.4.1 Temperature requirement

Temperature plays an important role, among the external factors which influence the growth and reproduction of fungi. Each fungus has its own temperature range. In the present study, maximum growth of *S. rolfsii* (255.90 mg), *F. solani* (365.00 mg) and *R. bataticola*. (225.00 mg) was obtained at the temperature of 30^0C, whereas optimum range was 30^0C to 35^0C for *S. rolfsii* and 25^0C to 30^0C for *F. solani* and *R. bataticola*. respectively.

Togashi (1949) reported that a number of plant pathogenic fungi have optimum temperature range of 20^0C to 30^0C and about half of these have their optimum temperature between 25^0C and 30^0C. The present findings are in conformity with the results of Uppal (1936); Waseer *et al*. (1990); Rodrigues *et al. (*1997) and Kulkarni (2000) in case of *R. botaticola*. Neal (1927); Chaung and Su (1988); Raghuwanshi (1995); Rawal, *et al.* (2003); Ramprasad (2005) in case of species of *Fusarium.* and Gondo (1964); Manjappa (1979); Sulladmath *et al. (*1977) and Ramprasad (2005) in case of *S. rolfsii* infecting *C. forskohlii .*

5.2.4.2 Hydrogen ion concentration

pH of the medium has profound effect on the rate and the amount of growth and many other life processes (Lilly and Barnett, 1951). The fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This shows importance of hydrogen ion concentration for the better fungal growth.

Results in the present study indicated that maximum growth of *F. solani* was observed at pH 6.5 (352.70 mg). The present findings are in agreement with the reports of Moore (1924), Neal (1927). Yogeshwari (1948), Singh and Chaube (1975), Sowmya (1993), Sataraddi (1998), Ramprasad (2005), Sachidananda (2005), Sumitra (2006) in *Fusarium* sp.

Maximum growth of *R. bataticola* (364.77 mg) was obtained at pH 7.0. The present findings are in conformity with the results of Ramprasad (2005), Sachidananda (2005) and Ammajamma (2010)

Maximum growth of *S. rolfsii* was observed at acidic pH of 4.0. These findings are in accordance with the reports of Lingaraju (1977) and Hari *et al.* (1988). At reduced pH, the cell membrane becomes studded with H^+ions which limit the passage of cations. The reverse condition could be obtained when the medium is alkaline and the accumulated H^+ions prevent the passage of essential anions, Enzyme activity is also conditioned by the reaction of the medium, as a result, the reduced growth of fungi was observed at extremities.

5.3 Studies on cultural, morphological and molecular variability among isolates of *Sclerotium rolfsii*

5.3.1 Collection of isolates of *Sclerotium rolfsii*

During the survey, wilted plant samples were collected from different places and isolated. *Sclerotium rolfsii* was isolated from all the places collected and proved to be most predominant pathogen among the wilt causing pathogens recorded. These isolates were designated as SrNID, SrHOS, SrGKVK, SrTHI, SrKAL, SrSAI, SrSIR, SrTHIR, SrRIP, SrGAN, SrBGM, SrGDG, SrRCR and SrMYS. These 14 isolates of *S. rolfsii* were used for further variability studies.

5.3.2 Cultural and morphological variability of isolates of *Sclerotium rolfsii*

5.3.2.1 Growth rate

Wide range of variation was noticed among fourteen isolates of *S. rolfsii* with respect to growth rate which ranged from 0.63 to 1.25mm per hour. Based on growth rate the isolates were grouped into three groups. Group I consisted of isolates with faster growth *viz*., SrGKVK, SrKAL, SrSAI, RCR and SrBGM (1.25 mm h^{-1}). Group II consisted four isolates with medium growth rate (0.94 mm h^{-1}) *viz*., SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS. Group III had isolates like SrRIP, SrTHIR and SrGAN with slow growth rate (0.63 mm h^{-1})

Barnett (1968) observed that growth and branching of *S. rolfsii* occurred at the apex of mycelium and pointed out that growth was regulated by a delicate balance between cell wall synthesis and degradation. Further, it was noted that, two enzymes *viz.,* β 1-3 glucanase and glucan synthetase were responsible for this activity. However, equilibrium of these two enzymes controlled the hyphal growth and branching in *S. rolfsii* as studied by Kritzman *et al.* (1978). Similar results with respect to variation in radial mycelial growth rate have been reported by many workers Sulladmath *et al*. (1977), Manjappa (1979), Mishra and Tewari (1990), Palaiah (2002) Jyothi (2006), Darakhshanda *et al. (*2007) and Shwetha (2011).

5.3.1.2 Time taken for sclerotial initiation

In the present study isolates varied with respect to time taken for sclerotial formation. Highly virulent isolates took less number of days for sclerotial initiation, whereas less virulent isolates needed more number of days for sclerotial initiation and it had positive correlation with virulence of isolates.

Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) which took just four days for sclerotial initiation. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS) in which sclerotial initiation was noticed on 5th day. Group III consisted of three isolates (SrRIP, SrTHIR and SrGAN) which took more number of days (7) for sclerotial initiation. In all isolates, sclerotial bodies were initially white, with time turned dark brown. The change in color of sclerotia might also be due to utilization/exhaustion of nutrients. Similar work conducted by Shwetha (2011) observed three groups of isolates of *S. rolfsii* based on the time taken for sclerotial initiation. Group I consisted of three highly virulent isolates (SrNID, SrKAL and SrSAI) which took minimum number of days (4) for sclerotial initiation. Group II consisted of four isolates (SrSIR, SrHOS, SrBEN and SrTHI) in which sclerotial initiation was noticed on 5th day. Group III consisted of three isolates (SrRIP, SrTHIR and SrGAN) took maximum number of days (7) for sclerotial initiation. Similar work has also been done by Palaiah (2002), Jyothi (2006) and Darakhshanda (2007).

5.3.1.3 Colony colour and colony type

All the isolates showed light white colonies except the isolates SrGAN, SrTHIR and SrRIP which showed extra whtie colony. Isolates *viz*., SrKAL, SrNID, SrTHI, SrGKVK, SrSIR, SrSAI, SrHOS, SrMYS, SrRCR, SrGDG and SrBGM showed compact mycelium while the isolates SrGAN, SrTHIR and SrRIP showed fluffy mycelium. Similar study was conducted by Sarma and Singh (2002), Shukla and Pandey (2007), Rakholiya and Jadeja (2011) and Bagwan (2011).

5.3.1.4 Number of sclerotia per plate and shape

With regard to number of sclerotia produced per plate, the isolates, SrKAL, SrNID, SrTHI, SrGKVK, SrSIR produced more sclerotia (241 to 324/plate) compared to others. Medium number of sclerotia were produced by the isolates (SrGAN, SrTHIR, SrSAI, SrHOS, SrMYS, SrRCR, SrGDG and SrBGM) *viz.,* 152 – 244 and 103 to 190/plate and very less number of sclerotia were produced in SrRIP isolate (142/plate). Isolates SrNID, SrTHIR, SrMYS and SrRIP showed oval shaped sclerotia whereas, others were round. Similar work was carried out by Sarma and Singh (2002) Shukla and Pandey (2007), Bagwan (2011), Shwetha (2011) and Shantalakshmi *et al*. (2012).

5.3.1.5 Position of sclerotia in Petri plate

In isolates, SrSIR, SrTHI, SrGKVK, SrRIP, SrSAI, SrHOS, SrBGM and SrGDG sclerotia were found near the edges of Petri plates. SrGAN and SrSIR isolates produced sclerotia at centre in concentrated form, while isolates SrKAL, SrNID, SrTHIR, SrMYS and SrRCR produced sclerotia irregularly all over Petri plate. Similar studies were conducted by Sarma and Singh (2002), Rakholiya and Jadeja (2011) and Bagwan (2011), Shwetha (2011), Shantalaksmi *et al*. (2012).

5.3.1.6 Size of sclerotial bodies

In the present study, size of the sclerotia exhibited wide variation with a range of 0.82 -2.00 mm. sclerotial size and virulence was directly related. Highly virulent isolates produced smaller sized sclerotia (0.82-0.95 mm) while sclerotial size was big in less virulent isolates (1.43-2.00 mm). However, range of 0.60-1.44 mm (Manjappa, 1979), 0.50-0.80 mm (Wolf, 1914), 0.40-2.50 mm (Singh and Srivastava, 1953) and 1.08-2.23 mm (Palaiah, 2002), 1.20 to 2.40 mm (Prabhu, 2003), 1.30-3.40 mm (Jyothi, 2006) was reported by earlier workers.

5.3.1.7 Hundred sclerotial weight

Hundred sclerotial weight of different isolates showed remarkable variation which ranged from 272 to 436 mg. This may be due to strainal variation in *S. rolfsii*. Similar type of study was conducted by Sulladmath *et al*. (1977), Manjappa (1979), Palaiah (2002), Prabhu (2003) and Jyothi (2006). They reported that, variation existed in sclerotial weight among the isolates of *S. rolfsii*. Based on sclerotial weight the different isolates were grouped in to three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrRCR, SrSAI and SrBGM) with test weight ranging from 272-304 mg. Group II consisted of five isolates (SrTHI, SrNID. SrSIR, SrMYS, SrGDG AND SrHOS) with test weight ranging from 210-342 mg and Group III consists of the isolates (SrRIP, SrGAM and SrTHIR) test weight ranging from 426 to 436 mg. Results clearly indicated variability among isolates with respect to sclerotial weight.

5.3.2 Mycelial compatibility/incompatability reaction on PDA plates

Vegetative compatibility in fungi reflects phenotypic differences (or similarity) among individuals representing the population of a species (Leslie, 1993). Thus individuas of fungal species having the same heterokaryaon or vegetative incompatibility loci can fuse to form a heterokaryon (Glass *et al.,* 2000). Fungal isolates that form stable heterokaryons are then considered to belong to the same vegetative compatible group (VCG). In contrast, isolates that are different at one or some or more of these loci will not anastomose. Rather, programmed cell death or apoptosis occurs in the mycelia cells that are in contact with an isolate representing a different VCG (Leslie, 1993)

In the present study, based on the mycelial compatibility seven vegetatively compatible groups (VCG) were found among the fourteen isolates. In the compatible reaction, mycelia of the two isolates intermingled at the zone of interaction. Whereas, in the incompatible reactions, a clear barriage zone of dead mycelia was formed. In all the antagonistic reactions, Sclerotia were formed only in the border of the lytic zone of the two isolates. However, a few sclerotia produced later on such lytic zone in some combinations failed to develop to the full size as those produced on the border of such barrages. The high rate of antagonistic reactions in the mycelia compatibility shows the extent of the diversity among these isolates of *S. rolfsii*. The death of mycelia at the interaction zone is attributed to the heterokaryotic condition of the nuclei, but the involvement of toxin(s) cannot be ruled out (Punja 1985). A detailed study in this regard may reveal more information about the cause of mycelial death in the incompatible reactions.

5.3.3 Oxalic acid production by isolates of *S. rolfsii*

Isolates of *S. rolfsii* were tested for oxalic acid production in culture filtrate which showed significant variation for the production of oxalic acid and it had positive correlation with virulence. Less virulent isolates produced low (1.30 to 2.00 mg/ml) and highly virulent isolates produced high (5 to 6 mg/ml) quantity of oxalic acid, while, other isolates produced intermediate quantity of oxalic acid in the culture. These findings are in conformation with the findings of Bateman and Beer (1965), Punja and Jenkins (1984), Palaiah (2002), Shukla and Pandey (2006), Shukla (2007) and Shwetha (2011) stated that difference in oxalic acid production among isolates can be correlated with the pathogenic potential.

5.3.4 Virulence index

The variation in pathogenicity among the isolates in relation to virulence was studied and found relationship between virulence and pathogenicity. Maximum degree of virulence was recorded in SrKAL (10.00) which took less number of days (10) for complete wilting of plant followed by SrGKVK (8.33), SrBGM (7.64), SrSAI (6.41) and SrRCR (6.11) required 12, 13 and 15 days for wilting. SrRIP was least virulent with minimum virulence index of 1.98 and required maximum period for wilting of plant. Isolates with maximum virulence index required less period for causing wilting of plants.

Based on virulence index, the isolates were classified into three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) with high virulence index ranging from 6 to 10. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS) with virulence index ranging from 3 – 5. Group III consisted of three isolates (SrGAN, SrTHIR and SrRIP) with low virulence index ranging from 1 –3.

Variation in the virulence among the *S. rolfsii* isolates was found significant. Epps *et al*. (1951) in a comparative study of four isolate of *S. rolfsii* found, difference in virulence of the pathogen. Subramaniam (1964) observed isolates of *S. rolfsii* from *Capsicum sp., Amorphaphallus* and groundnut varied in their pathogenicity.

Manjappa (1979) also reported significant variation in virulence of pathogen collected from different host. Ansari and Agnihotri (2000) observed differences in pathogenicity of *S. rolfsii* isolates collected from soybean from different locations and grouped isolates into 12 races. The race number 1 was found to be more dominant than other races and highly virulent to all the varieties.

Many workers have reported significant variation in the virulence and grouped isolates into different categories (Dhingra and Sinclair, 1973; Anilkumar and Sastry, 1980; Byadagi and Hegde, 1985; Than *et al*., 1991, Subramaniam, 1994; Ansari and Agnihotri, 2000; Palaiah, 2002; Shwetha, 2011).

5.3.5 Grouping of *S. rolfsii* isolates based on different characters

In the present study, virulence was related to the sclerotial size, virulence index, oxalic acid production. Based on these characters the isolates were divided into three groups. Group I consisted of SrGKVK, SrKAL, SrSAI, RCR and SrBGM isolates with small sized sclerotia, high virulence index and oxalic acid production and high virulence. Group II consisted of SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS isolates medium sized sclerotia, moderate virulence index and oxalic acid production were moderately virulent. While Group III consisted of SrTHIR, SrGAN, SrRIP isolates with big sized sclerotia, low virulence index and oxalic acid production and least virulent. These findings are in confirmation with the findings of Palaiah (2002), Prabhu (2003), Shwetha (2011).

5.4 Molecular variability among isolates of *Sclerotium rolfsii*

It is difficult to distinguish these species using traditional morphological and cultural differences. To understand better, the existence of variation among the isolates of *S. rolfsii*, PCR based technique, *i.e*. RAPD (Random Amplified Polymorphic DNA) and Internal Transcribed Spacer (ITS) were used in the present investigation.

The suitability of RAPD used was to detect the variations among the isolates of *S. rolfsii*. In the present investigation, OPA, OPB and OPF series primers were used to determine genetic distance between isolates and to construct a dendrogram. Of the 24 primers used for amplification, OPA02, OPA 04, OPA06, 0PA09, OPB02, OPB4 and OPF 02 showed cent per cent polymorphism and OPA03, OPA07, OPA0, OPB05, OPB 10 and OPF03, OPF 05 showed more than 80 per cent polymorphism among isolates.

The dendrogram by RAPD data revealed that, fourteen isolates were differentiated into three major clusters I, II and III and irrespective of geographical locations, the isolates are related. The suitability of random amplified polymorphic DNA for identification of *S. rolfsii* was investigated by Punja and Sun (2000). They screened 132 isolates of *S. rolfsii* from 36 different host species using oligo primers by RAPD. Some mycelial compatibility group in *S. rolfsii* and *S. delphinii* that had identical RAPD pattern were considered to be clonally derived. The extent of genetic diversity among the isolates of *S. delphinii* was lower than that observed in *S. rolfsii*. Tyson *et al*. (2002) studied a subset of 51 *S. cepivorum* Berk. Isolates and investigated for genetic diversity using universally primed PCR and random amplified polymorphic DNA analysis. Jyothi (2006) reported that a ten isolates of *S. rolfsii* were grouped in to two major clusters A and B. Major clusters A composed of isolates of wheat, chilli, cotton, groundnut,lucerne, onion, potato, soybean, sunflower and tomato. Hence, it is conformed that cluster group composed of isolates which showed very less variability. The isolates from different location which were studied for cultural studies could be differentiated further by molecular variability studies.

The results of the present investigation revealed that, isolates of same geographical locations were closely related. So, the results obtained from the cluster analysis revealed that sub cluster group composed of isolates belonging to same geographical location with very less variability.

5.4.1 Characterization of *S. rolfsii* by ITS-PCR

Internal Transcribed Spacer (ITS) region is now perhaps most widely sequenced DNA region in fungi. It has typically been most usefull for molecular systematic at the species level and within the species. The ribosomal DNA unit contains genetic and non-genetic or spacer region. Each repeat unit consists of a copy of 18S, 5.8S and 28S like rDNA and its spacer like Internal Transcribed Spacer (ITS) and intergeneric spcacer (IGS). The ribosomal DNA (rDNA) have been employed to analyze evolutionary events because it is highly conserved, where as ITS rDNA is more variable. Hence, it has been used for investigation of these species level relationships.

In the present investigation, ITS region of rDNA amplification with specific ITS1 and ITS4 universal primers produced approximately 650 to 700 bp in all the isolates confirmed that all the isolates obtained are *Sclerotium rolfsii*. Nucleotide sequences of fourteen *S. rolfsii* isolates of 18S, 5.8S and 28S rDNA region were analyzed using NCBI (National Center for Biotechnology Information) BLAST programme. Based on sequence comparison, all the fragment of 18S, 5.8S and 28S rDNA gene sequences of fourteen isolates were confirmed as *Athelia rolfsii* (Anomorph: *Sclerotium rolfsii*)

Comparision of fragment of 18S, 5.8S and 28S rDNA gene sequences of SrHOS, SrKAL, SrMYS, SrTHIR, SrGAN, SrGDG, SrNID, SrGKVK, SrRIP, SrTHI, SrBGM, SrRCR SrSIR and SrSAI showed 98 to 100 per cent similarity with the isolates published sequences of *Athelia rolfsii* downloaded from NCBI gene bank. Harlton *et al.* (1995) screened a worldwide collection of *S. rolfsii* which revealed variation in ITS regions of 12 sub-groups of *S. rolfsii.* Almeida *et al*. (2001) studied variability among 30 isolates of *S. rolfsii* by RAPD and were differentiated into distinct groups by ITS-PCR.

5.5 Estimation of loss

5.5.1 Growth and yield parameters

Maximum suppression of the disease was observed in Hexaconazole treatment which also recorded significantly superior growth parameters, *viz.,* plant height and number of branches.

Hexaconazole treatment could reduce the disease incidence to an extent of 80.02 per cent and recorded the increase in yield of 10 t/ha (fresh weight) and 2.56 t/ha (dry herbage weight) during 2011-12. Yield loss of 65.12 per cent (fresh weight) and 64.84 per cent dry herbage weight was estimated in untreated control.

Studies on the estimation of loss indicated that various components such as growth parameters (plant height and number of branches) and yields are the determinants of loss due to wilt complex disease at different severity levels. All these determinants of loss are negatively related to disease severity. Carboxin+thiram treatment could reduce the disease incidence to the extent of 68.85 -69.99 per cent and recorded the increase in yield up of 218.86 per cent and 147.60 per cent during 2007-08 and 2008-09 respectively. The same treatment recorded significantly superior growth parameters, *viz.* plant height and number of branches Disease resulted in 40 to 45 per cent decrease in plant height and 15 to 25 per cent decrease in number of branches in untreated plots (Ammajamma, 2010)

5.5.2 Biochemical changes in healthy and infected plants

5.5.2.1 Changes in sugars

In general, the infection due to pathogens bring about changes in respiratory pathway and photosynthesis, which are vital processes taking place inside the plant leading to wide fluctuations in sugar (Farkas and Kiraly, 1962; and Klement and Goodman, 1967).

In the present study, in *Sclerotium rolfsii* infected stevia leaves, there was reduction in reducing, non-reducing and total sugar contents to the tune of 42.25, 18.32 and 34.05 per cen. Due to infection of *Fusarium solani* the reduction was 43.97, 28.70 and 38.74 percent in reducing, non reducing and total sugars. In *R. bataticola* infected leaves there was marked reduction of 31.94, 16.21 and 36.55 percent in reducing, non reducing and total sugars compared to healthy plants.

These results are in conformity with the reports of Basamma (2008) in potato due to Slerotium wilt pathogen; Ammajamma (2010) in coleus against *R. bataticola*. The decrease in sugar content indicates the utilization of sugars by the invading pathogens as pointed out by Krog *et al.,* 1961 and Thind *et al.,* 1981.

5.5.2.2 Changes in phenols

Phenolics have been found to play an important role in determining resistance or susceptibility of a host to parasitic infection. A resistant variety may contain more phenolics than a susceptible variety (Rubin and Aksenova, 1957 and Raghunathan *et al*., 1958). For realization of their protective action, phenolic compounds must be liberated from in active forms, since it is precisely in the free state that polyphenols manifest the higher fungi and cytotoxicity (Friend, 1979). The marked increase in phenolic content might be due to accumulation of phenolic from surrounding healthy tissue (Farkas and Kiraly, 1962 and Jaypal and Mahadevan, 1968). Net synthesis of phenolics as a response to infection on release of bound phenolics by the enzymatic activities of the pathogen (Oku, 1959).

In *Sclerotium* infected plants, 37.77 per cent increase in total phenol and 21.05 per cent increase in ortho dihydroxy (OD) phenols was recorded compared to healthy leaves. Forty one per cent increase in total phenol and 22.36 per cent increase in orthodihydroxy (OD) phenols was recorded in case of *Fusarium solani* and per cent increase of 40.74 of total phenol and 22.36 per cent of ortho dihydroxy (OD) phenols was recorded compared to healthy leaves.

The results are in conformity with the findings of Patil and Kulkarni (1977) who reported that the infected leaves of sunflower variety contained more polyphenols than *Puccinia helianthii* healthy leaves of the same variety. Reuveni and Cohen (1978) observed that tobacco plants systemically infected with downy mildew exhibited severe stunting contained higher amount of phenolic compounds than uninoculated healthy tobacco plants.

5.6 Epidemiological studies

5.6.1 Inoculum density

Inoculum density is bound to influence the disease incidence. Infection takes place only when minimum inoculum potential of the pathogen is present in the soil.

In the present investigation, the inoculum density of *S. rolfsii, F. solani* and *R. bataticola* at different levels influenced the development of wilt disease. Disease incidence was progressed as there was increase in inoculum concentrations.

The results of the study indicated that, a minimum of 10 per cent inoculum was found to be optimum to cause maximum infection. With increase in per cent inoculum level there was significant increase in the disease incidence. The results are in conformity with the findings of Shalini (2006) in *F. solani* causing rhizome rot of ginger and turmeric and Ammajamma (2010) in *F. chlamydosporum* of coleus. Rattink (1986) reported the influence of inoculum level of *F. oxysporum* f.sp*. dianthi* on wilt disease development, viability and pathogenicity.

S. rolfsii is soil inhabitant and its inoculum level or effective propogules in soil has direct effect on incidence and severity of the disease. In the present study, optimum inoculum level was detected at which there will be maximum infection. The current results revealed that two per cent inoculum level resulted in 25.00, 33.33 and 16.66 per cent disease incidence in *S. rolfsii, F. solani* and *R. bataticola* which increased with increase of inoculum level. Highest incidence of 100 per cent was noticed at ten per cent inoculum level. The results are in conformity with the findings of Palakshappa *et al. (*1987), Kulkarni (2007) and Ammajamma (2010) who reported higher inoculum density resulted in cent per cent wilt incidence which may be due to the fact that higher inoculum always ensures the certainty of the infection.

5.6.2 Effect of soil temperature on *Sclerotium rolfsii*

Soil temperature is one of the important factors in geographical distribution of the fungus. In the present study, significant growth was found at 25^0C and 30^0C indicating its optimum soil temperature for germination and growth. Fungal growth and sclerotial germination was completely ceased at 50^0C. The reduced activity of the fungus at higher temperatures may be due to lack of proper supply of oxygen needed for the optimum growth of the fungus. This is in agreement with the findings of Harlapur (1998) and Kulkarni (2007), Ammajamma (2010) in *S. rolfsii.*

5.6.3 Effect of Soil moisture on *Sclerotium rolfsii*

The fungus survived better at low soil moisture levels than at high soil moisture levels. In the present study, maximum saprophytic activity of the fungus was found at 30 per cent (84.13%) with

maximum germination of sclerotia (100%). There was a decreased saprophytic activity and per cent germination of sclerotia with increased soil moisture level. The reduced activity with increase in soil moisture might be due to the decreased aeration in soil which deprives the pathogen by inadequate oxygen supply which is much needed for the activity of the fungus. The present indicated that 30 per cent soil moisture is optimum for growth and activity of the pathogen. Abeygunawardena and Wood (1957) observed no sclerotial germination at high soil moisture levels (75% MHC and above) and heavy colonization of other soil microflora, which are antagonistic to *S. rolfsii*. Curl and Hanson (1958) reported that high soil moisture supported growth of bacteria and enhanced the multiplication of actinomycetes, which were antagonistic to *S. rolfsii*. Nargund (1981) reported least disease incidence of foot rot of wheat and high grain yield of wheat when 3-4 irrigations were provided. The present findings are also in conformity with the work of Kulkarni (2007) and Ammajamma (2010) in *Sclerotium rolfsii.*

The saprophytic ability of *S. rolfsii* was less in high moisture content of the soil. This may be due to the lack of adequate oxygen supply in the soils having high moisture content. It is also noticed that *S. rolfsii* is a highly aerobic pathogen. In addition, the antagonistic organisms present in soil also inhibited the growth and multiplication of the pathogen at high soil moisture level.

5.6.4 Effect of soil pH on *Sclerotium rolfsii*

Studies on the saprophytic activity and germination of sclerotia of the fungus at different pH levels indicated that the fungus can survive and germinate at varied soil pH levels. Maximum per cent colonization on sorghum seeds and germination of sclerotia (92.25%) were observed at pH level of 6.0. Chowdhury (1946) reported that there was no correlation between soil pH and the disease incidence in case of betel vine. The present findings are in accordance with the observations of Palakshappa (1986) and Harlapur (1988) in case of *S. rolfsii* on betelvine and wheat respectively. Hence any little difference in soil pH will not affect the incidence of the disease.

5.6.5 Viability of sclerotia of *Sclerotium rolfsii* at different depths and duration in the soil

Sclerotium rolfsii is known to survive for longer period in the sclerotial stage in soil (Bateman and Beer, 1965) and it might be influenced by soil environment. In the present study, the viability of sclerotia was studied at different depths and duration. The viability of the sclerotia was decreased as there was increase in the depth of burial. The per cent germination of *S. rolfsii* was 80.57 at one cm depth, while it was 10.00 per cent at 20 cm depth. Since, S. *rolfsii* is highly aerobic in nature, deeper layers in the soil may not be favourable for its growth and perpetuation. Gurjar *et al.* (2004) revealed that, there was reduction in germination percentage of sclerotia with increase in depths. But they observed the failure of germination of sclerotia at the depth of 15 cm and above.

In the present study, 2 per cent germination of the sclerotia was noticed even at the depth of 16 cm. There was failure of germination at the depth of 17 cm and above. With respect to duration, when there was increase in storage duration, the per cent germination of sclerotia decreased. The germination was 100 per cent till one month of storage. Thereafter, it decreased successively and recorded 6.00 per cent after 18 months of storage. *Sclerotium rolfsii* is known to survive for more than 20 years in soil (Agrios, 2005). In the present study, failure of germination of sclerotia was recorded after 19 months of storage in the present study. However, Gurjar *et al.* (2004) reported that, *S. rolfsii* lost its viability after 18 months of burial and Mustafee and Chattopadhyay (1983) reported loss of viability after 17 months. Similar results were also reported by Kulkarni (2007).

5.6.6 Survival of *F. solani* in infected debries of stevia at different environmental conditions

The survival of an organism depends on its capacity to survive in an adverse environmental condition and to act as primary sources of inoculum. *F. solani* survived in the infected plant debris upto 36, 30 24 and 22 weeks at refrigeration, laboratory (room temperature) glasshouse and field conditions respectively. Vishwakarma *et al. (*1998) reported that *F. solani* survived in soil for 20 months, indicating that they survived mainly as chlamydospores. Shyla (1998) reported that *F. chlamydosporum* survived more than 210 days in host debris under room temperature. *F. moniliformae* conidia and hyphae survived two Kansas winters in sorghum stalks without any loss of viability or pathogenicity (Manzo and Claflin, 1984). Nyvall and Kommedhal (1970) studied the survival of *F. moniliformae* for eight months in maize stalk residue in Iowa field.

5.6.7 Susceptible stage of the crop

Susceptible stage study is useful in understanding the stage of the crop at which it causes economic damage. In the present study, significant difference in wilting percentage among the different stages of plants was observed. Significantly highest per cent wilt of 91.66 and 83.33 per cent was recorded in plants 15 and 30 days old rooted stevia cuttings. 120 days old stevia plants showed 41.67 per cent wilt. However, it took a maximum of 54 days for complete wilting. Similar study was conducted by Vinod (2006) in groundnut and Kulkarni (2007) in potato.

5.6.8 Interaction among the pathogens

There is no doubt about pathogenic potential of *S. rolfsii*, *F. solani and Rhizoctonia bataticola.* However, whether these pathogens act alone or in combination as part of disease complexes are not certain. This has been an area of research in recent years. Such studies would help us target control measures against the appropriate pathogen.

Independent inoculation of *S. rolfsii, F. solani* and *R. bataticola,* exhibited symptoms within 15, 22 and 45 days of inoculation with 65.56, 55.56 and 44.45 per cent disease incidence respectively. Simultaneous inoculation of two pathogens i.e., *S. rolfsii + F. solani*, *F. solani + R. bataticola* and *S. rolfsii + R. bataticola* exhibited the symptoms at 12, 17 and 15 days after inoculation with per cent disease incidence of 88.89, 77.78 and 77.78 respectively. However, when three pathogens (*Sclerotium + Fusarium + Rhizoctonia*) were inoculated simultaneously, the plants exhibited the symptoms quickly within 10 days and recorded cent per cent disease incidence (100.00). When pathogens were isolated from these samples, *S. rolfsii, F. solani* and *R. bataticola* were recorded.

All the organisms were pathogenic in independent inoculations. But combined infection resulted in rapid wilting (within 10 days after inoculation) of the plants. Since environmental conditions required for the fore mentioned pathogens are different, it is possible that a succession of the two/more pathogens occur in the same field. This possibility is particularly interesting since agro climatic conditions are not uniform throughout the crop season. Periodic isolation of pathogens from the same field is necessary to test this hypothesis.

Similar result of disease syndrome between fungal pathogens was reported by Shalini (2006) in rhizome rot complex of ginger and turmeric. Disease complex between *Pythium* spp. and *Fusarium* sp.. was also known with other pathosystems (Hendrix and Campbell, 1973; Bhardwaj *et al*., 1988). Dake and Edison (1989) stated that *F. oxysporum* can form complex with *Pythium* spp. Survey conducted in Kerala showed that, there was association among *Pythium* spp*, Fusarium* spp. or *R.solanacearum* in rhizome rot complex of ginger. The results were also in conformity with Ammajamma (2010).

5.6.9 Host Range Studies

Host range studies were conducted to know the ability of the test pathogen to infect other hosts. Out of eighteen hosts tested, eleven hosts were susceptible to *S. rolfsii* and can be considered as collateral hosts *viz*., *Ocimum sanctum, Coleus forskohlii Aloe vera, mentha spicata, Asparagus racemosus*, *Rosmarinus officinalis, Centella asiatica* and *Pogostemon patchouli*. All these hosts expressed very early symptoms (5 to 10 days) whereas, *Pandanus amaryllifolius* and *Sauropus androgynous* took maximum days to exhibit the symptoms i.e. 40 and 20 days respectively. The symptoms of the disease observed were similar to that of stevia. *Withania somnifera, Cymbopogan flexuosus, Costus pictus, Kaempferia galangal, Ocimum gratissimum, Cissus quadrangularis and Vetiveria zizanioides* did not show any infection by *S. rolfsii.*

Hegde *et al*. (2010b) and Shwetha (2011) studied the host range of *Sclerotium rolfsii* and revealed that coleus, tulsi, shatavari, brahmi, rose mary, patchouli and aloe were collateral host. Aswagandha, lemon grass and basmati were non host. Similarly Palaiah (2002) and Jyothi (2006) have studied the host range of *S. rolfsii.*

5.7 Management studies

5.7.1 *In vivo* studies

5.7.1.1 *In vitro* evaluation of botanicals

Green plants appear to be the reservoir of effective chemotherapeutants and would constitute inexhaustible sources of harmless pesticides. In recent times, the importance of using plant products against seed borne pathogens has been evolved for replacing the existing pesticides for several reasons. Plant possessing chemical derivatives are being identified by several workers (Annapurna *et al*., 1983). Among the plant species screened so far, several of them were found to possess antimicrobial compounds. Plant extracts have been reported to act as elicitors or induce defence mechanisms (Vidyasekaran, 1999).

In the present investigation, efficacy of eighteen extracts were evaluated against *S. rolfsii* and *F. solani* at 5 and 10 per cent concentrations. Among 18 plant extracts, *Duranta repens* showed maximum inhibition of mycelial growth (72.54%) of *S. rolfsii* followed by *Glyrecidia maculata*. Among 18 plant extracts tested against *F. solani,* maximum inhibition of mycelial growth was noticed in *Bougainvillea spectabilis* (52.41%) followed by *Adathoda* (49.72%) and *Duranta repens* (47.59%). Multineem showed cent per cent inhibition of mycelial growth of *R. bataticola* followed by garlic bulb extract (87.24%) and *Lantana camera* (63.92%). Hegde *et al*. (2010c). also reported that, *Duranta repens* and *Glyrecidia maculata* were most effective in reducing mycelial growth of *S. rolfsii.* They also reported that Multineem showed cent per cent inhibition of mycelial growth of *R. bataticola* The findings are in confirmation with Ammajamma (2010) in case of *F. chlamydosporum*, Hegde *et al.* (2012).

5.7.1.2 *In vitro* evaluation of bioagents by dual culture technique

Besides chemical control biological method of control is an effective, eco-friendly and alternative approach for any disease management practice. These antagonistic organisms act on the pathogen by different mechanisms *viz.,* competition, lysis, antibiosis, siderophore production and hyperparasitism (Vidyasekaran, 1999)

In *S. rolfsii*, maximum inhibition of mycelial growth was noticed in Dharwad isolate of *T. harzianum* (78.11%) which was on par with *T. harzianum* Rafai (75.63 %). Among the bioagents tested against *F. solani*, maximum reduction in colony growth was observed in *T. harzianum* (Dharwad isolate) which was on par with *T. harzianum* Rifai (77.03%) followed by *T. viride* (76.01%). Maximum per cent inhibition of mycelial growth of *R. bataticola* was obtained when native isolate of *T. harzianum* (Dharwad isolate) was evaluated (69.11%). *B. subtilis* and *P. fluorescens* failed to inhibit the mycelial growth of *S. rolfsii.*

These results are also in agreement with results of Karthikeyan (1996) and Sreenivasaprasad and Rao (1990) in groundnut. It is due to the penetration of the antagonist hyphae into hyphae of pathogen at the place of contact as confirmed by Mukharjee *et al.* (2000). Similar results were also reported by Shwetha (2010). Similar trend was observed by Guffar (1968); Ayers and Adam (1981); Kowalik (1997); Indra *et al. (*2003), Ramprasad (2005) and Ammajamma (2010) in *R. bataticola*. Sivan and Chet (1986), Kempf and Wolf (1989), Thomashow and Weller (1990), Kavitha *et al. (*2004), Sreedevi (2007) and Mishra *et al. (*2004) reported the same in case of *Fusarium* sp.

5.7.1.3 *In vitro* evaluation of fungicides

Use of fungicides is an alternative method of controlling the diseases of crop in the absence of resistant cultivars or when there is sudden out break of the disease. Hence, they would continue to be one of the major tools of integrated disease management. Evaluation of fungicides *in vitro* is a handy tool to screen a large number of fungicides and thus can serve as a guide for field testing.

In the present investigation, the results of *in vitro* revealed that out of six non–systemic fungicides tested, all the fungicides inhibited the mycelia growth of *Sclerotium rolfsii*. The most effective fungicides were emisan, mancozeb, thiram and captan completely (100.00 %) inhibited the growth of *S. rolfsii* at all the concentrations (0.1, 0.2, 0.3%) tested. The least inhibition was noticed in zineb (56.91%) and chlorothalonil (50.24%). Vyas and Joshi (1977) and Choudhury *et al.* (1998) reported highest inhibition of mycelial growth of *S. rolfsii* by thiram and mancozeb at 2000 ppm. These findings are also in conformation with the findings of Shwetha (2011) and Hegde *et al*. (2010b).

Among the six systemic fungicides evaluated, carbendazim + mancozeb, carboxin + thiram, propiconazole, hexaconazole and benomyl found to be most effective and significantly superior over control which inhibited cent per cent growth of *S. rolfsii* at all the concentrations (0.025, 0.05 and 0.1%) tested. Carbendazim was least effective in inhibiting mycelial growth. Johnson and Subramanyam (2000) reported least mycelial inhibition with carbendazim and chlorothalonil. Urany *et al.* (1984), Prabhu (2003) and Kulkarni (2007) reported that, among the systemic fungicides tested, carbendazim was least effective. Basamma (2008) and Hegde *et al*. (2010a) reported that propiconazole, difenconazole and hexaconazole were effective against *S. rolfsii*.

Mancozeb at 0.2 and 0.3 per cent and propineb at 0.3 per cent concentration completely (100%) inhibited the growth of *F. solani.* Least inhibition of mycelial growth was recorded in Zineb at 0.1 per cent (49.33%). Among systemic fungicides, carbendazim, carbendazim + mancozeb, propiconazole, hexaconazole and benomyl were found to be most effective. These findings are in conformation with the findings of Hegde *et al*. (2010b).

In the present investigation, among non-systemic fungicides, thiram at 0.3 per cent and among systemic fungicides, combiproduct of carboxin+thiram at 0.05% and 0.1% were found to be most effective in inhibiting growth of the *R. bataticola*. Similar results were reported by Sobti and Sharma (1988); Peshney *et al* (1992) and Sachidananada (2005).

5.7.2 *In vivo* studies

5.7.2.1 Management by organic amendments

Addition of organic amendments favourably improves crop yield mainly by enhancing soil fertility rather than through provision of nutrients to the plants. Considerable improvements in soil structure, water retention capacity and aeration in different types of soils have been observed following the addition of green manures, farm yard manures and other organic matter. Another additional advantage of using organic matter is the activation of many beneficial microbes antagonistic to soil borne pathogens, leading to disease suppression.

In the present investigation, among six organic amendments tested against *S. rolfsii*, enriched FYM was found to be superior, which recorded least disease incidence (33.33%) and increased plant height, followed by neem cake and vermicompost (41.67 %,), cotton oil cake (50.00%). Safflower oil cake and groundnut cake showed 58.33 per cent disease incidence. The effect of organic amendments on pathogenic fungi may be attributed due to release of ammonia (NH3) that is directly toxic to sclerotia. These amendments having high C:N ratio after decomposition results in an increase in the microbial population and activity of antagonistic microbes. Hence, there is a considerable amount of carbon dioxide gas liberation by these soil saprophytes, which suppressed the pathogenic activity of *S. rolfsii* in the soil (Punja, 1985). Addition of oil cakes proved to be more effective in reducing the disease than crop residues. Various biochemicals, including antibiotics and phenols, released during decomposition of lignin containing materials, induce disease resistance on the root surface as well as in the tissues when absorbed. The results thus obtained are in accordance with the reports of Nishatkhalis and Manoharachary (1985), Padmodaya and Reddy (1999), Rajan *et al.* (1991), Goudar *et al.* (1998), Karunanithi *et al.* (2003) and Padmodaya (2003)

5.7.2.2 Integrated Disease Management (IDM) for Sclerotium wilt of stevia

The concept of integrated disease management including chemicals, organic amendments and biocontrol agents has become talk of the day. Compatibility among different management component is a part of integrated disease management. Irving (1970) defined integrated control as a compatible system of control in which various methods are used in proper sequences and timing so as to create least hazard to man and environment and to permit maximum assistance to natural control. Keeping this in mind, the present investigations were carried out using chemicals, bio agents, plant extract and organic amendments singly and in combination, in pot as well as in the field. Thus, Integration of the best components of the disease management has given encouraging results.

In the pot culture experiment conducted for Sclerotium wilt of stevia, results revealed that hexaconazole and carboxin followed by FYM + *T. harzianum* and duranta leaf extract reduced the disease incidence at 60 DAP. The plant height (90 DAP) was maximum in hexaconazole (62.66 cm) with more number of branches (18.40) which was significantly superior to all other treatments. This was followed by carboxin + thiram (58.70 cm) which was on par with FYM + *T. harzianum* (56.40 mm)

A field experiment was conducted in the year 2011 on the integrated management of sclerotium wilt of stevia at Medicinal and Aromatic plants Unit, Saidapur Farm, Department of Horticulture, UAS, Dharwad.

Maximum suppression of the disease was observed when drenching of hexaconazole @0.1 per cent was used 30 days after transplanting (DAT). This treatment recorded significantly superior growth (plant height, number of branches and number of leaves) and yield parameters (Fresh weight and dry weight). Minimum disease incidence of 13.33 per cent was recorded with a maximum yield of 10.78 t/ha and dry weight of 2.56 t/ha was recorded. This was followed by carboxin + thirum treatment with 15.56 per cent disease incidence 120 DAT respectively. Next best to these two treatments was FYM + *T. harzianum* which showed minimum per cent disease incidence (17.78 %) at 120 DAT. *T. harzianum* is well known as effective biological control agent for wilt complex in stevia. Use of FYM in this treatment provided a food base for the multiplication of *T. harzianum* (Garret, 1965)

Future line of work

1. Identification of pathogenic variability among isolates of *Sclerotium rolfsii* causing wilt and other pathogens of wilt complex by using improved markers such as ARDRA or /SSR.
2. The genetic basis of mechanism of interaction in the disease complex needs to be studied.
3. A multipronged integrated disease management system involving phytosanitation, cultural and biological methods of control coupled with moderately disease resistant variety would be the practical solution to check the crop losses.
4. Management of wilt complex disease of stevia under nursery.
5. The technology developed should be demonstrated in farmers field on a larger area to popularize it.

SUMMARY AND CONCLUSIONS

Stevia rebaudiana (Wild.) Briq. is an important medicinal plant belonging to the family Asteraceae. It is a calorie free natural sweetener for diabetic and health conscious people. Stevioside and Rebaudioside are natural sweet compounds present in stevia leaf which are not absorbed by human body during the process of digestion and hence it is calorie free. Stevia is commonly propagated by stem cuttings

Stevia is affected by wilt caused by *Fusarium solani, Sclerotium rolfsii* and *Rhizoctonia bataticola*. Among them wilt caused by *Sclerotium rolfsii* Sacc. is one of the limiting factors in commercial cultivation of stevia. Fusarium wilt is very severe in nurseries. Root rot is observerved in some pockets in Karnataka which may become severe in future. The information available on these diseases with respect to stevia crop is very scanty. Hence, it demanded an early investigation into various aspects of the pathogens and also the disease.

The present investigation was carried out during the period from 2007-2008 and 2011-12, Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad and Medicinal and Aromatic Plant Unit, Saidapur Farm, Dept. of Horticulture, UAS, Dharwad, which included different studies, *viz*. survey for the disease in Karnataka, isolation, proving the pathogenicity, identification, symptomatology, cultural, nutritional and physiological studies, loss estimation with respect to growth parameters and yield, biochemical changes (phenols and sugars), Epidemiological studies *viz.,* inoculum levels, survival, soil temperature, soil moisture, soil pH, interaction among pathogens and host range. Cultural and molecular variability among isolates of predominant pathogen. *In vitro* evaluation of the bioagents, botanicals and fungicides, management by organic amendments and integrated management studies under pot and field conditions. The salient features of the findings are summarized below.

A random survey conducted in major stevia growing areas of Karnataka revealed the prevalence of wilt causing pathogens in all the locations surveyed. The disease incidence of Sclerotium wilt ranged from 6.25 to35.80 per cent, Fusarium wilt ranged from 5.25 to 35.00 per cent. Root rot incidence was observed only in Saidapur (8.75 per cent) and Gangavati (6.25%).

The pathogens were isolated from infected parts of stevia by following standard tissue isolation method and pure cultures of *S. rolfsii, F. solani and Rhizoctonia bataticola* were maintained. On the basis of morphological and cultural studies, the pathogens were identified as *Sclerotium rolfsii* Sacc., *Fusarium solani* (Mart.) Sacc and *Rhizoctonia bataticola* (Taub.) Butler.

Sclerotium rolfsii caused discoloration of the stem at collar region with white mycelial growth. The leaves became flaccid and dropped off. In severe cases, sclerotial bodies were observed on fallen leaves or at collar region of infected plant. The characteristic symptoms of *Fusarium solani* started as yellowing of lower leaves extended upwards and whole leaves gradually turned brown coloured. The infected plants showed discoloration of roots and complete destruction of root system. *Rhizoctonia bataticola* initially expressed as water soaked areas and the affected tissues soon turned into a soft black, watery mass at the collar region of the plant. The infection was also found on roots and caused decay, which ultimately resulted in collapse of the plant. The bark of such plants was easily peeled off. The affected plants were killed finally due to severe rot.

S. rolfsii produced silky white mycelium which gradually lost its luster and became dull in appearance. Sclerotial bodies were initiated on fifth day after inoculation which were white in the beginning, turned to brown then to chocolate brown at maturity. *Fusarium solani* on potato dextrose agar put forth moderately rapid growth covering the Petriplate in 10 days. The mycelium was sparse to dense, pinkish in colour. Hyphae are septate and hyaline and produced both macro- and microconidia. Macroconidia are moderately curved, stout, thick-walled, usually 3-5 septate. *R. bataticola* grew rapidly and profusely on potato dextrose agar. Hyphae were hyaline at first and gradually turned grey to brownish black, septate and branched at right angles. As the culture grew old, abundant tiny black microsclerotia were produced.

Among the solid media tested, maximum and minimum growth of fungi like *S. rolfsii, F. solani* and *R. bataticola,* was supported by potato dextrose agar and Browns agar respectively. In growth phase studies, maximum growth was reached on 10th day in *S. rolfsii*, 12th in *F. solani* and 14th day in *R. bataticola.* This was taken as maximum growth period for further studies. Richard's broth and potato dextrose broth supported the maximum dry mycelial weight of *S. rolfsii, F.solani* and *R. bataticola.*

Among the carbon sources, sucrose was found to be the best for the growth of *S. rolfsii, F.solani* and *R. bataticola.* and potassium nitrate was best nitrogen source. Temperature range of 30 to 35^{0}C, 25 to 30^{0}C and 25 to 35^{0}C were found to be optimum for the growth of *S. rolfsii, F. solani* and *R. bataticola*. These three fungi grew at all pH levels tested. However, the maximum growth was obtained at pH 4.0 for *S. rolfsii* and 6.5 *for F. solani* and *R. bataticola* respectively. The optimum pH range was found to be between 6.0 to 7.0 and 6.5 to 7.5 for *R. bataticola* and *F. solani* respectively and 3.5 to 5.0 for *S. rolfsii*.

S. rolfsii was isolated from almost all the places surveyed and proved to be most predominant pathogen among the wilt causing pathogens recorded. It was collected from 14 places. Wide range of variation was noticed among 14 isolates of *S. rolfsii* with respect to various attributes studied growth rate ranged from 0.63 mm per hour. Based on growth rate, the isolates were grouped in to three groups. Group I consisted of isolates with faster growth viz., SrGKVK, SrKAL, SrSAI, RCR and SrBGM (1.25 mm h^{-1}). Group II consisted four isolates with medium growth rate (0.94 mm h^{-1}) *viz*., SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS. Group III had isolates like SrRIP, SrTHIR and SrGAN with slow growth rate (0.63 mmh^{-1})

The isolates varied with respect to time taken for sclerotial formation. Isolates were grouped into three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) which took just four days for sclerotial initiation. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS. Group) in which sclerotial initiation was noticed on 5th day. Group III consisted of three isolates (SrRIP, SrTHIR and SrGAN) took more number of days (7) for sclerotial initiation. In all isolates sclerotial bodies were initially white, with time turned dark brown.

All the isolates showed light white colonies except the isolates SrGAN, SrTHIR and SrRIP showed extra whtie colony. Isolates *viz*., SrKAL, SrNID, SrTHI, SrGKVK, SrSIR, SrSAI, SrHOS, SrMYS, SrRCR, SrGDG and SrBGM showed compact mycelium while the isolates SrGAN, SrTHIR and SrRIP showed fluffy mycelium

With regard to number of sclerotia produced per plate, isolates, SrKAL, SrNID, SrTHI, SrGKVK, SrSIR produced more sclerotia (241 to 324/plate). Medium number of sclerotia were produced by isolates SrGAN, SrTHIR,SrSAI, SrHOS, SrMYS, SrRCR and SrBGM (150 to 244/plate) and very less number of sclerotia were produced by SrRIP isolate (142/plate). Isolates SrNID, SrTHIR, SrMYS and SrRIP showed oval shaped sclerotia whereas, others were round.

With respect to position of sclerotia, maximum isolates (SrSIR, SrTHI, SrGKVK, SrRIP, SrSAI, SrHOS, SrBGM and SrGDG) produced sclerotia near the edges of Petri plates. SrGAN and SrSIR isolates produced sclerotia at centre in concentrated form. Whereas other isolates SrKAL, SrNID, SrTHIR, SrMYS and SrRCR) produced sclerotia irregularly all over Petri plate

Variation in size of sclerotial bodies of isolates under study was found significant. SrRIP, SrTHIR and SrGAN isolates produced biggest sclerotia with diameter of (> 1.25 mm). In isolates SrTHI, SrNID, SrSIR, SrMYS, SrGDG and SrHOS medium sized sclerotia ranging from 1.00mm to 1.25 mm were produced. Where as in SrGKVK, SrKAL, SrRCR, SrSAI and SrBGM isolates produced very small sized sclerotia (< 1 mm).

Maximum test weight was recorded in SrTHIR isolate (436 mg) and minimum in SrHOS isolate (272 mg). Based on test weight, the isolates were grouped into three categories. Group I (SrNID, SrHOS, SrTHI, SrKAL, SrBGM and SrSAI) with test weight ranging from 272 to 304 mg. Group II consisted of six isolates (SrNID, SrSIR. SrTHIR, SrRIP, SrMYS, SrHOS, SrGDG, and SrGAN) with test weight ranging from 210-342 mg and group III (SrTHIR, SrRIP and SrGAN) with test weight ranging from 426 to 436 mg.

Based on the mycelial compatibility, seven vegetatively compatible groups (VCG) were made among the fourteen isolates. Kalenahalli isolate was compatible with all other isolates.

Maximum oxalic acid production (5.90 mg/ml) was observed in SrKAL isolate followed by SrGKVK (5.80 mg/ml), SrBGM (5.12mg/ml) and SrRCR (4.93 mg/ml) isolate. However, least oxalic acid production (1.33 mg/ml) was observed in SrRIP isolate followed by SrGAN (1.45 mg/ml) and SrTHIR (1.80 mg/ml) isolate. Other isolates remained intermediate in oxalic acid production.

Based on virulence index, the isolates have been classified into three groups. Group I consisted of five isolates (SrGKVK, SrKAL, SrSAI, RCR and SrBGM) with high virulence index ranging from 6 to 10. Group II consisted of six isolates (SrTHI, SrNID, SrSIR, SrMYS, SrGDG and

SrHOS) with virulence index ranging from 3 – 5. Group III consisted of three isolates (SrGAN, SrTHIR and SrRIP) with low virulence index ranging from 1 –3.

PCR - based RAPD analysis revealed that OPA02, OPA 04, OPA06, 0PA09, OPB02, OPB4 and OPF 02 showed cent per cent polymorphism. The dendrogram by RAPD data indicated that the fourteen isolates differentiated into three major clusters. In the present investigation, the results revealed that, geographical locations of isolates were closely related.

ITS region of rDNA amplification with specific ITS1 and ITS4 universal primers produced approximately 650 to 700 bp in all the isolates confirmed that all the isolates obtained are *Sclerotium rolfsii.* Based on sequence comparison, all the fragment of 18S, 5.8S and 28S rDNA gene sequences of fourteen isolates were confirmed as *Athelia rolfsii* (Anomorph: *Sclerotium rolfsii*). All the isolates showed 98 to 100 per cent similarity with the isolates published sequences of *Athelia rolfsii* downloaded from NCBI gene bank.

Soil drenching with hexaconazole @ 0.1% could reduce the disease to the extent of 80 per cent and yield loss of 66 fresh yield and 65 per cent dry yield was estimated.

In *Sclerotium rolfsii,* maximum per cent increase of total phenol content (37.77), 40.74 per cent in *F. solani* and 35.18 percent was recorded. Increase in the OD phenols was to the tune of 21.05 per cent, 22.36 per cent and 21.84 percent in *S. rolfsii, F.solani* and *R. bataticola* respectively. There was reduction in reducing, non-reducing and total sugar contents due to infection.

The inoculum density of 8, 9 and 10 per cent is required for *S. rolfsii. F. solani* and *R. bataticola* to get 100 per cent disease incidence. The optimum soil temperature for germination and growth of S*. rolfsii* was observed at 30^0C. Growth completely ceased at 50^0C. Maximum saprophytic activity of *S. rolfsii* was found at 30 per cent soil moisture (82%) with cent per cent germination of sclerotia. There was a decreased saprophytic activity and per cent germination of sclerotia with increase soil moisture level. It was concluded that 30 per cent soil moisture is optimum for growth and activity of the pathogen.

F. solani survived in the infected plant debris upto 36, 30, 24 and 22 weeks at refrigeration, laboratory (room temperature) glasshouse and field conditions respectively, indicating that they could survive also as chlamydospores.

Pot culture tests were conducted to investigate the interaction among the wilt causing pathogens *viz*., *S. rolfsii, F. solani and R. bataticola.* When simultaneously three pathogens (*Sclerotium + Fusarium + Rhizoctonia*) were inoculated, the plants exhibited earlier symptoms within 10 days and recorded cent per cent disease incidence.

The results of host range studies revealed that among sixteen hosts tested, eight hosts *viz*., *Ocimum sanctum, Coleus forskohlii Aloe vera, Asparagus racemosus* , *Rosmarinus officinalis, Centella asiatica, Pandanus amaryllifolius* and *Pogostemon patchouli* were infected by *S. rolfsii* and can be considered as collateral hosts. The symptoms of the disease observed were similar to that of stevia. Others did not show any infection and are non hosts for *S. rolfsii.*

The results of the dual culture technique revealed that the fungal bioagents were better than the bacterial bioagents in inhibiting the growth of *S. rolfsii, F. solani and R. bataticola.* Stronger antagonistic activities of *T. harzianum* (Dharwad isolate) against *S. rolfsii, F. solani and R. bataticola* was observed

Among plant extracts, leaf extracts of *Duranta repens and Glyrecidia macullata*, were effective in inhibiting *S. rolfsii*. *Prosophis juliflora* showed least inhibition of mycelial growth. *Bougainvillea spectabilis* and Adathoda leaf extracts were effective against *F. solani.* Multineem and garlic bulb extract were very effective in inhibiting the mycelia growth of *R. bataticola .* Plant extracts at 10 per cent was found to be more effective than at five per cent concentration.

In vitro studies of fungicides revealed that among non–systemic fungicides, Emisan, mancozeb, thiram and captan completely inhibited the growth of *S. rolfsii* (100.00%) at all the concentrations (0.1, 0.2, 0.3%) tested. Mancozeb and propineb were effective against *F. solani and R.bataticola*. Among systemic fungicides, carbendazim + mancozeb, carboxin + thiram, propiconazole, hexaconazole and benomyl found to be most effective which inhibited cent per cent growth of *S.rolfsii* at all the three concentrations (0.025, 0.05 and 0.1%). Carbendazim, carbendazim + mancozeb, propiconazole, hexaconazole and benomyl recorded cent per cent inhibition of *F. solani.*

Thiram at 0.3 per cent and combi product, carboxin + thiram (0.05 and 0.1%) were found effective against *R. bataticola*.

Among the six organic amendments tested against *S. rolfsii* in pots, enriched FYM was found to be very effective which recorded least disease incidence followed by neem cake and vermicompost. Integrated disease management studies in pot experiment revealed that soil drenching of hexaconazole @ 0.1% recorded minimum disease incidence with increased plant height. In field experiment also, soil drenching of hexaconazole @ 0.1% of helps to manage the sclerotium wilt of stevia effectively and to increase the yield (184.4%) over control. Equally effective is the soil drenching of carboxin + thiram @ 0.1% followed by *T. harzianum* @ 25 kg/ha for obtaining maximum yield of stevia with minimum disease incidence.

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* - Originals are not seen _____

STUDIES ON SOILBORNE DISEASES OF STEVIA AND THEIR MANAGEMENT WITH SPECIAL REFERENCE TO SCLEROTIUM WILT

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ABSTRACT

Stevia rebaudiana an important medicinal crop is infected by soil borne fungal pathogens like *Sclerotium rolfsii, Fusarium solani* and *Rhizoctonia bataticola* among which *S. rolfsii* is becoming a major threat in establishment of crop on a commercial basis. Survey on disease incidence in Karnataka revealed 6.25 to 39.65 % incidence.

PDA, Richards's broth, sucrose and potassium nitrate supported maximum growth of all three pathogens. Maximum growth of *S. rolfsii* was observed at 10 days of incubation at 30^0C to 35^0C temperature and at 4.0 pH, *F. solani* at 12 days of incubation at 25^0C to 30^0C temperature and at 6.5 pH, *R. bataticola* at 12 days of incubation at 25^0C to 30^0C temperature and at 7.0 pH.

The study on variability indicated that all isolates showed marked differences in their growth rate, time taken for sclerotial initiation, colour, size and weight of sclerotial body. Virulence was correlated with many aspects; small sized sclerotia, high virulence index and more oxalic acid production (SrGKVK, SrKAL, SrSAI, SrRCR and SrBGM). RAPD analysis with 24 random primers grouped 14 isolates into three major clusters. ITS region of rDNA amplification with ITS1 and ITS4 universal primers produced approximately 650 to 700 bp confirmed the identification of *S. rolfsii*.

Increase in total phenol and decrease in sugar content was recorded due to infection of *S. rolfsii*. Host range studies revealed that coleus, aloe, mint, brahmi and patchouli were collateral hosts. In the interaction studies, simultaneous inoculation of all three pathogens recorded 100 % wilt incidence.

Under *in vitro* studies, *Trichoderma harzianum* among bioagents; duranta, glyrecidia and multineem among the botanicals; mancozeb, thiram and captan among contact fungicides, propiconazole and hexaconazole among systemic fungicides were effective against all three pathogens. In field, soil drenching of hexaconazole @ 0.1% helps to manage disease effectively and to increase the yield.