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PHENOTYPIC AND PATHOGENIC VARIABILITY OF *Sclerotium rolfsii* Sacc. INFECTING FRUIT CROPS AND ORNAMENTALS

By

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(2009-11-111)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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We, the undersigned members of the advisory committee of **Miss. Hajara, P. H. (2009-11-111)** a candidate for the degree of **Master of Science in Agriculture**, with major field in Plant Pathology, agree that the thesis entitled **"Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals"** may be submitted by **Miss. Hajara, P. H.**, in partial fulfillment of the requirement for the degree.

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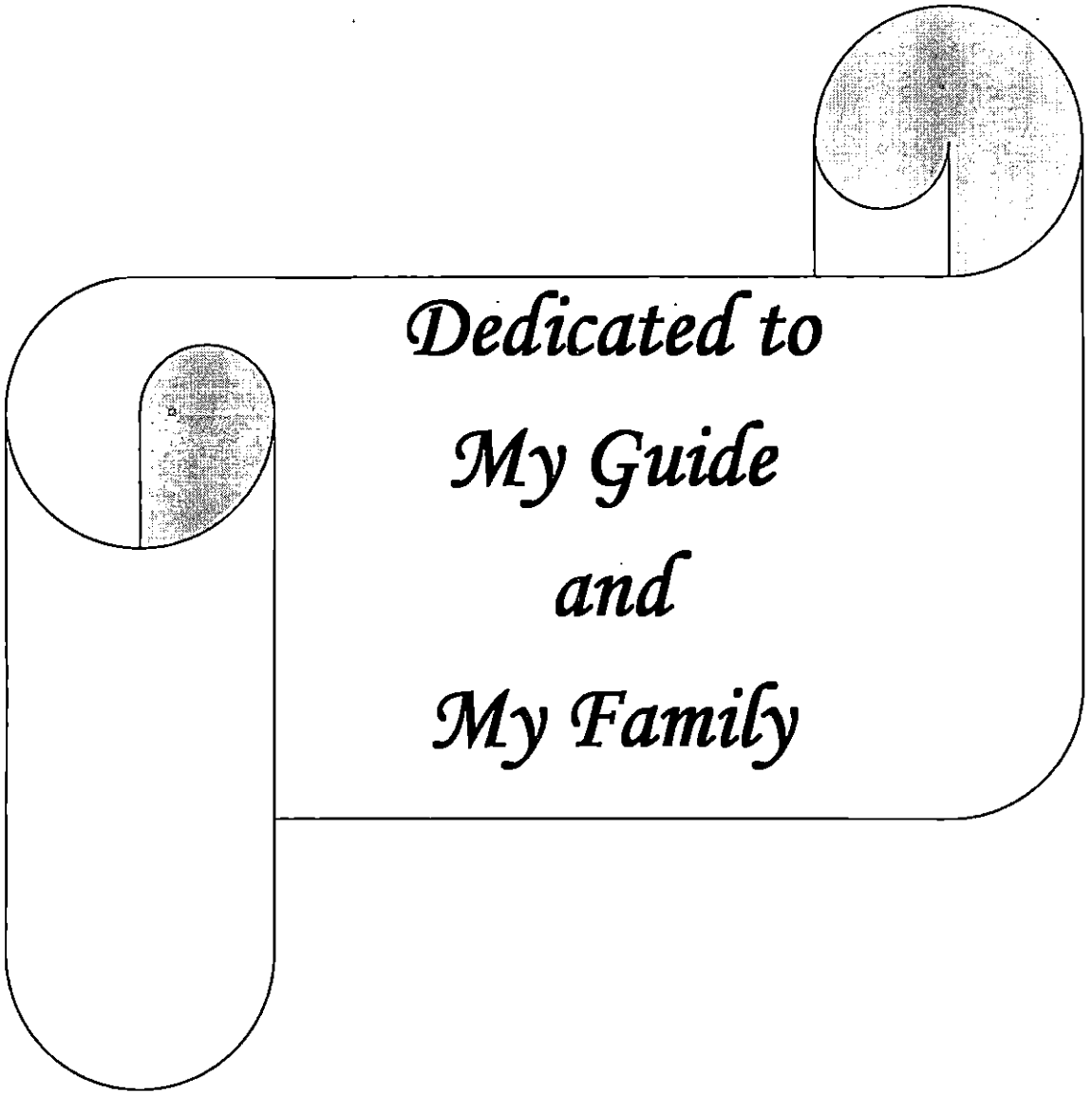
Dedicated to
My Guide
and
My Family

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Introduction

1. INTRODUCTION

The pathogen *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough) is a devastating soil borne fungus having wide host range and worldwide distribution (Aycock, 1966; Punja, 1988). It occurs in the tropics, sub-tropics and other warm temperate regions of the world. The fungus was placed in the form genus *Sclerotium* as it forms differentiated sclerotia and sterile mycelia (Saccardo, 1913). Since its sexual stage is not formed easily, this fungus is characterized by the morphological characters. Although there are several other sclerotium producing fungi, the fungi characterized by small tan to dark brown or black spherical sclerotia with internally differentiated rind, cortex and medulla were placed in the form genus *Sclerotium* (Punja and Rahe, 1992).

In 1931, Curzi discovered that the teleomorph (spore bearing state of *S. rolfsii*) was a corticioid fungus and accordingly placed the species in the form genus *Corticium* with a move to a more natural classification of fungi. *Corticium rolfsii* was transferred to *Athelia rolfsii* in 1978. Microscopically they consist of ribbon like hyphae with clamp connections. Small, brownish sclerotia are also formed arising from the hyphae (Tu and Kimbrough, 1978).

Rolfs (1892) first reported *S. rolfsii* as the causal organism of tomato blight in Florida. It usually causes root rot, leaf blight, stem rot, collar rot and wilt diseases in many economically important crops like vegetables, fruits, spices, ornamentals etc. The wide host range, prolific growth and ability to produce persistent sclerotia contribute to the large economic losses associated with the pathogen. Hence it is a serious problem and is very difficult to eradicate once established in a field. It occurs in the soil as a saprotroph, but can also attack living plants. It has

an almost indiscriminate host range, but its capacity to form sclerotia means that it particularly attacks seasonal crops.

Recently, in Kerala incidence of *S. rolfsii* has become a serious problem and severe infection has been observed on many horticultural crops like ornamental plants *viz.*, marigold, chrysanthemum, orchid, lilly, balsam, fruits crops *viz.*, mango, banana, jack, spices *viz.*, pepper, ginger and vegetables *viz.*, amorphophallus, solanaceous crops, curry leaves etc. and causing collar rot, leaf rot, leaf blight stem rot type symptoms. Prakash and Singh (1976) reported *S. rolfsii* as pathogen of basal rot of mango seedlings from Lucknow. Mohan and Lakshmanan (1989) observed pseudostem rot on *Musa* sp. caused by *S. rolfsii* from Tamil Nadu. Association of this pathogen with collar rot of mango seedlings and pseudostem rot of banana has also been noticed in Thrissur district of Kerala and isolated the pathogen from the diseased specimens. Several workers from Kerala also reported the incidence of this pathogen on canna, kodampuli, balsam etc. (Vilasini *et al.*, 1995; Cherian *et al.*, 1996; Girija and Umamaheswaran, 2003). But detailed information regarding the cultural, morphological and pathogenic characters of the pathogen is lacking.

In this context, the present study is taken up and is restricted to selected ornamental plants *viz.*, marigold, chrysanthemum and fruit crops *viz.*, mango and banana to know more about the diseases caused by this pathogen and also on variations in the phenotypic and pathogenic characters of *S. rolfsii*.

The research programme entitled "Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals" envisaged the following aspects.

1. Isolation and maintenance of *S. rolfsii* associated with selected fruits crops and ornamental plants.
2. Symptomatology of the disease.
3. Variability in phenotypic characters of different isolates of *S. rolfsii*
4. *In vitro* evaluation of compatibility of various isolates of *S. rolfsii*
5. Pathogenic variability of the isolates of *S. rolfsii*
6. Host range of different isolates of *S. rolfsii*
7. Estimation of IAA and total phenol content of different isolates of *S. rolfsii*

Review of Literature

2. REVIEW OF LITERATURE

Sclerotium rolfsii is a devastating soil borne fungal pathogen having a wide host range and worldwide distribution. It commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, leaf blight, stem rot, collar rot and wilt diseases in many economically important crops like vegetables, spices, fruit crops, ornamentals etc. The major ornamentals *viz.*, marigold and chrysanthemum and fruit crops *viz.*, mango and banana were found infected by this pathogen and causing collar rot and leaf blight diseases. The incidence of *S. rolfsii* on mango was observed in 2007 in the mango nursery of Agricultural Research Station, Mannuthy and on Kadali variety of banana in 2009 from Koottala area of Thrissur district.

2.1. Sclerotium *rolfsii* - A PLANT PATHOGEN

Sclerotium rolfsii Sacc.(telomporph: *Athelia rolfsii* (Curzi) Tu and Kimbrough, 1978) is an important plant pathogen, capable of attacking many crop plants. The fungus was first reported by Rolfs (1892) as a causal agent of tomato blight in Florida. Later, Saccardo (1911) named the fungus as *S. rolfsii*. The fungus was placed in the form genus *Sclerotium* by Saccardo (1913) since it produced differentiated sclerotia and sterile mycelia. In India, Shaw and Ajrekar (1915) isolated this organism from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. However, later studies revealed that the fungus involved was *S.rolfsii* (Ramakrishnan, 1930). Its perfect stage was first studied by Curzi (1931) and proposed generic name as *Corticium*. In the same year, Butler and Bisby reported *S. rolfsii* infection of *Amorphophallus companulatus* from Bengal and Bombay. Uppal *et al.* (1935) also reported *S. rolfsii* attack on *Amorphophallus companulatus* and a number of hosts from Bombay.

Chowdhury (1943) reported infection of *S. rolfsii* on *Piper nigrum* from Jaintpur, Assam. *S. rolfsii* infection was reported on *Lycopersicon esculentum* from Bombay (Anon., 1950). Mehrotra (1952) reported *S.rolfsii* infection on *Zingiber officinale* from Allahabad, U. P. Nambiar (1960) observed *S. rolfsii* infection on the collar region of chilli plants with whitish weft of mycelium and brown sclerotia. Jain (1962) observed seedling blight of apple caused by *S. rolfsii* and was the first report from Himachal Pradesh. They noticed wilt and death of apple seedlings. In 1965, Singh and Pavgi reported leaf rot on a number of cultivated ornamental plants growing in the fields and waste places. In the same year, Vir and Sharma reported *S.rolfsii* infection on mango from Delhi.

Rao (1966) observed *S. rolfsii* attack on *Lycopersicon esculentum* from Poona, Maharashtra. A severe wilt of *Alternanthera* sp. was noticed by Lim (1968). He observed large number of sclerotia and profuse, white mycelia around the stem, collar region and in the soil around the infected plants and also he identified the fungus responsible for sclerotial wilt of *Alternanthera* as *S. rolfsii* Sacc. The brinjal collar rot caused by *S. rolfsii* was first reported in India by Rao (1969) from Vijayawada district of Andhra Pradesh. Roy (1972) recorded *S. rolfsii* infection on *Chrysanthemum* sp. from Jorhat, Assam. Haware and Joshi (1973) reported *S. rolfsii* infection on stored rhizome of *Zingiber officinale* Rosc. from Madhya Pradesh. Prakash and Singh (1976) first recorded the basal rot of mango seedlings caused by *S. rolfsii* from Lucknow , U.P, India and they observed a severe and wide spread rotting of mango seedlings of the cultivar 'Dushehari' in the nursery of the central mango research station, Lucknow. Tyagi and Sharma (1977) recorded *S. rolfsii* attack on tuber of *Amorphophallus companulatus* Blume from Kota, Rajasthan. In the same year, Rath and Mohanan reported blossom blight of mango caused by *S.rolfsii* from Orissa. Sharma and Joshi (1977) also reported *S. rolfsii* infection on stored and freshly harvested rhizome of ginger from Jabalbur,

Madhyapradesh. Sridhar (1978) reported *S. rolfsii* infection on fruits of *Mangifera indica* Linn. from Banglore, Karnataka, India. In the same year, Mandal and Dasgupta recorded *S. rolfsii* infection on *Amorphophallus* sp. from West Bengal. Bisht (1982) observed *S. rolfsii* infects the potato plants at collar region which resulted in wilting and drying of the plants. He noticed brown sclerotia in the root and collar region of the infected plants and also noticed the presence of fused sclerotia with irregular shape. Kapoor (1988) investigated tomato wilt in different agro-climatic regions of India and he identified *S.rolfsii* (*Corticium rolfsii*) as one of the pathogens. Mishra *et al.* (1989) reported *S.* (*Corticium*) *rolfsii* as the causal organism of rotting of yams in Orissa. Mohan and Lakshmanan (1989) noticed a new pseudostem rot incited by *C. rolfsii* on 3-5 months old banana (*Musa* sp.) cv. 'Robusta' in the experimental plots at the university farm, Coimbatore. This was the first report of pseudostem rot on banana caused by *S. rolfsii* from India. Cherian *et al.* (1994) first reported the collar rot of *Murraya koenigii* L. by *C. rolfsii* from COH, Vellanikkara, Thrissur. In the same year, Osai and Ikotun also investigated microbial rot of yams and identified one of the pathogens involved was *S.* (*Corticium*) *rolfsii*. Vilasini *et al.* (1995) observed a new leaf blight and sheath blight disease on canna (*Canna indica*) grown in the homestead gardens of Thrissur District, Kerala. They identified the causal organism as *S. rolfsii* associated with disease. Hernandez and Aleman (1996) reported the foot rot and blight caused by *S.* (*Corticium*) *rolfsii* Sacc. in sunflower. In 1999, Arjunan *et al.* first reported sclerotial wilt of tuberose caused by *S. rolfsii* in India. Achbani and de Labrouhe (2000) reported collar rot on sunflower caused by *S. rolfsii* in N-W Morocco. In the same year, Naidu reported *S.rolfsii* infection on *Crossandra infundibuliformis*. He also noticed that *S.rolfsii* was pathogenic to tomato and chillies. Rivera *et al.* (2001) first reported the gladiolus corm rot caused by *S. rolfsii* in Argentina. In the same year, Tiwari first reported foliage blight in water chestnut caused by *S. rolfsii* from India. Curtis *et al.* (2001) also reported southern blight

caused by *S. rolfsii* (*C. rolfsii*) in field grown tomatoes in Southern Italy. Duff and Daly (2002) reported stem rot of orchids caused by *S. rolfsii* (*Athelia rolfsii*) from Northern Territory.

Pratt *et al.* (2003) first reported *S. rolfsii* (*Corticium rolfsii*) infection on Brassidium hybrid orchid. In the same year, Girija and Umamaheswaran first recorded the incidence of *S. rolfsii* on balsam plant from Kerala. Polizzi *et al.* (2003) observed blight of *Laurustinus* sp. caused by *S. rolfsii* from Eastern Sicily (Italy). Bag (2004) recorded a severe and fatal rotting disease on the orchid species (*Phaius flavus* and *Paphiopedilum venustum*) caused by *S. rolfsii* from Sikkim. Jeeva *et al.* (2005) reported the leaf spot disease of *Dioscorea alata* caused by *S.rolfsii* from Kerala. In the same year, Yadav also first reported a fruit rot disease of jack caused by *S. rolfsii* from India. According to Rajalakshmi *et al.* (2006), 30 per cent crop loss in tomato was due to *S. rolfsii* infection. She also noticed *S. rolfsii* occurrence on crossandra in the farmer's field at the Chittoor district of Andra Pradesh, India. Ashoka and Hegde (2006) reported *S. rolfsii* infection on *Vanilla planifolia* in Karnataka, India. Mesquita *et al.* (2007) observed a severe basal rot on arum lily (*Zantadeschia aethiopica*) caused by *S. rolfsii* in Brazil. Banyal *et al.* (2008) recorded the attack of tomato by a large number of fungal and bacterial pathogens. Of these, *S. rolfsii* Sacc. was the most dreaded one causing serious losses to crops and the incidence of collar rot ranged between 10.2 to 45.1 per cent. Gupta *et al.* (2008) recorded more than 50 per cent of the tulsi plants (*Ocimum sanctum*) in India were found affected by *S. rolfsii*. In the same year, Prakash *et al.* first reported *S. rolfsii* infection on begonia from Kerala, India. Sagar *et al.* (2008) also first reported the basal stem rot and bulb rot on lily (*Lilium* sp.) plants from India. Sharma and Sharma (2008) reported *S. rolfsii* infection on carnations. Tiwari and Sikarwar (2008) also observed that *S. rolfsii* caused rot of all plant parts including foliage, petiole, roots, fruit shoots and fruits

of chestnut. Occurrence of a new disease on Kadali variety of banana was reported from Thrissur district (KAU, 2008). A severe rotting of pseudostem of banana was observed and the symptoms were appeared as reddish coloured rotting on the pseudostem with numerous sclerotial formation. Pathogen was isolated and the pathogen was idenfied as *S.rolfsii*. Cating *et al.* (2009) first reported *S. rolfsii* affecting *Ascocendra* orchids. Wolcan and Grego (2009) recorded collar dry rot on busy lizzie (*Impatiens walleriana*) caused by *S. rolfsii* from Argentina. Thangavelu and Mustaffa (2010) reported a new wilt disease in cv. Rasthali variety of banana caused by *S. rolfsii* in the Thanjavoor district in Tamil Nadu, India.

2.2. SYMPTOMATOLOGY

In 1965, Singh and Pavgi observed chlorotic transluscent spot on a leaf of cultivated ornamental plants caused by *S. rolfsii* which became necrotic at its center and expanded to 5-10 mm in diameter. Later concentric zones invariably appeared on the necrotic tissues and occasionally showed a shot hole. They also reported that in few cases, root rot and stem rot with abundant sclerotia on the rotted stem. They noticed the initiation of spotted leaf rot with a single tiny sclerotium. Lim (1968) also observed large number of sclerotia and profuse white mycelia of *S.rolfsii* around the stem, collar region and in the soil around the infected *Alternanthera* plants. He also noticed that the young sclerotia were white and the older ones brown to dark brown in colour on the diseased plants. Prakash and Singh (1976) noticed the presence of a thick white weft of fungal mycelium with round sclerotia on the base of mango seedlings. Later a blackish brown spot developed around the growing mycelium which gradually girdles the stem partially or completely. At this stage, the leaves started dropping from the upper position and ultimately the seedlings collapsed and died. They also observed a vigorous growth of mycelium and sclerotia in the root zone of the infected tissues.

According to Mohan and Lakshmanan (1989), initially the pathogen, *S. rolfsii* produced a water soaked lesion at the base of pseudostem of banana, 5 - 7.5 cm above soil level. Later, these lesion developed into a necrotic zone with an yellowish white center and dark brown margin and at the plant base, brown ellipsoidal sclerotia along with white mycelium were found. In severe cases, the affected pseudostem rotted. The plants showed yellowing followed by wilting and drying resulting ultimately in death. They also observed the teleomorphic stage of the fungus (*Corticium rolfsii*) inside the pseudostem and sheath. Cherian *et al.* (1994) observed that the symptoms initiated at the collar region of curry leaf (*Murraya koenigii*) plants caused by *S. rolfsii* as a water soaked lesion. Later the infected region turned dark brown in colour and finally resulted in rotting. Under humid conditions, the infected area gets covered with white cottony growth of the fungal mycelium and small dark brown spherical sclerotia. In advanced stage, the whole plant defoliated and led to the death of the plant. Vilasini *et al.* (1995) explained that the symptoms of leaf and sheath blight of canna caused by *S.rolfsii* first appeared as brown coloured spots on the leaf and sheath measuring 0.3 to 0.5 cm. Later, the spots coalesced and resulted in blighting of the leaf sheath and leaves. In advanced cases, the lesions on the leaf sheath spread to other parts of the plant. According to Cherian *et al.* (1996), the leaf spot diseases caused by *S. rolfsii* in young plants of kodampuli (*Garcinia compogea*) initiated as small reddish brown spots on older leaves which enlarged to about 0.5 to 1.0 cm in size and became circular to irregular in shape. Later, the centrally grey coloured necrotic portion dried out leaving a shot hole. In severe condition, the infected leaves got blighted and dried.

Mc Grovern *et al.* (2000) observed the symptoms of stem blight in lisianthus (*Eustomia grandiflorum*) caused by *S. rolfsii* as stem necrosis at the soil line. Later yellowing and tan discolouration of leaves was noticed.

Ultimately infected plants wilted and died. In the same year, Holocomb observed white mycelial growth and small light brown sclerotia of *S.rolfsii* at the base of infected Madagascar periwinkle (*Catharanthus roseus* L.) plants which resulted in wilt, blight and stem necrosis. Tiwari (2001) recorded the symptoms of foliage blight in water chest nut caused by *S.rolfsii*. On the foliage, the symptoms appeared as dirty white to ashy brown circular to irregular spots from the margin of leaves which later covered total leaf area and expanded up to petiole and resulted in rotting of foliage within four days. White fluffy mycelial growth of *S. rolfsii* along with scattered brown round sclerotia were observed on the affected areas. According to Chand *et al.* (2003) the symptoms of *S.rolfsii* infection on lotus, water chest nut and chick weed initiated from the margin of leaves as rotting which later turned brown to black leaves. White mycelial strands of *S. rolfsii* were seen on the abaxial surface of leaves of all the hosts. They also noticed that the pathogen colonized on fruits and made small cavities which resulted in fruit rot. Girija and Umamaheswaran (2003) described the symptoms produced by *S. rolfsii* on balsam plant. They observed that water soaked lesions developed on the base of stem in contact with soil which later extended and caused total collapse of the base of the plant. White fan shaped mycelial growth of the pathogen and numerous small, brown, round mustard seed like sclerotia were also noticed at the base of the plant. The foliage turned yellow, premature leaf fall and root rot were noticed during advanced stage of infection. In the same year, Swart *et al.* first reported the wilting of kenaf plants caused by *S. rolfsii*. They also noticed that the stem at ground level had sunken tan lesion with white mycelial strands and small dark brown (1-2 mm diameter) sclerotia developed on the base of the stem. Bag (2004) explained the symptoms produced by *S. rolfsii* on orchid species (*Phaius flavus* and *Paphiopedilum venustum*).He noticed the symptoms on *Phaius flavus* initially as basal rot of pseudo bulbs with leaves became yellow and detached from the pseudo bulbs. Later the rot advanced to the death of

entire plant. He reported that the infection appeared as soft rot at the collar which later resulted in collapse of plant. He also noticed numerous small brown spherical sclerotia (0.5 -1.9 mm in diameter) with white mycelial growth on the pseudo bulbs, leaf base along the leaf sheath on *Paphiopedilum venustum*. In the same year, Koike explained the symptoms of southern blight of Jerusalem artichoke produced by *S. rolfsii* as wilting of new shoots and leaves followed by browning and collapse of all foliage. He noticed that the crown and lower stem tissues were colonized internally and externally with white, cottony mycelium with tan, spherical sclerotia on the infected areas.

In the study of crown rot of areca palm, Koike (2005) described the symptoms produced by *S. rolfsii* as light brown discolouration of stems near the soil line which later extended up the stem and down into the crown. Later, the foliage became grey green and the entire plant dried up which resulted in the death of the whole plant. He also observed extensive white, cottony mycelium and numerous tan spherical sclerotia were developed externally on the lower part of the stem, crown, palm seed and surrounding peat moss medium. In the same year, Khosla and Gupta observed the symptoms produced by *S. rolfsii* on Chinese gooseberry (*Actinidia deliciosa*). They reported that the symptoms started with water soaked lesions appeared on the base of the stem in contact with the soil. White fan shaped mycelial strands developed which later turned to brown and hard sclerotia of the size of mustard seed (0.5 -2 mm) in diameter. In advanced stage of infection, the lower most leaf of the plant drooped down first and later whole plant gave sickly and wilting appearance which led to the death of the plant. Yadav (2005) explained the symptoms produced by *S. rolfsii* on the surface of jack fruit as white mycelial growth of the pathogen with numerous minute whitish to brown mustard seed like sclerotial bodies. In advanced cases, softening of the surface tissues and

entire fruit was observed. He also noticed from the rotted fruits a fermented odour and dropping off of severely affected fruits.

Gutierrez and Cundom (2006) described the symptoms produced by *S. rolfsii* on *Chlorophytum comosum* plants as leaf and stem necrosis at the soil level with darkening and tissue disintegration advanced to apex which resulted in defoliation and death. They also found white mycelia and small sclerotia on the base of infected plants. In the study of basal rot of arum lily, Mesquita *et al.* (2007) described the symptoms as yellowing and drooping of leaves with wilting of plants. Thin white cottony mycelial growth with small brown, spherical sclerotia were also noticed on the infected collar region. Gupta *et al.* (2008) reported that the symptoms of *S.rolfsii* infection appeared as faint chlorotic lesions near the collar region which spreading downward to the root and partly to lower leaves. Later leaf petioles dropped, causing wilting and yellowing of lower leaves and infected stem tissues turned light to dark brown resulted in the collapse of entire plant. White thread like mycelium covered the soil surface. Many small mustard seed like spherical sclerotia developed on the soil and affected plant parts. Ultimately the affected plants died. In the same year, Prakash *et al.* also observed symptoms on begonia stems as water soaked areas near the soil line with white mycelial growth appeared in a fan like pattern at the collar region. The sclerotia are initially white turned dark brown when matured and were of the size of a mustard seed developed on collar region and finally leaf collapsed. In the same year, Tiwari and Sikarwar also observed rot of all plant parts including foliage, petiole, roots, fruit shoots and fruits of chestnut caused by *S. roifsii*. The symptoms observed on foliage as dirty white to ashy brown circular to irregular spots from the margin of the leaves. Later, these spots covered total leaf area and extended up to petiole resulted in complete foliage rot. They also noticed that affected area was covered by the white fluffy mycelia of the pathogen along with scattered, brown and round sclerotia. On the petiole,

white mycelial growth of the pathogen as well as sclerotia developed, which resulted in the softening of the petiole and finally disintegration of tissues. Symptoms on fruit shoot appeared as water soaked area on shoots which turned ashy- black and later these were covered by white mycelium and sclerotia of the fungus. Similar type of symptoms also appeared on fruits and roots.

In the study of southern blight of vanda orchids, Cating *et al.* (2009) described the symptoms produced by *S. rolfsii*. They reported that the symptoms started with tan, soft water soaked lesions on the base of the plants. As the lesions progressed, leaves around the base of the plants fell down and white mycelium covered the stem with numerous tan to brown sclerotia also developed on stem. Symptoms developed on busy lizzie (*Impatiens walleriana*) plants due to the infection of *S. rolfsii* were described by Wolcan and Grego in the same year. They reported that the infection appeared as dry rot at the collar region which enlarged to necrosis of the basal stem and finally the affected plants died. They also noticed dropping of leaves along with white and fluffy mycelial growth on the base of stem and numerous small brown spherical sclerotia on the soil surrounding the plants. Gupta and Gharde (2009) explained the symptoms of *S. rolfsii* infection on *Solidago virgaurea* plants as yellowing and wilting of leaves with white fluffy thread like mycelium and numerous small mustard seed like spherical sclerotia distributed on the collar region. Thangavelu and Mustaffa (2010) observed *S. rolfsii* infection on banana plants as yellowing of leaves from base to apex and pseudostem sheath rot covered with profuse mycelial growth of the pathogen. They also noticed that the colour of the rotted portion was yellowish red to reddish-brown with numerous brown sclerotia developed on pseudostem. Splitting of the basal portion or the pseudostem sheath was also noticed. The inner tissues of the corm were spongy which colonised with white mycelium. They also observed the symptoms of *S. rolfsii* in all stages of plant growth.

2.3. ISOLATION OF PATHOGEN

In India, Shaw and Ajrekar (1915) isolated *S. rolfsii* from rotted potatoes on Potato Dextrose Agar medium. In 1960, Nambiar used about six solid media for the isolation *S. rolfsii* from chilli plants and he reported oats agar as the best solid medium and Richards' solution as the best liquid medium for the isolation and culturing of the pathogen. Prakash and Singh (1976) isolated *S. rolfsii* from the basal portions of the mango seedlings on PDA medium and they maintained the pure cultures by a single sclerotium isolation on PDA medium. Similarly, Sulladmath *et al.* (1977) reported that out of the six media used, PDA and Malt extract agar were found supporting very good growth of *S. rolfsii* while the growth was least in Czapek's agar. In the same year, Lingaraju also reported potato dextrose broth as the best medium for getting maximum growth of *S. rolfsii* isolated from sunflower.

Mohanan and Lakshmanan (1989) isolated *S. rolfsii* from rotted pseudostem of Robusta banana and plated on PDA and they observed the fungus growth in a fan shaped manner and profuse development of sclerotia after 15 days at 25^0C. Cherian *et al.* (1994) isolated *S. rolfsii* from rotted collar region of *Murraya koenigii* plants on PDA medium. Vilasini *et al.* (1995) also isolated *S. rolfsii* from the blighted portions of canna on PDA medium. In the next year, Cherian *et al.* also isolated *S. rolfsii* from leaf spot infected tissues of kudampuli (*Garcinia compogea* Desr.) on PDA medium.

Cilliers *et al.* (2000) isolated *S. rolfsii* from various host plants showing the infection by transferring 1-3 sclerotia from each diseased plant to agar dishes. They sub cultured the isolates by hyphal tip method and maintained the permanent cultures on PDA plates. In the same year,

Mc Grovern *et al.* used Acidified Potato Dextrose Agar (APDA) medium for the isolation of *S. rolfsii* Sacc. from symptomatic stem sections of lisianthus (*Eustoma grandiflorum*) plants after surface sterilised with 0.5 per cent sodium hypochlorite whereas Holocomb (2000) used acidified water agar medium for the isolation of *S. rolfsii* from infected *Catharanthus roseus* plants and maintained the culture on Acidified Potato Dextrose Agar (APDA) medium.

Girija and Umamaheswaran (2003) isolated the *S. rolfsii* from the rotted basal stem of balsam plants on PDA and maintained the culture on PDA slants. In the same year, Hussain *et al.* used five solid media for the isolation of *S. rolfsii* and they recorded that corn meal agar and chickpea seed meal extract agar media were the best for the radial growth of the fungus. Polizzi *et al.* (2003) also isolated *S. rolfsii* from blight portions of laurustinus (*Viburnum tinus* L.) on acidified PDA by placing symptomatic tissues that were surface disinfested with 1.2 per cent sodium hypochlorite for one minute and rinsed in sterile water. Sarma *et al.* (2003) also isolated 12 isolates of *S. rolfsii* from seven hosts by single sclerotium isolation and the cultures were purified by growing single sclerotium from the cultures and maintained on PDA slants. They reported that sexual stage of the pathogen was observed in four isolates of *S. rolfsii* obtained from different hosts including *Amorphophallus companulatus* on CRMA medium.

Bag (2004) isolated *S. rolfsii* from two orchids *viz., Phaius flavus* and *Paphiopedilum venustum* on PDA medium. In the same year, Sonali and Gupta also isolated *S. rolfsii* from infected apple seedlings on PDA medium supplemented with streptomycin. Bhatia *et al.* (2005) isolated *S.rolfsii* from sunflower showing root or collar rot on PDA and pure culture of *S. rolfsii* was maintained on PDA medium. Khosla and Gupta (2005) isolated *S. rolfsii* from wilted Chinese gooseberry (*Actinidia deliciosa*) nursery plants with crown and root rot symptoms on PDA

medium. In the same year, Yadav also isolated *S. rolfsii* from rotted fruits of jack on PDA medium. Garibaldi *et al.* (2005) isolated *S. rolfsii* from southern blight infected *Dichondra repens* plants by disinfesting the diseased tissue in one percent sodium hypochlorite for one minute and plated on PDA medium amended with 100 mg/litre of streptomycin sulphate.

Gutierrez and Cundom (2006) isolated *S. rolfsii* from southern wilt incited *Chlorophytum comosum* plants on Potato Glucose Agar medium (PGA). In the same year, Rajalakshmi *et al.* isolated *S. rolfsii* from the infected plants of crossandra, ground nut and tomato by tissue segment method on PDA medium. The cultures were purified by hyphal tip method and maintained on PDA and they recorded that PDA has been supported maximum growth and sclerotial population in ground nut isolate of *S.rolfsii* whereas tomato isolate recorded excellent sclerotial population. Mesquita *et al.* (2007) used V_8 juice agar medium for the culturing of *S.rolfsii* from basal rot infected arum lily plants. Ohto *et al.* (2007) isolated *S. rolfsii* from damping off and root rot infected *Calophyllum brasiliensis* plants on PDA medium. Pane *et al.* (2007) isolated *S. rolfsii* from blight incited english ivy (*Hedera helix*) plants by disinfesting symptomatic basal stem tissues in one per cent sodium hypochlorite and plating on PDA medium amended with 100 mg/ litre of streptomycin sulphate.

Prasad and Naik (2008) cultured *S. rolfsii* on V_8 agar medium and also reported that the best media for colony growth of pathogen was V_8 agar medium. Cating *et al.* (2009) used acidified PDA medium for the culturing of *S. rolfsii* from southern blight infected. In the same year, Wolcan and Grego isolated *S. rolfsii* from collar dry rot infected *Impatiens walleriana* by isolating basal stem and sclerotia on PDA. In the same year, Gupta and Gharde also isolated *S. rolfsii* from infected *Solidago virgaurea* plants on PDA medium.

Bagwan (2010) cultured *S. rolfsii* on PDA medium from infected sunflower plants and rhizosphere soil samples. In the same year, Thangavelu and Mustaffa also isolated *S. rolfsii* from rotted corm of banana and cultured on PDA medium. Sennoi *et al.* (2010) used PDA medium for the culturing of *S. rolfsii* from Jerusalem artichoke (*Helianthus tuberosum*) plants showing typical symptoms of stem rot.

2.4. PHENOTYPIC CHARACTERS OF THE PATHOGEN

The phenotypic characters of the pathogen include colony characters, growth rate, production of sclerotia and its morphological characters.

Palo (1933) reported the sclerotial character as diameter of sclerotia of isolate of *S. rolfsii* from mango ranged from 2 to 5 mm. Singh and Srivastava (1953) recorded the sclerotial diameter of isolates of *S.rolfsii* from tomato was 2.5 mm. Townsend and Willetts (1954) observed isolate of *S. rolfsii* from pea nut when grown on agar medium, formed a submerged vegetative and aerial mycelium consisting of single hyphae and other aerial mycelium composing of both single hyphae and hyphal strands. They observed the sclerotial initials on these aerial hyphal strands and never in submerged mycelium. In the same year, Foucart noticed the sclerotial character like diameter of sclerotia of isolate of *S. rolfsii* from chrysanthemum ranged from 0.25 to 2 mm. West (1960) observed *Corticium* sp. having an areolate hymenium, short and stout hyphae, right angled branching of the mycelium and stout basidia have been segregated in the genus *Pellicularia*. In the same year, Nambiar noticed that mycelium of *S. rolfsii* from chilli plants was silky white, fluffy and aerial on media which were seldom growing singly but always appeared in groups of several branches giving out a fan like mycelial growth on the

media. He also observed clamp connections in mycelia. Small, white sclerotial initials with watery exudates were appeared mostly at the periphery of the culture. Sclerotia turn brown, indicating maturation and they are spherical, sometimes irregular with 0.81 to 1.13 mm in diameter on oats agar medium. The sclerotia were formed singly and sometimes two or more sclerotia coalesced to form bigger ones.

Boyle (1961) reported the two distinct phases of *S. rolfsii* isolated from peanut. First, there was mycelium with heavy white growth from which the fungus got the common name 'white mold' which was referred as the growth phase/pathogenic phase of the fungus and the less spectacular production of sclerotia which enabled the organism to survive adverse periods. Henis *et al.* (1965) noticed that sclerotium formation could be induced by cutting the mycelium with a cork borer, so the induction of sclerotia can also from mechanical damage. In the same year, Wheeler and Waller observed the sclerotium formed on agar plates when the mycelium reached the edge of the Petri dish. In the next year, Aycock reported that in *S. rolfsii* at least two types of hyphae are produced in coarse, straight and large cells measuring about 2-9 µm × 150-200 µm and have two clamp connections at each septa, but also exhibit branching in place of one of the clamps. Branching is common in the slender hyphae (1.5-2.5 µm in diameter) which tend to grow irregularly and lack clamp connections. Subramanian (1971) found mycelium of *S. rolfsii* as very floccose, not ropy, producing numerous sclerotia which are pinkish buff to olive brown to clove brown in colour, globose and 0.8 to 2.5 mm in diameter. Sclerotia are formed as elongate or globose, swollen or flattened often covering wide surfaces usually dark coloured, black, hard when dry, usually bright coloured internally.

Amma (1973) observed the mycelium of the *S. rolfsii* isolated from ground nut in culture was silky or cottony white, much branched and

spreading out in strands, producing fan like appearance. The mycelium sometime became aerial, fluffy and dense. The hyphae were septate and produced with clamp connections at frequent intervals. She observed the development of sclerotia within ten days and the sclerotia were spherical or irregular in shape, tannish red in colour and usually two or more sclerotia united to form a bigger one. She also noticed large drops of liquid on the surface of the sclerotia before their maturation and the size of the sclerotia varied from 0.5 to 3.00 mm in diameter. In the same year, Dhingra and Sinclair recorded variation in size of sclerotia among different isolates of *Macrophomina phaseolina* based on sclerotial diameter. In the next year, Kim grouped the isolates of *S. rolfsii* from *Mangolia kabus* DC. on the basis of difference in morphology, mode of growth in culture and pathogenicity. Mordue (1974) studied the cultural and morphological characters of *S. rolfsii* on PDA medium. He observed white coloured colony with many narrow mycelia strands in the aerial mycelium. Sclerotia were developed on colony surface, which were spherical with 1-2 mm diameter when fresh and shrinking of sclerotia when dry with smooth or shallow pitted shiny surface. The cross section of sclerotia showed sharply differentiated rind with evenly thickened strongly pigmented walls, cortex with faintly pigmented walls and medullae with colourless unevenly thickened walls, cortex and medulla contains vesicles of reserve materials.

Prakash and Singh (1976) recorded white, septate, branched mycelium of isolate of *S. rolfsii* from mango on PDA medium which covered the Petri dishes within 5 to 6 days and ultimately changed to mustard seed like dark brown sclerotia which turned chocolate brown at maturity and measured 1.0 to 2.6 mm in diameter. Backman and Kabana (1976) studied the growth of isolate of *S. rolfsii* from contaminated field and also from organic matter on solid media by measuring colony diameter and also on liquid media for growth characters during incubation.

In the next year, Sulladmath *et al.* reported significant variation in test weight of sclerotia of *S.rolfsii*. Manjappa (1979) found eight different isolates of S. *rolfsii* from sunflower showed marked differences in their rate of growth and also the time taken for sclerotial initiation on solid media. He observed isolate from ground nut took maximum time (6 DAI) for sclerotial initiation while isolate from niger took less time (4 DAI) on PDA. In the next year, Christias noticed that during the process of sclerotial development, numerous small droplets like exudates appeared on the sclerotial surface and later these droplets increased in size and often coalesced. But he observed these droplets only in the culture conditions and not under field.

Hadar *et al.* (1981) recorded induction of sclerotial production by transition from submerged mycelium to aerial growth of mycelium. They concluded that the removal of aerial mycelium of *S. rolfsii* have the potential for production of sclerotia long before mycelia growth had reached the edge of the Petri dish. Punja and Grogan (1983) reported that field isolates of *S. rolfsii* when grown on PDA medium varied in growth rate, number of sclerotia produced per plate and size. They observed that the S_1 strains varied morphologically and many isolates produced few sclerotia and 28 out of 100 isolates were asclerotial and these strains also differed from one another in radial growth rate, mycelia dry weight production, oxalic acid production, colour and arrangement of sclerotia on agar plates. Initially white coloured sclerotia were produced which later on changed into light brown and then dark brown. They also noticed that round shaped sclerotia with diameter ranging from 0.5 to 2.00 mm and number of sclerotia ranged from 91 to 1210 per plate. They also noted that strains with heavy mycelia growth produced more number of sclerotia. In the next year, Punja and Jenkins investigated on the standard liquid basal medium for the growth of *S. rolfsii* which contained Carboxy Methyl Cellulose combined with Dextrose for greater oxalic acid production and

mycelial dry weight. Mohan and Lakshmanan (1989) reported fan shaped growth of isolate of *S .rolfsii* from banana and the profuse growth of mycelium and sclerotial development within 15 days. The sclerotia were chocolate- brown, ellipsoidal and 0.4 to 1.2 mm in size.

Tsuno *et al.* (1990) observed the structure of sclerotia of *S .rolfsii* under SEM and reported that the development of sclerotia was strand type and their germination was myceliogenic. They studied about the internal mass of mature sclerotium and observed that it was differentiated into a thin culticle, a thicker rind consisting of densely arranged hyphae forming pseudoparenchymatous tissue with thick walled cells, and a medulla of loosely arranged ordinary filamentous hyphae forming pseudo parenchymatous tissue. They also noticed the sclerotial germination and found that it might have originated by resumption of hyphal growth from any location on the surface, cuticle, rind, cortex, medulla or inter medullary layer. In the next year, Rawn noticed when an isolate of *S.rolfsii* grown on two different agar media in Petri dishes , it produced sclerotia in different patterns predominantly at the colony centre on one medium but at the dish wall on the others. With the help of short cold treatment of colonies it was proved that the interruption of hyphal growth triggers sclerotial formation. Punja and Rahe (1992) recorded that the isolates of *S.rolfsii* characterised by small tan to dark brown or black spherical sclerotia with internally differentiated rind, cortex and medulla were placed in the form genus *Sclerotium*. Leslie (1993) reported that variation was observed in morphological characteristics of *S. rolfsii* originating from different geographical areas and hosts.

Vilasini *et al.* (1995) recorded that isolates of *S. rolfsii* from canna when grown on PDA medium formed mycelium which is floccose, not ropy which later changed into sclerotia. Sclerotia were pinkish buff to olive - brown to clove - brown, globose and measured 0.8 to 2.5 mm in

diameter. In the next year, Cherian *et al.* noticed that the isolate of *S.rolfsii* from kodampully (*Garcinia compogea*) produced white colony growth with narrow septate mycelial strands on PDA medium. On maturation, dark brown spherical sclerotia of size 1 to 2 mm were observed on colony surface. Hameed *et al.* (1999) determined the effect of six culture media (Richard's agar, chickpea agar, PDA, malt extract agar, Waksman's agar and corn meal agar) for the rapid multiplication of *S.rolfsii*. They observed maximum mycelia growth on corn meal agar medium while Richard's agar showed minimum multiplication of the pathogen. Sarma *et al.* (2002) reported that the isolates of *S. rolfsii* from various hosts and soil samples from diverse geographic regions varied in all of the characters evaluated such as colony morphology, mycelia growth rate, sclerotial production, basidiocarp induction, sclerotial size and colour etc. They also found that most of the isolates produced a very large number of sclerotia (>300 to 500 sclerotia/plate) while others produced fewer (<80 to 200 sclerotia/plate). Similarly variation in the size and colour of sclerotia of different isolates was observed. The average size of sclerotia of most of the isolates varied within 1-1.2 mm in diameter, whereas the largest up to 2.2 mm in diameter were produced by the *Pogostemon cablin* isolate. The colour of sclerotia was mostly dark to reddish brown at maturity with an exception in the soil isolate from Varanasi being very light brown even after maturity. In the same year, Palaiah reported that significant variation in test weight of sclerotia of *S.rolfsii*.

Chand *et al.* (2003) observed that all the isolates of *S. rolfsii* from aquatic plants *viz.*, lotus, water chestnut and duckweed produced white mycelium with different growth pattern. Radiate growth recorded in lotus isolate while in water chestnut and duckweed isolate there was fluffy growth. Brown to black, smooth and scattered sclerotia were observed on PDA medium in all the isolates. Growth of water chestnut and duckweed isolates were faster than that of lotus isolate whereas number of sclerotia

was higher in lotus isolate. They also concluded that analysis of colony character, radial growth, sclerotial colour, shape and numbers of sclerotia/plate showed differences and common features in all the aquatic isolates indicating the pathogen population consists of heterogeneous groups. Girija and Umamaheswaran (2003) reported that *S. rolfsii* isolated from balsam plant produced white, fan shaped mycelail growth that covered the 9 cm diameter Petri dish within four days on PDA and brown coloured mustard seed like sclerotia were formed in 8 days. Jimenez *et al.* (2004) recorded that variation in mycelial growth and sclerotia production of isolates of *S. rolfsii* from different regions in Mexico. It was determined on five different media *viz.*, PDA, V_8 juice agar, Malt Extract Agar (MEA), Onion Extract Agar (OEA) and Garlic Extract Agar (GEA) and observed that most isolates had their greatest growth rate in V_8 juice agar medium while sclerotia production was better in GEA and MEA. The size and weight of sclerotia were greater in GEA. MEA was the best medium to differentiate the morphological variations but it had the lowest mycelia growth rate.

Jeeva *et al.* (2005) recorded measurement of hyphae of *S. rolfsii* isolated from *Dioscorea alata* on PDA and it ranged from 1.5- 2.7µm. They also observed numerous round to ellipsoidal, dark brown to black, smooth sclerotia in the culture. In the same year, Pandey *et al.* observed that colonies of *S. rolfsii* causing spotted leaf rot in various hosts were slow growing with profuse, fluffy mycelium on *Cyperus rotundus* Rhizome Meal Agar (CRMA) medium while white, smooth, fast growing colonies were observed in PDA medium. The number of sclerotia produced on CRMA medium was less than PDA medium. They also observed wide variations in colony characters, growth and sclerotium formation in leaf spot causing isolates of *S.rolfsii* on CRMA medium. In the same year, Prabhu and Patil investigated morphological studies of 12 isolates of *S. rolfsii* from soya bean on PDA medium and revealed

significant difference with respect to colour, shape, diameter and test weight of sclerotia. They noticed sub spherical shape of sclerotia in three isolates whereas spherical shape in the remaining isolates. The isolates showed variation in colour of sclerotia as light brown, chocolate brown, dark brown and brown. They also noticed that difference in size of sclerotia ranged from 1.13 to 2.4 mm. Based on the mean diameter of sclerotia, they classified the isolates into three groups. Group I ranged from 2.2 to2.4 mm, group II ranged from 1.58 to 1.83 mm and group III ranged from 1.13 to 1.4 mm. They also noticed variations in the test weight of the sclerotium which were in the range of 69.2 to 147.5 mg.

Palaiah and Adiver (2006) observed isolate of *S. rolfsii* collected from ground nut plants from 12 different locations had significant difference with respect to growth and morphological characters of sclerotia *viz.*, shape, colour, size and test weight. The size of sclerotia ranged from 1.08 to 2.23 mm and test weight of sclerotia ranged from 91.80 to 125.20 mg. In the same year, Rajalakshmi *et al.* reported that isolate of *S.rolfsii* from crossandra, tomato and ground nut produced sclerotial population per plate was rated poor (1-100), fair (101-250), good (251-500) and excellent (>500). Among the solid media tested, they observed PDA as a good medium for maximum growth of *S. rolfsii.* Kokub *et al.* (2007) noticed that mycelial growth rate of different strains of *S. rolfsii* from chick pea plants varied considerably up to three days. Mycelia of most strains showed white coloured growth along the surface of the medium that was lying to the base while a few strains showed fluffy appearance. The formation of sclerotia started after three days of incubation and continued till seven days. Initially white coloured sclerotia were formed which later changed to off white, light brown and dark brown as they attained maturity. The highest number of scelrotia per plate was 1210 whereas lowest was 150 after seven days. In the same year, Okereke and Wokocha observed that isolates of *S. rolfsii* from cowpea, tomato,

cocoyam and pawpaw produced white cottony mycelia growth with ropy strands and the PDA media were completely covered by the mycelia of the pathogen on 7-8 DAI in all the isolates. They also noticed that the mycelia were silky white at early stage of growth but after ten days of inoculation, the pathogen lost its luster and became dull in appearance. Sclerotia formed on 11 DAI at the edges of the plates. They also recorded that size of sclerotia ranged from 0.5 to 1.2 mm at 14 DAI whereas 0.8 to 1.5 mm and 1.0 to 1.8 mm on 21 and 28 DAI repectively. The numbers of sclerotia ranged from 24-42, 86-136 and 102-178 at 14, 21 and 28 DAI respectively. They also noticed that no sclerotia were produced in all the isolates at seven DAI. Goldring *et al.* (2007) also noticed that sclerotia of isolate of *S. rolfsii* from rye grass (*Lolium perenne*) were nearly spherical, 1 to 2 mm in diameter, white but changed to brown with age and produced in large numbers over the entire colony surface.

Ohto *et al.* (2007) found the isolate of *S. rolfsii* from *Calophyllum brasiliensis* plants produced abundant whitish mycelia growth with clamp connections in each septum of the mycelium in addition to aerial mycelium with a great number of whitish spherical sclerotia. Pane *et al.* (2007) recorded the isolate of *S. rolfsii* from english ivy (*Hedera helix*) plants produced a densely, floccose, white mycelium on PDA. The mycelia were septate with clamp connection at hyphal septa. Numerous small sclerotia developed on the colony surface measuring 0.5 to 1.9 mm in diameter, spherical, slightly ellipsoidal with a smooth surface. The colour of sclerotia was initially white which turned to pinkish buff and then to olive brown and eventually to clove brown when mature. Sclerotia were produced mostly in the center as well as close to the edges of the Petri dishes. Prasad and Naik (2008) studied the morphological characters of mycelium and sclerotia and growth response to different temperature and mycelia of various field isolates of *S. rolfsii* and *S. delphinii* collected from diverse geographical areas and they reported that the best

medium for colony growth was V_8 agar for *S. rolfsii* whereas PDA for *S. delphinii.*

In the next year, Wolcan and Grego noticed the isolate of *S. rolfsii* from busy lizzie (*Impatiens walleriana*) plants produced white, fluffy mycelia growth with numerous small, brown spherical sclerotia measuring 0.5 to 2 mm in diameter. Gupta and Gharde (2009) recorded the isolate of *S. rolfsii* from *Solidago virgaurea* plants produced white, fan shaped, flaccose colony on PDA. They also found sclerotia were distributed around periphery or scattered on the Petri dish and they are initially white in colour later changed to brown measured about 0.6 to 1.2 mm in diameter. Thilagavathi (2009) recorded that the isolates of S. *rolfsii* from sugar beet varied in all of the phenotypic characters *viz.*, mycelia growth rate, colony morphology, sclerotial production, sclerotial numbers, size and colour. She recorded that among the 17 isolates, colonies of five isolates were fluffy whereas 12 were compact. She found that mycelia growth rate of the isolates also varied considerably up to three days ranged from 2- 2.8 cm. Most of the isolates reached complete growth between two to three days and the slow growing isolates took more than three days for their complete growth and she also noted that the number of days taken for the formation of slerotia varied among the isolates ranged from 5-15 days. She found that the production of sclerotia among various isolates also varied and grouped the isolates as large producer of sclerotia(1200-1800), fewer producer of sclerotia (100-250) and medium producer of sclerotia (400-1000) . Similarly the dry weight of 100 sclerotia also varied among the isolates which were ranged from less than 30 to 86 mg. She also grouped the isolates on the basis of variation in the size and colour of sclerotia among the various isolates ranged from 1.0 to 1.6 mm and light brown to dark brown respectively.

Kumar and Prasad (2010) observed isolates of *S. rolfsii* from finger millet (*Eleusine coracana*) when cultured on PDA produced white, fluffy with abundant aerial mycelia with fan shaped growth. The mycelium was very floccose and produced numerous sclerotia which were mustard like white to dark brown globose, tough and hard especially at maturity. In the same year, Thangavelu and Mustaffa observed isolates of *S. rolfsii* from banana produced white mycelial growth within a week after incubation and small, round, dark brown sclerotia were also developed in the culture. They also noticed that profuse growth of compact, septate, hyaline mycelium that later developed into sclerotia and number of sclerotia ranged from 47 to 199 per plate and sclerotial diameter ranged from 0.8 to 1.0 mm. Xu *et al.* (2010) tested the difference in morphological characters among three *Sclerotium* species *viz.*, *S. coffeicola*, *S. rolfsii* and *S. rolfsii* var. delphinii (Welch) isolated from various hosts *viz.*, *Lilium* sp., *Medicago sativa*, *Lens culinaris*, *Arachis hypogaea* and turf grass and they found that there is not much variation in number of sclerotia per Petri dish, shape and size of sclerotia among three *Sclerotium* species and they concluded that these three species are closely related and morphological characters, colour of mycelia, number and size of sclerotia alone were insufficient to delimit the species of *Sclerotium*.

2.5. COMPATIBILITY OF VARIOUS ISOLATES OF *S. rolfsii*

Taubenhaus (1919) first reported the aversion phenomena between different cultures of *S. rolfsii* from different host species representing different countries and he considered it as a sexual response. Nakata (1925) found 37 aversion strains in the 47 culture combinations and also noted that aversion associated with morphological differences and also he concluded that aversion was due to substances diffusing from the mycelium into the medium. Luke and Gayathri (1978) observed the

interaction among six isolates of *S. rolfsii* from hosts *viz.*, sorghum, ragi and brinjal showed aversion or non aversion between the isolates and also noted that there was complete intermingling of hyphae along the interfaces of the two colonies of isolates showing non aversion type of reaction whereas in aversion type of reaction, no intermingling of hyphae was observed. In non aversion type reaction of isolates, there was a clear demarcation line at the meeting point of the hyphae of the two colonies and along this line, sclerotia were formed. In most cases, a single line of demarcation was observed among the isolates.

Kaveriappa (1979) noticed the isolates of *S. rolfsii* from brinjal showed mutual aversion or non aversion as a result of interaction among the isolates. Hyphae bordering the averting colonies also showed a marked deviation in growth at the interphase resulting in a clear demarcation between the colonies. In the non- averting colonies the hyphae merged freely at the inter phase producing a confluent growth and sclerotia in the region. Punja and Grogan (1983) reported that out of 46 isolates of 72 field isolates of *S. rolfsii* from various locations, antagonism zone among 114 colonies was found and heterokaryons had not been formed. They also noted that initially intermingling of the mycelia of two incompatible strains took place but later on there was lysis of mycelia of two strains and the development of a clear zone at the region of interaction. They also found that hyphae of incompatible isolates lysed rapidly after mycelial contact and later on a macroscopic zone of mycelial clearing was developed. Gutierrez and Garcia (1988) recorded combinations of 15 *S.rolfsii* strains from various host species from different locations of Cuba and they noticed that out of the 105 reactions, 31 were compatible, one slightly incompatible, 36 moderately incompatible and 37 were strongly incompatible. Leslie (1993) reported that isolates of *S. rolfsii* from the same Mycelial Compatibility Group (MCG) or Vegetative Compatibility Group (VCG) were presumed to be more genetically similar than isolates

from different groups. Harlton *et al.* (1995) tested all the isolates of *S.rolfsii* and *S. delphinii* from different hosts of various locations and found that most pairings of *S. rolfsii* and *S. delphinii* developed the presence or absence of an aversion zone within 7-10 days. Hyphae of compatible isolates intermingled or produced a knitted ridge and the contact zone of hyphae was difficult to discern and sclerotia formed randomly over the colony surface of both isolates. Incompatible pairings were developed by thinning of hyphae with a distinct barrage or aversion zone of varying width and they identified 49 MCG within *S. rolfsii*. Powell (1995) observed the presence of an antagonistic (barrage or aversion) zone in the region of mycelial contact of isolates of *S. rolfsii*. In the same year, Nalim *et al.* reported that the isolates of *S. rolfsii* from ground nut in Texas were placed in different MCG based on the presence of an antagonistic zone which is the basis of incompatibility between two paired fungal colonies. They observed the mycelia of isolates in the same MCG formed a clear antagonism zone in the area of mycelial contact and sclerotia were formed on either side of this zone for most incompatible pairings. The isolates within the same MCG grew at similar rates and formed sclerotia in distinct patterns like many small sclerotia developed at the edge of the Petri plates whereas a few large sclerotia formed in the center of the Petri plates.

Okabe *et al.* (1998) described that mycelia of paired isolates of *S.rolfsii* from various hosts of diverse geographic origin came into contact with each other in 1 to 2 days after inoculation and hyphae anastomosed and mycelia of both isolates intermingled completely in 3 to 4 days. They also noticed that the hyphal cells in the contact zone died in every combination except self pairings and a barrage zone was formed in 5 to 14 days. Cilliers *et al.* (2000) observed that isolates of *S. rolfsii* from various host plants at different locations in South Africa appeared to group together in the same MCG, but this was not true for isolates from the same

host plant and found that some of MCG's appeared to be less host specific than others. Almeida *et al.* (2001) identified 13 MCG's among 23 *S. rolfsii* isolates collected from different hosts and regions of Brazil. Punja and Sun (2001) noted the development of aversion zone between various isolates of *S. rolfsii* from 36 host species representing 13 different countries on PDA on 14 days after incubation. Initial intermingling of hyphae of incompatible isolates was followed by lysis and a clearing zone developed in the region of interaction. In some cases, sclerotia were produced at the retreating edge of the mycelium. They also observed that isolates within a MCG did not show aversion when paired against all other isolates from that group, but were incompatible with isolates from all other MCG.

Sarma *et al.* (2002) observed the high rate of antagonistic reactions in the mycelial compatibility test showed the extent of the diversity among the isolates of *S.rolfsii* from various hosts and soil samples from diverse geographic origins. All of the four leaf spot causing isolates exhibited mycelial compatibility with each other but antagonistic reactions with collar rot or foot rot causing isolates. In all the antagonistic reactions, sclerotia were not formed at the interaction zone. These observations that distinguish the leaf spot causing isolates from others. Among the 325 pairings of the 26 isolates of *S. rolfsii* and out of all only 29 combinations showed a compatible reaction. Abandonon *et al.* (2005) reported that during the MCG test of 66 isolates of *S. rolfsii* from cowpea, peanut and bambara ground nut, antagonism zones developed between incompatible isolates and mycelium thinned out in the region of interaction. In all selfed pairings, the hyphae fused and no barrage zones developed which were developed between mycelium of isolates that were vegetatively incompatible, whereas compatible isolates had fused mycelia and dense growth associated with abundant sclerotia produced in the contact zone. They grouped the isolates which were vegetatively compatible in the same MCG. They also identified four MCGs from the 66 *S. rolfsii* isolates and

concluded that a low level of phenotypic differentiation existed between the isolates. Shukla and Pandey (2007) studied the mycelial compatibility of 32 isolates of *S. rolfsii* causing collar rot disease from various hosts and soil samples from diverse geographical locations. When paired against each other on PDA medium, they observed that out of 495 combinations, 81 combinations were compatible. Hyphae of compatible isolates intermingled or produced a knitted ridge of mycelia. Isolates that were vegetatively compatible were grouped in the same MCG. In some combinations, the antagonistic zone broadened on prolonged incubation with no sclerotia at lytic zone.

Akram *et al.* (2008) found 50 VCG based on mycelial compatibility among 16 isolates of *Sclerotinia sclerotiorum* from chick pea. They examined the pairings macroscopically after 10 to 15 days for the presence of an antagonistic zone in the region of mycelial contact and found that in all the antagonistic reactions, sclerotia where not formed. Sclerotia were formed only in the border of lytic zone of the two isolates. But later on, few sclerotia were produced on lytic zone in some combinations but failed to develop the size as those produced on the border of such barrage zones. On prolonged incubation, the antagonistic zone in some combinations was broadened at the interaction zone either parallel to both sides traversing to almost two by third of the mycelia growth or in some cases, lysis occurred completely on one isolate only. Prasad and Naik (2008) reported that when field isolates of *S. rolfsii* collected from diverse geographical area of the same MCG were paired, hyphae of the isolates intermingled with little or cell death, whereas isolates from different groups formed antagonistic zones that were accompanied by plasmolytic killing of hyphal cells. They identified 25 MCG's from 366 isolates of *S. rolfsii* from ground nut based on the formation of an antagonistic zone between incompatible mycelia of paired isolates. Shinde and Reddy (2009) noticed that in isolates of *S. rolfsii* from

ground nut, onion and pigeon pea, the existence of strain variation based on both aversion and pathogenicity among the isolates of *S. rolfsii* isolated from different hosts and also from the same host growing in the same locality. Both aversion and non aversion interactions were found among these isolates. There was complete intermingling of hyphae along the interfaces of the two colonies and between two pairings of the isolates which were considered as non aversion type. They also noticed that clear aversion between the hyphae of the two colonies between five isolate pairings. They determined the inter relationship among the isolates of *S.rolfsii* isolated from different host and of the same host based on the aversion reaction.

2.6. PATHOGENIC VARIABILITY

Roy (1977) tested pathogenicity of isolate of *S. rolfsii* from carrot (*Daucus carota*) on pea, cauliflower, cabbage, potato and arum. He observed that infection appeared two days later as light brown lesions on which white radial growth of mycelium was developed. Rotting in all the crops, except arum was more than 50 per cent in four to five days. On arum, infection did not reach up to the core. Thiribhuvanamala *et al.* (1999) recorded the inoculum level required to cause successful *S. rolfsii* (*C. rolfsii*) infection on tomato. They observed that the disease incidence (97.5%) was longest when plants were inoculated 30 DAI with 15 Sclerotia per plant. Sarma and Singh (2002) collected 20 isolates of *S.rolfsii* from various hosts and tested their infection causing ability to 14 plants. They observed variation among isolates in their pathogenic ability. No difference was observed between the collar rot as well as leaf rot causing isolates of *S. rolfsii* in their pathogenicity. They did not observed infection by any isolates on four crop plants in the family Gramineae.

Yaqub and Shahzad (2005) observed the effects of different inoculum densities of the pathogen on root colonization and growth of mung bean and sunflower plants. They also recorded that *S. rolfsii* was highly pathogenic on sunflower, mung bean and sugar beet whereas mild pathogenic on tomato, lentil, sweet pumpkin and cabbage and non pathogenic on cauliflower plants. They concluded that increased inoculum density of *S. rolfsii* caused gradual reduction in growth parameters of sunflower and mung bean plants whereas a positive correlation was observed between root colonization and population of *S. rolfsii* in soil. In the same year, Ellil reported the degree of pathogenicity of four isolates of *Rhizoctonia solani* infecting *Phaseolus vulgaris* seedlings and observed that the dark isolate was the most virulent while albino isolate was the least virulent.

In the year 2007, Basha and Chatterjee and Ghosolia and Shivpuri observed variation in potential of isolates of *Sclerotinia sclerotiorum* causing infection in various hosts. Shukla and Pandey (2008) studied the pathogenic diversity of *S. rolfsii* isolates as biocontrol agent against *Parthenium hysterophorus*. They identified four distinct pathogenicity reactions among the ten isolates tested against *Parthenium* and reported that disease reactions varied with the isolates which ranged from yellowing of entire plant and formation of sclerotia to wilting and finally death of plant. Goswami *et al.* (2008) investigated the pathogenic variability of different isolates of *Sclerotinia sclerotiorum* on pea, brinjal and parthenium. They observed that the isolate from *Brassica rapa* var. toria was highly virulent and isolates from *Brassica napus* and parthenium were the least virulent. They also noted that the inoculation study showed prevalence of pathogenic variability among various isolates of *Sclerotinia sclerotiorum* on different hosts.

2.7. HOST RANGE

S. rolfsii is a devastating soil borne fungal pathogen having a wide host range and worldwide distribution. There are reports from several workers on the host range of *S. rolfsii*. Rolfs (1893) listed a number of plants including weeds and garden plants as host of this pathogen. Taubenhaus (1919) reported *Lycopersicon esculentum, Gossypium hirsutum, Vigna sinensis, Solanum melongena* etc. as affected by *S.rolfsii*. Weber (1931) listed 189 host species from many different plant families covering a fern family, monocot and 42 dicot families. He reported that majority of plant species listed as host were annuals or herbaceous perennials. Gram and Webber (1940) reported that *S. rolfsii* was estimated to attack at least 150 plant species. Nambiar (1960) studied the host range of isolate of *S. rolfsii* from chilli and he also observed that it could able to infect a variety of hosts belonged to the dicotyledons and the monocotyledons. Cross inoculation studies showed that chilli was completely susceptible to infection by the isolate of *S. rolfsii* from *Arachis hypogoea* and highly susceptible to an isolate from *Amorphophallus companulatus*. In the same year, West reported wide host range to the vegetative stage of the fungus *S. rolfsii*. Singh and Pavgi (1965) observed that several hosts of *S. rolfsii* belonged to both monocots and dicots and these include *Jasminum pubescens, Commelina* sp. etc. Aycock (1966) recorded *S. rolfsii* was a devastating soilborne plant pathogenic fungus with a wide host range and caused rot in all hosts. The pathogen *Corticium rolfsii* is principally soil borne in tropical and subtropical regions and has a wide host range (Misra and Khare, 1970). *S. rolfsii* has a wide host range, but it more commonly observed to attack ornamental plants (Punja, 1985). Nnodu and Emehute (1988) observed rhizome and stem rot of ginger (*Zingiber officinale*) caused by *S. rolfsii* as a non specialised soil borne fungal pathogen of worldwide importance and has a wide host range of over 500 species. Mohan and Lakshmanan (1989) reported the soil

inhabitant nature of *S. rolfsii* with a wide host range and they listed out the diseases caused by pathogen as damping off of seedlings, collar rot or stem rot of older palnts, spotted leaf rot or leaf sheath blight.

Hall (1991) reported that *S. rolfsii* infect more than 500 species of monocotyledons and dicotyledonous plants and he observed severe infection on legumes, solanaceous crops, cucurbits and other vegetables grown in rotation with beans. *S. rolfsii* is a cosmopolitan pathogen that has a wide host range and includes many field, vegetable and ornamental crops (Punja and Rahe, 1992). Mishra *et al.* (1994) tested the host specificity of two isolates of *S. rolfsii* from *Parthenium hysterophorus* in pot and field experiments. Out of 73 hosts tested, only eight plants were found susceptible to both isolates. They also reported that the pathogen incited severe infection in some wild plants including *Lantana camera, Clerodendron incerme, Cassia tora* and *Xanthium strumarium.* Wall (2000) listed out some of the common plants infected by *S. rolfsii* which include beans, cucurbits, pepper, tomatoes, yams and various ornamental plants. Girija and Umamaheswaran (2003) observed the wide host range of *S. rolfsii* and they noticed infection on all the ornamental plants and vegetable crops inoculated with the sclerotia of the pathogen. They reported that the succulent stemmed plants like tomato, begonia, marigold, gerbera showed typical basal rot symptom within two to three days whereas the hard stemmed plants like brinjal, bhindi, ixora and anthurium showed delayed lesion development followed by partial yellowing of the foliage and stunted growth. Moctezuma *et al.* (2005) reported that the host plants of *S. rolfsii* include garlic, amaranthus, cauliflower, cabbage, pepper, dahlia, sunflower, tomato, African marigold and chrysanthemum. In the same year, Khosla and Gupta tested the host range of *S. rolfsii* on a number of vegetable plants, fruit crops and ornamental plants. They observed rotting symptom within 4-6 days in all the vegetable plants *viz.,* spinach, brinjal, tomato and chilli while in ornamental plants, marigold

showed basal rot in three days but lilium and gladiolus showed delayed symptoms on seven to nine days.

2.8. ESTIMATION OF IAA AND PHENOLIC COMPOUNDS

The presence of IAA and phenolic compounds in *S. rolfsii* induce or activate the growth and defense mechanism against other microbes during its survival in soil. In the case of *S. rolfsii*, IAA synthesis may have a function in sclerotia formation as well as the infection process.

Bray and Thorpe (1954) estimated that phenols in *S. rolfsii* with Folin-Ciocalteau reagent was based on the reaction between phenols and an oxidizing agent phosphomolybdate which resulted in the formation of a blue colour complex. Mahadevan and Sridhar, 1986; Mahto *et al.,* 1987 also estimated total phenols by using the same method. The role of auxins on fungal metabolism and on the synthesis of auxins by different fungi has been reviewed in detail by Gruen (1959). In the year 1972, Gunasekaran and Weber assayed auxin syntheis of *S. rolfsii* in culture filtrate with tryptophan (0.2 %) and without adding tryptophan. They observed that IAA production was twice the amount in medium supplemented with tryptophan (7.95 mg/ml) as compared to without tryptophan (3.13 mg/ml) but there was no variation in rate of growth in both the media. They also noted that no endogenous auxin was produced in the uninoculated medium incubated under the same conditions. They observed that vegetative growth of *S. rolfsii* was not stimulated by tryptophan. In the absence of tryptophan also *S. rolfsii* produced IAA. They concluded that *S. rolfsii* has all the enzymes for synthesizing tryptophan as well as indole acetic acid.

Hasan (2002) estimated IAA in culture filtrate of *Fusarium oxysporum* collected from melochia, sesame and soyabean plants spectrophotometrically and showed IAA content of isolates of *F.oxysporum* from thee crops recorded 100 µg/ml, 120 µg/ml and 140

µg/ml respectively. He observed maximum quantity of GA and IAA after ten days and declined after 15 days in mycelium whereas in culture filtrate, GA and IAA reached their maximum production after 15 days. In the same year, Saram *et al.* recorded oxalic acid, IAA and cinnamic acids were detected in culture filtrate of all isolates of *S. rolfsii* by using HPLC analysis. They also observed that by HPLC analysis of sclerotial exudates, sclerotia, mycelia and culture filtrate of *S.rolfsii* revealed the IAA, phenolic acids and oxalic acids of all these samples have wide variation in their content among the isolates. Oxalic acid was the major component in culture filtrate of *S. rolfsii* ranged from 0.29 to 10.2 µg/ml followed by IAA measured about 0.09 to 0.52 µg/ml. IAA was detected only in exudates and culture filtrate. They listed the phenolic acids and IAA amount in culture filtrate of different isolates of *S. rolfsii*. The amount of IAA present in culture filtrate of *Amorphophallus companulatus* was 0.13 µg/ml whereas 0.23 µg/ml in culture filtrate of isolate from *Colocasia variegata* and 0.19 µg/ml in culture filtrate of isolate from *Piper betle*. The amount of IAA produced in culture filtrate of isolate from *Mangifera indica* was 0.22 µg/ml whereas in *Lycopersicon esculentum* was 0.31 µg/ml. They also reported ferulic acid, chlorogenic acid and cinnamic acid in culture filtrate of *Amorphophallus companulatus* were 0.007, 0.012 and 0.002 µg/ml whereas in *Piper betle* was 0.005, 0.01 and 0.002 µg/ml, in culture filtrate of *Mangifera indica* were 0.01, 0.002 and 0.001 µg/ml respectively. In the culture filtrate of *Lycopersicon esculentum,* only cinnamic acid was present which measured about 0.01 µg/ml. The presence of relatively higher amounts of individual phenolic acids in sclerotia than in mycelia indicated that sclerotia needed higher amounts of phenolic acids for their survival than mycelia. They also reported that in *S.rolfsii*, phenolic acids protect the fungus from the attack of other soil inhabiting microbes. Ellil (2005) reorded the amount of phenols in culture filtrate of four isolates of *Rhizoctonia solani* grown on different nitrogen sources ranging from 28.8 to 133.5mg/ml. Pandey *et al.* (2005) recorded

HPLC analysis of mycelia mat of *S. rolfsii* obtained from Potato Dextrose broth revealed the presence of 0.14 µg/g chlorogenic acid, 1.01 µg/g ferulic acid and 0.05 µg/g o-coumaric acid after 10 days of inoculation. They noted that the mycelia of *S. rolfsii* contain some phenolic acids which seemed to be necessary for their growth and resistance against adverse conditions of the environment. They found phenolic acids decreased in 20 day old mycelia mat. They concluded that this might be due to conversion of one phenolic acid into another and increase of phenolic acid was also observed in different concentrations after 20 days. Singh *et al.* (2010) recorded by doing HPLC analysis of mycelia of *S.rolfsii* grown on neem cake and *Zephyraenthes citrina* bulb supplemented PDA media showed gallic acid was seen in the control as 18.7 µg but increased several times at five per cent concentration and gradually decreased with an increase in concentration. Cinnamic acid was not detected in control but was noticed at five per cent concentration (1.98 µg) which decreased to 0.25 µg at seven per cent and was completely absent at ten per cent concentration. The phenolic compounds increased when mycelia treated with neemcake and *Zephyraenthes citrina*. They also concluded that the enhancement of mycelia growth in media supplemented with neemcake and *Zephyraenthes citrina* bulb is because of increased synthesis and accumulation of phenolics in mycelia of *S. rolfsii* and this might have a bearing on the prolonged pathogenicity of *S. rolfsii* causing serious diseases in plants.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals' was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the year 2009-2011. The details of the materials used and the techniques adopted for the investigation are described below.

3.1. SURVEY AND COLLECTION OF DISEASED SAMPLES

Purposive sampling surveys were conducted in nurseries and research plots of KAU. The locations surveyed for the collection of diseased specimens are given in Table 1. From these locations specimens of collar rot, stem rot, flower rot, leaf blight and pseudostem infections of selected ornamental plants and fruit crops were collected periodically and brought to laboratory for isolation of the pathogen and the symptomatology of the disease was also recorded.

3.2. ISOLATION AND MAINTENANCE OF PATHOGEN ASSOCIATED WITH THE DISEASED SPECIMENS

The pathogen associated with the leaves, stems and flowers of the selected fruit crops and ornamental plants showing the symptoms was isolated on Potato Dextrose Agar (PDA) medium.

3.2.1. Isolation of *S. rolfsii* from leaves, collar region and flowers of marigold

The infected leaves, flowers and stems collected from different locations were brought to the laboratory and washed under tap water to remove dust particles. Small bits of infected portions along with some healthy portions were

Table 1. Survey for the collection of diseased samples

Sl. No	Locations	Host
1	Central nursery of KAU, Vellanikkara	Mango, Marigold and Chrysanthemum
2	Research plots of COH, Vellanikkara	Marigold and
3	Mango orchard of Agricultural Research Station, Mannuthy	Mango and Chrysanthemum
4	Mango orchard of COH, Vellanikkara	Mango
5	Garden centre, Vellanikkara	Marigold, Chrysanthemum and Mango
6	South Indian Agri farm, Mullakara	Marigold and Mango
7	South Indian Agri farm, Mannuthy	Marigold and Mango
8	Shalimar nursery, Mannuthy	Marigold and Chrysanthemum
9	Sreelakshmi gardens, Mannuthy	Marigold and Chrysanthemum
10	National Rose garden, Mannuthy and Pandiparampu	Marigold, Chrysanthemum and Mango
11	EASF nursery, Panancheri	Marigold, Chrysanthemum and Mango
12	Farmer's field, Mannuthy	Marigold
13	Farmer's field, Koottala	Banana
14	Farmer's field, Vellanikkara	Chrysanthemum
15	Farmer's field, Cherpu	Banana

surface sterilized with one per cent sodium hypochlorite solution, washed in three changes of sterile water and then transferred to sterile Petri dishes containing solidified PDA medium. The dishes were incubated at room temperature (26±2° C) and observed for the growth of pathogen from next day onwards. The isolates obtained from different locations were purified by hyphal tip method. The pure cultures were maintained on PDA slants by periodical sub culturing.

3.2.2. Isolation of *S. rolfsii* from leaves and collar region of chrysanthemum

The pathogen associated with the infected leaves and collar region of chrysanthemum was isolated and maintained the pure cultures on PDA slants by using the same procedure as described in 3.2.1.

3.2.3. Isolation of *S. rolfsii* from mango seedlings

The mango seedlings and grafts showing the symptom at collar region were collected during the survey and brought to the laboratory. The pathogen associated with the disease specimens was isolated and maintained the pure cultures on PDA slants by using the same procedure as described in 3.2.1.

3.2 .4. Isolation of *S. rolfsii* from pseudostem of banana

The infected pseudostem of banana variety Kadali collected from different locations were brought to the laboratory and washed under tap water to remove dust particles. Portions of the pseudostem showing white mycelial strands were cut into small pieces of about 1-1.5 cm along with some healthy portions. The pathogen associated with the diseased specimens was isolated and maintained the pure cultures on PDA slants by using the same procedure as described in 3.2.1.

3.3. PATHOGENICITY TEST

The pathogenicity of different isolates of *S. rolfsii* was studied by artificial inoculation on respective hosts under *in vivo* conditions. For this, tissue culture banana plants, young seedlings and grafts of mango, seedlings of chrysanthemum and marigold were grown and maintained in polythene bags containing potting mixture. Three types of inocula *viz.*, mycelium alone, sclerotium alone and mycelium with sclerotium of different isolates of pathogen were used for the inoculation.

The different parts of the plants were inoculated separately with the above mentioned three different inocula of each isolate with and without pinpricks. Tissue culture plants of banana were inoculated separately on collar region, pseudostem, and leaves. The seedlings and grafts of mango, seedlings of chrysanthemum and marigold were inoculated separately on two middle leaves and also on the collar region. The pathogenicity of isolate obtained from the flowers of marigold was proved by inoculating this isolate on the flowers. Plants inoculated with sterile water served as control. Five replications were maintained for each inoculation. The inoculated plants were kept in humid chamber under room temperature (26±2° C) and observed for the symptom expression. The pathogen was reisolated from the different parts of the inoculated plants showing symptoms and compared with the original culture.

After the pathogenicity test, a total of eleven isolates were selected for further study. The name of isolate, it's host and location from where it collected are given in Table 2.

Table 2. Various isolates of *S. rolfsii* used in the study

Sl. No	Host	Isolates	Locations
1	Marigold	MG-1	Farmer's field, Mannuthy
		MG-2	Research plots of COH, Vellanikkara
2	Chrysanthemum	CH -1	Farmer's field, Nadathara
		CH-2	Farmer's field, Vellanikkara
		CH -3	National Rose garden, Pandiparampu
		CH-4	Shalimar nursery, Mannuthy
3	Mango	MN-1	National Rose garden, Pandiparampu
		MN-2	South Indian Agri farm, Mannuthy
		MN-3	EASF nursery, Panancheri
4	Banana	BA-1	Farmer's field, Koottala
		BA-2	Farmer's field, Cherpu

3.4. SYMPTOMATOLOGY

Symptom developed on the leaves, stem and flowers of the selected fruit crops and ornamentals by *S. rolfsii* under natural conditions were recorded during the survey at various locations. To study the symptomatology under artificial condition, the different isolates were inoculated on the above mentioned parts of the respective hosts as described in 3.3. The symptoms developed on the inoculated plants were compared with that in natural conditions.

3.5. VARIABILITY IN PHENOTYPIC CHARACTERS OF DIFFERENT ISOLATES OF *S. rolfsii*

The phenotypic characters *viz.*, cultural and morphological characters of different isolates of *S. rolfsii* obtained from the selected fruit crops and

ornamentals were studied to find out the variations in characters, existing among them.

3.5.1. Cultural characters of different isolates of *S. rolfsii*

The colony characters of the different isolates of *S. rolfsii* such as colour, texture, mycelial type, growth rate and characters of sclerotia such as time taken for the initiation and maturation of sclerotia, number of sclerotia at 15, 30 and 45 days after incubation (DAI), weight of 100 sclerotia, sclerotial position and exudate production on sclerotia were studied on four different media. Potato Dextrose Agar (PDA) medium, Czapek (Dox) Agar (CDA) medium, Richard's Agar (RA) medium and Special medium for *S. rolfsii* (SM) were used for this study.

3.5.2. Morphological characters of different isolates of *S. rolfsii*

The morphological characters of the hyphae and sclerotia of *S. rolfsii* in pure culture were studied. Permanent slides were prepared from the pure culture of virulent isolates. Using micromctry, measurements on the size of hyphae and sclerotia of each isolate were recorded. Other morphological characters of sclerotia, like colour, texture and shape were also recorded. Photomicrographs of hyphae and sclerotia were made. These characters were compared with the characters given in CMI descriptions of Pathogenic Fungi and Bacteria to identify the pathogen. The cultural and morphological characters of different isolates of *S.rolfsii* were analyzed with Euclidean co- efficient and was clustered by the Unweighed Pair Group Average Method (UPGMA: Sneath and Sokal, 1973) using NTSYS pc 2.02 software to produce grouping. The genetic dissimilarity matrix was also computed.

3.6. *IN VITRO* EVALUATION ON COMPATIBILITY OF DIFFERENT ISOLATES OF *S. rolfsii*

An *in vitro* evaluation on the compatibility between different isolates of *S.rolfsii* obtained from the selected fruit crops and ornamentals from different locations was conducted on PDA medium by dual culture technique (Dennis and Webster, 1971). Mycelial disc of 10 mm diameter taken from the tip of an actively growing culture of *S. rolfsii* isolated from same host from different locations and different hosts from different locations were placed at 4.5 cm apart at the centre of each half of a Petri dish containing PDA medium. The control plates were maintained by placing mycelial disc of each of the isolate separately on one half of the Petri dish at 2.25 cm from the periphery of the dish. Each combination and the control plates were replicated three times. All these Petri dishes were incubated at room temperature and observed next day onwards for the growth of the isolates. The radial growth of each isolate was recorded till the growth attained 4.5 cm in control plates. Per cent inhibition of growth of each isolate over control was calculated by the following formula.

$$\text{Per cent Inhibition (PI)} = \frac{C-T}{C} \times 100$$

C-Growth of pathogen in monoculture (cm)

T- Growth of pathogen in dual culture (cm)

The cultures were kept at room temperature for prolonged incubation and the type of reaction between the isolates was recorded by following the method given by Webber and Hedger (1986).

Types of reaction

1. Intermingling of hyphae (I)
2. Overgrowth (O)

3. Mutual inhibition with thick mycelial strand (MT)
4. Mutual inhibition with clear zone (MC)
5. Extreme inhibition of pathogen(E)

3.7. STUDY ON PATHOGENIC VARIABILITY OF DIFFERENT ISOLATES OF *S. rolfsii*

An *in planta* experiment was conducted to study the pathogenic variability of different isolates of *S. rolfsii* by cross inoculating each isolate from one of the selected host on other selected hosts. For this, tissue culture banana plants, seedlings plants of mango, chrysanthemum and marigold were maintained in polythene bags filled with potting mixture. The inoculum used for the cross inoculation includes both mycelium and sclerotium of each isolate of *S. rolfsii*. The inoculation was given on the leaves and collar region of each host plant. Plants inoculated with sterile water served as control. For each isolate, five replications were maintained. To maintain high humidity, the inoculated plants were covered with polythene cover for 24 h after inoculation and observed for the symptom expression from next day onwards. The pathogen was reisolated from the inoculated plants showing symptoms and pure cultures were compared with the original culture obtained from its host.

3.8. HOST RANGE

The host range of different isolates of *S. rolfsii* was studied by inoculating only the virulent isolates obtained from the selected fruit crops and ornamentals on the vegetables *viz.*, tomato and amorphophallus and spices *viz.*, black pepper and ginger by following the same procedure explained in 3.7. The pathogen was reisolated from the inoculated plants showing symptoms and pure cultures were compared with the original culture obtained from its host.

3.9. ESTIMATION OF IAA OF DIFFERENT ISOLATES OF *S. rolfsii*

The amount of IAA in the mycelia, culture filtrate and sclerotia of various isolates of *S. rolfsii* was estimated by using colorimetric procedure modified by Bric *et al.* (1991).

3.9.1. Estimation of IAA in mycelia

One centimeter mycelial disc of each isolate of *S. rolfsii* was inoculated separately in 100 mL of Potato Dextrose broth amended with 100µg mL $^{-1}$ of tryptophan in 50 per cent ethanol as precursor and incubated at room temperature. When the mycelial growth completely covered the surface of the broth, one gram of mycelia was transferred into 10 mL of 50 per cent ethanol in a centrifuge tube and centrifuged at 10,000 rpm for 10 min. The supernatant was collected in another tube after centrifugation. To 5 mL of supernatant 20-25 drops of O-phosphoric acid and 25 mL of Salkowsky reagent (1mL of 0.5M $FeCl_3$ in 50 mL of 35 per cent $HClO_4$) were added and incubated at 28^{0}C for 30 min and the absorbance was measured at 530 nm (Spectronic-20D$^+$). For each treatment three replications were kept. A standard curve was prepared with different concentrations of IAA (2, 4, 6, 8 and 10 µg mL^{-1}), quantified the IAA production in the mycelia of each isolate and expressed the concentration as µg g^{-1} mycelia.

3.9.2. Estimation of IAA in culture filtrate

As described in 3.9.1. the culture growth of each isolate of *S. rolfsii* was made in Potato Dextrose broth. The mycelial growth was separated from the broth culture by filtering it through Whatman No: 1 filter paper. One millilitre of the culture filtrate was transferred to 10 mL of 50 per cent ethanol in a centrifuge tube and allowed for centrifugation at 10,000 rpm for 10 min. The amount of IAA in the culture filtrate was estimated using the same procedure as described in 3.9.1. and expressed as µg mL^{-1} culture filtrate.

3.9.3. Estimation of IAA in sclerotia

Culture growth of each isolate of *S. rolfsii* was prepared in Potato Dextrose broth as described in 3.9.1. and incubated the broth culture for six days for the development of sclerotia. From this culture, one gram of sclerotia was transferred into a sterilized pestle and mortar and ground it in 10 mL of 50 per cent ethanol. The IAA content of sclerotia was estimated using the same procedure as described in 3.9.1 and expressed as $\mu g\ g^{-1}$ sclerotia.

3.10. ESTIMATION OF TOTAL PHENOL IN DIFFERENT ISOLATES OF *S. rolfsii*

The amount of total phenol in the mycelium, culture filtrate and sclerotia of various isolates of *S. rolfsii was* estimated by using standard protocol developed by Malick and Singh (1980).

3.10.1. Estimation of total phenol in mycelia

One centimetre mycelial disc of each isolate of *S. rolfsii* was inoculated separately in 100 mL of Potato Dextrose broth and incubated at room temperature. When the mycelial growth completely covered the surface of the broth, two gram weight of mycelial growth was ground with 20 mL of 80 per cent methanol, using a sterilized pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was taken and the residue was re-extracted with 80 per cent of methanol (five times the volume), centrifuged, the supernatants were pooled together, evaporated to dryness and the residue was dissolved in 25 mL of distilled water. One millilitre of residue was pipetted into a test tube and the volume was made up with 3 mL distilled water. One millilitre Folin-Ciocalteau reagent was added to the test tube. After 3 min, 4mL of 20 per cent Na_2CO_3 solution was added to each tube and mixed thoroughly. The tubes were placed in a boiling water bath for exactly one minute, cooled and the absorbance was measured at 650nm (Spectronic-20D+). A standard curve was prepared with different concentrations of phenol at 2,4,6,8 and 10 $\mu g\ mL^{-1}$ levels and was used

to quantify the total phenol production in the mycelia of each isolate and expressed the concentration as $\mu g\ g^{-1}$ mycelia.

3.10.2. Estimation of total phenol in culture filtrate

As described in 3.10.1 the culture growth of each isolate of *S. rolfsii* was made in Potato Dextrose broth. The mycelial growth was separated from the broth culture by filtering it through Whatman No1 filter paper. One millilitre of the culture filtrate was transferred to a centrifuge tube containing ten times the volume of 80 per cent of methanol .The amount of total phenol in the culture filtrate was estimated using the same procedure as described in 3.10.1. and expressed as $\mu g\ mL^{-1}$ culture filtrate.

3.10.3. Estimation of total phenol in sclerotia

Culture growth of each isolate of *S. rolfsii* was prepared in Potato Dextrose broth as described in 3.10.1 and incubated the broth culture for six days for the development of sclerotia. From this culture, one gram of sclerotia was transferred into a sterilized pestle and mortar and ground it ten times the volume of 80 per cent of methanol. The total phenol content of sclerotia was estimated using the same procedure as described in 3.10.1. and expressed as $\mu g\ g^{-1}$ sclerotia.

3.11. STASTICAL ANALYSIS

Analysis of variance was performed on the data using the statistical package MSTATC (Freed, 1986). Multiple comparisons among the treatments were done using DMRT (Duncan, 1951).

Results

4. RESULTS

The present investigation was carried out to find out the variability existing among the different isolates of *Sclerotium rolfsii* causing collar rot, leaf blight, stem rot and wilt diseases in selected fruit crops and ornamentals. For this, studies were conducted on the symptomatology, cultural and morphological characters of the different isolates, compatibility among various isolates, pathogenic variability, host range and estimation of indole acetic acid and total phenol content of mycelia, culture filtrate and sclerotia of various isolates of *S. rolfsii*. The results of this study are presented below.

4.1. ISOLATION OF *S. rolfsii* ASSOCIATED WITH SELECTED FRUIT CROPS AND ORNAMENTAL PLANTS

The pathogen associated with the diseased specimens of marigold, chrysanthemum, mango and banana from different locations was isolated on PDA medium. The isolation yielded total eleven isolates of *S. rolfsii*, two from marigold (MG-1 and MG-2), four from chrysanthemum (CH-1, CH-2, CH-3 and CH-4), three from mango seedlings (MN-1, MN-2 and MN-3) and two from banana (BA-1 and BA-2). All the eleven isolates were brought into pure culture on PDA medium and sub cultured at monthly intervals. The cultures were primarily identified as *S. rolfsii* Sacc. based on the colony morphology, white thick mycelial growth with numerous light to dark brown, small, spherical sclerotia which showed the characteristic cultural growth of *S. rolfsii*.

4.2. PATHOGENICITY TEST

Pathogenicity of 11 isolates of *S. rolfsii* were proved by inoculation under *in vivo* conditions in their respective hosts. The plants inoculated with three different inocula after giving pinprick showed early infection in their respective host plants. Among the three inocula, mycelia along with sclerotia recorded early infection on all the plant parts inoculated. The other inocula *viz.*, mycelia alone and sclerotia alone took more time to initiate the infection on the inoculated area. In all the four selected crops, the isolates inoculated without pin prick recorded initial infection

on 4- 7 DAI. The pathogen was reisolated from the inoculated area after the development of symptoms and compared with the original culture.

4.2.1. Pathogenicity test of isolates from marigold

The isolate MG-2 showed first infection on 2 DAI on leaves and collar region of marigold plants which were inoculated with mycelia and sclerotia together. The isolate MG-1 recorded the infection of leaves on 3 DAI whereas the same isolate recorded infection on collar region on 4 DAI. Among the isolates inoculated on flowers of marigold, the second isolate (MG-2) showed first infection on flowers on three DAI whereas the other isolate (MG-1) initiated the infection on 4 DAI. The other two inocula *viz.*, mycelia alone and sclerotia alone took 5-6 days for initiating infection on leaves, collar region and flowers of marigold (Plate 1a).

4.2.2. Pathogenicity test of isolates from chrysanthemum

The inoculation of leaves with mycelia and sclerotia together with pin prick resulted in first infection on three DAI by the isolate CH-2. The other two isolates CH-1 and CH-4 recorded the infection on leaves and collar region on four and five DAI respectively whereas CH-3 showed the infection on leaves and collar region on 5DAI. The other two inocula *viz.*, mycelia alone and sclerotium alone took 6-8 days for initiating infection on leaves and collar region of chrysanthemum (Plate 1b).

4.2.3. Pathogenicity test of isolates from mango

On three days after inoculation, the isolate MN-2 recorded first infection on leaves of mango seedlings on inoculation with mycelia and sclerotia together after giving pin prick. The other two isolates MN-1 and MN-3 recorded infection on leaves on five and six DAI respectively whereas the same isolates showed infection on collar region on seven DAI when inoculated with the same inoculum. The other two inocula of these isolates took six to eight days for initiating

infection on leaves and collar region of mango seedlings after giving pinprick (Plate 1c).

4.2.4. Pathogenicity test of isolates from banana

On leaves and collar region, the initial infection was observed on 2 and 3 DAI respectively by BA-2. The other isolate BA-1 showed the infection on leaves and collar region on 3 and 4 DAI respectively. On pseudostem, the initial infection was noticed on 3 DAI by BA-2 whereas BA-1 recorded on 4 DAI. The other two inocula *viz.*, mycelia alone and sclerotium alone took 7-8 days for initiating infection on leaves and collar region of banana (Plate 1d).

4.3. SYMPTOMATOLOGY

Symptomatology of the diseases developed on the selected fruit crops and ornamental plants by different isolates of *S. rolfsii* was conducted under natural and artificial conditions. The study revealed a slight variation in symptom expression by the pathogen in different host plants. But the symptoms developed in the same host at different locations were found similar.

4.3.1. Symptoms on fruit crops and ornamental plants under natural conditions

4.3.1.1. Marigold

Infection on marigold with *S. rolfsii* was observed on leaves, flowers and collar region. The infection was first initiated on the collar region where white weft of mycelial growth was seen. The numerous mustard seed like reddish tan to dark brown sclerotia were produced around the stem and complete rotting of collar region was seen under high humidity. On leaves, the symptoms started from the middle leaves. It initiated as small dark brown water soaked lesion which later enlarged to large lesion with yellow halo. Infection spread to other leaves and complete rotting of leaves was noticed. Infection also observed on flowers and complete rotting and drying up of the flowers were observed. In the advanced stage, white coloured mycelial growth of the pathogen was observed on leaves

Plate 1. Pathogenicity test

a. Marigold

b. Chrysanthemum

c. Mango

d. Banana

and flowers which later produced large number of dark brown to black coloured mustard like sclerotia without exudates on flowers and collar region. Ultimately the plant was wilted and died (Plate 2a).

4.3.1.2. Chrysanthemum

Infection by *S. rolfsii* on chrysanthemum was observed on leaves and collar region of plants. Initially it formed a thick white mycelial growth at the base of the stem. Later as small dark brown lesion which enlarged to large lesions and resulted in complete rotting of the basal part of the plants. In the advanced stage, the pathogen produced large number of dark brown coloured mustard like sclerotia. Symptoms observed on middle leaves as small dark brown water soaked lesion which later enlarged to large lesion with yellow halo. Infection spread to other leaves and complete blighting of leaves was observed under high humidity. General yellowing of leaves was also noticed. In the advanced stage, large numbers of dark brown coloured mustard like sclerotia was observed on leaves. No exudates were observed on the surface of sclerotia. Finally the severely infected plant was wilted and died (Plate 2b).

4.3.1.3. Mango

Infection on mango with *S. rolfsii* was first observed on the collar region of seedlings and grafts. Initially it developed as dark brown discolouration on the base of stem with the presence of thick weft of white fungal mycelium up to a height of 5 cm on which round sclerotia were observed. Dark brown to black spots developed around the growing mycelium, which gradually girdles the stem partially or completely. At this stage, the leaves started dropping from upper portion and finally the seedlings collapsed and died. A vigorous growth of mycelium and sclerotia were found in the root zone of the infected tissues. Sclerotial exudates production was not observed (Plate 2c).

4.3.1.4. Banana

Infection of *S. rolfsii* was observed on Kadali variety of banana. It was observed on pseudostem at the base of leaf petiole. The symptom initiated as dark brown water soaked lesions which later enlarged and resulted in rotting of that area. The rotting was spread to the inner tissues of the pseudostem and also to the basal portion of the leaf petiole. Later, white thick mycelial growth was observed on the surface of the infected area. The thick mycelial growth spread to the inner side of the leaf sheath and it was observed when the leaf sheath get separated from the pseudostem. Large number of sclerotial formation without exudates was also observed on the surface and inner side of the pseudostem and leaf sheath. In advanced stage, the pseudostem broken at the rotted area due to the weight of the foliage. Yellowing of leaves and wilting of plant was not noticed (Plate 2d).

4.3.2. Symptoms on fruit crops and ornamental plants under artificial conditions

Under artificial conditions, slight variation in the symptom expression by the different isolates of *S. rolfsii* on their respective hosts were observed as compared to those noticed under natural conditions.

4.3.2.1. Marigold

White mycelial growth was observed on middle leaves and collar region of plants inoculated with mycelia and sclerotia together. The infection started as dark brown water soaked lesion with yellow halo on leaves which advanced to complete rotting of the leaves under high humidity. The infection spread to other leaves and complete rotting with white mycelial growth was observed. On collar region, dark brown water soaked lesion developed which later enlarged and resulted in collar rot. White mycelial growth of the pathogen was observed on the infected area. Infection on flowers developed as dark brown lesion which advanced to blackening and drying up of the flowers. In the advanced stage, the pathogen produced large number of dark brown coloured mustard like sclerotia on collar region. Sclrerotial exudates were not seen on the surface of sclerotia.

Plate 2. Symptomatology under natural conditions

a. Marigold

Leaf rot, collar rot

Flower rot

b. Chrysanthemum

Leaf blight

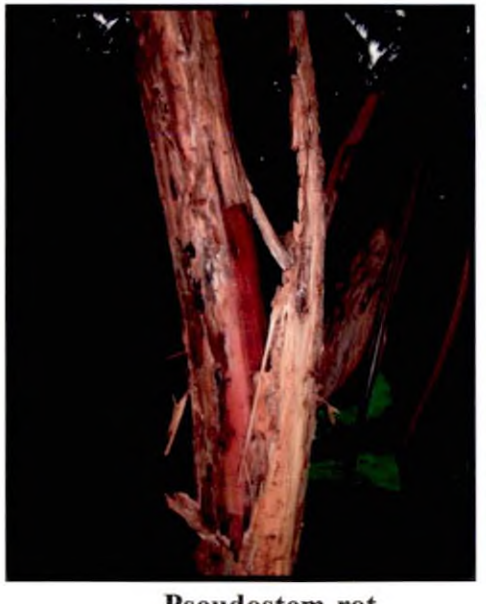
Collar rot

c. Mango

Collar rot

Sclerotia on collar region

d. Banana



Pseudostem rot

Mycelial growth

Ultimately the plant wilted, dried up and death of seedlings was observed. Inoculation of mycelia and sclerotia together recorded faster symptom development followed by inoculation with mycelia and sclerotia separately. No variation in symptoms were noticed when isolates from marigold from two different locations were inoculated (Plate 3a).

4.3.2.2. Chrysanthemum

On the leaves of chrysanthemum, the symptoms developed as dark brown water soaked lesion which later enlarged to leaf blight with yellow halo. White mycelial growth of the pathogen was seen on the surface of the leaves. Dark brown discolouration on collar region with white mycelial growth was noticed in plants inoculated with mycelia and sclerotia together. Infection spread to other leaves and complete rotting of leaves was observed. Production of large number of dark brown coloured mustard like sclerotia was seen on leaves and collar region of plant. On the surface of sclerotia exudates were not found. Collar infection was not observed in plants inoculated separately with mycelia and sclerotia (Plate 3b).

4.3.2.3. Mango

Symptoms developed as circular, dark brown water soaked spot on leaves of mango seedlings inoculated with mycelia and sclerotia together. Later these spots enlarged to large lesion with white mycelial growth on under surface of leaves. The lesion enlarged to 2.5 cm in length and 2.0 cm in width with yellow halo. Sclerotial development was observed as thick white mycelial rolls on both sides of leaves. On the collar region of mango seedlings dark brown discoluration with thick strand of white mycelial growth was observed. In advanced stage, round dark brown mustard like sclerotia were developed on collar region. At this stage, the seedlings showed wilting, defoliation and drying up of the twigs from tip portion. Finally the seedlings collapsed and died. Collar infection was not observed on plants inoculated with mycelia and sclerotia separately (Plate 3c).

4.3.2.4. Banana

On tissue culture plants of Kadali banana, the symptoms developed as dark brown water soaked lesion on pseudostem where the leaf petiole is attached and covered with white mycelial growth. On leaves, infection developed as dark brown coloured water soaked lesion with white mycelial growth on both surfaces of leaf inoculated with mycelia and sclerotia together. Later the lesion developed into a necrotic lesion with yellow halo. On the necrotic lesions, dark brown mustard like sclerotia were seen. General yellowing of infected leaves was also noticed. Ultimately yellowing and drying up of leaves and toppling down of plants at the point of infection on pseudostem were observed. White mycelial growth and sclerotia were developed on collar region, pseudostem and leaves (Plate 3d).

4.4. VARIABILITY IN PHENOTYPIC CHARACTERS

The variability in phenotypic characters of different isolates of *S. rolfsii* were studied in detail. The cultural and morphological characters of different isolates of *S. rolfsii* from selected fruit crops and ornamental plants were studied to find out the variations among the isolates.

4.4.1. Cultural characters

Cultural characters of different isolates of *S. rolfsii* such as colony characters, growth rate, number of days taken for sclerotial initiation and maturation, position of sclerotia, number of sclerotia per plate on 15, 30 and 45 DAI and weight of 100 sclerotia were studied in detail and the results are presented in Table 3 to 6.

4.4.1.1. Colony characters of different isolates of *S. rolfsii*

The colony characters such as colour, texture and mycelial type of the fungal colony, number of days required for sclerotial initiation and maturation, number of sclerotia per plate at 15, 30 and 45 DAI, weight of 100 sclerotia, sclerotial position and production of exudates on sclerotia were studied on four

Plate3. Symptomatology under artificial conditions

a. Marigold

b. Chrysanthemum

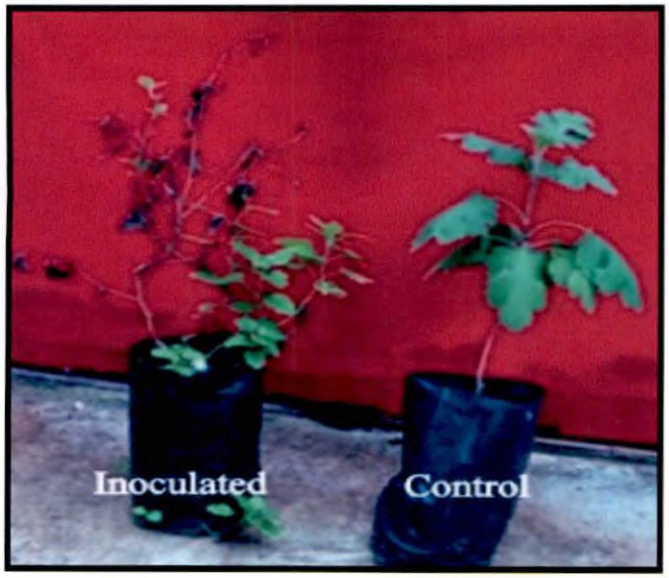


Plate3. Continued

c. Mango

d. Banana

different media *viz.*, PDA medium, Czapek (Dox) Agar medium, Richard's Agar medium and special medium for *S. rolfsii*. The data are presented in Table 3 to 6.

4.4.1.1.1. Colony characters of different isolates of *S. rolfsii* on PDA medium

All the isolates of *S. rolfsii* produced white coloured mycelial growth on PDA medium (Table 3). A smooth textured colony was observed in all isolates of *S. rolfsii* except in the three isolates from mango and the first isolate from marigold, where a rough textured colony was observed. Among the eleven isolates, two isolates from marigold (MG-1and MG-2) and all the three isolates from mango showed a fluffy growth in the colony whereas all the four isolates from chrysanthemum and banana showed a compact type of colony. A slight variation in the sclerotial characters was observed among the isolates. In all isolates except the isolates from banana, the sclerotial initials were developed on the mycelia present on the surface of the medium. The isolates from banana showed the sclerotial formation both from the aerial hyphal strands and mycelia present on the medium. The number of days required for the initiation of sclerotia in the culture of each isolate showed variation. It ranged from 7 to 9 days. Variation in the number of days required for the maturation of sclerotia in the culture of each isolate was recorded. It ranged from 10 to 13 days. The isolate CH-4 from chrysanthemum, MN-2 and MN-3 isolates from mango took the maximum days (9) for the initiation of sclerotia. It was also observed that the isolate CH-4 from chrysanthemum and MN-2 and MN-3 isolates from mango took the maximum days (13days) for the maturation of sclerotia.

The number of sclerotia developed in the culture of each isolate of *S. rolfsii* was recorded at 15, 30 and 45 DAI. From the date given in Table 3, it was observed that there was a gradual increase in the number of sclerotia in all the isolates from 15 to 45 DAI. The isolate from banana (BA-1) recorded the maximum number of sclerotia at 15 (112), 30 (387) and 45 (402) DAI. The isolate from marigold (MG-1) recorded the minimum number of sclerotia at 15 (45) and 45 (103) whereas the isolate from chrysanthemum (CH-2) recorded the minimum

Table 3. Cultural characters of different isolates of *S.rolfsii* on Potato Dextrose Agar medium

Sl. No	Host	Isolate	Colony character*			Sclerotial character*							
			Colour	Texture	Mycelial type	Sclerotial initiation DAI	Sclerotial maturation DAI	No of sclerotia per plate DAI*			Weight of 100 sclerotia (mg)	Sclerotial position	Production of exudates
								15	30	45			
1	Marigold	MG-1	White	Rough	Fluffy	7	10	45	93	103	109	Centre &periphery	++
		MG I2	White	Smooth	Fluffy	7	10	52	97	112	95	Centre &periphery	++
2	Chrysanthemum	CH-1	White	Smooth	Compact	7	11	69	109	145	99	Centre &periphery	++++
		CH-2	White	Smooth	Compact	7	11	63	82	108	97	Centre &periphery	++++
		CH-3	White	Smooth	Compact	7	11	68	90	110	92	Centre &periphery	++++
		CH-4	White	Smooth	Compact	9	13	75	124	178	86	Centre &periphery	++++
3	Mango	MN-1	White	Rough	Fluffy	8	11	65	117	134	104	Centre &periphery	+++
		MN-2	White	Rough	Fluffy	9	13	76	126	180	98	Centre &periphery	+++
		MN-3	White	Rough	Fluffy	9	13	95	136	186	84	Centre &periphery	+++
4	Banana	BA-1	White	Smooth	Compact	8	11	112	387	402	60	Centre & periphery of lid and base	+
		BA-2	White	Smooth	Compact	8	12	105	376	398	68	Centre & periphery of lid and base	+

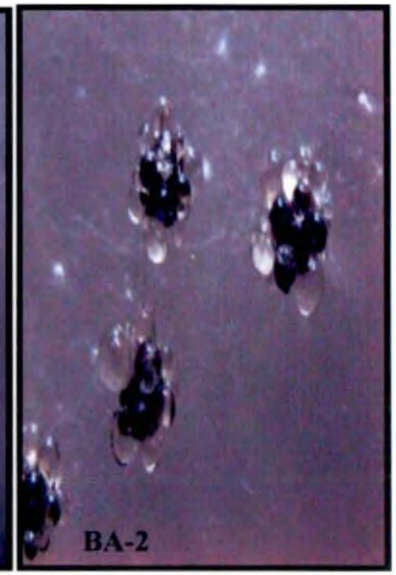
*Mean of 5 replications DAI-Days After Incubation ++++-very high +++-high ++-medium +-low

Plate 4. Production of exudate by different isolates of *S. rolfsii* on Potato Dextrose Agar medium

a. Marigold

b. Chrysanthemum

c. Mango



d. Banana

number of sclerotia at 30 (82) DAI. The isolate from marigold (MG-1) recorded the maximum weight of sclerotia (109 mg) whereas the isolate from banana (BA-1) recorded the minimum weight (60 mg) for 100 sclerotia. The production of sclerotial exudates was very high in all the four isolates from chrysanthemum whereas low in the two isolates from banana. The three isolates from mango and the two isolates from marigold showed high and medium production of sclerotial exudates respectively (Plate 4a to 4d). It was observed that the exudates present on the sclerotia produced by all isolates dried up on 15-17 DAI. The matured sclerotia were observed on the centre and periphery of the culture of isolates from marigold, chrysanthemum and mango. In the culture of isolates from banana, it was noticed on the centre and periphery of the lid of Petri dishes (Plate 5a).

4.4.1.1.2. Colony characters of different isolates of *S. rolfsii* on Czapek (Dox) Agar medium

White mycelia growth of all the eleven isolates of *S. rolfsii* was observed on Czapek (Dox) Agar medium (Table 4). The first isolate of marigold (MG-1) showed a rough texture whereas in all other isolates the texture of the colony was smooth. All isolates from marigold, chrysanthemum, mango and banana showed a compact type of mycelia growth. Variation in the number of days required for the initiation of sclerotia was noted. It ranged from 3 to 5 days. The isolates from chrysanthemum (CH-1, CH-2, CH-3 and CH-4) took the minimum days (3) for the initiation of sclerotia. A slight variation was also recorded in the number of days required for the maturation of sclerotia in the culture of isolates from different crops. It ranged from 7 to 11 days. The isolates from chrysanthemum (CH-1, CH-2, CH-3 and CH-4) took the minimum days (7) for the maturation of sclerotia.

The sclerotial developement in the culture of each isolates of *S. rolfsii* was recorded at 15, 30 and 45 DAI. An increase in the number of sclerotia in all the isolates from 15 to 45 DAI was observed. The isolate from banana (BA-2) recorded the maximum number of sclerotia at 15 (32), 30(68) and 45 (96) DAI.

Table 4. Cultural characters of isolates of *S.rolfsii* on Czapek (Dox) Agar medium

Sl. No.	Host	Isolate	Colony character *			Sclerotial character*							
			Colour	Texture	Mycelial type	Sclerotial initiation	Sclerotial maturation	No of sclerotia per plate			Weight of 100 sclerotia (mg)	Sclerotial position	Productio of exudate
						DAI	DAI	DAI					
								15	30	45			
1	Marigold	MG-1	White	Rough	Compact	4	9	4	9	16	103	Centre &periphery	++
		MG-2	White	Smooth	Compact	4	9	6	10	19	91	Centre &periphery	++
2	Chrysanthemum	CH-1	White	Smooth	Compact	3	7	12	26	38	97	Centre	+++
		CH-2	White	Smooth	Compact	3	7	18	32	52	93	Centre	+++
		CH-3	White	Smooth	Compact	3	7	14	28	42	90	Centre	+++
		CH-4	White	Smooth	Compact	3	7	10	23	34	84	Centre	+++
3	Mango	MN-1	White	Smooth	Compact	4	9	8	18	30	101	Centre &periphery	++
		MN-2	White	Smooth	Compact	4	9	12	26	45	96	Centre &periphery	++
		MN-3	White	Smooth	Compact	4	9	10	22	36	81	Centre &periphery	++
4	Banana	BA-1	White	Smooth	Compact	5	11	26	54	82	51	Periphery	+
		BA-2	White	Smooth	Compact	5	11	32	68	96	67	Periphery	+

* Mean of 5 replications DAI-Days After Incubation +++-high ++-medium +-low

The isolate from marigold (MG-1) recorded the minimum number of sclerotia at 15 (4), 30 (9) and 45 (16) DAI. The isolate from marigold (MG-1) recorded the maximum weight of sclerotia (103 mg) whereas the isolate from banana (BA-1) recorded the minimum weight of sclerotia (51 mg). The production of sclerotial exudates was recorded as high in all the four isolates from chrysanthemum and medium in all the three isolates from mango and two isolates from marigold. Low production of sclerotial exudates was observed in the two isolates from banana. The sclerotial formation was observed in the centre and periphery of Petri dishes containing isolates *viz*., MG-1, MG-2, MN-1, MN-2 and MN-3. The sclerotial formation was noticed in the centre of Petri dish containing all the four isolates from chrysanthemum whereas in the periphery of Petri dish containing two isolates from banana (Plate 5b).

4.4.1.1.3. Colony characters of different isolates of *S. rolfsii* on Richard's Agar medium

The colour of mycelium observed was white in all the eleven isolates of *S. rolfsii* on Richard's Agar medium. All the three isolates from mango and the first isolate from marigold (MG-1) showed a rough textured colony whereas a smooth textured colony was noticed in all isolates from chrysanthemum, banana and second isolate from marigold (MG-2). Two isolates from marigold (MG-1and MG-2) and all the three isolates from mango showed a fluffy growth in the colony whereas all the four isolates from chrysanthemum and two isolates from banana showed a compact type of mycelial growth. No variation was observed in the number of days (7 days) required for the initiation of sclerotia in the culture of each isolates whereas a slight variation was recorded in the number of days required for the maturation of sclerotia in the culture of isolates from different hosts. It ranged from 12 to 14 days. The isolates from banana (BA-1 and BA-2) took the maximum days (14 days) for the maturation of sclerotia whereas two isolates from marigold (MG-1and MG-2) and all the three isolates from mango took the minimum days (12) for the maturation of sclerotia.

Table 5. Cultural characters of isolates of *S. rolfsii* on Richard's Agar medium

Sl. No	Host	Isolate	Colony character*			Sclerotial character*							
			Colour	Texture	Mycelial type	Sclerotial initiation DAI	Sclerotial maturation DAI	No of sclerotia per plate DAI			Weight of 100 sclerotia (mg)	Sclerotial position	Production of exudates
								15	30	45			
1	Marigold	MG-1	White	Rough	Fluffy	7	12	39	48	48	108	Centre &periphery	++
		MG-2	White	Smooth	Fluffy	7	12	45	56	56	93	Centre &periphery	++
2	Chrysanthemum	CH-1	White	Smooth	Compact	7	13	56	68	68	98	centre	+++
		CH-2	White	Smooth	Compact	7	13	68	76	76	96	centre	+++
		CH-3	White	Smooth	Compact	7	13	58	72	72	90	centre	+++
		CH-4	White	Smooth	Compact	7	13	72	85	85	82	centre	+++
3	Mango	MN-1	White	Rough	Fluffy	7	12	48	62	62	102	Centre &periphery	+++
		MN-2	White	Rough	Fluffy	7	12	54	64	64	94	Centre &periphery	+++
		MN-3	White	Rough	Fluffy	7	12	10	22	22	81	Centre &periphery	+++
4	Banana	BA-1	White	Smooth	Compact	7	14	102	356	356	57.5	Lid centre& periphery	+
		BA-2	White	Smooth	Compact	7	14	108	363	363	66.5	Lid centre& periphery	+

*-Mean of 5 replications DAI-Days After Incubation +++-high ++-medium +-low

Sclerotial development in the culture of each isolate of *S. rolfsii* was recorded at 15, 30 and 45 DAI. From the Table 5, it was noticed that an increase in the number of sclerotia was not noticed in any of the isolates from 30 to 45 DAI. The isolate from banana (BA-2) recorded the maximum number of sclerotia at 15(108) and 30 (363) DAI. The isolate from mango (MN-3) recorded the minimum number of sclerotia at 15(10) and 30 (22) DAI. The isolate from marigold (MG-1) recorded the maximum weight of sclerotia (108 mg) whereas the isolate from banana (BA-1) recorded the minimum weight of sclerotia (57.5 mg). The production of sclerotial exudates was high in all the four isolates from chrysanthemum and three isolates from mango whereas medium in two isolates from marigold. Production of sclerotial exudates was recorded as low in the two isolates from banana. The sclerotial formation was observed in the centre and periphery of Petri dishes containing isolates from marigold and mango. The sclerotial formation was noticed only in the centre of Petri dish containing all the four isolates from chrysanthemum whereas in the centre and periphery of lid and base of Petri dish containing two isolates from banana (Plate 5c).

4.4.1.1.4. Colony characters of different isolates of *S. rolfsii* on special medium

White coloured mycelial growth of all the eleven isolates of *S. rolfsii* was observed on special medium (Table 6). Smooth texture of the colony was recorded in all the eleven isolates of *S. rolfsii*. All the isolates from marigold (MG-1and MG-2), mango (MN-1, MN-2 and MN-3) and chrysanthemum (CH-1, CH-2, CH-3 and CH-4) showed a fluffy mycelial growth whereas the two isolates from banana (BA-1 and BA-2) showed a compact type of mycelial growth. The time taken for sclerotial initiation by the isolates ranged from 8 to 10 days. Two isolates from marigold (MG-1and MG-2) and two isolates from banana (BA-1 and BA-2) took the maximum days (10) for the initiation of sclerotia whereas all the three isolates from mango took the minimum days (8) for the initiation of sclerotia. A slight variation was also recorded in the number of days required for the maturation of sclerotia in the culture of isolates from different hosts. It ranged

Table 6. Cultural characters of isolates of *S.rolfsii* on special medium

Sl. No	Host	Isolate	Colony character*			Sclerotial character*							
			Colour	Texture	Mycelial type	Sclerotial initiation DAI	Sclerotial maturation DAI	No of sclerotia per plate DAI			Weight of 100 sclerotia (mg)	Sclerotial position	Production of exudates
								15	30	45			
1	Marigold	MG-1	White	Smooth	Fluffy	10	14	12	26	40	102	Periphery	++
		MG-2	White	Smooth	Fluffy	10	14	7	18	34	93	Periphery	++
2	Chrysanthemum	CH-1	White	Smooth	Fluffy	9	14	9	23	46	97	Periphery	+++
		CH-2	White	Smooth	Fluffy	9	14	16	34	58	94	Periphery	+++
		CH-3	White	Smooth	Fluffy	9	14	21	45	72	90	Periphery	+++
		CH-4	White	Smooth	Fluffy	9	14	12	28	48	82	Periphery	+++
3	Mango	MN-1	White	Smooth	Fluffy	8	12	7	16	31	98	Periphery	++
		MN-2	White	Smooth	Fluffy	8	12	16	38	60	93	Periphery	++
		MN-3	White	Smooth	Fluffy	8	12	26	48	75	81	Periphery	++
4	Banana	BA-1	White	Smooth	Compact	10	14	325	380	454	48	Centre & periphery	+
		BA-2	White	Smooth	Compact	10	14	432	496	542	65	Centre & periphery	+

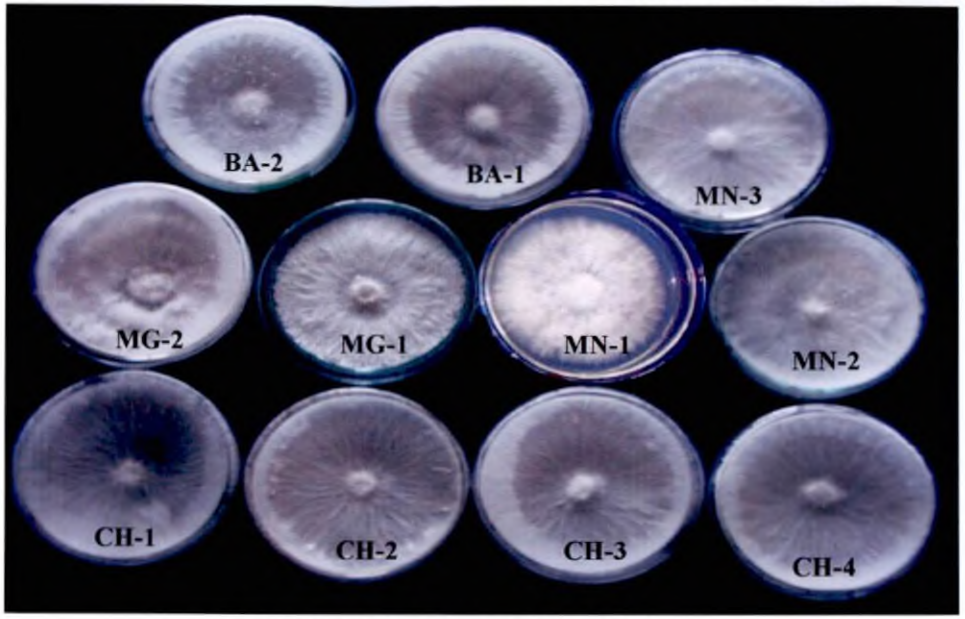
*-Mean of 5 replications DAI-Days After Incubation +++-high ++-medium +-low

from 12 to 14 days. All the isolates from marigold, chrysanthemum and banana took 14 days for the maturation of sclerotia whereas the isolates from mango took 12 days for the maturation of sclerotia.

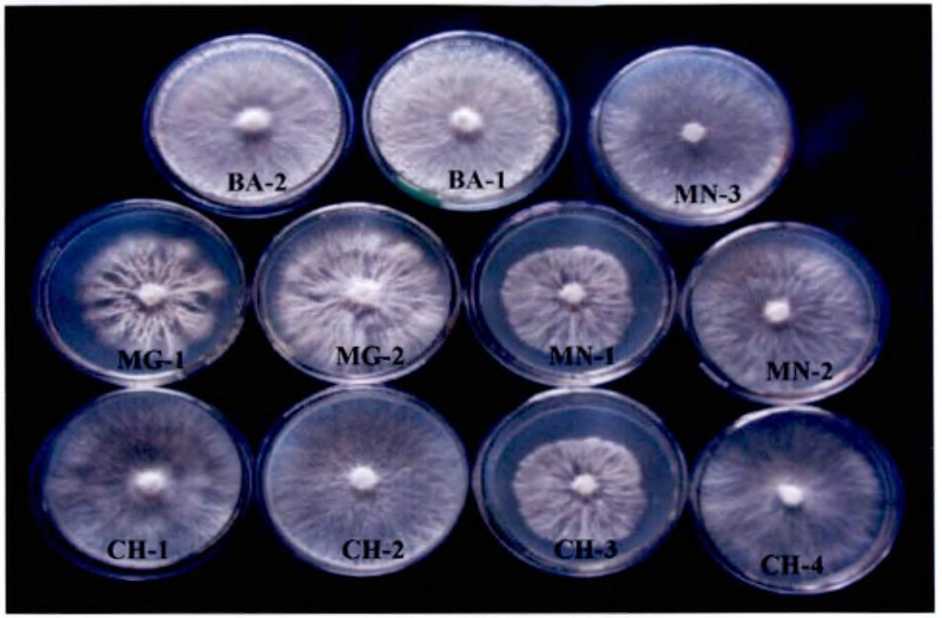
The number of sclerotia developed in the culture of each isolates of *S. rolfsii* was recorded at 15, 30 and 45 DAI. A gradual increase in the number of sclerotia in all the isolates from 15 to 45 DAI was noticed. The second isolate from banana (BA-2) recorded the maximum number of sclerotia at 15 (432) and 30 (496) and 45 (542) DAI. The isolate from mango (MN-1) recorded the minimum number of sclerotia at 15 (7) and 30 (16) and 45 (31) DAI. The isolate from marigold (MG-1) recorded the maximum weight of sclerotia (102 mg) whereas the isolate from banana (BA-1) recorded the minimum weight of sclerotia (48 mg). The production of sclerotial exudates was high in all the four isolates from chrysanthemum and was found to be medium in all the three isolates from mango and two isolates from marigold. The sclerotial exudates production was recorded low in the two isolates from banana. The position of sclerotia was observed in the centre and periphery of Petri dish containing banana isolates *viz.*, BA-1 and BA-2 whereas sclerotial position was observed in the periphery of Petri dish containing all isolates from marigold, chrysanthemum and mango (Plate 5d).

In all the four media, white coloured mycelial growth was produced by all the isolates. Slight variation was observed in the texture and mycelial type. Variation was also observed among the isolates in number of days required for the sclerotial initiation and sclerotial maturation and it ranged from 3-10 and 7-14 DAI respectively. The sclerotial production was recorded in all media except in Richard's Agar medium up to 45DAI. In all media, the maximum number of sclerotia was recorded by the isolates from banana (BA-1 and BA-2). Regarding the weight of 100 sclerotia, all isolates recorded the maximum weight on PDA medium and it ranged from 60 to 109 mg. Production of exudates on the surface of sclerotia was observed in all isolates grown on all media. Very high exudate formation was observed in all the four isolates from chrysanthemum on PDA medium whereas it was low in both isolates from banana.

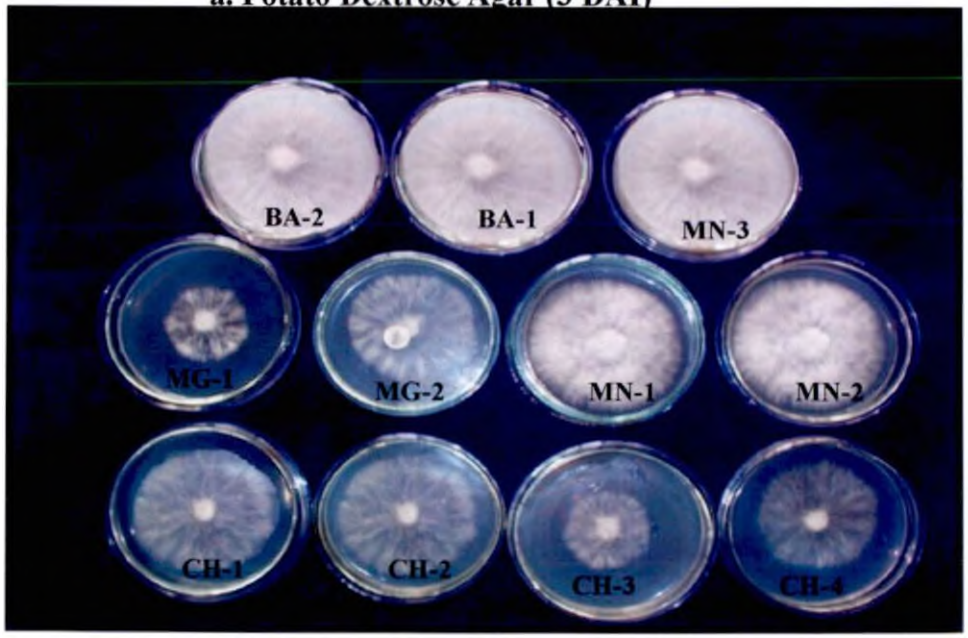
Plate 5. Colony characters of *S. rolfsii* isolates on different media



a. Potato Dextrose Agar (3 DAI)



b. Czapek (Dox) Agar (4 DAI)



c. Richard's Agar (8 DAI)

d. Special Medium (6 DAI)

DAI- Days After Inoculation

4.4.1.2. Growth rate of different isolates of *S. rolfsii* on selected media

Growth rate of different isolates of *S. rolfsii* was studied on four different media *viz.*, PDA medium, Czapek (Dox) Agar medium, Richard's Agar medium and special medium. The colony diameter of different isolates of *S. rolfsii* was recorded daily till the colony completely covered the Petri dish. The growth rate in different media was presented in Tables from 7 to 10.

4.4.1.2.1. Growth rate of different isolates of *S. rolfsii* on PDA medium

The data on the growth rate of different isolates of *S. rolfsii* on PDA medium is presented in Table 7. Eventhough there was variation in growth rate, all the eleven isolates produced good growth on PDA medium and visible mycelial growth was observed from second day onwards. The isolates from same host from different locations showed variation in the growth rate and the time taken for full growth in Petri dishes. Among the eleven isolates, the four isolates from chrysanthemum and isolate from mango (MN-2) took minimum time(3 days) to complete 9 cm growth in Petri dish whereas the first isolate from mango, MN-1 took 6 days to complete the full growth in Petri dish.

Among the isolates from marigold, the isolate MG-2 took 4 days to complete full growth in Petri dish whereas MG-1 took 5 days to complete full growth in dishes. The three mango isolates showed variation in growth rate. MN-2 and MN-3 took 3 and 4 days respectively whereas MN-1 took the maximum time to complete the full growth in Petri dish. The isolates from banana *viz.*, BA-1 and BA-2 completed the 9 cm growth on the same day (4 days) in Petri dish (Fig. 1).

4.4.1.2.2. Growth rate of different isolates of *S. rolfsii* on Czapek (Dox) Agar medium

The data presented in Table 8 on the growth rate of different isolates of *S.rolfsii* on Czapek (Dox) Agar medium revealed that there was variation in growth rate of isolates from different locations and visible mycelial growth was observed from second day onwards. The isolates of same host from different

Table 7. Mycelial growth of different isolates of *S. rolfsii* on PDA medium

Sl. No	Host	Isolate	Colony diameter* (cm)					
			Days after incubation					
			1	2	3	4	5	6
1	Marigold	MG-1	1.44	4.34	6.44	7.86	9.00	
		MG-2	1.67	4.62	7.34	9.00		
2	Chrysanthemum	CH-1	1.85	6.47	9.00			
		CH-2	1.71	6.69	9.00			
		CH-3	1.94	6.37	8.89	9.00		
		CH-4	1.76	6.56	9.00			
3	Mango	MN-1	1.47	2.42	4.41	6.39	8.73	9.00
		MN -2	1.54	6.23	9.00			
		MN -3	1.87	5.68	8.86	9.00		
4	Banana	BA-1	1.88	6.66	8.72	9.00		
		BA-2	2.15	6.12	8.56	9.00		

*Mean of 5 replications

Table 8. Mycelial growth of different isolates of *S. rolfsii* on Czapek (Dox) agar medium

Sl. No	Host	Isolate	Colony diameter * (cm)								
			Days after incubation								
			1	2	3	4	5	6	7	8	9
1	Marigold	MG-1	1.56	2.73	4.61	5.54	5.97	7.29	7.63	8.51	9.00
		MG-2	1.55	3.19	5.38	6.79	7.65	8.36	9.00		
2	Chrysanthemum	CH-1	2.08	4.41	6.73	8.73	9.00				
		CH-2	1.96	5.08	7.47	9.00					
		CH-3	1.4	3.67	5.5	7.46	8.31	8.88	9.00		
		CH-4	1.56	4.52	7.29	8.44	9.00				
3	Mango	MN-1	1.00	1.12	1.6	3.16	4.55	5.87	7.19	9.00	
		MN-2	1.48	3.3	5.18	7.39	9.00				
		MN-3	1.46	3.66	6.19	8.26	9.00				
4	Banana	BA-1	1.57	4.22	7.27	8.47	9.00				
		BA-2	1.59	5.23	8.8	9.00					

*Mean of 5 replications

locations showed variation in the growth rate. Among the eleven isolates, the isolates from chrysanthemum (CH-2) and isolate from banana (BA-2) recorded the shortest time of 4 days to complete 9 cm growth in Petri dish whereas the first isolate from marigold (MG-1) took the longest time of 9 days to complete the full growth in Petri dish. Among the four isolates from chrysanthemum, the isolates *viz.*, CH-1 and CH-4 took 5 days whereas CH-2 took 4 days and CH-3 took 7 days to complete 9 cm growth in Petri dish. The isolates from mango *viz.*, MN-2 and MN-3 took 5 days whereas MN-1 isolate took 8 days to complete the full growth in Petri dish. In the case of isolates from banana, BA-2 recorded less time to complete the full growth in Petri dish compared to BA-1 (Fig. 2).

4.4.1.2.3. Growth rate of different isolates of *S. rolfsii* on Richard's Agar medium

From the data on the growth rate of different isolates of *S. rolfsii* on Richard's Agar medium given in Table 9, it was observed that there was variation in growth rate of isolates from different locations. Among the eleven isolates, the two isolates from chrysanthemum (CH-1 and CH-2) recorded the minimum time of 8 days whereas the other two isolates of chrysanthemum CH-3 and CH-4 and isolate from marigold MG-1 took 10 days to complete the full growth in Petri dish. The three isolates from mango *viz.*, MN-1, MN-2 and MN-3, second isolate from marigold (MG-2) and the two isolates from banana (BA-1 and BA-2) recorded 9 days to complete the full growth in Petri dish (Fig. 3).

4.4.1.2.4. Growth rate of different isolates of *S. rolfsii* on special medium

The data on the growth rate of different isolates of *S. rolfsii* on special medium is presented in Table 10. There was no variation in the growth rate of different isolates from the same host except in marigold. The three isolates from mango *viz.*, MN-1, MN-2 and MN-3 recorded the shortest time of 6 days to complete 9 cm growth in Petri dish whereas the second isolate from marigold (MG-2) and two isolates from banana (BA-1 and BA-2) took the longest time of 8 days to complete the full growth in Petri dish. The four isolates from

Table 9. Mycelial growth of different isolates of *S.rolfsii* on Richard's Agar medium

Sl. No	Host	Isolate	Colony diameter * (cm)									
			Days after incubation									
			1	2	3	4	5	6	7	8	9	10
1	Marigold	MG-1	1.47	2.27	3.31	3.91	4.82	5.28	6.59	7.65	8.33	9.00
		MG-2	1.52	2.51	4.19	5.36	6.57	7.53	8.15	8.73	9.00	
2	Chrysanthemum	CH-1	1.44	2.21	3.81	5.02	6.66	7.32	8.69	9.00		
		CH-2	1.47	2.58	4.63	6.09	7.22	8.00	8.56	9.00		
		CH-3	1.2	1.4	2.07	2.78	4.14	5.11	6.29	7.4	8.72	9.00
		CH-4	1.48	2.87	4.44	5.07	5.52	6.26	7.35	7.92	8.81	9.00
3	Mango	MN-1	1.58	2.14	3.46	4.71	6.07	6.87	7.92	8.51	9.00	
		MN-2	1.59	2.37	3.62	4.73	6.13	6.9	7.94	8.56	9.00	
		MN-3	1.49	2.00	3.82	5.05	6.39	7.33	7.9	8.69	9.00	
4	Banana	BA-1	1.51	2.55	3.69	4.81	6.04	7.12	7.66	8.71	9.00	
		BA-2	1.43	2.68	3.98	4.77	5.79	6.89	7.49	8.56	9.00	

*Mean of 5 replications

Table 10. Mycelial growth of different isolates of *S. rolfsii* on special medium

Sl. No	Host	Isolate	Colony diameter * (cm)							
			Days after incubation							
			1	2	3	4	5	6	7	8
1	Marigold	MG-1	1.09	1.28	2.43	3.52	4.63	7.21	9.00	
		MG-2	1.04	1.14	1.47	2.86	4.24	5.45	7.86	9.00
2	Chrysanthemum	CH-1	1.25	2.06	3.85	5.60	7.43	8.51	9.00	
		CH-2	1.36	2.33	4.02	5.61	7.33	8.36	9.00	
		CH-3	1.35	2.35	4.05	5.62	6.88	8.02	9.00	
		CH-4	1.00	1.16	1.43	3.07	6.33	7.84	9.00	
3	Mango	MN-1	1.23	1.57	3.78	7.18	8.53	9.00		
		MN-2	1.00	1.10	3.93	6.17	7.38	9.00		
		MN-3	1.26	2.08	4.47	7.46	8.53	9.00		
4	Banana	BA-1	1.00	1.16	1.92	2.97	4.23	6.44	8.59	9.00
		BA-2	1.00	1.00	1.21	2.41	4.11	6.23	8.35	9.00

*Mean of 5 replications

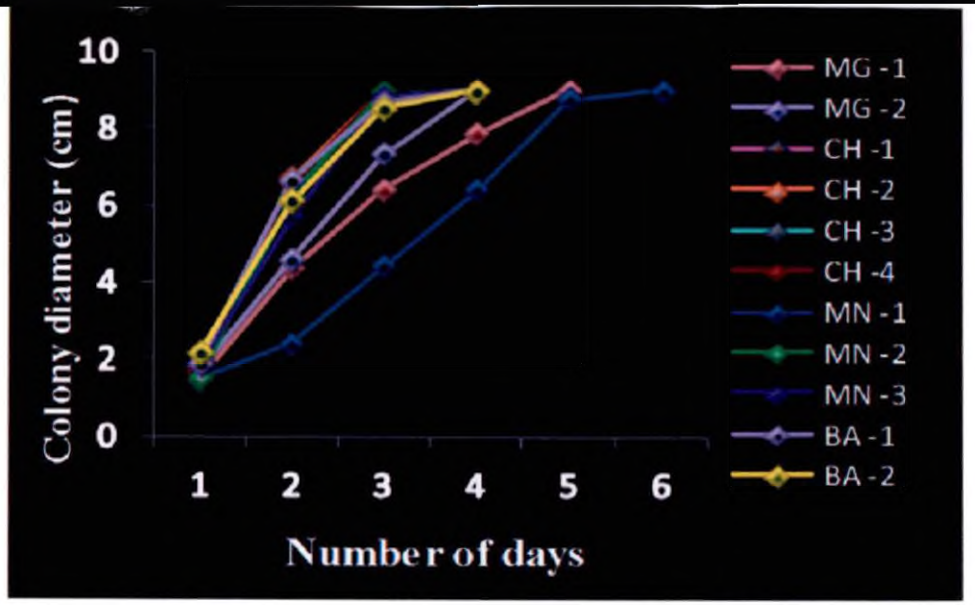


Fig 1. Growth rate of different isolates of *S. rolfsii* on Potato Dextrose Agar medium

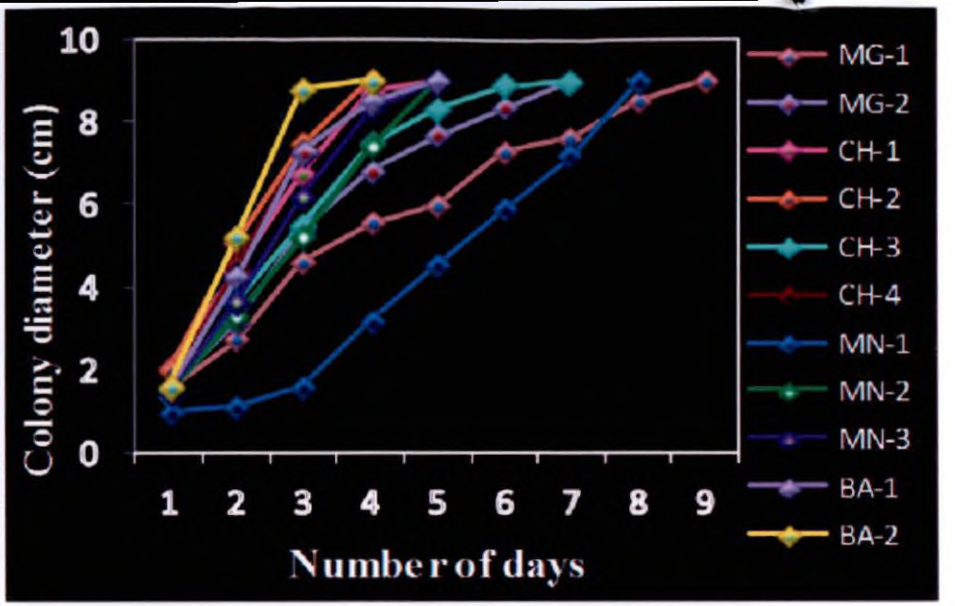


Fig 2. Growth rate of different isolates of *S. rolfsii* on Czapek (Dox) agar medium

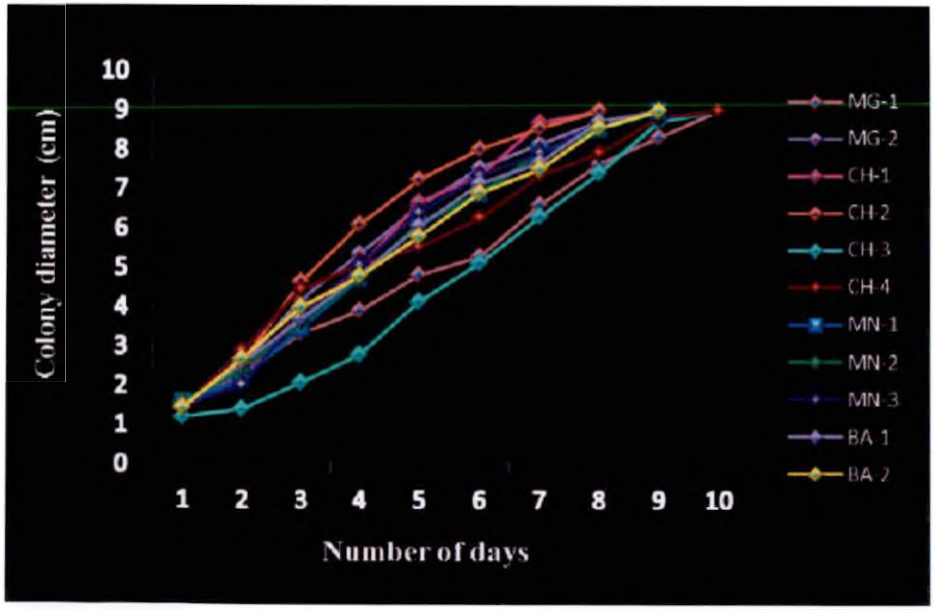


Fig 3. Growth rate of different isolates of *S. rolfsii* on Richard's agar medium

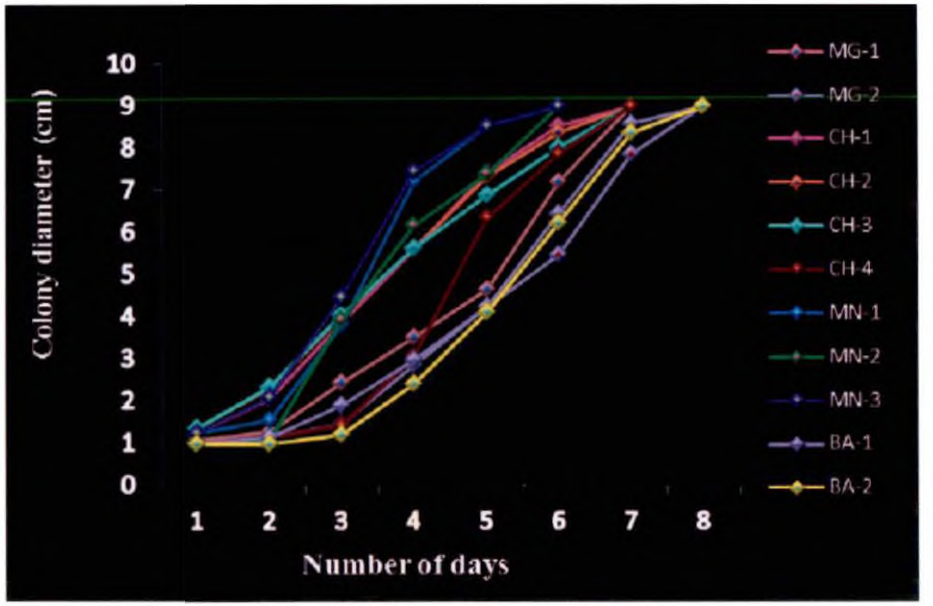


Fig 4. Growth rate of different isolates of *S. rolfsii* on special medium

chrysanthemum (CH-1, CH-2, and CH-3 and CH-4) and first isolate from marigold (MG-1) recorded 7 days to complete the full growth in Petri dish (Fig. 4).

On comparing the growth rate in four media, it was observed that all 11 isolates took minimum days to complete the full growth in Petri dish on PDA medium and was found to be the best one for supporting the fast growth of all isolates.

4.4.2. Morphological characters of different isolates of *S. rolfsii*

The morphological characters of different isolates of *S. rolfsii* such as colour and size of hyphae, colour, shape, texture and size of sclerotia were studied in detail on PDA medium. The size of hyphae and sclerotia were taken using micrometry. The results are presented in Table 11.

4.4.2 .1. Morphological characters of hyphae

Hyphae branched, hyaline and septate in all the isolates of *S. rolfsii.* The breadth of hyphal cell ranged from 4.25 to 8.93 µm among the various isolates of *S. rolfsii.* The minimum breadth of hyphal cell recorded in isolate from banana (BA-1) whereas maximum width of hyphal cell observed in mango isolate (MN-3). The length of hyphal cell ranged from 52.6 (CH-1) to 196.32 µm (BA-2) (Plate 6a).

4.4.2.2. Morphological characters of sclerotia

No variation was recorded in the morphological characters of sclerotia *viz.,* colour, texture and shape produced by different isolates. All isolates showed the formation of dark brown coloured smooth and spherical sclerotia on PDA medium. Variation was recorded in the size of sclerotia of different isolates. The size of sclerotia ranged from 0.53 to 2 mm. The maximum size was recorded in MN-2 (1.17-2.00 mm) whereas BA-1 recorded the minimum size of sclerotia (0.53-0.94 mm) (Plate 6b).

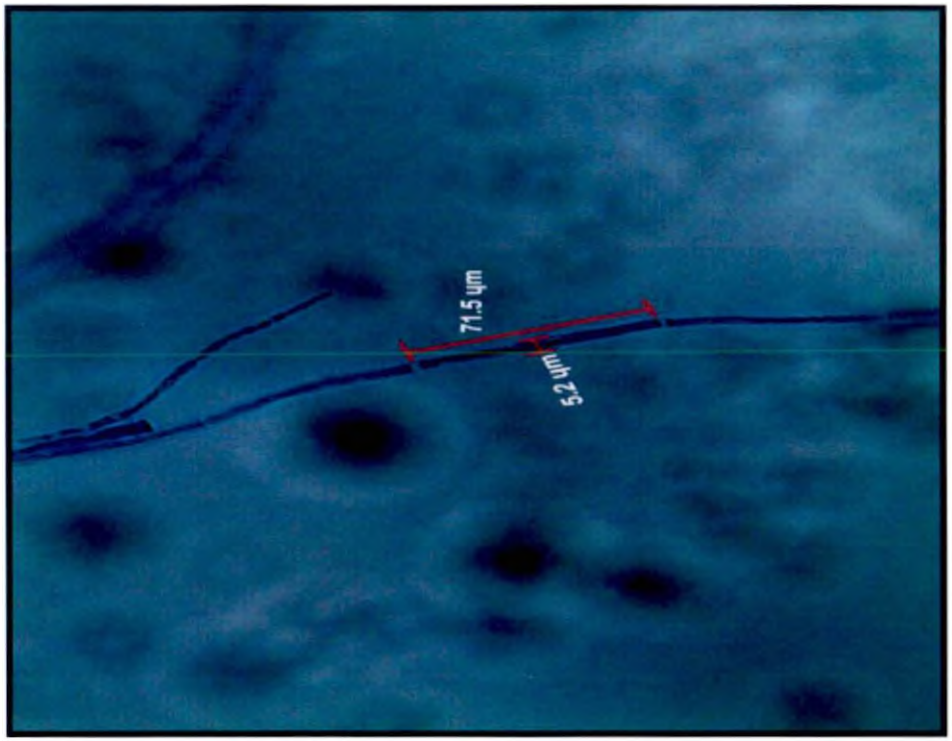
Table 11. Morphological characters of different isolates of *S. rolfsii* on PDA medium

Sl. No	Host	Isolate	Hyphal cell (µm) *		Sclerotia*			
			Distance between two septa	Breadth	Colour	Texture	Shape	Size (mm)
1	Marigold	MG-1	99.64-149.63	4.93-7.48	Dark brown	Smooth	Spherical	0.60-1.85
		MG-2	126.42-154.48	5.34-7.63	Dark brown	Smooth	Spherical	1.08-1.50
2	Chrysanthemum	CH-1	52.6-142.35	4.76-8.35	Dark brown	Smooth	Spherical	0.92-1.72
		CH-2	64.65-146.34	4.89-8.46	Dark brown	Smooth	Spherical	0.99-1.95
		CH-3	67.45-152.38	5.14-8.57	Dark brown	Smooth	Spherical	0.64-1.55
		CH-4	65.73-149.65	5.26-8.64	Dark brown	Smooth	Spherical	0.66-1.65
3	Mango	MN-1	70.57-164.42	5.46-8.85	Dark brown	Smooth	Spherical	1.17-1.95
		MN-2	71.7-168.32	5.23-8.74	Dark brown	Smooth	Spherical	1.17-2.00
		MN-3	73.45-172.43	5.85-8.93	Dark brown	Smooth	Spherical	1.06-1.35
4	Banana	BA-1	89.46-194.23	4.25-7.52	Dark brown	Smooth	Spherical	0.53-0.94
		BA-2	91.12-196.32	4.34-8.48	Dark brown	Smooth	Spherical	0.58-0.96

*Mean of 20 measurements

Plate 6. Morphological characters of hyphae and sclerotia of *S. rolfsii*

a. Hyphae (450X)



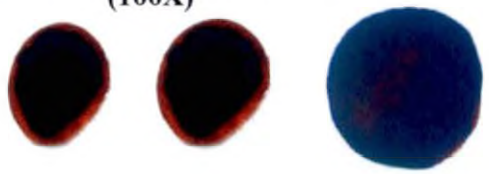
Development of sclerotia on PDA medium

b. Sclerotia

Small sized sclerotia (0.53-0.94 mm) (100X)

PDA-Potato Dextrose Agar

Large sized sclerotia (1.17-2.00 mm) (100X)



4.4.3. Cluster analysis of *S. rolfsii* isolates based on cultural and morphological characters

Genetic dissimilarity index (DI) of isolates of *S. rolfsii* from different hosts was computed from cultural and morphological characters as Euclidean co-effeicient using NTSYS pc 2.02 software. The results are presented in Table 12. The dendrogram was constructed by using Unweighed Pair Group Average Method (UPGMA) as shown in Fig. 5. The lowest dissimilarity index of 1.00 was observed between isolates of CH-1 and BA-2. It was followed by 1.03 between MG-1 and BA-1. Highest dissimilarity index of 9.91 was observed between isolates CH-4 and BA-2 followed by 9.80 between isolates MG-2 and BA-1. The isolates were grouped into two clusters A and B based on the cultural and morphological characters (Table 13). In cluster A, the highest dissimilarity coefficient of 6.71 was recorded between isolates CH-2 and MN-3. Cluster A was further divided into two sub clusters A_1 and A_2. In sub cluster A_1, the highest dissimilarity coefficient of 5.88 was recorded between isolates MG-1 and MN-2 whereas lowest dissimilarity index of 1.19 was recorded between isolates MG-1 and MG-2. In sub cluster A_2, the lowest dissimilarity index of 3.84 was recorded between isolates MN-2 and MN-3 whereas highest dissimilarity index of 6.71 was recorded between isolates CH-2 and MN-3. Cluster B had one sub cluster B_1. In sub cluster B_1, the lowest dissimilarity index of 1.00 was recorded between isolates CH-1 and BA-2 whereas highest dissimilarity index of 9.91 was observed between isolates of CH-4 and BA-2.

4.5. *IN VITRO* EVALUATION ON THE COMPATIBILITY OF VARIOUS ISOLATES OF *S. rolfsii* FROM SELECTED FRUIT CROPS AND ORNAMENTAL PLANTS

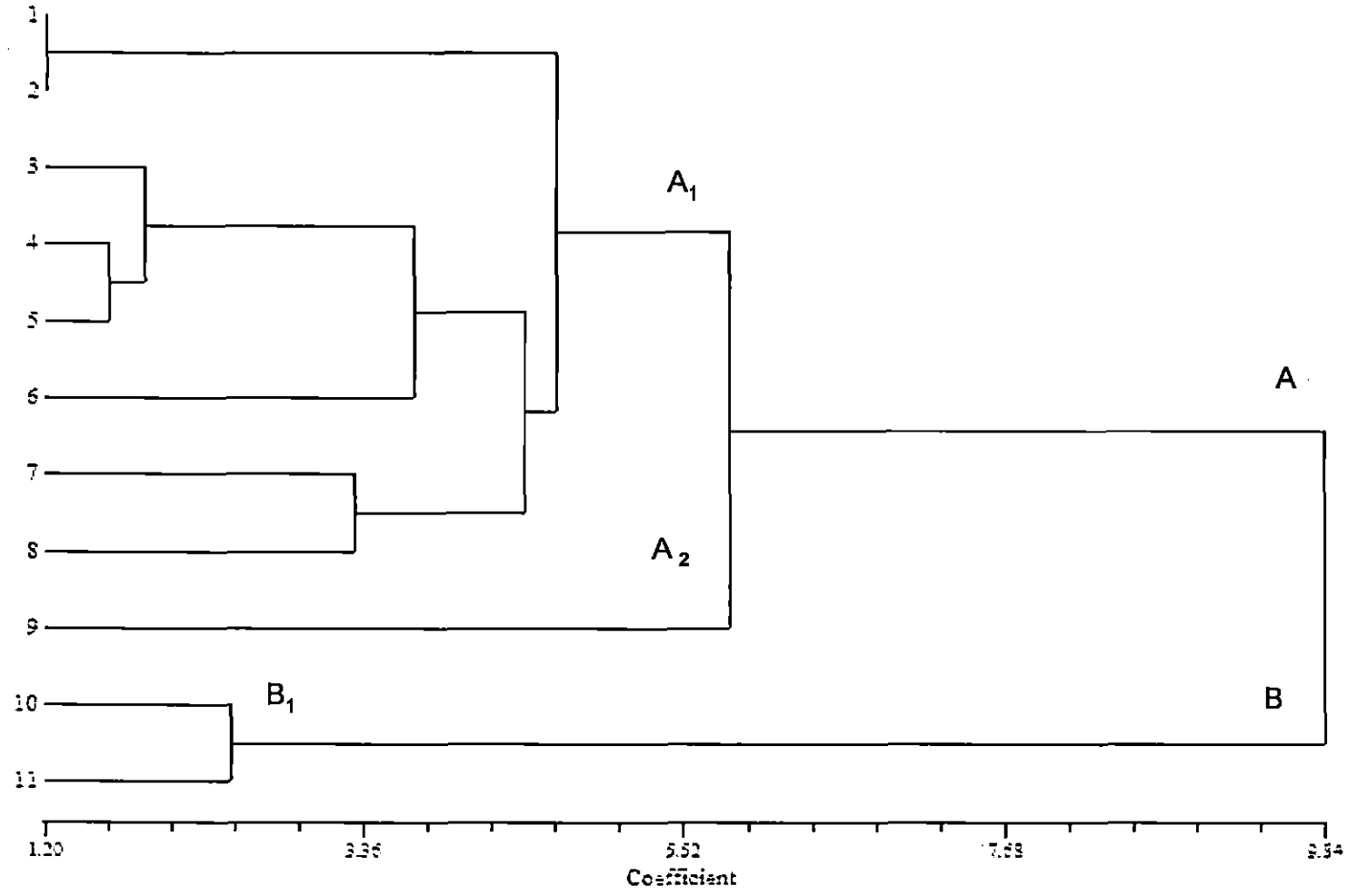
An *in vitro* experiment was carried out to study the mycelial compatibility of various isolates of *S. rolfsii* obtained from the selected fruit crops and ornamental plants by dual culture method on PDA medium. Per cent reduction in the growth of individual isolate over control was calculated when 4.5 cm growth

Table 12. Dissimilarity matrix of *S. rolfsii* isolates from selected fruit crops and ornamental plants based on cultural and morphological characters

	MG-1	MG -2	CH-1	CH-2	CH-3	CH -4	MN-1	MN-2	MN-3	BA-1	BA-2
MG -1	0										
MG -2	1.19	0									
CH -1	3.90	3.60	0								
CH -2	4.96	4.71	1.79	0							
CH -3	4.49	4.33	1.94	1.63	0						
CH-4	5.44	5.01	3.23	3.92	3.92	0					
MN-1	4.06	3.84	3.82	4.73	4.75	4.40	0				
MN-2	5.88	5.72	4.59	4.70	4.60	3.98	3.29	0			
MN-3	6.57	6.44	6.21	6.71	5.83	5.84	5.11	3.84	0		
BA-1	1.03	9.80	8.95	8.67	8.68	8.85	9.79	8.90	9.71	0	
BA-2	1.16	1.12	1.00	9.43	9.65	9.91	1.09	9.71	1.08	2.46	0

Table 13. Grouping of *S. rolfsii* isolates from selected fruit crops and ornamental plants based on dissimilarity index for cultural and morphological characters

Cluster	Sub cluster	Isolates
A	A_1	MG-1, MG-2, CH-1, CH-2, CH-3, CH-4, MN-1, MN-2
	A_2	MN-3
B	B_1	BA-1, BA-2



1. MG-1
2. MG-2
3. CH-1
4. CH-2
5. CH-3
6. CH-4
7. MN-1
8. MN-2
9. MN-3
10. BA-1
11. BA-2

Fig. 5. Unweighed Pair Group Average Method (UPGMA) dendrogram based on cultural and morphological characters of various isolates of *S. rolfsii*

recorded by the monoculture of the respective isolate. The compatibility / incompatibility reaction of different combinations of isolates was recorded when the isolates showed the development of sclerotia in the dual culture. The data are given in the Table 14 to 23 and the result revealed that there are compatible and incompatible reactions among the various isolates tested.

4.5.1. Compatibility of different isolates of *S. rolfsii* from the same host

The data on the compatibility test of different isolates from the same host are given in Table 14. From the data, it was found that all the isolates started growth on next day after inoculation in the dual culture and monoculture plates and completed 4.5 cm growth on mono culture plates on 3 DAI except the two isolates from banana. The isolates from banana (BA-1 and BA-2) completed the 4.5 cm growth on 2 DAI in the Petri dish.

Among two marigold isolates, MG-1 recorded 3.86 cm growth compared to MG-2 which recorded 4.5 cm growth on 3 DAI. An inhibition zone of width 0.7cm was developed in between the two isolates which recorded 14.07 per cent reduction in growth over control. Both the isolates produced sclerotia on the edges of the culture on either side of the inhibition zone (Table 15) (Plate 7). The compatibility test of four different isolates of chrysanthemum revealed that maximum reduction in growth over control (38.51 %) was recorded in CH-1when it was paired with CH-2. The minimum reduction in growth over control (10.37 %) was observed in CH-3 in the combination ofCH-1 × CH-3. Statistically it was on par with per cent reduction in growth recorded by the fourth isolate of chrysanthemum (CH-4) in combination with CH-2 (11.85%), CH-1 (12.59%) and CH-3 (12.59%).

Among the various combinations of isolates of *S. rolfsii* from chrysanthemum, except in CH-2 × CH-3 and CH-3 × CH-4 a clear zone of 0.4 cm to 2.6 cm width was observed on 6-7 DAI in all combinations. Sclerotial development was observed on 9-10 DAI in all combinations and were produced in a layer along the margin of the zone developed between two isolates.

Table 14. ***In vitro*** **evaluation on compatibility of different isolates of** ***S. rolfsii*** **from the respective hosts**

Sl. no	Isolates			Colony diameter (cm)*						Per cent reduction in growth	
				Days after incubation							
	1st	2nd		1		2		3			
				1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	MG-1	MG-2	D	1.76	2.10	2.30	2.60	3.86	4.50	14.07[a]	0 †
			M	2.50	2.20	3.20	2.90	4.50	4.50		
2	CH-1	CH-2	D	1.46	2.00	1.86	2.40	2.76	3.40	38.51[e]	24.44[e]
			M	2.00	2.24	2.50	3.64	4.50	4.50		
3	CH-1	CH-3	D	1.46	1.86	1.80	2.56	2.93	4.03	32.22[d]	10.37[b]
			M	2.00	2.56	2.50	3.40	4.33	4.50		
4	CH-1	CH-4	D	1.46	2.06	1.80	2.50	3.10	3.93	35.37[de]	12.59[b]
			M	2.00	2.50	2.50	3.53	4.33	4.50		
5	CH-2	CH-3	D	2.06	2.40	2.60	2.93	3.40	3.73	26.66[bc]	17.03[c]
			M	2.30	2.56	3.63	3.20	4.50	4.50		
6	CH-2	CH-4	D	2.00	2.06	2.40	2.50	3.40	3.96	26.66[bc]	11.85[b]
			M	2.23	2.50	3.63	3.53	4.50	4.50		
7	CH-3	CH-4	D	1.86	2.26	2.56	2.60	3.46	3.93	22.96[b]	12.59[b]
			M	2.30	2.70	3.20	3.53	4.50	4.50		
8	MN-1	MN-2	D	1.46	1.23	2.93	2.30	3.83	3.50	11.57[a]	22.22[de]
			M	3.86	2.93	4.13	3.86	4.33	4.50		
9	MN-1	MN-3	D	1.46	1.30	2.93	2.60	3.83	3.50	11.57[a]	19.26[cd]
			M	3.86	3.60	4.13	4.00	4.33	4.50		
10	MN-2	MN-3	D	1.50	1.80	1.83	3.00	2.06	3.93	55.55[f]	12.59[b]
			M	2.93	3.90	4.10	4.26	4.50	4.50		
11	BA-1	BA-2	D	1.50	1.73	3.26	3.46	-	-	27.35[C]	22.96[e]
			M	2.46	3.00	4.50	4.50	-	-		

†- Not included in statistical analysis

* Mean of 3 replications

D- Dual culture

M-Mono culture

In each column figures followed by same letter do not differ significantly according to DMRT

Table 15. Compatibility reactions of different combinations of *S. rolfsii* isolates from the respective hosts

Sl. no	Isolates	Observations recorded	Type of reaction
1	MG-1×MG-2	Mutual inhibition with a clear zone (0.7 cm). Sclerotia with exudate at interaction point.	MC
2	CH-1×CH-2	Mutual inhibition with a clear zone (2.6 cm). Sclerotia with exudate at interaction point.	MC
3	CH-1×CH-3	Mutual inhibition with a clear zone (2.0 cm). Sclerotia with exudate at interaction site.	MC
4	CH-1×CH-4	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
5	CH-2×CH-3	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
6	CH-2×CH-4	Mutual inhibition with a clear zone (0.4 cm). Sclerotia with exudate at interaction point.	MC
7	CH-3×CH-4	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
8	MN-1×MN-2	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
9	MN-1×MN-3	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
10	MN-2×MN-3	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
11	BA-1×BA-2	Mutual inhibition with a clear zone (1.5 cm). Sclerotia with exudate at interaction zone point.	MC

I-Intermingling of hyphae MC-Mutual inhibition with clear zone

Among the isolates from mango, the maximum reduction in growth (55.55%) over control was observed in MN-2 when it was paired with MN-3 and the minimum reduction in growth (11.57%) was recorded by MN-1 in combination with MN-2 and MN-3. Mutual inhibition was observed in dual culture of MN-1 × MN-2, MN-2 × MN-3 and MN-1 × MN-3. Thick mycelial band was noticed at the interaction point of MN-1 and MN-3. Sclerotia of the paired isolates were developed near the thick band of mycelia.

In the dual culture of the isolates from banana BA-1 and BA-2 recorded 27.35 and 22.96 per cent inhibition over control respectively. A clear demarcation of 1.5 cm width was noticed between these isolates. The sclerotial formation was observed on either side of the inhibition zone (Table 15).

4.5.2. Compatibility of different isolates from the selected ornamental plants

The mycelial compatibility of two isolates from marigold and four isolates from chrysanthemum was studied under *in vitro* conditions. In all the combinations, except inCH-3 × MG-1, the mono cultures of the various isolates completed 4.5cm growth on 3rd DAI. Among the various combinations the third isolate of chrysanthemum, CH-3 recorded the maximum reduction in growth over control (56.85 %) when paired with marigold isolate, MG-1. The same isolate when paired with MG-2 recorded 54.07 per cent inhibition over control and statistically both were on par with each other. The isolate from chrysanthemum, CH-1 in both combinations (CH-1× MG-1 and CH-1 × MG-2) recorded the minimum inhibition in growth (22.96%) over control (Table 16).

Among the isolates from marigold, the maximum inhibition in growth was noticed in MG-1, when paired with CH-1 (43.70%) and the minimum value was recorded by MG-2 when paired with CH-2 (21.47%). In other combinations, marigold isolates showed the reduction in growth within a range of 24.44 to 38.51 per cent over control.

In all the combinations of four isolates from chrysanthemum and two isolates from marigold, except in one combination (CH-2 × MG-2) an inhibition

Table 16. ***In vitro*** **evaluation on compatibility of different isolates of** ***S. rolfsii*** **from the selected ornamental plants**

Sl. no	Isolates			Colony diameter (cm)* Days after incubation						Per cent reduction in growth	
	1st	2nd		1		2		3			
				1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	CH-1	MG-1	D	2.00	1.53	2.50	2.06	3.46	2.53	22.96[a]	43.70[d]
			M	2.00	2.16	2.66	2.30	4.50	4.50		
2	CH-1	MG-2	D	2.00	1.56	2.60	2.50	3.46	2.76	22.96[a]	38.51[c]
			M	2.00	2.16	2.83	2.73	4.50	4.50		
3	CH-2	MG-1	D	1.16	1.56	1.63	1.86	3.03	3.30	32.59[b]	26.66[b]
			M	2.36	2.16	3.63	2.30	4.50	4.50		
4	CH-2	MG-2	D	1.16	1.46	1.63	1.96	3.13	3.53	30.36[b]	21.47[a]
			M	2.36	2.16	3.63	2.73	4.50	4.50		
5	CH-3	MG-1	D	1.13	1.66	1.36	1.96	1.86	2.93	56.85[d]	34.81[c]
			M	2.10	2.16	3.23	2.30	4.33	4.50		
6	CH-3	MG-2	D	1.26	1.43	1.66	2.53	2.06	3.40	54.07[d]	24.44[ab]
			M	2.10	2.16	3.23	2.73	4.50	4.50		
7	CH-4	MG-1	D	1.70	1.76	2.06	2.13	2.56	2.93	42.96[c]	34.81[c]
			M	2.50	2.00	3.16	2.96	4.50	4.50		
8	CH-4	MG-2	D	1.43	1.70	2.00	2.26	2.63	2.73	42.96[c]	34.81[c]
			M	2.50	2.16	3.16	2.96	4.50	4.50		

* Mean of 3 replications D- Dual culture M- Mono culture

In each column figures followed by same letter do not differ significantly according to DMRT

Table 17. Compatibility reactions of different combinations of *S. rolfsii* isolates from selected ornamental plants

Sl. no	Isolates	Observations recorded	Type of reaction
1	CH-1×MG-1	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
2	CH-1×MG-2	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
3	CH-2× MG-1	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction point.	MC
4	CH-2× MG-2	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
5	CH-3× MG-1	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction point.	MC
6	CH-3× MG-2	Clear demarcation with inhibition zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
7	CH-4× MG-1	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
8	CH-4× MG-2	Clear demarcation with inhibition zone (0.4 cm). Sclerotia with exudate at interaction point.	MC

I-Intermingling of hyphae MC-Mutual inhibition with clear zone

zone having width of 0.3-0.5 cm was noticed on 6-7 DAI and the sclerotial development was observed on either side of the inhibition zone as a thin layer on 9-10 DAI. In the combinations of CH-2 × MG-2 the growth of the both isolates met at the centre of the Petri dish. The sclerotial formation was observed in a line at the meeting point of the two isolates (Table 17).

4.5.3. Compatibility of different isolates from the selected fruit crops

An *in vitro* study was carried out to know the mycelial compatibility of three isolates of *S. rolfsii* from mango and two isolates from banana. The monoculture of isolates from banana took two days and isolates from mango took three days to complete full growth of 4.5 cm in the Petri dish. Among the combinations, the maximum reduction in growth over control (47.4 %) was observed in MN-2 isolate when paired with first isolate from banana (BA-1). A fluffy mycelial growth was also noticed in the culture of MN-2 when paired with BA-1.

The isolate MN-3 recorded the minimum reduction of growth (24.44 %) over control when paired with banana isolate BA-2. Among the isolates from banana BA-2 when paired with MN-3 recorded the minimum reduction of growth over control (5.92 %). The maximum reduction of growth of BA-1 was observed when it was paired with MN-1 and also paired with MN-2 which recorded 33.33 per cent reduction over control in both combinations (Table 18).

In the dual culture of all these isolates, intermingling and overgrowth of the hyphae were absent. Clear zone of 0.2 cm width was observed in all combinations on 6-7 DAI. The isolates from banana (BA-1 and BA-2) produced large number of small sized sclerotia on the surface of the culture and also observed on the periphery of the Petri dishes on 9-10 DAI. A fluffy mycelial growth was noticed in the culture of MN-2 when paired with BA-2 (Table 19).

Table 18. ***In vitro*** **evaluation on compatibility of different isolates of** ***S. rolfsii*** **from the selected fruit crops**

Sl. no	Isolates			Colony diameter (cm)* Days after incubation						Percent reduction in growth	
				1		2		3			
	1st	2nd		1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	MN-1	BA-1	D	1.50	2.50	1.75	3.00	2.50	3.33		
			M	3.80	2.46	4.00	4.50	4.23	4.50	40.80^{c}	33.33^{d}
2	MN-1	BA-2	D	1.83	3.00	2.20	2.93	2.53	3.93		
			M	3.80	3.00	4.00	4.50	4.23	4.50	40.06^{c}	12.59^{b}
3	MN-2	BA-1	D	1.30	2.50	1.65	2.25	2.36	3.00		
			M	2.93	3.00	3.20	4.50	4.50	4.50	47.40^{d}	33.33^{d}
4	MN-2	BA-2	D	1.93	2.93	2.40	3.45	2.93	4.06		
			M	2.93	3.00	3.20	4.50	4.50	4.50	34.81^{b}	9.627^{ab}
5	MN-3	BA-1	D	2.13	2.36	2.65	2.75	3.00	3.63		
			M	3.60	2.46	3.95	4.50	4.50	4.50	33.33^{b}	19.26^{c}
6	MN-3	BA-2	D	1.93	3.00	2.60	3.65	3.40	4.23		
			M	3.60	3.00	3.95	4.50	4.50	4.50	24.44^{a}	5.92^{a}

* Mean of 3 replications D- Dual culture M- Mono culture

In each column figures followed by same letter do not differ significantly according to DMRT

Table 19. Compatibility reactions of different combinations of *S. rolfsii* isolates from the selected fruit crops

Sl. no	Isolates	Observations recorded	Type of reaction
1	MN-1×BA-1	Mutual inhibition with a clear zone (0.2cm). Sclerotia with exudate at interaction point.	MC
2	MN-1×BA-2	Mutual inhibition with a clear zone (0.2cm). Sclerotia with exudate at interaction site.	MC
3	MN-2×BA-1	Mutual inhibition with a clear zone (0.3 cm). Sclerotia with exudate at interaction point.	MC
4	MN-2×BA-2	Mutual inhibition with a clear zone (0.2cm). Sclerotia with exudate at interaction site.	MC
5	MN-3×BA-1	Mutual inhibition with a clear zone (0.2cm). Sclerotia with exudate at interaction point.	MC
6	MN-3×BA-2	Mutual inhibition with a clear zone (0.2cm). Sclerotia with exudate at interaction site.	MC

MC- Mutual inhibition with clear zone

4.5.4. Compatibility of different isolates from the selected ornamental plants and fruit crops

The results of the *in vitro* study carried out to know the mycelial compatibility of different isolates obtained from ornamental plants *viz.*, marigold and chrysanthemum and fruit crops *viz.*, mango and banana are presented in Table 20. The results showed that the monocultures of the two isolates from banana (BA-1 and BA-2) completed the 4.5 cm growth in Petri dishes on 2 DAI whereas the monoculture of two isolates from marigold (MG-1 and MG-2), four isolates from chrysanthemum (CH-1, CH-2, CH-3 and CH-4) and three isolates from Mango (MN-1, MN-2 and MN-3) took 3 days to complete 4.5 cm growth in Petri dishes.

In all the combinations of MG-1 and MG-2 with BA-1 and BA-2, it was observed that the growth of banana isolates was inhibited by the isolate from marigold. The maximum inhibition in growth of banana isolates over control (67.40 %) was noticed in the combination of MG-1 × BA-2 and the minimum reduction in growth was observed in the combination of MG-2 × BA-1. Per cent reduction in growth of isolates from marigold was found in the range of 26.61 to 43.70 per cent with minimum and maximum values were observed in the combinations of MG-1 × BA-2 and MG-2 × BA-1 respectively. In the dual culture plates of all these combinations except in MG-2 × BA-2 a clear inhibition zone of 0.5 cm width was observed on 6-7 DAI. Fluffy mycelial growth was observed in the culture plates of isolates from marigold and sclerotial formation by the isolates from marigold and banana was observed on the peripheral layer of the culture on 9-10 DAI. In the combinations of MG-2 × BA-2, the inhibition zone and overgrowth were absent and at the point of contact of the mycelium of the two isolates, large sized sclerotial formation was observed on 9-10 DAI. Large number of small sized sclerotial formation was observed on the surface of the mycelial growth of BA-2 culture.

Table 20. *In vitro* evaluation on compatibility of different isolates of *S. rolfsii* from the selected ornamental plants and fruit crops

Sl. no	Isolates			Colony diameter (cm) *						Per cent reduction in growth	
				Days after incubation							
				1		2		3			
	1st	2nd		1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	MG-1	BA-1	D	1.56	1.23	1.92	1.50	3.20	1.93	28.80^{ef}	57.03^{de}
			M	2.50	2.46	3.20	4.50	4.50	4.50		
2	MG-1	BA-2	D	1.53	1.13	2.40	1.25	3.30	1.46	26.61^{de}	67.40^{g}
			M	2.50	3.00	3.20	4.50	4.50	4.50		
3	MG-2	BA-1	D	1.50	1.26	1.75	1.85	2.53	2.06	43.70^{h}	54.07^{d}
			M	2.50	2.46	3.45	4.50	4.50	4.50		
4	MG-2	BA-2	D	1.50	1.23	2.35	1.55	3.06	1.76	31.85^{fg}	60.74^{ef}
			M	2.50	3.00	3.15	4.50	4.50	4.50		
5	CH-1	BA-1	D	2.13	1.86	2.85	2.15	3.93	2.46	12.59^{a}	45.18^{c}
			M	2.26	2.46	3.25	4.50	4.50	4.50		
6	CH-1	BA-2	D	2.13	1.90	2.75	2.65	3.93	3.66	12.59^{a}	18.52^{a}
			M	2.26	3.00	3.25	4.50	4.50	4.50		
7	CH-2	BA-1	D	2.36	1.86	2.95	2.25	3.63	2.46	19.26^{b}	45.18^{c}
			M	2.80	2.46	3.45	4.50	4.50	4.50		
8	CH-2	BA-2	D	2.36	2.13	2.86	2.45	3.50	2.73	21.48^{bc}	39.99^{b}
			M	2.80	3.00	3.45	4.50	4.50	4.50		
9	CH-3	BA-1	D	2.20	1.30	2.85	1.50	3.43	1.60	23.70^{cd}	64.44^{fg}
			M	2.33	2.46	3.55	4.5	4.50	4.50		
10	CH-3	BA-2	D	2.20	1.93	2.35	2.05	2.46	2.10	45.18^{h}	53.33^{d}
			M	2.33	3.00	3.55	4.50	4.50	4.50		
11	CH-4	BA-1	D	2.16	1.86	2.65	2.25	2.93	2.43	34.81^{g}	45.92^{c}
			M	2.70	2.46	3.65	4.50	4.50	4.50		
12	CH-4	BA-2	D	2.16	1.73	2.65	1.80	2.93	1.93	34.81^{g}	57.03^{de}
			M	2.70	3.00	3.65	4.50	4.50	4.50		

* Mean of 3 replications D- Dual culture M- Mono Culture

In each column figures followed by same letter do not differ significantly according to DMRT

Reduction in the growth of banana isolates was observed in all the combinations of four isolates from chrysanthemum and two isolates from banana, with maximum and minimum values noticed in the combinations of CH-3 × BA-2 and CH-1 × BA-2 respectively. Reduction in growth over control of four different isolates from chrysanthemum was observed in the range of 12.59 to 45.18 per cent. An inhibition zone of width 0.2 to 0.5 cm was observed in all combinations of these isolates in the dual culture plates on 6-7 DAI. The isolate from banana were found to produce sclerotia on the surface and on the outer periphery of the culture on 9-10 DAI. Sclerotial formation by the isolates from chrysanthemum was observed near the zone in the centre of the Petri dishes on 9-10 DAI (Table 21).

When the isolates from marigold were paired with isolates from mango, the reduction of growth of mango isolates was found more compared to marigold isolates. The maximum inhibition in growth of mango isolates (57.03 %) was noticed in combinations of MG-2 with MN-2 and MN-3 whereas the maximum reduction in growth (28.80 %) of marigold isolates was observed in the combinations of MG-1 with MN-1, MN-2 and MN-3. The minimum inhibition in growth of marigold (10.37 %) was observed in the combinations of MG-2 × MN-3 (Table 22). In all combinations except in MG-2 × MN-2 an inhibition zone of 0.3 cm to 0.7 cm width was observed in between the paired isolates on 6-7 DAI and the sclerotial formation was observed mainly along the curved inhibition zone on 9-10 DAI depending on the inhibition of growth. In the combination of MN-2 × MG-2, the inhibition zone was absent. The mycelia of both isolates were met at the centre and thickening of mycelium of MN-2 isolate was observed. MN-2 × MG-2 produced sclerotia at the interaction point of mycelia and the number of sclerotia produced by each isolate was found very less on 9-10 DAI. The second isolate from mango (MN-2) produced fluffy mycelial growth in the dual and mono cultures (Table 23).

In the combinations of four isolates from chrysanthemum and three isolates from mango, a uniform type of reaction was not observed. The isolate

Table 21. Compatibility reactions of various combinations of isolates of *S. rolfsii* from selected ornamental plants and fruit crops

Sl. no	Isolates	Observations recorded	Type of reaction
1	MG-1×BA-1	Clear demarcation with inhibition zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
2	MG-1×BA-2	Clear demarcation with inhibition zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
3	MG-2×BA-1	Clear demarcation with inhibition zone (0.5 cm). Sclerotia with exudate at interaction site.	MC
4	MG-2×BA-2	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
5	CH-1×BA-1	Mutual inhibition with a clear zone (0.4 cm). Sclerotia with exudate at interaction point.	MC
6	CH-1×BA-2	Mutual inhibition with a clear zone (0.2 cm). Sclerotia with exudate at interaction point.	MC
7	CH-2×BA-1	Mutual inhibition with a clear zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
8	CH-2×BA-2	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
9	CH-3×BA-1	Mutual inhibition with a clear zone (0.4 cm). Sclerotia with exudate at interaction point.	MC
10	CH-3×BA-2	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
11	CH-4×BA-1	Clear demarcation with inhibition zone (0.2 cm). Sclerotia with exudate at interaction site.	MC
12	CH-4×BA-2	Mutual inhibition with a clear zone (0.2 cm). Sclerotia with exudate at interaction point.	MC

I- Intermingling of hyphae MC- Mutual inhibition with clear zone

Table 22. ***In vitro*** **evaluation on compatibility of different isolates of** ***S. rolfsii*** **from selected ornamental plants and fruit crops**

Sl. no	Isolates			Colony diameter (cm)*						Per cent reduction in growth	
				Days after incubation							
				1		2		3			
	1st	2nd		1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	MG-1	MN-1	D	1.50	1.23	3.00	2.50	3.20	2.93	28.80[b]	34.81[a]
			M	2.50	3.86	3.16	4.13	4.50	4.50		
2	MG-1	MN-2	D	1.50	1.06	3.00	2.06	3.20	2.60	28.80[b]	42.22[b]
			M	2.50	2.93	3.16	3.86	4.50	4.50		
3	MG-1	MN-3	D	1.50	1.33	3.00	2.43	3.20	2.86	28.80[b]	36.29[a]
			M	2.50	3.6	3.16	4.00	4.50	4.50		
4	MG-2	MN-1	D	1.80	1.50	3.33	1.93	4.00	2.13	11.11[a]	52.59[c]
			M	2.50	3.86	2.96	4.13	4.50	4.50		
5	MG-2	MN-2	D	1.80	1.33	2.93	1.63	4.00	1.93	11.11[a]	57.03[c]
			M	2.50	2.93	2.96	3.86	4.50	4.50		
6	MG-2	MN-3	D	1.80	1.50	2.93	1.83	4.06	1.93	10.37[a]	57.03[c]
			M	2.50	3.60	2.96	4.00	4.50	4.50		
7	CH-1	MN-1	D	1.46	2.06	1.86	2.56	2.86	3.36	36.29[cd]	25.18[b]
			M	2.00	3.86	2.50	4.13	4.50	4.50		
8	CH-1	MN-2	D	1.46	1.86	1.86	2.33	2.73	2.26	38.51[d]	31.85[de]
			M	2.00	3.16	2.50	3.50	4.50	4.50		
9	CH-1	MN-3	D	1.46	1.43	1.86	1.73	2.73	2.26	39.25[d]	49.63[f]
			M	2.00	3.66	2.50	4.06	4.50	4.50		

10	CH-2	MN-1	D	2.06	1.53	2.43	2.06	3.06	2.86	31.84^{bc}	36.29^{e}
			M	2.36	2.73	3.63	3.50	4.50	4.50		
11	CH-2	MN-2	D	2.50	1.20	3.33	1.53	3.66	3.13	18.51^{a}	30.36^{cd}
			M	2.50	2.90	3.50	3.93	4.50	4.50		
12	CH-2	MN-3	D	2.06	1.93	2.43	2.20	3.13	2.96	30.36^{cd}	34.07^{de}
			M	2.36	3.60	3.63	4.00	4.50	4.50		
13	CH-3	MN-1	D	1.26	1.53	1.40	2.06	1.46	3.43	66.01^{e}	23.70^{ab}
			M	2.10	2.93	3.06	3.50	4.50	4.50		
14	CH-3	MN-2	D	1.26	1.76	1.40	2.33	1.46	2.93	66.01^{e}	32.59^{de}
			M	2.10	3.10	3.06	3.66	4.50	4.50		
15	CH-3	MN-3	D	1.26	2.06	1.40	2.50	1.46	3.53	64.72^{e}	21.48^{ab}
			M	2.10	3.60	3.06	4.00	4.16	4.50		
16	CH-4	MN-1	D	1.93	1.50	2.50	1.86	3.53	3.06	21.48^{a}	26.29^{bc}
			M	2.50	3.00	3.16	3.66	4.50	4.50		
17	CH-4	MN-2	D	1.93	1.20	2.50	1.50	3.53	2.10	21.48^{a}	51.39^{f}
			M	2.50	2.93	3.50	3.86	4.50	4.33		
18	CH-4	MN-3	D	1.93	2.20	2.43	2.60	3.06	3.60	31.85^{bc}	20.00^{a}
			M	2.50	3.60	3.16	4.00	4.50	4.50		

* Mean of 3 replications D- Dual culture M- Mono culture

In each column figures followed by same letter do not differ significantly according to DMRT

Table 23. Compatibility reactions of various combinations of *S. rolfsii* isolates from different ornamental plants and fruit crops

Sl. no	Isolates	Observations recorded	Type of reaction
1	MG-1× MN-1	Clear demarcation with inhibition zone (0.7 cm). Sclerotia with exudate at interaction point.	MC
2	MG-1× MN-2	Clear demarcation with inhibition zone (0.4 cm). Sclerotia with exudate at interaction site.	MC
3	MG-1× MN-3	Mutual inhibition with a clear zone (0.6 cm). Sclerotia with exudate at interaction site.	MC
4	MG-2× MN-1	Mutual inhibition with a clear zone (0.4 cm). Sclerotia with exudate at interaction point.	MC
5	MG-2× MN-2	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
6	MG-2 × MN-3	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
7	CH-1× MN-1	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction point.	MC
8	CH-1× MN-2	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction site.	MC

9	CH-1 × MN-3	Clear demarcation with inhibition zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
10	CH-2 × MN-1	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction site.	MC
11	CH-2 × MN-2	Clear demarcation with inhibition zone (0.5 cm). Sclerotia with exudate at interaction site.	MC
12	CH-2 × MN-3	Mutual inhibition with a clear zone (0.3 cm). Sclerotia with exudate at interaction site.	MC
13	CH-3 × MN-1	Clear demarcation with inhibition zone (1.0 cm). Sclerotia with exudate at interaction site.	MC
14	CH-3 × MN-2	Mutual inhibition with a clear zone (0.5 cm). Sclerotia with exudate at interaction site.	MC
15	CH-3 × MN-3	Clear demarcation with inhibition zone (1.0 cm). Sclerotia with exudate at interaction site.	MC
16	CH-4 × MN -1	Mutual inhibition with a clear zone (0.2 cm). Sclerotia with exudate at interaction site.	MC
17	CH-4 × MN-2	Intermingling of hyphae and sclerotia with exudate at interaction point.	I
18	CH-4× MN-3	Clear demarcation with inhibition zone (0.2 cm). Sclerotia with exudate at interaction site.	MC

I-Intermingling of hyphae

MC- Mutual inhibition with clear zone

CH-2 inhibited the growth of three isolates from mango and the maximum reduction in growth (36.29 %) was observed in CH-2 × MN-1 combination. The isolate from chrysanthemum CH-3 was found inhibited by the three isolates from mango *viz.*, (MN-1, MN-2 and MN-3) and they recorded 66.01, 66.01 and 64.72 per cent inhibition respectively. Among all the combinations, the minimum reduction in growth over control (18.51 %) was recorded by the isolate CH-2 when it was paired with MN-2. In dual culture plates, an inhibition zone of 0.2 to 1.0 cm width was observed on 6-7 DAI in all combinations except CH-4 × MN-2, in which overgrowth ofCH-4 on MN-2 was noticed.

Based on the *in vitro* evaluation on the mycelial compatibility of various isolates of selected fruit crops and ornamental plants, a compatibility chart was prepared and is given in the Table 24. From this, it was revealed that eight combinations of isolates of *S. rolfsii* were found compatible with each other and the remaining 47 combinations were incompatible (Plate 7a). It was observed that the isolate MN-2 was compatible with MG-2; the isolate BA-2 was compatible with MG-2; CH-4 and MN-2; MN-1 and MN-2; MN-1 and MN-3 recorded compatible reactions when paired with each other. The isolate CH-3 showed mycelial compatibility with two other isolates from chrysanthemum *viz.*, CH-2 and CH-4 (Plate 7b).

4.6. PATHOGENIC VARIABILITY

The variability in pathogenicity of different isolates of *S. rolfsii* were studied under *in vivo* conditions by cross inoculating the different isolates on selected fruit crops and ornamental plants. The observations are presented in Table 25.

4.6.1. Pathogenic variability of different isolates of *S. rolfsii* from chrysanthemum, mango and banana on marigold

The different isolates of *S. rolfsii* obtained from chrysanthemum, mango and banana were cross inoculated on leaves and collar region of marigold and it was noticed that all the isolates were pathogenic to marigold (Plate 8a). There was

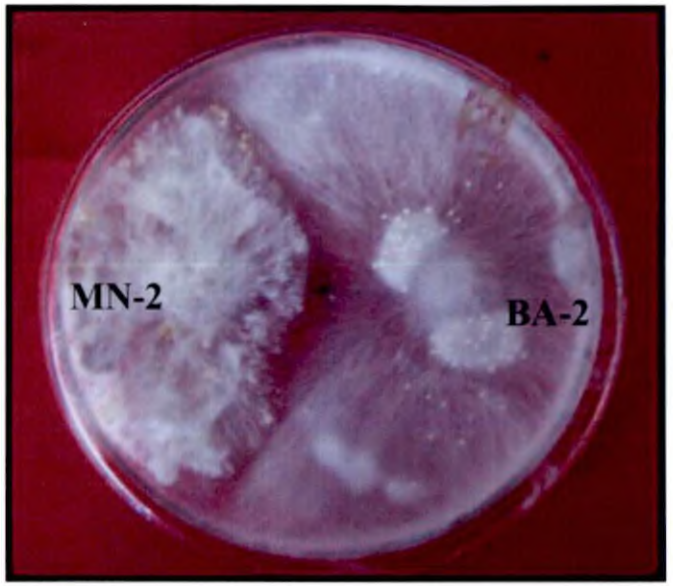
Table 24. Compatibility of various isolates of *S. rolfsii*

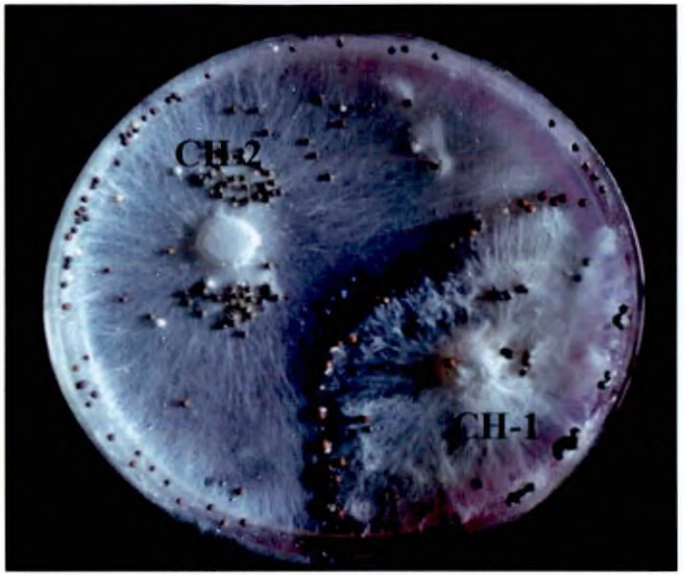
Isolate	MG-1	MG-2	CH-1	CH-2	CH-3	CH-4	MN-1	MN-2	MN-3	BA-1	BA-2
MG-1		IC	IC	IC	IC	IC	IC	IC	IC	IC	IC
MG-2			IC	C	IC	IC	IC	C	IC	IC	C
CH-1				IC	IC	IC	IC	IC	IC	IC	IC
CH-2					C	IC	IC	IC	IC	IC	IC
CH-3						C	IC	IC	IC	IC	IC
CH-4							IC	C	IC	IC	IC
MN-1								C	C	IC	IC
MN-2									IC	IC	IC
MN-3										IC	IC
BA-1											IC
BA-2											

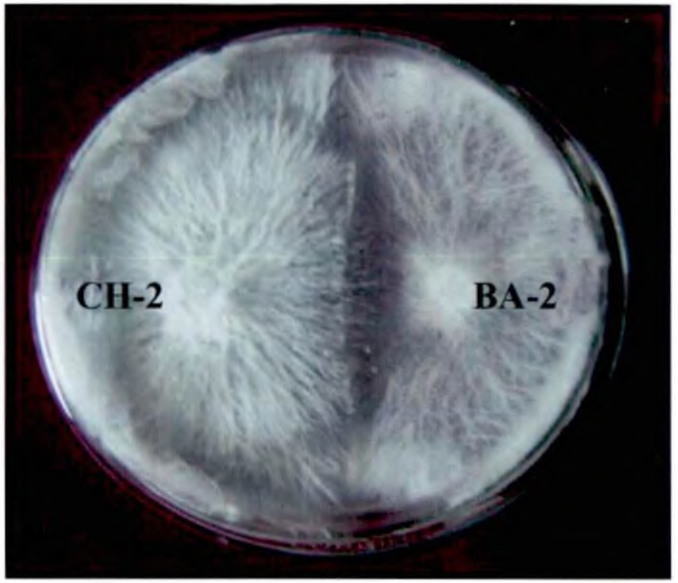
C-Compatible IC- Incompatible

Plate 7. Mycelial compatibility reaction between isolates of *S. rolfsii*

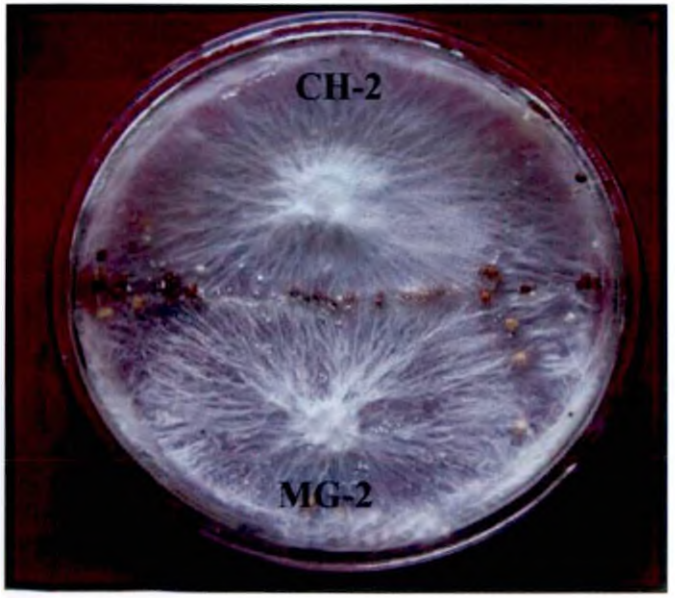
a.Incompatible isolates

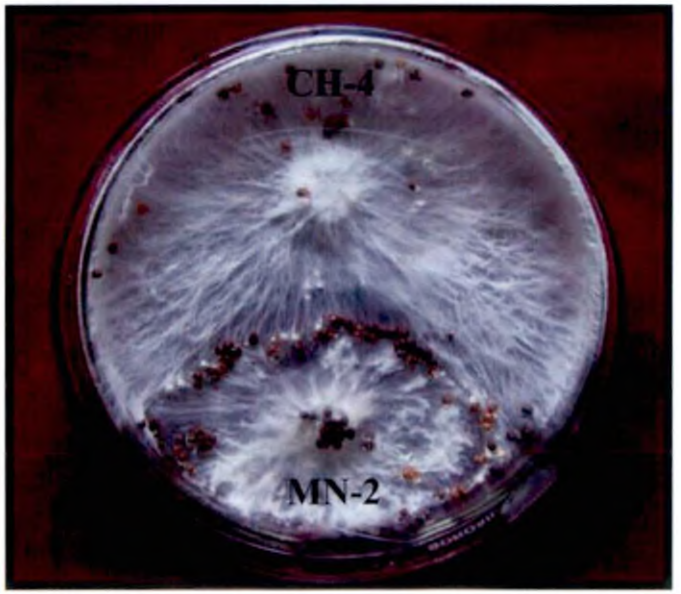






b. Compatible isolates





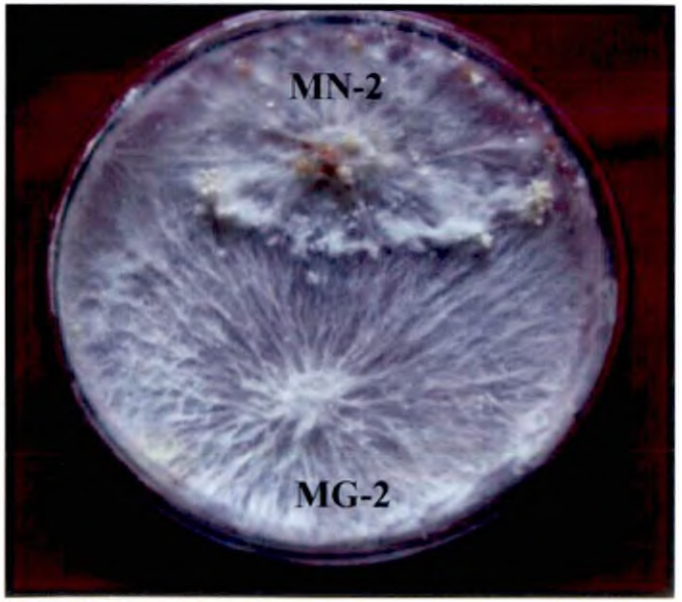


Table 25. Pathogenic variability of different isolates of *S. rolfsii*

Host	Isolate	Symptom initiation			Presence of mycelial growth	Presence of sclerotia
		Leaves	Collar region	Pseudostem		
		DAI	DAI			
Marigold	CH-1	3	4		+	+
	CH-2	3	4		+	+
	CH-3	3	4		+	+
	CH-4	3	4		+	+
	MN-1	4	4		+	+
	MN-2	3	3		+	+
	MN-3	4	4		+	+
	BA-1	3	3		+	+
	BA-2	3	3		+	+
Chrysanthemum	MG-1	3	5		+	+
	MG-2	3	5		+	+
	MN -1	3	4		+	+
	MN -2	3	4		+	+
	MN-3	3	4		+	+
	BA-1	3	-		+	+
	BA-2	3	-		+	+
Mango	MG-1	3	-		+	+
	MG-2	3	-		+	+
	CH-1	3	-		+	+
	CH-2	3	-		+	+
	CH-3	3	-		+	+
	CH-4	3	-		+	+
	BA-1	3	-		+	+
	BA-2	3	-		+	+
Banana	MG-1	3	3	5	+	+
	MG-2	3	3	5	+	+
	CH-1	3	5	5	+	+
	CH-2	3	5	5	+	+
	CH-3	3	5	5	+	+
	CH-4	3	5	5	+	+
	MN -1	3	5	5	+	+
	MN -2	3	5	5	+	+
	MN-3	3	5	5	+	+

+ = Present - = Absent

no variation in the symptom expression on marigold by different isolates from the symptoms developed on their respective hosts. All the isolates except MN-1 and MN-3 recorded initial infection on leaves of marigold on 3 DAI which observed as small, water soaked dark brown lesions with yellow halo. Later, the infection enlarged and resulted in leaf rot. The isolates from mango (MN-1 and MN-3) recorded infection on leaves on 4 DAI. White thick mycelial growth of the pathogen was noticed on leaves 4-5 DAI. Sclerotial formation by all the isolates was not observed on the inoculated leaves on 6-7 DAI. Variation in collar infection by the different isolates of *S.rolfsii* was noticed on marigold. The isolate MN-2 and the two isolates from banana (BA-1 and BA-2) recorded collar infection on 3 DAI whereas all the four isolates from chrysanthemum, two isolates from mango (MN-1 and MN-3) recorded collar infection on 4 DAI. The infection initiated as small water soaked dark brown lesion on collar region. Later, infection advanced to collar rot with white mycelial growth of the pathogen and numerous dark brown coloured sclerotia. On 7-9 DAI shredding of the collar region was observed in plants inoculated on collar region with the different isolates. Collar infection resulted in wilting and drying of whole plant and was observed in all isolates.

4.6.2. Pathogenic variability of different isolates of *S. rolfsii* from marigold, mango and banana on chrysanthemum

The cross inoculation of different isolates of *S. rolfsii* obtained from marigold, mango and banana on chrysanthemum showed that all the isolates were pathogenic to chrysanthemum (Plate 8b). There was no variation in the symptom developed in chrysanthemum by the different isolates from the same host. All isolates recorded initial infection on leaves on 3 DAI which appeared as small dark brown coloured, water soaked lesion. Later this lesion enlarged with yellow halo and covered 75 per cent of the leaf area resulted in leaf blight symptom. The blighted area fully covered with thick white mycelial growth and was observed on both surfaces of leaves. On 6 DAI brown coloured sclerotial development was observed on infected leaves. Variation in the infection by the different isolates on

collar region of chrysanthemum was noted. Collar infection was not recorded in chrysanthemum inoculated with the two isolates from banana. The two isolates from marigold and three isolates from mango initiated the infection on collar region on 5 and 4 DAI respectively. Dark brown coloured water soaked lesions were developed on the collar region which later coalesced and developed as large lesions. Under high humid condition white thick mycelial growth with dark brown, round, smooth sclerotial formation was noticed. Wilting and drying was observed in plants infected at the collar region.

4.6.3. Pathogenic variability of different isolates of *S. rolfsii* from marigold, chrysanthemum and banana on mango

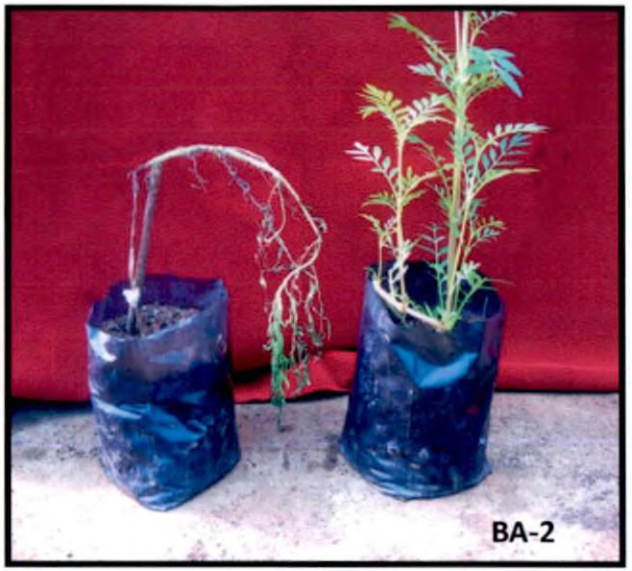
The isolates of *S. rolfsii* from marigold, chrysanthemum and banana, when cross inoculated on mango, it was observed that all the isolates were pathogenic to mango (Plate 8c). The different isolates from the same host showed no variation in the symptom expression on mango. All the isolates produced infection only on leaves and the initial infection was recorded on 3 DAI. Infection on collar region was not recorded in any of the mango seedlings inoculated with different isolates. On the leaves, the initial infection appeared as small, round, dark brown coloured, water soaked spot. The infection enlarged to leaf blight symptom with yellow halo. White thick mycelial growth was observed on lower surface of leaves on 5-6 DAI. White coloured sclerotial formation was noticed on leaves on 6-7 DAI. Finally the leaf blight symptom advanced to half of the leaf area and such leaves blighted and dried.

4.6.4. Pathogenic variability of different isolates of *S. rolfsii* from marigold, chrysanthemum and mango on banana

Tissue culture banana plants were inoculated separately on leaf, pseudostem and collar region with different isolates from marigold, chrysanthemum and mango. It was observed that banana plants was susceptible to all the isolates and showed infection on all parts inoculated (Plate 8d). The isolate from marigold (MG-1and MG-2) recorded initial infection on leaves, pseudostem

Plate 8. Pathogenic variability of different isolates of *S. rolfsii*

a. Marigold

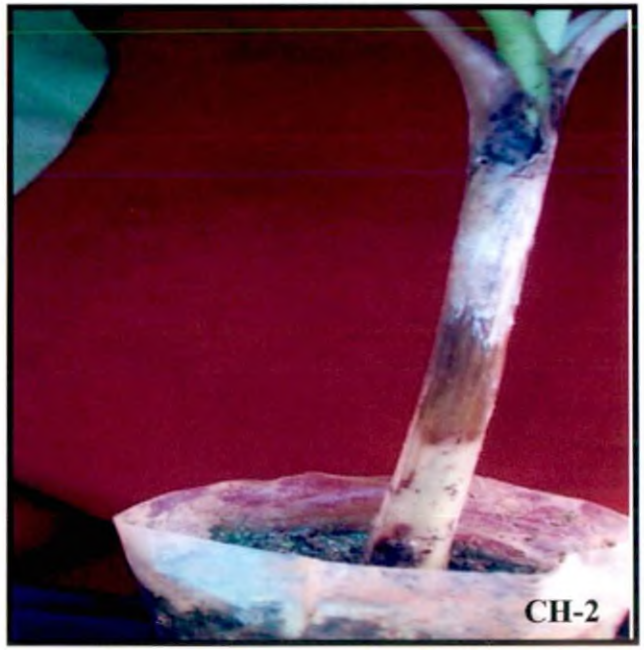


b. Chrysanthemum

Plate 8. Continued

c. Mango

d. Banana



and collar region on 3 DAI. The isolates from other two crops *viz.*, chrysanthemum and mango recorded initial infection only on leaves on 3 DAI and on other parts of the plant, the infection initiated on 5 DAI. The infection appeared as small, dark brown water soaked lesion on both surfaces of the leaves which enlarged to leaf blight symptom with yellow halo. Water soaked lesion was observed on pseudostem and collar region inoculated with the different isolates. On all the parts inoculated, white mycelial growth of the pathogen with dark brown sclerotia were observed on 5 to 7 DAI. In advanced stage yellowing, drying up of the leaves and death of the plant were observed in plants inoculated on the collar region and pseudostem.

4.7. HOST RANGE

An *in vitro* study was conducted to know the host range of different isolates of *S.rolfsii* obtained from the selected fruit crops and ornamental plants. The pure culture of the most virulent isolate from each crop was used for inoculation on selected vegetables *viz.*, tomato and amorphophallus and spices *viz.*, black pepper and ginger. The plants were inoculated with mycelia and sclerotia of each isolate together after giving pinprick. The results of the study revealed that all the inoculated plants recorded infection on inoculation with different isolates of *S. rolfsii.* The observations are presented in Table 26.

4.7.1. Pathogenicity of various isolates of *S. rolfsii* on tomato

The tomato plants inoculated with the virulent isolates from marigold (MG-2), chrysanthemum (CH-2), mango (MN-2) and banana (BA-2), showed initial infection on leaves and collar region on 2 DAI. The initial symptom developed by the four different isolates recorded not much variation in the symptom expression. A slight difference in the size of lesion produced by the different isolates was noticed. All the isolates produced small, round to irregular dark brown coloured water soaked lesion on the leaves and collar region. On the leaves and collar region, the initial size of the lesion developed by all the isolates was found to be 0.5-1 cm except in CH-2 which initiated large sized lesion (1.5-2

Table 26. Host range of different isolates of *S. rolfsii*

Host	Isolate	Plant part inoculated	Initial infection(DAI)	Initial size of lesion (cm)	Final size of lesion (cm)	Colour of lesion	Presence/absence of mycelial growth	Presence/absence of sclerotia
Tomato	MG-2	Leaves	2	0.5-1	2.5-3.00	Brown	+	-
		Collar region	2	0.5-1	3.5-4.5			
	CH -2	Leaves	2	0.5-1	2.5-3.00	Brown	+	-
		Collar region	2	1.5-2	3.5-4.5			
	MN -2	Leaves	2	0.5-1	2.5-3.00	Brown	+	-
		Collar region	2	0.5-1	3.5-4.5			
	BA -2	Leaves	2	0.5-1	2.5-3.00	Brown	+	-
		Collar region	2	0.5-1	3.5-4.5			
Amorphophallus	MG-2	Leaves	-	-	-	Brown	-	-
		Collar region	2	1.5-2	2.5-5.0			
	CH-2	Leaves	-	-	-	Brown	+	+
		Collar region	2	2-2.5	2.5-5.0			
	MN-2	Leaves	-	-	-	Brown	-	-
		Collar region	2	1.5-2	2.5-5.0			
	BA-2	Leaves	-	-	-	Brown	+	+
		Collar region	2	2-2.5	2.5-5.0			

Black pepper	MG-2	Leaves	3	2.5-3	3.5-4.5 cm	Brown	+	+
		Collar region	7	2.5-3	3.5-4.5 cm			
	CH -2	Leaves	3	1-1.5	3.5-4.5 cm	Brown	+	+
		Collar region	7	1-1.5	3.5-4.5 cm			
	MN-2	Leaves	3	1-1.5	3.5-4.5 cm	Brown	+	+
		Collar region	7	1-1.5	3.5-4.5 cm			
	BA -2	Leaves	3	2.5-3	3.5-4.5 cm	Brown	+	+
		Collar region	7	2.5-3	3.5-4.5 cm			
Ginger	MG-2	Leaves	4	2.0-2.5	3.0-4.5	Brown	+	-
		Collar region	4	2.0-2.5	3.0-4.5			
	CH-2	Leaves	2	2.0-2.5	3.0-4.5	Brown	+	-
		Collar region	4	2.0-2.5	3.0-4.5			
	MN-2	Leaves	2	2.0-2.5	3.0-4.5	Brown	+	-
		Collar region	4	2.0-2.5	3.0-4.5			
	BA-2	Leaves	2	1.5-2.0	3.0-4.5	Brown	+	-
		Collar region	4	1.5-2.0	3.0-4.5			

DAI-Days After Inoculation

cm) on collar region of tomato. Later the water soaked lesion on leaves of tomato enlarged in size (2.5-3 cm) and covered half of the leaf area. On the collar region, the infection spread upwards and lesion extended up to a height of 3.5-4.5 cm. Thick, white mycelial growth was observed on all the inoculated parts of the plants on 3-4 DAI. Thinning of infected collar region was noticed in all the tomato plants inoculated by different isolates. Sclerotial formation was not observed on the inoculated area of leaves and collar region. The infected plants showed general yellowing and drooping of leaves, wilting and drying up of the plants was noticed within 6-7 DAI (Plate 9a).

4.7.2. Pathogenicity of various isolates of *S. rolfsii* on amorphophallus

The amorphophallus plants inoculated with the virulent isolates from marigold (MG-2), chrysanthemum (CH-2), mango (MN-2) and banana (BA-2) on collar region showed initial infection on 2 DAI. All the four isolates produced almost same type of lesions on collar region and variation in the size of the lesion was noticed. The infection appeared as small, irregular dark brown coloured water soaked lesion on collar region. The lesion produced by the isolates CH-2 and BA-2 recorded 2-2.5 cm in length whereas the lesions developed by the other isolates MG-2 and MN-2 recorded 1.5-2 cm in length on 2 DAI. Later the water soaked lesion enlarged in size and infection spread to the inner tissues and disintegration of tissues of collar region was noticed. The infection spread upwards and lesion showed the size of 2.5-5.0 cm length. The isolates CH -2 and BA-2 produced white mycelial growth on the collar region near the base of plants whereas mycelial growth was not noticed in plants inoculated with MG-2 and MN-2. All the inoculated plants showed yellowing of leaves on 4-5 DAI and within 6-7 DAI all the inoculated plants wilted, dried and fell down (Plate 9b).

4.7.3. Pathogenicity of various isolates of *S. rolfsii* on black pepper

Variation in initiating infection on leaves and collar region of black pepper was recorded when inoculated with virulent isolates from marigold (MG-2), chrysanthemum (CH-2), mango (MN-2) and banana (BA-2). The leaves of black

pepper inoculated with these isolates showed initial infection on leaves on 3 DAI whereas the initial infection on collar region was recorded on 7 DAI. All the isolates produced small, water soaked dark brown coloured lesion with yellow halo on all the leaves inoculated. Variation in the size of the lesion developed by different isolates was noticed. The lesion produced by the isolates CH-2 and MN-2 recorded 1-1.5 cm in length whereas the lesions produced by the other isolates MG-2 and BA-2 recorded 2.5-3 cm in length on 3 DAI. White mycelial growth of the pathogen was noticed on lower side of the inoculated leaves and sclerotial formation by each isolate was noticed in large numbers on upper surface of the leaves. Later, the water soaked lesion produced by all the isolates enlarged in size (3.5-4.5 cm) and showed leaf blight symptoms by covering half of leaf area with yellow halo and dark brown margin. The isolate BA-2 produced concentric zonations on the infected leaves with its characteristic sclerotia on the upper surface of leaves. Infection on leaf petiole was noticed in plants inoculated with the isolate BA-2, which showed black coloured discolouration with white mycelial growth on 7 DAI. Dark brown water soaked lesion developed on collar region which later enlarged to collar rot. White mycelial growth and sclerotial formation by all the isolates was noticed on all plants inoculated on collar region. Defoliation of inoculated leaves was noticed on 8-9 DAI (Plate 9c).

4.7.4. Pathogenicity of various isolates of *S. rolfsii* on ginger

Artificial inoculation of young ginger plants with the virulent isolates of *S.rolfsii* obtained from the selected hosts revealed the susceptibility of ginger to these isolates. All the four isolates except MG-2 recorded initial infection on leaves of ginger on 2 DAI whereas the collar infection by these isolates was noticed on 4 DAI. The isolate from marigold MG-2 recorded the initial infection on 4 DAI on the leaves and collar region of ginger. Slight difference in the size of lesion produced by the four isolates was observed. All the four isolates produced small, irregular water soaked lesion on the leaves and collar region. Initially the lesion produced on the leaves and collar region by BA-2 isolate recorded 1.5-2.0 cm length whereas the lesion produced by all other three isolates (MG-2, CH-2

Plate 9. Host range of different isolates of *S. rolfsii*

a. Tomato

b. Amorphophallus

c. Black pepper

d. Ginger

and MN-2) recorded 2.0-2.5cm length. In advanced stages, the water soaked lesion enlarged in size and increased to half of the leaf area. On the collar region, the infection spread upwards to about 3.0-4.5 cm height. The infection on leaves spread to nearby leaves resulted in leaf blight with grey centre and yellow halo. White mycelial growth was observed on undersurface of the leaves showing the infection. On 7-9 DAI yellowing, drooping and drying up of the leaves were noticed (Plate 9d).

4.8. ESTIMATION OF IAA IN VARIOUS ISOLATES OF *S. rolfsii*

The amount of IAA in mycelia, culture filtrate and sclerotia of the eleven isolates of *S. rolfsii* was estimated and the data are presented in Table 27. From the data, it was observed that all the isolates were able to produce IAA in mycelia, culture filtrate and sclerotia. Statistical analysis of the data showed significant difference in the IAA content of mycelia, culture filtrate and sclerotia recorded separately by the different isolates of *S. rolfsii* (Fig. 6, 7 &8).

4.8.1. IAA in mycelia

From the data given in Table 27, it was observed that there was significant difference in IAA content in mycelia of the various isolates from different hosts. The highest amount of IAA was recorded by the isolate from marigold, MG-1 (158.33μg g^{-1}) followed by MG-2 (141.66 μg g^{-1}) and statistically which were on par with each other. The IAA content in the mycelia of isolates from mango was in the range of 108.33-133.33 μg g^{-1} which are also on par with the IAA content recorded by the isolates from marigold. The lowest amount of IAA in mycelia was recorded by the isolate from chrysanthemum, CH-1 (50 μg/g) and no significant difference was observed in the IAA content in mycelia of four different isolates from chrysanthemum. The IAA in mycelia of two isolates from banana BA-1 (91.66 μg g^{-1}) and BA-2 (87.50 μg g^{-1}) recorded no significant difference between each other.

Table 27. Estimation of IAA in different isolates of *S. rolfsii*

Sl. No	Isolate	Mycelia* ($\mu g\ g^{-1}$)	Culture filtrate* ($\mu g\ mL^{-1}$)	Sclerotia* ($\mu g\ g^{-1}$)
1	MG-1	158.33^{a}	191.66^{a}	258.33^{a}
2	MG-2	141.66^{ab}	183.33^{ab}	245.83^{ab}
3	CH-1	50.00^{d}	100.00^{bcd}	179.16^{d}
4	CH-2	54.16^{d}	108.33^{abcd}	195.83^{abc}
5	CH-3	66.66^{cd}	100.00^{bcd}	191.66^{bc}
6	CH-4	62.50^{d}	104.16^{bcd}	183.33^{bc}
7	MN-1	133.33^{ab}	179.16^{abc}	237.50^{abc}
8	MN-2	129.16^{abc}	175.00^{abc}	229.16^{abc}
9	MN-3	108.33^{abcd}	170.83^{abcd}	216.66^{abc}
10	BA-1	91.66^{bcd}	95.83^{cd}	175.00^{d}
11	BA-2	87.50^{bcd}	87.50^{d}	170.83^{d}

* Mean of 3 replications

In each column figures followed by same letter do not differ significantly according to DMRT

4.8.2. IAA in culture filtrate

Significant difference was observed in IAA content in the culture filtrate of various isolates from different hosts (Table 27). The maximum amount was noticed in MG-1 isolate (191.66 μg mL^{-1}) which was followed by MG-2 (183.33 μg mL^{-1}) and were on par with each other. All the three isolates from mango and the second isolate from chrysanthemum recorded IAA content in the range of 108.33- 179.16 μg mL^{-1} and were statistically on par with the IAA content recorded by the isolates from marigold.

4.8.3. IAA in sclerotia

From the data presented in Table 27, it was noticed that there was significant difference in the content of IAA in the scleorotia of various isolates from different hosts. The maximum amount of IAA in sclerotia was recorded by MG-1 isolate (258.33 μg g^{-1}) followed by MG-2 (245.83 μg g^{-1}) which were on par with each other. The IAA content in the sclerotia of CH-2, MN-1, MN-2 and MN-3 were also statistically on par with the IAA content of sclerotia of the isolates from marigold. The minimum amount of IAA was recorded by the isolate from banana BA-2 (170.83 μg g^{-1}) which was on par with BA-1 (175.00 μg g^{-1}) and CH-1 (179.16 μg g^{-1}).

4.9. ESTIMATION OF TOTAL PHENOL IN VARIOUS ISOLATES OF *S.rolfsii*

The amount of total phenol in mycelia, culture filtrate and sclerotia of eleven different isolates of *S. rolfsii* was estimated and the data are presented in Table 28. It was observed that all isolates of *S. rolfsii* from different hosts produced phenolic compounds in mycelia, culture filtrate and sclerotia. Significant difference was recorded in amount of total phenol in the mycelia, culture filtrate and sclerotia of different isolates of *S. rolfsii* (Fig. 6, 7&8).

4.9.1. Total phenol in mycelia

There was significant difference in total phenol content in mycelia of various isolates from different hosts (Table 28). The maximum amount of total

Table 28. Estimation of total phenol in different isolates of *S. rolfsii*

Sl. no	Isolate	Mycelia* ($\mu g\ g^{-1}$)	Culture filtrate* ($\mu g\ mL^{-1}$)	Sclerotia * ($\mu g\ g^{-1}$)
1	MG-1	139.66 ^{a}	143.33 ^{a}	160.00 ^{ab}
2	MG-2	145.00 ^{a}	150.66 ^{a}	176.66 ^{a}
3	CH-1	64.00 ^{bc}	97.00 ^{abc}	146.66 ^{b}
4	CH-2	65.00 ^{bc}	99.00 ^{abc}	140.00 ^{b}
5	CH-3	61.00 ^{bc}	123.00 ^{ab}	132.33 ^{b}
6	CH-4	62.00 ^{bc}	80.66 ^{abc}	132.33 ^{b}
7	MN-1	91.00 ^{b}	80.66 ^{abc}	90.00 ^{c}
8	MN-2	88.00 ^{b}	93.33 ^{abc}	83.33 ^{c}
9	MN-3	80.00 ^{b}	75.66 ^{abc}	80.00 ^{c}
10	BA-1	44.33 ^{c}	47.66 ^{bc}	46.33 ^{d}
11	BA-2	42.66 ^{c}	44.00 ^{c}	44.33 ^{d}

* Mean of 3 replications

In each column figures followed by same letter do not differ significantly according to DMRT

phenol was noticed in the isolate MG-2 (145.00 μg g^{-1}) which was on par with MG-1 (139.66 μg g^{-1}) and statistically isolates from marigold was found superior. All the four isolates from chrysanthemum and three isolates from mango recorded total phenol in the range of 61.00-91.00 μg g^{-1} and were on par with each other. The minimum amount of total phenol was recorded in mycelia of BA-2 isolate (42.66 μg g^{-1}) which was on par with BA-1(44.33 μg g^{-1}).

4.9.2. Total phenol in culture filtrate

From the data presented in Table 28, it was observed that the total phenol in culture filtrate of various isolates from different hosts recorded significant difference between each other. The highest amount of total phenol was recorded by the isolate from marigold MG-2 (150.66 μg mL^{-1}) followed by MG-1 (143.33 μg mL^{-1}) and these isolates were statistically on par with each other. There was no significant difference in total phenol content in culture filtrate of isolates from chrysanthemum and mango and were statistically on par with IAA content recorded by the isolates from marigold. The lowest amount of total phenol was recorded by the isolate from banana, BA-2 (44.00 μg mL^{-1}) which was on par with BA-1(47.66 μg mL^{-1}).

4.9.3. Total phenol in sclerotia

Significant difference was observed in total phenol content in the sclerotia of various isolates from different hosts (Table 28). But there was no significant difference in total phenol content in the sclerotia of various isolates from same host. The maximum amount of phenol was recorded in isolate from marigold MG-2 (176 .66 μg g^{-1}) followed by MG-1 (160.00 μg g^{-1}) which are on par with each other. All the four isolates from chrysanthemum recorded total phenol in the sclerotia in the range of 132.33-146.66 μg g^{-1} and were statistically on par with each other. Similarly, the total phenol content in sclerotia of three isolates from mango recorded in the range of 80.00 – 90.00 μg g^{-1} which were statistically on par with each other. The minimum amount of total phenol was recorded by BA-2 (44.33 μg g^{-1}) which was on par with BA-1(46.33 μg g^{-1}).

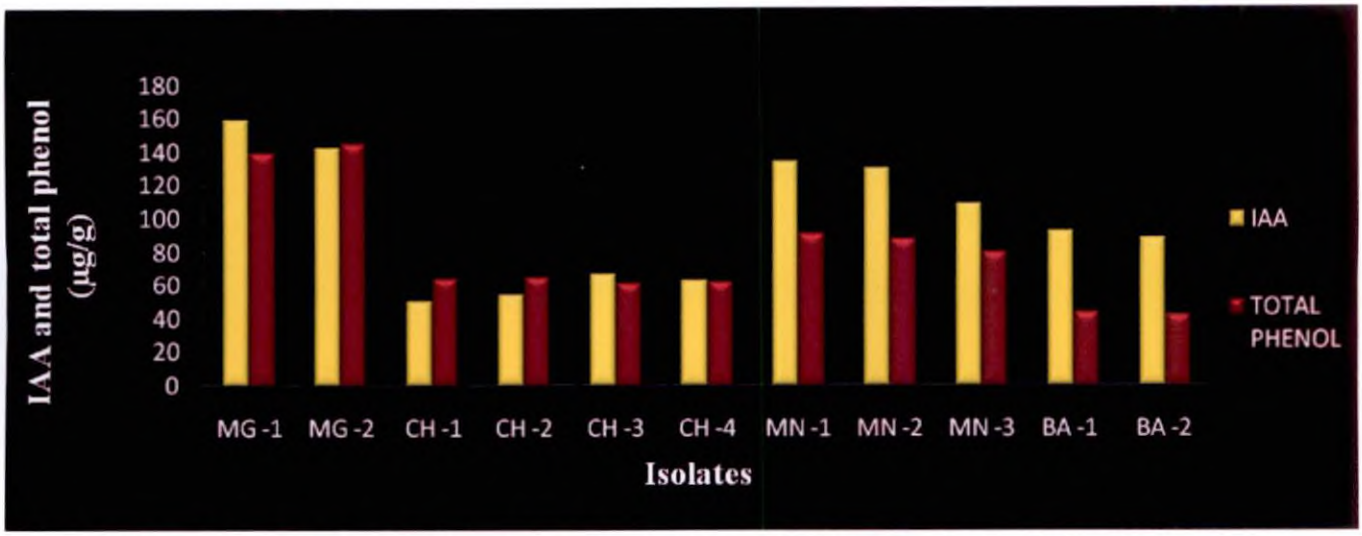


Fig.6. Estimation of IAA and total phenol in mycelia of different isolates of *S. rolfsii*

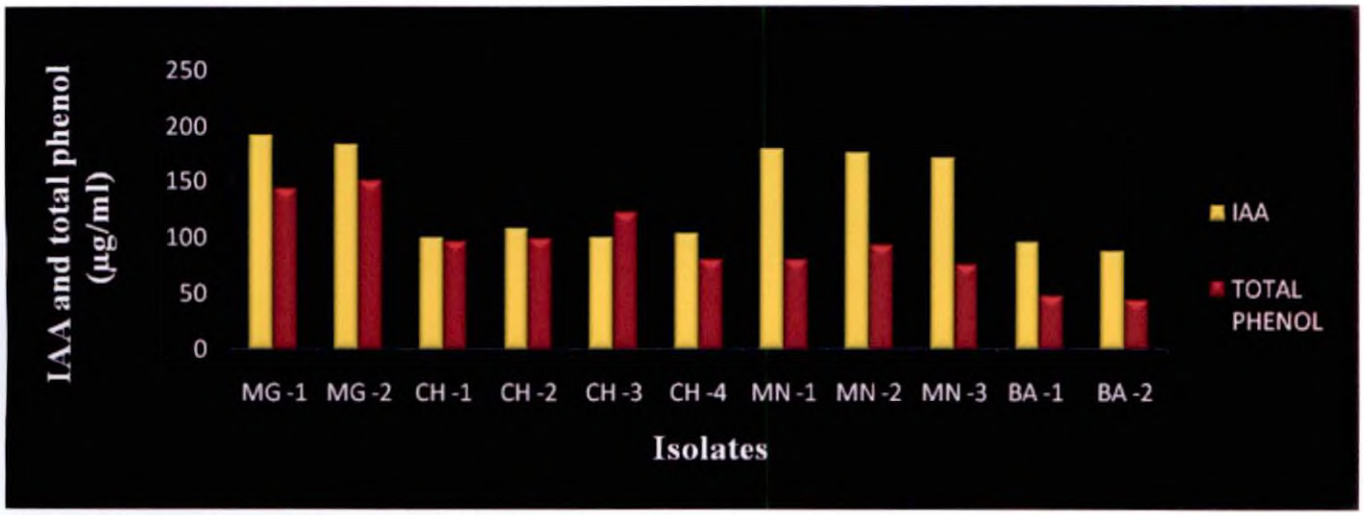


Fig.7. Estimation of IAA and total phenol in culture filtrate of different isolates of *S. rolfsii*

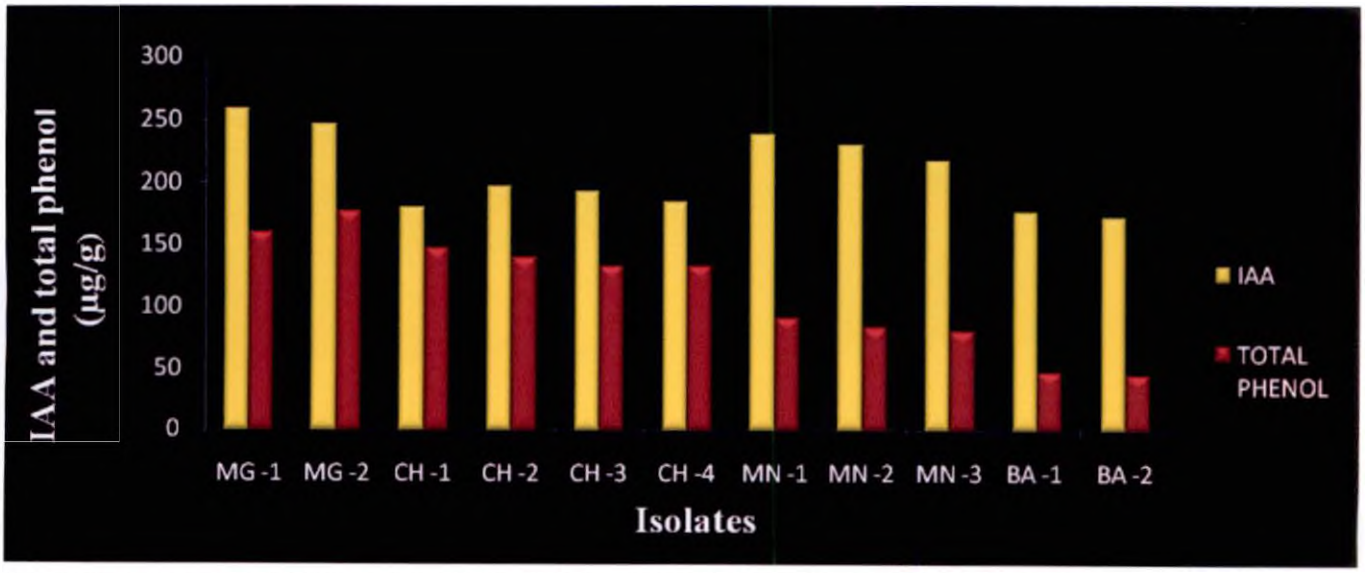


Fig.8. Estimation of IAA and total phenol in sclerotia of different isolates of *S. rolfsii*


Discussion

5. DISCUSSION

The pathogen *Sclerotium rolfsii* Sacc. is a polyphagous, non-target ubiquitous facultative parasite and a major root pathogen of many cultivated economic crops of the world. According to Pandey *et al.* (1999) it is a universally distributed soil borne pathogen with high competitive saprophytic ability, high lethal potentiality and wide host range that makes the fungus economically important. This pathogen is widely distributed in tropics, sub tropics and also in warmer parts of temperate zone of the world. In India, it is wide spread and reported from almost all the states, causing economic losses in many crops. It has a wide host range infecting cultivated crops *viz.*, tomato, potato, ground nut, soya bean, sunflower, chilli, cotton, wheat, lucerne and onion etc. It is documented that, *S. rolfsii* infects more than 500 plant species (Rupe, 1999). Due to this wide spread distribution and extensive host range, several morphological, physiological and pathogenic strains have been reported from different countries by a number of workers (Celino, 1936; Obee, 1937; Goto, 1952 and Sulladmath *et al.*, 1977). Therefore in the present study, attempts were made to study the variability in phenotypic and pathogenic characters of the different isolates, compatibility of different isolates of pathogen and also estimation of IAA and total phenol content in various isolates of *S. rolfsii*. The results obtained on the above aspects are discussed in detail below.

To find out the occurrence of disease caused by *S. rolfsii* in the selected ornamental plants *viz.*, marigold, chrysanthemum and fruit crops *viz.*, mango, banana and for the collection of diseased specimens, surveys were conducted in 15 various locations including research plots, orchards and nurseries of KAU, private nurseries and farmer's fields in Thrissur district. During the survey various types of infections by this pathogen were noticed on the above selected crops. In marigold, infection was noticed on the collar region, leaves and flowers whereas in chrysanthemum the leaves were mainly infected by the pathogen causing leaf blight symptoms but in some locations collar infection was also observed. Infection by the pathogen was observed in mango seedlings and grafts mainly on

the collar region and in some instances, infection on leaves resulting in leaf spot symptom was noticed. In banana, infection was seen on the pseudostem at the base of leaf petiole, resulted in pseudostem rot. The pathogen associated with the above disease specimens was isolated on PDA medium and yielded a total of 11 isolates of *S. rolfsii*. Among them, two isolates from marigold, four from chrysanthemum, three from mango seedlings and two from banana were obtained. All these different isolates produced white mycelial growth with small dark brown, spherical sclerotia on the medium which were primarily identified as *S. rolfsii* Sacc. Isolation of pathogen from the infected plants from different locations showed the association of *S.rolfsii* Sacc. with the diseased specimens.

Pathogenicity of the different isolates was proved by artificial inoculation on their respective hosts with mycelia alone, sclerotia alone and mycelia with sclertoia together under *in vivo* conditions. Among them, the mycelium with sclerotia recorded the early infection on all plant parts inoculated with pinprick. The other two inocula took more time to develop infection on the inoculated plant parts. It was also noticed that injury, by pinprick favoured quick symptom expression. In this test, it was noticed that the isolates of the pathogen *viz.*, MG-2, CH-2, MN-2 and BA-2 obtained from each host took the minimum time (2-3 days) to initiate the infection on the leaves of their respective host. The same isolates took 2-6 days to initiate the infection on collar region. So variation in the pathogenic character of these isolates to their respective host was observed. The isolates which took the shortest time to cause infection were selected as virulent isolates of *S. rolfsii* and were used for the host range study. The symptoms produced under artificial conditions showed a slight variation as that observed under natural conditions. The symptoms produced by the same pathogen vary depending upon the host and no variation in symptom development was noticed in case of infection caused by isolates of same host from different locations. Therefore a detailed study on the symptoms of disease caused by *S.rolfsii* in selected fruit crops and ornamental plants were carried out under natural conditions.

The successful infection results in the appearance of symptoms. The visible and detectable changes in the infected plant make up the symptoms of the disease. Development of symptom is an important stage in the disease cycle and is placed in the later stage of the chain of events in disease cycle. The symptoms produced by the isolates of pathogen on their host were studied in detail under natural and artificial conditions. Under natural conditions, a slight variation in the symptom produced by the pathogen in different hosts was observed. But in the same host at different locations, the symptom expression by the pathogen was found similar. In marigold and chrysanthemum infection was observed on leaves and collar region. Flowers of marigold were also infected by the pathogen. In these plants infection started from the middle leaves as small dark brown water soaked lesion which later enlarged to large lesion with yellow halo. On the collar region, thick white mycelial growth was first observed near the basal part of the stem. The infected flowers of marigold were also exhibited the same type of symptoms with white mycelial growth. In all the infected areas, the pathogen produced thick white mycelial growth and brown to black coloured mustard like sclerotia in large numbers. Earlier workers also reported the same type of symptoms developed by *S. rolfsii* on different ornamental plants (Girija and Umamaheswaran, 2003; Bag, 2004; Mesquita *et al.*, 2007). Mango seedlings and grafts were found infected by the pathogen near the collar region (Prakash and Singh, 1976). A dark brown discolouration was first observed in this area followed by the formation of a thick weft of white mycelial growth up to a height of five centimeter with deep brown sclerotia. In advanced stage, the seedlings died. Whereas in banana, the infection was seen on the pseudostem at the base of leaf petiole as water soaked lesions. Later lesions enlarged and resulted in rotting of that area. Thick white mycelial strands with sclerotial formation were also observed on the infected area. The pseudostem broken at the point of infection. The symptom developed on fruit crops due to infection by *S. rolfsii* was earlier reported by many workers. (Mohan and Lakshmanan, 1989; Yadav, 2005; Thangavelu and Mustaffa, 2010). The symptoms observed in the selected fruit

crops and ornamental plants were found same as those described by earlier workers.

Symptoms developed by the artificial inoculation of the different isolates on their respective host showed a slight variation as compared to the natural condition. Inoculation of mycelia and sclerotia together recorded early symptom expression followed by inoculation with mycelia and sclerotia alone. On artificially inoculated marigold plants, sclerotial development was observed only on the collar region whereas in chrysanthemum dark brown water soaked lesion observed on collar region which were not developed into complete rot. On artificial inoculation on mango, thick white mycelial growth and brown mustard like sclerotia were observed throughout the stem and leaves. In banana, infection on collar region and leaves were observed with thick white mycelial growth and sclerotia during the artificial inoculation.

Detailed study on the phenotypic characters of various isolates of the pathogen was carried out to find out any variability existing among the different isolates. The phenotypic characters *viz.,* cultural and morphological characters of plant pathogen are the most important criteria for the correct identification of the plant pathogens. The cultural characters of the 11 isolates were studied on four different media *viz.,* PDA, Czapek (Dox) Agar, Richard's Agar and Special medium for *S. rolfsii.* In all the four media, white coloured mycelial growth was produced by all the isolates. Slight variation was observed in the texture and mycelial type. A smooth texture and fluffy growth of mycelium was observed in all isolates except banana which showed compact mycelial type on special medium whereas smooth and rough textured with fluffy and compact mycelial type on the other three media. The three isolates from mango (MN-1, MN-2 and MN-3) recorded a rough and fluffy mycelial growth on PDA and Richard's Agar media. Isolate MG-1 produced rough textured mycelial growth in all the media except in special medium. All isolates from chrysanthemum and banana produced smooth and compact colony in all the media except in special medium where all the isolates from chrysanthemum produced a fluffy type of mycelial growth.

Many workers reported white coloured smooth and rough textured, compact and fluffy nature of the colony characters of *S. rolfsii*. The present observations on the cultural characters of *S. rolfsii* is in conformity with the earlier reports (Chand *et al.*, 2003; Kokub *et al.*, 2007; Thilagavathi, 2009; Wolcan and Grego, 2009; Kumar and Prasad, 2010).

Variation was also observed among the isolates in number of days required for the sclerotial initiation and sclerotial maturation and it ranged from 3-10 and 7-14 DAI respectively. Czapek (Dox) Agar medium recorded minimum days for the sclerotial initiation (3-5 days) and maturation (7-11 days) in all the 11 isolates. Among them, the four isolates from chrysanthemum took the shortest time for sclerotial initiation (3 days) and maturation (7 days). Kokub *et al.* (2007) also reported the early initiation of sclerotia in different strains of *S. rolfsii*.

Observation on the number of sclerotia produced per plate at 15, 30 and 45 DAI revealed that all the isolates recorded sclerotial formation on 30 DAI in all media except Richard's Agar medium. In this medium new sclerotium formation was not recorded on 30 DAI by all the isolates. In all media except in PDA the maximum number of sclerotia at 45 DAI were recorded in the isolate from banana (BA-2) whereas in PDA medium, the isolate BA-1 recorded the maximum number of sclerotia (402) on 45 DAI. On PDA medium, all isolates recorded more than 100 sclerotia per plate on 45 DAI. In the other three media, except in Czapek (Dox) Agar medium, only the two isolates from banana (BA-1and BA-2) recorded more than 100 sclerotia per plate on 45 DAI. The isolate BA-2 recorded the maximum number of sclerotia (542) on special medium for *S. rolfsii,* on 45 DAI. So the rate of sclerotial formation was found very high in the isolates from banana, especially in BA-2. Regarding the weight of 100 sclerotia, all isolates recorded the maximum weight on PDA medium and it ranged from 60 to 109 mg. Eventhough the isolates from banana recorded the maximum number of sclerotia per plate, the weight of 100 sclerotia was found to be very less. The maximum weight of 109 mg was recorded by MG -1on PDA medium and was followed by MN-1. These isolates recorded the same ranking in all the media. Sclerotial

distribution in Petri dish showed that, almost all isolates produced the sclerotia in the centre of the culture and also along the edges of Petri dishes. In the isolates from banana, sclerotial formation was observed in the centre, periphery and also on the inner side of upper lid of Petri dish. In the special medium, those isolates which showed fluffy growth of mycelium produced the sclerotia only at the periphery of Petri dishes. Production of exudates on the surface of sclerotia was observed in all isolates grown on all media. But its intensity varied with the isolates. Very high exudate formation was observed in all the four isolates from chrysanthemum on PDA medium whereas it was low in both isolates from banana. Similar observations on the cultural characters of *S. rolfsii* were reported by earlier workers and hence the observations made in this study were also in line with the earlier reports (Sulladmath *et al.*, 1977; Punja and Grogan, 1983; Rawn, 1991; Palaiah, 2002; Sarma *et al.*, 2002; Okereke and Wokocha, 2007; Akram *et al.*, 2008; Thangavelu and Mustaffa, 2010).

The growth rate of different isolates of *S. rolfsii* was studied in the four media. It was observed that, all isolates showed highest growth rate on PDA medium compared to other three media. The isolates *viz.*, CH-1, CH-2, CH-4 and MN-2 took only three days to complete the full growth on PDA medium whereas MN-1recorded the maximum days (6 days) for the completion of full growth. In the other three media, all isolates recorded 4-10 days to complete the 9 cm growth in Petri dish. On comparing the growth rate in four media, PDA medium was found to be the best one for supporting the fast growth of all 11 isolates. Moreover, from the observations made on the cultural characters of these isolates on four media, it was concluded that PDA medium was the most suitable culture medium for the growth of *S. rolfsii*. Hence this medium was selected for the study of morphological characters of the isolates. This finding was supported to the reports of some earlier workers (Hari *et al.*, 1991; Girija and Umamaheswaran, 2003; Okereke and Wokocha, 2007). Sulladmath *et al.*, 1977 and Rajalakshmi *et al.*, 2006 reported PDA as the best medium for the growth and sclerotial population of *S. rolfsii*.

Observations on the morphological characters of various isolates of *S. rolfsii* showed a slight variation in the size of the hyphal cell. But no variation was recorded in the morphological characters of sclerotia except in the size of sclerotia. All isolates produced dark brown coloured smooth and spherical scerotia on PDA medium. The size of the sclerotia varied in different isolates. The average size of sclerotia in most of the isolates varied within 0.53 to 2.00 mm in diameter. Sclerotia produced by MN-2 recorded the maximum size of 1.17-2.00 mm whereas BA-2 produced the minimum sized sclerotia (0.53-0.94 mm). Early reports by many workers also showed variation in the size of sclerotia from different isolates of S. *rolfsii* (Prabhu and Patil, 2005; Okereke and Wokocha, 2007; Thilagavathi, 2009).

The cultural and morphological characters of various isolates of *S. rolfsii* were subjected to cluster analysis. Clustering was done as per UPGMA method of Sneath and Sokal (1973) and the clustering pattern of isolates revealed a degree of variability. The 11 isolates were grouped into two clusters A and B. The lowest dissimilarity index was noticed in sub cluster B_1 between isolates CH-1 and BA-2. The cluster A consisted of two sub clusters A_1 and A_2, the lowest dissimilarity index was noticed in sub cluster A_1 between isolates MG-1 and MG-2. The third isolate from mango MN-3 included in sub cluster A_2. The isolates from banana stands separate and showed more dissimilarity with all other isolates.

The next part of the investigation was to evaluate the mycelial compatibility between various isolates of *S. rolfsii* obtained from the selected fruit crops and ornamental plants. The results revealed compatible and incompatible reactions between the various isolates. Out of the 55 combinations tested, eight combinations were found compatible with each other and the 47 combinations were incompatible. It was observed that the three isolates from mango (MN-1, MN-2 and MN-3) showed compatible reaction when paired with each other. Similarly the isolate CH-3 from chrysanthemum showed mycelial compatibility with two other isolates from chrysanthemum *viz.,* CH-2 and CH-4. These were the

compatible reactions observed between the isolates obtained from the same host. Whereas, the compatible reactions observed between the isolates from different hosts were recorded in the combinations of MN-2×MG-2, BA-2×MG-2, CH-2×MG-2 and CH-4×MN-2. From the compatibility chart, it was clear that the virulent isolate from marigold (MG-2) showed mycelial compatibility with virulent isolates from chrysanthemum (CH-2), mango (MN-2) and banana (BA-2). On comparing the groups of isolates based on dissimilarity index and MCG's, it was observed that all the isolates which showed mycelial compatibility were grouped under the cluster A. Among them the isolates MG-2, CH-2, CH-3, CH-4, MN-1 and MN-2 were again grouped under the sub cluster A_1 of cluster A. But the isolate MN-3 which showed compatible reaction with MN-1 and MN-2 was grouped in a separate sub cluster A_2 of cluster A. This is because of the highest dissimilarity index (6.71) recorded between MN-3 and CH-2 based on the cultural and morphological characters. The isolates from banana (BA-1 and BA-2) showed variation in sclerotial characters (number, size, position, production of exudate and weight of sclerotia) compared to other isolates. Similarly in compatibility study, only BA-2 recorded compatible reaction with only one isolate ie., MG-2. Hence in the dissimilarity grouping, BA-1 and BA-2 were grouped under a separate cluster B. Many workers also have prepared cluster analysis (dendrogram) based on the molecular characters of various isolates of *S. rolfsii* and grouped the isolates in different clusters (Punja and Sun, 2001; Adandonon *et al.*, 2005; Kokub *et al.*, 2007). Leslie (1993) suggested that the isolates from the same Mycelial Compatibility Group (MCG) or Vegetative Compatibility Group (VCG) were more genetically similar than isolates from different groups. Harlton *et al.* (1995) screened a worldwide collection of *S. rolfsii* isolates and identified 49 MCG's from 119 isolates. Similarly, Punja and Grogan (1983) and Almeida *et al.* (2001) reported several MCG's in *S.rolfsii* isolates.

In the compatibility reactions, complete overgrowth of the mycelium of two isolates and inhibition zone formation were absent. At the point of contact, the mycelia of two isolates were intermingled and produced sclerotia with

exudates in a line at the contact zone. Sclerotial formation on the surface of both the cultures was also noticed. In all the incompatible reactions, thinning of hyphae was observed that resulted in the development of a clear inhibition zone of 0.2-2.6 cm in between the two isolates. These reactions were observed in combinations of isolates from same host and different host. In relation to inhibition zone formation, these isolates were found distinct and stable and showed the same behaviour in all the replicated plates. Based on the inhibition reaction the inter relationship among the various isolates of *S. rolfsii* isolated from different hosts and the same host can be determined. Isolates showed incompatible reactions produced sclerotia with exudate on either side of the inhibition zone and also on the surface of the cultures. But sclerotial formation was not observed at the inhibition zone. Earlier many workers studied on the mycelial compatibility of various isolates of *S. rolfsii* and reported compatible and incompatible reactions. Shukla and Pandey (2007) observed 81 combinations as compatible reactions out of 495 combinations of different isolates of *S. rolfsii* obtained from different localities. Similar results were reported by many workers (Punja and Sun, 2001; Adandonon *et al.,* 2005). Shinde and Reddy (2009) reported the strain variation based on both aversion and pathogenicity among the isolates of *S. rolfsii* isolated from different hosts and also from the same host growing in the same locality.

In the paired combinations of isolates from the same host or different hosts, the per cent reduction in the growth of individual isolates over the control was calculated. Out of 55 combinations of isolates tested for the mycelial compatibility, in 17 combinations more than 50 per cent reduction in growth over control was shown by any one of the isolates. It was noticed in three compatible combinations and 14 incompatible combinations. Eventhough the paired isolates showed compatible reaction, more than 50 per cent reduction in growth was exhibited by any one of the paired isolates. The compatible pairs which showed this specific reaction are MG-2 × MN-2, CH-4 × MN-2 and MG-2 × BA-2 in which, MN-2 in the above combinations showed 57.03 and 51.39 per cent reduction in growth over control respectively. The isolate BA-2 when paired with

MG-2 recorded 60.74 per cent reduction in growth over control. Among the incompatible combinations the maximum reduction of growth over control was recorded by the isolate BA-2 (67.40 %) when it was paired with MG-1. It was followed by the third isolate from chrysanthemum (CH-3) which recorded 66.01 per cent reduction when paired with MN-1 and MN-2. The same isolate when paired with MN-3 recorded 64.72 per cent reduction in growth over control. It was observed that reduction in growth rate of CH-3 which might be due to some inhibitory substances produced by the isolates from mango. The isolate MG-2 recorded cent per cent growth in dual culture when it was paired with MG-1. It was followed by BA-2 which recorded 5.92 per cent reduction when paired with MN-3. From this it was concluded that different isolate perform in different ways when they came in contact with other isolates from the same host or different host.

Another important aspect studied in the investigation was the pathogenic variability of different isolates of *S. rolfsii* obtained from the selected fruit crops and ornamental plants. Each isolate from a host was cross inoculated on other selected hosts, to know any variation in the pathogenic character among the isolates. Cross inoculation studies revealed that all the isolates were pathogenic to other hosts but variation in the pathogenic character of these isolates was observed. Variation in the symptom expression was not noticed on the inoculated plants by different isolates obtained from the same host. All the isolates, when cross inoculated on the leaves of other hosts, produced the infection on 3 DAI except inMN-1 and MN-3 on leaves of marigold, which recorded the infection on 4 DAI. Variation was observed in the pathogenicity of various isolates to initiate infection on the collar region of cross inoculated hosts. Different isolates took 3-5 days to initiate infection on collar region. Among them, MN-2, BA-1 and BA-2 on marigold, MG-1 and MG-2 on banana took only three days whereas MG-1 and MG-2 on chrysanthemum and all isolates from chrysanthemum and mango on banana took five days to initiate infection on collar region. Inoculation with the isolates from marigold, chrysanthemum and banana on the collar region of mango

seedlings failed to produce any infection. Similarly the isolates from banana, BA-1 and BA-2 did not show infection on collar region of chrysanthemum. All the isolates inoculated on the pseudostem of banana showed delayed infection and they took five days to initiate the infection. In all the plant parts inoculated with different isolates showed the presence of white mycelial growth and also showed the formation of brown coloured smooth sclerotia. From these observations, it was concluded that different isolates obtained from the selected fruit crops and ornamental plants showed variability in their pathogenic character. It was noticed that, the pathogenic variability was based on the nature of host plant and the plant parts infected as is evidenced by the isolates BA-1 and BA-2 recorded infection on leaves and collar region of marigold whereas the same isolates could not cause infection on collar region of chrysanthemum and mango. Similarly the isolatesMG-1and MG-2 showed infection on 3 DAI on the leaves of chrysanthemum, mango and banana, but they took three and five days for the collar infection on banana and chrysanthemum respectively. At the same time they could not cause collar infection on mango seedlings. The pseudostem infection on banana by the same isolates appeared only on 5 DAI. All isolates from marigold, chrysanthemum and mango were found less virulent to infect pseudostem and collar region of banana except marigold isolates, MG-1 and MG-2 which showed infection on collar region of banana on 3 DAI. So based on the host infected and time taken to initiate the infection, the isolates were grouped into three categories *viz.,* high virulent, moderate virulent and less virulent. Among the isolates inoculated on marigold, MN-2, BA-1 and BA-2 were found high virulent, CH-1, CH-2, CH-3 and CH-4 were grouped as moderate virulent and MN-1 and MN-3 were grouped as less virulent. In chrysanthemum, MG-1, MG-2, MN-1, MN-2 and MN-3 were found moderately virulent and BA-1 and BA-2 were less virulent. Inoculation on mango seedlings showed infection only on leaves and hence all isolates were grouped as less virulent. In banana, only MG-1 and MG-2 were found as high virulent and all the isolates inoculated were found moderate virulent. Similarly many earlier workers reported significant variation in the virulence and they grouped the isolates of *S. rolfsii* in different

categories (Than *et al.*, 1991; Subramaniam, 1994; Ansari and Agnihotri, 2000; Adandonon *et al.*, 2005; Shukla and Pandey, 2008; Thilagavathi, 2009). Hence the results of the pathogenic variability are supportive to the earlier reports.

Survival and spread are the distinct events in the chain of disease cycle of any plant pathogen. The main source of survival is host plant which includes cultivated host, collateral host and alternate host. So the study on the host range of a pathogen will help to know more about the perpetuation and dissemination of the pathogen. To find out the host range of *S.rolfsii* isolated from selected fruit crops and ornamental plants, cross inoculation was carried out on selected vegetables *viz.*, tomato and amorphophallus and spices *viz.*, ginger and black pepper. The result of the study revealed that all the selected vegetables and spices were susceptible to virulent isolates of S. *rolfsii* and produced typical collar rot on all the crops inoculated and leaf blight symptoms in black pepper, ginger and tomato. Symptoms appeared as small, water soaked brown discolouration on the inoculated leaves and collar region. Later it enlarged in size and covered with white mycelial growth of the pathogen in all the hosts except in the collar region of amorphophallus inoculated with MG-2 and MN-2. It was also observed that the initial infection was first noticed on leaves and collar region inoculated with pinprick which indicate the easy entry of the pathogen into the host through injury and it took less incubation period for the expression of the symptoms.

In this study variation among the isolates was observed in the production of sclerotia on the inoculated area. It was noticed that sclerotial development was completely absent in ginger and tomato on both leaves and collar region. On the leaves of pepper, white mycelial growth was noticed on the lower side of the leaves and each isolate produced its sclerotia in large number on the lower side of the leaves. The isolate BA-2 produced concentric zonations on the inoculated leaves of pepper with characteristic sclerotia on the lower side. Petiole infection was also noticed on pepper inoculated by BA-2 isolate on leaves whereas in other plants, the different isolates produced small brown water soaked lesion on the leaves, but variation only in the size of the lesion was noticed. In ginger, the

infection on leaves was found to spread to the nearby leaves and resulted in leaf blight with grey centre and yellow halo. From this study, it was concluded that the four virulent isolates are pathogenic to the plants tested except on the leaves of amorphophallus, where these isolates could not cause infection. So these four crop plants *viz.*, tomato, amorphophallus, black pepper and ginger could act as collateral host to the various isolates of *S. rolfsii* obtained from the selected fruit crops and ornamental plants. There were several reports on the host range of *S. rolfsii.* Girija and Umamaheswaran (2003) and Khosla and Gupta (2005) reported tomato and marigold as hosts of *S. rolfsii.* Moctezuma *et al.* (2005) also studied the host range of *S. rolfsii* and they found pepper, tomato, marigold and chrysanthemum susceptible to *S. rolfsii* and reported as hosts of this pathogen. So the results of host range study are in line with the earlier reports.

The last part of this investigation was the estimation of IAA and total phenol content in the mycelia, culture filtrate and sclerotia of the various isolates of *S. rofsii.* IAA is a growth regulator and has an important role in pathogenesis. Like plants, the pathogens also produce growth regulators during infection, which in turn stimulate or retard the production of growth regulators by the plant. The more IAA a pathogen produces, the more severe the symptom it causes. The IAA content of mycelia, culture filtrate and sclerotia of various isolates of *S. rolfsii* was estimated and it was observed that all isolates produced IAA in all the three samples of the pathogen. The data showed significant difference in the IAA content of various isolates. All isolates recorded more amount of IAA in sclerotia than in mycelia and culture filtrate. The first isolate from marigold (MG-1) recorded the maximum IAA content (258.33 $\mu g\ g^{-1}$) in sclerotia. The same isolate also recorded maximum IAA in mycelium (158.33 $\mu g\ g^{-1}$) and culture filtrate (191.66 $\mu g\ mL^{-1}$). Statistically this isolate was significantly superior in IAA production in all the three samples. The lowest amount of IAA in sclerotia (170.83 $\mu g\ g^{-1}$) and culture filtrate (87.50 $\mu g\ mL^{-1}$) was observed in BA-2 and that in mycelia (50 $\mu g\ g^{-1}$) was observed in CH-1. The IAA production by different parts of fungal pathogen was studied by many workers. The earlier reports proved

the ability of sclerotia of *S. rolfsii* to produce more IAA than any of the other parts of the pathogen (Gunasekaran and Weber, 1972; Sarma *et al.*, 2002). Hence the results of this IAA estimation are found to be supportive to the earlier reports.

The phenolic compounds have an important role in the defence mechanism and survival of pathogen during adverse conditions. It includes chlorogenic acid, ferulic acid, cinnamic acid, coumaric acid etc. In this study the total phenol content of mycelia, culture filtrate and sclerotia of the different isolates was estimated by colorimetric method. Significant difference occurred in amount of total phenol in the mycelia, culture filtrate and sclerotia of the various isolates of *S. rofsii*. All isolates recorded more amount of total phenol in sclerotia than in mycelia and culture filtrate. The highest total phenol content of 176.66 $\mu g\ g^{-1}$ in sclerotia was recorded by second isolate from marigold (MG-2) and was followed by first isolate from marigold (MG-1) which recorded 160.00 $\mu g\ g^{-1}$. The lowest quantity of total phenol in sclerotia was observed in isolate from banana (BA-2) with 44.33 $\mu g\ g^{-1}$. Also MG-2 recorded the highest total phenol in culture filtrate (150.66 $\mu g\ mL^{-1}$) and mycelia (145.00 $\mu g\ g^{-1}$). The presence of phenolic acids in *S. rolfsii* might be for it's self defense against other microbes during its survival in adverse soil conditions. The presence of relatively higher amount of total phenol in sclerotia than in mycelium suggested that sclerotia need higher amounts of phenolic acids for their survival than mycelia. Sclerotia are the survival structure of this pathogen because this pathogen is not producing any asexual and sexual spores / structures for their survival. The present result is in conformity with the earlier reports. Sarma *et al.* (2002) reported higher amount of phenolic acids in sclerotia of *S. rolfsii*. In 2005, Ellil estimated phenolic content in culture filtrate of four isolates of *Rhizoctonia solani* which comes under same systemic position of *S. rolfsii* and he reported the amount of phenols ranging from 28.8 to 133.5 mg mL^{-1}. Pandey *et al.* (2005) estimated the amount of phenolic acids in mycelia of *S. rolfsii* and they reported 0.14 $\mu g\ g^{-1}$ chlorogenic acid, 1.01 μg g-1 ferulic acid and 0.05 $\mu g\ g^{-1}$ coumaric acid.

The data on the estimation of IAA and total phenol content showed their highest amount in sclerotia of all the isolates. Among the isolates, MG-1 and MG-2 recorded the highest amount of IAA and total phenol in mycelia, culture filtrate and sclerotia. The IAA is a growth regulator and phenolics are inhibitory to the pathogen which has an important role in the defence mechanism of plants. In the present study, the individual phenolic compounds present in the pathogen are not estimated. The phenolic compounds which are not self inhibiting to the pathogen might be present in the various samples of pathogen. The increased IAA content in sclerotia was also noticed. Actually the phenolics are known to suppress the activity of IAA oxidases, the enzyme required for the oxidation of IAA. This might be one of the reasons for the increased level of IAA in sclerotia because the high level of phenolics suppress the IAA oxidising enzyme that regulate the production and accumulation of auxin (Singh, 2009).

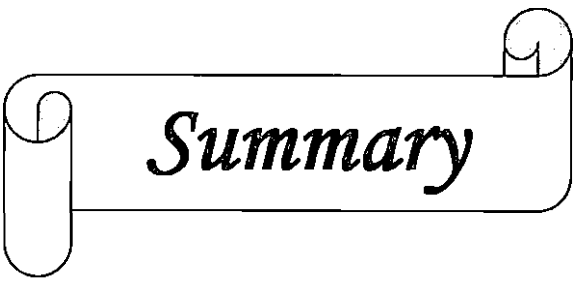
From the results of the study discussed so far, it may be concluded that variability in symptom expression by various isolates of *S. rolfsii* on different hosts was observed but not much variation in symptom expression by isolates from the same hosts from different locations. Different isolates of *S. rolfsii* showed variation in growth rate in different media tested. Variability was observed in colony characters and morphological characters of different isolates of *S. rolfsii*. The microscopic measurement of hyphae and sclerotia of various isolates of *S. rolfsii* were comparable to the description given by earlier workers.

Cluster analysis on cultural and morphological characters of the pathogen revealed a degree of variability among the isolates. The isolates from banana stands separate and showed more dissimilarity with all other isolates, whereas isolates from marigold, chrysanthemum and two isolates from mango showed more similarity and they were grouped under sub cluster A_1 of cluster A.

Among the 55 combinations, eight MCG's were identified which showed intermingling of mycelia at the zone of contact. Thinning of mycelium and inhibition zone formation was observed in incompatible combinations.

The pathogenic variability test and host range study of various isolates of S. *rolfsii* showed that all the isolates were pathogenic to hosts tested and there is variation in symptom expression by the isolates on different hosts.

Significant differences were noticed in IAA and total phenol content of various isolates of *S. rolfsii*. The lowest quantity of IAA and total phenol was observed in isolates from banana whereas maximum in isolates from marigold.


Summary

6. SUMMARY

The present investigation on "Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals" was carried out to study various aspects particularly symptomatology, cultural and morphological of the different isolates, compatibility between various isolates, pathogenic variability, host range and estimation of Indole Acetic Acid and total phenol content of mycelia, culture filtrate and sclerotia of various isolates of *S. rolfsii*. Surveys were conducted in 15 various locations including research plots, orchards and nurseries of KAU, private nurseries and farmer's fields in Thrissur district. Isolation of diseased specimens yielded fungal growth on PDA and isolated a total of 11 isolates of *S. rolfsii*. The patLogenicity of these isolates was proved by artificial inoculation under *in vivo* condition.

Under natural conditions, a slight variation in the symptom produced by the pathogen in different host plants was observed. In marigold and chrysanthemum infection was observed on leaves and collar region. Flowers of marigold were also infected by the pathogen. In these plants infection started from the middle leaves as small dark brown water soaked lesion which later enlarged to large lesion with yellow halo. On the collar region, thick white mycelial growth was first observed. The infected flowers of marigold were also exhibited the same type of symptoms with white mycelial growth. On mango, a dark brown discolouration was first observed near the collar region followed by the formation of a thick weft of mycelial growth upto a height of five centimetre with dark brown sclerotia. In advanced stage, the seedlings died. In banana, infection seen on the pseudostem at the base of leaf petiole as dark brown water soaked lesions. Later lesions enlarged and resulted in rotting of that area. Thick white mycelial strands with sclerotial formation were also observed on the infected area. The pseudostem broken at the point of infection. The

symptoms produced under artificial conditions showed a slight variation when compared with that observed under natural conditions. The symptoms produced by the same pathogen vary depending upon the host and no variation in symptom development was noticed in case of infection caused by isolates from same host from different locations.

To find out the variation existing among the isolates of *S. rolfsii*, cultural and morphological characters of all the isolates were studied in detail on PDA medium, Czapek (Dox) agar medium, Richard's agar medium and special medium for *S. rolfsii*. White coloured mycelial growth was produced by all the isolates in all the four media. Slight variation was observed in the texture and mycelial type. A smooth texture and fluffy growth of mycelium was observed in all isolates except banana which showed compact mycelial type on special medium whereas smooth and rough textured with fluffy and compact mycelial type on the other three media. The three isolates from mango (MN-1, MN-2 and MN-3) recorded a rough and fluffy mycelial growth on PDA and Richard's Agar media. All isolates from chrysanthemum and banana produced smooth and compact colony in all the media except in special medium whereas all the isolates from chrysanthemum produced a fluffy type of mycelial growth. Variation was also observed among the isolates in number of days required for the sclerotial initiation and sclerotial maturation and it ranged from 3-10 and 7-14 DAI respectively. Czapek (Dox) Agar medium recorded minimum days for the sclerotial initiation (3-5 days) and maturation (7-11 days) in all the 11 isolates and among them, the four isolates from chrysanthemum took the shortest time for sclerotial initiation (3 days) and maturation (7 days). Observation on the number of sclerotia produced per plate at 15, 30 and 45 DAI revealed that all the isolates recorded sclerotial formation even on 30 DAI in all media except Richard's Agar medium. Except in PDA, all media recorded the maximum number of sclerotia at 45 DAI in the isolate from banana (BA-2) whereas in PDA medium, the isolate BA-1 recorded the maximum number of sclerotia (402) on 45 DAI. On PDA medium, all

isolates recorded more than 100 sclerotia per plate on 45 DAI. The isolate BA-2 recorded the maximum number of sclerotia (542) in special medium for *S. rolfsii,* on 45 DAI. So the rate of sclerotial formation was found very high in the isolates from banana, especially in BA-2. Regarding the weight of 100 sclerotia, all isolates recorded the maximum weight in PDA medium and it ranged from 60 to 109 mg. Eventhough the isolates from banana recorded the maximum number of sclerotia per plate, the weight of 100 sclerotia was found to be very less in these isolates. The maximum weight of 109 mg was recorded by MG -1on PDA medium and was followed by MN-1. These isolates recorded the same ranking in all the media. Sclerotial distribution in Petri dish showed that almost all isolates produced the sclerotia in the centre of the culture and also along the edges of Petri dishes. In the isolates from banana, sclerotial formation was observed in the centre, periphery and also on the underside of upper lid of Petri dish. In the special medium, those isolates which showed fluffy growth of mycelium produced the sclerotium only on the periphery of Petri dishes. Production of exudates on the surface of sclerotia was observed in all isolates grown on all media. Very high exudate formation was observed in all the four isolates from chrysanthemum on PDA medium whereas it was low in both isolates from banana.

Variation in growth rate of different isolates of *S. rolfsii* was studied in four different media. The results revealed that PDA supported maximum growth of all isolates. They took 3-6 days to complete 9 cm growth in Potato Dextrose Agar medium. In this medium, only MN-1recorded the maximum days (6 days) for the completion of full growth in Petri dish. In the other three media, all isolates recorded 4-10 days to complete the 9 cm growth in Petri dish. From the observations made on the cultural characters of these isolates on four media, it was concluded that PDA medium was the most suitable culture medium for the growth of *S. rolfsii*.

Observations on the morphological characters of various isolates of *S. rolfsii* showed a slight variation in the size of the hyphal cell. But no variation was recorded

in the morphological characters of sclerotia except in the size of sclerotia. All isolates produced dark brown, smooth and spherical scerotia on PDA medium. The average size of sclerotia in most of the isolates varied within 0.53 to 2.00 mm in diameter.

Cluster analysis of cultural and morphological characters revealed a degree of variability among the isolates. The lowest dissimilarity index was noticed in sub cluster B_1 between isolates CH-1 and BA-2. The isolates from marigold, chrysanthemum and two isolates from mango (MN-1 and MN-2) were found more similar in their cultural and morphological characters. The isolates from banana stands separate and showed more dissimilarity with all other isolates.

Mycelial compatibility between various isolates of *S. rolfsii* obtained from the selected fruit crops and ornamental plants revealed compatible and incompatible reactions among the various isolates. Out of the 55 combinations tested, eight combinations were found compatible with each other and the 47 combinations were incompatible. From the compatibility chart, it was clear that the virulent isolate from marigold (MG-2) showed mycelial compatibility with virulent isolates from chrysanthemum (CH-2), mango (MN-2) and banana (BA-2). In the compatible reactions, complete overgrowth of the mycelium of two isolates was not observed. Lysis of mycelium and inhibition zone formation was also not recorded. At the point of contact, the mycelia of two isolates were intermingled and became difficult to recognize the contact zone. The isolates produced sclerotia with exudates in a line at the centre of the Petri dish in between the isolates. Sclerotial formation on the surface of both the cultures was also noticed. In all the incompatible reactions, development of a clear inhibition zone 0.2-2.6 cm was observed in between the two isolates. These reactions were observed in combinations of isolates from same host and different host. Out of the 55 combinations of isolates tested for the mycelial compatibility, in 17 combinations, one of the isolates recorded more than 50 per cent reduction in growth over control. It was noticed in three compatible combinations and 14 incompatible reactions. It was concluded that different isolate perform in different

ways when they came in contact with other isolates from the same host or different host.

Cross inoculation studies revealed that all the isolates were pathogenic to other hosts but variation in the pathogenic character of these isolates was observed. Variation in the symptom expression was not noticed on the inoculated plants by different isolates obtained from the same host. All the isolates, when cross inoculated on the leaves of other hosts, produced the infection on 3 DAI except in MN-1 and MN-3 on leaves of marigold, which recorded the infection on 4 DAI. Variation was observed in the pathogenicity of various isolates to initiate infection on the collar region of cross inoculated hosts. Different isolates took 3-5 days to initiate infection on collar region. Inoculation with the isolates from marigold, chrysanthemum and banana on the collar region of mango seedlings failed to produce infection. Similarly, the isolates from banana, BA-1 and BA-2 did not show infection on collar region of chrysanthemum. In all the plant parts inoculated with different isolates showed the presence of white mycelial growth and also showed the formation of dark brown coloured smooth sclerotia. From these observations, it was concluded that different isolates obtained from the selected fruit crops and ornamental plants showed variability in their pathogenic character which is based on the nature of host plant and the plant parts infected.

Host range study was conducted by cross inoculating different isolates of *S.rolfsii* on selected vegetables *viz.,* tomato, amorphophallus and spices *viz.,* black pepper, ginger. All the isolates produced symptom on all the crops tested. Symptom appeared as small, water soaked brown discolouration on the inoculated leaves and collar region. Later it enlarged in size and covered with white mycelial growth of the pathogen in all the hosts except in amorphophallus inoculated with MG-2 and MN-2 on collar region. On the leaves of pepper, white mycelial growth was noticed on the lower side of the leaves and each isolate produced it's sclerotia in large number on the lower side of the leaves. The isolate BA-2 produced concentric zonations on the

inoculated leaves of pepper and petiole infection was also noticed whereas in other plants, the different isolates produced small dark brown water soaked lesion on the leaves, but variation only in the size of the lesion was noticed. In ginger, the infection on leaves was found spread to the nearby leaves and resulted in leaf blight with grey centre and yellow halo. Thinning of infected collar region was noticed in all the tomato plants inoculated by the isolates. Variation among the isolates was observed in the production of sclerotia on the inoculated area. It was noticed that sclerotial development was completely absent in ginger and tomato on both the leaves and collar region. All isolates were pathogenic to the plants tested except the leaves of amorphophallus where the four isolates could not able to cause infection.

Estimation of IAA and total phenol content of mycelium, culture filtrate and sclerotia of the different isolates by colourimetric method showed significant difference in the IAA content of various isolates. All isolates recorded more amount of IAA in sclerotia than in mycelia and culture filtrate. The first isolate from marigold (MG-1) recorded the maximum IAA content in mycelia, culture filtrate and sclerotia. Not much difference in total phenol content was observed in all the three samples of various isolates of *S. rolfsii* from different hosts. The highest total phenol content in mycelia, culture filtrate and sclerotia was recorded by second isolate from marigold (MG-2).

It was concluded from the above study that phenotypic and pathogenic variability were exhibited by the soilborne pathogen *S. rolfsii*. The investigation can be extended to other important fruit crops and ornamentals of Kerala. Different methods of survival and dispersal of the pathogen can be studied that will give a thorough knowledge about the life cycle of the pathogen. Role of sexual stage of the pathogen on pathogenesis and eco-friendly management of the disease can also be studied.

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*Originals not seen

Appendices

Appendix-1

MEDIA COMPOSITION

1. POTATO DEXTROSE AGAR

Potato	: 200g
Dextrose	: 20.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

2.CZAPEK (DOX) AGAR

Sucrose	: 30.00 g
Sodium nitrate	: 2.00 g
Dipotassium phosphate	: 1.00 g
Magnesium sulphate	: 0.50 g
Potassium chloride	: 0.50 g
Ferrous sulphate	: 0.01 g
Agar	: 20.00 g
Distilled water	: 1000 ml

3. RICHARD'S AGAR

Potassium nitrate	: 10.00 g
Potassium dihydrogen phosphate	: 5.00 g
Magnesium sulphate	: 2.50 g

Ferric chloride	: 0.02 g
Sucrose	: 50.00 g
Agar	: 20.00 g
Distilled water	: 1000 ml
p^H	: 6.6-7.2

4. SPECIAL MEDIUM FOR *SCLEROTIUM ROLFSII*

Ammonium dihydrogen phosphate	: 4.12g
Carboxy methyl cellulose	: 6.00g
Dipotassium hydrogen phosphate	: 1.70 g
Magnesium sulphate	: 0.40 g
Manganese sulphate	: 14.00 mg
Ferric chloride	: 14.00 mg
Thiamine HCl	: 1.00 mg
Dextrose	: 8.00g
Sodium succinate	: 6.00 mg
Potassium chloride	: 0.15 g
Zinc sulphate	: 12 mg
Initial p^H	: 6.00

PHENOTYPIC AND PATHOGENIC VARIABILITY OF *Sclerotium rolfsii* Sacc INFECTING FRUIT CROPS AND ORNAMENTALS

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study on "Phenotypic and pathogenic variability of *Sclerotium rolfsii* Sacc. infecting fruit crops and ornamentals" was undertaken in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2009-2011 with an aim to isolate the pathogen associated with the disease and to study the symptomatology of the disease, variability in phenotypic and pathogenic characters of isolates, compatibility of the different isolates, host range of the pathogen and estimation of IAA and total phenol content in mycelia, culture filtrate and sclerotia.

Isolation of pathogen associated with the diseased specimens of selected ornamental plants *viz.*, marigold and chrysanthemum and fruit crops *viz.*, mango and banana yielded a total of 11 isolates of *S. rolfsii* from different locations. Among them, two isolates from marigold (MG-1, MG-2), four from chrysanthemum (CH-1, CH-2, CH-3, CH-4), three from mango (MN-1, MN-2, MN-3) and two from banana (BA-1 and BA-2) were obtained. The pathogenicity of the isolates was proved by artificial inoculation on their respective host.

Symptomatology of disease revealed slight variation in the symptoms produced by the pathogen in different host plants. In marigold and chrysanthemum infection was observed on leaves and collar region. Flowers of marigold were also infected by the pathogen. Mango seedlings and grafts were infected by the pathogen on the collar region. In banana var. Kadali, infection was observed on pseudostem at the base of leaf petiole and pseudostem broken at the point of infection. Dark brown water soaked lesions were present on infected areas of all the plants. Later it was covered with thick white mycelial growth and brown sclerotia.

Detailed study on the phenotypic characters of the isolates revealed variations in the cultural and morphological characters. All the isolates produced

white coloured colony on all the selected media. Variation in the texture and type of mycelium, sclerotial initiation and maturation, number, position, weight of 100 sclerotia and production of exudates was recorded. Among the different media tested, Potato Dextrose Agar was found to be the best medium for the growth of pathogen. In all the isolates, hyphae are hyaline, branched, septate and the hyphal cell size 52.6-196.32 µm×4.25-8.93 µm. Sclerotia were dark brown, smooth and spherical, 0.5-2.00 mm diameter in size. The maximum size of sclerotia was recorded in MN-2 and minimum in BA-2.

Cluster analysis of cultural and morphological characters revealed a degree of variability among the isolates. The lowest dissimilarity index was noticed between isolates CH-1 and BA-2. The isolates *viz.,* MG-1, MG-2, CH-1, CH-2, CH-3, CH-4, MN-1 and MN-2 were found more similar and were grouped under the sub cluster A_1 of cluster A. The isolates from banana stands separate and showed more dissimilarity with all other isolates.

Mycelial compatibility between the different isolates of *S. rolfsii* was tested by dual culture method. Out of 55 combinations, eight Mycelial Compatibility Groups (MCG's) were identified. In the compatible reactions, intermingling of the mycelia and sclerotial formation was noticed at the site of interaction between isolates. Complete overgrowth of the mycelium of two isolates was not observed in compatible reactions. The isolates produced sclerotia with exudates in a line at site of interaction between the isolates. Sclerotial formation on the surface of both the cultures was also noticed. In the incompatible reactions, formation of inhibition zone of size 0.2 - 2.6 mm and development of sclerotia on either side of inhibition zone were observed. From the study, it was clear that the virulent isolate from marigold (MG-2) showed mycelial compatibility with virulent isolates from chrysanthemum (CH-2), mango (MN-2) and banana (BA-2).

Cross inoculation studies revealed that all the isolates were pathogenic to other hosts but variation in the pathogenic character of these isolates was observed. Variation in the symptom expression was not noticed on the inoculated plants by different isolates obtained from the same host. All the isolates produced infection on the leaves of other hosts, but failed to produce collar infection on mango seedlings. The isolates BA-1 and BA-2 did not initiate infection on collar region of chrysanthemum. It was concluded that different isolates of *S.rolfsii* obtained from the selected fruit crops and ornamental plants showed variability in their pathogenic character.

Host range of *S. rolfsii* was studied by cross inoculating different isolates of *S. rolfsii* on selected vegetables *viz.*, tomato, amorphophallus and spices *viz.*, black pepper, ginger. All the plants tested were found susceptible to the pathogen. Symptom appeared as small, water soaked brown discolouration on the inoculated leaves and collar region. Later it enlarged in size and covered with white mycelial growth of the pathogen in all the hosts except in the collar region of amorphophallus inoculated with MG-2 and MN-2. It was also observed that the initial infection was first noticed on leaves and collar region where pinprick have been given before inoculation. Variation among the isolates was observed in the production of sclerotia and was noticed that sclerotial development was completely absent in ginger and tomato on both the leaves and collar region. On the leaves of black pepper, white mycelial growth and sclerotial formation were noticed on the lower side of the leaves. The isolate BA-2 produced concentric zonations on the inoculated leaves of pepper.

Estimation of IAA and total phenol in mycelia, culture filtrate and sclerotia of various isolates revealed that all the isolates produced highest IAA and total phenol in the sclerotia. Among the isolates, MG-1 and MG-2 recorded the highest IAA and total phenol in mycelia, culture filtrate and scloerotia of various isolates of the pathogen respectively.