

**INTEGRATED MANAGEMENT OF
FOOT-ROT DISEASE OF *PIPER BETLE* L.
CAUSED BY *SCLEROTIUM ROLFSII***



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BY
MD. MONTAZ ALI SARKAR

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**MYCOLOGY AND PLANT PATHOLOGY LABORATORY
INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI
RAJSHAHI-6205
BANGLADESH**

*Dedicated
To
My Parents*

DECLARATION

I do hereby declare that the whole work submitted as a thesis entitled **“INTEGRATED MANAGEMENT OF FOOT-ROT DISEASE OF *PIPER BETLE* L. CAUSED BY *SCLEROTIUM ROLFSII*”** in the Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh, for the degree of **Doctor of Philosophy** is the result of my own investigation and was carried out under the supervision of Dr. M. Shahidul Alam, Professor, Department of Botany, University of Rajshahi, Bangladesh. The thesis has not been submitted in the substance for any other degree.

Date: March, 2016

Md. Montaz Ali Sarkar

Dr. M. Shahidul Alam
 Professor
 Department of Botany
 University of Rajshahi
 Rajshahi-6205
 Bangladesh

ইব্রাহীম আলী



University of Rajshahi

W. Gg. কবীর আলী

Associate Professor
 Department of Botany
 University of Rajshahi
 Rajshahi-6205
 Bangladesh

Phone: Office 880-721-711118, Fax: Office-721-750064, HP: 88-01715-138818, E-mail: alam.shahidul89@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled “INTEGRATED MANAGEMENT OF FOOT-ROT DISEASE OF *PIPER BETLE* L. CAUSED BY *SCLEROTIUM ROLFSII*” is an original work done by Md. Montaz Ali Sarkar for the degree of Doctor of Philosophy in the Institute of Biological Sciences, University of Rajshahi, Bangladesh. The references cited in it have duly been acknowledged. The style and contents of the thesis have been approved and recommended for submission.

Date: March, 2016

Supervisor

(Dr. M. Shahidul Alam)
 Professor
 Department of Botany
 Rajshahi University
 Rajshahi

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ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
°C	:	Degree Celsius
2,4-D	:	2, 4-Dichlorophenoxy acetic acid
BBS	:	Bangladesh Bureau of Statistics
cm	:	Centimeter(s)
e. g.	:	(exempli gratia) for example
<i>et. al.</i>	:	and other people
etc	:	et cetra (and all the others)
Fig	:	Figure
gm	:	Gram
h	:	Hour
ha	:	Hectare
HS	:	Highly Susceptible
i e	:	(idiest) in the other words
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
L	:	Liter
Lb	:	Pound(s) (weight)
m	:	Meter
mg	:	Milligram(s)
ml	:	Millilitre(s)
mm	:	Millimetre(s)
MS	:	Moderately Susceptible
mt	:	Metric ton(s)
NAA	:	α -Naphthylacetic acid
pH	:	Negative logarithm of hydrogen ion (-log H ⁺)
PPM	:	Parts per million(s)

R	:	Resistant
RH	:	Relative humidity
S	:	Susceptible
sp.	:	Species (Singular)
spp	:	Species (Plural)
sq	:	Square
t	:	Ton
T	:	Treatments(s)
viz.	:	Videli (namely).

ABSTRACT

Foot rot disease of betelvine is a serious fungal disease caused by *Sclerotium rolfsii*. Eight cultivars of betelvine were identified on survey period and found the highest and the lowest disease incidence in cultivars Bangla and Sanchi. Among the places, Mohanpur areas of Rajshahi district showed the highest disease infected areas than that of other places in different districts. The disease incidence also varied from place to place and year to year. During survey period, loamy soil planted borojes showed the highest disease incidence and sandy loamy soil borojes showed the lowest disease incidence. Flood systems irrigated borojes showed the highest and drip system irrigated borojes showed the lowest disease incidence of betelvine. Positive correlation existed between the disease incidence and the weather factors like rainfall, number of rainy days and relative humidities. The morphological variation was observed among ten isolates of *S. rolfsii* and isolates-5 showed the highest virulence. The highest per cent mycelial growth was observed in cellulose containing carbon sources medium and in sodium nitrate, nitrogen sources medium. Phytohormone 2, 4 D inhibited hundred per cent mycelial growth of the fungus. Clove and Cinnamum oil inhibited hundred per cent mycelial growth of the fungus at all concentrations at PDA medium. Urea fertilizer inhibited hundred percent mycelial growth of the fungus. Neem cake at 3% concentration inhibited hundred percent mycelial growth of the fungus. Cow dung and cow urine also had inhibitory effect on mycelial growth of the fungus. Nicotinic acid showed inhibitory effect of the fungus. At all concentrations NaHCO_3 and NH_4HCO_3 showed complete inhibition mycelial growth of the fungus. Complete inhibition of mycelial growth of *S. rolfsii* was observed with treatment after *A. sativum* and *Lawsonia inermis* plant extracts. Hundred per cent mycelial growth inhibition was observed in *Allium sativum* and *Lawsonia inermis*, extracts at all concentrations in dried plant extracts. Hundred

per cent mycelial growth inhibition was observed in *Allium sativum* ethanolic extracts. Complete inhibition of mycelial growth was observed in folicure and score fungicides. Herbicides Logran and 2, 4-D inhibited hundred per cent mycelial growth at all concentrations. Insecticides Desis and Moventa at all concentrations showed the same results. The highest per cent mycelial growth inhibition was observed in *Trichoderma* isolates-3 and the lowest in *Trichoderma* isolates-14. Among the ten *Trichoderma* isolates culture filtrates, isolates-3 had shown maximum per cent mycelial growth inhibition. The highest per cent disease incidence observed in 03 months old betelvine plantation. The lowest viability of sclerotia was observed at 5 cm depth with black plastic mulching. The lowest per cent disease incidences were recorded after treatment with 60 days solar heating. Maximum reduction in sclerotial germination was observed in soil amended with neem cake. No viability of the fungus was recorded upto 30 minutes hot water treatment at 52°C and above. The lowest disease incidences were recorded after treatment hot water 0.5 Lit/kg cultural practices. The lowest disease incidence was recorded after treatment with neem cake, *A. sativum*, Score, *Trichoderma* iso-3, N₂₀₀P₁₀₀K₁₀₀ respectively in field. The effect of crop rotation by sugarcane cultivation found the lowest disease incidence. Out of eight cultivars, only one cultivar, namely, sanchi showed moderately resistance reaction. Incase of IDM effect, the lowest disease incidence was recorded in treatment T₉ by (sanitation + neem cake 6t/h + application *A. sativum* extract 5% + application *T. harzianum* + score 1.0% drenching (neem cake, *A. sativum* and *Trichoderma* at quarterly intervals and score at monthly intervals) and increasing production number of leaves in lakh/hac/year.

Chapter One

INTRODUCTION

The betel leaf generally known as 'Pan' (*Piper betle* L.) is a perennial climber cultivated mainly for its leaves, which are used as a masticatory. The betelvine grows best under tropical conditions having a cool, shade, considerable humidity and a good supply of soil moisture. Geographically it belongs to region bounded by 68°E to 118°W longitudes and 30°N to 12°S latitudes. It is grown from sea level to an altitude of about 900 m (Chaurasia, 2001).

Betelvine is extensively grown as an important commercial crop throughout the tropical and subtropical regions like India, Bangladesh, Srilanka, Malaysia and to some extent of Singapore, Thailand, Burma (Myanmar), Indonesia, Philippines, Nepal, Bhutan, Pakistan and Papua New Guinea (Plate 2A). Betelvine has been referred in the Indian history religion and culture dating back to 3000 B.C (Balasubrahmanyam *et al.*, 1994).

The major betelvine (Pan) producing districts of Bangladesh are Rajshahi, Khulna, Barisal, Chittagong, Sylhet, Mymensingh, Chuadanga, Meherpur, Cox's Bazar, Kustia, Jessore, Nilphamary, Bhola, Bagerhat and Sathkhira (Plate 2B). In addition to that small-scale production is also found in the district of Rangamati, Bandarban, Khagrachari, Noakhali, Feni, Lakshmipur, Comilla, Brahmanbaria, Chandpur, Moulvibazar, Habiganj, Narsingdi, Narayanganj, Faridpur, Pabna, Gopalganj, Madaripur, Sariatpur, Rajbari, Jhennaidah, Magura, Narail, Pirojpur, Jhalokati, Patuakhali, Barguna, Panchogarh, Rangpur, Dinajpur, Lalmonirhat and Nawabganj (Roy, 1993). India is the largest producer of betel leaves in the world (Arulmozhiyan *et al.*, 2005).

Betelvine cultivation is a century old tradition in Bangladesh. Bangladesh has flourished betel leaf garden with 12,700 ha area (Hossain *et al.*, 1986). Total area

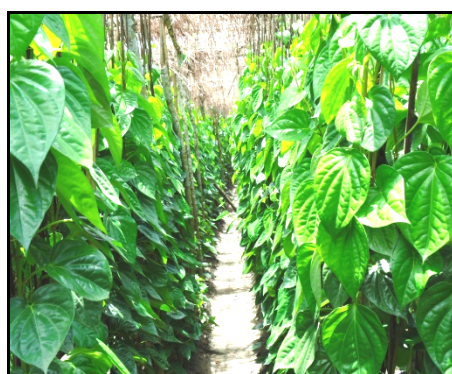
and production of betel leaf in Bangladesh are 56,605 acres and 15, 1814 metric tons, respectively in 2011-2012 (BBS, 2012). Betelvine belongs to the dicotyledones family Piperaceae that comprises of 12 genera and 300 species (Chaurasia, 2001). Betel leaf is aromatic, digestive, stimulant and carminative. Medicinally it is useful in catarrhal and pulmonary affections. It is also used as poultices (Anonymous, 1976).

Betel leaf chewing is considered as a good and chief source of dietary calcium (Anonymous, 1976). The leaves act as deodorant and tend to remove the fetid smell in the mouth. The fresh crushed leaves are used as antiseptic for cuts and wounds. It is also good for respiratory systems and is used in treatment of bronchitis, cough, cold and chillies (Chopra *et al.*, 1954; Balasubrahmanyam *et al.*, 1994). Leaf juice is used to relieve cerebral congestion and allay thirst and also act as a tonic to the brain, liver and heart (Chaurasia, 2001). Juice of leaf is used as an eye drop in painful affections and night blindness (Ghani, 1998). It is antiseptic astringent, mildly stimulant, expectorant and exhalent in nature and rich with a good number of essential oils and alkaloids (Chaurasia, 2001). Betel leaves have antimicrobial activity in mouth. Essential oils of the plant contained phenol compounds such as cavicol, cavibetol, carvacrol, eugenol and allylpyrocatechol, which are assumed, could inhibit food borne pathogens, as well as food spoilage microorganisms (Jenie *et al.*, 2001). Betel leaf also possesses the important food value. The leaf is fairly rich in vitamin B and C (Chaugle, 1960). From chemical analysis it is reported that in every 100 g betel leaf having water 85.4 g, mineral 2.3 g, fiber 2.3 g, protein 3.1 g, fat 0.8 g, carbohydrate 6.1 g, calcium 0.23 g, iron 0.007 g, vitamin A 0.00576 g, vitamin B 0.00007 g, vitamin B₂ 0.00003 g and C 0.005 g respectively and 4.4 Kcal energy is released from every 100 g betel leaf (Ali *et al.*, 1977). In Bangladesh most of the betel vine cultivation takes place in close conservatories called 'boroj' (Plate 1A, B&C) but in Nilphamari, Rangpur, Dinajpur and hilly areas it grows in open field condition with the support of Areca nut or other tree plant (Plate 1D).

Plate 1



A



B



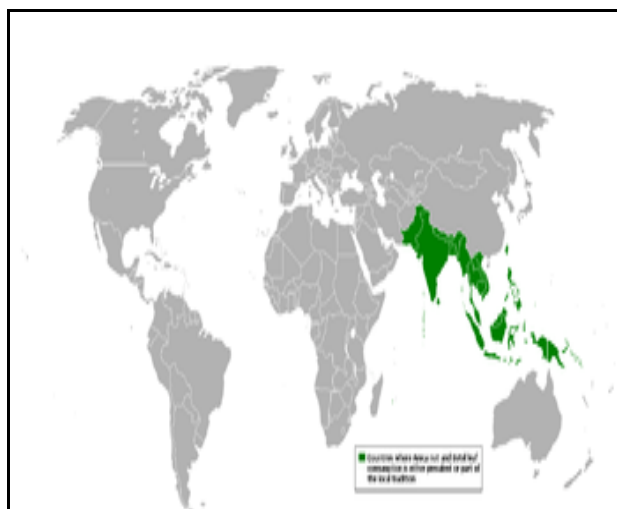
C



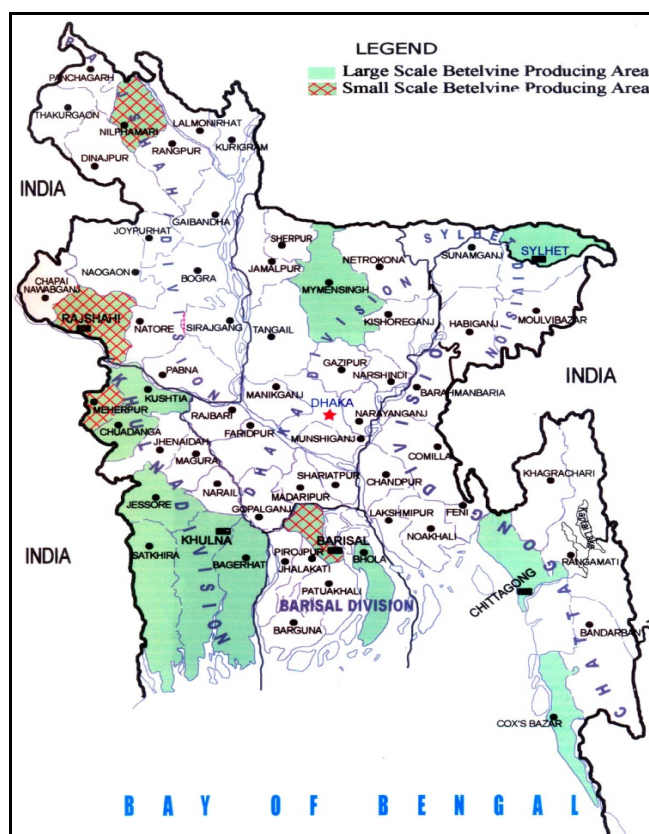
D

A- Betelvine cultivation in close thrives, B-Healthy betelvine boroj
B- New betelvine plantation, D- Betelvine cultivation in open condition.

Plate 2



A



B

A- World wide betelvine cultivation, B- Betelvine cultivation in Bangladesh

Plate 3



A



B



C

**A= Infected betelvine plantation, B = Rotting of stems near the soil level,
C= Severely damaged betelvine plantation**

Betelvine belongs to the dicotyledones family Piperaceae that comprises of 12 genera and 300 species (Chaurasia, 2001). Malayan archipelago may be the home of betel vine and considered that it has been under cultivation for more than 200 years. Betelvine is native of central and eastern Malaysia (Burkhill, 1966; Balasubrahmanyam *et al.*, 1994; Chaurasia, 2001 and Roy, 1993).

Betel leaf is one of the important cash crops. Its cultivation is highly intensive and particularly suited to smallholdings. A baroj (betelvine garden) 15 decimals (0.15 acre) of land can be economically viable and can sustain a family of five members, with an average net return of about Rs. 500 per month, over a period of 15 years an average life of the plantation in west Bengal of India. The practice is similarly applied also for Bangladesh; ones established a betelvine garden becomes a perennial source of employment for the growers.

Betel leaf has been and is likely to remain a potential trade item in the domestic and foreign markets. Bangladesh earn Tk. 12.84 crore in 1987-88 fiscal year by exporting betel leaf to Pakistan, United kingdom, Canada, Hong Kong, Saudi Arabia, Qatar, United Arab Emirates, Oman and Bahrain (Roy, 1993; Hossain *et al.*, 1986).

The moist humid shaded conditions of "pan" gardens (Barojs) favour betelvine growth but also serve as an optimal condition for fungal disease. This crop has been found to be affected by a number of serious fungal diseases reducing its productivity considerably and limit economic cultivation of the crop. Foot rot is a major disease among other diseases in betelvine cultivation. This disease caused by *Sclerotium rolfsii* (Sacc.). It is found in all the seasons in Bangladesh but severely effect in the rainy season. If this disease gets favourable weather conditions of its own, it attacks betelvine plantation and damages entire cultivation with in week (Plate 3 A&C). Several workers from different pan growing areas of the world studied different aspects of "Pan" diseases. Several workers from' time to time (< biblio>) have reviewed fungal diseases of "pan"

and presented good review type on fungal diseases of pan along with other aspect of pan diseases. Information on the diseases of pan in Bangladesh appears to be limited with the reports by Anonymous (1973), Alamgir (1985), Hossain *et al.* (1985), Jalil (1986).

Soil borne plant pathogens pose potential threats for crop plants. Among these, *Sclerotium rolfsii* (Sacc.) Curzi is an unspecialized soil-inhabiting parasite and is very widespread in distribution. It survives in soil in absence of the host for a considerable length of time. The sclerotia of the fungus can survive in soil alone and also with the crop debris for a long period of time and can migrate from one place to another with agricultural appliances and irrigation water (Rangaswami, 1979). During its survival resting structures like sclerotia may plays a major role in the ability of such fungi to attack and colonize dead plant materials, which enables them to remain active as saprophyte for some time on such substrates and develop their mycelium. Antagonism by other soil microflora reduces their ability to continue saprophytic activity unless they have strong competitive saprophytic ability and can colonize organic substrates more successfully than other soil saprophytes. Based on nutritional requirements and mode of infection, *S. rolfsii* has been termed as necrotroph, which kills advance of penetration and then lives saprophytically.

The systematic position of the *S. rolfsii* is Division- Mycota, Sub-Division- Deuteromycotina, Class- Deuteormycetes, Order-Myelia Sterilia, Genus- *Sclerotium* and Species- *Sclerotium rolfsii*. The fungus produces inter and intra-cellular mycelium, which invades the host tissue and weakens it. The sclerotial bodies, 100-200 μ in diameter, are formed on the host as well as in culture media (Aycock, 1966). The perfect stage of the fungus is *Pellicularia rolfsii* (Sacc.). We belong to the Sub-Division- Basidiomycotina and basidia and basidiospore (Webster, 1993).

Most of the soil-borne plant pathogens like *S. rolfsii* grow only to a limited extent in soil and therefore are immobile. However, the mycelium grows to some distance depending on the size of food base from which they have originated and also up the extent to which soil antagonists permit them to grow. The fungus frequently produces sclerotia. Sclerotia are the pseudoparenchymatous aggregations of hyphae, which serve the purpose of survival in unfavourable situation. Mature sclerotia can be differentiated into four zones; a fairly thick skin or cuticle, a rind made up of 2-4 layers of tangentially flattened cells, a cortex of their walled cells with densely staining contents and a medulla of loose filamentous hyphae with dense contents (Chet *et al.*, 1969). It is characterized by the formation of hard sclerotia, which are brown in colour and about a size of mustard seed with smooth or shallow pitted slimy surface. The fungus may attack the plant at any stage of growth. Under favorable condition the disease can destroy entire plantation within one crop season.

The fungus causes cuticle decay/rot at ground level and the conspicuous white mycelium appears and spreads over the soil and on any organic debris. Such decay or rotting is initiated by two groups of pectinolytic enzymes, such as pectinoesterases and polygalacturonases. These are responsible for degradation of pectic substances, which are major chemical constituents of middle lamella that binds the cell together. Besides the enzymes, pathogens produce substances like impedin which aid in infection and development of the disease syndrome. Impedin is a substance produced by a pathogen, which inhibits the development of defense reaction of plants. Oxalic acid produced by *S. rolfsii* is an impedin as the acid reduces tissue pH at which toxic steroid alkaloids are ineffective against *S. rolfsii* (Rangaswami, 1979).

The fungus *S. rolfsii* possesses wide host range (Aycok, 1966). The major diseases caused by this fungus in the tropics are root rot of groundnut, damping off of cotton and leaf sheaths of sugarcane. It severely attacks black pepper and sweet potato. In

the sub-tropical countries, it is a virulent pathogen of beans, maize, chickpea, lentil, potato, tomato, cabbage and cowpea etc (Mehrotra and Aneja, 1990).

Yield loss due to stem rot might be as great as 80% (Porter *et al.*, 1984). About 72% yield loss due to stem rot in groundnut in India, 70% from Northern Transvaal (Aycock, 1966) were reported. The United State Department of Agriculture (USDA) estimated losses from \$10 to 20 million annually in the southern peanut growing areas of the country (Aycock, 1966). Under favorable condition about 12 to 15.8% yield loss in potato and 84% loss in pulse have been reported by Ali and Dey (1994) due to foot rot/collar rot/stem rot caused by *S. rolfsii*.

Plant pathogens exhibit variation in their morphological, cultural/physiological, biological characteristics as well as pathogenicity or resistance in appropriate environment. Pathogenic and morphogenic variations are well documented in many fungal pathogens (Kumar *et al.*, 1995). However, a very few reports are available in respect of variability in the population of *S. rolfsii*, pathogenic to different host and within the same host population (Kim, 1974 and Punja, 1985).

Plant diseases caused by soil-borne pathogens are by far the most difficult and expensive to manage. It has been reported from various countries that numerous fungicides inhibit the germination of sclerotia or mycelial growth of the fungus. Most of them effectively control the diseases of various crop plants in the field (Agnihotri *et al.*, 1975; Backman *et al.*, 1975). The major constraints in using fungicides to control *S. rolfsii* are that a large amount of chemicals is usually required. The efficacy of particular compound may vary depending on the nature of the chemical, environment and methods of to minimize the inoculums of *S. rolfsii*. Moreover, most of the farmers are not serious to adopt cultural approaches due to lack of proper knowledge. Again, most of the methods are not effective unless used in conjunction with fungicides or fertilizer application (Gurkin and Jenkins, 1975 and Punja *et al.*, 1985).

Control measures through creation of host resistance have not yet become a viable method because of frequent failure of resistance in the host and formation of new virulent strains/races/biotypes among the pathogens. Biological control can be one of the options or one of the integrated methods for the management of disease. Biocontrol with beneficial microbes are capable to manipulate the environment around a crop plant to favour organisms that contribute to plant growth and vigour, rather than simply applying fungicides, which may also destroy a range of nontarget beneficial microorganisms. The organisms used as biocontrol agents are less disruptive to ecosystem than that of chemical pesticides. Among the different biocontrol agents *Trichoderma* spp. are known antagonist to soil-borne pathogenic fungi and have been reported as potential biocontrol agents under both *in vitro* and *in vivo* condition (Papavizas, 1983 and Ruppei, *et al.*, 1983). *Trichoderma* spp. were also found to be effective against different sclerotial fungi including *S. rolfsii* (Labakhqua and Donadze, 1975; Santos and Dhingra, 1982; Inbar *et al.*, 1994).

In the light of present days demand integrated disease management is considered as the most rational and effective approach where fungal or bacterial antagonist and fungicides could be the common components. In most cases single weapon for disease management is not enough or adequate to suppress the disease. So in such cases integration of management practices are indispensable. Thus one of the most desirable characteristics of an antagonist would be its compatibility in using with other methods. Insensitivity of *Trichoderma* to different fungicides has been reported earlier by several researchers (Hadar *et al.*, 1979; Swant and Mukhopadhyay, 1990).

Behaviors of a fungus depends upon its nutritional response, Phytopathogenic organisms express a similarity in broader behavior of their basic nutritional needs yet, they maintain their individuality for the choice of specific substances (Lilly and Barnett, 1951). Microorganisms possess a remarkable capacity to utilize a

wide range of organic and inorganic substances. Now a day, most of the organisms are grown in *in vitro* by supplying proper nutrients.

The growth of fungi is profoundly affected by environment. Temperature is one of the important environmental factors, which influences the growth and development of fungi. Some fungi multiply severely at low temperature, others at high temperature. So fungi/pathogens differ in their optimum temperature for growth, sclerotia formation and infection (Bruehl and Cunfer, 1971). Humidity is another important environmental factor, which influences disease development and sclerotia formation of fungi and occur over a wide range of relative humidity (Jacome *et al.*, 1991).

The role of plant hormones in plant disease is not clearly identified (Al- Masri *et al.*, 2002). NAA reduced mycelium growth rate of fungi (Al-Marsi *et al.*, 2002). To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant disease, the interest on essential oils has been increased. Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants (Isman, 2000). They were previously known to have biological activities such as antifungal (Soliman and Badeaa, 2002), antibacterial (Dorman *et al.*, 2000), insecticidal and nematicidal effects. Cow urine and cow dung are the two important bio matters that are extensively used by the growers as manure for production of different crops. Cow urine and cow dung was more effective in suppressing mycelial growth. Cow urine had comparatively better efficacy than that of cow dung in suppressing mycelial growth.

The growth of some organisms has been inhibited by herbicides which are meant to destroy weeds while some fungi have been affected by application of insecticides. The inhibitory effects of some pesticides on non-target organisms have already been confirmed. Reports have shown that lambda cyhalothrin (active ingredient in Karate) insecticide significantly reduced *Fusarium* ear rot of maize caused by *F. verticilloides* (formerly known as *F. monilliforme*).

Crop rotation is one of the economical and ecofriendly management practice which helps in reducing the disease incidence (Rodriguez *et al.*, 1991). It is an age old practice followed by the farmers. This operation has effect on the starvation of pathogen in the absence of proper host. To take advantage of this phenomenon in the eradication of soil borne, *S. rolfsii* of betelvine, different crops were tried as rotation crops with betelvine. This may perhaps to starve *S. rolfsii* in the absence of proper host.

Cultural practices can be harnessed for the management of foliar and soilborne diseases by creating an environment which is favorable for the crop and unfavorable for the pathogen. Certain cultural practices e.g. flooding and sanitation are used mainly for pest control while others, e.g. irrigation, can be used for both crop management and pest control. Some cultural practices, e.g. deep plowing, crop rotation, flaming sanitation, soil solarization and biofumigation. Neglecting crop rotation results in the enrichment of pest populations, the accumulation of toxic substances, the depletion of mineral nutrients, degradation in soil fertility and destruction of soil structure.

Crop rotation deals with plant health in general rather than with controlling a specific disease, although a certain crop sequence might be more effective in reducing the incidence of a specific disease than others. It can also be regarded as insurance against the rise of unknown pathogens. In tomatoes, the incidence of the southern blight disease was lower after deep plowing (25 cm depth), compared with shallow tillage by means of a harrow. Deep plowing results in exposure of propagules to elevated temperatures and physical killing of the pathogen. This can be regarded as dry soil solarization. Summer plowing was effective at reducing populations of cyst nematodes and increasing wheat yield. The two principal aims of sanitation are to prevent the introduction of inoculum, by whatever means, into the field, greenhouse, farm or community, and to reduce or eliminate the inoculums that is already present at these sites. This can be achieved by flooding, flaming, solarization, plowing, chemical treatments to

destroy resting structures, mechanical removal of residues, controlling alternate hosts (weeds, volunteer plants), rouging (removing diseased plants from host populations), pruning and other means.

Application of balanced fertilizers is very important for the management of many diseases. Severity of disease of many crops can be reduced by applying nutrients at suitable doses. The plants take up the nutrient from fertilizers and utilize them in the plant metabolic system for strengthening the defense mechanism of plant through formation of different kinds of barriers (both mechanical and biochemical), which ultimately opposes the infection by plant pathogenic organisms. Khatun *et al.* (2011) reported that fertilizer dose 120-30-90 kg/ha appeared to be the best combination of N, P, and K in reducing the disease incidence and to increase seed yield of mustard.

The utilization of organic amendments for control of soil-borne plant pathogens has often been considered at best variable. Organic amendments containing high nitrogen, significantly reduced populations of a wide spectrum of soil-borne plant pathogens. Pathogen control was shown to arise from the ammonia and (or) nitrous acid generated, the concentrations of which are controlled by pH, organic matter content, soil buffering capacity, and nitrification rate. Organic amendments, such as vermicompost, mustard cake and neem cake were commonly used in agricultural production for their fertility value prior to the availability of chemical fertilizers. It is likely that these amendments also provided other benefits such as improved plant health due to reduction of pathogens. It also increased soil beneficial microorganism. Organic matter added to soil may increase soil fertility, stimulate growth of crops, and suppress plant pathogens. When properly used, amendments can not only reduce populations of soil-borne pathogens but also induce soil suppressiveness by stimulating activities of antagonists in soil.

Of the various methods used to control plant diseases, the use of chemical fungicides is very common. However, in view of the complexities arising from

the use of chemical pesticides, such as harmful effect on environment and non-target organisms including man, domestic animals, beneficial insects, wild life, the use of microorganisms as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control. Antagonists may act against pathogens in one or more of the following mechanisms: competition, antibiosis, parasitism, and predation or induce resistance in plant; a hydrolytic enzyme excreted by antagonists is a well-known feature of mycoparasitism. Though, fungicides have enormous killing capacity but, indiscriminate use of fungicides is not only hazardous to living being but disrupt the natural ecological balance by killing the beneficial soil microbe. However, biological control of plant pathogens is a potential non-chemical means for plant disease control and can serve as an alternative for costly chemical treatment. *T. harzianum* was found to be an effective biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* under both greenhouse and field conditions (Elad *et al.*, 1980). *T. harzianum* is a common soil species and is used in biological control of a variety of plant-pathogenic fungi. The application of fungi as biological control agents, especially *T. harzianum*, parasitized *S. rolfsii* (Desai and Schlosser, 1999). Significant reduction of mycelial growth of *S. rolfsii* in the presence of *Trichoderma* spp. were also reported by many other workers (Mondal, 1999; Akhter, 1999; Khan, 2003).

However, to reduce toxic hazards of human being as the betel leaves are directly chewed immediately after harvest, attempts were made by many workers to replace application of fungicides with biological control. Control of these plant pathogens is very difficult because of their survival capacity. It is also not practicable owing to high cost of chemicals besides problems of environmental pollution and development of resistance in target fungus as opined by Rudresh *et al.*, 2005.

Biological control offers a novel approach, as an alternative solution for long term sustainability and is a promising disease management technology against

soil borne plant pathogens, when applied either alone or in combination with other management practices.

Trichoderma sp., which is a common saprophytic filamentous fungus, in almost all soil and rhizosphere microflora, it is recognized as a biocontrol agent against various plant pathogenic fungi. They are commercially applied as biological control agents based on different mechanisms, such as the production of antifungal metabolites, competitor for space and nutrients and through mycoparasitism (Pandey *et al.*, 2005).

Chemical control can reduce the disease to some extent but it is not so economical for the farmers. With this view in mind, integrated crop management (ICM) approach has played special role by the researchers. It can also reduce the indiscriminate use of chemical fertilizers and pesticides. The ICM practices not only save crop from the above field diseases but also reduce the possibility of attack by the others pathogens. Therefore, the present study was under taken to workout the effect of integrated management combined with different fertilizer doses, soil treatment (neem cake) and fungicide (Score) on the intensity of foot disease and yield of betelvine.

Findings of the present study clearly demonstrated that any individual disease control techniques may not be effective but satisfactory control is possible through integrated approach. Effectiveness of fungicide may be improved if fungicides are applied in combination with recommended chemical fertilizer, soil treatments and other control measures. Many investigators also demonstrated the benefit of integrated disease management practices to control plant diseases. Till the year 1999 very much little information has been published about the Integrated Diseases Management (IDM) of betelvine. The constraints to IPM and IDM development are identified as lack of forecasting system, non availability of tools for breeding either conventionally or biotechnologically, and a sketchy understanding of the biological control system. The present investigation was

undertaken for the management of foot rot of betelvine by using sanitation, soil amendment, biocontrol agent and folicure in combination to reduce the toxic hazards of chemicals as the betel leaves are chewed directly after harvest.

Soil solarization has proved as an effective and environmentally safe alternate to chemicals that are applicable to various agricultural situations for the control of various soil-borne pests, including phytopathogens and weeds (De Vay *et al.*, 1990; Stapleton *et al.*, 1987).

The present work has been carried out to give detail information of *Sclerotium rolfsii* occurring on betelvine plants of 8 different betelvines plantations from different areas of Bangladesh.

Keeping all this view, detail studies with following objectives were under taken:

1. Survey of different betelvine plantations.
2. Isolation, purification, identification, its pathogenecity, cultural and morphological characteristics of the fungus.
3. Effect of temperature, relative humidity and rainfall on the incidence of foot rot disease.
4. Effect of carbon and nitrozen sources on the mycelial growth of *S. rolfsii*
5. Factors affecting development of the fungus, viz.
 - (a) Nutritional requirements of the fungus on the growth of mycelium and sclerotia formation was carried out.
6. Effect of Phytohormones, oils, fertilizers, oilcake, cowdung, cow urine, vitamin and salts on the per cent of inhibition of *S. rolfsii*.
7. Effect of` different plant extracts and fungicides on the inhibition of fungal mycelium growth.
8. Effect of herbicides and insecticides on the inhibition of fungal mycelium growth.

9. Effect of` soil solarization, hot water treatment, cultural practice and crop rotation to manage the disease.
10. Antagonistic effect of *Trichoderma* strains and culture filtrate test on the inhibition of mycelium growth.
11. Effect of organic amendment, plant extract, fungicides, *Trichoderma* and NPK fertilizer for the management of the disease in field.
12. Screening different betelvine cultivars against foot rot disease.
13. Integrated management of foot- rot disease of betelvine.

Chapter Two

MATERIALS AND METHODS

The fungus was isolated from collar part of stem of betelvine. The diseased materials were collected throughout the season in June 2010 to December 2012.

Samples were collected after proper noting down from the field and kept separately in polythene bags (6 × 4 inch), which were labeled properly with date for future reference. After collection, the samples were brought in laboratory for the study.

After proper sterilization disease plant samples were incubated in humid chamber for 5 days at 27°C.

Examination were made within 5-10 days under stereoscopic binocular microscope and finally slides were prepared using lacto phenol as mounting medium for morphological studies under compound microscope, whenever necessary cotton-blue was used to stain the fungal materials.

Slides were also prepared for morphological studies of the fungus from its culture grown on PDA medium. Isolation of the fungus was made either by direct method or by plate culture method.

2.1 Origin of isolates

Sclerotium rolfsii was obtained from the diseased plants of betelvine.

2.2 Method of sterilization and incubation

In laboratory the advancing or water soaked portion of the lesions were cut into small pieces (approximately 5×5 mm per section) and surface-sterilized by soaking for 15 seconds in 5% commercial chlorox (commercial chlorox usually consists of 5.25% sodium hypochlorite by weight), solution contained in a

beaker, by rotating the beaker frequently. These were then given several washes in sterilized distilled water so as to remove sodium hypochlorite from them. Excess water from the surface of the sterilized materials was removed by gently pressing them between two flaps of previously sterilized filter papers. The materials were removed with a flamed forceps and placed within a humid chamber. The materials were placed sufficiently apart, so as to prevent them from touching each other. The materials under this condition when incubated were found to maintain their good health for longer period and support profuse mycelia and sclerotia of the fungus.

2.3 Identification of isolated fungus

The fungus isolated from infected tissues of stem on betelvine describe above. The fungus was transferred to PDA slants and subculture on PDA for the identification. The fungus was identified with the help of keys outlined by Subramaniam (1971).

2.4 Screening of the pathogen:

10 isolates of *S. rolfsii* were isolated from different locations of infected betelvine borojes. The isolates were cultured in Petri dishes on PDA medium and their cultural, morphological variation was studied.

2.5 Pathogenicity tests

2.5.1 Raising of host plants

For pathogenicity test, betelvine were grown, in earthen pot (29.5 cm breath & 21.5 cm height) containing loamy soils. Two to three cuttings were raised in each pot from treated cuttings of a particular variety of betelvine at about 2 months old.

2.5.2 Preparation of sclerotial suspension

Sterilized distilled water was used for the preparation of sclerotial suspension. 20 ml sterilized distilled water was added on a plate and shaken well to remove maximum number of sclerotia from 14-15 days old culture of *S. rolfsii* grown on PDA.

2.5.3 Method of inoculation

Cuttings of betelvine were inoculated by placing 3-5 sclerotia/plant. The inoculated plants were covered with polythene bags and incubated at 27°C for two days. Plants were then transferred to the borj with special arrangement. Water was added every day to keep them under moist condition after removing the polythene bags. Observations were made after 10-12 days when the stem of the betelvine had developed characteristic lesion and compared with the earlier symptoms recorded before.

2.5.4 Reisolation of pathogen

The artificially infected stems of betelvine were collected and after sterilization, isolation was made following the usual procedure. In most of the cases pathogen was successfully isolated from the infected plants to PDA plates. The morphological characters of the isolated organism were compared with the original isolates.

2.6 Preparation of lacto phenol

Lacto phenol solution used as mounting medium consisted of the following composition (Ainsworth, 1963).

Phenol (crystals)	20.00 g
Lactic acid	20.00 ml
Glycerol	40.00 ml
Distilled water	20.00 ml

After weighting the constituents were taken in a conical flask to which distilled water was added. The flask was shaken well till a homogenous solution was obtained.

2.7 Preparation of lacto phenol-cotton blue stain

One gram of cotton-blue added to 100 ml of lactophenol and shaken well till it was dissolved. The solution of lactophenol and lactophenol cotton-blue were stored in a cool dark place.

2.8 Preparation of slides

A suitable portion of culture of *S. rolfsii* from PDA plate was selected under a stereoscopic binocular microscope and was taken out with the help of forceps and needles and on cleaned slide and poured 1 or 2 drops of lactophenol on clean slide. It was then gently warmed by heating and cooling over low flame of spirit lamp for 6 to 8 times, but was never allowed to boil. Whenever needed, the material was stained with small quantity of cotton blue. A clean cover glass was then placed over the materials, excess fluid was removed by soaking with blotting paper and examined under compound microscope.

2.9 Preparation of clearing reagent

Throughout the present investigation corning glassware's and chemicals supplied by E. Mark and BDH were used. All glassware's were cleaned with a solution of potassium dichromate and sulphuric acid in the following proportions (Ricker and Ricker, 1936).

Potassium dichromate	60.00 g
Sulphuric acid	60.00 ml
Water (tap)	1000.00 ml

2.10 Preparation of 0.1% mercuric chloride (HgCl₂) solution

Mercuric chloride (HgCl₂) solution is used for surface sterilization of the diseased sample.

Mercuric chloride 100.00 g

Distilled water 100.00 ml

Shaken well till it was dissolved.

2.11 Preparation of culture media

Potato Dextrose Agar (PDA) medium:

Peeled and sliced potatoes	200.00 g
Dextrose	15.00 g
Agar	20.00 g
Distilled water	1000.00 ml

The liquid media were solidified with the addition of 2% agar, wherever required.

Solid media were autoclaved at 15 lb/inch² pressure for 20 minutes. In case the medium contained any substance liable to decomposition or denaturation, it was subjected to fractional sterilized for three successive days. Petri dish and other glassware's were sterilized for three hours in hot air oven at 160°C.

Garrett's (1936) agar disc method was used for inoculation, except where otherwise stated. The inocula were taken from 14-16 days old culture and sizes of the inocula were the same in all cases. After inoculation the culture were incubated at 27°C for 15 days for mycelial growth and sclerotia formation.

2.12 Survey and surveillance of disease

To estimate the incidence of foot rot disease of betelvine survey was carried out in Rajshahi, Natore, Nilphamary, Pabna, Chuadanga, Jessore, Rangpure and Pirozpure districts during 2010-11 and 2011-12. In each plot random sampling were made from which the number of healthy and wilted plants were counted and expressed as per cent disease incidence. The disease incidence was calculated by using following formula.

$$\text{Disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

2.13 Survey and surveillance of incidence of disease in different betelvine cultivars on survey period

To estimate the incidence of foot rot disease of different betelvine cultivars on survey period was carried out in Rajshahi, Natore, Nilphamary, Pabna, Chuadanga, Jessore, Rangpure and Pirozpure districts during 2010-11 and 2011-12. In each plot random cultivars sampling were made from which the number of healthy and wilted plants were counted and expressed as per cent disease incidence. The disease incidence was calculated by using following formula.

$$\text{Disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

2.14 Survey and surveillance of disease incidence variation in betelvine with soil type from various districts

To estimate the incidence of foot rot disease of betelvine with soil type from various districts survey was carried out in Rajshahi, Natore, Nilphamary, Pabna, Chuadanga, Jessore, Rangpure and Pirozpure during 2010-11 and 2011-12. In each plot random sampling were made with soil types from which the number of healthy and wilted plants were counted and expressed as per cent disease incidence. The disease incidence was calculated by using following formula.

$$\text{Disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

2.15 Survey and surveillance of means for disease incidence on the basis of influence of irrigation system from different districts

To estimate the incidence of foot rot disease of betelvine disease on the basis of influence of irrigation system of different districts was recorded in Rajshahi, Natore, Nilphamary, Pabna, Chuadanga, Jessore, Rangpure and Pirozpure during 2010-11 and 2011-12. In each plot random sampling were made on the basis of influence of irrigation from which the number of healthy and wilted plants were counted and expressed as per cent disease incidence. The disease incidence was calculated using following formula.

$$\text{Disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

2.16 Method the studying the effect of metrological parameter (temperature, relative humidity and rainfall) on the incidence of foot-rot disease

The experiment was conducted at the village of Mohanpure upazilla, Rajshahi district. Fortnightly data were recorded from an established betelvine cultivation boroj starting from the month of June, 2010 and continued till December, 2012. Disease data were correlated with the metrological data to find out the critical

weather condition for the epidemiology of the disease. Metrological data were collected from the nearest Metrological Centre (Rajshahi weather office).

2.17 Method of studying morphological and cultural studies

The following experiment was conducted to study the variation in the morphological characters of 10 isolates of *S. rolfsii* collected from different locations. Twenty ml of potato dextrose agar was poured into Petri plates. Mycelial discs (5 mm) of four days old culture of the respective isolates were placed at the centre of the plate. Three replications were maintained for each treatment at temperature of 27°C for 4 days and colony characters were recorded. To get matured sclerotial bodies the cultures were further incubated up to fifteen days. Diameter of ten sclerotial bodies was recorded in each treatment with the help of screw gauge and observations were statistically analysed. Colony diameter, dry mycelium weight, the total number of sclerotia produced per cm², weight of 100 sclerotia and shape of sclerotia of individual isolates were also recorded and data were analysed statistically.

2.18 Method of studying the effect of pathogenicity of different isolates of *S. rolfsii* on Bangla cultivar

10 isolates of *S. rolfsii* were tested for their ability to cause foot rot disease of betelvine by soil infestation method in pot culture experiment under boroj condition in Badehajrapara village, Mohanpur upazilla and Rajshahi district. Each earthen pot was filled with 1.0 kg of sterilized soil. *S. rolfsii* was thoroughly mixed with sterilized soil at the rate of 20 g/kg of soil. Controls were prepared using sterilized soil only. Two cuttings of betelvine were planted in each pot. Disease development was observed regularly and recorded at 15 days after plantings to estimate the effect of pathogens in causing cuttings mortality. The causal agent of cuttings mortality was confirmed after re-isolation of the pathogen from collar region of stems.

2.19 Method of studying of carbon and nitrozen utilization

The carbon requirements of the fungi *i.e.*, *S. rolfsii* was studied by replacing sucrose in Richards's solution with different carbon compounds. The carbon sources used in present study were D-glucose, D-fructose, mannitol, glycerol, starch, cellulose, sorbitol, and sucrose. The quantity of each carbon compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of carbon as that of sucrose present in the basal medium. Each treatment was replicated thrice. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. Separate sets of flasks were maintained for each pathogen. Cultures of *S. rolfsii* were inoculated separately to the respective sets of flasks and incubated at $26\pm 2^\circ\text{C}$ for ten, twelve and sixteen days respectively. Dry mycelial weight of the fungus was recorded and results were analyzed statistically. The nitrogen requirements of the fungi was studied by replacing potassium nitrate of Richards's medium. With different nitrogen sources viz., calcium nitrate, potasium nitrate, amonium nitrate, peptone, urea, amonium sulphate, asparagine and Sodium nitrate. The quantity of each nitrogen compound to be added was determined on the basis of their molecular weight, so as to provide equivalent amount of nitrogen as that of potassium nitrate present in the basal medium. They were sterilized at 1.1kg/cm^2 pressure for 15 minutes. The flasks were inoculated and incubated as described earlier. The dry mycelial weight of the fungus was recorded and results were analyzed statistically.

2.20 Method of studying the effect of phytohormones on mycelial growth inhibition of *S. rolfsii*

Five phytohormones namely IAA, IBA, 2,4-D, NAA, and BA at five different concentrations viz. 50, 100, 250, 500 and 1000 ppm were tested *in vitro* to evaluate their effect on radial colony growth using poison food technique (Dingra and Sinclair, 1985). The amount required for obtaining a known concentration of the desired phytohormons was calculated and added aseptically to a known

amount of sterilized PDA medium immediately before pouring in plates into each 90 mm diameter Petri dish. After solidification, the inocula (5 mm disc) from three days old culture of test organism *S. rolfsii* were placed at the center of the test plate. There are three replications for each treatment. The plates were incubated in the laboratory having ambient temperature of 28°C. Data on radial colony diameter was recorded after three days of incubation when the whole plate under control was covered with the growth of test organism. Inhibition of radial growth based on colony diameter was calculated following the formula as suggested by Sundar *et al.* (1995).

2.21 Method of studying the effect of essential oils on mycelial growth inhibition of *S. rolfsii*

Commercial essential oils of neem, mustard, clove, black cumin, cinnamon, and sesame oils were used in the present work. Essential oils used in the study were obtained from local market, Rajshahi. The inhibitory effect of the essential oils was evaluated against the linear growth of the pathogen. For each of the essential oil, five concentrations, *i. e.* 0.25, 0.50, 1, 2 and 3% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.22 Method of studying the effect of oilcakes and organic manure on mycelial growth inhibition of *S. rolfsii*

Different oil cakes like Sesame, Neem, Mustard, Vermicompost, Farmyard manure and Poultry manure were used in the present work. Oil cakes and organic manure used in the study were obtained from local market, Rajshahi. The inhibitory effect of the oil cakes and organic manure were evaluated against the linear growth of the pathogen. For each of the fertilizers, three concentrations, *i. e.* 1, 2 and 3% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.23 Method of studying the effect of fertilizers on mycelial growth inhibition of *S. rolfsii*

Different fertilizers like urea, murate of potash, zipsum, phosphate were used in the present work. Fertilizers used in the study were obtained from local market, Rajshahi. The inhibitory effect of the fertilizers was evaluated against the linear growth of the pathogen. For each of the fertilizers, three concentrations, *i. e.* 1, 2, 3% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.24 Method of studying the effect of cow dung and cow urine on mycelial growth inhibition of *S. rolfsii*

To study the effect of cow dung and cow urine on mycelial growth of *S. rolfsii*, the experiment was set up as described below. Cow dung and cow urine were collected and added with PA (Potato Agar) medium at the concentrations of 5, 10, 15, 20 and 25%. The media were sterilized at 15lb/ins² in 120°C. The experiment was set up in laboratory following the methods of Brown (1923). 20 ml of media were poured after sterilization under aseptic condition in sterilized 90 mm Petri dishes and inoculated as described earlier. The measurement of radial growth of the colony was taken at intervals of 24 hours up to 4th day.

2.25 Method of studying the effect of vitamins on mycelial growth inhibition of *S. rolfsii*

Different vitamins were used in the present work. Vitamins used in the study were obtained from local market, Rajshahi. The inhibitory effect of the vitamins was evaluated against the linear growth of the pathogen. For each of the vitamins, one concentration, *i.e.* 0.25% was prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.26 Method of studying the effect of salts on mycelial growth inhibition of *S. rolfsii*

Three different salts were used in the present work. Salts used in the study were obtained from laboratory. The inhibitory effect of the salts was evaluated against the linear growth of the pathogen. For each of the salts, five concentrations, *i.e.* 0.10, 0.20, 0.30, 0.40 and 0.50% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.27 Method of studying the effect of plant extracts on inhibition of mycelial growth of *S. rolfsii*

To study the effect of *Allium sativum*, *Lawsonia inermis*, *Azadirachta indica*, *Swietenia mahagoni*, *Curcuma longa*, *Nicotiana tabacum*, *Parthenium hysterophorus*, *Lantana camara*, *Clerodendrum viscosum*, *Cinnamomum tamala*, *Zingiber officinale*, *Polygonum hydropiper*, *Syzigium aromaticum*, *Ucalipotus globulus*, *Calotropis procera*, *Bryophyllum pinnatum*, *Corchorus capsularis* and *Cynodon dactylon* extracts against the growth of *S. rolfsii*, experiment was set up in laboratory following the methods of Brown (1923). 20 ml of media at the concentration of 5, 10, 15, 20 and 25% of each leaf extract were poured after sterilization under aseptic condition in sterilized 90 mm Petri dishes and inoculated as described earlier. The measurement of radial growth of the colony were taken at intervals of 24 hours and expressed in mm. For the preparation of plant extracts (cold water extracts), fresh plant leaves were washed with tap and sterile water. The leaf samples were cut into small pieces (1-2 cm) and were crushed. It was then processed with sterile distilled water at the rate of 1ml per gram of tissue (1:1w/v) and filtered through Whatman No. 1 filter paper. For the evaluation of antifungal effect of plant extracts on mycelial growth of *S. rolfsii*, PDA medium containing 25% of each plant extracts were prepared. PDA medium without any extracts served as control. The medium was poured into 90 mm Petri plates in 20 ml/plate and three replicates were maintained.

2.28 Method of studying the effect of dried plant aqueous extracts on inhibition of mycelial growth of *S. rolfsii*

Leaves or seeds of *Allium sativum*, *Lawsonia inermis*, *Azadirachta indica*, *Swietenia mahagoni*, *Nicotiana tabacum*, *Syzigium aromaticum*, *Parthenium hysterophorus*, *Lantana camara*, *Cinnamomum tamala*, *Corchorus capsularis*, *Zingiber officinale*, *Polygonum hydropiper*, *Clerodendrum viscosum*, *Eucalyptus globulus* and *Cynodon dactylon* were surface sterilized for 2 minutes in 70% ethanol. Samples were then rinsed twice in sterilized distilled water, dried under room temperature for 21 days and ground to powder separately. For preparation of aqueous extracts of crushed dry sample of each plant was soaked in sterilized distilled water at 1:1 w/v, vigorously stirred and left for 24 hrs. The suspensions were passed separately through thin cloth, filtered through Whitman's filter paper No. 41. The filtrates were further passed through Millipore filter of 0.2 μ m pore size to avoid the bacterial contamination and stored at 4°C until use. The extracts thus obtained were arbitrarily termed as 'S' (100%). Further dilutions (5%, 10% and 15%) were prepared by the addition of requisite amount of distilled water.

2.29 Method of studying the effect of plant extracts (ethanol) on inhibition of mycelial growth of *S. rolfsii*

Leaves or seeds of *Allium sativum*, *Lawsonia inermis*, *Azadirachta indica*, *Swietenia mahagoni*, *Nicotiana tabacum*, *Parthenium hysterophorus*, *Lantana camara* and *Cinnamomum tamala* were surface sterilized for 2 minutes in 70% ethanol. Samples were then rinsed twice in sterilized distilled water, dried under room temperature for 21 days and ground to powder separately. For preparation of aqueous extracts of crushed dry sample of each plant was soaked in sterilized distilled water at 1:1 w/v, vigorously stirred and left for 24 hrs. The suspensions were passed separately through thin cloth, filtered through Whitman's filter paper No. 41. The filtrates were further passed through Millipore filter of 0.2 μ m pore size to avoid the bacterial contamination and stored at 4°C until use. The extracts thus obtained were arbitrarily termed as 'S' (100%). Further dilutions (5%, 10% and 15%) were prepared by the addition of requisite amount of distilled water.

2.30 Method of studying the effect of fungicides on inhibition on mycelial growth of *S. rolfsii*

Twelve fungicides namely Dithane M-45, Antacole, Thiovit, Bavistin, Ridomil, Cupravit, Secure, Rovral, Heconazole, Tilt, Folicure and Score at five different concentrations viz. 50, 100, 250, 500 and 1000 ppm were tested *in vitro* to evaluate their effect on radial colony growth using poison food technique (Dingra and Sinclair, 1985). Requisite quantity of individual fungicides was added to melt PDA to have concentration of 50, 100, 250, 500 and 1000 ppm. After thorough mixing, amended medium was autoclaved at 121°C under 1 kg/cm² for 20 min. Approximately 20 ml of melted PDA mixed with fungicides was poured into each 90 mm diameter Petri dish. After solidification, the inocula (5 mm disc) from three days old culture of test organism *S. rolfsii* were placed at the center of the test plate. There are three replications for each treatment. The plates were incubated in the laboratory having ambient temperature of 27°C. Data on radial colony diameter was recorded after three days of incubation when the whole plate under control was covered with the growth of test organism. Inhibition of radial growth based on colony diameter was calculated following the formula as suggested by Sundar *et al.* (1995).

2.31 Method of studying the effect of herbicides on the inhibition of mycelial growth of *S. rolfsii*

Five different herbicides were used in the present work. Herbicides used in the study were obtained from local market, Rajshahi. The inhibitory effect of the herbicides was evaluated against the linear growth of the pathogen. For each of the herbicides, five concentrations, *i. e.* 0.10, 0.20 and 0.30% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.32 Method of studying the effect of insecticides on inhibition of mycelial growth of *S. rolfsii*

Five different insecticides were used in the present work. Insecticides used in the study were obtained from local market, Rajshahi. The inhibitory effect of the insecticides was evaluated against the linear growth of the pathogen. For each of the insecticides, five concentrations, *i. e.* 0.10, 0.20 and 0.30% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

2.33 Method of studying the efficacy of antagonists of *Trichoderma* and *S. rolfsii*

In vitro tests were conducted to evaluate the antagonistic effect of 20 isolates of *Trichoderma* sp. against *S. rolfsii* on PDA medium by dual culture technique (Dhingra and Sinclair, 1985). The medium was prepared by mixing infusion of 200 g peeled potato, 20 g dextrose and 20 g agar per liter of medium. One mycelial disc (5 mm) of individual isolate of *Trichoderma* sp. and one disc (5 mm) of test pathogen was placed simultaneously on the edge of each PDA Petri dish plate at opposite direction. Three plates were used for each isolate of *Trichoderma* sp. and the test pathogen. The plates that received only mycelial disc of *S. rolfsii* served as control. The plates were incubated in the laboratory having temperature of 27°C. Thereafter, inhibition percentage of *S. rolfsii* was calculated on the growth of pathogen on PDA plate after four days of incubation following the formula as suggested by Sundar *et al.* (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Inhibition of growth (%) = Where, X = mycelial growth of pathogen in the absence of *Trichoderma* sp. (control), Y = mycelial growth of pathogen in the presence of *Trichoderma* sp.

2.34 Method of studying the effect *Trichoderma* spp. culture filtrates on per cent inhibition of *S. rolfii*

The ability of *Trichoderma* sp. to inhibit the mycelial growth of *S. rolfii* through production of fungitoxic metabolites at different temperatures was tested according to the method mentioned by Dennis and Webster (1971 a&b). Fifty ml of potato dextrose broth (PDB) at pH 6 in 250 ml Erlenmeyer flasks was inoculated with 6 mm-agar disk from 7-day old PDA cultures of three *Trichoderma* isolates incubated at 25°C without shaking. After 10 days of incubation, the cultures were filtrated through Millipore membrane filter (0.45 μ m) and were autoclaved at 121°C for 15 minutes. The culture filtrate (1.2 ml) was placed in Petri dishes (90 mm diameter) and approximately 12 ml of PDA was added and mixed with the filtrate (10% v/v). The filtrate amended PDA plates were then centrally inoculated with 5-mm mycelial plugs of *S. rolfii*. Plates were incubated at 26 $\pm$ 2°C with four replicates; unamended PDA served as control. The linear growth rate of *S. rolfii* was measured after 48 hour and calculated as cm²/day and per cent inhibition was calculated. The experimental design was completely randomized with four replicates (plates) for each treatment.

2.35 Method of studying the effect of inoculums levels and disease incidence

The soil, sand and farmyard manure were sieved by passing through 2 mm mesh and sterilized separately and then mixed in 1:1:0.5 per cent proportion, respectively. After mixing it is filled in 15×20 cm surface sterilized earthen pots. *S. rolfii* culture grown on sand-wheat meal medium for 30 days was mixed to each pot as to give different inoculums levels viz., 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 per cent. The pot filled with sterilized soil without inoculums served as control. Each treatment was replicated four times. Apparently healthy cuttings of betelvine were planting in pots. Water was added to the pots at regular intervals to maintain 30 per cent soil moisture. The observations on per cent survival of vines were recorded.

2.36 Method of studying the effect of identification of the susceptible stage of betelvine

To know the susceptible stage of the betelvine, an experiment was conducted under glass house condition. Five stages of the betelvine crop were taken for their susceptible reaction with the foot rot causal pathogen *S. rolfsii*. These stages of crop were maintained in the earthen pots of 25 cm diameter filled with sterilized soil. After raising all the stages, finally inoculums were added at time to all. After 15 days number of plants showing wilting symptoms due to inoculation of *S. rolfsii* and data were recorded. The treatments were as follows: T₁= 3 months aged betelvine, T₂= 6 months aged betelvine, T₃= 12 months aged betelvine, T₄= 18 months aged betelvine and T₅= 24 months aged betelvine.

2.37 Method of studying the effect of soil solarization with clear plastic mulch and black plastic mulch on the viability of sclerotia

Soil solarization was used to observe its effect on the viability of sclerotia and the experiment was carried out in earthen pots (29.5 cm breadth & 29.5 cm height). The sclerotia were filled in cotton bags and buried at 5, 10, 15, 20 & 25 cm depths in pots soil filled which were later covered with single and double layer of clear plastic mulch (CPM) and black plastic mulch. Temperatures were recorded at different depth.

2.38 Method of studying the effect of solarization on the disease incidence of *S. rolfsii* infested pot soil

An earthen pot (29.5 cm breadth & 29.5 cm height) containing loamy soils were taken for this experiment. A portion (150 g) of soil was infested with sclerotia (100 sclerotia per 10 g soil). The treatments were as follows: T₁= 10 days solar heating, T₂= 20 days solar heating, T₃= 30 days solar heating, T₄= 40 days solar heating, T₅= 50 days solar heating and T₆= 60 days solar heating. After treatments, two to three cuttings of betelvine bangla cultivar was planted each pot. Water was added every day to keep them under moist condition. Observations were made after 10-12 days when the stem of the betelvine had developed characteristic lesion.

2.39 Method of studying the effect of amendment soil on germination of sclerotia of *S. rolfsii* at different intervals

A loamy sandy soil was used in this experiment. Soils were collected from the boraj of Mohanpur, Rajshahi. Each of ash, vermicompost, cowdung (5% w/w), sesame cake, mustard oil cake and neem cake (1% w/w) were add to amend the soil. A portion (150 g) of amended soil was infested with sclerotia (100 sclerotia per 10 g soil). After thorough mixing the amended soils containing sclerotia, the soils were transferred to earthen pots. The pots were then placed in baroj covered with sterilized papers to maintain moisture. After various time intervals (7, 14, 21, 28, 35 and 42 days), the soils from the pot were washed in running tap water with a 850 millimicrone-mesh sieve. Sclerotia were recovered with a fine forceps from the residue remained in the sieve. Sclerotial germination was determined by planting out 50 sclerotia per replicate on PDA. Plates were incubated at room temperature and germination of sclerotia was assessed visually after 36 hours.

2.40 Method of studying the viability of sclerotia on agar disks of *S. rolfsii* as affected with hot water temperatures and exposure time

Six mm diameter mycelia were cut from the grown edge of 15 days old cultures of *S. rolfsii* with sclerotia on PDA medium and transferred to Screw-cap glass vials, 20.0 cm long and 20.0 mm in diameter, containing 20.0 ml sterilized water placed in water bath at 25°, 50°, 51°, 52°, 53°, 54°, 55°, 56°, 57°, 58°, 59° and 60 °C, for different exposures time *i. e.* 1, 5, 10, 15, 20, 25 and 30 minutes. Treated agar disks mycelia with sclerotia were dried using sterilized filter paper and transferred into Petri plates containing PDA medium. Six Screw-cap glass vials and 3 disks were used for each treatment. Viability of sclertia that had been subjected to previous temperatures was assessed by planting treated disks on PDA medium and incubated at 25°C for 5 days. Disks showing growth or non-growth were recorded.

2.41 Method of studying the effect of different degree hot water treatment at the rate of 0.5 L/kg soil on the per cent of disease incidence in *S. rolfsii* infested pot soil

Boiling water (100°C) were addition to pot soil to get different degrees of hot water such as 65, 70, 80, 90 and 100 °C were tested to study their effect on soil temperatures, total count of foot rot disease incidence of betelvine under pot experiments. Twenty four hour later of soil treatments with hot water betelvine cuttings were planted at the rate of 4 cuttings/pot. Foot rot incidence was recorded as per cent of disease after 15 days of planting.

2.42 Method of studying the effect of cultural practice on the incidence of disease in *S. rolfsii* infested soil

The trial was arranged in a land of 475 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district Bangladesh. The experiment was laid out following randomized complete block design with three replications. The land was prepared for cultural treatments. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. The treatments were as follows: T₁= Deep ploughing, T₂= Deep ploughing + solarization, T₃= Hot water 0.5 Lit/kg, T₄= Earthing up soil 25 cm height one time/year, T₅= Earthing up soil 25 cm height two time/year and control. After cultural treatments, planted with a cultivar, Bangla cuttings. Two rows of 3.75 m² represented an experimental unit. Each treatment was separated with buffer rows. Data on per cent disease incidence, (PDI) were recorded at 15 days after application of treatment.

2.43 Method of studying the effect of organic amendment in controlling foot-rot disease in field

The trial was carried out in two borojes (the borojes were of same size and of same aged) of 475 m², 450 m² and 350 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district, planted with a cultivar, Bangla for two consecutive years. The experiment was laid out following randomized complete block design with three replications. Two rows of 3.75 m² represented an

experimental unit. The amendments were made with the application of mustard cake, vermicompost, neem cake, sesame cake and ash. The amendments were incorporated into the soil 45 days before planting the cuttings. After established the cuttings, the amendments were then applied within the rows of vines at monthly intervals. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. Before application of treatment, all the infected leaves and plants were removed from the experimental plot. Each treatment was separated with buffer rows. The treatments were as follows: T₁= mustard cake, T₂= vermicompost, T₃= neem cake, T₅= sesame cake and control. Data on plant height, stem girth, no. of leaves, length of internodes, fresh weight of 100 leaves, yield in lak ha⁻¹ye⁻¹ and per cent disease incidence, (PDI) were recorded at 15 days after application of treatment.

2.44 Method of studying the efficacy of plant extracts in controlling foot-rot disease in field

The trial was carried out in two borojes (the borojes were of same size and of same age) of 475 m², 450 m² and 350 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district, planted with a variety, Bangla for two consecutive years. The experiment was laid out following randomized complete block design with three replications. Two rows of 3.75 m² represented an experimental unit. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. Before application of treatment, all the infected leaves and plants were removed from the experimental plot. Each treatment was separated with buffer rows. The treatments were as follows: T₁= *Swietenia mahagoni*, T₂= *Allium sativum*, T₃= *Lawsonia inermis* T₄= *Azadirachata indica*, at the rate of 5% and control. Data on plant height, stem girth, no. of leaves, length of internodes, fresh weight of 100 leaves, yield//lak/ha/year and per cent disease incidence (PDI) were recorded at 15 days after application of treatment.

2.45 Method of studying the efficacy of fungicides in controlling foot-rot disease in field

The trial was carried out in two borojes (the borojes were of same size and of same age) of 475 m², 450 m² and 350 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district, planted with a cultivar, Bangla for two consecutive years. The experiment was laid out following randomized complete block design with three replications. Two rows of 3.75 m² represented an experimental unit. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. Before application of treatment, all the infected leaves and plants were removed from the experimental plot. Each treatment was separated with buffer rows. The treatments were as follows: T₁= Rovral, T₂= Tilt, T₃= Hecanazol, T₄= Folicure, T₅= Score at the rate of 0.1% and control. Data on plant height, stem girth, no. of leaves, length of internodes, fresh weight of 100 leaves, yield in lakh/ha and per cent disease incidence, (PDI) were recorded at 15 days after application of treatment.

2.46 Method of studying the effect of *Trichoderma* isolates in controlling foot-rot disease in field

The trial was conducted in two borojes (the borojes were of same size and of same age) of 475 m², 450 m² and 350 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district, planted with a cultivar, Bangla for two consecutive years. The experiment was laid out following randomized complete block design with three replications. Two rows of 3.75 m² represented an experimental unit. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. Before application of treatment, all the infected leaves and plants were removed from the experimental plot. For field testing *Trichoderma* isolates were grown in oil cake medium for mass production and incubated at 27°C for 30 days. The *Trichoderma* containing oilcakes were mixed with neem cake previously soaked in water for 7 days at the ratio of 1: 10 and kept for another seven days covering with Polyethylene sheet. The

antagonists were then applied within the rows of vines and tightly covered with the soil at monthly intervals. Each treatment was separated with buffer rows. The treatments were as follows: $T_1 = \textit{Trichoderma}$ iso-3, $T_2 = \textit{Trichoderma}$ iso-10, $T_3 = \textit{Trichoderma}$ iso-11, $T_4 = \textit{Trichoderma}$ iso-12, $T_5 = \textit{Trichoderma}$ iso-13 and control. Data on plant height, stem girth, no. of leaves, length of internodes, fresh weight of 100 leaves, yield in lakh/ha and per cent disease incidence (PDI) was recorded at 15 days after application of treatment.

2.47 Method of studying the effect of NPK fertilizer controlling foot-rot disease of betelvine in field

The trial was conducted in two borojes (the borojes were of same size and of same age) of 475 m², 450 m² and 350 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district, planted with a cultivar, Bangla for two consecutive years. The experiment was laid out following randomized complete block design with three replications. Two rows of 3.75 m² represented an experimental unit. The soil was incorporated by spraying with the mycelial suspension of *S. rolfsii* to make the soil sick. Before application of treatment, all the infected leaves and plants were removed from the experimental plot. Each treatment was separated with buffer rows. The treatments were as follows: $T_1 = N_0P_{100}K_{100}$, $T_2 = N_{200}P_{100}K_{100}$, $T_3 = N_{150}P_{100}K_{100}$, $T_4 = N_{100}P_{100}K_{100}$, $T_5 = N_{200}P_0K_{100}$, $T_6 = N_{200}P_{50}K_{100}$, $T_7 = N_{200}P_{100}K_0$, $T_8 = N_{200}P_{100}K_{50}$, $T_9 = N_0P_0K_0$ (control). Data on plant height, stem girth, no. of leaves, length of internodes, fresh weight of 100 leaves, yield in lakh/ha and per cent disease incidence (PDI) were recorded after 15 days of application of treatment.

2.48 Method of studying the effect of crop rotation

Six crops were selected for the study of crop rotation study. Under pot culture, the betelvine was grown in sick pots and crop rotation practices were carried out with crops like, *Zea mays*, *Solanum tuberosum*, *Allium sativum*, *Saccharum officinarum*, *Allium cepa* and *Triticum aestivum* and only betelvine served as

control. Four replications were maintained for each crop rotation practices. Observations on per cent wilt incidence were recorded.

2.49 Method of studying the reaction of betelvine cultivars against foot-rot disease

Six cultivars of Pan were collected from different areas of Bangladesh. The cultivars were namely Bangla, Chaltaguta, Rangpuri, Methapan, Sanchi, Gachpan, Jhalpan, and Mistypan. The betelvine cuttings were planted in line on raised bed in double row system. Each bed was maintained 2.5 m long 30 cm wide and plot to plot distance was 50 cm. Plant to plant distance was 12 cm. The design was randomized complete block having three replications. The plants were inoculated by spraying with the mycelial suspension of *S. rolfsii* during the month of May. The tested cultivars were graded as follows: (Ahmed and Bakre, 1991)

Types of reaction

Resistance	0 to 10
Moderately resistance (MR)	11 to 20
Moderately susceptible (MS)	21 to 40
Susceptible (S)	41 to 60
Highly susceptible (HS)	61 to above

2.50 Integrated disease management (IDM) for foot-rot disease of betelvine using different biocontrol agents, organic amendments and chemicals

An integrated disease management (IDM) strategy was tested to manage foot rot disease of betelvine. The trial was conducted in two Borojes (the borojes were of same size and of same age) of 500 m² and 300 m² situated at Badehajrapara village, Mohanpur upazilla, Rajshahi district planted with a moderately resistant cultivar, Bangla for two consecutive years *i.e.* during 2010-11 and 2011- 12. The experiment was setup in early June and continued up to June next year for both the years. In the experimental plot, the soil was inoculated by spraying with the mycelial and sclerotial suspension of *S. rolfsii* to make the soil sick. The soil amendments, plant extracts, antagonist and fungicides which are found effective

under *in vitro* studies were evaluated in field conditions. Nine treatments were laid out in randomized block design. Three replications were maintained for each treatment. Betelvine cuttings of 15-20 cm with 3-5 nodes of cuttings were taken for planting. Fertilizers were applied @ 200: 100: 100 of N: P: K kg/ha. Six rows of 3.75 m² represented an experimental unit. Data on plant growth parameters like yield in lakh ha⁻¹, fresh weight of 100-leaves and per cent disease incidence were recorded at 30 days after application of treatment. Results were analyzed statistically. The soil was previously mixed with neem cake. Before the start of the experiment all the infected plants in treatment rows were removed. Six rows containing 400-450 vines were considered as treatment. Each treatment was separated by a buffer row. For field testing the selected biocontrol agents *Trichoderma* iso-3 were grown in oil cake medium for mass production and incubated at 27°C for 30 days. They were mixed with neem cake previously soaked in water for seven days and kept for another seven days after covering it with Polyethylene sheet. The antagonists were then applied within the rows of vines and slightly covered with the soil. The components were used in nine different combinations to have nine treatments. The components of the IDM strategy were as follows: T₁ = sanitation, T₂ = neem cake 6t/h at quarterly intervals, T₃ = application *Allium sativum* extract 5% at quarterly intervals, T₄ = application *T. harzianum* at quarterly intervals, T₅ = score 1.0% drenching at quarterly intervals, T₆ = sanitation + neem cake 6t/h at quarterly intervals, T₇ = sanitation + neem cake 6t/h + *A. sativum* extract 5% at quarterly intervals, T₈ = sanitation + neem cake 6t/h + *A. sativum* extract 5% + *T. harzianum* at quarterly intervals, T₉ = sanitation + neem cake 6t/h + *A. sativum* extract 5% + *T. harzianum* + score 1.0% drenching (neem cake, *A. sativum* and *Trichoderma* at quarterly intervals and score at monthly intervals), T₁₀ = Control.

Chapter Three

OBSERVATIONS AND RESULTS

3.1 Field investigations of foot- rot disease of betelvine (*Piper betle* L.)

A survey was conducted during 2010-11 and 2011-12 in eight betelvine growing districts such as Rajshahi, Natore, Nilphamary, Pabna, Chuadanga, Jessore, Rangpur and Pirozpur. The results obtained during survey are presented in Table 1, 2 and Fig 1. The data indicated that the disease appeared in the entire betelvine plantation during both the years (2010-11 and 2011-12). During 2010-11, the highest foot disease incidence (38.53%) of betelvine was observed in Rajshahi district followed by Pabna (36.63%), Natore (35.73%) and the lowest disease incidence (28.25%) of betelvine was recorded in Pirozpur district. Among the betelvine plantation of different upazilla, Mohonpur upazilla in Rajshahi district recorded maximum disease incidence (42.02%) and the lowest incidence (28.25%) was in Pirozpure Sadore upazilla (Table 1). During 2011-12, the highest disease incidence (42.88%) of betelvine was observed in Rajshahi district followed by Pabna (37.35%), Natore (36.99%) and the lowest disease incidence (26.71%) was recorded in Pirozpur district. Among the different upazilla, Mohonpur upazilla in Rajshahi district recorded maximum disease incidence (42.88%) and the lowest incidence (26.71%) was recorded in Pirozpure Sadore upazilla of Pirozpur district of betelvine in this study (Table).

3.2 Incidence of disease in different betelvine cultivars on survey period

The incidence of disease in different betelvine cultivar was tested and results are presented in Table 3 Fig 2. In both the year Bangla cultivar of betelvine showed the highest disease incidence and Sanchi cultivar showed the lowest disease incidence. It is observed from the data that in the year (2010-11), the highest foot-rot disease incidence (38.53%) was observed in Bangla cultivar followed by

Mistypan (36.57%), Methapan (35.12%) and the lowest (23.26%) in Sanchi. In the year (2011-12), the highest foot rot disease incidence (41.15%) was observed in Bangla cultivar followed by Mistypan (40.51%), Methapan (39.35%) and the lowest (19.73%) in Sanchi. It is observed from the data foot rot disease incidence varied from cultivar to cultivar and cultivar Sanchi pan showed fewer sufferers than other tested cultivars during the study. LSD (0.05) showed more significant results for the incidence of foot rot disease from cultivar to cultivar (Table 3).

3.3 Disease incidence (%) variations in betelvine with soil type from various districts

The incidence of foot rot disease of betelvine was recorded in different soil types borjoes from different districts and results are presented in Table 4. It is indicated from the data that loamy soil planted borjoe showed the highest disease (25.99%) incidence of betelvine and sandy loamy soil planted borjoe showed the lowest disease (7.49%) incidence. No disease was noted in sandy and clay soil planted betelvine borjoe during the study. It is evident from the experiment that though the soil type is same, but the incidence of disease recorded different from place to place. This deviation may be due to initiation of cultivation of betelvine in that area and statistically showed LSD (0.05) significant from place to place.

3.4 Means for disease incidence on the basis of influence of irrigation system from eight districts

Foot rot disease incidences of betelvine on the basis of influence of irrigation system practices in different districts were recorded and results are presented in Table 5, Fig 3 and plate 4 A & B. In the case of flood irrigation system, data indicated that in the year (2010-11), the highest disease incidence (33.96%) of betelvine was observed in Rajshahi and the lowest (23.19%) in Pirozpur district. In the year (2011-12), the highest disease incidence (36.93%) of betelvine was observed in Rajshahi and the lowest (26.28%) in Pirozpur district. In the case of drip irrigation system (2010-11), the highest disease incidence (18.24%) was observed in Pabna and the lowest Pirozpur (12.39%) district. In the year (2011-

12), the highest disease incidence (16.22%) of betelvine was observed in Chuadanga and the lowest (9.63%) in Pirozpur district. The data was recorded on the basis of flood system irrigation where used shallow machine and drip system irrigation where used hand spraying. Flood irrigation system means that where shallow machines are used for irrigation in betelvine plantation and drip system of irrigation followed with hand spraying in the boraj. This evidences showed that foot rot disease occurred more in flood system of irrigation than drip system. LSD (0.05) showed more significant results in the incidence of foot rot disease of betelvine.

3.5 Effect of metrological parameter (temperature, relative humidity and rainfall) on the incidence of foot-rot disease of betelvine caused by *S. rolfsii* during 2010- 2011 & 2011-2012.

The Effect of temperature, relative humidity and rainfall on the incidence of foot rot disease was recorded and results are presented in Table 6 and Fig 4. In the year 2010-11, the highest foot rot disease (40.80%) incidence was observed in September (16-30) and the lowest (16.22%) in December (16-30). In the year 2011-12, the highest foot rot disease (41.67%) incidence was observed in September (16-30) and the lowest (15.23%) in December (16-30). It is evident from table 7 that temperature ranges from 28-29°C with heavy rainfall from 243 mm to 285 mm and RH between 80 to 86% are favourable for foot rot disease of betelvine development in *in vivo* condition with moderate rainfall between 127-188 mm, temperature varied from 28-30°C, RH from 75 to 87% showed at least 77.35% disease incidence of foot rot of betelvine in this experiment (Table 7). Very little disease incidence was noted like low rainfall, less RH and with low temperature. It is clear from the study that foot rot of betelvine increased with the increase of rainfall, RH and temperature. July to September is very favourable period for the growth and multiplication of foot rot pathogen and makes havoc damage betelvine cultivation in Bangladesh. Though there is a little deviation recorded in the incidence of foot rot disease of betelvine in this study from data collected in the year 2010-11 and 2011-12 cropping seasons but it is clear that this disease occurrence depends on the role of rainfall, RH and temperatures (Table).

Table 1. Observed and recorded the incidence of foot-rot disease of betelvine during 2010 to 2012 cropping seasons.

Name of districts	Upazillas	Name of location	2010-11		2011-12	
			No. of fields visited	Disease incidence	No. of fields visited	Disease incidence
Rajshahi	Mohonpur	Duroil	26	44.14 (41.61)	26	46.70 (43.11)
		Sampur	28	43.54 (41.27)	28	44.98 (42.07)
		Sakoa	25	42.45 (40.63)	25	45.56 (42.48)
		Batupara	24	44.33 (41.73)	24	45.23 (42.25)
		Vaturia	27	48.53 (44.14)	27	49.34 (44.60)
		Naonogar	32	45.78 (42.19)	32	46.13 (42.76)
	Mean			44.80 (42.02)		46.32 (42.88)
	Bagmara	Nordas	24	45.67 (42.53)	24	46.23 (42.82)
		Gobindopara	25	43.34 (41.15)	25	45.45 (42.36)
		Kunda	23	46.32 (42.88)	23	48.13 (43.91)
		Sripur	25	41.36 (40.05)	25	44.87 (42.02)
		Dipnagor	26	44.55 (41.84)	26	47.86 (43.74)
		Bigacha	24	42.30 (40.57)	24	45.12 (42.19)
		Madaimuri	25	43.67 (41.38)	25	41.85 (40.28)
	Mean			43.89 (41.50)		45.64 (42.48)
	Durgapur	Alipur	26	43.22 (41.09)	26	45.65 (42.53)
		Kanpara	23	45.77 (42.59)	23	47.87 (43.74)
		Daoudkandy	27	42.54 (41.27)	27	43.15 (41.03)
		Naopara	26	42.33 (40.57)	26	44.64 (41.90)
		Bhabanipur	25	34.45 (35.91)	25	37.60 (37.82)
		Pananagar	26	43.67 (41.38)	26	45.87 (42.59)
		Maria	24	45.50 (42.42)	24	46.42 (42.94)
		Joinagar	26	47.94 (43.80)	26	49.33 (44.60)
	Mean			43.18 (41.09)		45.07 (42.19)
	Putia	Mollapara	24	32.40 (34.70)	24	33.56 (35.37)
		Bhalukgaci	26	30.05 (33.21)	26	33.23 (35.18)
		Mahindra	23	34.23 (35.79)	23	36.75 (37.29)
		Nandonpur	27	32.65 (34.82)	27	33.78 (35.55)
		Silmaria	28	34.33 (35.85)	28	35.77 (36.75)

Name of districts	Upazillas	Name of location	2010-11		2011-12	
			No. of fields visited	Disease incidence	No. of fields visited	Disease incidence
		Palpara	24	31.23 (33.96)	24	33.75 (35.55)
		Hariapara	28	35.89 (36.81)	28	37.62 (37.82)
		Sadonpur	22	33.38 (35.30)	22	34.22 (35.79)
		Mean		28.94 (32.52)		34.84 (36.15)
	Paba					
		Borgaci	38	37.35 (37.64)	38	39.21 (38.76)
		Naohata	33	36.55 (37.17)	33	38.44 (38.29)
		Parila	30	31.24 (33.96)	30	30.36 (33.40)
		Damkura	27	29.45 (32.83)	27	31.66 (34.20)
		Huzripura	22	32.68 (34.88)	22	33.60 (35.43)
		Mean		33.45 (33.30)		34.65 (37.11)
Natore	Sador	Sador	25	34.89 (36.21)	25	36.55 (37.17)
	Gurudaspur	Gurudaspur	20	33.38 (35.30)	20	35.92 (36.81)
	Mean			34.14 (35.73)		36.24 (36.99)
Nilphamary	Domar	Domar	36	26.61 (31.05)	36	35.84 (36.75)
	Chilahati	Chilahati	23	23.88 (29.27)	23	31.40 (34.08)
	Dimla	Dimla	22	21.38 (27.56)	22	22.31 (28.18)
	Mean			23.96 (29.27)		29.85 (33.09)
Pabna	Atgoria	Atgoria	21	33.87 (35.61)	21	36.94 (37.41)
	Chatmohore	Chatmohore	16	36.47 (37.17)	16	38.75 (38.47)
	Esordi	Esordi	15	36.50 (37.17)	15	34.65 (36.03)
	Mean			35.61 (36.63)		36.78 (36.75)
Chuadanga	Alomdanga	Alomdanga	17	32.45 (34.70)	17	35.19 (36.39)
	Horinakundu	Horinakundu	14	38.80 (38.53)	14	40.35 (39.41)
	Damurhuda	Damurhuda	16	27.04 (31.31)	16	29.55 (32.90)
	mean			32.76 (34.94)		35.03 (36.27)
Zessore	Monirampur	Monirampur	15	28.25 (32.08)	15	31.67 (34.20)
Rangpure	Mitapukure	Mitapukure	18	29.82 (33.09)	18	32.28 (34.63)
Pirozpure	Sadore	Sadore	17	22.45 (28.25)	17	20.16 (26.71)

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 2. Mean disease incidence of foot-rot of betelvine in different districts as observed and recorded during 2010 to 2012 crop seasons.

District	Upazilla	Average disease incidence*		
		2010-11	2011-12	Mean
Rajshahi	Mohonpur	44.80 (42.02)	46.32 (42.88)	40.08 (39.29)
	Bagmara	43.89 (41.50)	45.64 (42.48)	
	Durgapur	43.18 (41.03)	45.07 (42.19)	
	Putia	28.94 (32.52)	34.84 (36.15)	
	Paba	33.45 (35.30)	34.65 (36.03)	
	Mean	38.85 (38.53)	41.30 (39.99)	
Natore	Sador	34.89 (36.21)	36.55 (37.17)	35.19 (36.39)
	Gurudaspur	33.38 (35.30)	35.92 (36.81)	
	Mean	34.14 (35.73)	36.24 (36.99)	
Nilphamary	Domar	26.61 (31.05)	35.84 (36.75)	26.91 (31.24)
	Chilahati	23.88 (29.27)	31.40 (34.08)	
	Dimla	21.38 (27.56)	22.31 (28.18)	
	Mean	23.96 (29.27)	29.85 (33.09)	
Pabna	Atgoria	33.87 (35.61)	36.94 (37.41)	36.20 (36.99)
	Chatmohore	36.47 (37.17)	38.75 (38.47)	
	Esordi	36.50 (37.17)	34.65 (36.03)	
	Mean	35.61 (36.63)	36.78 (37.35)	
Chuadanga	Alomdanga	32.45 (34.70)	35.19 (36.39)	33.90 (35.61)
	Horinakundu	38.80 (38.53)	40.35 (39.41)	
	Damurhuda	27.04 (31.31)	29.55 (32.90)	
	mean	32.76 (34.27)	35.03 (36.27)	
Zessore	Monirampur	28.25 (32.08)	31.67 (34.27)	29.96 (33.15)
Rangpure	Mitapukure	29.82 (33.09)	32.28 (34.63)	31.05 (33.85)
Pirozpure	Sadore	22.45 (28.25)	20.16 (26.71)	21.31 (27.49)
	Grand Mean	33.08 (35.12)	32.91 (35.00)	32.99 (35.00)

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 3. Incidence of foot-rot disease of betelvine in different betelvine cultivars.

Cultivars	Disease incidence*	
	2010- 2011	2011- 2012
Bangla	38.85 (38.53)	43.30 (41.15)
Chaltaguta	26.45 (30.92)	22.16 (28.11)
Rangpuri	29.82 (33.09)	32.28 (34.63)
Methapan	33.14 (35.12)	40.24 (39.35)
Sanchi	15.57 (23.26)	11.45 (19.73)
Gachpan	23.96 (29.27)	21.35 (27.49)
Jhalpan	20.25 (26.92)	25.67 (30.46)
Mistypan	35.54 (36.57)	42.17 (40.51)
LSD (0.05)	0.1615	0.01931

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 4. Variations of foot-rot disease of betelvine with soil type from different districts.

S. No.	Districts	Disease Incidence*				
		Clay	Clay Loam	Loamy	Sandy Loamy	Sandy
1	Rajshahi	-	15.37 (23.1)	19.23 (25.99)	4.92 (12.79)	-
2	Natore	-	13.89 (21.89)	17.44 (24.65)	3.19 (10.31)	-
3	Nilphamary	-	10.65 (18.91)	14.56 (22.46)	2.09 (8.33)	-
4	Pabna	-	14.34 (22.22)	18.62 (25.55)	3.22 (10.31)	-
5	Chuadanga	-	12.50 (20.70)	16.23 (23.73)	2.78 (9.63)	-
6	Zessore	-	11.48 (19.82)	15.75 (23.34)	2.36 (8.91)	-
7	Rangpure	-	12.64 (20.79)	17.88 (25.03)	2.66 (9.46)	-
8	Pirozpure	-	7.39 (15.79)	12.53 (20.70)	1.73 (7.49)	-
	LSD (0.05)		0.9995	0.6826	0.6715	

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 5. Foot-rot disease incidence of betelvine on the basis of influence of irrigation system from eight districts.

Districts	Disease Incidence*			
	Flood system		Drip system	
	2010-11	2011-12	2010-11	2011-12
Rajshahi	31.20 (33.96)	36.06 (36.93)	7.65 (16.00)	5.25 (13.18)
Natore	26.81 (31.18)	30.33 (33.40)	7.32 (15.68)	5.92 (14.06)
Nilphamary	18.23 (25.25)	21.17 (27.42)	8.16 (16.64)	6.26 (14.54)
Pabna	24.73 (29.80)	30.01 (33.21)	9.81 (18.24)	7.85 (16.22)
Chuadanga	21.68 (27.76)	27.66 (31.76)	9.77 (18.24)	8.69 (17.16)
Zessore	20.55 (26.92)	25.17 (30.33)	7.77 (16.22)	6.43 (14.65)
Rangpure	22.18 (28.11)	28.12 (32.01)	7.05 (15.34)	4.75 (12.52)
Pirozpure	15.46 (23.19)	19.64 (26.28)	4.64 (12.39)	2.88 (9.63)
LSD (0.05)	0.01931	0.01763	0.01825	0.01840

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 6. Effect of metrological parameter (temperature, relative humidity and rainfall) on the incidence of foot-rot disease of betelvine caused by *S. rolfsii* during 2010- 2011 and 2011-12.

Disease incidence Date	RH% (Average)		Temp.°C (Average)		Rain fall (Average)		Disease Incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
June (1-15)	87	79	28.7	27.9	127	123	29.24 (32.71)	30.04 (33.21)
June (16-30)	86	80	30.3	27.3	130	125	31.45 (34.08)	32.23 (34.57)
July (1-15)	84	84	29.6	26.7	185	184	33.35 (35.30)	34.22 (35.79)
July (16-30)	83	85	28.4	26.4	188	184	35.15 (36.33)	35.87 (36.81)
Aug (1-15)	80	88	29.2	26.8	243	234	36.22 (36.99)	37.66 (37.88)
Aug (16-30)	81	89	28.8	26.2	247	239	38.66 (38.47)	38.45 (38.29)
Sept (1-15)	84	87	28.3	26.3	283	278	40.56 (39.58)	41.35 (39.99)
Sept (16-30)	86	86	28.1	26.2	285	274	42.66 (40.50)	44.25 (4.67)
Oct (1-15)	83	82	27.5	24.4	47	39	35.25 (36.39)	33.79 (35.55)
Oct (16-30)	84	81	26.9	23.2	49	31	28.40 (32.20)	31.59 (34.20)
Nov (1-15)	78	77	24.2	16.6	05	03	16.37 (23.89)	17.11 (24.43)
Nov (16-30)	76	75	23.3	15.7	03	02	14.07 (22.55)	12.66 (20.88)
Dec (1-15)	74	78	17.9	12.2	00	00	10.14 (18.53)	9.55 (17.95)
Dec (16-30)	75	79	16.2	11.1	00	00	7.79 (16.22)	6.96 (15.23)
Jan (1-15)	78	82	15.9	11.5	01	01	9.33 (17.76)	11.10 (19.46)
Jan (16-30)	82	81	14.4	12.4	01	01	10.52 (18.91)	12.68 (20.88)
Feb (1-15)	68	70	17.7	13.6	06	06	12.46 (20.62)	13.93 (21.89)
Feb (16-28)	67	68	18.3	15.4	07	08	14.28 (22.22)	16.87 (24.27)
Mar (1-15)	60	65	25.4	18.8	26	14	17.75 (24.88)	18.67 (25.62)
Mar (16-30)	62	63	25.2	20.3	28	16	20.40 (26.85)	22.45 (28.25)
Apr (1-15)	66	62	26.5	23.9	36	32	21.62 (27.69)	23.04 (28.66)
Apr (16-30)	68	60	27.9	24.6	38	33	23.86 (29.20)	25.23 (30.14)
May (1-15)	75	76	28.8	35.8	133	140	24.16 (29.47)	26.77 (31.18)
May (16-30)	76	78	28.6	36.6	135	138	27.42 (31.56)	29.17 (32.75)
LSD (0.05)							0.0383	0.0145

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

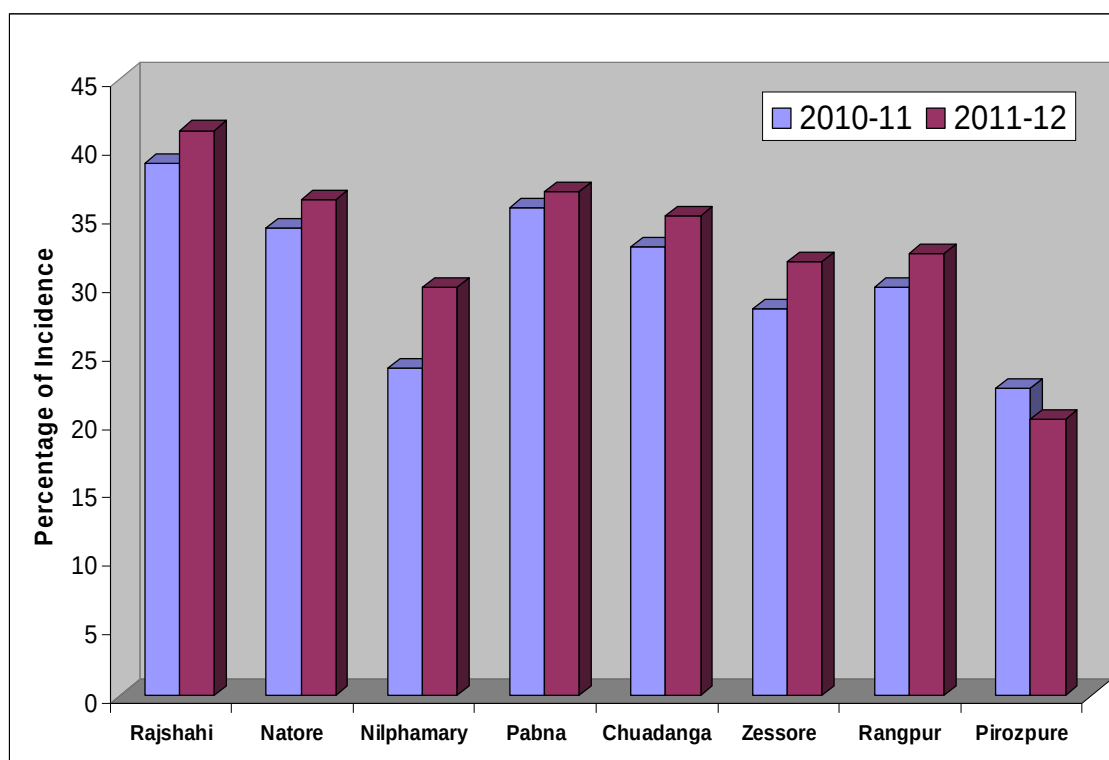


Figure 1. Incidence of foot-rot disease of betelvine in different districts during 2010-2011 and 2011-12 cropping seasons.

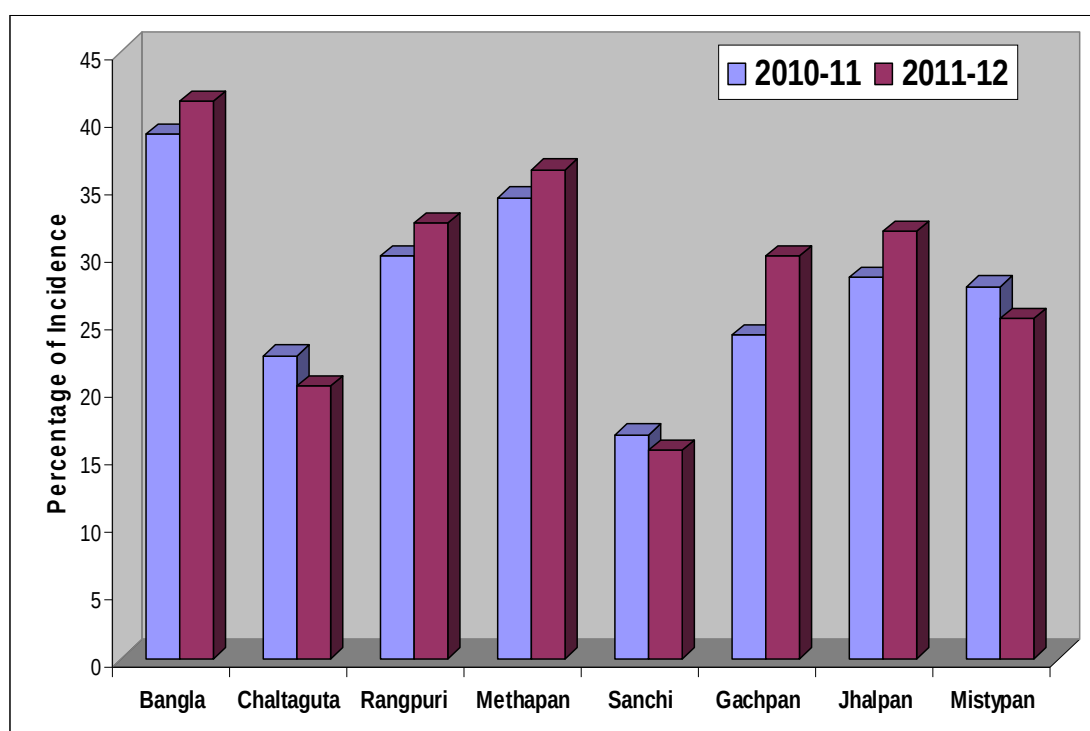


Figure 2. Incidence of foot-rot disease in different cultivars of betelvine during 2010-2011 and 2011-12 cropping seasons.

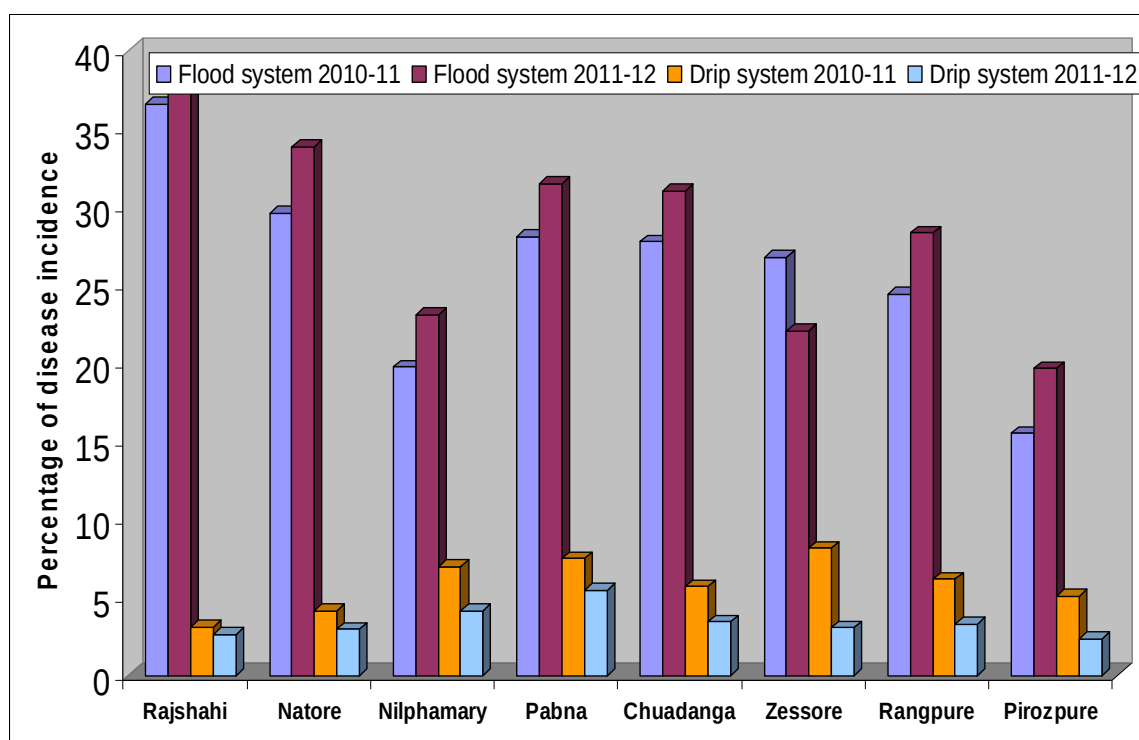


Figure 3. Incidence of foot-rot disease on the basis of influence of irrigation system from eight districts.

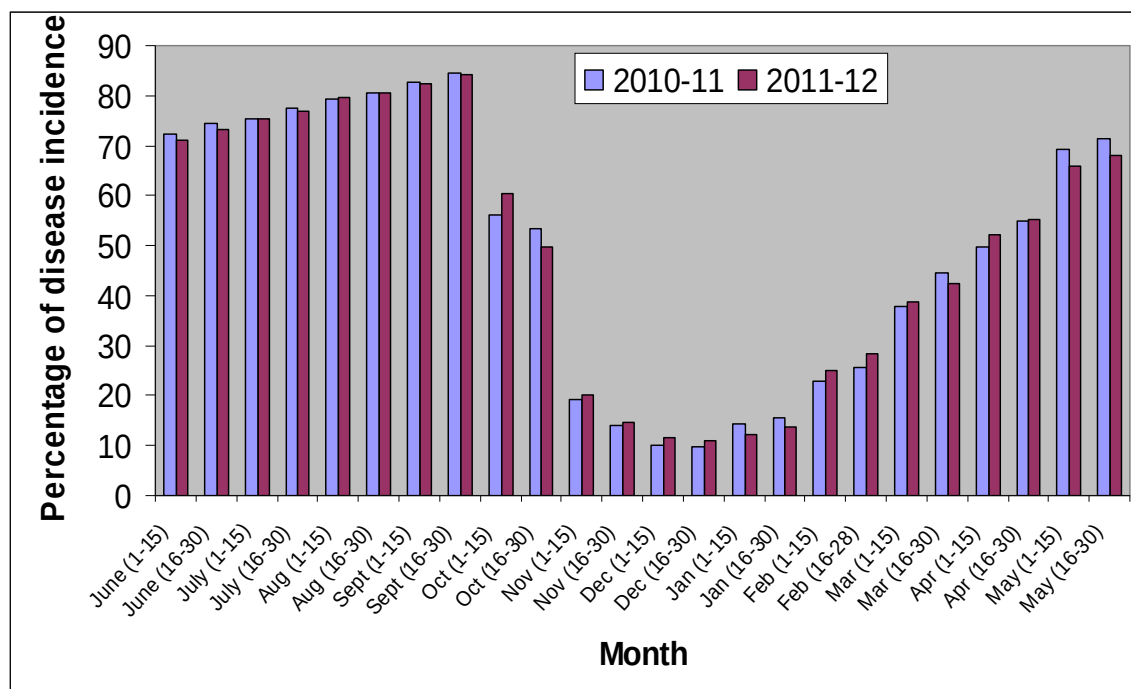


Figure 4. Incidence of foot-rot disease in metrological parameter (temperature, relative humidity and rainfall) during 2010-2011 and 2011-12 cropping seasons.

3.6 Symptom of foot rot disease

Foot rot disease is observed through out the seasons but severely in the rainy season and spreads widely over all the borojs. The affected plants show general loss of tissue and dropping of leaves and tender shoots. There is rotting of stems near the soil level producing black discoloration on the affected region (Plate 3B). After rotting at the foots of stems the plants wilt (Plate 3A). In the wilted plants the disease causes hollowing out of the pith of the first internodes. The cortex is found under a soft and some times slimy rotting over about three nodes and internodes. The fungus forms dense white cottony mycelial mass around the base of stem. Numerous sclerotia form of the affected parts.

3.7 Morphology of the organism (*Sclerotium rolfsii*) and its taxonomic position

10 isolation from the diseased part of the host (*Piper betle* L.) from different location of betelvine cultivation area of Bangladesh revealed *S. rolfsii* and its morphological characters are given below:

3.8 Taxonomic Position:

Division	:	Eumycota
Sub-division	:	Deuteromycotina
Form-class	:	Hyphomycetes
Form-order	:	Agonomycetales/Mycelia sterilia
Form-family	:	Agonomycetaceae
Form-genus	:	<i>Sclerotium</i>
Form-species	:	<i>S. rolfsii</i>

3.8.1 Morphological characters

Mycelium highly variable in appearance depending on its age, composed at first of colourless hyphae, later becoming yellowish and then deep brown in colour. The mycelial stage producing sclerotia is the commonly encountered stage. In artificial culture media, the fungus produces a fast growing stout mycelium with

septate hyphae. Young colonies may look white but older mycelium is invariably same shade of brown (Plate 5 A & C). Sclerotia may be formed. In some cases (primary stages) the sclerotia are light white (Plate 5 B) while in some (finally) they are so dark as to look black. The diameter of hyphae ranges between 5 and 14 microns. The septa are typically of dolipore type. The lateral branches from the main hyphae are invariably constricted at the point of origin and a septum occurs in the branches near the junction with the main axis. In young advancing hyphae the branching almost invariably occurs near the distal septum but it may occur at any point of the hyphae. The mycelium produces monilliod or barrel-shaped cells, which have been termed as chlamydospores. The cells are as rule multinucleate. The sclerotia of the fungus are distinct from many other fungi in that there is no differentiation of the sclerotial tissue into a rind and internal medulla although outer cells may be darker and thicker walled.

On the host, the mycelial stage of the fungus produces superficial, irregular, scab-like, black sclerotia growing on the surface of the usually subterranean parts of the plant. The sclerotia are flattened on the lower side.

This is preceded or accompanied by a superficial, dark colored, short-celled, abundantly branched, stout mycelium. This mycelium is entirely different in appearance from the mycelium inside the host tissues, which is slender hyaline, and longer celled. No other spores are produced in the asexual stage of the fungus.

3.8.2 Variation in the different isolates of *S. rolfsii*

Ten isolates of *S. rolfsii* have been tested their mycelial growth, dry mycelial weight, sclerotia size, no. of sclerotial body/cm² and test weight of 100 sclerotial body (mg) and results are presented in Table 7 and Plate 6. It is evident from the experiment that Isolate no, S-5 showed the highest mycelial growth (90 mm) followed by S-8 (89 mm), S-2 (88.22 mm), S-3 (87.34 mm) and S-7 showed the lowest mycelial growth (75.44 mm) in the PDA medium. The highest dry

mycelial weight (249.44 mg) was observed in S-5 and the lowest (159.94 mg) in S-7. The highest sclerotial size (1.98 mm) was measured in S-5 and the lowest (1.30 mm) in isolate-7. The highest no. of sclerotial bodies/cm² (9) was counted in isolate-5 and the lowest (3) in S-7. The highest test weight of 100 sclerotial bodies (101.66 mg) was measured in S-5 and the lowest (72.54 mg) in S-7.

3.9 Pathogenecity of different isolates of *S. rolfsii* on betelvine cultivar Bangla

The pathogenicity has been tested using ten isolates of *S. rolfsii* and results are presented in Table 8 and Plate 4 C. It is observed from the experiment that the highest per cent disease incidence (71.85%) of betelvine was noted when using the isolate no S-5 and followed by S-9 (70.00%), S-4 (69.12%), S-8 (67.78%) and S-1 (66.34%) after 7 days inoculations and the lowest (45.56%) was observed in S-7. In this study isolate no (S-5) was used in every case. Among the ten isolates, there occurred significant differences on their virulence and producing disease.

3.10 Environmental and nutritional factors affecting the mycelial growth of *Sclerotium rolfsii*

Nutritional factors influence the growth of mycelium and sclerotia formation of *S. rolfsii*. Nutritions are important factors for mycelial growth. The mycelium of *S. rolfsii* was allowed to grow separately under different nutrients media as described under materials and methods.

3.10.1 Effect of carbon and nitrozen sources

The effect of different carbon and nitrozen sources on the mycelial growth of *S. rolfsii* was tested and results are presented in Table 9 and Plate 7 A & B. It is indicated from the experiment that the highest mycelial growth (90 mm) of *S. rolfsii* was observed in cellulose contained medium followed by sorbitol (88 mm), starch (82 mm), manitol (68 mm) and the lowest (30 mm) in glycerol. In the case of nitrozen, the highest mycelial growth (90 mm) of *S. rolfsii* was

observed in sodium nitrate contained medium followed by asparagine (86 mm), ammonium sulphate (73 mm), urea (70 mm) and the lowest (45 mm) in calcium nitrate.

3.11 *In vitro* control measures

3.11.1 Effect of Phytohormones on per cent mycelial growth inhibition of *S. rolfsii*

The effect of Phytohormones on per cent of inhibition *S. rolfsii* was tested and results are presented in Table 10 and Plate 8. It is noted from the experiment that 2, 4-D hormone inhibited hundred per cent mycelial growth of *S. rolfsii* in PDA medium at all concentrations. IAA and IBA hormones inhibited hundred per cent mycelial growth at 500 ppm concentrations respectively. NAA and BA hormone inhibited 94.44 and 47.14% mycelial growth at 1000 ppm concentrations respectively.

3.11.2 Effect of oils on per cent mycelial growth inhibition of *S. rolfsii*

Six oils were used in this experiment to study their inhibitory effect against *S. rolfsii* and results are presented in Table 11 and Plate 9. It is evident from the experiment that all the oils have inhibitory effect against the pathogen. Among them, clove oil and cinnamon oil inhibited 100% mycelial growth of the fungus at all the concentrations. Mustard oil, neem oil, black cumin oil and sesame oil inhibited 88.89, 86.67, 81.12 and 68.89% mycelial growth of the fungus at 2.5% concentrations respectively.

3.11.3 Effect of oilcake on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different oilcake the inhibition *S. rolfsii* was tested and results are presented in Table 12 and Plate 10. It is evident from the experiment that poultry manure completely inhibited mycelial growth of the fungus at 3% concentration. Til cake, neem cake, mustard cake, vermicompost and farmyard manure inhibited 18.89, 38.89, 36.67, 41.11 and 28.89% mycelial growth of the fungus at 3% concentration respectively.

3.11.4 Effect of fertilizers on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different fertilizers on per cent inhibition of *S. rolfsii* was tested and results are presented in Table 13 and 11 (A-D). It is indicated from the experiment that hundred per cent mycelial growth inhibition was noticed with urea fertilizer at all concentrations. Zipsum at 3% concentration completely inhibited mycelial growth of the fungus. Murate of Potash and Phosphate inhibited 42.22 and 68.89% mycelial growth of the fungus at 3% concentration.

3.11.5 Effect of cowdung and cow urine on per cent mycelial growth inhibition of *S. rolfsii*

The effect of cowdung and cow urine on per cent inhibition of mycelial growth of *S. rolfsii* was tested and results are presented in Table 14 and Plate 7 C & D. It is observed from the experiment that 22.22% and 42.22% inhibition of mycelial growth was observed in cowdung and cow urine contain media at 25% concentration. With the increase of concentrations, the rate of inhibition per cent also increased.

3.11.6 Effect of vitamins on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different vitamin on per cent inhibition mycelial growth of *S. rolfsii* was tested and results are presented in Table 15 and Plate 7 E. It is evident from the experiment that the highest per cent mycelial growth inhibition (55.56%) was observed in Nicotinic acid followed by Pyridoxin (48.89%), Thiamine (33.33%), Ascorbic acid (27.78%) and Riboflavine (25.56%). The lowest inhibition was recorded in as Folic acid (22.22%).

3.11.7 Effect of salts on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different salts on per cent inhibition of *S. rolfsii* was tested and results are presented in Table 16 and Plate 11(E-G). It is indicated from the experiment that hundred per cent mycelial growth was observed in NaHCO_3 and NH_4HCO_3 salt at all concentrations. It is evident from the experiment that at 0.4 to 0.5% concentration of ZnSO_4 inhibited 100% mycelial growth of *S. rolfsii*.

3.11.8 Effect of plant extracts on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different plant extracts on per cent inhibition of *S. rolfsii* was tested and results are presented in Table 17 Plate (12-14). In this experiment eighteen indigenous medicinal plant have been tested and it is evident from the experiment that 100% inhibition of mycelial growth of *S. rolfsii* was observed with *A. sativum* and *L. inermis* extracts at all concentrations. *A. indica* and *S. mahagoni* extracts caused 100% inhibition mycelial growth at 25% concentration. Next to this extracts, *C. longa*, *N. tabacum*, *L. camara* and *Z. officinale* inhibited (90.54, 88.12, 86.30, 83.64 and 85.52%) mycelial growth of *S. rolfsii* at 25% concentrations after 4 days of incubation. *C. tamala*, *S. aromaticum*, *C. viscosum*, *U. globulus*, and *P. hydropiper* extracts inhibited moderate to good (76.64, 75.88, 75.22, 74.92 and 68.40%) mycelium growth inhibition of the fungus. The lowest mycelial growth inhibition (22.70%) was observed at 25% concentration of *C. dactylon* extract. Rest of the plant extracts had poor inhibitory effect on mycelial growth of *S. rolfsii*.

3.11.9 Effect of dried plant extracts on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different dried plant extract on the per cent of inhibition of *S. rolfsii* was tested and results are presented in Table 18 Plate (15 & 16). Hundred per cent mycelial growth inhibition of *S. rolfsii* was observed in *A. sativum*, *L. inermis* extracts at all concentrations. Next to this extracts, *S. mahagoni*, *S. aromaticum*, *N. tabacum*, and *P. hystrophorus* extracts inhibited (88.33, 86.20, 84.50, and 83.89%) mycelial growth of *S. rolfsii* at 25% concentrations after 4 days of incubation. *L. camara*, *C. tamala*, *C. capsularis*, and *Z. officinale* extracts inhibited moderate to good (77.78, 75.67, 71.74 and 70.33%) mycelium growth inhibition of the fungus. The lowest mycelial growth inhibition (14.50%) was observed at 25% concentration of *C. dactylon* extract. Rest of the plant extracts had poor inhibitory effect on mycelial growth of *S. rolfsii* (Table 18).

3.11.10 Effect of ethanol plant extracts on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different ethanol plant extract on the per cent of inhibition of *S. rolfsii* was tested and results are presented in Table 19 Plate 17 & 18. Hundred per cent mycelial growth of *S. rolfsii* inhibition was measured in *A. sativum* extract treated medium at all concentrations. *L. inermis*, *A. indica* and *S. mahogoni* extracts inhibited 100% mycelial growth of *S. rolfsii* at 10, 15% concentrations. *N. tabacum* and *P. hysterophorus* extracts inhibited 100% mycelial growth of *S. rolfsii* at 15% concentrations. The lowest inhibition (84.44%) mycelial growth of the fungus was observed in *C. tamala* at 15% concentration.

3.11.11 Effect of fungicides on per cent mycelial growth inhibition of *S. rolfsii*

The effect of fungicides on the per cent of inhibition of *S. rolfsii* was tested and results are presented in Table 20 Plate 19 & 20. Complete inhibition of mycelial growth of the fungus was observed when treated in Folicure and Score at all concentrations. Rovrol and Tilt showed complete inhibition from 250 ppm concentration to rest of the concentrations. Dithan M-45 showed complete inhibition from 1000 ppm concentration to rest of the concentrations. Hecnazol inhibited from 500 ppm concentration. Secure, Antacle, Thiovit, Bavistin and Ridomil showed 74.22, 71.11, 58.89, 57.78 and 53.33% mycelial growth inhibition at 1000 ppm concentration of the fungus. The lowest mycelial growth inhibition (5.56%) was measured when treated in Cupravit at 1000 ppm.

3.11.12 Effect of herbicides on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different herbicides on per cent inhibition of *S. rolfsii* was tested and results are presented in Table 21 and Plate 21. Data indicated that Logran and 2, 4-D at all concentrations completely inhibited mycelial growth of the fungus. Sunraise at 0.3% concentration completely inhibited mycelial growth of the

fungus. Rifit and Topsatar at 0.3% concentration inhibited 86.67 and 71.11% mycelial growth of the fungus.

3.11.13 Effect of insecticides on per cent mycelial growth inhibition of *S. rolfsii*

The effect of different insecticides on per cent inhibition of *S. rolfsii* was tested and results are presented in Table 22 and Plate 22. It is evident from the experiment that Desis and Moventa at all concentrations complete inhibited mycelial growth of the fungus. Sunraise at 0.3% concentration inhibited completely mycelial growth of the fungus. Confidor and Fifanon at 0.3% concentration inhibited completely mycelial growth of the fungus. Ripcord at 0.3% concentrations inhibited 86.67% mycelial growth of the fungus. It is clear from the experiment that insecticides have inhibitory effect against the fungus.

3.11.14 Effect of *Trichoderma* spp. on per cent mycelial growth inhibition of *S. rolfsii*

The effect of *Trichoderma* sp. on per cent inhibition mycelial growth of *S. rolfsii* was tested and results are presented in Table 23 and Plate 23(A-C). The highest per cent mycelial growth inhibition (81.11%) was observed in *Trichoderma* isolates, Tr-3 and the lowest (54.44%) in Tr-14. On the other hand 78.23%, 75.78% and 74.44% mycelial growth inhibition was recorded in Tr-11, Tr-12 and Tr-10 respectively.

3.11.15 Effect of *Trichoderma* spp. culture filtrate on per cent mycelial growth inhibition of *S. rolfsii*

The effect of *Trichoderma* spp. culture filtrate on per cent mycelial growth inhibition of *S. rolfsii* was tested and results are presented in Table 24 and Plate 23 D. Among the ten isolates, Tr-3 had shown maximum per cent mycelial growth inhibition (84.44%) followed by Tr-11 (71.11%), Tr-12 (63.33%) and lowest in Tr-12 (5.56%).

Table 7. Variation in the different isolates of *S. rolfsii*.

Sl. No	Isolates	Colony diameter (mm) *				Dry mycelial wt (mg) *	Sclerotial size (mm) *	No. of sclerotial bodies*/cm ²	Test weight of 100 sclerotial bodies* (mg)
		24 hr	48 hr	72 hr	96 hr				
1	S-1	25.67	38.56	59.43	82.45	185.45	1.55	4	78.94
2	S-2	31.25	48.90	72.66	88.22	192.68	1.38	5	82.43
3	S-3	32.20	49.55	69.84	87.34	238.55	1.63	6	88.27
4	S-4	21.23	32.58	54.32	77.66	246.89	1.95	8	95.79
5	S-5	34.24	52.35	72.98	90.00	249.44	1.98	9	101.66
6	S-6	27.92	44.79	66.44	84.67	176.37	1.48	7	85.72
7	S-7	18.94	29.45	52.77	75.44	159.94	1.30	3	72.54
8	S-8	32.48	50.96	71.50	89.00	160.73	1.40	4	73.41
9	S-9	30.66	47.88	67.46	86.33	171.24	1.32	5	79.86
10	S-10	23.25	34.72	59.43	82.45	187.88	1.56	6	84.64
	LSD (0.05)	2.86	6.97	7.33	9.12	4.64			

*Average of three replications

Table 8. Pathogenecity test of different isolates of *S. rolfsii* on betelvine cultivar Bangla.

<i>Sclerotium</i> isolates	Disease incidence*
S-1	83.88 (66.34)
S-2	48.72 (44.25)
S-3	78.15 (62.10)
S-4	87.34 (69.12)
S-5	90.28 (71.85)
S-6	67.90 (55.49)
S-7	45.56 (42.48)
S-8	85.76 (67.78)
S-9	88.30 (70.00)
S-10	58.65 (49.95)
Control.	82.67 (65.42)
LSD (0.05)	0.8494

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 9. Effect of carbon and nitrogen sources on the mycelial growth of *S. rolfsii*.

SL No.	Carbon sources	Mycelial growth (mm)*
1	D-glucose	51.00
2	D-fructose	54.00
3	Manitol	68.00
4	Glycerol	30.00
5	Starch	82.00
6	Cellulose	90.00
7	Sorbitol	88.00
8	Sucrose	60.00
	LSD (0.05)	2.293
	Nitrozen sources	
1	Calcium nitrate	45.00
2	Potassium nitrate	51.00
3	Amonium nitrate	53.00
4	Peptone	55.00
5	Urea	70.00
6	Amonium sulphate	73.00
7	Aspargine	86.00
8	Sodium nitrate	90.00
	LSD (0.05)	1.437

*Average of three replications

Table 10. Effect of Phytohormones on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Phytohormones	Per cent inhibition of mycelial growth*				
	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	1000 (ppm)
IAA	17.78	38.89	88.89	100	100
IBA	14.56	34.45	62.22	100	100
2.4. D	100	100	100	100	100
NAA	12.11	25.56	42.22	71.11	94.44
BA	5.56	11.11	24.67	34.44	57.14
LSD (0.05)	0.01485	0.04650	0.01474	0.06232	0.06269

*Average of three replications

Table 11. Effect of different oils on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Oils	Per cent inhibition of mycelial growth*				
	0.5%	1%	1.5%	2.0%	2.5%
Mastard oil	26.67	48.89	68.89	75.56	88.89
Neem oil	86.67	94.45	100	100	100
Clove oil	100	100	100	100	100
Black cumin oil	100	100	100	91.12	81.12
Cinnamon oil	100	100	100	100	100
Sesame oil	6.67	18.89	37.78	54.45	68.89
LSD (0.05)	0.01208	0.01205	0.01198	0.01208	0.01378

*Average of three replications

Table 12. Effect of different oilcake on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Oilcakes	Per cent inhibition of mycelial growth*		
	1%	2%	3%
Sesame cake	4.44	13.33	18.89
Neem cake	74.44	83.33	100.0
Mustard cake	13.33	30.00	36.67
Vermicompost	14.44	22.22	41.11
Farmyard manure	8.89	15.56	28.89
Poultry manure	16.67	27.78	38.89
LSD (0.05)	1.901	1.798	2.504

*Average of three replications

PLATE-4



A

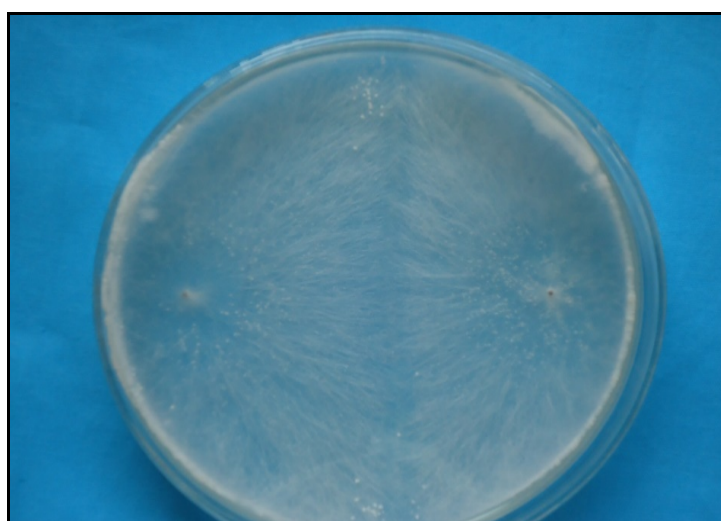


B



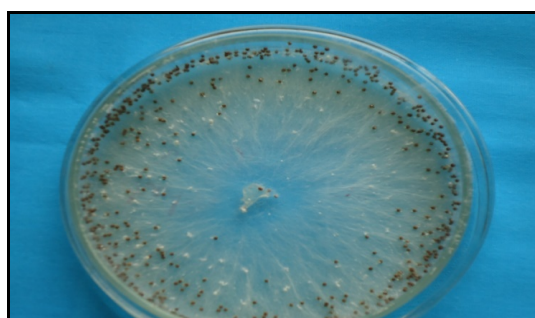
C

A- Drip system irrigation, B- Flood system irrigation, C- Pathogenicity test

PLATE 5**A****B****C**

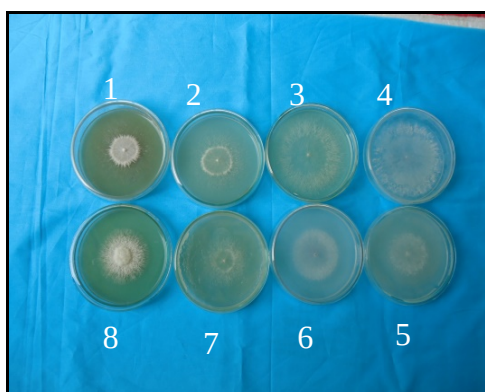
A- Mycelial growth of *S.rolfsii*, **B-** Formation of sclerotia, **C-** mycelial compatibility of two isolates of *S.rolfsii*.

PLATE 6

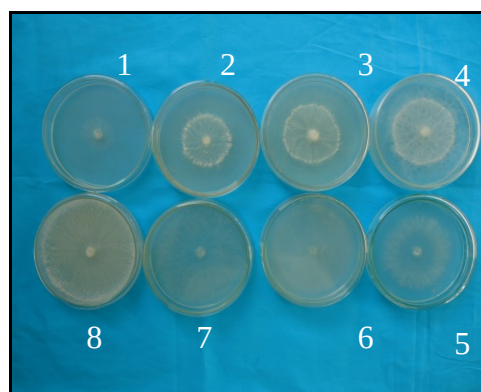


Different type isolates of *S. rolfsii*

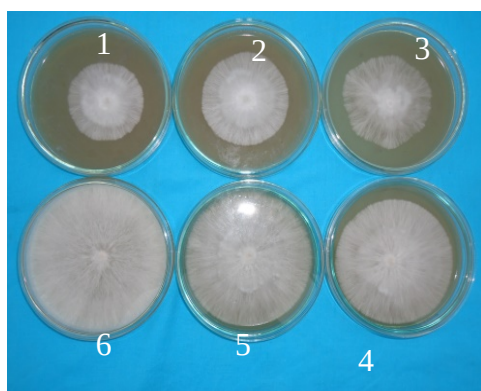
PLATE 7



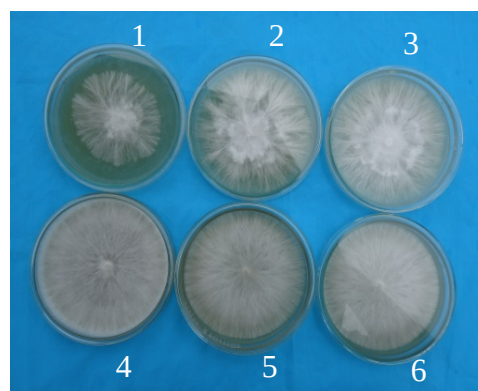
A



B



C

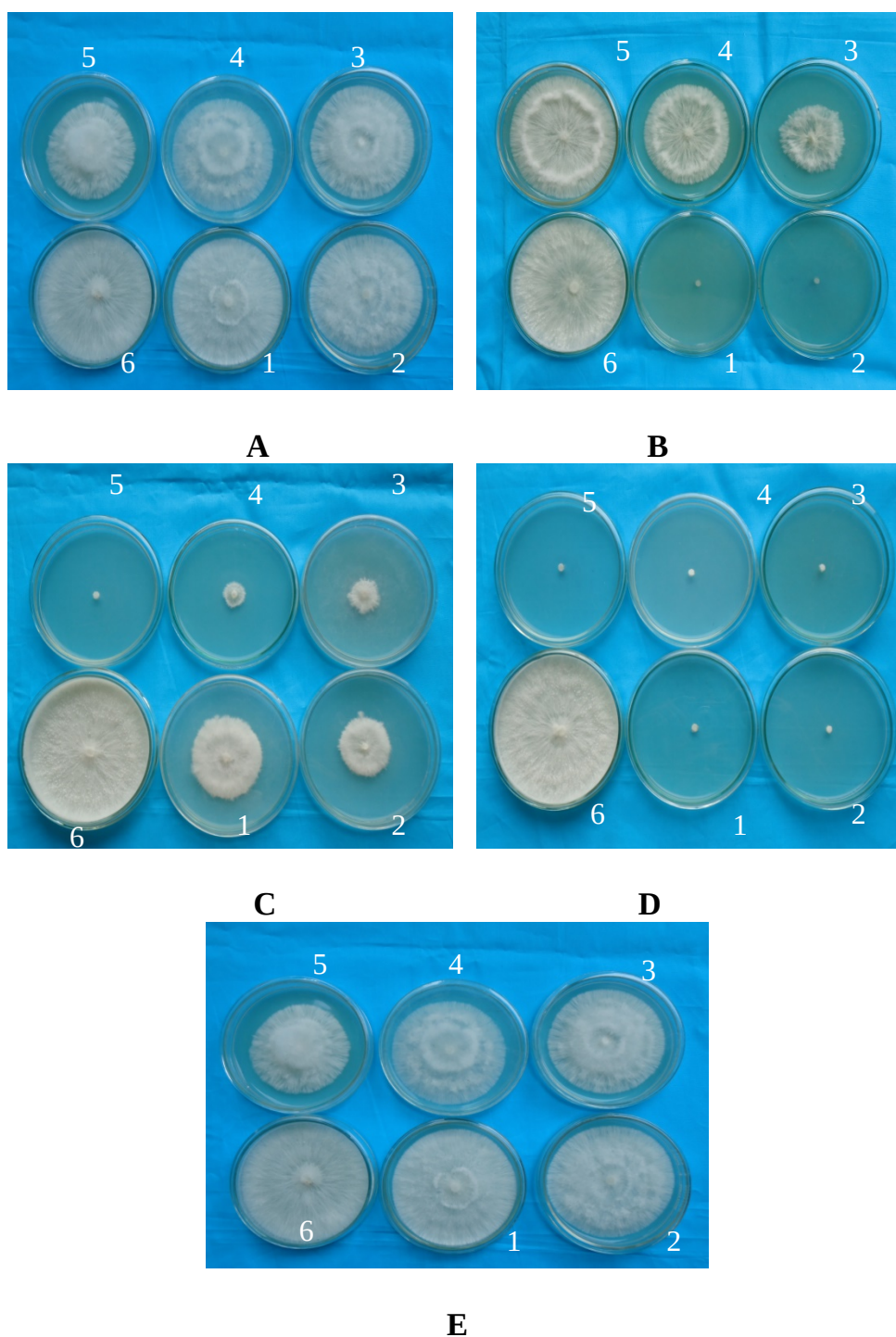


D

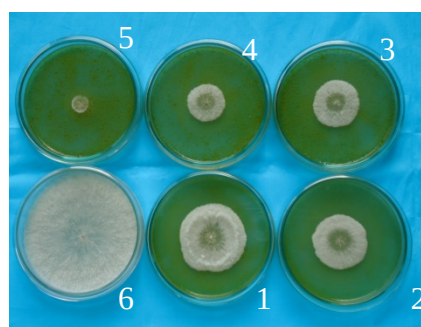
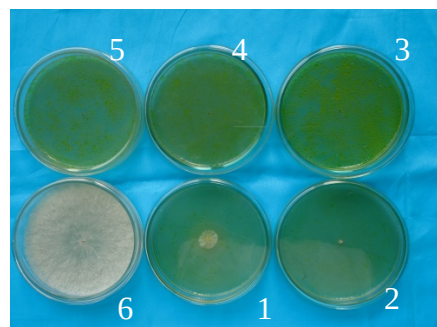
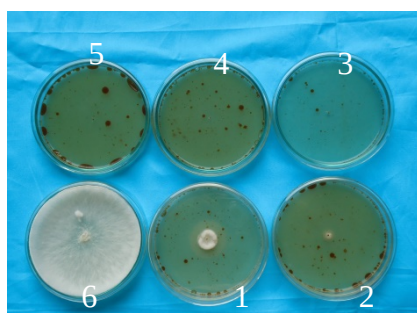
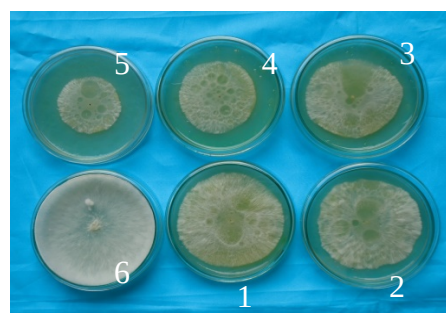
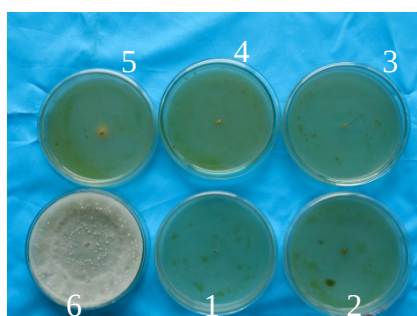
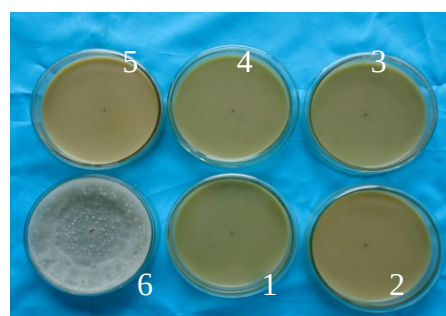


E

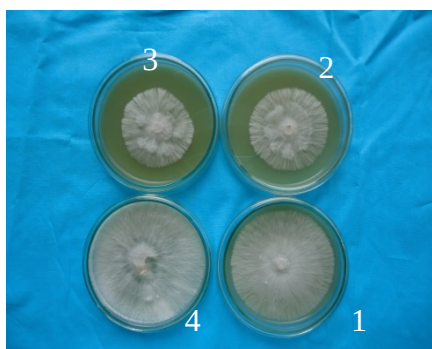
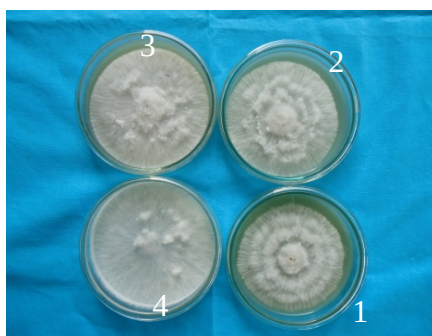
A- 1. Glycerol, 2. D-fructose, 3. Mannitol, 4. D-glucose 5. Starch, 6. Cellulose 7. Sorbitol, 8. Sucrose; B- 1. Calcium nitrate, 2. Potassium nitrate, 3. Ammonium nitrate, 4. Peptone, 5. Urea; C. Cow dung; D. Urine; E- Vitamin (1. Nicotinic acid, 2. Pyridoxin, 3. Thiamine, 4. Folic acid, 5. Riboflavin, 6. Ascorbic acid)

PLATE 8

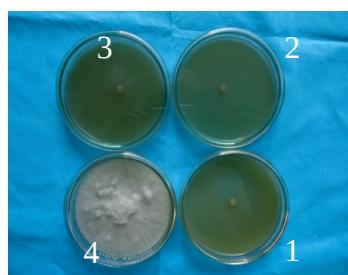
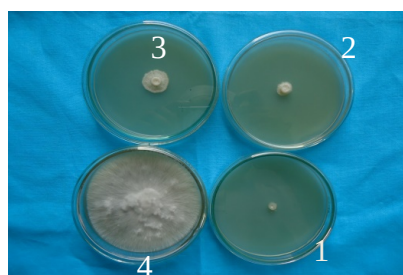
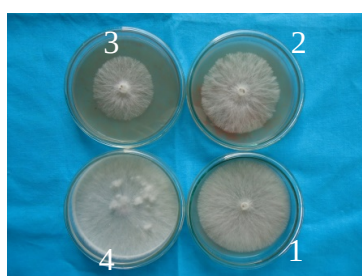
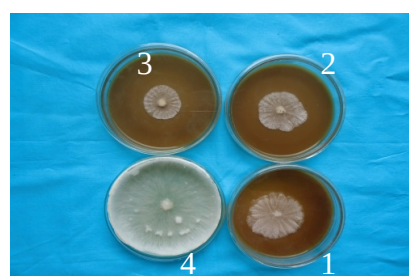
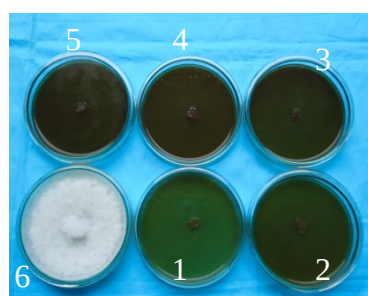
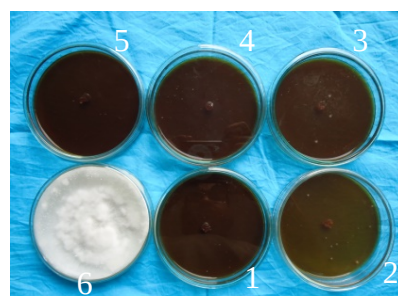
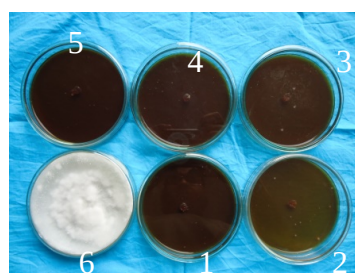
A- I.A.A, B- I.B.A, C- N.A.A, D- 2-4.D, E- B.A. (1=50 ppm, 2=100ppm, 3= 250 ppm, 4= 500ppm, 5= 1000ppm and 6= control)

PLATE 9**A****B****C****D****E****F**

A- Mastard oil, B- Neem oil, C-Kaloziira oil, D-Til oil, E- Clove oil, F-Cinnamon oil. (1=0.5%, 2=1.0%, 3= 1.5%, 4= 2.0%, 5= 2.5% and 6= control)

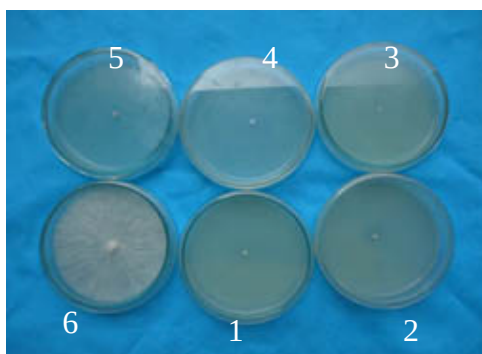
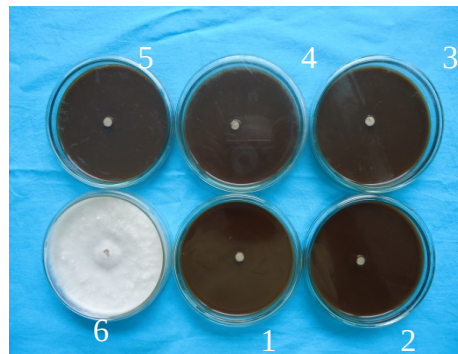
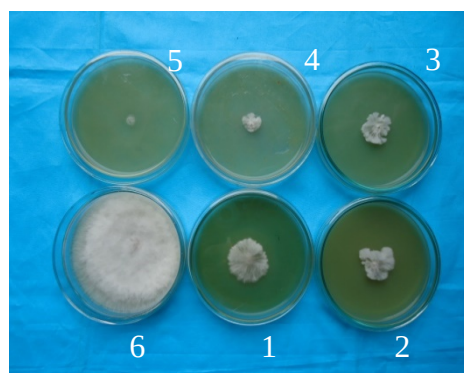
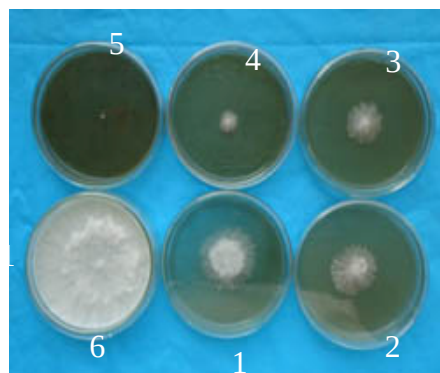
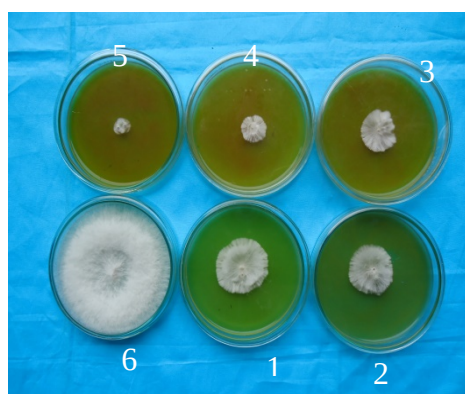
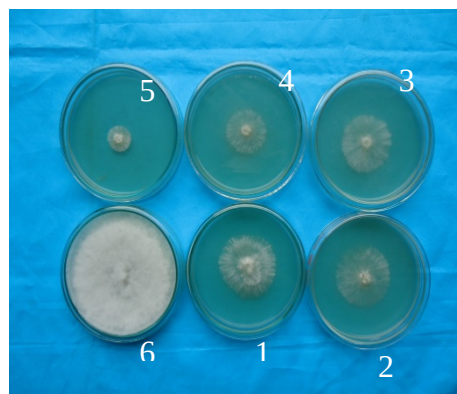
PLATE 10**A****B****C****D****E**

A. Tilcake, B. Neem cake, C. Mastard cake, D. Vermicompost, E. Farmyard manure, F. Poultry manure. (1=1%, 2=2%, 3= 3% and 4= control)

PLATE 11**A****B****C****D****E****F****G**

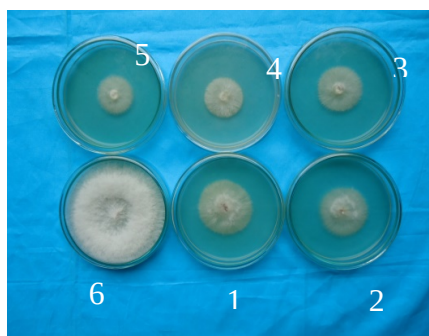
A. Urea, B. Zipsum, C. Potash, D. Phosphate, E. ZnSO_4 , F. NH_4HCO_3 ,
G. NaHCO_3 (Conc. 1=1%, 2=2%, 3= 3% and 4=control)

Plate 12

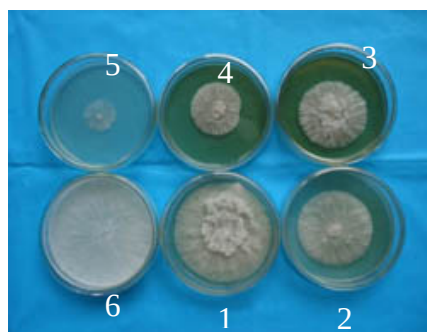
**A****B****C****D****E****F**

A- *A. sativum*, B- *L. inermis*, C- *A. indica*, D- *S. mahagoni*, E- *C. longa* F- *N. tabacum*, (1=5, 2=10, 3=15, 4=20 5=25% and 6=control)

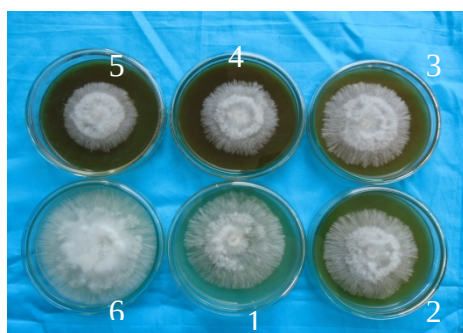
Plate 13



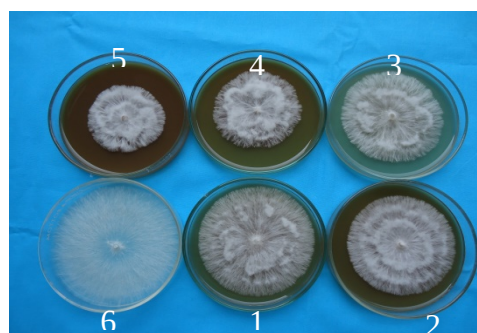
G



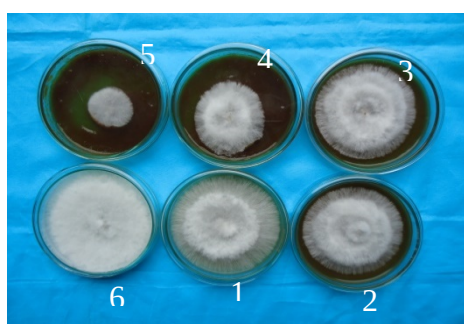
H



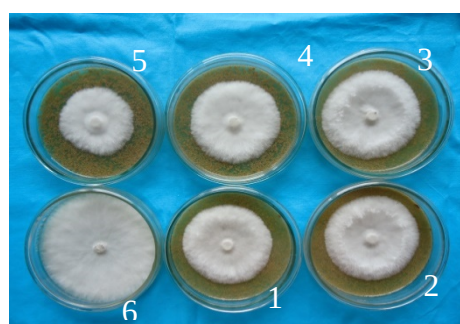
I



J



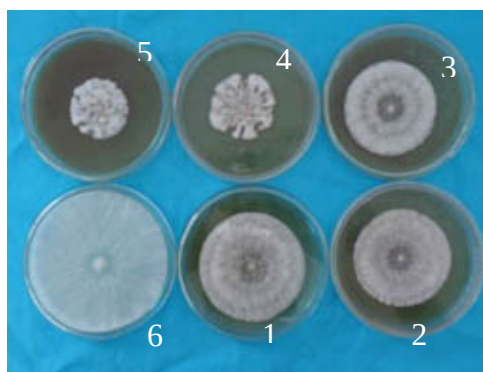
K



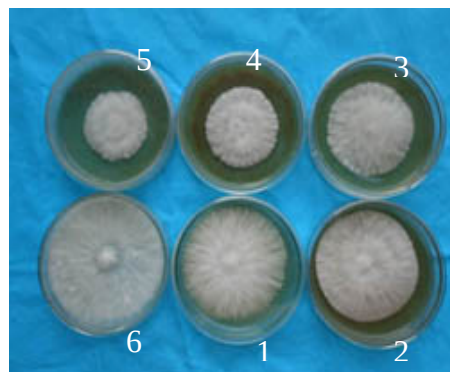
L

G- *P. hysterophorus*, H- *L. camara*, I- *C. viscosum*, J- *C. tamala*, K- *Z. officinale*,
L- *P. hydropiper* (1=5, 2=10, 3=15, 4=20 5=25% and 6=control)

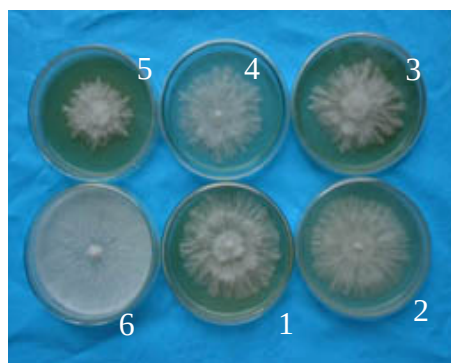
Plate 14



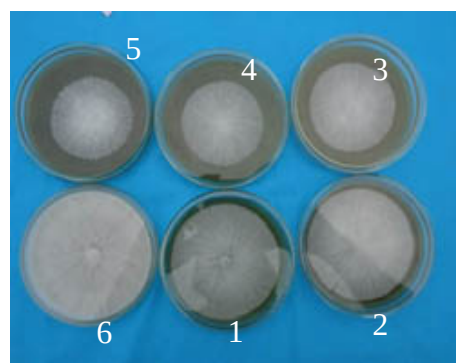
M



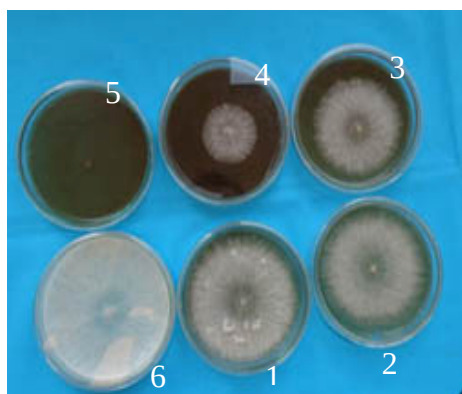
N



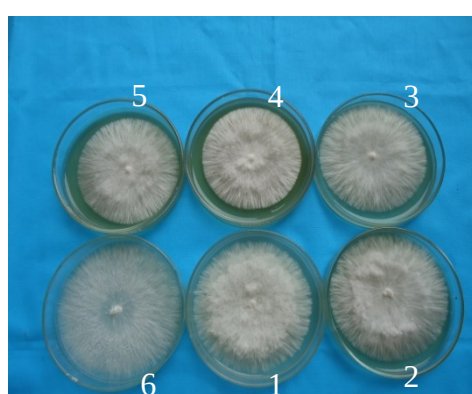
O



P



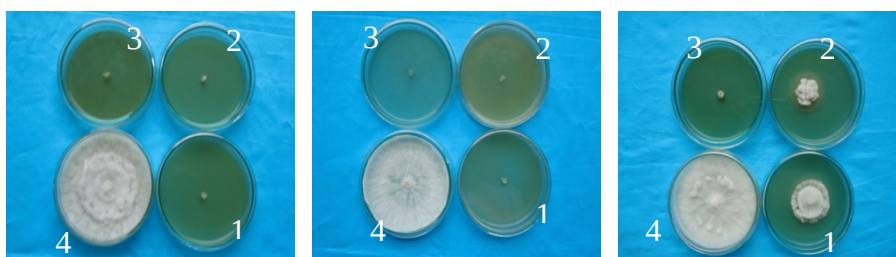
Q



R

M- *S. aromaticum*, N- *U. globulus*, O- *C. procera*, P- *B. pinnatum*, Q- *C. capsularis*, R- *C. dactylon*. (1=5, 2=10, 3=15, 4=20, 5=25 % and 6= control)

PLATE 15



A

B

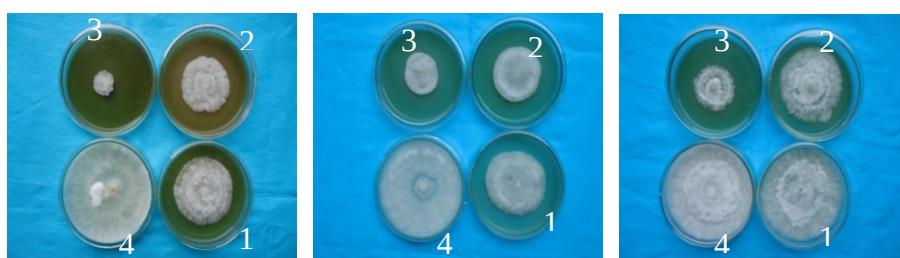
C



D

E

F



G

H

I

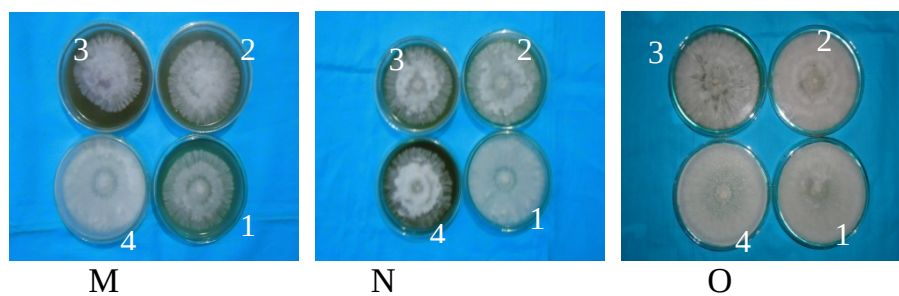


J

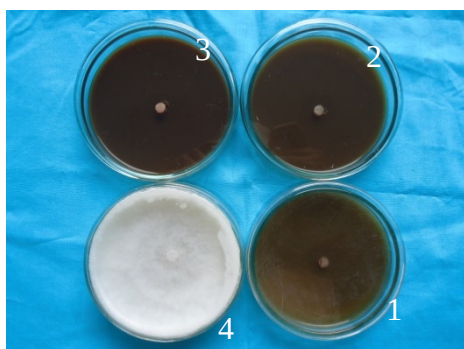
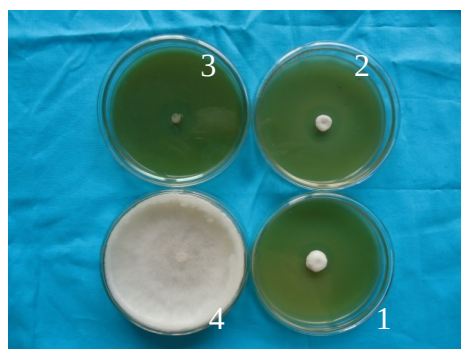
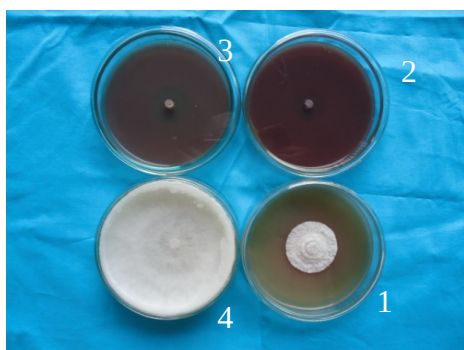
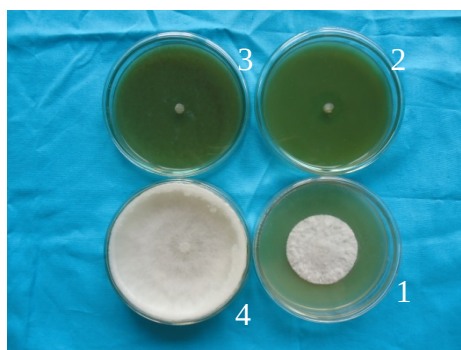
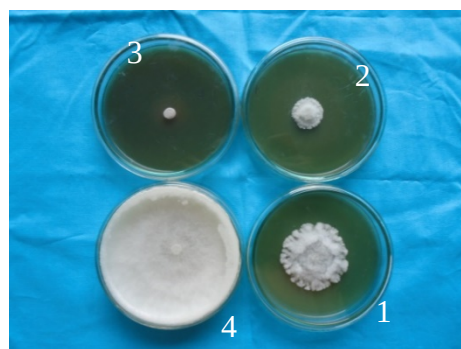
K

L

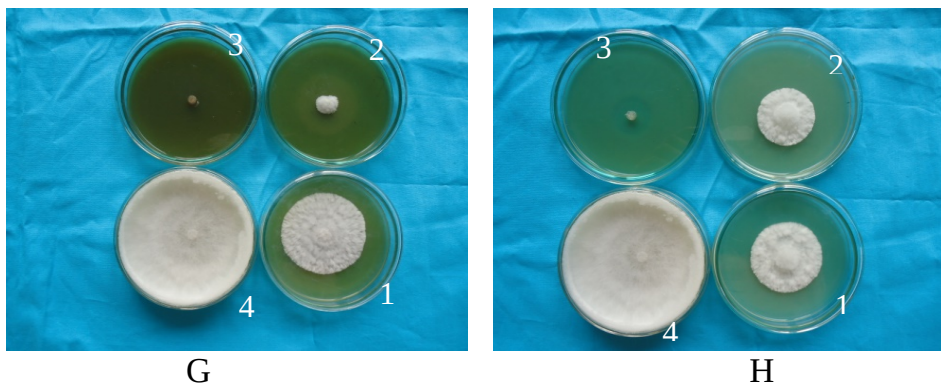
A- *A. sativum*, B- *L. inermis*, C- *A. indica*, D- *S. mahagoni*, E- *N. tabacum*, F- *S. aromaticum*, G- *P. hysterophorus*, H- *L. camara*, I- *C. tamala*, J- *C. capsularis*, K- *Z. officinale*, L- *P. hydropiper* (1=5, 2=10, 3=15 and 4=control)

PLATE 16

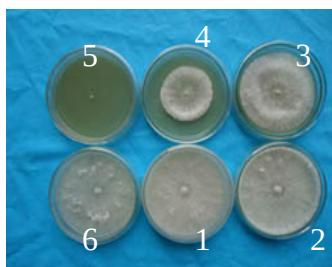
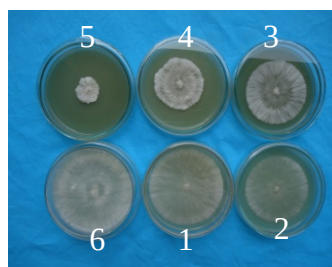
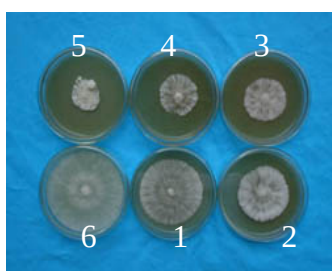
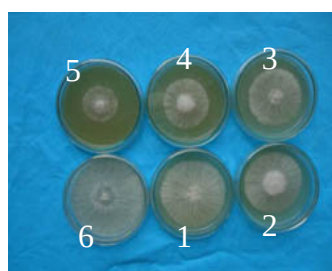
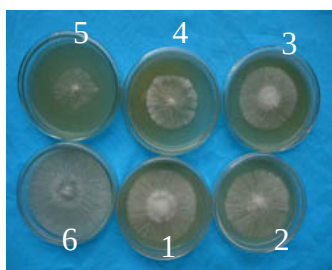
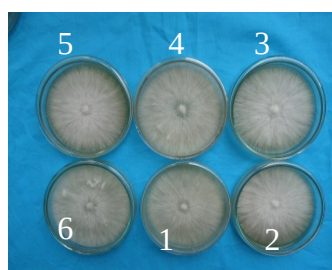
M- *C. viscosum*, N- *U. globulus*, O- *C. dactylon*, (1=5, 2=10, 3=15 and 4=control).

PLATE 17**A****B****C****D****E****F**

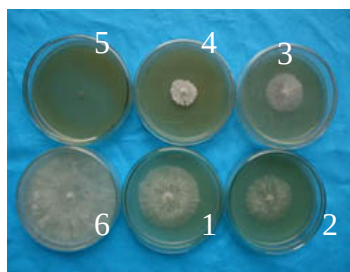
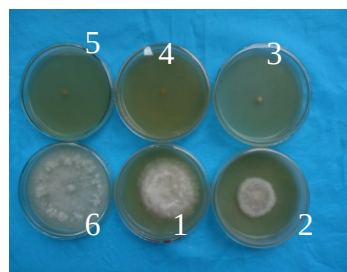
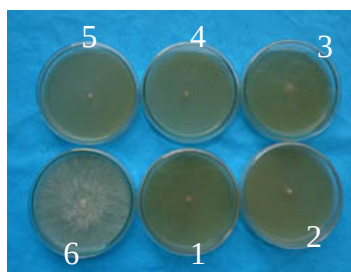
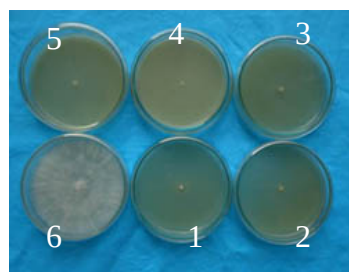
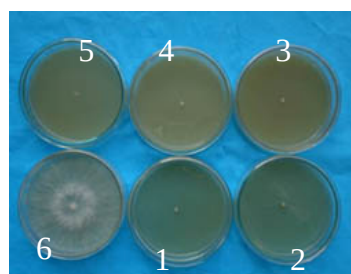
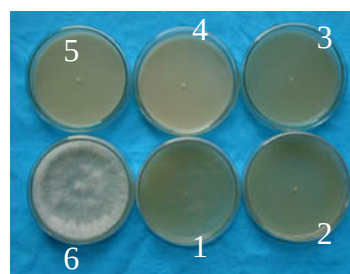
A- *A. sativum*, B- *L. inermis*, C- *A. indica*, D- *S. mahagoni*, E- *N. tabacum*, F- *P. hystrophorus*, (1=5, 2=10, 3=15 % and 4=control)

PLATE 18

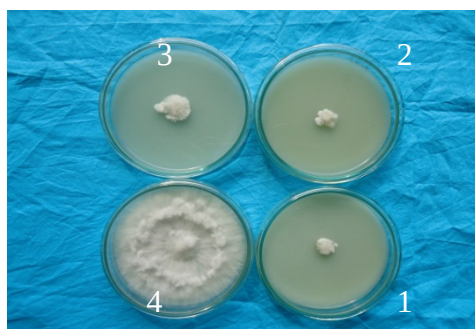
G- *L. camara*, H- *C. tamala*, (1=5, 2=10, 3=15 % and 4=control)

PLATE 19**A****B****C****D****E****F**

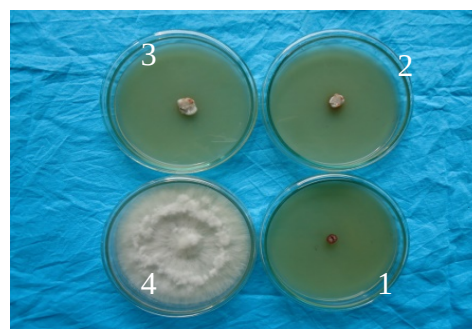
A-Dithan M- 45, B-Antacle, C- Thiovit, D- Bavistin, E- Ridomil, F- Cupravat
(1=50, 2=100, 3=250, 4=500, 5=1000 ppm and 6=control)

PLATE 20**G****H****I****J****K****L**

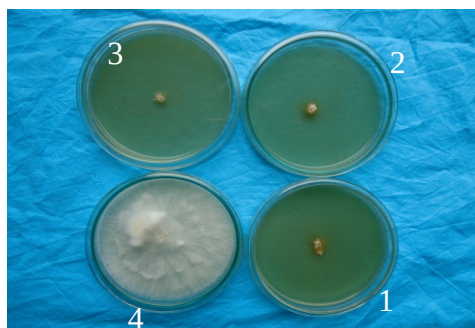
G- Secure, H- Rovrol, I- Heconazole, J- Tilt, K- Folicure, L- Score (1=50, 2=100, 3=250, 4=500, 5=1000 ppm and 6=control).

PLATE 21

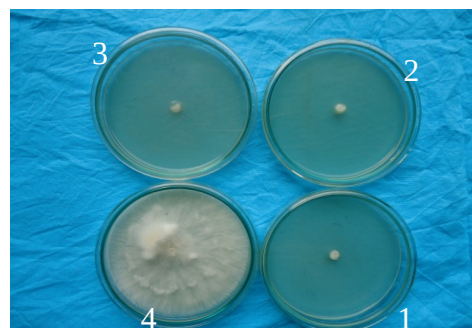
A



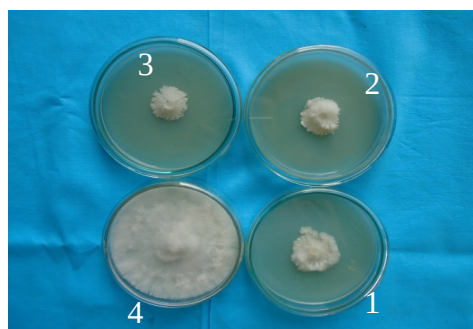
B



C

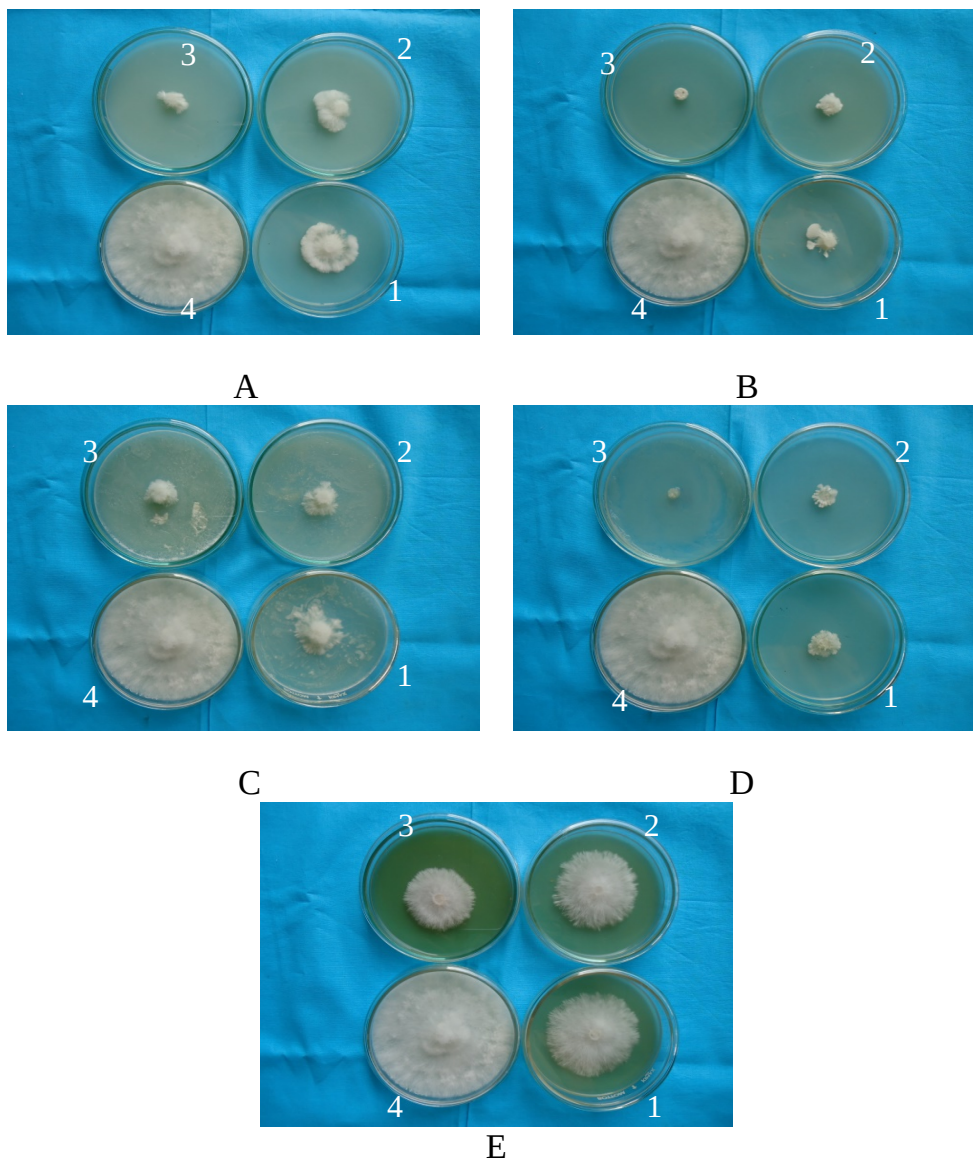


D



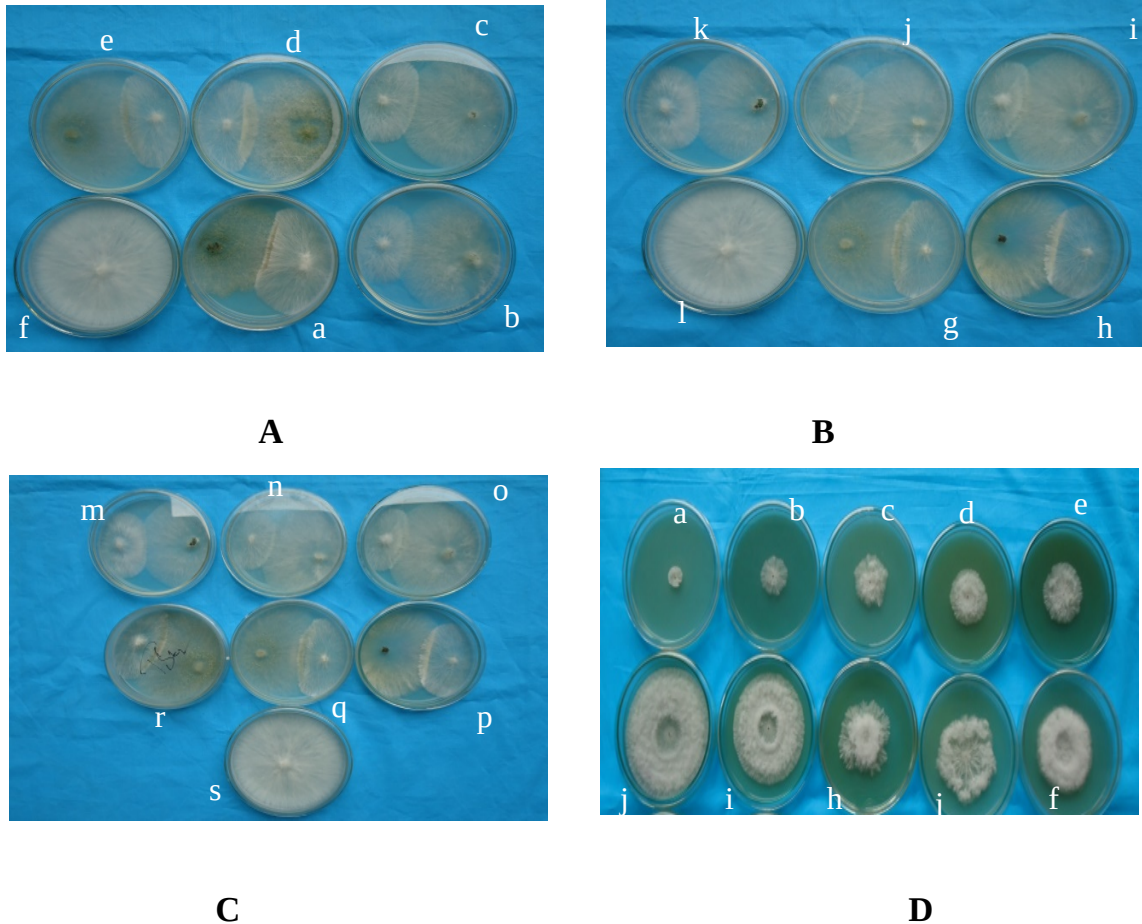
E

A- Rifit, **B-** Sunraise, **C-** Lograan, **D-2. 4- D, E-Topstar** (1=0.1, 2=0.2, 3=0.3% and 4=control).

PLATE 22

A- Ripcord, B-Desis, C- Moventa, D-Fifanon, E- Confidor (1=0.1, 2=0.2, 3=0.3%and 4=control).

PLATE 23



A-a.*Trichoderma* sp.-1, b.*Trichoderma* sp.-2, c.*Trichoderma* sp.-3, d.*Trichoderma* sp.-4, e.*Trichoderma* sp.-5, f.Control.

B-g.*Trichoderma* spp.-6,h.*Trichoderma* sp.-7, i.*Trichoderma* sp.-8, j.*Trichoderma* spp.-9, k.*Trichoderma* sp.-10, l.control

C- m.*Trichoderma* sp.-11, n. *Trichoderma* sp.-12, o.*Trichoderma* sp.-13, p.*Trichoderma* sp.-14,q. *Trichoderma* sp.-15, r. Control.

D-a.*Trichoderma* sp.-3, b.*Trichoderma* sp.-11, c.*Trichoderma* sp.-12, d.*Trichoderma* sp.-10, e.*Trichoderma* sp.-13, f.*Trichoderma* sp.-16, g.*Trichoderma* spp.-8, h.*Trichoderma* sp.-6, i.*Trichoderma* sp.-2, j- Control.

Table 13. Effect of different fertilizers on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Fertilizers	Per cent inhibition of mycelial growth*		
	1%	2%	3%
Urea	100	100	100
Murate of Potash	15.44	24.44	42.22
Zipsum	67.78	86.67	100
Phosphate	24.44	51.11	68.89
LSD (0.05)	3.041	3.438	3.206

*Average of three replications

Table 14. Effect of cowdung and cow urine on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Substarates	Per cent inhibition of mycelial growth*				
	5%	10%	15%	20%	25%
Cowdung	2.26	5.56	11.11	15.56	22.22
Cow Urine	13.33	22.22	27.79	32.22	42.22
LSD (0.05)	0.01309	0.01295	0.01325	0.01215	0.01346

*Average of three replications

Table 15 Effect of different vitamin on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Vitamins	Mycelial growth*	% inhibition
Nicotinic acid	40	55.56
Pyridoxine	46	48.89
Thiamine	60	33.33
Ascorbic acid	65	27.78
Riboflavin	67	25.56
Folic acid	70	22.22
Control	90	
LSD (0.05)		0.8577

*Average of three replications

Table 16. Effect of different salts on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Salts	Per cent inhibition of mycelial growth*				
	0.1%	0.2%	0.3%	0.4%	0.5%
NaHCO ₃	100	100	100	100	100
NH ₄ HCO ₃	100	100	100	100	100
ZnSO ₄	86.67	91.11	93.11	100	100
LSD (0.05)	3.635	3.898	3.594	3.463	0.02598

*Average of three replications

Table 17. Effect of different plant extracts (water) on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation

Plant extract	Used Plant parts	Per cent inhibition of mycelial growth*				
		5%	10%	15%	20%	25%
<i>Allium sativum</i>	Leaf	100.00	100.00	100.00	100.00	100.00
<i>Lawsonia inermis</i>	Leaf	100.00	100.00	100.00	100.00	100.00
<i>Azadirachata indica</i>	Leaf	33.24	54.20	66.33	88.40	100.00
<i>Swietenia mahagoni</i>	Seed	31.00	52.74	63.67	83.11	100.00
<i>Curcuma longa</i>	Leaf	28.56	55.44	64.89	71.25	90.54
<i>Nicotiana tabacum</i>	Leaf	24.75	42.88	66.78	81.66	88.12
<i>Parthenium hysterophorus</i>	Leaf	27.66	45.28	66.89	77.60	86.30
<i>Lantana camara</i>	Leaf	24.44	43.33	59.11	71.00	83.64
<i>Clerodendrum viscosum</i>	Leaf	22.89	35.44	48.00	62.42	75.22
<i>Cinnamomum tamala</i>	Leaf	14.44	23.33	38.24	57.36	76.64
<i>Zingiber officinale</i>	Tuber	9.25	27.12	34.67	62.33	85.52
<i>Polygonum hydropiper</i>	Leaf	6.18	28.00	40.56	51.88	68.40
<i>Syzygium aromaticum</i>	bud	17.78	33.28	52.56	62.22	75.88
<i>Ucalyptus globulus</i>	Leaf	10.96	16.50	27.68	48.26	74.92
<i>Calotropis procera</i>	Leaf	9.33	17.11	43.67	33.33	60.00
<i>Bryophyllum pinnatum</i>	Leaf	11.35	21.56	31.80	43.24	55.75
<i>Corchorus capsularis</i>	seed	7.22	22.89	42.58	78.72	100.00
<i>Cynodon dactylon</i>	Leaf	2.26	5.72	11.25	15.56	22.70
LSD (0.05)		0.516	0.548	0.633	0.521	0.372

*Average of three replications

Table 18. Effect of different dried plant extracts on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation

Plant extract	Used Plant parts	Per cent inhibition of mycelial growth*		
		5%	10%	15%
<i>Allium sativum</i>	Tuber	100.00	100.00	100.00
<i>Lawsonia inermis</i>	Leaf	100.00	100.00	100.00
<i>Azadirachata indica</i>	Leaf	34.56	68.26	100.00
<i>Swietenia mahagoni</i>	Leaf	25.00	58.96	88.33
<i>Nicotiana tabacum</i>	Leaf	32.33	77.78	84.50
<i>Syzygium aromaticum</i>	Flower bud	34.15	65.22	86.20
<i>Parthenium hysterophorus</i>	Leaf	22.22	46.11	83.89
<i>Lantana camara</i>	Leaf	28.44	58.56	77.78
<i>Cinnamomum tamala</i>	Leaf	11.22	37.90	75.67
<i>Corchorus capsularis</i>	Seed	24.33	44.00	71.44
<i>Zingiber officinale</i>	Tuber	20.89	39.44	70.33
<i>Polygonum hydropiper</i>	Leaf	14.67	27.78	44.44
<i>Clerodendrum viscosum</i>	Leaf	11.20	20.00	36.67
<i>Ucalyptus globulus</i>	Leaf	8.56	18.00	24.44
<i>Cynodon dactylon</i>	Leaf	3.33	8.88	14.50
LSD (0.05)		0.532	0.484	0.321

*Average of three replications

Table 19. Effect of different ethanol plant extracts on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation

Plant extract	Used Plant parts	Per cent inhibition of mycelial growth*		
		5%	10%	15%
<i>Allium sativum</i>	Leaf	100.00	100.00	100.00
<i>Lawsonia inermis</i>	Leaf	74.44	100.00	100.00
<i>Azadirachata indica</i>	Leaf	58.67	100.00	100.00
<i>Swietenia mahagoni</i>	Leaf	52.78	100.00	100.00
<i>Nicotiana tabacum</i>	Leaf	36.56	85.44	100.00
<i>Parthenium hysterophorus</i>	Leaf	32.66	78.33	100.00
<i>Lantana camara</i>	Seed	32.12	62.22	88.89
<i>Cinnamomum tamala</i>	Leaf	30.75	56.67	84.44
LSD (0.05)		5.865	0.502	0.001

Table 20. Effect of fungicides on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Fungicides	Per cent inhibition of mycelial growth*				
	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	1000 (ppm)
Dithan M-45 (Mancozeb 80%)	00	00	4.44	35.56	100
Antacle (Probineb)	8.89	20.00	26.56	38.89	71.11
Thiovit (Sulphur 80%)	12.00	23.33	43.33	50.00	58.89
Bavistin (Carbendazim 50%)	11.11	20.00	32.22	42.22	53.33
Ridomil (Metalaxyl+Mancozeb)	12.22	18.89	27.78	42.22	57.78
Cupravit (Copper Oxychoride)	00	00	00	2.22	5.56
Secure (Fenamedon+Mancozeb)	14.22	34.44	56.67	60.76	74.22
Rovrol (Eprodion)	27.78	53.33	100	100	100
Heconazole (Hexaconazole)	56.67	78.89	91.11	100	100
Tilt (Propiconazole)	67.78	88.89	100	100	100
Folicure (Tebuconazole)	100	100	100	100	100
Score (Difenaconazole)	100	100	100	100	100
LSD (0.05)	0.01013	0.01123	0.009315	0.01347	0.01088

*Average of three replications

Table 21. Effect of different herbicides on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Herbicides	Per cent inhibition of mycelial growth*		
	0.1%	0.2%	0.3%
Rifit (Pretilaclore 500 E.C)	75.56	82.22	86.67
Sunraise (Ethoxisalphuran 150 W.G)	83.33	87.78	100
Logran (Triasulfuran)	100	100	100
2. 4-D(2, 4-Dicholorophenoxy acetic acid)	100	100	100
Topsatar (Oxadiazine)	57.78	65.56	71.11
LSD (0.05)	3.449	3.154	3.252

*Average of three replications

Table 22. Effect of different insecticides on per cent mycelial growth inhibition of *S. rolfsii* at 28±2°C on 4 days of incubation.

Insecticides	Per cent inhibition of mycelial growth*		
	0.1%	0.2%	0.3%
Ripcord (Cypermethine 10 E.C)	60.00	68.89	81.11
Confidor (Emidachlorpide 150 W.G)	63.33	87.78	100
Desis (Deltamethirne 2.5 150 W.G)	100	100	100
Moventia (Spirotetramate)	100	100	100
Fifanon (Melathine)	75.56	82.22	100
LSD (0.05)	3.392	3.628	3.074

*Average of three replications

Table 23. Antagonistic effect of *Trichoderma* isolates on per cent mycelial growth inhibition of *S. rolfsii*.

<i>Trichoderma</i> isolates	Per cent of inhibition*
<i>Trichoderma</i> sp.-1	64.44
<i>Tricoderma</i> sp. - 2	67.67
<i>Trichoderma</i> sp. - 3	81.11
<i>Trichoderma</i> sp. - 4	61.11
<i>Trihoderma</i> sp. - 5	66.02
<i>Trichoderma</i> sp. - 6	68.22
<i>Trichoderma</i> sp. - 7	64.66
<i>Trichoderma</i> sp. - 8	69.61
<i>Trichoderma</i> sp. - 9	58.56
<i>Trichoderma</i> sp.- 10	74.44
<i>Trichoderma</i> sp.- 11	78.23
<i>Trichoderma</i> sp.- 12	75.78
<i>Trichoderma</i> sp. - 13	73.39
<i>Trichoderma</i> sp. - 14	54.44
<i>Trichoderma</i> sp.- 15	62.33
<i>Tricoderma</i> sp. -16	70.88
LSD (0.05)	0.01470

*Average of three replications

Table 24. Effect of *Trichoderma* spp. culture filtrates on per cent mycelial growth inhibition of *S. rolfsii*.

<i>Trichoderma</i> filtrates	Per cent of inhibition*
<i>Trichoderma</i> sp.-3	84.44
<i>Trichoderma</i> sp.- 11	71.11
<i>Trichoderma</i> sp.- 12	63.33
<i>Trichoderma</i> sp.- 10	58.89
<i>Trichoderma</i> sp. - 13	55.56
<i>Trichoderma</i> sp.-16	42.22
<i>Trichoderma</i> sp. - 8	36.67
<i>Trichoderma</i> sp. - 6	34.56
<i>Trichoderma</i> sp.- 2	20.00
<i>Trichoderma</i> sp. - 5	5.56
LSD (0.05)	0.01499

*Average of three replications

3.12 Field Experiment

3.12.1 Effect of inoculum level of *S. rolfsii* on incidence of foot-rot of betelvine

The effect of inoculums level of *S. rolfsii* on incidence of foot rot disease infested with *S. rolfsii* sick soil was tested and results are presented in Table 25. Data indicated that hundred per cent disease incidence was recorded in 8, 9 & 10 per cent inocula level and the lowest (35.79%) in 1 per cent level. On the other hand 82.73, 78.91 and 76.82% disease incidence were found at 7, 6 and 5% inoculums level respectively. It is evident from the study that increase of inocula level occurred much foot rot disease incidence.

3.12.2 Identification of the susceptible stage of the crop

The effect of identification of susceptible stage of the betelvine on incidence of disease of foot rot containing *S. rolfsii* sick soil was tested and results are presented in Table 26 and Plate 24 A. It is evident from the experiment that the

highest disease incidence was observed in treatment T₁ (03 month old crop) as (76.44%) and the lowest (61.27%) in T₅ (24 month old crop) treatment. It is clear from the experiment that the fungus cause disease on young plants than that of old aged plants.

3.12.3 Effect of soil solarization on the viability of sclerotia of *S. rolfsii* with clear and black plastic mulch after 30 days of incubation

The effect of soil solarization on the viability of sclerotia of *S. rolfsii* in different depths such as 5 cm, 10 cm, and 15 cm depth without mulching, clear plastic mulch and black plastic mulch were tested and the results are presented in Table 27. It was observed that the highest (82.03%) viability of sclerotia was observed at 15 cm without mulching and the lowest (28.27%) viability of sclerotia was observed at 5 cm depth with black plastic mulch. It is clear from the experiment that black plastic mulching layer has much effect on the viability of sclerotia of *S. rolfsii*. It is noted that 10 cm depth without mulching showed 88.66% viability of sclerotia, whereas at the same depth, clear plastic mulch and black plastic mulch recorded 80.42% and 78.67% viability of sclerotia of the fungus. It was recorded that 15 cm depth with clear plastic mulch and black plastic mulching recorded 83.87% and 81.03% viability of sclerotia of the fungus but with increase of depth, viability of sclerotia of *S. rolfsii* also reduced.

3.12.4 Effect of solarization on per cent disease incidence in *S. rolfsii* infested pot soil

The effect of solarization on *S. rolfsii* infested soil was tested and results are presented in Table 28. In the year (2010-11), the highest (62.80%) and the lowest (54.39%) disease incidence were recorded in comparison with control (66.66%) after treatment with 10 days solar heating and 60 days solar heating respectively. In the year (2011-12), the highest (64.60%) and the lowest (53.25%) disease incidence were recorded in comparison with control (68.36%) after treatment with 10 days solar heating and 60 days solar heating respectively. It is clear from

the study that with the increase of time of sclerotial germination failed and disease incidence also reduced (Table 28).

3.12.5 Effect of amendment soil on germination of sclerotia of *S. rolfsii* at different intervals (42 days)

The effect of amendment soil on germination of sclerotia of *S. rolfsii* was tested and the results are presented in Table 29. All the treatments reduced sclerotial germination little or more except the control. Sclerotial germination reduction was observed in soil amended with neem cake (16.39%) followed by sesame cake (29.72%), vermicompost (32.18%), mustard oil cake (37.54%) and the highest in ash (55.24%) in comparison with control (95.00%) on 30 days of incubation. Inhibition of germinability of propagules of soil borne pathogen in soil is well known. The result of the present study confirmed that sclerotia of *S. rolfsii* are subject to soil fungistasis.

3.12.6 Viability of growth sclerotia with agar disks of *S. rolfsii* as affected with hot water temperatures and exposure time (minute)

The effect of viability of sclerotia of *S. rolfsii* as affected with hot water temperatures and exposure time was tested and the results are presented in Table 30. It was observed that there were no viable sclerotia when treated in hot water at 52 °C temperature up to with 30 minutes and above 52 °C temperature. Viability of *S. rolfsii* was recorded up to 52 °C temperatures to below up to 25 °C.

3.12.7 Effect of different degree hot water treatment at rate 0.5 l/kg soil on the per cent disease incidence in *S. rolfsii* infested pot soil

The effect of different degrees hot water treatment @ 0.5 L/kg soil on foot rot disease incidence of betelvine with *S. rolfsii* infested pot soil was tested and the results are presented in Table 31. This experiment was setup during 2010-11 and 2011-12 cropping seasons and the highest (43.11%) and the lowest (17.56%) disease incidence were recorded in comparison with control (68.28%) after treatment with 65°C and 100°C respectively during 2010-11 cropping seasons.

On the other hand, during 2011-12 cropping seasons, it recorded as 40.51% and 18.63% at the same temperatures. When the hot water are treated as 70°, 80° and 90° C and in respect to soil temperatures maintained as 59°, 68° and 77°C, the foot rot diseased incidence of betelvine in both the crop seasons noted as 38.53, 28.18 and 24.20% and 36.57, 25.33 and 18.63%. It is evident from the Table that, with the increase of temperature in soil, the infestation may decreased and occurrence of foot rot disease was reduced (Table 31).

3.12.8 Effect of cultural practice on the incidence of disease in *Sclerotium rolfsii* sick soil

The effect of cultural practices on the incidence of foot rot disease of betelvine with *S. rolfsii* sick soil was tested and results are presented in Table 32 and Plate 24(B-D). It is observed from the experiment that in the year (2010-11), the lowest (26.28%) and the highest (62.64%) disease incidence were recorded in comparison with control (64.52%) after treatment with earthing-up soil up to 25 cm height one time/year and hot water 0.5lit/kg respectively. In the year (2011-12), the lowest (21.47%) and the highest (62.87%) disease incidence were recorded in comparison with control (68.28%) after treatment with earthing-up soil up to 25 cm height one time/year and hot water 0.5lit/kg respectively. In this experiment, hot water treated showed better performance to reduce the intensity of *S. rolfsii* and incidence of disease. Hot water treatment performed better to reduce the infestation of pathogen and reduce the incidence of disease from cropping year 210-11 and 2011-12. (Table 32)

3.12.9 Effect of organic amendment on incidence of foot-rot disease in field during 2010-2011 and 2011-12

Effect of organic amendment on incidence of foot rot disease in field condition was tested and results are presented in Table 33 and Plate 25 A. In year (2010-11), the highest (54.33%) and the lowest (51.24%) foot rot disease incidence of betelvine were recorded in comparison with control (63.87%) after treatment

with mustard and neem cake respectively. The highest (228.10 cm) and the lowest (219.60 cm) plant height were recorded in comparison with control (203.10 cm) after treatment with neem cake and mustard cake respectively in boroj. The highest (2.55 cm) and the lowest (2.33 cm) stem girth of betelvine were recorded in comparison with control (2.01 cm) after treatment with neem and sesame cake respectively. The highest (32) and the lowest (28) no. of leaves/plants were recorded in comparison with control (24) after treatment with neem cake and mustard cake treated vines respectively. The highest (7.76 cm) and the lowest (6.84 cm) length of internodes were recorded in comparison with control (8.40 cm) after treatment with sesame cake and neem cake respectively. The highest (406.98 g) and the lowest (360.82 g) fresh weight of 100 leaves of betelvine were recorded in comparison with control (245.34 g) after treatment with neem cake and sesame cake respectively. The highest (18.67 ha) and the lowest (13.77 ha) yield/ha were recorded in comparison with control (6.26 ha) after treatment with neem cake and mustard cake respectively. More or less similar and statistically non-significant results are recorded in rest of the treatments in this experiment.

In year (2011-12), the highest (53.31%) and the lowest (47.58%) disease incidence were recorded in comparison with control (66.03%) after treatment with sesame cake and neem cake respectively. The highest (231.60 cm) and the lowest (214.70 cm) plant height were recorded in comparison with control (199.30 cm) after treatment with neem cake and sesame cake respectively. The highest (2.71 cm) and the lowest (2.27 cm) stem girth were recorded in comparison with control (2.02 cm) after treatment with neem cake and sesame cake respectively. The highest (33) and the lowest (27) no. of leaves were recorded in comparison with control (22) after treatment with neem cake and sesame cake respectively. The highest (7.84 cm) and the lowest (6.82 cm) length of internodes were recorded in comparison with control (8.16) after treatment with sesame cake and neem cake respectively. The highest (408.16 g) and the

lowest (365.28 g) fresh weight of 100 leaves were recorded in comparison with control (251.57 g) after treatment with neem cake and sesame cake respectively. The highest (21.89 ha) and the lowest (14.10 ha) yield/ha was recorded in comparison with control (5.27 ha) after treatment with neem cake and sesame cake respectively.

3.12.10 Efficacy of plant extracts in controlling foot-rot disease in field during 2010-11 and 2011-12

Efficacy of plant extracts in controlling foot rot disease in field was tested and results are presented in Table 34 and Plate 25 B. In year (2010-11), the highest (43.05%) and the lowest (39.70%) disease incidence were recorded in comparison with control (64.08%) after treatment with the extract of *S. mahagoni* and *A. sativum* respectively. The highest (224.2 cm) and the lowest (210.6 cm) plant height were recorded in comparison with control (192.8 cm) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (2.46 cm) and the lowest (2.32 cm) stem girth were recorded in comparison with control (1.86 cm) after treatment with the extract of *A. sativum* and *L. inermis* respectively. The highest (29) and the lowest (27) no. of leaves were recorded in comparison with control (21) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (7.86 cm) and the lowest (7.60 cm) length of internodes were recorded in comparison with control (8.61 cm) after treatment with the extract of *S. mahagoni* and *A. sativum* respectively. The highest (313.30 g) and the lowest (265.60 g) fresh weight of 100 leaves were recorded in comparison with control (220.90 g) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (23.62 ha) and the lowest (20.81 ha) yield/ha was recorded in comparison with control (5.72 ha) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively.

In year (2011-12), the highest (43.17%) and the lowest (37.05%) disease incidence were recorded in comparison with control (66.66%) after treatment with the extract of *S. mahagoni* and *A. sativum* respectively. The highest (227.4 cm) and the lowest

(215.6 cm) plant height were recorded in comparison with control (194.0 cm) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (2.55 cm) and the lowest (2.29 cm) stem girth were recorded in comparison with control (2.01 cm) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (30) and the lowest (27) no. of leaves were recorded in comparison with control (23) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (7.75 cm) and the lowest (7.31 cm) length of internodes were recorded in comparison with control (8.31 cm) after treatment with the extract of *S. mahagoni* and *A. sativum* respectively. The highest (320.90 g) and the lowest (275.30 g) fresh weight of 100 leaves were recorded in comparison with control (241.57 g) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively. The highest (25.51 ha) and the lowest (20.93 ha) yield/ha were recorded in comparison with control (5.40 ha) after treatment with the extract of *A. sativum* and *S. mahagoni* respectively.

3.12.11 Efficacy of fungicides in controlling foot-rot disease in field during 2010-11 and 2011-12

Efficacy of fungicides in controlling foot rot disease of betelvine was tested and results are presented in Table 35 and Plate 25 C. In year (2010-11), the highest (34.70%) and the lowest (27.06%) disease incidence were recorded in comparison with control (65.27%) after treatment with Rovral and Score respectively. The highest (222.6 cm) and the lowest (211.0 cm) plant height were recorded in comparison with control (199.3 cm) after treatment with Rovral and score respectively. The highest (2.42 cm) and the lowest (1.87 cm) stem girth were recorded in comparison with control (1.82 cm) after treatment with Rovral and Score respectively. The highest (30) and the lowest (27) no. of leaves were recorded in comparison with control (23) after treatment with Rovral and Score respectively. The highest (7.59 cm) and the lowest (7.27 cm) length of internodes were recorded in comparison with control (8.40 cm) after treatment with Rovral and Score respectively. The highest (370.5 g) and the lowest (305.5 g) fresh

weight of 100 leaves were recorded in comparison with control (295.3 g) after treatment with Rovral and Score respectively. The highest (34.07 ha) and the lowest (23.81 ha) yield/ha were recorded in comparison with control (6.21 ha) after treatment with Rovral and Score respectively. In this experiment Folicure, Hecnazol and Tilt showed more or less similar and better performance to control the foot rot disease of betelvine and improve yield and yield contributing characters of betelvine.

In year (2011-12), the highest (37.11%) and the lowest (26.06%) disease incidence were recorded in comparison with control (63.01%) after treatment with Rovral and Score respectively. The highest (228.4 cm) and the lowest (216.6 cm) plant height were recorded in comparison with control (205.4 cm) after treatment with Rovral and Score respectively. The highest (2.40 cm) and the lowest (1.81 cm) stem girth were recorded in comparison with control (1.82 cm) after treatment with Rovral and Score respectively. The highest (31) and the lowest (28) no. of leaves were recorded in comparison with control (24) after treatment with Rovral and Score respectively. The highest (7.54 cm) and the lowest (7.25 cm) length of internodes were recorded in comparison with control (8.20 cm) after treatment with Rovral and Score respectively. The highest (375.0 g) and the lowest (291.9 g) fresh weight of 100 leaves were recorded in comparison with control (292.5 g) after treatment with Rovral and Score respectively. The highest (33.90 ha) and the lowest (21.77 ha) yield/ha were recorded in comparison with control (8.53 ha) after treatment with Rovral and Score respectively.

3.12.12 Effect of *Trichoderma* isolates in controlling foot-rot disease of betelvine in field condition during 2010-11 and 2011-12

Effect of *Trichoderma* isolates in controlling foot rot disease of betelvine was tested and results are presented in Table 36 and Plate 26 A. In year (2010-11), the highest (38.23%) and the lowest (35.30%) disease incidence were recorded in comparison with control (63.94%) after treatment with Tr-11 and Tr-3

respectively. The highest (226.2 cm) and the lowest (217.5 cm) plant height were recorded in comparison with control (201.0 cm) after treatment with Tr-3 and Tr-13 respectively. The highest (2.65 cm) and the lowest (2.34 cm) stem girth were recorded in comparison with control (1.87 cm) after treatment with Tr-11 and Tr-13 respectively. The highest (32) and the lowest (29) no. of leaves were recorded in comparison with control (25) after treatment with Tr-10 and Tr-11 respectively. The highest (7.21 cm) and the lowest (7.09 cm) length of internodes were recorded in comparison with control (8.36 cm) after treatment with Tr-13 and Tr-11 respectively. The highest (380.5 g) and the lowest (305.8 g) fresh weight of 100 leaves were recorded in comparison with control (298.5 g) after treatment with Tr-11 and Tr-13 respectively. The highest (29.88 ha) and the lowest (26.21 ha) yield/ha were recorded in comparison with control (8.17 ha) after treatment with Tr-11 and Tr-13 respectively.

In year (2011-12), the highest (39.58%) and the lowest (33.71%) disease incidence were recorded in comparison with control (61.96%) after treatment with Tr-13 and Tr-3 respectively. The highest (230.7 cm) and the lowest (220.5 cm) plant height were recorded in comparison with control (207.8 cm) after treatment with Tr-3 and Tr-13 respectively. The highest (2.71 cm) and the lowest (2.36 cm) stem girth were recorded in comparison with control (1.95 cm) after treatment with Tr-3 and Tr-13 respectively. The highest (33) and the lowest (29) no. of leaves were recorded in comparison with control (25) after treatment with Tr-3 and Tr-13 respectively. The highest (7.37 cm) and the lowest (7.12 cm) length of internodes were recorded in comparison with control (8.36 cm) after treatment with Tr-13 and Tr-3 respectively. The highest (385.3 g) and the lowest (310.3 g) fresh weight of 100 leaves were recorded in comparison with control (302.3 g) after treatment with Tr-11 and Tr-13 respectively. The highest (31.70 ha) and the lowest (25.69 ha) yield/ha were recorded in comparison with control (7.17 ha) after treatment with Tr-3 and Tr-13 respectively.

3.12.13 Efficacy of NPK fertilizer in controlling foot-rot disease in field during 2010-11 and 2011-12

Efficacy of NPK fertilizers in controlling foot rot disease was tested and results are presented in Table 37 and Plate 26 B. In year (2010-11), the highest (59.47%) and the lowest (53.91%) disease incidence were recorded in comparison with control (62.37%) after treatment with $N_0P_{100}K_{50}$ and $N_{200}P_{100}K_{100}$ respectively. The highest (223.8 cm) and the lowest (212.6 cm) plant height were recorded in comparison with control (191.4 cm) after treatment with $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (2.51 cm) and the lowest (1.89 cm) stem girth were recorded in comparison with control (1.58 cm) after treatment with $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (29) and the lowest (24) no. of leaves were recorded in comparison with control (20) after treatment with $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (8.38 cm) and the lowest (6.97 cm) length of internodes were recorded in comparison with control (8.52 cm) after treatment with $N_0P_{100}K_{100}$ and $N_{200}P_{100}K_{100}$ respectively. The highest (398.9 g) and the lowest (265.5 g) fresh weight of 100 leaves were recorded in comparison with control (215.0 g) after treatment with $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (16.10 ha) and the lowest (8.83 ha) yield/ha were recorded in comparison with control (6.56 ha) after treatment with $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively.

In year (2011-12), the highest (61.75%) and the lowest (51.30%) disease incidence were recorded in comparison with control (64.16%) after treatment with the fertilizer dose $N_0P_{100}K_{100}$ and $N_{200}P_{100}K_{100}$ respectively. The highest (230.5 cm) and the lowest (210.4 cm) plant height were recorded in comparison with control (197.0 cm) after treatment with the fertilizer dose $N_0P_{100}K_{100}$ and $N_{200}P_{100}K_{100}$ respectively. The highest (2.56 cm) and the lowest (2.06 cm) stem girth were recorded in comparison with control (1.65 cm) after treatment with the fertilizer dose $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (32) and the

lowest (24) no. of leaves were recorded in comparison with control (24) after treatment with the fertilizer dose $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (8.20 cm) and the lowest (7.06 cm) length of internodes were recorded in comparison with control (8.36 cm) after treatment with the fertilizer dose $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (405.8 g) and the lowest (251.6 g) fresh weight of 100 leaves were recorded in comparison with control (213.4 g) after treatment with the fertilizer dose $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively. The highest (17.88 ha) and the lowest (7.89 ha) yield/ha were recorded in comparison with control (5.5 ha) after treatment with the fertilizer dose $N_{200}P_{100}K_{100}$ and $N_0P_{100}K_{100}$ respectively.

3.12.14 Effect of crop rotation on the incidence of foot-rot disease of betelvine

The effect of effect of crop rotation on the incidence of foot rot disease of betelvine which infested with sick soil was tested and results are presented in Table 38. It is indicated from the experiment that in the year (2010-11), the lowest disease incidence (56.35%) was recorded in *Z. mays* cultivated field followed by *S. officinarum* (57.04%) and the highest in *P. betle* (66.97%). In the year (2011-12), the lowest disease incidence (56.23%) was recorded in *A. sativum* followed by *S. officinarum* (55.98%) and the highest in *Piper betle* (69.21%) cultivated field.

3.12.15 Reaction of betelvine cultivars against foot-rot disease

The effect of reaction of betelvine cultivars against foot-rot disease was tested and results are presented in Table 39. It is evident from the experiment that out of eight cultivarss only one cultivar, namely; Sanchi showed moderately resistance reaction (MR), three cultivars namely, Bangla, Rangpuri, Methapan and Mistypan showed highly susceptible (HS). The other three cultivars, namely Chaltaguta, Gachpan and Jhalpan were found to be susceptible (MS) in 2010-

11 and 2011-12 crop seasons. No cultivars showed resistant against foot rot disease of betelvine. In most of the cases, PDI value showed increasing tendency of disease from one crop year to another crop year.

3.12.16 Efficacy of different treatment combinations in the management of foot-rot disease betelvine caused by *S. rolfsii* in field condition during 2010 -2011 and 2011-2012

Efficacies of different treatment combinations in the management of foot rot disease of betelvine were tested and results are presented in Table 40 Plate 27 A. The result revealed that, in crop year (2010-11), the maximum foot and root rot incidence (65.57%) was recorded in untreated control plots. The lowest disease incidence (19.73%) was recorded in treatment T₉ followed by T₈ (26.21%). The third lowest disease incidence (36.09%) was observed in treatment T₇. The highest (426.30 g) and the lowest (314.80 g) fresh weight of 100 leaves were measured in comparison with control (218.60 g) after treatment with T₉ and T₁ respectively. The highest (41.10 lakh/ha/year) and the lowest (10.86 lakh/ha/year) yield/ha were counted in comparison with control (7.09 lakh/ha/year) after treatment with T₉ and T₁ respectively. In the year (2011-12), the highest disease incidence was found in treatment T₁₀ (Control). The lowest disease incidence was observed in treatment T₉ (17.66%) followed by T₈ (28.59%). The third lowest disease incidence was observed in treatment T₇ (33.71%). The highest (431.7 g) and the lowest (299.9 g) fresh weight of 100 leaves were recorded in comparison with control (227.6 g) after treatment with T₉ and T₁ respectively. The highest (41.10 lakh/ha/year) and the lowest (9.88 lakh/ha/year) yield/ha were recorded in comparison with control (5.76 lakh/ha/year) after treatment with T₉ and T₁. Statistically no much significant difference of disease incidence was observed during 2010-11 and 2011-12 cropping year but significant positive difference was noted in yield may be due to management of betelvine plantation.

PLATE 24**A****B****C****D**

A- Susceptible stage of betelvine B- Soil solarization technique for new betelvine plantation, C- Deep ploughing system for new betelvine plantation, D-Earthing up soil in borj to avoid soil infested pathogen,

PLATE 25



A



B



C

A- Treatment with organic amended cake, B-Treatment with Plant extract, C-Treatment with fungicides

Plate 26



A



B

A-Treatment with *Trichoderma* spp., B- Treatment with NPK

PLATE 27



A

A= Integrated management with different types treatment

Table 25. Effect of inoculums level of *S .rolfsii* on incidence of foot-rot of betelvine.

Inoculum levels (%)	Disease incidence* (%)
1	34.23 (35.79)
2	63.76 (53.55)
3	74.48 (59.67)
4	92.66 (74.32)
5	94.76 (76.82)
6	96.33 (78.91)
7	98.45 (82.73)
8	100.00 (90.00)
9	100.00 (90.00)
10	100.00 (90.00)
LSD (0.05)	1.617

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 26. Identification of the susceptible stage of betelvine against foot-rot disease of betelvine.

Tr. No.	Treatments	Disease incidence*
T ₁	03 months old crop	94.55 (76.44)
T ₂	06 months old crop	88.96 (70.54)
T ₃	12 months old crop	83.08 (65.73)
T ₄	18 months old crop	80.22 (63.58)
T ₅	24 months old crop	76.87 (61.27)
LSD (0.05)		0.02267

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 27. Effect of soil solarization on *S. rolfsii* with plastic mulch after 30 days of incubation.

Treatment	Soil temperature average(°C)	Per cent of viability*
5 cm depth without mulching	36.3	86.33
5 cm depth with clear plastic mulch	48.8	75.58
5 cm depth with black plastic mulch	52.4	28.27
10 cm depth without mulching	33.6	88.66
10 cm depth with clear plastic mulch	42.5	80.42
10 cm depth with black plastic mulch	45.6	78.67
15 cm depth without mulching	32.8	89.15
15 cm depth with clear plastic mulch	37.2	83.87
15 cm depth with black plastic mulch	40.6	81.03
Air temperature	36.5	
LSD (0.05)		0.6273

*Average of three replications

Table 28. Effect of solarization on foot-rot disease incidence of betelvine *S. rolfsii* infested pot soil.

Treat. No.	Treatment	Disease incidence*	
		2010-11	2011-12
T ₁	10 days solar heating	79.07 (62.80)	81.66 (64.60)
T ₂	20 days solar heating	77.74 (61.82)	80.24 (63.58)
T ₃	30 days solar heating	75.80 (60.53)	74.50 (59.67)
T ₄	40 days solar heating	73.34 (58.89)	72.33 (58.24)
T ₅	50 days solar heating	70.76 (57.29)	69.35 (56.35)
T ₆	60 days solar heating	66.15 (54.39)	64.22 (53.25)
T ₇	Control	84.26 (66.66)	86.45 (68.36)
	LSD (0.05)	0.01998	0.01898

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 29. Effect of amendment soil on germination of sclerotia of *S. rolfsii* at different intervals (42 days).

Treatment	Germination (%) at different intervals (days)*					
	7	14	21	28	35	42
Ash	95.66	82.45	75.35	66.42	60.24	55.24
Vermicompost	92.35	87.16	71.24	54.58	46.48	32.18
Cowdung	96.45	88.56	79.80	68.44	57.34	47.23
Sesame cake	78.46	71.56	67.21	60.67	56.79	29.72
Mustard oil cake	77.08	70.63	65.65	62.72	58.05	37.54
Neem cake	73.57	63.44	60.67	56.17	51.94	16.39
Control	100	100	100	98	97	95
LSD (0.05)	0.0171	0.0401	0.0127	0.0908	0.0470	0.0674

*Average of three replications

Table 30. Viability of growth agar disks of *S. rolfsii* as affected with hot water temperatures and exposure time (minute).

Hot water °C	Exposure times (minutes) *						
	1	5	10	15	20	25	30
25	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+
51	+	+	+	+	+	+	+
52	+	+	+	+	+	+	-
53	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-

(+), mean viable sclerotia, (-), mean non viable sclerotia

*Average of three replications

Table 31. Effect of different degrees hot water treatment at rate of 0.5 L/kg soil on foot-rot disease incidence of betelvine with *S. rolfsii* infested pot soil during 2010-11 and 2011-12 cropping seasons.

Hot water (°C)	Soil temperature(°C)	Disease incidence*	
		2010-11	2011-12
65	53	46.74 (43.11)	42.24 (40.51)
70	59	38.80 (38.53)	35.50 (36.57)
80	68	22.34 (28.18)	18.33 (25.33)
90	77	16.76 (24.20)	12.35 (20.53)
100	84	9.15 (17.56)	10.22 (18.63)
Control	28	86.26 (68.28)	89.45 (71.00)
LSD (0.05)		1.3271	0.8577

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 32. Effect of cultural practice on the incidence of foot-rot disease of betelvine on *S. rolfsii* sick soil.

Treatment	Disease incidence*	
	2010-11	2011-2012
Deep ploughing	78.57 (62.64)	79.17 (62.87)
Deep ploughing + solarization	68.16 (55.67)	64.94 (53.67)
Hot water 0.5 Lit/kg (100°C)	19.66 (26.35)	13.38 (20.62)
Earthing-up soil 25 cm height one time/year	75.40 (60.27)	75.27 (60.20)
Earthing-up soil 25 cm height two time/year	71.38 (57.67)	74.14 (59.41)
Control	81.54 (64.52)	86.26 (68.28)
LSD (0.05)	0.0208	0.0210

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 33. Effect of organic amendment on incidence of foot-rot disease of betelvine in field during 2010-2011 and 2011-12

Treatment	Plant Height (cm)*		Stem Girth (cm)*		Length Internodes (cm)*		No. of leaves/plant*		Fresh weight of 100 leaves (g)*		Yield*/ lakh/ ha/ Year		Disease incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Mustard cake	219.60c	226.40b	2.42 b	2.49 b	7.54 c	7.21 c	28c	30bc	382.45b	385.38b	13.77 d	16.25 c	66.04b (54.33)	64.16 b (53.25)
Vermicompost	224.80b	223.40b	2.43 b	2.66 a	7.14 d	7.14 c	31ab	31b	398.77a	405.99a	17.63 b	18.93 b	62.34b (52.12)	56.45 c (48.68)
Neem cake	228.10a	231.60a	2.55 a	2.71 a	6.84 e	6.82 d	32 a	33a	406.98a	408.16a	18.67 a	21.89 a	63.46b (52.83)	54.49 c (47.58)
Sesame cake	221.40c	214.70c	2.33 b	2.27 c	7.76 b	7.84 b	29bc	27c	360.82c	365.28c	15.56 c	14.10 d	60.77b (51.24)	64.27 b (53.31)
Control	203.10d	199.30d	2.01 c	2.02 d	8.40 a	8.16 a	24d	22d	245.34d	251.57d	6.26 e	5.27 e	80.58a (63.87)	83.53 a (66.03)
LSD (0.05)	2.434	3.846	0.1014	0.1434	0.2028	0.1756	2.41	1.93	10.70	8.120	0.9068	0.6414	6.383	2.217

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 34. Efficacy of plant extracts on controlling foot-rot disease of betelvine in field during 2010-11 and 2011-12

Treatment	Plant Height (cm)*		Stem Girth (cm)*		Length Internodes (cm)*		No. of leaves/plant*		Fresh weight of 100 leaves (gm)*		Yield*/ lak/ ha/year		Disease incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
<i>Swietenia mahagoni</i>	210.6 c	215.6 b	2.39 ab	2.29 c	7.72bc	7.75 b	27ab	27c	265.60d	275.30d	20.81 d	20.93 c	46.57 b (43.05)	46.80 c (43.17)
<i>Azadirachata indica</i>	213.5 c	221.6 b	2.34 b	2.32 c	7.62c	7.55 c	27ab	28b	283.80b	290.90b	22.24 c	23.45 b	44.59 c (41.90)	41.64 d (40.16)
<i>Lawsonia inermis</i>	219.1 b	220.0 a	2.32 b	2.40 b	7.86c	7.71 b	28b	28b	272.90a	282.88c	22.73a	23.51 b	41.35 d (39.99)	42.63 e (40.74)
<i>Allium sativum</i>	224.2 a	227.4 c	2.46 a	2.55 a	7.60b	7.31 d	29a	30a	313.30c	320.90a	23.62 b	25.51 a	40.78 e (39.70)	36.33 b (37.05)
Control	192.8 d	194.0 d	1.86 c	2.01 d	8.61a	8.31 a	21c	23d	220.90e	241.57e	5.72 e	5.40 d	80.98 a (64.08)	84.30 a (66.66)
LSD (0.05)	4.048	3.280	0.1242	0.0716	0.2028	0.1014	2.03	0.50	4.895	5.370	0.3926	0.3206	0.5599	0.4180

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 35. Efficacy of fungicides in controlling foot-rot disease in field condition during 2010-11 and 2011-12

Treatment	Plant Height (cm)*		Stem Girth (cm)*		Length Internodes (cm)*		No. of leaves/plant*		Fresh weight of 100 leaves (gm)*		Yield*/ lakh/ ha/year		Disease incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Rovral	211.0d	216.6e	1.87c	1.81d	7.59 b	7.54 b	27e	28e	305.5 e	291.9	23.81d	21.77d	32.42b (34.70)	36.42b (37.11)
Tilt	214.2c	220.5c	2.20b	2.13c	7.51 b	7.44 c	28d	29d	309.6 d	320.0	28.21c	28.43c	25.50d (30.33)	30.28d (33.40)
Hecanazol	213.5c	218.7d	2.22b	2.21bc	7.35 c	7.35 cd	28c	29c	315.4 c	326.4	28.41c	28.83c	27.80c (31.82)	31.77c (34.27)
Folicur	217.4b	224.5b	2.24ab	2.36ab	7.35 c	7.30 de	29b	30b	331.8 b	338.3	32.69b	33.58a	20.67f (27.06)	25.36e (30.26)
Score	222.6a	228.4a	2.42a	2.40a	7.27 c	7.25e	30a	31a	370.5 a	375.0	34.07a	33.90b	23.52e (29.00)	19.32f (26.06)
Control	199.3e	205.4f	1.82c	1.82d	8.40 a	8.20a	23f	24f	295.3 f	292.5	6.21e	8.53e	82.50a (65.27)	79.37a (63.01)
LSD (0.05)	1.302	0.5433	0.1756	0.1484	0.1484	0.9386	0.942	0.18	2.237	9.844	0.4250	0.4250	0.3252	0.4967

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 36. Effect of *Trichoderma* isolates in controlling foot-rot disease of betelvine in field during 2010-11 and 2011-12.

Treatment	Plant Height (cm)*		Stem Girth (cm)*		Length Internodes (cm)*		No. of leaves/plant*		Fresh weight of 100 leaves (gm)*		Yield*/ lakh/ ha/year		Disease incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Tr-11	223.4 b	230.7a	2.51ab	2.50b	7.12 b	7.13c	29a	31ab	346.2 c	355.1 c	27.64ab	30.42a	38.36 b (38.23)	32.21 c (34.57)
Tr-10	218.5 c	224.4b	2.53ab	2.58b	7.21 b	7.17c	29a	29bc	364.8 b	370.5 b	28.63ab	26.61b	38.22 b (38.17)	37.91 b (38.00)
Tr-3	226.2 a	220.5c	2.65a	2.71a	7.09 b	7.12c	31a	33a	380.5 a	385.3 a	29.88a	31.70a	33.36 c (35.30)	30.85 c (33.71)
Tr-13	217.5 c	224.5b	2.34b	2.36c	7.14 b	7.37b	32a	29ab	305.8 d	310.3 d	26.21b	25.69b	34.16 c (35.79)	40.63 b (39.58)
Control	201.0 d	207.8d	1.87c	1.95d	8.36 a	8.36a	25b	25c	298.5 e	302.3 d	8.17c	7.17c	80.67 a (63.94)	77.89 a (61.96)
LSD (0.05)	2.378	1.898	0.1897	0.1242	0.1897	0.0716	3.16	3.69	6.682	10.42	2.941	1.784	3.900	4.427

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 37. Efficacy of NPK fertilizer in controlling foot-rot disease in field during 2011-12 and 2011-12

Treatment	Plant Height (cm)*		Stem Girth (cm)*		Length Internodes (cm)*		No. of leaves/plant*		Fresh weight of 100 leaves (gm)*		Yield*/ lakh/ ha/year		Disease incidence*	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
N ₀ P ₁₀₀ K ₁₀₀	212.6 c	210.4e	1.89f	2.06d	8.38 b	8.20 a	24b	24d	265.5 f	251..6 f	8.83h	7.89h	74.17b (59.47)	77.64b (61.75)
N ₁₀₀ P ₁₀₀ K ₁₀₀	215.1bc	218.4d	2.12e	2.25bc	7.49 d	7.78 b	26ab	24d	289.7 e	305..7 e	11.60e	9.41f	70.33de (56.98)	74.35cd (59.54)
N ₁₅₀ P ₁₀₀ K ₁₀₀	215.6abc	225.7b	2.27b	2.35b	7.01 e	7.10 ef	28ab	30ab	380.4 ab	377..3 b	13.52b	12.36c	69.33ef (56.35)	71.48e (57.73)
N ₂₀₀ P ₁₀₀ K ₁₀₀	223.8a	212.5e	2.51a	2.56a	6.97 e	7.06 r	29a	32a	398.9 a	405..8 a	16.10a	17.88a	65.33g (53.91)	60.99 f (51.30)
N ₂₀₀ P ₀ K ₁₀₀	219.8abc	230.5a	2.20cd	2.13cd	7.57 d	7.60 bc	29a	27c	384.3 a	366..3 c	10.89f	9.20g	71.83cd (57.92)	75.51c (60.33)
N ₂₀₀ P ₅₀ K ₁₀₀	221.3ab	222.2c	2.28b	2.23bc	7.51 d	7.27de	28ab	29bc	343.8 d	375.9 b	12.81d	12.13d	69.65def (56.54)	71.39e (57.67)
N ₂₀₀ P ₁₀₀ K ₀	213.8bc	217.4d	2.23bc	2.18cd	7.72 c	7.43 cd	24b	27c	359.6 c	345.4 d	9.88g	9.78e	72.57bc (58.44)	72.89de (58.33)
N ₂₀₀ P ₁₀₀ K ₅₀	216.4abc	224.4bc	2.15de	2.23bc	7.71 c	7.56 c	25ab	28c	370.8 bc	376.3 b	13.15c	14.48d	67.80f (55.43)	61.86f (51.88)
N ₀ P ₀ K ₀	191.4d	197.0f	1.58g	1.65e	8.52 a	8.36 a	20c	24d	215.0 g	213.4 g	6.56i	5.52i	78.54a (62.37)	81.04a (64.16)
LSD (0.05)	7.931	2.708	0.595	0.1191	0.08420	0.1883	3.70	2.14	11.93	9.382	0.1458	0.1883	2.143	1.737

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 38. Effect of crop rotation on the incidence of foot-rot diseases of betelvine.

Crops	Disease incidence*	
	2010-11	2011-12
<i>Zea mays</i> L.	69.34 (56.35)	73.06 (58.76)
<i>Solanum tuberosum</i> L.	75.82 (60.53)	78.26 (62.24)
<i>Saccharum officinarum</i>	70.38 (57.04)	68.66 (55.98)
<i>Allium sativum</i>	71.76 (57.92)	69.12 (56.23)
<i>Allium cepa</i>	74.17 (59.47)	73.79 (59.21)
<i>Triticum aestivum</i> L.	77.47 (61.68)	81.97 (64.90)
<i>Piper betle</i> L.	84.67 (66.97)	87.41 (69.21)
LSD (0.05)	0.01967	0.01998

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 39. Reaction of betelvine cultivars against foot-rot disease of betelvine.

Cultivars	PDI value and Reaction*				Final grading
	2010-11		2011-12		
	PDI	Reaction	PDI	Reaction	
Bangla	78.34 (62.24)	HS	83.68 (66.19)	HS	HS
Chaltaguta	36.17 (36.99)	MS	32.72 (34.88)	MS	MS
Rangpuri	67.26 (55.12)	HS	74.91 (59.93)	HS	HS
Methapan	72.06 (58.12)	HS	81.57 (64.60)	HS	HS
Sanchi	16.35 (23.81)	MR	17.44 (24.65)	MR	MR
Gachpan	38.60 (38.41)	MS	33.66 (35.49)	MS	MS
Jhalpan	34.25 (35.79)	MS	32.86 (34.94)	MS	MS
Mistypan	75.73 (60.47)	HS	79.16 (62.87)	HS	HS
LSD (0.05)	0.01931		0.3957		

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

Table 40. Efficacy of different treatment combinations in the management of foot-rot disease betelvine caused by *S. rolfsii* in field condition during 2010 -2011 and 2011-2012.

Tr. No.	Treatments	Disease incidence*		Yield* lakh/ ha/year		Fresh weight of 100- leaves(gm)*	
		2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
T ₁	Sanitation	72.69b (58.50)	77.88b (61.96)	10.86g	9.88e	314.8h	299.9h
T ₂	Neem cake 6t/h	61.58c (51.71)	55.06c (47.93)	31.84d	32.24c	396.4d	410.9d
T ₃	<i>A. sativum</i> extract 5%	43.12e (41.03)	35.46f (36.57)	24.83f	30.39c	321.1g	327.5g
T ₄	Application Tr-3	37.94f (38.00)	32.08f (34.51)	29.57e	31.62c	383.4e	386.0e
T ₅	Score 1.0% drenching	24.25g (29.53)	19.13h (25.92)	33.63c	36.90b	375.8f	362.6f
T ₆	T ₁ +T ₂	49.61d (44.77)	51.01d (45.57)	23.30f	21.39d	406.3c	416.5c
T ₇	T ₁ +T ₂ +T ₃	34.75f (36.09)	30.76f (33.71)	30.04e	31.57c	411.6c	422.2b
T ₈	T ₁ +T ₂ +T ₃ +T ₄	19.51h (26.21)	22.95g (28.59)	38.49b	35.41b	419.2b	427.4ab
T ₉	T ₁ +T ₂ +T ₃ +T ₄ +T ₅	11.45i (19.73)	9.16i (17.66)	41.16a	41.10a	426.3a	431.7a
T ₁₀	Control	82.87a (65.57)	85.19a (67.37)	7.09h	5.76f	218.6i	227.6i
	LSD (0.05)	3.567	1.877	1.658	1.905	5.928	5.303

*Average of three replications, Figure in parentheses are the angular transformed values of per cent disease incidence.

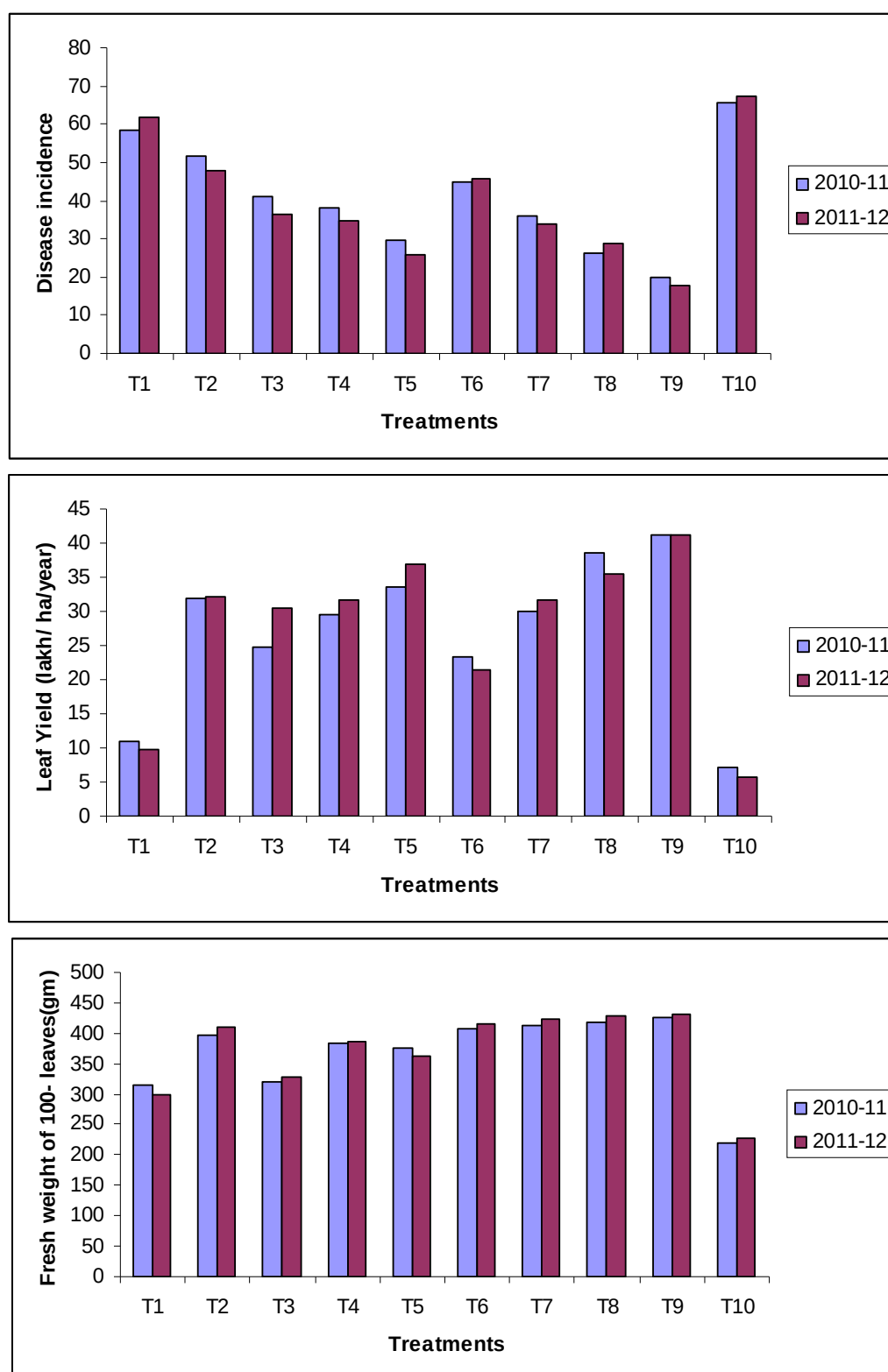


Figure 5. Efficacy of different treatment combinations in the management of foot-rot disease betelvine caused by *S. rolfsii* in field condition during 2010 -2011 and 2011-2012

T₁ = Sanitation, T₂ = Neem cake 6t/h, T₃ = *A. sativum* extract 5%, T₄ = Application Tr-3, T₅ = Score 1.0% drenching T₆ = T₁+T₂, T₇ = T₁+T₂+T₃, T₈=T₁+T₂+T₃+T₄ T₉ = T₁+T₂+T₃+T₄+T₅, T₁₀= Control

Chapter Four

DISCUSSION

Foot-rot disease of betelvine is a serious fungal disease which is caused by *Sclerotium rolfsii* in Bangladesh and betelvine growing countries. During the survey period, the disease was recorded from different areas of Bangladesh from June 2010 to December 2012. It is evident from the survey that the highest and the lowest disease incidence of betelvine were showed in Rajshahi and Pirozpur district. Hossain *et al.* (1985) recorded that in a survey of betelvine (*P. betel*) gardens at Babuganj and Gournadi areas of Barisal district, four severe diseases were recorded: leaf spot caused by *C. capsici*; leaf rot caused by *P. palmivora*; stem rot caused by *S. rolfsii*; and foot rot caused by *Fusarium* spp. *Colletotrichum* leaf spot was found to be widely distributed, deteriorating the quality and quantity of betel leaf, and reducing the market value. The extents of losses vary from 5 to 90 per cent (Dasgupta and Sen 1999). Mrida and Alamgir (1987) stated that four diseases of betelvine (*P. betle* L.) were recorded during the survey of betelvine growing areas of Chittagong district of Bangladesh. They were leaf rot, foot rot, stem rot and leaf spot caused by *Phytophthora parasitica*, *S. rolfsii*, *Fusarium* sp. and *C. piperis* respectively. Among the recorded diseases foot rot caused by *S. rolfsii* was widespread and damaging. Maiti and Sen (1982) surveyed of *Piper betle* plantations in 3 districts of West Bengal, India and showed major diseases was foot rot (*S. rolfsii*) may cause losses of up to 25-90%. Palakshappa *et al.* (1986) surveyed the incidence of *S. rolfsii* on *Piper betle* in different areas of Karnataka state during 1984-85 and recorded 35 to 39 per cent disease incidence.

In both the year, during the study period the highest and the lowest disease incidence of betelvine were showed in Bangla and Sanchi cultivars of betelvine. Rao (1996) mentioned that during periodical surveys of the crop betelvine, a foot

and leaf rot was consistently observed in some plantations around Bangalore, India. The incidence of the foot rot phase was recorded under natural conditions on 36 varieties of *P. betle*, belonging to 4 of the 5 morphological groups. The results suggested that variety Awamipan under Bangla group was resistant to foot rot phase and other varieties in all 4 groups showed varying degrees of susceptibility to the disease. The variety Halisaharsanchi was found to be highly susceptible to this pathogen. This is the first report of *P. capsici* causing foot and leaf rot of *P. betle* in India.

Soil type played important role in causing disease incidence. It was observed that during survey period, loamy soil boroj showed the highest disease incidence and sandy loamy soil boroj showed the lowest disease incidence. Soil texture may also affect survival of sclerotia (Merriman, 1976). Alexander and Stewart (1994) showed more rapid sclerotial degradation and lower sclerotia survival in higher clay content soil and this could have resulted from higher water retention and higher levels of microbial activity in clay soil. Kataria and Grover (1987) reported that the disease incidence was higher in adequately moist sandy loam and less in soil under moisture stress. Incidence of cowpea seedling rot was higher in heavy-textured loam and silt loam soils than in light-textured sandy and loamy sand.

In this study, it was observed that irrigation system played important role on disease development. The highest and the lowest disease incidence of betelvine were observed in flood and drip system irrigated borojes. Goldmayer *et al.* (2002) obtained that when compared with a traditional flood irrigation system, the buried drip system reduced orchard humidity and dew duration and increased temperature. This significantly reduced leaf symptoms of *Alternaria* late blight of pistachios. Using buried drip irrigation to control fungal diseases may also be possible in other orchard crops where irrigation-related high humidity has been identified as the primary factor contributing to the disease (Teviotdale *et al.*, 2001).

It is evident that high incidence of foot rot was detected during the months of July, August and September. It started from June and gradually increased till August of each year under observation. From the first fortnight of October, the severity of infection decreased gradually and continued till second fortnight of May. Data showed the relationship between different meteorological factors and disease. It was seen from data that the disease was maximum during June and September in both the years. Disease peaks were always preceded by heavy and optimum rainfall conditions. A positive correlation existed between the disease incidence and the weather factors like rainfall, number of rainy days and relative humidity. It is known that the effects of the individual meteorological factors on the disease occurrence are only relative and the unfavorable effect of any factor may be compensated for by the highly favorable factors (Rotem, Cohen and Putter, 1971). Sindhan and Bose (1980) showed that atmospheric temperature and humidity are important meteorological factors for development of different diseases and increase in relative humidity due to rainfall enhances initiation of diseases. From the present study, it is apparent that foot rot of betelvine required adequate more temperature and relative humidity for its development compared to leaf spot. It was observed that starting from first fortnight of December to second fortnight of December there was no rainfall. Under this environmental condition, severity of foot rot was lowest. So, it may be concluded that rainfall is an important factor for spreading foot rot disease. Meteorological parameters like atmospheric temperature, relative humidity and rainfall are congenial for the disease (Marimathu and Rabindran, 1988). Ramachandra *et al.* (1988) studied the effect of climatic factors on the occurrence of foliar infection in black pepper caused by *Phytophthora palmivora*. Maiti and Sen (1982) found that foot rot was more related to mean min. temp. ($> 22^{\circ}\text{C}$) than to rainfall, the leaf diseases to mean av. daily rainfall of 6 mm or above accompanied by moderate to high temperatures. The results concluded that with the increase of temperature, relative humidity and rainfall the foot rot disease incidence increased maximum in the month of August and lowest disease incidence showed in the month of December. We forecast farmer to epidemic situation.

In this study ten number isolates have been isolated from different areas of Bangladesh. The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, and sclerotial size and colour. Among ten isolates, S-5 showed the highest mycelial growth, dry mycelial weight, sclerotial size, sclerotial bodies/cm² and weight of 100 sclerotial bodies. Sarma *et al.* (2002) observed that variability among 26 isolates of *Sclerotium rolfsii* collected from various hosts/soil samples and localities in India is reported. Akram *et al.* (2007) reported morphological variability and mycelial incompatibility among twelve *S. rolfsii* isolates collected from various localities of chickpea growing areas of Punjab. Kokub *et al.* (2007) observed considerable variation in the growth rate of eight *S. rolfsii* isolates and mycelial incompatibility.

Pathogenicity depended on virulency of isolates. All isolates were not equally virulent to occur disease incidence. Isolate, S-5 showed the highest disease incidence and S-7 showed the lowest disease incidence in this experiment.

Carbon sources influence greatly on the mycelial growth of *S. rolfsii*. Among the tested eight carbon sources, the highest per cent mycelial growth was observed in cellulose and the lowest in glycerol. Hussain (2003) observed that starch was the best carbon sources for growth of *S. rolfsii*. The fungus may utilize certain simple form of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al.*, 1970). Ramprasad (2005) studied that *S. rolfsii* fungus grew well utilizing potassium nitrate (323.67 mg dry mycelial weight) followed by ammonium nitrate (219.33 mg dry mycelial weight). This was similar to the findings of Xiao *et al.*, (2012).

Nitrogen sources influence greatly on the mycelial growth of *S. rolfsii*. The highest per cent mycelial growth was observed in sodium nitrate and the lowest in calcium nitrate. Khattabi *et al.* (2004) reported the effect of urea, sulfate ammonium, nitrate potassium and horse manure on *S. rolfsii* was tested *in vitro*, tests on liquid culture media showed that *S. rolfsii* did not utilize urea as a source of nitrogen while

sulfate ammonium and nitrate potassium allowed the growth of the fungus. On a solid medium, the fertilizers at rates of $12 \text{ g N}\cdot\text{m}^{-2}$ and $18 \text{ g N}\cdot\text{m}^{-2}$ had an inhibitory effect on the growth of *S. rolfsii* mycelium. The highest effect was observed for urea. Hussain *et al.* (2003) showed that peptone was found to be the best source of nitrogen for *S. rolfsii*. It was followed by Potassium nitrate. Basamma (2008) stated that potassium nitrate and peptone recorded the maximum mycelial growth of *S. rolfsii*. Nitrogen being a component of protein is an essential element and like carbon, it is used by fungi for functional as well as structural purposes. But all the sources of nitrogen are not equally good for the growth of fungi.

Phytohormones influenced greatly on the inhibition mycelial growth of *S. rolfsii*. 2. 4-D hormones inhibited hundred per cent mycelial growth of the fungus at all concentrations. IAA and IBA inhibited mycelial growth hundred per cent at 500 ppm concentrations respectively. Alam *et al.* (2004) studied that four phytohormone e.g I.A.A, N.A.A, 2.4-D and BAP were used to find out the role over mycelial growth of *C. gloeosporioides*. All the concentrations of BAP hormones have good inhibitory effect against mycelial growth of fungus. Some investigations indicated that Naphtholene acetic acid (NAA) is a potential antifungal agent (Nakamura *et al.*, 1978; Tomita *et al.*, 1984). Auxin strongly inhibited mycelial growth, sporulation and spore germination of *Fusarium culmorum in vitro* (Michniewicz and Rozej, 1987). Michniewicz and Rozej, 1987 and Melinda and Stevenson (1991) have pointed out that auxins act as a fungal growth and development controlling factor. NAA increase the resistance of potato plants to early blight (Melinda and Stevenson, 1991).

Oil had an inhibitory effect on the mycelial growth of *S. rolfsii*. Hundred per cent mycelial growths inhibited at all concentrations at clove oil and cinnamon oil. Hamad *et al.* (2015) showed that fungitoxic activity of eleven different oils was tested by the poisoned food technique using potato dextrose agar medium at six different concentrations on radial growth of guava decline pathogenic fungi named *R. solani*; *B. theobromae* and *F. oxysporum*. All tested volatile oils showed toxicity

to the three decline fungi. The highest inhibition of mycelial radial growth was 78.44% in case of clove oil. In the case of *B. theobromae*, the linear growth was completely inhibited by the clove and judean wormwood oils at concentration of 250 ppm. Singh and Dwivedi (1989) stated that, the viability of sclerotia was reduced when treated with neem oil. Huv *et al.* (2000) showed that *Eucalyptus unigera* oil inhibited mycelial growth of three phytopathogenic fungi such as *C. gloeosporioides*, *R. solani* and *Pythium* spp.

Neem cake at 3% concentration completely inhibited mycelium growth of the fungus. Dubey (1998) showed that oil cake inhibit about 86% of mycelial growth followed by Karanj cake 83% and Neem cake 82%.

Fertilizer had an inhibitory effect on the mycelial growth of *S. rolfsii*. Hundred per cent mycelial growth was inhibited with urea fertilizer at all concentrations. Zipsum at 3% concentration completely inhibited mycelial growth of the fungus. Khattabi *et al.* (2004) observed that the effect of urea, sulfate ammonium, nitrate potassium and horse manure on *S. rolfsii* was tested *in vitro*, tests on liquid culture media showed that *S. rolfsii* did not utilize urea as a source of nitrogen. On a solid medium, the fertilizers at rates of 12 g N·m⁻² and 18 g N·m⁻² had an inhibitory effect on the growth of *S. rolfsii* mycelium.

Cowdung and cow urine were tested and showed inhibitory effect on mycelial growth of the fungus. In PDA medium, cow urine and cowdung inhibited mycelial growth respectively at 25% concentrations. Amin *et al.* (2013) found that no growth of *S. rolfsii* was observed at 70%, 60%, 50%, 40% concentration of cow's urine alone. Kannan *et al.* (2007) observed that in pot and field studies, foliar spray of combined application of buffalo urine and sheep urine (1:1 v/v) at 5 per cent concentration on peanut have completely inhibited the mycelial growth, production and germination of sclerotia of *Sclerotium rolfsii* when compared to control and chemical fungicide, mancozeb (0.05%). Basak and Lee (2001a) reported that cow dung is very effective for controlling *Fusarium* wilt of

cucumber. In another study, Basak and Lee (2001b) observed that cow urine and cow dung are capable of suppressing conidial germination and mycelial growth of *F. oxysporum* f. sp. *cucumerinum* causing *Fusarium* wilt of the crop.

The highest per cent mycelial growth inhibition was observed in nicotinic acid followed by pyridoxine and the lowest was in folic acid. Ahn *et al.* (2005) demonstrated that thiamine induces SAR and vitamin B1 functions as an activator of plant disease resistance. They described that thiamine treated rice, *A. thaliana* and vegetable crops showed resistance to fungal, bacterial and viral infections.

Complete inhibition mycelial growth of the fungus was observed in NaHCO_3 and NH_4HCO_3 at all concentrations. Erper *et al.* (2011) observed that the mycelial growth of *S. sclerotium* significantly reduced with the increasing concentrations of KHCO_3 . Mycelial growth of the fungus was inhibited especially at concentrations greater than 25 mM. Moreover, it completely inhibited when exposed to 100 mM bicarbonate. Mrida and Alamgir reported (1987) that a preliminary observation on the control of foot rot of betelvine caused by *S. rolfsii* showed that ammonia solution of 0.125% and 0.5% gave effective control in soil.

Indiscriminate use of the fungicides has been cautioned as some of these are found to have various harmful residual effects on the surrounding environment. Some researchers have already demonstrated the successful use of extracts of plants against some important disease of crop plants. Thus an attempt was made to evaluate some available plant extracts from different parts of plant with a view to comparing their value as fungitoxicants against *S. rolfsii*. Out of eighteen plant extracts, complete inhibition of mycelial growth was observed with *A. sativum* and *L. inermis* extracts at all concentrations. The minimum inhibition was showed by *C. dactylon* extracts. Singh and Dwivedi (1989) noticed that, leaf extracts of twelve plants were found toxic against *S. rolfsii*. Jalal and Ghaffar (1992) studied antifungal characteristics of *Ocimum sanctum* L. and found that its leaf extract

completely inhibited the growth of *S. rolfsii* and other fungi. On the basis of present study, it is concluded that leaf extract of the plants which have been found effective against collar rot can be recommended against the disease after in field trials. Sivakadacham (1988) reported that, leaf extracts of *Adathoda vasica* and *Cullen corylifolium* suppressed the mycelial growth of *Sclerotium rolfsii*. Further, they also observed the morphological changes and significant reduction in mycelial growth of *S. rolfsii*. However, root extract of *Parthenium hysterophorus* L. exhibited maximum inhibition of mycelial growth of *S. rolfsii*.

Dried plant extract inhibited mycelial growth of the fungus. Hundred per cent mycelial growth inhibition was observed in *A. sativum* and *L. inermis* treated plant extracts at all concentrations. The minimum inhibition was showed by *C. asiatica* extracts. Farook *et al.*, (2010) observed that maximum inhibition was recorded by *Azadirachta indica* (73.8%) followed by *Cassia fistula* (73.5%) and *Cannabis sativa* (67.1%) mycelial growth of *S. rolfsii*.

Dried ethanol plant extract inhibited mycelial growth of the fungus *S. rolfsii*. Eight ethanol plant extract screened for their antifungal activity. In this experiment, among the tested eight plant extract, it was observed that hundred per cent inhibition was observed in *A. sativum*. The minimum inhibition was showed by *S. aromaticum* extract. Venkateswarlu and Sreeramulu (2013) showed that 2% of *Nyctanthes arbor-tristis* was found effectively control mycelial growth of *S. rolfsii*.

Out of twelve fungicides tested, two were the most effective against *S. rolfsii*. Complete mycelial growth inhibition was observed in folicure and score at all concentrations. Rovrol and Tilt showed complete inhibition at 250 ppm concentration and Hecnazol at 500 ppm concentration. Several reports on the management of collar rot disease were reported earlier. Vyas and Joshi (1977) studied that carboxin was highly effective on *Sclerotium rolfsii*. Siddaramaiah *et al.* (1979) found that, calixin (Triademorph) completely inhibited the mycelial growth of *Sclerotium rolfsii* under *in vitro* condition. They also found the inhibition of

sclerotial germination by Bayletan and Panoram at 100 ppm. Effectiveness of plantavax and vitavax was recorded in inhibiting the vegetative growth of *S. rolfsii* while working with foot rot of various crop plants (Palakashppa, 1986). Harlapura (1988) showed the efficacy of thiram in inhibiting the growth of *S. rolfsii*, the casual agent of foot rot of wheat. Further, Harlapur (1988) noticed the complete inhibition of mycelial growth of *Sclerotium rolfsii* by agallol and Diathans M-45. Propiconazole was found highly effective in inhibiting the mycelial growth of *Sclerotium rolfsii* (Hagans *et al.*, 1991). Hagans *et al.* (1991) also reported the efficacy of tebuconazole, diniconazole and flutatanil against *S. rolfsii* a causal organism of stem rot of peanut. Complete inhibition of tuberosc isolate of *S. rolfsii* at 100 ppm concentration was reported by Das and Panda (1997). Ikra and Javaid reported (2015) that the inhibitory effect of the fungicide Thiophanate Methyl was ranging from 91-100% over control. The concentration 150 ppm and higher completely controlled the fungal growth.

Herbicides were tested and showed inhibitory effect on fungal mycelial growth. Logran and 2, 4-D at all concentration complete inhibition mycelial growth of the fungus. Growth of *Sclerotium rolfsii* was retarded on culture plates amended with benomyl or glyphosate, each at the commercial rate of application. Both amended media reduced the radial growth of *S. rolfsii* compared to the control; however, glyphosate-amended medium had the greater inhibitory effect (Westerhuis *et al.*, 2007). Beam *et al.* (1977) demonstrated that enzyme activities and mycelial growth of *Rhizoctonia solani* were significantly reduced by prometryn and sclerotium production of *Sclerotium rolfsii* was reduced or even repressed by atrazine. 2, 4-D and fluchloralin drastically inhibited the growth of *S. rolfsii* and *R. bataticola* (Tripathi *et al.*, 1988). Lal and Nagarajan (1988) reported the evaluation of herbicides alachlor, basalin and trifluralin against *S. rolfsii* causing collar rot disease of tobacco at 125, 250, 500, 1000 and 2000 ppm concentrations. The per cent growth inhibition was 67.30 – 88.14 upto 500 ppm concentration. While cent per cent inhibition was noticed at higher concentrations (1000 and 2000 ppm). The

effect of Dicuran MA on the colony growth of five soil borne fungal pathogens of chickpea (*Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum*) was tested under *in vitro* conditions and the results showed that the treatment decreased the growth of all the pathogens tested, but to different extents. *S. rolfsii* was completely inhibited and *M. phaseolina* showed low sensitivity and the growth of other fungi was inhibited by 50- 62.4 per cent (Iqbal *et al.*, 1994). The effects of acetochlor, imazethapyr, metachlor, pendimethalin, trifluralin and mixture acetochlor and imazethapyr on the production and viability of *S. rolfsii* sclerotia were evaluated *in vitro* by Pastro and March (1999). Trifluralin and pendimethalin were the most efficient compounds because they notably reduced the production of viable sclerotia. Madhuri and Reddy (2013) evaluated eight herbicides tested for their efficacy on *S. rolfsii* by poisoned food technique and found that oxyflourfen, alachlor, quizalofop-p-ethyl and 2, 4-D Sodium salt completely inhibited the growth of *S. rolfsii*. Among all the five herbicides, Tribunil (methabenythiazuron) was found to inhibit the sclerotialgermination completely at 100 ppm while, isoproturon (3-(4-isopropyl phenyl)-1, 1-diamethyl urea) and 2, 4-D ethyl Sodium salt (2, 4-D dichloro phenoxy acetic acid) wereeffective at 250 and 500 ppm, respectively (Harlapur, 1988). Susha and Naseema (1998) also observed that 2, 4-D at 0.1 % inhibited the growth of *F. pallidroseum* mildly.

Five insecticides were tested and showed that Desis and Moventa at all concentration completely inhibited mycelial of growth the fungus. Thiruchcchelvan *et al.* (2013) reported that insecticides chlorantraniliporole inhibited 59.29% mycelial growth under *in-vitro* condition. Among them Monocrotophos, 2, 4-D and Nuvan completely inhibited the mycelial growth and sclerotial production of the the fungus *S. rolfsii*.

Among the tested sixteen different *Trichoderma* isolates screened for their antagonistic effect on the growth and per cent inhibition of *S. rolfsii*. The highest per cent mycelial growth inhibition was observed in *Trichoderma* isolates, Tr-3

and the lowest in Tr-14. Weinding (1932) was first to demonstrate the antagonistic effects of a soil fungus to *Sclerotium rolfsii*. He found over growth of hyphae of *Trichoderma viride* Pers on *S. rolfsii*. It secreted some substance which was found lethal to *Sclerotium rolfsii*. Later, Brain (1951) identified the antagonistic substance as gliotoxin. Many workers reported that, *T. viride* was found to be antagonistic to *S. rolfsii* (Hino and Endo, 1940. *Trichoderma harzianum* Rifai was found antagonistic to *Sclerotium rolfsii* (Agrawal *et al.*, 1977). Madhavi and Bhattiprolu (2011) evaluated five bio-agents against *S. rolfsii* revealed that; there is significant difference in per cent inhibition of mycelial growth of *S. rolfsii* by all the bioagents tested. Per cent mycelial inhibition of *Sclerotium rolfsii* was found highest (57.5%) with *Trichoderma harzianum* was significantly superior over the rest of bioagents tested. This was followed by *Trichoderma viride* I (55.8%) followed *Trichoderma viride* II (53.63%), *T. hamatum* caused 44.46% inhibition and 40.7 % inhibition was recorded with *Pseudomonas fluorescens*. Padmaraj *et al.* (2013) studied that the antagonistic potential of native *Trichoderma* isolates was investigated *in vitro* against *S. rolfsii* the causal agent of stem rot disease in groundnut. Ten native *Trichoderma* isolates and a commercial formulation of *Trichoderma* were screened. Eight isolates inhibited the pathogen from 60 to 80%. Two isolates showed maximum growth inhibition (80.78%). Amin *et al.* (2010) reported 67% growth inhibition of *S. rolfsii* by the *Trichoderma* isolates from dual culture studies and rated it as the best isolate. Ratnakumari *et al.* (2011) tested the volatile and non-volatile metabolites of *Trichoderma* against *S. rolfsii* and shown 42-63% inhibition in dual culture and 33-46% with culture filtrate studies. Montealegre and Henrique (1990) reported that *T. harzianum* inhibited the mycelial growth of *S. rolfsii* by 32 per cent and degrade mycelium and Sclerotia of *S. rolfsii*. Synergistic and positive effects on disease control was observed when *T. harzianum* was applied to soil in integration Similar findings were reported by Singh and Thapliyal (1998), Patibanda *et al.*, (2002), Thakare *et al.*, (2002). Results of the experiment showed that all the tested isolates were

effective in reducing mycelial growth of *S. rolfsii* on culture media. Significant reduction of mycelial growth of *S. rolfsii* in the presence of *Trichoderma* spp. were also reported by many other workers (Khan, 2003) and the results of the present investigations are in agreement with that of the above mentioned investigators.

Among the tested ten culture filtrates, *Trichoderma* isolates-3 had shown maximum per cent inhibition and Isolate Tr-14, had shown minimum per cent inhibition. Nawar (2013) reported that maximum inhibition was observed in the culture filtrate of *T. harzianum* drawn from potato dextrose broth with mean reduction of 52.33%. Murtaza *et al.* (2012) tested the effect of *Trichoderma* culture filtrate with different concentrations grown in different media and reported 60 to 80% inhibition at 100% concentration and very less effect at lower concentrations. *Trichoderma* spp. produces over 40 different secondary metabolites that may contribute to their mycoparasitic and antibiotic action. Large number of volatile secondary metabolites produced by *Trichoderma* such as Ethylene, Hydrogen cyanide, Aldehydes and Ketones play an important role in controlling plant pathogens and metabolites such as harzianic acid, alamethicins and tricholin that hinders the growth of other microorganisms. The inhibitory effects found here were mainly attributed to the antibiosis by volatile metabolites and culture filtrates.

Inocula level were the important factor to occur disease in crops. In this experiment, hundred per cent disease incidence observed in 10, 9 and 8 per cent inocula level and the lowest in one percent level. The results of the present study corroborate well with the report made by Khalequzzaman (2003) where soybean plants inoculated with different inoculum levels of *S. rolfsii* and *M. javanica*, showed a gradual reduction in plant growth, nodulation and yield per plant with a gradual increase in inoculum levels. Yaquband and Shazad (2005) showed that increase in inoculum density of *S. rolfsii* caused gradual reduction in growth parameters of sunflower and mungbean plants whereas a positive correlation was

observed between root colonization and population of *S. rolfsii* in soil. The symptoms caused by *S. rolfsii* are in agreement with the description given by Chang (1994) also evaluated the effect of inoculum density on disease incidence of *Sclerotium* rot of *Edgeworthia papyrifera* by using four inoculum levels viz., 8, 4, 2 and 1 sclerotium per seedling and reported that time for causing plants death was reversely in proportion to number of sclerotia inoculated. Jomduang (1995) reported that high concentration of *S. rolfsii* propagules was needed to cause foot rot disease in the older chilli plants. Cent per cent disease was noticed in four and five per cent inoculum levels. Narqund (1981) considered two per cent inoculum level as sufficient to cause considerable infection even though hundred per cent disease incidence was noticed at six percent and above inoculum level of *S. rolfsii* in wheat seedlings. Palakshappa *et al.* (1987) observed considerable foot rot infection, when betelvine was inoculated with two and three per cent inoculum *S. rolfsii*. Further, they recorded 100 per cent infection at four per cent and above inoculum levels. Harlapur (1988) stated that two per cent inoculum was essential for infection. But, maximum infection (100%) was noticed in inoculum level of more than four per cent in foot rot disease of wheat. Singh and Thapliyal (1998) reported inoculum density levels of 2.5 to 10 g kg⁻¹ soil significantly increased the emergence rot which ranged from 36.7 to 90 per cent seed and seedling rot of soybean caused by *S. rolfsii*. Hanumanthegowda (1999) observed 92.50 per cent pre-emergence collar rot of groundnut at two per cent inoculum level and above two per cent lead to 100 per cent seedling mortality. Prabhu (2003) studied the effect of inoculum density on foot rot of soybean which indicated cent per cent pre-emergence disease incidence in four per cent and above inoculum levels and maximum post emergence disease incidence was noticed in three per cent inoculum level. The disease intensity ranged from 36.70 to 90.00 per cent in seed and seedling rot of soybean caused by *S. rolfsii*.

Susceptible stage of the crop influenced greatly on incidence of disease of *S. rolfsii*. In this experiment, the highest per cent disease incidence was observed in

T₅ (03 month old plantation) treatment and the lowest in T₁ (24 month old plantation) treatment. Kulkarni *et al.* (1994) while studying the most susceptible growth stage of groundnut to *S. rolfii* maximum infection, colonization, disease development and mortality was recorded in 15 days old plants and least, mortality in 105 days old plants. It is clear from the study that foot rot of betelvine affects much in young plants than that of old aged plant.

Viability of *S. rolfii* depends on the depth of soil and temperature of the respective area. It was observed that the highest viability of sclerotia was observed at 15 cm depth without mulching and the lowest viability of sclerotia was observed at 5 cm depth with black layer mulching. Bhardwaj and Raj (2004) reported that soil solarization for 40 days completely inhibited the germination of sclerotia of the fungus buried at 7 cm soil depth. Dubey *et al.* (2009) showed that soil solarization effectively caused a decline in propagules of *M. phaseolina* by 20% in comparison to unsolarized control soil after 30 days. Soil solarization or solar heating is a relatively recent method for controlling *S. rolfii* inoculum. Sclerotia grown *in vitro* are still viable after 12 hours at 45°C, but are killed in 4-6 hours at 50°C and in 3 hours at 55°C. Covering soil with transparent polyethylene sheets during the hot season increases soil temperatures and kills *sclerotia* when the temperature under the sheets get hot enough for an appropriate length of time. Most field trials have achieved *sclerotia* degradation at 1 cm, but eradication at greater depths usually did not occur. In addition, this method requires immediate planting, which excludes crops that are planted in spring because temperatures are not high enough to affect sclerotia. Mulching of the irrigated plots with the polyethylene sheet resulted in 10.2°C and 10.6°C higher temperature at 7 cm soil depth with an average maximum temperature of 38.4 and 39.6°C, respectively. Soil solarization for 40 days completely inhibited the germination of sclerotia of the fungus buried at 7 cm soil depth. Mulching with polyethylene sheets proved highly effective in raising the soil temperatures as compared to non-mulched soils. However, mulching heated the soils more at the

depth of 5 cm than at 10 cm. Singh *et al.* (1990) also recorded maximum temperature of 53°C in a tarped plot at 1 cm depth. Similar results have also been recorded by Usmani & Ghaffar (1982), who evaluated the effect of mulching on *R. solani*, *Sclerotium oryzae* and *Macrophomina phaseolina*, respectively. Soil solarization by polyethylene mulching significantly reduced the sclerotial viability. The mulching at both depths produced more or less similar results in terms of sclerotial mortality, however, more sclerotial mortality was observed after 15 days than 7 days. The heat generated in soil by solar radiation and the resultant death of pathogens encompasses the major principles of soil solarization. Singh *et al.* (1990) observed that after 45 days of soil solarization at 1 cm depth, only 10% sclerotia of *S. rolfsii* germinated while in our experiment similar sclerotial mortality was achieved after 15 days at 5 cm depth.

In hot summer solarization play important role to reduce disease incidence. The lowest per cent disease incidence was recorded after treatment with 60 days solar heating. Soil solarization has been demonstrated to control diseases caused by many fungal pathogens such as *Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Verticillium* spp., *Sclerotium rolfsii* etc. in many crops (Katan *et al.*, 1983). Dubey *et al.* (2009) showed that soil solarization effectively caused a decline in propagules of *M. phaseolina* by 20% in comparison to unsolarized control soil after 30 days. Soil solarization during summer months increases soil temperatures to levels lethal to many soil-borne plant pathogens, weeds, nematodes, and some soil residing mites. Brown *et al.* (1989) observed that black plastic and nylon mulch can limit the incidence of southern blight by providing a physical barrier that prevents pathogen and host contact. Bhardwaj and Raj (2004) reported that soil solarization with transparent polyethylene mulch (25 µm) for 40 days was found effective for the control of collar and root rot of strawberry caused by *Sclerotium rolfsii*. Consequently, less infection results in the better plant growth and maximum plant height and weight were recorded in plants grown in soils removed from 5 cm depth from mulched plots. Our results

are in confirmation to those reported by Ristaino *et al.* (1996) who observed that solarization of fallow soil in bell pepper raised beds significantly reduced incidence of *S. rolf sii*. Our results are also in accordance with the findings of Grinstein *et al.* (1979) who observed that soil solarization effectively reduced the infection of *S. rolf sii* in crop plants. The results of the present study suggest that a eight week mulching period could provide almost 100% reductions in population of *S. rolf sii* in soil.

Effect of amendmended soil were used for the germination of sclerotia at different intervals was studied and it was observed that after 42 days, neem cake amendmend soil showed the lowest germination sclerotia. The role of organic amendmends in reducing the propagules of soil borne plant pathogens has been well established (Lewis and Papavizas, 1977). Additions of organic amendmends were also found to increase antagonistic population in soil and thereby decrease the inoculum of soil borne pathogens (Linderman, 1989). The addition of an amendmend may increase populations of antagonistic soil microorganisms. Khattabi *et al.* (2004) noticed that the horse manure at high rates inhibited the growth of *S. rolf sii*.

In PDA medium, mycelial growth of *S. rolf sii* effectively inhibited with hot water temperatures and exposure time. In this experiment, it was observed that there was no mycela growth when exposed hot water up to 52 °C temperature treatments with 30 minutes and above 52 °C temperature. Viability of *S. rolf sii* was showed 51 °C temperatures. Mahdy *et al.*, (2011) showed that the lethal temperatures to *S. rolf sii* 53.0°C when exposed to hot water temperatures for one minutes as agar disks with mycelia.

The hot water soil treatment has recently received special attention to control plant disease. In this experiment, hot water treatment at the rate 0.5 L/kg soil on the per cent disease incidence in *S. rolf sii* infested pot soil was tested and observed that the highest and the lowest disease incidence was recorded after

treatment with 65° and 100°C respectively. The hot water treatment has recently been receiving special attention in Japan as the most promising Methyl bromide alternative (Kita *et al.*, 2003). Mahdy *et al.*, (2011) reported that as for the disease incidence the highest reduction in root rot disease was obtained with boiling water at rate 0.4 & 0.5 l / kg soil and hot water at 90.0 & 100°C (0.5 L/kg soil) which reduced the disease incidence of cucumber more than 88.4 and 92.9 % for pre and post emergence respectively. Kita *et al.* (2003) reported when *Fusarium oxysporum* f.sp. *lycopersici* present within the 30 cm depth, soil was exposed to the lethal temperature (55°C) complete disinfestations was successfully achieved leading to the effective suppression of the wilt disease. Applications of hot water (95 to 100°C) on to soil surface raise the soil temperature up to the lethal level to the plant pathogens as well as pests and weed seeds (Noling, 1995; Fujinaga *et al.*, 2005 and Ogawara *et al.*, 2006). Promising effects of hot water treatment of the soil-borne disease control have been confirmed in various crops such as tomato, melon, strawberry, spinach, rose, sweet pea, and carnation (Uematsu *et al.*, 2003 and 2005).

Among the tested five cultural practices, hot water 0.5 L/kg treatment reduced the disease incidence than other tested cultural practices. Deep ploughing (at least 20 cm) with a moldboard extention inverts soil so that organic matter, sclerotia, and plant debris is buried at least 10 cm beneath the surface. This helps to eliminate inoculum when plowing occurs just prior to planting. Buried soil must not be re-surfaced during the growing season. Earhing up of sandy loam soil once or twice in a year before the onset of monsoon appreciably reduces the disease incidence.

Four organic amendments such as mastard cake, vermicompost, neem cake, and seasamum cake were used for the purpose of disease management in field condition in this experiment. It is evident from the experiment that neem cake amendment barojas showed the lowest disease incidence and the highest yielding. Bailey and Lazarovits (2003) demonasted that organic amendments, manures and composts with high nitrogen contents, suppress soil-borne diseases by releasing

allelochemicals produced during product storage or by ultimate microbial decomposition. Additions of organic amendments were also found to increase antagonistic population in soil and thereby decrease the inoculum of soil borne pathogens. Organic matter added to soil may increase soil fertility, stimulate growth of crops and suppress plant pathogens. When properly used, amendments can not only reduce populations of soil-borne pathogens but also induce soil suppressiveness by stimulating activities of antagonists in soil. Islam and Toyata, (2004) studied that poultry manure and farm yard manure suppressed bacterial wilt of tomato caused by *Ralstonia solanacearum*. Compost, oat or straw added to the soil has been shown to limit disease incidence. The addition of an amendment may increase populations of antagonistic soil microorganisms (see biocontrol section). Oil cakes have been found to inhibit the growth of *Sclerotium rolfsii* (Ramaraio & Raja, 1983). Dutta and Deb (1986) studied the effect of organic and inorganic amendments on the soil and *Rhizosphere microflora* in relation to the biology and control of *Sclerotium rolfsii*.

It is very difficult to control even by the use of chemical fungicide. Moreover, most of the chemicals are costly. So the farmers have to spend a large amount of money to buy these chemicals. Now-a-days use of chemical for management of crop disease is being discouraged due to health hazards and environmental pollution. So, control of the pathogen through botanical pesticides or plant extracts and natural biocides might be a good alternative. Four plants extract such as *Swietenia mahagoni*, *Azadirachata indica*, *Lawsonia inermis* and *Allium sativum* were used for the purpose of disease management in field condition in this experiment. It is evident that *Allium sativum* extract treated barojes showed the lowest disease incidence and the highest yielding. Botanical extracts are biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. These botanical pesticides are affordable by low income farmers and they have the potentiality for use in agriculture, especially with the dramatic increase towards the consumption of organically produced plants and ensure the

sound ecology and friendly environment without any pollution. Dayaram and Tewari (1994) found that, the soil application of green leaves of *Adathoda vasica*, *Aegle marchelos*, *Anisomeles ovata*, *Azadirchta indica*, *Cymbopogon flexuous*, rhizomes of *Curcuma amada* and resin of *Ferula foetida* at 2 to 5 per cent concentration reduced both pre and post emergence collar rot of chickpea caused by *Sclerotium rolfsii*.

Five fungicides were tested in controlling foot rot disease in field conditions. All the tested fungicides significantly controlled the disease effectively. But Score appeared to be the most effective fungicide to reduce disease incidence and increase yield of betelvine. Patil *et al.* (1986) reported effectiveness of captan in reducing the mortality of *Piper betel* L. due to *S. rolfsii*. The foot rot caused by *P. parasitica* was claimed to be ameliorated by soil application of Bordeaux mixture (Dasgupta and Sen, 1999 and Dasgupta *et al.*, 2008. Anilkumar and Pandurangegowda (1984) observed the efficacy of vitavax as soil drenching fungicide against *S. rolfsii* in reducing the sunhemp seedling mortality. Patil *et al.* (1986) observed that, soil drenching of captan, copperoxychloride and difolaton were found effective in controlling wilt of betelvine caused by *S. rolfsii*. *In vivo* soil drenching was done with nine fungicides and found that tebuconazole and combination of carbendazim+mancozeb proved effective in controlling the pathogen at 1000, 2000 and 3000 ppm (Madhavi and Bhattiprolu, 2011).

Four *Trichoderma* isolates were tested in controlling foot rot disease in field conditions. All the tested *Trichoderma* isolates significantly controlled the disease effectively. But *Trichoderma* iso-3 appeared to be the most effective isolate to reduce disease incidence and increase yield of betelvine. Mehrotra and Tiwari, (1976) showed that dipping of cutting in a *Trichoderma viride* cell suspension effectively reduced the foot rot disease of betelvine. D'Souza *et al.* (2001) reported significant reduction foot rot of disease of betelvine with the use of an isolate of *Trichoderma harzianum*. Dasgupta *et al.* (2011) studied that in the 2 year, the minimum foot rot disease was recorded in the treatment where

four applications of the *Trichoderma* preparation at quarterly intervals were given. *Trichoderma* spp. is known mycoparasites of a number of plant pathogens. *T. harzianum* colonizes *S. rolfsii* hyphae, disrupts mycelial growth, and kills the organism. Field studies where effective control was obtained, involved application rates in the range of 140 to 600 to 1500 kg/ha. Patibanda *et al.* (2002) reported the efficiency of *T. harzianum* alone and in combination with fungicides against *sclerotium* wilt of groundnut. Yedidia *et al.* (2001) showed that treatment of cucumber plants in soil with *T. harzzianum* (T-203) resulted in large increase in root area and cumulative root lengths, and significant increase in dry weight, shoot length and leaf area over that of the untreated control. *T. harzianum* has also been used in Nigeria in control of basal stem rot diseases of tomato caused by *Sclerotium* (*Corticium rolfsii*) (Wokocha *et al.*, 1986). The antagonists have also been found to increase the yield, plant height, fresh weight and vigour index in different crops (Begum *et al.*, 1999; Chowdhury *et al.*, 2000; Hossain and Samsuzzaman, 2003; Yeasmin, 2004).

Based on findings of the present investigation, it may be concluded that combination of NPK fertilizer decreases the disease incidence and the yield increases. The NPK dose of 200-100-100 kg/ha was the most effective to reduce the disease and increase yield of betelvine. Deb and Dutta (1992) recorded that in field, urea (80 kg/ha) and ZnSO₄ (10 kg/ha) initially increased *S. rolfsii* population which decreased slightly at a later stage. A significant reduction in the disease was recorded with all the fertilizers used, of which ZnSO₄ produced the best result. Urea (80 kg/ha) and ZnSO₄ (10 kg/ha) stimulated plant growth, which was reflected on the increased plant height and yield as compared to others. Brahmankar *et al.* (2011) stated that NPK @ 100:50:50 kg/ha was significantly management the wilt or root rot disease of betel cultivation. Singh (2002) also reported that 120 kg N/ha and 45 kg P/ha significantly increased the seed and yield attributes of mustard. Maiti *et al.* (1995) stated that application of 200 kg N ha⁻¹ through organic and inorganic sources in 1:1 ratio is the best way for obtaining higher yield in betelvine. Saikia *et al.* (1995) in a

field experiment with cv. local Bangla reported that 200 kg N ha⁻¹ in the form of urea: mustard oil cake (1:1) had produced maximum leaf vine (24.15). Adverse effects of chemical fertilizers on the keeping quality of leaves and aggravating foliage diseases have also been reported (Das *et al.*, 1989).

Five crops were selected for crop rotation studies and the results revealed that, the lowest per cent disease incidence was recorded in *Saccharum officinarum* followed by *Zea mays*, *Allium sativum* and the highest incidence was noticed in betelvine which served as control. *Zea mays* and *Saccharum officinarum* recorded better crop stand over all other crops. Crop rotation is one of the economical and ecofriendly management practice which helps in reducing the disease incidence (Rodriguez *et al.*, 1991). It is an age old practice followed by the farmers. This operation has effect on the starvation of pathogen in the absence of proper host. To take advantage of this phenomenon in the eradication of soil borne, *S. rolfsii* of betelvine, different crops were tried as rotation crops with betelvine. Plant rotation with maize and wheat resulted in a decrease in the stem rot of peanut by 50% (Bicici *et al.*, 1994).

Cultivars screening against foot-rot disease was tested and eight locally available cultivars were carried out in this study. Among the tested cultivars, Sanchi cultivar of betelvine showed resistant against *S. rolfsii*. On the other hand, Chaltaguta, Metha pan, Mistypan and Gachpan showed moderately susceptible reaction. Incase of cultivars Bangla, Rangpuri, and Jhalpan, they showed highly susceptible reaction against *S. rolfsii*. Singh (2002) reported that of the different cultivars screened under *in vivo* and *in vitro* conditions for disease resistant cv. Desawari was found to be the most susceptible with Ayurvedic Bangla being least susceptible. Therefore cv. Desawari was selected as host for foot rot disease management trials caused by *S. rolfsii*.

Efficacies of different treatment combinations (IDM) in the management of foot rot disease of betelvine were tested and it was observed that treatment T₉

(sanitation+ neem cake 6t/h+ application *Allium sativum* extract 5%+ application Tr-3+ Score 1.0% drenching) was found to be effective in management of disease in comparison with individual treatments. The individual application of sanitation, neem cake, *Allium sativum* extract, *T. harzianum*, Folicure were found less effective to reduce the disease. For the soil-borne pathogens, use of fungicides is not practical due to exorbitant cost and environmental hazards involved (Arunasri *et al.*, 2011). The methods employed to manage groundnut root rot (*S. rolfsii*) includes: fungicide seed treatment, soil solarisation, use of antagonistic microorganisms, deep plowing, crop rotation, and incorporation of organic residues (Punja, 1985). Organic matter added to soil may increase soil fertility, stimulate growth of crops and suppress plant pathogens. When properly used, amendments can not only reduce populations of soil-borne pathogens but also induce soil suppressiveness by stimulating activities of antagonists in soil. Where organic matter levels are adequate the activity of these and other cellulase and laminarinase producing microorganisms would lead to an accumulation of these enzymes in the soil. Brahankar *et al.* (2011) reported that soil solarization, *Trichoderma* @ 10 kg/ha (set and soil application), Neem cake @ 2000 kg/ha, NPK @ 100:50:50 kg/ha and drip system of irrigation) was found significantly controlling the wilt causing fungus *S. rolfsii* in betlvine. Mohanty *et al.* (2011) founded that integrated use of sanitation, Bordeaux mixture and *T. harzianum* gave the best control of foot rot and leaf rot of betelvine. Sollorin and Tangonan (1990) reported that disease control by *Trichoderma* sp. was from 25.67 to 71.00%. *Trichoderma* sp. thus has good potential as bio-control agent against *S. rolfsii*. Khattabi *et al.* (2004) observed that the horse manure at high rates inhibited the growth of *S. rolfsii*, favored the development of *T. harzianum* and enhanced its antagonistic effect on *S. rolfsii*. Particularly, the manure in combination with *T. harzianum* induced high mortality of sclerotia of the fungus. Brahmankar *et al.* (2011) stated that wilt or root rot is a major constraint to betel cultivation in Vidarbha. In present investigation, integrated disease management module (M-III) (soil solarization, *Trichoderma* @ 10 kg/ha (set and soil

application), Neem cake @ 2000 kg/ha, NPK @ 100:50:50 kg/ha and drip system of irrigation) was found significantly superior not only in controlling the wilt causing fungus and nematode but also had a favourable effect on growth parameters. Wokocha (1986) reported that tomato disease caused by *S. rolfsii* was controlled very effectively by using *Trichoderma viride* and some fungicides. Mukthapadhyay *et al.* (1992) reported biological seed treatment for control of soilborne pathogens by integration of biological (*Trichoderma* and *Gliocladium* species) and fungicidal (Carboxin (0.1%) seed treatments which has been found to improve the disease control potential to a great extent. Bicici *et al.* (1994) mentioned that rotation of maize and wheat with groundnut spacing of 90 cm between rows, application of quintozone (PCNB) in combination with urea and calcium ammonium nitrate, soil solarisation with polythene sheet and application of biocontrol agent (*T. harzianum*) effectively reduced stem rot of groundnut. Hanumanthegouda (1999) reported that, seed treatment *T. harzianum* and soil application of *T. harzianum* recorded the low incidence of groundnut stem rot caused by *Sclerotium rolfsii*. Anahosur (2001) conducted an experiment on integrated disease management in farmer's field during the rainy season of year 1999-2000. The results were encouraging and concluded that, IDM comprising the components viz., crop rotation in rabi with rabi sorghum varieties for 2 years + application of FYM to the soil + Tuber treatment with *T. viride* or *T. harzianum* prior to planting @ 4 g kg has helped in reducing the sclerotium wilt of potato in the field. Vanitha and Suresh (2002) conducted a study to investigate efficacy of biological control agents and organic amendments in controlling collar rot of brinjal caused by *Sclerotium rolfsii*, where *Trichoderma viride* + FYM + dry adathoda leaf powder were found effective. Integration of different treatments including seedling dip with carbendazim+mancozeb, addition of vermicompost, drenching with fungicide and application of *Trichoderma harzianum* (7%) were found to be effective in management of disease in comparison with individual treatments (Madhavi and Bhattiprolu, 2011).

Chapter Five

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