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Studies on the Bio-pesticidal Properties of Some Selected Flora for Eco-friendly Control of Fungal Diseases of Brinjal Field

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University of Rajshahi

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By

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October 2015

DEDICATED TO
MY BELOVED
PARENTS AND WIFE

DECLARATION

I declare that the thesis entitled "Studies on the Bio-pesticidal Properties of Some Selected Flora for Eco-friendly Control of Fungal Diseases of Brinjal Field" submitted for the degree of Doctor of Philosophy in the Institute of Environmental Science, University of Rajshahi, Bangladesh, is the record of work carried out by me during the period from July 2011 to June 2015 under the guidance of Prof. Dr. Md. Abul Kalam Azad and this work not submitted earlier for the award of any degree at Rajshahi University or any other university or any other similar institution of higher degree or prize.

Date: October 2015 **(Shanjit Kumar Sarker)**

CERTIFICATE

This is to certify that Mr. Shanjit Kumar Sarker has carried out Ph. D. dissertation entitled **"Studies on the Bio-pesticidal Properties of Some Selected Flora for Eco-friendly Control of Fungal Diseases of Brinjal Field"** in the Institute of Environmental Science University of Rajshahi. This dissertation or part thereof has not been submitted for the award of any degree, diploma or associated with any other similar title.

The data presented in this thesis produced by Mr. Shanjit Kumar Sarker are new, genuine and original. Through this research work, Mr. Sarker has fulfilled all the requirements required for submission of a dissertation for the Ph.D. degree in the field of environmental science under the University of Rajshahi.

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Shanjit Kumar Sarker

ABSTRACT

The present study was carried out to determine the effect of some medicinal plants and bio-agents on plant diseases like foot rot, root rot and fusarial wilt of brinjal (*Solanum melongena*). Twenty six medicinal plants and two bio-agents were applied for the control of diseases and antifungal effect was tested against *Sclerotium rolfsii* and *Fusarium oxysporum* that cause foot /collar rot, root rot and fusarial wilt diseases in *Solanum melongena.* These medicinal plants are Datura (*Datura stramonium*), Alamanda (*Allamanda cathartica*), Babla (*Acacia nilotica*) Hazarmony (*Phyllanthus niruri*), Telachcha (*Coccinia cordifolia*), Vat (*Clerodendrum viscosum*), Akando (*Calotropis procera*), Ata (*Annona squamosal*), Lantana (*Lantana camara*), Mahagony-Seed (*Swietenia macrophylla*), Jute-Seed (*Corchorus caspsularis*), Khoksha (*Ficus hispida*), Tobacco (*Nicotiana tabacum*), Chirota (*Swertia chirata*), Nishinda (*Vitex negundo*), Soj (*Carum roxburghianum),* Polygonum (*Polygonum orientale*), Mehedi (*Lawsonia inermis*), Tulsi (*Ocimun sanctum*), Ganda (*Tagetes patula*), Holde hurhurae (*Cleome viscosa*), Bell (*Aegle marmelos*), Arhar (*Cajanus cajan*), Neem (*Azadirachta indica*), Apang (*Achyranthes aspera*) and Garlic(*Allium sativum*). Extract of *Acacia nilotica* (babla) was found efficient in inhibiting the sclerotial and conidial germination of *S. rolfsii* and *F. oxysporum* correspondingly. The whole mycelial growth of *S. rolfsii* & *F. oxysporum* was inhibited by *Acacia nilotica*.

The antifungal effect of crude medicinal plant extracts of 26 plants species was determined by *in vitro* study using water and methanol as a solvent following Poisoned Food Technique. The leaf extract of *Nicotiana tabacum, L. camara, S. macrophylla* and *Corchorus capsularis* completely inhibited the mycelial growth of *S. rolfsii* at 15% concentration. The *in vitro* study revealed that aqueous extract was more effective than methanol extract.

Aqueous extracts of 13 plants datura (*Datura stramonium*), lantana (*Lantana camara*), babla (*Acacia nilotica*), tulsi (*Ocimum sanctum*), akondo (*Calotropis procera*), garlic (*Allium sativum*), jute seed (*Corchorus caspsularis*), mahogany seed (*Swietenia macrophylla*), tobacco (*Nicotiana tabacum*), telachucha (*Coccinia cordifolia*), alamonda (*Allamanda cathartica*), hazarmony (*Phyllanthus niruri*) and khoksha

(*Ficus hispida*) were tested in sterile soil and PDA media against the sclerotial germination. Extracts of *Acacia nilotica*, *Datura stramonium*, *Swietenia macrophylla* were found to inhibit *in vitro* germination of sclerotia of *Sclerotium rolfsii* compare to control. At 20% concentration *Acacia nilotica*, extract showed a high inhibition against sclerotial germination in both PDA and sterilized soil.

Out of the 26 plant extracts in water, higher inhibition was noticed in 5 plants extracts namely *Acacia nilotica, Allium sativum, Swietenia macrophylla, Datura stramonium* and *A. indica.* Whereas, in methanol solvent, at 15% concentration complete inhibition was noticed in 3 plants extracts namely *Acacia nilotica, Nicotiana tabacum* and *Swietenia macrophylla*. *Acacia nilotica* at all concentration alone recorded 100% inhibition that showed maximum inhibition in water and methanol. Combination of *Acacia nilotica* with *Corchorus capsularis* and *Nicotiana tabacum* with *Corchorus capsularis* revealed excellent inhibition compare to control at 20% concentration against *Fusarium oxysporum.*

The extract of *A. sativum* at highest concentration proved highly effective in reducing the spore germination of *Fusarium oxysporum* followed by extract of *A. nilotica* and *Lawsonia inermis,* respectively in ethanol extract, and the low efficiency was recorded in *Lantana camara* in all solvents. The inhibition in spore germination increases with corresponding increase in the extract concentration.

Six isolates of *Trichoderma* spp. namely TRU-5, TRU-14, TB-23, TR-37, TD-44 and TTG-50 showed significant difference in mycelial growth among them after 24 hours, 48 hours and 72 hours. The *Trichoderma* isolate, TB-23 caused the maximum growth on PDA. Antagonistic activity of *Trichoderma harzianum* was conducted on PDA following dual culture plate technique against *S. rolfsii* and *F. oxysporum.*

Aqueous extract of ten plants, one antagonist and two fungicides were tested on the vegetative growth and disease control of brinjal plant (*Solanum melongena* L.). *Acacia nilotica, Allium sativum, Datura stramonium extracts* and *Trichoderma* formulation were found most effective agent in controlling foot rot and wilt in comparison to control under field conditions.

The formulated *T. harzianum* and *Rhizobium* bio-fungicide was tested against soil-borne pathogens of brinjal like *Sclerotium rolfsii* and *Fusarium oxysporum* causing foot and root rot and wilt diseases. All the substrates formulated with *Trichoderma* had significant effect in seed germination and decreasing seedling mortality as well as increasing plant growth over control.

The result suggests that the application of plant extracts are growth promotive and effective in control of fungal diseases of brinjal field.

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ABSTRACT

The present study was carried out to determine the effect of some medicinal plants and bio-agents on plant diseases like foot rot, root rot and fusarial wilt of brinjal (*Solanum melongena*). Twenty six medicinal plants and two bio-agents were applied for the control of diseases and antifungal effect was tested against *Sclerotium rolfsii* and *Fusarium oxysporum* that cause foot /collar rot, root rot and fusarial wilt diseases in *Solanum melongena.* These medicinal plants are Datura (*Datura stramonium*), Alamanda (*Allamanda cathartica*), Babla (*Acacia nilotica*) Hazarmony (*Phyllanthus niruri*), Telachcha (*Coccinia cordifolia*), Vat (*Clerodendrum viscosum*), Akando (*Calotropis procera*), Ata (*Annona squamosal*), Lantana (*Lantana camara*), Mahagony-Seed (*Swietenia macrophylla*), Jute-Seed (*Corchorus caspsularis*), Khoksha (*Ficus hispida*), Tobacco (*Nicotiana tabacum*), Chirota (*Swertia chirata*), Nishinda (*Vitex negundo*), Soj (*Carum roxburghianum),* Polygonum (*Polygonum orientale*), Mehedi (*Lawsonia inermis*), Tulsi (*Ocimun sanctum*), Ganda (*Tagetes patula*), Holde hurhurae (*Cleome viscosa*), Bell (*Aegle marmelos*), Arhar (*Cajanus cajan*), Neem (*Azadirachta indica*), Apang (*Achyranthes aspera*) and Garlic(*Allium sativum*). Extract of *Acacia nilotica* (babla) was found efficient in inhibiting the sclerotial and conidial germination of *S. rolfsii* and *F. oxysporum* correspondingly. The whole mycelial growth of *S. rolfsii* & *F. oxysporum* was inhibited by *Acacia nilotica*.

The antifungal effect of crude medicinal plant extracts of 26 plants species was determined by *in vitro* study using water and methanol as a solvent following Poisoned Food Technique. The leaf extract of *Nicotiana tabacum, L. camara, S. macrophylla* and *Corchorus capsularis* completely inhibited the mycelial growth of *S. rolfsii* at 15% concentration. The *in vitro* study revealed that aqueous extract was more effective than methanol extract.

Aqueous extracts of 13 plants datura (*Datura stramonium*), lantana (*Lantana camara*), babla (*Acacia nilotica*), tulsi (*Ocimum sanctum*), akondo (*Calotropis procera*), garlic (*Allium sativum*), jute seed (*Corchorus caspsularis*), mahogany seed (*Swietenia macrophylla*), tobacco (*Nicotiana tabacum*), telachucha (*Coccinia cordifolia*),

alamonda (*Allamanda cathartica*), hazarmony (*Phyllanthus niruri*) and khoksha (*Ficus hispida*) were tested in sterile soil and PDA media against the sclerotial germination. Extracts of *Acacia nilotica*, *Datura stramonium*, *Swietenia macrophylla* were found to inhibit *in vitro* germination of sclerotia of *Sclerotium rolfsii* compare to control. At 20% concentration *Acacia nilotica*, extract showed a high inhibition against sclerotial germination in both PDA and sterilized soil.

Out of the 26 plant extracts in water, higher inhibition was noticed in 5 plants extracts namely *Acacia nilotica, Allium sativum, Swietenia macrophylla, Datura stramonium* and *A. indica.* Whereas, in methanol solvent, at 15% concentration complete inhibition was noticed in 3 plants extracts namely *Acacia nilotica, Nicotiana tabacum* and *Swietenia macrophylla*. *Acacia nilotica* at all concentration alone recorded 100% inhibition that showed maximum inhibition in water and methanol. Combination of *Acacia nilotica* with *Corchorus capsularis* and *Nicotiana tabacum* with *Corchorus capsularis* revealed excellent inhibition compare to control at 20% concentration against *Fusarium oxysporum.*

The extract of *A. sativum* at highest concentration proved highly effective in reducing the spore germination of *Fusarium oxysporum* followed by extract of *A. nilotica* and *Lawsonia inermis,* respectively in ethanol extract, and the low efficiency was recorded in *Lantana camara* in all solvents. The inhibition in spore germination increases with corresponding increase in the extract concentration.

Six isolates of *Trichoderma* spp. namely TRU-5, TRU-14, TB-23, TR-37, TD-44 and TTG-50 showed significant difference in mycelial growth among them after 24 hours, 48 hours and 72 hours. The *Trichoderma* isolate, TB-23 caused the maximum growth on PDA. Antagonistic activity of *Trichoderma harzianum* was conducted on PDA following dual culture plate technique against *S. rolfsii* and *F. oxysporum.*

Aqueous extract of ten plants, one antagonist and two fungicides were tested on the vegetative growth and disease control of brinjal plant (*Solanum melongena* L.). *Acacia nilotica, Allium sativum, Datura stramonium extracts* and *Trichoderma* formulation were found most effective agent in controlling foot rot and wilt in comparison to control under field conditions.

The formulated *T. harzianum* and *Rhizobium* bio-fungicide was tested against soil-borne pathogens of brinjal like *Sclerotium rolfsii* and *Fusarium oxysporum* causing foot and root rot and wilt diseases. All the substrates formulated with *Trichoderma* had significant effect in seed germination and decreasing seedling mortality as well as increasing plant growth over control.

The result suggests that the application of plant extracts are growth promotive and effective in control of fungal diseases of brinjal field.

Chapter - 1
General Introduction

1.1 Introduction

Brinjal (*Solanum melongena* L. fam. Solanaceae) or eggplant or aubergine in an important widely consumed nutritious vegetable crop in Bangladesh or cultivated commercially throughout the tropical and subtropical region of the world. The name brinjal is popular in India subcontinents and is derived from Arabic and Sanskrit. It is considered a native of China or major Asia where the major domestication of large fruited cultivars occurred. It has been cultivated in India for the last 4,000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable.

Brinjal is important in the warm areas of Far East, being grown intensively in India, Bangladesh, Pakistan, China and Philippines. It is also popular in Egypt, France, Italy and US. In Bangladesh and India, it is popular and principal vegetable crops grown throughout the country except higher altitudes. It is a perennial but grown commercially as an annual crop. The major producing areas are East Bengal, West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Karnataka, Uttar Pradesh and Andhra Pradesh. In Bangladesh, it is grown well in Rangpur, Rajshahi, Bogra, Mymensingh-Gaphorgaon, Jamalpur, Khulna and Sylhet.

In a subtropical country like Bangladesh, eggplant is grown all over the country in medium high land to high land in both Rabi and Kharif seasons. It is positioned the 2nd in acreage, production, yield, and in consumption next to potato. In the year 2008-2009, total 360,000 acres land were under eggplant cultivation, total production was 338,000 MT and yield was 21.5 MT/acre (BBS, 2010).

Eggplant is the sixth most widely grown vegetables produced in the world after tomato, water melons, cabbage, onions and cucumbers. Over the last 15 years, world production of eggplants rose approximately three-fold, with a steady annual growth of around 1.3 million MT reaching a global total of 30.5 million MT in 2005 (FAO, 2006). The top four producers China, India, Egypt and Turkey hold about 89% of world production, while China alone takes as much as 55% of the total.

The global area under brinjal cultivation has been estimated at 1.85 million ha with total production of brinjal fruit of about 32 million MTs (Anonymous, 2005). India accounts for about 8.7 million MTs with an area of 0.53 million hectors under cultivation. It is also exported in the fresh or frozen form. In 2007-2008, 34 million kg worth of Rs. 19 million was imported mainly to UK, Netherlands, Saudi Arabia and Middle East countries (Anonymous, 2008). Rajasthan accounts for about 20339 MTs with an area of 7 ha.

The composition per 100g of edible portion of brinjal constitutes Calories (24.0), Sodium (mg) (3.0), moisture content (%) (92.7), Copper (mg) (0.12), Carbohydrates (%)(4.0), Potassium (mg) (2.0), Protein (g) (1.4), Sulphur (mg) (44.0), Fat (g) (0.3), Chlorine (mg) (52.0), Fiber (g) (1.3), Vitamin A (I.U.) (124.0), Oxalic acid (mg) (18.0), Folic acid (μg) (34.0), Calcium (mg) (18.0), Thiamine (mg) (0.04), Magnesium (mg) (15.0), Riboflavin (mg) (0.11), Phosphorus (mg) (47.0), B-Carotene (μg) (0.74), Iron (mg) (0.38), Vitamin C (mg) (12.0), Zinc (mg) (0.22) and Amino acid (0.22)(Gopalan *et al*.,2007).

Botanically, it is an herbaceous prickly perennial herb or undershrub; flowers purple, solitary; berries large green or purple, globular or oblong, acrid-sweet in taste. The fruit contains arginin, aspartic acid, solanin, histidine, leucine, methionine, pipecolic acid, phenylanine, theonone, tryptophane, valine, choline, nicotinic acid, riboflavin, vit-A & C, fructose, glucose, sucrose, anthocynine, lycoxanthin, caffic acid and chlorogenic acid. Taxonomically there are 3 main botanical varieties under the species Melongena. The common brinjal, to which large, round or egg shaped fruited forms belong, or grouped under *Solanum melongena* var. *esculentum*. The long, slender types are included under *Solanum melongena* var. *serpentinum* and the dwarf brinjal plants are put under *Solanum melongena* var. *depressum* (Choudhury, 1976). It has been reported that on an average, the oblong-fruited eggplant cultivars are rich in total soluble sugars, whereas the long-fruited cultivars contain a higher content of free reducing sugars, anthocyanin, phenols, glycoalkaloids (solasodine), dry matter and amide proteins (Bajaj *et al*., 1979).

Brinjal fruit (unripe) is primarily consumed as cooked vegetables in various ways and dried shoots are used as fuel in rural areas. It is known to have ayurvedic medicinal

properties, good for diabetic patients and recommended as an excellent remedy for those suffering from liver complaints. The fruit contains no endogenous toxins or a significant level of anti-nutritional factors have been found till date and is not capable of causing any disease in human, animals or plants (Shukla *et al*., 1993)**.** In Brazil, eggplant is consumed extensively and believed that infusion of a powdered preparation of the fruit may reduce serum cholesterol. The results suggest that infusion of brinjal has modest and transitory effect which was not different from the obtained with standard and orientation for dyslipidemia patients (diet and physical activities) (Guirmaraes *et al*., 2000)**.** It concluded that water extracts of peduncles used frequently as mouth wash may have benefited effect against periodontal disease (Diab *et al*., 2011)**.** Ethno-botanically the plant used for cure of several diseases by using different parts of plants such as fruit stalk used for cure of fistula and piles (Tarafder *et al.,* 1987), mature fruit for stomach pain (Rajan *et al.,*2003) and leaf used for burns (Thomas *et al.,* 2003)**.**

Comparing with production and yield rate in other countries, the position of Bangladesh is very poor for the lacking of appropriate knowledge of sound production. The yield is around 9 ton ha^{-1} in 2006; a very low yield compared to that the other countries like India and japan (20 & 30 tons ha^{-1}, respectively). The most devastating disruption in sound eggplant production, usually farmers suffer is the disease management problem. In Bangladesh, eggplant suffers from 12 diseases among which most serious diseases are foot/collar rot, root rot and *Fusarium* wilts.

1.2 The fungus *Sclerotium rolfsii*

1.2.1 History, Host Range, and Distribution of *Sclerotium rolfsii*

The fungus had been named *Sclerotium rolfsii* by Saccordo in 1911. It is an economically important pathogen on numerous crops worldwide (Aycock, 1966). It is an omnivorous and destructive soil borne pathogen of many plants. It has a very extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf, 1960).

It commonly occurs in the tropics, subtropics, and other warm temperate regions (Punja, 1985). Although no statistical data are available, disease caused by this

pathogen lead to heavy losses in vegetable crop yield especially during the wet season (May to October) when weather conditions are favorable for both crop production and for the growth and dissemination of the sclerotia of the pathogen (Wokocha *et al.,* 1986). Its growth is optimal at 27-30°C and rarely occurs where average winters temperatures fall below 0°C.

1.2.2 Biology of *Sclerotium rolfsii*

S. rolfsii is an imperfect fungus and belongs to Form class Deuteromycetes. It does not produce spores. Growth of *S. rolfsii* on all organic-based and inorganic synthetic media is accompanied by forming of spherical, brown to tan colored sclerotia measuring 0.3 to 3.0 mm in diameter (Edelstein *et al.,* 1983). They are initially developed as white aggregates or knots of mycelium, and then it differentiates to form the mature sclerotium within 2-3 weeks. Sclerotia form abundantly on potato dextrose agar (PDA) and can also be produced on a substrate such as autoclaved oat kernels moistened with 1.5% water agar (Punja and Grogan, 198la). These sclerotia resemble those that form in soil (Punja and Jenkins, 1984). The prevalence of *S. rolfsii* in warm regions of the world is a reflection of the high temperature optimal for its growth and sclerotial production. The temperature range for hyphal extension and dry weight production is 8-40°C (Zoberi, 1980); maximum growth and sclerotial formation occur at 27-30°C (Mihail and Alcorn, 1984). Punja (1985) found that exposing sclerotia to temperature above 50°C for extended period was lethal. The linear growth rate of hyphae on agar media at 27°C ranged from 0.85-0.97 mm per hour, depending on the isolate. The optimal temperatures for growth in sterile soil are similar to those in culture (Mustafee and Chatlopadhyay, 197la). Although the effects of diurnal temperature fluctuations on growth in culture or in non-sterile soil have not been studied, it is unlikely that the general response differs between them. Temperature fluctuations may, however, affect the shape and size of the sclerotia (Aycock, 1966).

Mycelial growth was progressively less with increasing moisture content (Mustafee and Chattopadhyay, 1971b), and disease incidence was greater in well-drained, sandy soils (Weerapat and Schroeder, 1966) and at soil water contents below saturation (Ramarao and Raja, 1980). Punja (1985) also found that sclerotia fail to germinate

when the relative humidity is much below saturation, while it germinates best at relative humidity of 25-35%.

Growth and sclerotial formation were greater under continuous light especially blue light than in continuous darkness (Miller and Liberia, 1977). Humpherson and Cooke (1977) suggested that the sclerotial number of young (1-7 days old) cultures increased after exposure low light more than those of older cultures (8-14 days old).

Punja and Grogan (1981b) suggested that the optimum pH range for mycelial growth is 3.0-5.0, and sclerotial germination occurs between 2.0- 5.0. Germination of sclerotia is inhibited at a pH above 7.0 (Sharma and Kaushal, 1979).

Punja and Grogan (198la) have described two forms of germination, hyphal and eruptive. Hyphal germination is characterized by the growth of individual strands from the sclerotium surface; these hyphae originate from cells of the medulla (Chet *et al.,* 1977) but their growth is not extensive unless an external source nutrient is available. In contrast, eruptive germination is characterized by plug(s) or aggregates of mycelium bursting through the sclerotial rind. Sclerotia can germinate eruptively only once, since internal stored materials arc utilized during the growth of the mycelium. The rind is all that remains following eruptive germination, although in many cases secondary sclerotia form (Punja and Grogan 198la and Smith, 1972). Thus, reports of sclerotia germinating more than once (Smith, 1972) refer only to the hyphal form. Eruptive germination is induced by drying sclerotia (Punja and Grogan 198la. Smith, 1972), exposing them to volatile compounds (primarily alcohols and aldehydes) (Punja and Grogan 198la; Beute and Rodriguez-Kabana, I979a; Beute and Rodriguez-Kabana, 1979b; Linderman and Gilbert, 1973; Linderman and Gilbert, 1969), and to a lesser extent by brief sodium hypochlorite treatment (Punja and Grogan 198la; Linderman and Gilbert, 1973). Mature sclerotia germinate readily and appear to have a very brief, if any, dormancy period.

Both the amount of mycelial growth and the energy available for infection are affected by the form of sclerotial germination, which could have a significant bearing on infection and disease incidence (Punja and Grogan 1981b). Therefore, in ecological epidemiological studies, distinguishing between the two types of germination is extremely important. Germination should also be distinguished from

viability; in the latter case, presence or absence of mycelial growth is evaluated on nutrient media.

Eruptive germination on soil and agar is greatest at 21-30°C and is less common below 15°C and above 36°C. Germination is not significantly retarded by carbon dioxide levels in the range of 0.5-9%, oxygen levels between 15 and 20.5%, and ethylene concentrations in the range of l-40 ug/ml. Levels of carbon dioxide above 20% and oxygen below 3% are inhibitory to germination (Punja and Jenkins, 1984).

Although percentage of germination is lower at soil depths below 2.5 centimeters than it is at the soil surface and is nil below 8 centimeters, results from controlled gaseous studies suggest that the inhibition is not due to oxygen depletion or ethylene and carbon dioxide buildup (Punja and Jenkins, 1984). While this finding is consistent with the contentions of earlier investigators (Coley-Smith and Cooke, 1971; Griffin and Nair, 1968), it does not corroborate reports on the inhibitory effects of ethylene (Smith, 1976 and Smith, 1973). Physical pressure exerted by the weight of soil over deeply buried sclerotia could in part account for the inhibition of germination, since metal weights placed over sclerotia at the soil surface give similar results (Punja and Jenkins, 1984).

1.2.3 Epidemiology and Ecology of *Sclerotium rolfsii*

The fungus attacks all plant parts in contact with soil under favorable environmental conditions including stems, roots, leaves, and fruits (Farr *et al.,* 1989). Sclerotia serve as the principle primary inoculums for disease (Aycock, 1966), and may exist free in the soil near the soil surface or in association with plant debris (Backman and Brenneman, 1984). The mature sclerotia contain amino acids, sugars, fatty acids and lipids, and its wall contains chitin, laminarin, and β-1, 3 glucan (Mathur and Sarbhoy 1977, Jones *et al.,* 1972).

Disease incidence due to *S. rolfsii* may increase following periods of temperature and moisture fluctuations; cycles of drying and wetting have been reported to stimulate germination of sclerotia (Punja and Grogan, 1981b and Smith, 1972). Initial infection by the fungus occurs at the soil surface, where sclerotia are most likely to be stimulated to germinate by drying and remoistening (Punja and Grogan, 1981b).

Before the pathogen penetrates host tissue it produces a mass of mycelium on the plant surface, a process which can take 2-10 days (Punja, 1985, Punja and Grogan, 1981b). Penetration of host tissues occurs when the pathogen produces an enzyme, which deteriorates the host's outer cell layer (Sadana *et al.,* 1983).

S. rolfsii produces extracellular enzymes including pectin methyl esterase (Bateinan and Beer, 1965), cutinase (Baker and Bateman, 1978), phosphatidase (Kaveriappa, 1979), arabanase (Cole and Bateman, 1969), galatanase, mannanase, xylanase (Sadana *et al.,* 1980), oxalic acid and polygalacturonases (Bateman, 1972).

The presence of an organic substrate for mycelial growth, e.g. senescing leaves, may enhance disease severity (McCarter and Kays, 1984; Beckman and Finch, 1980; Beute and Rodriguez-Kabana, 1979b). Methods aimed at preventing the defoliation of peanut, which should have reduced disease if a food base is a requisite for infection, paradoxically, however, increased disease incidence (Shew and Beute, 1984; Backman *et al.,* 1975). It was postulated that preventing defoliation made microenvironmental conditions within the intact canopy more conducive to infection (Shew and Beute, 1984; Backman *et al.,* 1975). Alternatively, foliar fungicides may have reduced the levels of antagonistic *Trichoderma* spp. in soil (Backman *et al.,* 1975). On carrots, disease seldom is apparent until the canopy is fully developed, which also coincides with the time at which temperatures are optimal for growth of the pathogen (Punja, 1985). Cultural practices such as dirtying that bring the inoculum into closer contact with the host can enhance disease incidence (Gurkin and Jenkins, 1985; Aycock, 1966). While high temperatures and moist conditions both appear to enhance disease development regardless of whether an organic substrate is present, there have been no attempts to precisely monitor temperature and moisture in the field and relate them to disease incidence or severity. Such information could be useful in predicting when severe outbreaks of disease might occur.

Free moisture apparently is not required for infection (Punja and Jenkins, 1984). Extensive plant-to-plant (secondary) spread occurs in closely spaced crops such sugar beet and carrot and results in the formation of disease foci (Punja, 1985). In these crops, relatively few initial foci can result in extensive disease and failure to yield. In peanut, in which plant-to-plant spread is not extensive, yield is indirectly correlated

with an increase in the numbers of disease foci (Rodriguez-Kabana *et. al.,* 1975a). Host density and the proximity of roots may thus be major factors influencing the rate of disease progression.

The role of volatile compounds (in particular methanol) in initiating disease epidemics on peanut (Shew and Beute, 1984; Beute and Rodriguez-Kabana, 1979a) or on other crops is undetermined. Although many compounds result in greater mycelial growth rates in vitro (Punja *et al.,* 1984.), but their presence in soil at biologically active concentrations has not been demonstrated.

There is also no convincing evidence of the importance of biological or physical stress factors (Shew and Beute, 1984; Beute and Rodriguez-Kabana, 1979b; Minton *et al.,* 1975) in predisposing plants to infection by *S. rolfsii.* To establish whether stress is important in disease development, micro environmental conditions, in particular moisture, need to be more precisely monitored. The effects of low-moisture stress on the host should be carefully distinguished from its effects on the pathogen (Shew and Beute, 1984).

Disease incidence can be directly or indirectly affected by non-target pesticides. Disease may be more severe following applications of fungicides (Shew and Beute, 1984; Backman *et al.,* 1975) and a nematicide (Rodriguez-Kabana *et al.,* 1979.) or less severe following insecticide (Hackman and Hammond, 1981) nematicide (Rodriguez-Kabuna *et al.,* 1976) applications. The direct effects of many other pesticides (mainly herbicides) on *S. rolfsii* have been evaluated in culture media (Ereegovich *et al*., 1973; Bozarth and Tweedy, 1971; Rodriguez-Kabana *et al.,* 1969; Rodriguez-Kabana *et al.,* 1975b) and in soil (Rodriguez-Kabana *et al.,* 1979; Rodriguez-Kabana *et al.,* 1969; Curl *et al.,* 1968; Rodriguez-Kabana *et al.,* 1968). Continuous rotation with crops highly susceptible to *S. rolfsii* may increase disease incidence in subsequent years; a one-year rotation of corn has been reported to reduce inoculum density (Rodriguez-Kabana *et al.,* 1974).

Punja (1985) found that the survival of *S. rolfsii* is affected by abiotic and biotic factors. Exposure of sclerotium to temperature above 50 °C for long periods is lethal (Yuen and Raabe, 1984). Beute and Rodriguez (1981) found that the effect of temperature is modified by moisture; while survival of sclerotia is poor in moist than

in dry soil. Abiotic factors include drying (Smith, 1972), heating (Lifshitz *et al.,* 1983), deep burial (Punja *et al.,* 1984), exposing sclerotia to chemicals (Linderman and Gilbert, 1973) and inducing changes in the integrity of the sclerotial rind (Lifshitz *et al.,* 1983). Antagonistic microorganisms such as *Trichoderma* spp. (Henis and Papavizas, 1983) and *Aspergillus* can penetrate the rind and destroy the inner sclerotial tissues; for *Trichoderma,* this is facilitated by the production of the enzymes β-1, 3 glucanase and chitinase (Elad *et al*., 1984).

1.2.4 Symptoms of Foot Rot Caused by *Sclerotium rolfsii*

S. rolfsii primarily attacks host stems, although it may infect any part of a plant under favorable environmental conditions including roots, fruits, petioles, leaves, and flowers. The first signs of infection, though usually undetectable, are dark-brown lesions on the stem at or just beneath the soil level; the first visible symptoms are progressive yellowing and wilting of the leaves. Following this, the fungus produces abundant white, fluffy mycelium on infected tissues and the soil. Sclerotia of relative uniform size are produced on the mycelium roundish and while when immature then becoming dark brown to black. Mature sclerotia resemble mustard seed. The fungus occasionally produces basidiospores (the sexual stage of reproduction) at the margins of lesions and under humid conditions, though this form is not common (Agrios. 2005).

Seedlings are very susceptible and die quickly once they become infected. Older plants that have formed woody tissue are gradually girdled by lesions and eventually die. Invaded tissues are pale brown and soft, but not watery (Meah, 2007; Lievens *et al.,* 2004; Dutta and Das, 2002).

1.2.5 Control of *Sclerotium rolfsii*

Control of Sclerotium diseases is difficult and control efforts have often met with limited success, due in part to the extensive host range, prolific growth, and ability to produce large numbers of sclerotia that may persist in soil for several years. Furthermore, control measures effective for a particular crop area may not be adaptable elsewhere due to regulatory or economic constraints. Good cultural practices including rouging, eliminating weed hosts, and avoiding crop injury during

cultivation lower the disease incidence. A dense canopy increases disease incidence, thus increasing crop spacing can help keep infection down. A delayed planting date may also help reduce disease incidence if planting is timed so that the dense canopy forms after temperatures fall so that infection is not as likely. Also, keeping plant bases free of dead leaves (and weeds) will deny the pathogen a food source, helping to keep disease incidence down.

1.2.5.1 Cultural Control

Solar heating of moistened soils under polyethylene tarp is reported to reduce both sclerotial numbers and limit disease due to *S. rolfsii* (Mihail and Alcorn, 1984; Porter and Merriman, 1983; Elad *et al.,* 1980; Grinstein *et al.,* 1979b; Griffin, 1972). The pathogen was effectively eliminated from soil to a depth of 6-20 centimeters, depending on location and time of year (Mihail and Alcorn, 1984; Porter and Merriman, 1983; Elad *et al.,* 1980). Solar heating combined with *T. harzianum* application resulted in less disease than either method alone (Elad *et al.,* 1980). Another effective method of removing inoculum (sclerotia and infested crop debris) from the infectious zone (upper 8-12 centimeters) is deep plouwing (Gurkin and Jenkins, 1985; Punja *et al*., 1985). Early investigators recommended this method in conjunction with nondrying practices to control *S. rolfsii* on peanut. Plouwing may be done in the fall or in the spring prior to planting. On processing carrots, however, where low inoculum levels result in high disease incidence (Punja, 1985), plouwing alone has no effect on disease unless used in conjunction with fungicide or fertilizer applications (Gurkin and Jenkins, 1985; Punja *et al.,* 1985).

Since *S. rolfsii* has a wide host range and can persist on virtually all types of crop debris, crop rotation is not likely to be an effective or practical control method. On the other hand, rotating away from very susceptible crops to those not seemingly affected by the pathogen, such as corn or wheat, may result in less disease incidence in subsequent years by lowering inoculum levels. Adding organic amendments such as compost (Mathur and Sinha, 1970) or oat or corn straw (Gaulum and Kolte, 1979) to soil limits disease incidence, possibly due to the release of toxic NH_3 or to increases in levels of resident antagonistic soil microorganisms. This may be an economical

approach to disease control in small scale sustenance agricultural systems, but it is not likely to be practical in large scale production unless used in conjunction with crop rotation wherein the straw from a preceding cereal crop is disked under.

An approach that can minimize the effects of *S. rolfsii* on yield in areas where the availability of land is not limiting is to avoid planting in heavily infested fields. Several possible approaches to sampling fields to determine inoculum density have been described (Punja *et al.,* 1985a).

Although host resistance has not yet become a viable control measure for diseases caused by *S. rolfsii,* it may be used as a component of an integrated control effort in cases where some level of tolerance has been identified. Combining cultural with chemical or biological control may be the most economical and effective approach to control diseases caused by *S. rolfsii.*

1.2.5.2 Chemical Control

Numerous fungicides inhibit the germination of sclerotia or the mycelial growth of *S. rolfsii* (Agnihotri *et al.,* 1975; Anilkumar *et al.,* 1979; Brown and Hendrix, 1980; Fellman and Toumeau, 1983). A number of these have effectively controlled disease on various crops in the field (Agnihotri *et al.,* 1975; Brown and Hendrix, 1980; Punja *et al.,* 1982a; Punja *et al.,* 1982b). The major limitations to the widespread use of fungicides to control *S. rolfsii* are that large amounts of the chemicals usually are required, the efficacy of a particular compound may vary depending on the crop, and results may not be consistent from one growing season to another.

Fumigation of soils with methyl bromide, chloropicrin, or metham-sodium may limit disease incidence, since these compounds are toxic to sclerotia (Elad *et al.,* 1980; Munnecke *et al.,* 1982). The application of a general biocide such as potassium aside (Rodriguez-Kahuna *et al.,*1972) resulted in less disease, as do nematicides (Rodriguez-Kabana *et al.,*1976), insecticides (Backman and Hammond, 1981), herbicides (Grinstein *et al.,*1979a), but the usefulness (both practical and economic) of these compounds specifically to control, *S. rolfsii* has not been evaluated.

Disease incidence was lower in response to applications of ammonium fertilizer such as urea and ammonium bicarbonate (Bakr and Khan, 1981; Punja *et al.,* 1985a; Punja *et al.,* 1982a). These compounds could be used to control disease provided that the increased nitrogen levels are not detrimental lo crop growth or yield. Ammonium fertilizers may directly inhibit sclerotial germination and retard mycelial growth of *S. rolfsii,* especially if NH_3 is released (Punja and Grogan, 1982a), or they may indirectly limit disease by altering host susceptibility or populations of antagonistic soil microorganisms (Henis and Chet, 1968). Increased calcium levels in tissue following the application of calcium fertilizers such as calcium nitrate or calcium sulfate may provide some level of control under low disease pressure (Punja *et al.,* 1982a). Babar (1999) found that higher amount of vitamin-C and phenol in tissues of sunflower resulted lower infection by *S. rolfsii* than the varieties with lower amount of these compounds. Higher calcium levels in tissue may partially offset the action of oxalic acid and cell wall-degrading enzymes (Punja *et al.,*1982b). The application of hydrated lime, however, had no effect on disease (Punja *et al.,* 1982a).

1.3 Causal organism of *Fusarium* wilt

A survey of aubergine (eggplant) production conducted in Adana and Mersin, southern Turkey, in 2002 revealed leaf chlorosis and slight vein clearing on the outer leaflets followed by yellowing and dropping of leaves, discoloration of the stem and death of the above ground plant parts of some aubergine (eggplant) plants. Isolations were made from the discolored stem vascular tissue. A *Fusarium* species was the only fungus isolated. Single spore isolates were obtained and the culture characteristics and micromorphology were investigated. All isolates obtained were identified as *Fusarium oxysporum* due to the production of characteristic 3 to 5 septet, sickle shaped macro conidia with a foot shaped basal cell, ellipsoid micro conidia borne in false heads on short monophialides and chlamydospores. This is thought to be the first report of *F. oxysporum* f. sp. *melongenae* in Turkey (Altinok, 2005). *Fusarium* wilting in eggplant is caused by *Fusarium oxysporum* f. sp. *melongenae.* (Thurston, 1998; Miller *et al.,* 1996).

In 1989, plants of cv. Monstaro showed symptoms of leaf yellowing and wilting followed by death of the plant. *F. oxysporum* was isolated from the diseased plants and was highly pathogenic to the cv. Monstaro, Kyoryoku-beiju No.2 and Ponderosa (Kuwata *et al.,* 1994).

1.3.1 Symptoms of *Fusarium* Wilt

Fusarium wilting in eggplant includes leaf chlorosis and slight vein clearing on the outer leaflets followed by yellowing and dropping of leaves, discoloration of the stem and death of the above ground plant parts of some aubergine plants (Altinok, 2005). *Fusarium* wilting in eggplant causes wilting plus yellowing of leaves. The leaves turn lifeless immature or brownish red in tone plus bend upon the stems. They are not tumble off plus sojourn trustworthy to the plant (Thurston, 1998).

In eggplant, wilting are progresses from lower to upper leaves, followed by collapse of the plant. The symptoms of fungal wilt of eggplant infected by *F. oxysporum* f. sp. *melongeriae* as the yellowing of lower leaves that appears first, usually affecting the leaflets unilaterally. The affected leaves die and the symptoms continue to appear on successive younger leaves. The plant as a whole is stunted and commonly it eventually goes into permanent wilt of the leaves, which die as they cling of the upright woody stems (Agrios, 1997).

Fusarium wilt symptoms begin in tomato and potato as slight vein clearing on outer leaflets and drooping of leaf petioles. Later the lower leaves wilt, turn yellow turn die and the entire plant may be killed, often before the plant reaches maturity. In many cases a single shoot wilts before the rest of the plant shows symptoms or one side of the plant is affected first. If the main stem is cut, dark, chocolate-brown streaks may be seen running lengthwise through the stem. This discoloration often extends upward for some distance and is especially evident at the point where the petiole joins the stem. Potato tubers may show browning of the vascular ring as well as browning at the stem end and decay where stolon’s are attached. In pepper, lower leaves do not begin to wilt until roots and the base of the stem have already started to decay. Wilting of the entire plant soon follows. Dark brown, sunken, and eventually girdling cankers may be seen at the base of the pepper plant (Agrios, 1997).

Fusarium wilt starts out looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. On older plants, symptoms are more distinct between the blossoming and fruit maturation stages (Miller *et al.,* 1996).

Affected eggplants show yellowing of leaves that progressively wilt and die from bottom upwards. Woody stem and root tissue of diseased plants turn brown (Critchley, 1995).

In general, *Fusarium* wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988).

Browning of the vascular tissue is strong evidence of *Fusarium* wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Smith *et al.,* 1988).

The entire plant is stunted and the infected leaves do not abscise but remain attached to the stem (Beckman *et al.,* 1981).

1.3.2 Host range of *Fusarium oxysporum*

The pathogen is a soil-invader, mostly survives on plant refuses at mycelial or chlamydospiral stage (Singh, 2002).

Infection through the seed can occur at temperatures as low as 14°C. *F. oxysporum* f. sp. *asparagi* has a narrow host range as a pathogen but a broad host range as a parasite (Block and Bolbn, 1997).

Fusarium wilt disease occurs on cotton, pea, tomato, bean, melons, banana, cabbage spinach, and more than 60 other plant hosts, as well as sugar beet. *Fusarium* wilt is prevalent in tomato, eggplant and cucurbits. It might cause yield loss up to 10-50%

(Luckyanenco, 1991). Over twenty four *Fusarium* species have been reported to occur in Hawaii (Raabe *et al.,* 1981).

F. oxysporum is a common soil saprophyte that infects a wide host range of plant species around the world. It has the ability to survive in most soil arctic, tropical, desert, cultivated and non-cultivated (Snyder and Hansen, 1940).

Though *F. oxysporum* may be found in many places and environments, development of the disease is favored by high temperatures and warm moist soils. The optimum temperature for growth on artificial media is between 25-30°C, and the optimum soil temperature for root infection is 30°C or above (Russ, 1936).

1.3.3 Distribution of *F. oxysporum*

The fungus survives and may actually increase in a number of soil types for many years, independent of any host plants. This ability to survive eliminates any normal, rotation program or general sanitation as an effective control measure. *Fusarium* wilt is almost completely checked when the average soil temperature is below 61°F (16°C). The appearance and severity of the disease are increased when air and soil temperature averages rise above this point. The fungus grows most rapidly and the disease is most severe at temperatures from 80° to 85°F (26° to 29°C). Growth is inhibited at 90° to 95°F (32° to 35°C). Soil moisture and soil reaction (pH) have little effect on *Fusarium* wilt. However, soil nutrient status can critically affect symptom expression; potassium deficiency leads to a much intensified syndrome. The fungus invades the plant through the young rootlets or wounds in the older roots at transplanting time or later. The fungus then moves directly to the water conducting tissues (xylem) and then progresses up the stem into the leaves. The fungus colonizes the xylem tissues and does not invade other tissues until part or all of the plant dies. The fungus then produces its spores both inside and outside affected stems. Dissemination of the fungus takes place from seedbed to field or from one field or garden to another by infected transplants, infested soil clinging to transplants, farm equipment, plant refuse, animals, surface-drainage water, and wind. Occasionally, this is carried long distances in seeds. The fungus produces three kinds of microscopic spores: small, colorless, one-celled, oval to elliptical microconidia; large, slightly

curved, septet macroconidia and rounded, thick-walled chlamydospores that can survive long periods in the soil being resistant to unfavorable environmental conditions (Agrios, 2005).

1.3.4 Disease cycle of *F. oxysporum*

The *Fusarium* spp. that causes vascular wilts can be spread in soil, dust and irrigation water (Smith *et al,* 1988).

Wind, rain, farm equipment and decaying plant tissue can also help to spread the fungus. *Fusarium* is a soil inhabitant which over winters between crops in infected plant debris as mycelium and in its three spore forms. It can remain in the soil for long periods of time, including fallow periods. Healthy plants can become infected through their root tips; either directly, through wounds or at the point of formation of lateral roots (Agrios, 1988).

The fungus grows as mycelium through the root cortex intercellularly, ultimately advancing to the vascular tissue. As the mycelium continues to grow usually upward toward the stem and crown it branches and produces micro conidia. The proliferation of fungal growth in the plant's vascular tissue eventually causes the plant to wilt and die. The fungus can continue to grow on the decaying tissue where it can sporulate profusely. At this point, the spores can be spread to other plants or areas by wind, water or through the movement of soil (Agrios, 1988).

On occasion, the fungus can reach the fruit and contaminate the seed. This occurs when the soil moisture is high and the temperature is relatively low (Agrios, 1988).

In addition the vascular wilting, the fungus can infect other parts of the plant close to the soil to induce root, stem and corm rots. When seedlings are infected with *Fusarium,* damping-off may occur. If harvested fruits are contaminated with the fungus, post-harvest diseases such as "pink or yellow molds" on vegetables and ornamentals can develop. This is especially important on root crops (tuber and bulbs), as well as on low-lying crops like cucurbits and tomatoes (Agrios, 1988).

1.3.5 Soil-borne nature of *F. oxysporum*

Fusarium oxysporum is a common soil-borne pathogen and saprophyte that feeds on dead and decaying organic matter. It survives in the soil debris as mycelium and all spore types, but is most commonly recovered from the soil as chlamydospores (Snyder and Hansen, 1940).

Fusarium oxysporum spreads in two basic ways: it spreads short distances by water splash, and by planting equipment, and long distances by infected transplants and seeds. *F. oxysporum* infects a healthy plant by means of mycelium or by germinating spores penetrating the plant's root tips, root wounds or lateral roots. The mycelium advances intracellularly through the root cortex and into the xylem. Once in the xylem the mycelium remains exclusively in the xylem vessels and produce micro conidia (asexual spores) (Agrios, 2005).

The micro conidia are able to enter into the sap stream and are transported upward. Where the flow of the sap stops the micro conidia germinate. Eventually the spores and the mycelium clog the vascular vessels, which prevent the plant from up-taking and trans locating nutrients. In the end the plant transpires more than it can transport, the stomata close, the leaves wilt and the plant dies. After the plant dies the fungus invades all tissues, sporulates and continues to infect neighboring plants (Agrios, 2005).

1.4 Bio Control

Synthetic fungicides are currently used as primary means for the control of plant disease. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicide among fungal pathogens, and high development cost of new chemicals. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee *et al*., 2007). In agriculture, the crop loss due to plant pathogens has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agro-based products, increased resistance for chemicals in target pathogens and environmental pollution. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases. Crude extracts of

some well-known medicinal plants are used to control some of the plants pathogens (Kubo *et al*., 1981).

Many plant species therefore contain antifungal compounds. Bio pesticides are natural plant products that belong to the so called secondary metabolites, which include alkaloids, terpenoids, phenolics, and minor secondary chemicals. Plants are rich source of bioactive organic chemicals. It is estimated that the plants may contain as many as 4000,000 secondary metabolites (Mamun, 2011). Certain products derived from plant are uses for tea pest control. As many as 2121 plant species have been reported to possess pest control properties (Jacobson, 1989); 25 of these plants species possess the characteristics required for an ideal botanical insecticide and are therefore more promising for use in organic pest control programmes (Radhakrishnan, 2005). The anti-pest plants documented included, *Capsicum frutescens, Tagetes* spp, *Nicotiana tabacum, Cypressus* spp*., Tephrosia vogelii, Azadirachta indica, Musa* spp, *Moringa oleifera, Tithonia diversifolia, Lantana camara, Phytollacca dodecandra, Vernonia amygdalina, Aloe* spp*., Eucalyptus* spp*., Cannabis sativa, Cofea* species and *Carica papaya* (Mugisha-Kamatenesi *et al.*, 2008). Botanical pesticides are extracted from various plant parts (leaves, stems, seeds, roots, bulbs, rhizomes, unripe fruits and flower heads etc.) of different plant species. Botanical pesticides are hailed for having a broad spectrum of activity, being easy to process and use, having a short residual activity and for not accumulating in the environment or in fatty tissues of warm blooded animals, (Philip and Robert, 1998). Some plants have been scientifically tested and have been found to have good pesticidal properties. Botanicals like Bonkalmi, Bazna, Bishkatali, Datura, Durba, Eucalyptus, Ghora-neem, Hijal, Karanja, Mahogany, Marigold, Neem, Nishinda, Pithraj, and many others may be grown by farmers with minimum expense and extracted by indigenous methods (Mamun *et al.*, 2009). These botanical materials can be used as an alternative to chemical pesticides.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and

large number of diverse types of plants grows in different parts of the country. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al.,* 1998). The effects of plant extracts on bacteria have been studied part of the world (Reddy *et al*., 2001; Ateb and Erdoural, 2003). Much work has been done on ethno medicinal plants in india (Maheswari *et al.,* 1986: Negi *et al.,* 1993). Interest in a large number of traditional natural product has increased (Taylor *et al.,* 1996; Negi *et al.,* 1993). Medicinal plants were used as excellent antimicrobial agents because it possess a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of medical agents.

In biological control, living micro-organisms are employed as antagonists, parasites or predators (Kwok *et al.,* 1987). *Trichoderma* have gained considerable recognition as biological agent (Papavizas *et al.,* 1985). Several strains of *Trichoderma* have been found to be effective as biocontrol agent of various soil borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoctonia* etc. (Chet and Inbar, 1994). *Trichoderma* produces chemicals called trichodermin which is responsible for its antagonistic properties (Tverdyukov *et al.,* 1994). Thus, *T. harzianum* may ecofriendly be used as a bio control agent and the nature will relatively be undisturbed and many beneficial micro-organisms in the soil will be saved. This bio control agent has the potential to protect seedlings against several diverse plant pathogenic fungi. As a consequence, plant growth may be increased (Burr *et al.,* 1978 and Baker, 1988).

Little work has been done on the management of plant diseases with biocontrol microorganism in Bangladesh. Kashem (2005) reported that soil treatment with *Trichoderma harzianum* and *Trichoderma viride* was effective for controlling foot and root rot *(Fusarium oxysporum)* and collar rot *(Sclerotium rolfsii)* in the artificially inoculated field. Sultana and Hossain (1999) reported that root rot of lentil was decreased significantly under field condition when seeds were treated with *Trichoderma* spp. before sowing. Begum *et al.,* (1998) reported the antagonistic activity of *Trichoderma harzianum* against *Sclerotium rolfsii.* However, intensive

work on the management of soil-borne diseases with microbial antagonists had been carried out in India and other developed countries (Mukhopadhyay, 1987; Kehri and Chandra, 1991; Laha *et al.,* 1992; Lewis *et al.,* 1996; Roberts *et al,* 1997 and Lewis and Larkin, 1997).

The major limitation of biological control by *Trichoderma* spp. is the production of inoculum on large scale. To overcome the limitation, many researchers have worked on these problems. Papavizas and Lewis (1985), Kenny and Couch (1981) and Churchill (1982) have discussed the mass production of biocontrol agents. It has frequently been pointed out that if the biological control of plant pathogens is to be accomplished on the field scale it will be necessary to produce biocontrol agents in the form of spores or other propagules. The viable inocula must be produced in an inexpensive medium and the cost of production for treatment of large areas must be competitive with that of the chemical pesticides. They also suggested that the economic mass production of antagonists could be achieved by using readily available crude agricultural product. Various substrates have been used to produce inoculum, e.g., grain bran (Wells *et al.,* 1972), celadon and molasses (Backman and Rodriguez-Kabana, 1975), wheat straw (Akhtar, 1977), wheat bran (Hader *et al.,* 1979), cereal meal and sand (Mangenot and Diem, 1979), barley grain (Moity and Shatla, 1981), wheat bran and saw dust (Lewis and Papavizas, 1980), sand and corn meal (Lewis and Papavizas, 1980) and ten substrates, based on combinations of wheat bran, pulse bran, sugarcane bagasse, rice straw, wheat straw, cow dung, poultry manure, groundnut shell, and sawdust (Dubey and Patel, 2002). *Trichoderma* spp. need some incubation period for their establishment in soil to act as an antagonist.

1.5 Objectives of the research

Considering the fact described above, the present work was undertaken to achieve the following objectives.

1. To survey the different brinjal plants to identify the fungal diseases,
2. Isolation, purification, identification and characterization of the pathogenic fungi *Sclerotium rolfsii* and *Fusarium oxysporum* from brinjal plant,
3. To identify the Bangladeshi herbs, shrubs and trees having fungicidal activities,
4. To verify the effect of different plant extracts on the inhibition of fungal growth and germination of sclerotia of *Sclerotium rolfsii* and spore of *Fusarium oxysporum* and
5. To study the efficacy of *Trichoderma* spp. and *Rhizobium* spp. as bio-control agents against *Sclerotium rolfsii* and *Fusarium oxysporum* causing foot/collar rot and wilt diseases of brinjal.

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Chapter - 2

Antifungal Activity of Some Plant Extracts Against *Sclerotium rolfsii* Causing Foot Rot Disease in Brinjal

Abstract

Sclerotium rolfsii is an omnivorous aggressive soil borne pathogen infecting large number of plants at different categories. The present study was conducted to determine the efficacy of 26 plants extracts against *S. rolfsii.* Pure culture of *S. rolfsii* was isolated from foot rot of brinjal. The powdered plant materials were extracted using water and methanol. Antifungal activity was tested by Poisoned Food Technique. All plants were found to effective against the fungus and inhibitory activity from varied form 5.55 to 100% except *Phylanthus niruri and Annona squamosa* at 15% concentration of aqueous extract in 5 days incubation period. The leaf extract of *Nicotiana tabacum, L.camara, S. macrophylla and Corchorus capsularis* completely inhibited the mycelial growth of *S. rolfsii whereas* the leaf extract of *Clerodendrum viscosum* exhibited low inhibitory activity. Therefore, it is concluded that the plants selected in this study have promising future as alternatives of toxic synthetic fungicids. *In vitro* study revealed that aqueous extract of these plants was more effective than methanol extract.

Key words: Foot rot of brinjal, *Sclerotium rolfsii*, Aqueous extract, Methanol extract, Poisoned food technique, Antifungal activity.

2.1 Introduction

Sclerotium rolfsii Sacc. is ubiquitous soil borne pathogens with a broad host range (Sneh *et al*., 1995; Sarma & Singh, 2002). They survive forming resting structures that remain free or embedded in plant debris (Shlevin *et al*., 2003). *Sclerotium rolfsii* Sacc. is a soil borne polyphagous fungal pathogen distributed in tropical and sub-tropical regions of the world where high or warm temperature prevails. It attacks more than 500 species of plants in about 100 families **(**Aycock, 1966; Keyser, Ferreira, 1988; Mustafee, 2004; Stephen, Rebecca, 1992 Madhavi, Bhattiprolu, 2011**)** The fungus is characterized by white fluffy, branched, septate mycelium, and spherical or irregular shaped brown sclerotia, which range from 0.5-2.0 mm in diameter and at maturity, resemble mustard seed. The mycelium of *S. rolfsii* survives best in sandy soils, whereas, the sclerotia survive best in moist, aerobic conditions found at the soil surface (Punja,1985; Arunasri *et al*., 2011) Mycelia developed from sclerotia initiate infection causing pre or post emergence damping off (Bedendo, 1995). These fungi are known to cause diseases such as damping-off of seedlings, stem canker, crown blight, root, crown, bulb, tuber and fruit rots. Sclerotial diseases affect a wide variety of plants viz., vegetables, legumes, cereals, forage plants and weeds. *Sclerotium rolfsii* is an omnivorous, soil-borne pathogen causing various kinds of diseases in numerous crops worldwide in the tropics, subtropics, and other warm temperate regions. It has an extensive host range. Crops such as cocoyam, brinjal, tomato, chili, lentil, turmeric, ground nut, betel vine and many others are drastically infected by this fungus resulting in significant crop loss. *S. rolfsii* can maintain continuity of generation under adverse situation by the formation of sclerotia. The sclerotia constitute the primary inoculum and overwintering stage of *S. rolfsii*. The most distinctive effect of this pathogen is rottening of affected tissues that are directly attacked by the fungus. However, the mass of mycelium it produce, secretes oxalic acid as well as pectinolytic, cellulolytic, and some other enzymes which kill and disintegrates tissues before it actually penetrates the host (Farooq *et al*., 2010). Once established in the plants, the fungus progress and generate both mycelium and sclerotia quite rapidly, especially at high moisture and high temperature i.e. between 30 and 35°C (Agrios, 2005).

Eggplant suffers from many diseases. Ahmed and Hossain (1985) and Meah (2003) listed a number of diseases of eggplant caused by fungi, bacteria, virus, nematode and mycoplasma. Of them, collar rot caused by *Sclerotium rolfsii* is damaging to the crop. Collar rot of eggplant caused by *Sclerotium rolfsii* occurs at any growth stage of the plant (Begum *et al.,* 1985). Existence and occurrence of the disease has been reported from South Eastern Nigeria by Nwufo and Onysagba (1989) and from India by Sugha *et al.* (1990). *Sclerotium rolfsii,* the causal agent of foot rot or collar rot of many crops (Aycock, 1966) having a wider host range (Talukdar, 1974; Battacharrya *et al.,* 1977) attracted the attention of plant pathologist and professional researcher throughout the world. The pathogen is known to cause diseases of cereals, pulses, oil crops, potatoes, vegetables, ornamentals, and nursery seedlings of fruits and forest trees.

In Bangladesh, diseases caused by *S. rolfsii* in different crops have been reported among many others by Miah (1973), Talukdar (1974) and Meah and Khan (1987). The wide host range, fastidious growth and ability to produce persistent sclerotia contribute to their involvement in losses of economic crops (fruits and vegetables) and difficulty in their control. The management of disease is based on the use of synthetic fungicides which are not so beneficial because the fungus displays strong ability to survive in soil through the formation of spherical sclerotia (remain free or embedded in plant debris) that have strong resistance to chemicals. Besides, these chemical fungicides are costly and often suffer from several other drawbacks such as development of resistance in fungal pathogens, residual effect, destruction of non-target organisms and toxic effects on humans. In Benin, the only registered fungicide used on edible crops, such as cowpea, is Super-Homai 70% PM (active ingredient: methylthiophanate 35%, thiram 20%, and diazinon 15%) (SPV, Benin). Unfortunately, there is a problem regarding the efficacy of this product (Kakpo-Zannou, Pers. comm.). Despite the effectiveness of synthetic fungicides, there are potential harmful effects on human health and the environment (Demoz and Korsten, 2006). Hence, search for alternative disease control strategies are utmost needed. Plants, their extracts and plant based formulations have shown to be promising alternates for synthetic fungicides as they have shown to possess marked inhibitory activity against *S. rolfsii* (Kwon and Park, 2002; Barakat *et al*., 2006; Khanzada *et al.,* 2006; Singh *et al*., 2007; Nwachukwu and

Osuji, 2008; Jagtap and Kamble, 2010; Osemwegie *et al*., 2010; Pattanapipitpaisal and Kamlandharn, 2012; Darvin, 2013; Amin *et al.,* 2013; Rivera *et al.,* 2013).

Foot rot and damping-off diseases are major cause of economic loss in vegetables and crops throughout the globe. Maximum countries are still using very much toxic pesticides despite their harmful effects. They have no alternatives. Thus, there is a need to search for alternative approaches to protection of crops from fungal diseases. Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Much work has been done on ethno medicinal plants in India (Maheshwari *et al*., 1986; Negi *et al.,* 1993). Plants extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Satish *et al*., 1999, Okigbo and Ogbonnaya, 2006; Shariff *et al.,* 2006; Bouamama *et al.,*2006; Ergene *et al*., 2006; Kiran and Raveesha, 2006; Mohana , 2006). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al*., 2008). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different zone of the world (Reddy *et al*., 2001; Ateb and Erdourul, 2003). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Arokiyaraj *et al*.,2008; Gangadevi *et al*., 2008; Satish *et al.,* 2008; Brinda *et al*., 2009; Jagadish *et al*., 2009; Milind Panda *et al*.,2009; Shanmugavalli *et al*., 2009; Swarna Lata and Neelakanta Reddy, 2009; Vetrivel Rajan *et al*.,2009).

As alternative to synthetic fungicides, plant extracts with fungicidal properties could be used (Stoll, 1988; SIBAT, 1993; Obagwu *et al*., 1997). Moringa oleifera leaf extracts have been successfully used as a seed treatment against some soil borne fungi in cereals (Stoll, 1988). The difficulties with plant extracts are that the active ingredients break down easily, thereby reducing persistence of the compound (Adandonon *et al*., 2006). Panda *et al.,* (1996) tested the efficacy of leaf extracts from *Polyalthia longifolia, Aegle mermelos, Azadirachta indica, Carthemus roseus, Ocimum sanctum* and *Allamanda cathertica* for control of *Phomopsis* blight (caused by *P. vexans).* Leaf extract of *Allamanda cathertica* had excellent potential as a fungicide. *Allium* species have a widespread use in folk medicine (Keusgen *et al*., 2006). Their antimicrobial properties have been studied on human pathogens (Aala *et al*., 2010), food

degrading fungi (Yin and Tsao, 1999) and poisonous mushrooms (Auger and Thibout, 2005). Zeidan *et al*., (1986) found that the inclusion of onion (*A. cepa* L.) in crop rotation significantly reduced losses caused by *S. rolfsii* in subsequent productions. Onion bulb extracts inhibited the growth of *R. solani* and *Sclerotium* sp. (Navrekar and Patil, 1986; García and Padilla, 1994; Sindhan *et al.,* 1999). Plant extracts are eco-friendly, display structural diversity and complexity and infrequently contain halogenated atoms. (Duke *et al.,* 2000).Protective, curative and antagonistic activity of different plants against variety of diseases has been reported by several workers (Kanda *et al*., 1974; Hale and Mathers, 1977; Rahber-Bhatti, 1986; Kalo and Taniguchi, 1987). The present study was ascertain to investigate the inhibitory effect of leaf extracts of various plant species on the mycelial growth of *S. rolfsii under in vitro* conditions.

Therefore, the current experiment was carried out with the following objectives.

- To survey of different brinjal host plant,
- To identify the fungal disease in brinjal,
- Isolation, purification, identification and characterization of pathogenic fungi from brinjal,
- To identify the herbs, shrubs and trees of Bangladesh having fungicidal activities and
- To evaluate the efficacy of selective fungicides plant extracts against *S. rolfsii*.

2.2 Materials and Methods

2.2.1 Experimental site

All the experiments were conducted during July 2011 to June 2014 in IES - Laboratory, Rajshahi University and Plant Pathology Laboratory, Dept. of Botany, Rajshahi University. The pot house and field experiment was committed out in Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

2.2.2 Collection of isolates

The *Sclerotium rolfsii* isolates were collected from different brinjal fields of Rajshahi area during January to June, 2012 from infected brinjal plant. Then the specimens were taken to the Plant Pathology Laboratory of BARI and were subjected to the process of isolation.

2.2.3 Origin of Isolates

Sclerotium rolfsii were collected from the diseased plants of brinjal *(Solanum melongena* L.).

2.2.3.1 Method of Sterilization and Incubation

After collection the diseased plant materials were brought to the laboratory as soon as possible so that stems are still fresh during the isolation of the pathogen. In the laboratory, advancing or water soaked portion of the lesion were cut into small sections (approximately 5x5 mm per section) and sterilized the surface by soaking for 15 seconds in 5% commercial Clorox (5.25% sodium hypochlorite by weight). This was done to kill the microorganisms or pathogens on the surface of the stem tissues that may complete with the growth of *S. rolfsii.* 5.25% sodium hypochlorite solution contain in a beaker, surface sterilization of materials was continued for 15 seconds 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 materials was removed by gently pressing them between to 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. In the early practices, the sterilized materials were placed over the moist filter paper. 2-3 glass bars (90 mm x 2 mm x 2 mm) were placed on the moist filter paper of the humid chamber, which prevented them from coming in contact with the moisture of the filter papers. 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.

2.2.3.2 Preparation of Humid Chamber

The humid chamber used for incubation of the diseased plant parts in Petri plate (90 mm dia.) with lids. Before use of, the petri dishes were sterilized by flaming. The linear walls of the petri dish and its lid were lined previously with sterilized filter paper, which were then moistened with sterilized distilled water. The excess water was poured out and the plate with its lid was used as humid chamber. Desired number

of diseased materials from each of the samples lots collected from the field was incubated using a separate humid chamber.

2.2.4 Isolation of The *Sclerotium rolfsii*

Isolation of *Sclerotium rolfsii* from diseased eggplant plants noted above was made following either (i) by direct method or (ii) by plant method.

2.2.4.1 Direct Method of Isolation

The method consisted of sterilization and incubation of the diseased plant parts in humid chamber following the technique described before. The affected plant parts were incubated for 15 days at 28±2°C. During this period, the fungus was found to grow mycelia and sclerotia on the necrotic areas. The structure of sclerotia could be seen easily under steroscopic binocular microscope and from the mycelia and sclerotia were collected on a very small agar block (about 2 mm in length), mounted at the tip of a flamed spearheaded tungsten needle. Sclerotia were collected by touching them with the agar block and could be seen lying attached to transparent surface of agar, which was then transferred to a freshly prepared PDA plate. After incubation fungal colonies were found to develop and free from contamination.

2.2.4.2 Plating Method of Isolation

In this period of isolation, diseased plants parts were cut into small pieces, about 0.5 cm in length, in such a manner so as to include both healthy and necrotic tissues in each piece. The pieces were then washed in running tap water, sterilized in 5.25% sodium hypochlorite solution and washed repeatedly for several times in sterilized distilled water to remove sodium hypochlorite solution. Three pieces were taken out using flamed forceps and dried between filter papers and finally transferred to a PDA plate. Plates were incubated at 28±2°C for 15 days, during which period the fungal colonies appeared on the PDA plates. Often the colonies were found to become contaminated due to unwanted growth of bacteria and other fungi. No doubt *Sclerotium* species was obtained in pure form but not so as it was with the direct method. Moreover, this method was found to be time consuming and it required

further transfer to get pure culture. Where as in the direct method pure colony of *Sclerotium* could be obtained by single transfer. Unless otherwise stated direct method has been used throughout this work for the isolation of *Sclerotium* species (Plate 2.1).

2.2.5 Identification of Isolated Fungus

The fungus isolated from infected tissues of stem on brinjal plant 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 Subramanian (1971).

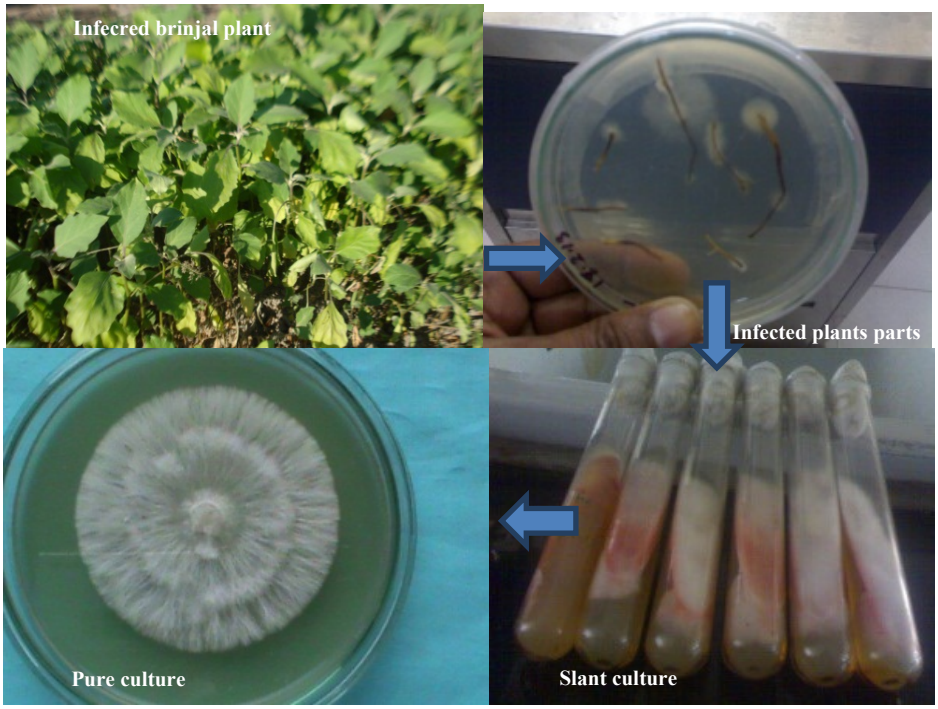

Plate 2.1 Collection, isolation, purification and identification of *S. rolfsii*

2.2. 5.1 Pathogenicity Tests

2.2.5.1.1 Raising of Host Plants

For pathogenicity test, the host plants are grown, in earthen pot (9 inches) containing loam soils. Five to ten seedlings were raised in each pot from treated seed of a particular verity of eggplant. The hosts of *Solanum melongena L.* were inoculated with a dense sclerotial suspension of *Sclerotium rolfsii* its stem by artificial inoculation.

2.2.5.1.2 Preparation of Sclerotial Suspension

Sterilized distilled water was used for the preparation of sclerotial suspension. Sclerotia were washed in sterilized distilled water separately from 10-16 days old culture of *S. rolfsii* grown on PDA. At first, 20 ml water was added on a plate and shaken well to remove maximum number of sclerotia in it. Using about 4-5 plates a total of 100 ml of sclerotial suspension was prepared for each culture. Before being used the density of sclerotia in water was adjusted to $40x10^4$ - $50xl0^4$/ml from haemocytometer count.

2.2.5.1.3 Method of Inoculation

Seedlings of most plants (in eggplant) were inoculated by spraying a sclerotial suspension with an atomizer. The inoculated plant was covered with polythene bags and incubated at 28±2°C for two days. Plant was then transferred to the pot house. 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 host plant had developed characteristic rot and compared with the maturity-developed symptoms recorded before.

2.2.5.1.4 Re-isolation of Pathogens

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

2.2.6.1 Preparation of Lactophenol

Lactophenol solution used as mounting medium consisted of the following composition (Aniswarth, 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.

2.2.6.2 Preparation of Lactopiienol 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 lactorphenol-cotton blue were stored in a cool dark place.

2.2.6.3 Preparation of Slides

A suitable portion of culture of *Sclerolium rolfsii* on from PDA plate was selected under a stereoscopic binocular microscope and was taken out with the help of forceps and needles and put in 1 or 2 drops of lactophenol of clean slide. It was then gently waned 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.

2.2.6.4 Preparation of Clearing Reagent

Throughout the present investigation corning glassware's and chemicals supplied by E. Merck 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 ml

2.2.6.5 Preparation of 0.1% Mercuric Chloride ($HgCl_2$) Solution

Mercuric chloride ($HgCl_2$) solution is used for surface sterilization of the diseased sample.

Mercuric chloride	l00.00mg
Distilled water	100.00ml

Shaken well till it was dissolved.

2.2.6.6 Preparation of Culture Media

The following culture media were used for laboratory.

(a) Potato Dextrose Agar (PDA) medium:

Peeled and sliced potatoes	200.00 g
Dextrose	I5.00g
Agar	20.00 g
Distilled water	1000ml

The liquid media were solidified with the addition of 2% agar, wherever required. All solid media were autoclaved at 15 lb/inch^2 pressure for 20 minutes. In case the medium contained any substance liable to decomposition or denaturation ion, 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 inoculum was taken from 7-10 days old culture and size of the inoculum was the same in all cases. After inoculation the culture were incubated at 25±2°C for 15 days for mycelial growth and sclerotia formation.

2.2.7 Control Measure

In order to find out the control of mycelial growth of *Sclerotium rolfsii* different fungicides and plant extracts were used in *in vitro.*

2.2.7.1 Method of studying *in vitro* Fungicidal effect

The effect of fined amount (2 g/100 ml) of two fungicide's namely; Bavstin and Provax on mycelial growth and sclerotia formation of *S. rolfsii* was tested in this experiment. The medium was sterilized at 15 Ib/ins in 121°C. The experiment was setup in laboratory. 20 ml of media were poured after sterilization under specific condition in sterilized 90 mm Petri dishes and inoculated as described earlier. The measurements of radial growth of the colony were taken at intervals of 24 hours and days of first's sclerotia formation and total sclerotia at final day of incubation were recorded and expressed in mm.

2.2.7.2 Collection of Plant Materials

The plant materials of twenty six species (Table1) were collected from different places in Rajshahi University campus and Rangpur district. The collected plants were identified and authenticated by a botanist in the Department of Botany, University of Rajshahi, Bangladesh.

2.2.8 Method of Studying Plant Extracts Preparations:

2.2.8.1 Preparation of powder from leaves and seeds:

The selected parts (leaves and seeds) of different medicinal plants were cut into small pieces and dried in shade at room temperature for15 days. Dried leaves and seeds were grinded to produce powder using an electric grinder. The powders were put in plastic containers with tightly fitted lids, which were kept in the laboratory before use. The powders were used, within one week of preparation.

2.2.8.2 Methanol Extract:

Finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using sox let apparatus. For extraction, 50 gm. leave and seed powder of each of the selected plants was transferred into separate conical flasks containing 200 ml of methanol and mixed well. The flasks were left for two days with occasional stirring. The extracts were then filtered (Whatman No.1 filter paper), concentrated in vacuum under reduced pressure and dried in the desiccator (Manasa *et al.*, 2013). These crude extracts were kept under low temperature (4°C) in the refrigerator until use.

2.2.8.3 Aqueous Extract:

After collection, the leaves were washed in distilled water clearly and surface sterilized by rubbing with absolute ethyl alcohol (95%) soaked sterilized absorbent cotton. Then it washed thoroughly with sterilized water. The solutions of the materials were prepared by blending 100g of leaves in 100 ml of sterilized water. The sap thus extracted was first passed through four layers of muslin cloth and then filtered through Whitman's filter paper No.1 in 250 ml Pyrex flasks. The flasks were tightly wrapped with aluminum foil. The extracts were used for screening their antifungal activity.

2.2.8.4 Dose used in the experiment:

Four types of concentration viz. 0%, 5%, 10%, 15% and 20% of water and methanol extracts were prepared during this study by dissolving 5mg of botanical extract in 100 ml PDA media. Dry methanol extract was dissolved in DMSO before adding to PDA media.

2.2.9 Mycelial Growth Inhibition Test /Antifungal Activity of Selected Plants:

2.2.9.1 *In vitro* tests with water leaf extracts:

In order to determine the inhibitory potential of extracts against mycelial growth of *S. rolfsii*, we employed Poisoned Food Technique (Mishra and Tiwari, 1992, Nene and Thapliyal, 1993, Basher *et al.* 1991). Here, Potato dextrose agar (HiMedia, Mumbai) was prepared, poisoned with extracts. To obtain 5 percent plant extracts medium, 95 ml PDA was poured in 100 ml sterilized conical flask and 5 ml of plant extract was poured in each flask with the help of sterilized pipette. Desired concentration of individual plant extracts 5%, 10% and 15 % (v/v). After thorough mixing with plant extracts the medium was autoclaved and approximately 20 ml of melted PDA mixed with extracts was poured into each 90 mm petri dish. The pH of the medium was adjusted to 6.5 by using pH meter with the help of 1N HCl or 1N NaOH. After solidification, the plates were inoculated by placing 5 mm mycelial disc of *S. rolfsii* from the periphery of 7 days old culture and sclerotia from one month culture. Sterile Potato dextrose agar (HiMedia, Mumbai) medium containing streptomycin (to prevent bacterial contamination).The inoculums (5 mm mycelial disc of *S. rolfsii*) were placed upside down on agar media at the Centre of the petriplates and incubated at 25±1ºC. Control plates maintain without plant extract. The plates were arranged in Completely Randomized Design (CRD) with three replications. Mycelial Growth of the fungus was measured by taking the diameter in two directions and the average was recorded at an interval of 24 hours. Final growth reading was recorded when the growth of the fungus in control plate was full. Then these plates were kept for 30 days for sclerotia formation. Inhibition of radial growth was computed based on colony diameter on control plate using the specified formula (Sultana *et al*., 2012):

$$\% \text{ Inhibition} = \frac{x - y}{x} \times 100$$

Where,

X = Average growth (cm) of *S. rolfsii* in control petri dishes.

Y = Average growth (cm) of *S. rolfsii* in each plant extract and biocides treated petri dishes.

After 30 days the sclerotia of each petri dish were separated by using camel hair brush and number of sclerotia of each petri dish was counted manually. Here a petri dish was maintained as control to compare it with others.

Average radial growth of the fungus (cm) after 3days, 5days and 7days was computed from the data recorded and per cent inhibition of radial growth over control was calculated using the average value. The data were subjected to statistical analysis following CRD (Gomez *et al.,* 1984)

2.2.9.2 *In vitro* tests with methanol extracts:

The methanolic extracts of thirteen different plant extracts (Table 2.6) were screened for antifungal activity by "Poisoned Food Technique" at three different Concentrations (5mg/100ml, 10mg/100ml and 15mg/100ml of medium) with three replicates were inoculated with fresh 7 days old culture of *Sclerotium rolfsii*. About 5 mm agar disc of fungal culture was prepared with the help of sterile cork borer and kept upside down on agar plates, these plates were incubated at 25 ± 1ºC. Plates with DMSO served as control and standard (Bavistin 5µg/ml) was maintained in agar plate. The colony diameter in mutual perpendicular directions was measured using a ruler. Antifungal activity of extracts (in terms of inhibition of mycelial growth) was calculated using the formula:

Mycelial growth inhibition (%) = (x-y/x) × 100, where 'x' is average colony diameter (CD) in control plate and 'y' is average colony diameter in poisoned plates (Kambar *et al*., 2014). The experiment was repeated two times and the result was presented as Mean ± Standard deviation.

2.3 Results and Discussion

2.3.1 Efficacy of botanicals on mycelial growth of *Sclerotium rolfsii*

2.3.2 Aqueous extract

Antifungal activity of twenty six botanical extracts was assayed and data on effect of plant extracts on the growth of *Sclerotium rolfsii* is presented in Tables (2.1 & 2.2). The data revealed that significant reduction in growth of *Sclerotium rolfsii* was observed with extracts of twenty six medicinal plants and the extracts showed significant differences in their efficacy. Among all the twenty six plant aqueous extracts, 85% plants showed inhibition of mycelial growth, over control.

Table 2.1 Antifungal activity of the plant extracts against *Sclerotium rolfsii*

Botanicals		**Mycelial Growth (cm) of *S. rolfsii***						
Local Name	Scientific name	Control	5%	% inhibition	10%	% inhibition	15%	% inhibition
Datura	*Datura stramonium*	9.00±.00a	5.06±.06b	47.90	4.03±.03c	58.89	1.06±.06d	88.67
Alamanda	*Allamanda cathartica*	9.00±.00a	5.40±.10b	54.69	3.63±.06c	67.15	1.26±.06d	88.59
Babla	*Acacia nilotica*	9.00±.00a	9.00±.00a	00	4.12±1.90b	68.88	.72±.04c	89.22
Hazarmony	*Phyllanthus niruri*	9.00±.00a	9.00±.00a	00	9.00±.00a	00	9.00±.00a	00
Telachcha	*Coccinia cordifolia*	9.00±.00a	9.00±.00a	00	7.52±.13b	27.11	5.36±.05c	44.77
Vat	*Clerodendrum viscosum*	9.00±.00a	9.00±.00a	00	8.71±.28a	3.77	9.00±.00a	5.55
Akando	*Calotropis procera*	9.00±.00a	9.00±.00a	00	9.00±.00a	17.66	9.00±.00a	31.66
Ata	*Annona squamosa*	9.00±.00a	9.00±.00a	00	9.00±.00a	00	9.00±.00a	00
Lantana	*Lantana camara*	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100
Mahagony (Seed)	*Swietenia macrophylla*	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100
Jute (Seed)	*Corchorus caspsularis*	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100
Khoksha	*Ficus hispida*	9.00±.00a	9.00±.00a	19.84	7.30±.23b	49.48	1.92±.14c	100
Tobacco	*Nicotiana tabacum*	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

Efficacy of botanicals on mycelial growth of *S. rolfsii* varied with different botanicals at different concentration viz., 5%, 10% and 15 % (Tables 2.1 & 2.2). At 5 percent concentration. *Lantana camera, Swietenia macrophylla* (seed), *Corchorus capsularis* and *Nicotiana tabacum* recorted complete inhibition (100%) in mycelial growth followed by *Allamanda cathartica* (54.69%). *Datura stramonium* (47.90%) and *Lawsonia inermis* (44.88%) and minimum/least inhibition was recorded in *Aegle marmelos* and *Cajanus Cajun*(0.77%) while *Polygonum orientale, Clerodendrum visccosum, Acacia nilotica, Phyllanthus niruri, Coccinia cordifolia, Allium sativum, Calotropis proecra* and *Annona squamosa* showed no any efficacy (Table 2.3 & Fig. 2.1).

Table 2.2 Antifungal activity of the plant extracts against *Sclerotium rolfsii*.

Botanicals		**Mycelial Growth(cm) of *S. rolfsii***						
Local name	Scientific name	Control	5%	% inhibition	10%	% inhibition	15%	% inhibition
Chirota	*Swertia chirata*	9.00±.00a	9.00±.00a	3	8.66±.08a	23	7.31±.20b	54.88
Nishinda	*Vitex negundo*	9.00±.00a	9.00±.00a	1.88	8.93±.06a	3.33	8.83±.08a	6.33
Soj	*Carum roxburghianum*	9.00±.00a	9.00±.00a	5.22	8.76±.03b	16	8.33±.08c	24.11
Polygonum	*Polygonum orientale*	9.00±.00a	9.00±.00a	00	9.00±.00a	3.77	8.80±.05b	6
Mehedi	*Lawsonia inermis*	9.00±.00a	9.00±.00a	44.88	8.76±.14ab	68.55	8.70±.05b	88.88
Tulsi	*Ocimun sanctum*	9.00±.00a	8.83±.08a	12.66	8.50±.25a	15.22	7.63±.32b	41.11
Ganda	*Tagetes patula*	9.00±.00a	9.00±.00a	8.22	8.66±.16a	20	6.36±.72b	46.33
Holde hurhurae	*Cleome viscosa*	9.00±.00a	9.00±.00a	4.11	8.66±.08a	15.55	7.46±.17b	42.22
Bell	*Aegle marmelos*	9.00±.00a	9.00±.00a	.77	9.00±.00a	1.11	8.66±.08b	10
Arhar	*Cajanus cajan*	9.00±.00a	9.00±.00a	.77	9.00±.00a	2.66	8.66±.12b	13.33
Neem	*Azadirachta indica*	9.00±.00a	9.00±.00a	2.66	9.00±.00a	6.33	2.73±.31b	83
Apang	*Achyranthes aspera*	9.00±.00a	6.40±.11b	36.61	5.13±.13c	48.15	2.76±.03d	76.93
Garlic	*Allium sativum*	9.00±.00a	5.40±.10b	00	3.23±.14c	6.11	1.06±.06d	89.22

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

Similar trend was recorded at 10 per cent concentration. *Lantana camera, Swietenia macrophylla* (seed), *Corchorus capsularis* (seed), and *Nicotiana tabacum* recorded total/complete/maximum inhibition (100%) in mycelial growth, followed by *Acacia nilotica* (68.88%), *Lawsonia inermis* (68.55%) and *Allamanda cathartica* (67.15%) and minimum/least inhibition was recorded in *Aegle marmelos* (1.11%). *Phyllanthus niruri* and *Annona squamosa* did not show activity at 15 percent concentration (Plate 2.2). *Lantana camera, Swietenia macrophylla* (seed), *Corchorus capsularis* (seed), *Nicotiana tabacum* and *Ficus hispida* recorded complete inhibition (100%) in mycelial growth followed by *Acacia nilotica* (89.22%) and *Allium sativum* (89.22%) and *minium* inhibition was recorded in *Clerodendrum viscosum*(5.55%). At all the concentration, there is significant difference between the treatments (Fig. 2.1 & Appendices Table-2.3)

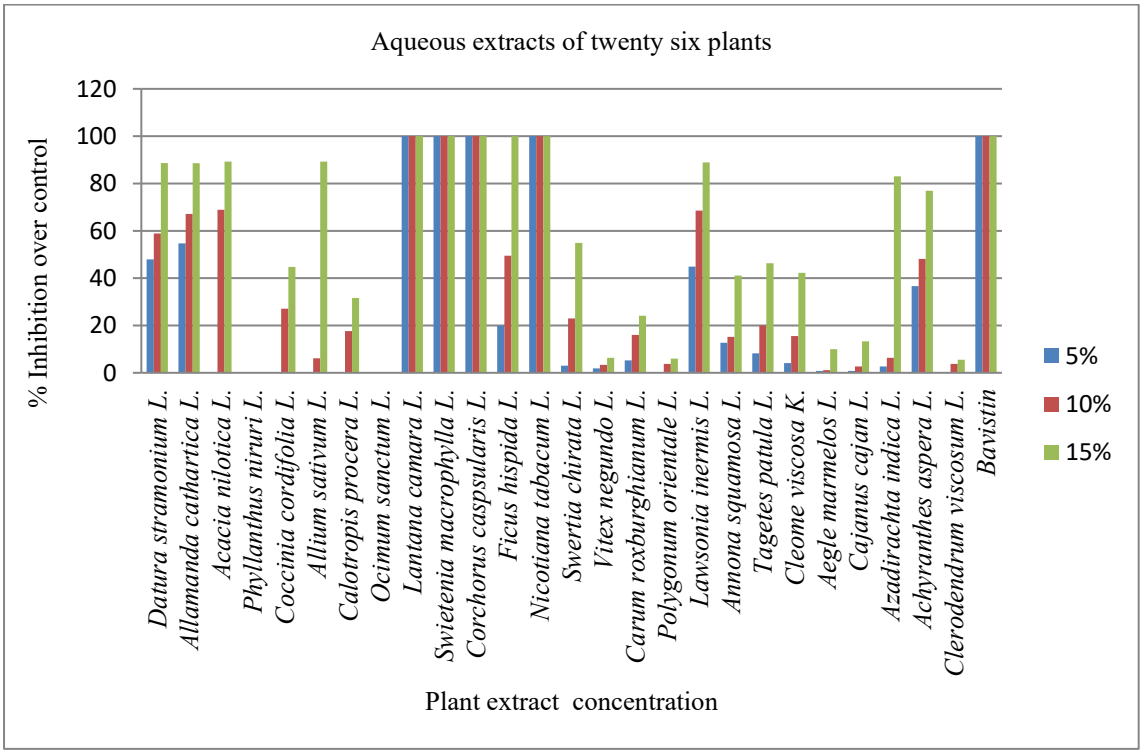

Fig. 2.1 Aqueous extract of twenty six plants against plant pathogenic fungus- *S. rolfsii.*

However, there is no significant difference among *Lantana camera, Swietenia macrophylla* (seed), *Corchorus capsularis*(seed), *Nicotiana tabacum and Ficus hispida* at 15 percent concentration. As a whole, extract of *Lantana camera, Swietenia macrophylla*(seed), *Corchorus capsularis* (seed) and *Nicotiana tabacum* were found highly effective and inhibited the mycelial growth of the test fungus. The findings are in agreement with other researchers. Satish *et al*., (2007) tested the aqueous extract of 52 plants from different families for their antifungal potential against eight important species of *Aspergillus* such as *Aspergillus candidus*, *A. columnaris*, *A. flavipes, A. flavus*, *A. fumigatus, A. niger, A. ochraceus,* and *A. stamarii*. Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum, Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Sygigium cumini* have recorded significant antifungal activity against one or the other *Aspergillus* species tested. *Aspergillus flavus* recorded high susceptibility.

Aqueous extract of 30 plants namely *Allium sativum, Solanum nigrum ,Clerodendron infortunatum , Lawsonia inermis, Azadirachta indica* etc. were tested for their antifungal potential against *Ganoderma lucidium* (Curtis ex. Fr.) isolated from arecanut. Among the plant species tested, *Allium sativum* corn extract exerted maximum inhibition followed by extract of *Solanum nigrum* (chakrabarty *et al.,* 2014).

Garlic was found to be fungi toxic to a number of plant pathogen (Iyer *et al*., 2004, Gowda and Nambiar, 2006, Chakrabarty *et al*., 2013). Crude extract of different plant parts of *Solanum nigrum* obtained using solvents viz., petroleum ether, chloroform, acetone, ethanol and methanol showed that leaf aqueous extract was more active against all the microbes tested (Ramya *et al.,* 2012). In another study , Farooq M.A. *et al.,* (2010) expressed that aqueous extract of twenty different plant species inhibited mycelial growth of *Sclerotium rolfsii* causing sclerotium rot in sugar beet but the leaf extract of *Azadirachta indica* showed maximum inhibition. Aqueous leaf extract of *Allium sativum, Datura alba* and *Withana somnifera* inhibited the growth of *Alternaria alternate, A. brassicola* and *Myrothecium roridum* (Mughal *et al*.,1998). Jalal and Ghaffar (1992) used aqueous plants extracts of *Allium cepa* (onion),

Calotropis procera (Akk), *Chenopodium album* (Bathu), *Chenopodium murale* (Karund), *Azadirachta indica* (Neem) and *Cannabis sativa* (Bhang) for antifungal activity against *Macrophomina phaseolina*, *Alternaria radicina, Helminthos-porium tusricum* and *Ascochyta rabiei*.

Among the plant oils and plant extracts, karanja oil (88.94%) and Murraya exotica leaf extract(86.15%) were found effective in reducing the growth of *S .rolfsii* (Mahato, A. *et al*., 2014). The present findings were also corroborated with the earlier report made by S. Masuduzzaman *et al.,* (2008). Allamanda extract in water has been found to be very much effective against nursery diseases. This was established by Khan (1999) and others. Hawlader (2003) reported that seed treatment with allamanda leaf extracts effectively increased germination of eggplant seeds and tremendously decreased nursery diseases. This has conformity with the findings of Panda *et al*. (1996) who reported leaf extracts of *Allamanda cathertica* as an excellent potential fungicide for control of nursery diseases. Aqueous leaf extract of *Lawsonia inermis* is completely inhibited the growth of *Malassezia* than methanolic and chloroformic extracts (Berenji, F. *et al*., 2010). Sanguri, S. *et al*., (2012) found antimicrobial property of aqueous extract of *Achyranthus aspera*. Yeni I.J. (2011) revealed that cold water extracts of *Allium sativum* and *Nicotiana tabacum* have proved effective against mycelial growth of *B. theobromae*. Taiga *et al.,* (2008) showed that *N. tabacum* cold extract inhibited the mycelia of *F. oxysporum* yam rot organism. The active principle present in plants are influenced by many factors which include the age of plant extracting solvent method of extraction and time of harvesting plant materials (Quasem and Abu – blan 1996: Okigbo *et al.,* 2005; Amadioha and Obi 1999; Okigbo and Ajalie 2005). The presence of fungicidal compounds in *A. sativum* and *N. tabacum* which caused the inhibition of mycelia growth in vitro agreed with the reports of other investigators (Okigbo and Nmeka 2005, Quasem and Abu-blan, 1996 and Amadioha 2000). Rahmoun *et al*., (2013) observed antifungal activity of *Lawsonia inermis* leaf extract against filamentous fungi. Aqueous extract of *L. inermis* leaves was tested for antifungal activity against eight important isolated species of *Aspergillus* from sorghum, maize and paddy seed samples. *A. lavus* recorded high susceptibility and hence solvent extracts, viz., petroleum ether, benzene, chloroform, methanol and ethanol extract of the plant showed significant

antifungal activity (Raveesha *et al.,* 2007). Mohan and Raveesha, 2007) reported that among the eight plants tested for their antifungal activity against *Fusarium solani* and *Aspergillus flavus.* 50% concentration of *Decalepis hamiltonii* aqueous extract totally inhibited both storage and field fungi. All the tested *Nicotiana tabacum* aqueous extract concentrations and 100% concentration of *Acacia indica* completely inhibited radial mycelial growth of *Fusarium oxysporium*(Taiga *et al*., 2008). Bowers, *et al.,* 2000 reported that *Syzygyum aromaticum* (clove) produced good results with other soil borne fungus such as *Fusarium oxysporum.*Varaprasad Bobbarala *et al.,* 2009 reported that significant reduction in growth of *Aspergillus niger* was observed with extracts of forty three medicinal plants and the extracts showed significant differences in their efficacy. Aggarwall *et al.,* 2000 reported antimitotic activity of *cymbopogon martinii* against *Aspergillus niger.*

Table 2.4 Effect of combinations of Tobacco extract with several fungistatic extracts at different concentrations on *Sclerotium rofsii*.

Extracts combination and concentration (%)	Mycelial growth (cm)	Number of Sclerotia /plate (14days)
N. tabacum/*S. macrophylla* (20)	.00 ± .00 d	.00 ± .00 c
N. tabacum /*S. macrophylla* (15)	.00 ± .00 d	.00 ± .00 c
N. tabacum /*C. capsularis* (20)	.00 ± .00 d	.00 ± .00 c
N. tabacum / *C. capsularis* (15)	.00 ± .00 d	.00 ± .00 c
N. tabacum /*L. camara* (20)	1.33 ± .16 c	.00 ± .00 c
N. tabacum / *L. camara* (15)	1.86 ± .06 c	27.33 ±6.38 c
N. tabacum /*A. cathartica* (20)	.97 ± .03 c	142.0 ± 24.9 b
N. tabacum / *A. cathartica* (15)	1.30 ± .10 c	136.66 ± 12.7b
N. tabacum /*D. stramonium* (20)	.93 ± .06 c	135.00 ± 14.0 b
N. tabacum/ *D. stramonium* (15)	1.31 ± .15 c	172.33 ± 12.12 b
Bavistin (2g/L)	.00 ± .00 d	.00 ± .00 c
Control	9.00 ± .16 a	309.13 ± 14.0 a

In each column, numbers followed by the same letter are not significantly different according to Duncun multiple range test (P = 0.05)

Table 2.5 Effect of combinations of Babla extract with several fungistatic extracts at different concentrations on *Sclerotium rolfsii*.

Extracts combination and concentration (%)	Mycelial growth (cm)	Number of Sclerotia /plate (14days)
A. nilotica/ *S. macrophylla* (20)	.00 ± .00 d	.00 ± .00 c
A. nilotica / *S. macrophylla* (15)	.00 ± .00 d	.00 ± .00 c
A. nilotica / *C. capsularis* (20)	.60 ± .10 bc	.00 ± .00 c
A. nilotica / *C. capsularis* (15)	.90 ± .05 b	.00 ± .00 c
A. nilotica / *N. tabacum* (20)	.00 ± .00 d	.00 ± .00 c
A. nilotica / *N. tabacum* (15)	.00 ± .00 d	.00 ± .00 c
A. nilotica /*D.stramonium* ((20)	.56 ± .13 bc	136.0 ± 7. 6 c
A. nilotica /*D.stramonium* ((15)	.75 ± .07 bc	164.3 ± 15.4 b
A. nilotica / *A. cathartica* (20)	.53 ± .08 c	128.3 ± 26.6 b
A. nilotica / *A. cathartica* (15)	.71 ± .12 bc	153.66 ± 17.8 b
Bavistin (2g/L)	.00 ± .00 d	.00 ± .00 c
Control	9.10 ± .12 a	320.39 ± 20.3 a

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

When the extracts (Fungicidal and Fungistatic) were combined by pairs different types of action were observed (Table 2.4 and 2.5). Antagonistic effect when the combination lost the fungicidal effect of one of the extracts this was observed in all combination between *N. tabacum*(Tobacco) - *L. camara*(Lantana), *N. tabacum*(Tobacco) - *D.stramonium* (Datura), *N. tabacum*(Tobacco) - *A. cathartica* (Allamonda) as well as single fungicidal effect when the combinations retained the fungicidal effect, this was observed in *N. tabacum* (Tobacco) - *S. macrophylla* (Mahogany) and *N. tabacum* (Tobacco) - *C. capsularis* (Jute) showed fungicidal effect against *Sclerotium rolfsii* (Table 2.4). Likewise antagonistic effect when the combination lost the fungicidal effects but delayed mycelial growth this was observed between *A. nilotica* (Babla) - *C. capsularis* (Jute). Otherwise, *A. nilotica* (Babla) - *S. macrophylla* (Mahogany) and *A. nilotica* (Babla) - *N. tabacum* (Tobacco) showed fungicidal effect. Besides that *A. nilotica* (Babla) - *D.stramonium* (Datura) and *A. nilotica* (Babla) - *A. cathartica* (Allamonda) combination of extract increase antagonistic effect was observed against *Sclerotium rolfsii* (Table-2.5). Montes-Belmont and Prados-Lingero, 2006 had showed similar results about *Pachirhyzus erosus* and *Connamomum zeylanicum* extract on *Sclerotium cepivorum.* A study done by Suprata and Khalimi (2009) showed that the plant extract formulation containing a mixture of *E. aromatica* and *Piper betle* extracts significantly suppressed the radial growth of *F. oxysporum* f. sp. vanilla on PDA medium, and in the soil of vanilla seedlings. Concentration of 6% and more of the two extracts in combination were able to cause complete growth inhibition of the tested fungus (Nehal and Rokayah, 2009). Tobacco leaf extract in cow's urine was more effective in both of mycelial growth inhibition and sclerotia formation (Amin *et al.,* 2013). Bhardwaj (2012) carried out test of aqueous extract of twenty plants for their antifungal activity against *Fusarium solani,* the causal organism of dry rot disease of potato. The combined leaf extracts of *Lawsonia inermis* and stem extracts of *Acacia catechu* in general showed a strong enhancement in activities over the individual extract of each against the mycelial growth of the fungus.

It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of extracts. It also supports the earlier investigation (Banso and Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume

pharmacological importance. R. Montes *et al.,* 2006 reported that aqueous extract of *Pimenta dioica* (Allspice), *Syzygyum aromaticum* (clove) showed a fungicidal effect while *Medicago sativa* (Alfalfa), *Piper nigrum* (Black pepper), *Connamomum zeylanicum* (cinnamon), *Rosmarinus officinalis* (Rosemary), *Pachirhyzus erosus* (Yambean), *Petroselinium crispum* (Parsley), *Origanum vulgare* (Marjoram), *Teloxys ambrosioides* (worm seed) and *Pithecellobium dulce* (Guamuchil) reduced growth rate of *selerotium cepivorum* and effects on sclerotial production. It also supports presents studies.

2.3.3 Methanolic Extract on *Sclerotium rolfsii*

All the medicinal plant extracts of *Acacia nilotica, A.indica, Nicotiana tabacum, Lantana camara, Swietenia macrophylla, Corchorus capsularis, lawsonia inermis, Ocimum sanctum, Allium sativum, Datura stramonium, Allamunda cathartica, Clerodendrum viscosum and Ficus hispida* at 5% .10% & 15% inhibited mycelial growth to different degrees (Table-2.6). Different species of fungi isolated and identified to be associated with disease of brinjal. In case of *Sclerotium rolfsii* methanolic extract of *Nicotiana tabacum, Lantana camara, Swietenia macrophylla, Ocimum sanctum* showed complete inhibition of mycelial growth. *A. nilotica, Allium sativum, Datura stramonium, Allamunda cathartica* showed moderate and *Corchorus capsularis* showed low inhibition of mycelial growth in all concentration after five days of incubation at 5% concentration. Rest of the plants were less effective in reducing the mycelial growth of *Sclerotium rolfsii* in comparison to *Nicotiana tabacum, Lantana camara, Swietenia macrophylla, Ocimum sanctum.* Maximum inhibition recorded at 15% concentration in all treatments. Highest percent inhibition observed was 100 % (.00±.00) and lowest per cent inhibition observed was 30.78% (6.16 ±.12).

The extract of plant *Acacia nilotica* showed complete inhibition at 10% and 15% concentrations (Table 2.6 and Fig. 2.2). The control plate representing DMSO did not exhibit inhibition on the tested fungi where as standard antifungal drug bavistin have antifungal activity even at 2mg/L.

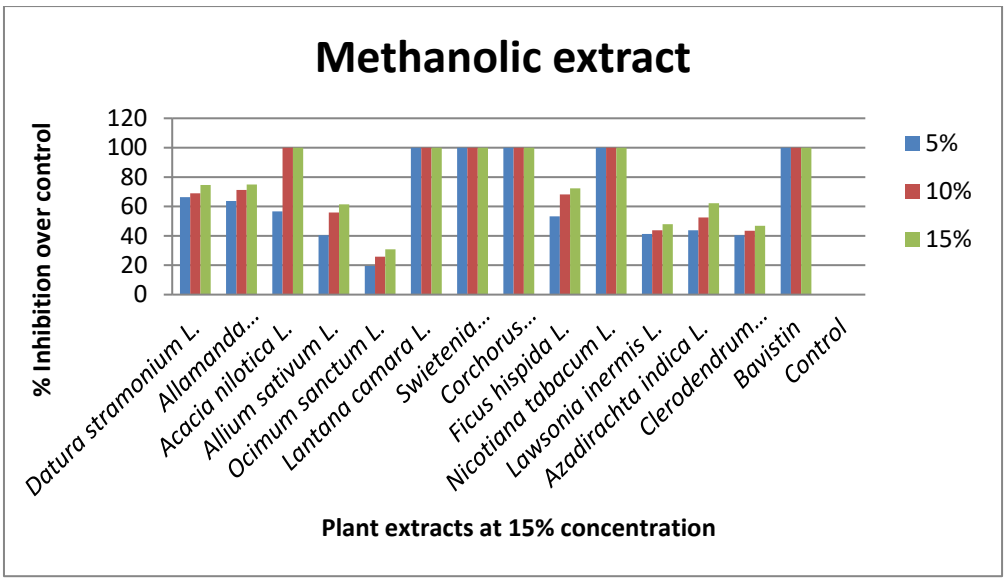

Fig.2.2 Methanolic extracts of thirteen plant against plant pathogenic fungus *S. rolfsii.*

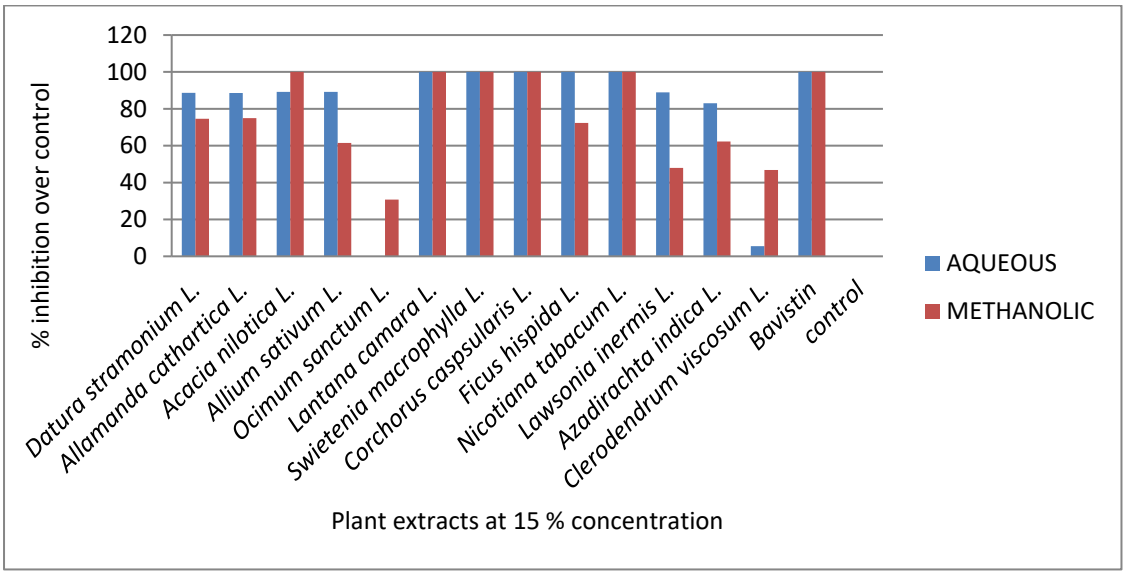

Fig.2.3 Aqueous and Methanolic plant extracts against *S. rolfsii*.

In particular, the authors may recommend that the methanolic extract of *Acacia nilotica, Nicotiana tabacum, Lantana camara, Swietenia macrophylla and Ocimum sanctum* to be used as potent biocide to treat diseases in plants caused by *Sclerotium rolfsii* as it showed maximum activity even at lower concentrations nearly equal to the standard antifungal agent (Fig. 2.3). It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts (Fig. 2.2 and 2.1). It also supports the earlier investigation (Banso and Adeyemo, 2007) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Extensive bioprocess parameter studies should be undertaken for the methanolic extract of *Acacia nilotica, Nicotiana tabacum, Lantana camara, Swietenia macrophylla* and *Ocimum sanctum* as a strong antifungal agent against *Sclerotium rolfsii* causing brinjal diseases (Fig. 2.2 & 2.3).

The present study, the tested plant extract showed antifungal activity against *Sclerotium rolfsii* damping off and foot/collar rot disease in brinjal field. The activity can be positively correlated to the dose, as there is decreased radial growth of fungi with increased dose. These plant extracts showed less antifungal activity when compared with standard antifungal Bavistin (2g/L) except *Nicotiana tabacum, Lantana camara, Swietenia macrophylla, Ocimum sanctum and Acacia nilotica.*

The efficacy of different plant extracts belonging to different species other than the tested botanical extracts against the pathogenic fungi either under laboratory, greenhouse conditions or pot house have been reported Tegegne *et al.,* 2008; Anyanwu and Dawat, 2005; Chutia *et al*., 2009; Anand *et al*., 2009; Zaker *et al*., 2010; Jagtap and Kamble (2010) reported that 10% alcoholic leaf extracts of *Melia azedarach, Clerodendrum inerme, Hyptis suaveolens* and *Swietenia macrophylla* were completely inhibited growth of both the sensitive and resistant isolates of *Sclerotium rolfsii.* Methanolic leaf and seed extract of all above plants were effective and some of them completely inhibited growth of *Sclerotium rolfsii*. (Table 2.6 and fig. 2.2 & 2.3). The findings are in agreement with other works Misra and Dixit (1977b) observed antifungal activity of *Allium sativum* leaf extract against eleven pathogenic fungi. Dubey and Dwivedi (1991) found fungicidal property of *Allium cepa* against *Macrophomina phaseolina.* Leaf extract of *Ocimum sanctum* is quite effective in

reducing the growth of *Rhizoctonia solani* (Tiwari and Nayak, 1991). Dargan and Saxena (2003) observed the leaf extract of *Withania somnifera* had potentiality to control *Aspergillus niger* causing fruit rot of tomato. 10% alcoholic leaf extract of *Semecarpus anacardium, Azadirachta indica* and *Commiphora stoksiana* completely inhibited the growth of both the sensitive and resistant isolates of *Fusarium oxysporum* f. *spinaceae* (Bhale *et al.,* 2005). Among the fifteen plants tested for their antifungal activity against *Alternaria solani* causing early blight of tomato, the leaf extract of *Allium sativum* showed maximum inhibition of spore germination and biomass production in the test fungus (Gachande, 2009).

Efficacy of 24 extracts of 18 plants (belonging to 13 botanical families) of Western Ghats of Shivamogga district, Karnataka, India against *S. rolfsii* isolated from foot rot of finger millet. All plants were effective against the fungus but to a varied extent (8.92 to 100%) have been reported (Kekuda *et al*., 2014). Extract of *M. malabaricum* was more effective against *C. capsici* when compared to extract of *M. talboltianum* (Vivek *et al.,* 2014). The study of Hullatti and Rai (2004) showed the antifungal efficacy of methanol extract of leaves of *M. malabaricum*. Tatiya *et al.* (2014) found dose dependent antifungal effect of stem bark extracts of *P. macrantha*. Sahu R. K. *et al* .,(2012) reveled the potential of *A .conyzoides, A. indica ,A. marmelos, P. pinatta* extracts to control the alternaria canker, earlyblight, leafspot, fruitspot, blossom end rot and sunscold disease of tomato. The efficacy of leaf extracts of *Taphrosia puepurea* and *Catharanthus roseus* against *Macrophomina phaseolina* and *Sclerotium rolfsii* the casual agents of root rot diseases of chickpea plants respectively were studied (Wadikar *et al.,* 2010). In this study, it is found that the antifungal activities of methanolic extract of leaf of *Lantana camara, Swietenia macrophylla, Nicotiana tabacum, Acacia nilotica, Ocimum sanctum* were significantly active against the tested organism, while *Corchorus capsularis* extract does not show good activities against tested organism. Rest of the plant extracts showed moderate activities. There are some articles indicating more effectiveness of plant extracts performed with pure solvents such as ethanol in comparison with aqueous extracts, for instance Hassanien *et al.* (2008) screened ethanol, ethyl acetate extracts and aqueous extracts of neem and chinaberry against two fungal pathogens and found that ethanol and ethyl acetate extracts of these plants suppressed the growth of *F. oxysporum* and *A. solani* in comparison to aqueous extracts which were less effective. In connection Shirzadin *et al.* (2009) compared antifungal properties of ethanol, petroleum ether and

water extracts of some plants against some pathogenic fungal pathogens including *Alternaria alternata* and found highest antifungal activity in ethanolic extract. For the present study different plants were selected on the basis of some reports such as Patel *et al.* (2007) screened the antimicrobial activity of weed *Lantana camara* extract; in this report it showed antifungal activity against *Aspergillus niger* and *Aspergillus awamori.* Similar in a study conducted by Sharma and Kumar, (2009), they reported that *Lantana camara* showed antifungal potential against *F. oxysporum*. Srivastava and Singh (2012) confirmed that ethanol and acetone extract of *Lantana camara* can be used as bio fungicide to control this phytopathogenic fungus . Prabhahar *et al*. (2012) reported that the extract of *Turnera subulata* showed maximum zone of inhibition against fungal pathogens when compared to *Acacia nilotica*. Hossain *et al.,* (2014) reported that fruit extract of *Ficua racemosa* linn (methanolic extract) exhibited both antimicrobial activity and antifungal activity against the tested microorganism and fungi. Prince *et al.,* (2011) reported that chloroform, ethanol and aqueous extract of eight different plants against *Colletotrichum falcatum. Vitex negundo* showed maximum antifungal activity. David *et al*. (2009) suggested that the ethanol and methanol extracts of the leaves of *Achyranthes aspera* Linn revealed a significant scope to develop a novel broad spectrum of antifungal herbal formulation. Evidentely Mathur *et al*. (2011) reported that hydro-alcohol extract of *Valeriana jatamansi,Coleus barbatus, Berberis aristata, Asparagus racemosus, Andrographis paniculata, Achyranthes aspera, Tinospora cordifolia* and *Plantago depressa* showed maximum antifungal activity against *Aspergillus niger* and *candida albicans*. Similarly Sule *et al*. (2010) evaluated the antifungal activity of *Senna alata linn*. Crude leaf extract exhibited moderate activity against *Microsporum canis, Trichophyton jirrucosum, T. mentagrophytes* and *Epidermophyton jlorrcosum*. Abera *et al*. (2011) reported that the antifungal potential of aqueous and ethanol extracts of eight different plants species *in vitro* and *in vivo* against *Colletotrichum kahawae* in completely randomized design with three replication. The extracts were from *Hogenia abyssinica, Allium sativum, Phytolacca dodcandera, Croton macrostachyus,* Al-Hazmi, (2013) reported that the water and ethanolic seeds and leaves extracts of *A. indica* inhibited the growth of the studied six pathogenic fungi at different percentages. Ambikapathy *et al.* (2011) reported that among five different medicinal plants the methanolic extracts of *Lawsonia inermis* showed maximum antifungal activity against *Pythium debaryanum.*

Table 2.6 Efficacy of methanolic plant extracts on radial growth of *Sclerotium rolfsii.*

S. No	Botanicals (Scientific name)	Control	5 % Mycelial growth (cm)	Percent inhibition over control	10 % Mycelial growth (cm)	Percent inhibition over control	15 % Mycelial growth (cm)	Percent inhibition over control
1	*Datura stramonium* L.	8.90±.10a	3.00±.05b	66.29	2.76±.23bc	68.98	2.26±.16c	74.60
2	*Allamanda cathartica* L.	8.90±.10a	3.23±.06b	63.70	2.56±.16c	71.23	2.23±.03c	74.91
3	*Acacia nilotica* L.	8.90±.10a	3.86±.13b	56.62	.00±.00c	100	.00±.00c	100
4	*Allium sativum* L.	8.90±.10a	5.30±.10b	40.44	3.93±.06c	55.84c	3.43±.21d	61.46
5	*Corchorus caspsularis*	8.90±.10a	7.16±.16b	19.55	6.60±.11c	25.84	6.16±.12d	30.78
6	*Lantana camara* L.	8.90±.10a	.00±.00b	100	.00±.00b	100	.00±00b	100
7	*Swietenia macrophylla* L.	8.90±.10a	.00±.00b	100	.00±.00b	100	.00±.00b	100
8	*Ocimum sanctum* L.	8.90±.10a	.00±.00b	100	.00±.00b	100	.00±.00b	100
9	*Ficus hispida* L	8.90±.10a	4.16.08b	53.25	2.83±.38c	68.20	2.46±.41c	72.35
10	*Nicotiana tabacum* L.	8.90±.10a	.00±.00b	100	.00±.00b	100	.00±.00b	100
11	*Lawsonia inermis* L.	8.90±.10a	5.23±.13b	41.23	5.00±.00b	43.82	4.63±.03c	47.97
12	*Azadirachta indica* L.	8.90±.10a	5.00±.00b	43.82	4.23±.18c	52.47	3.36±.06d	62.24
13	*Clerodendrum viscosum* L.	8.90±.10a	5.30±.20b	40.44	5.03±.23b	43.48	4.73±.16b	46.85
14	Bavistin	8.90±.10a	.00±.00b	100	.00±.00b	100	.00±.00b	100

In each column, numbers followed by the same letter are not significantly different according to Duncun multiple range test (P = 0.05)

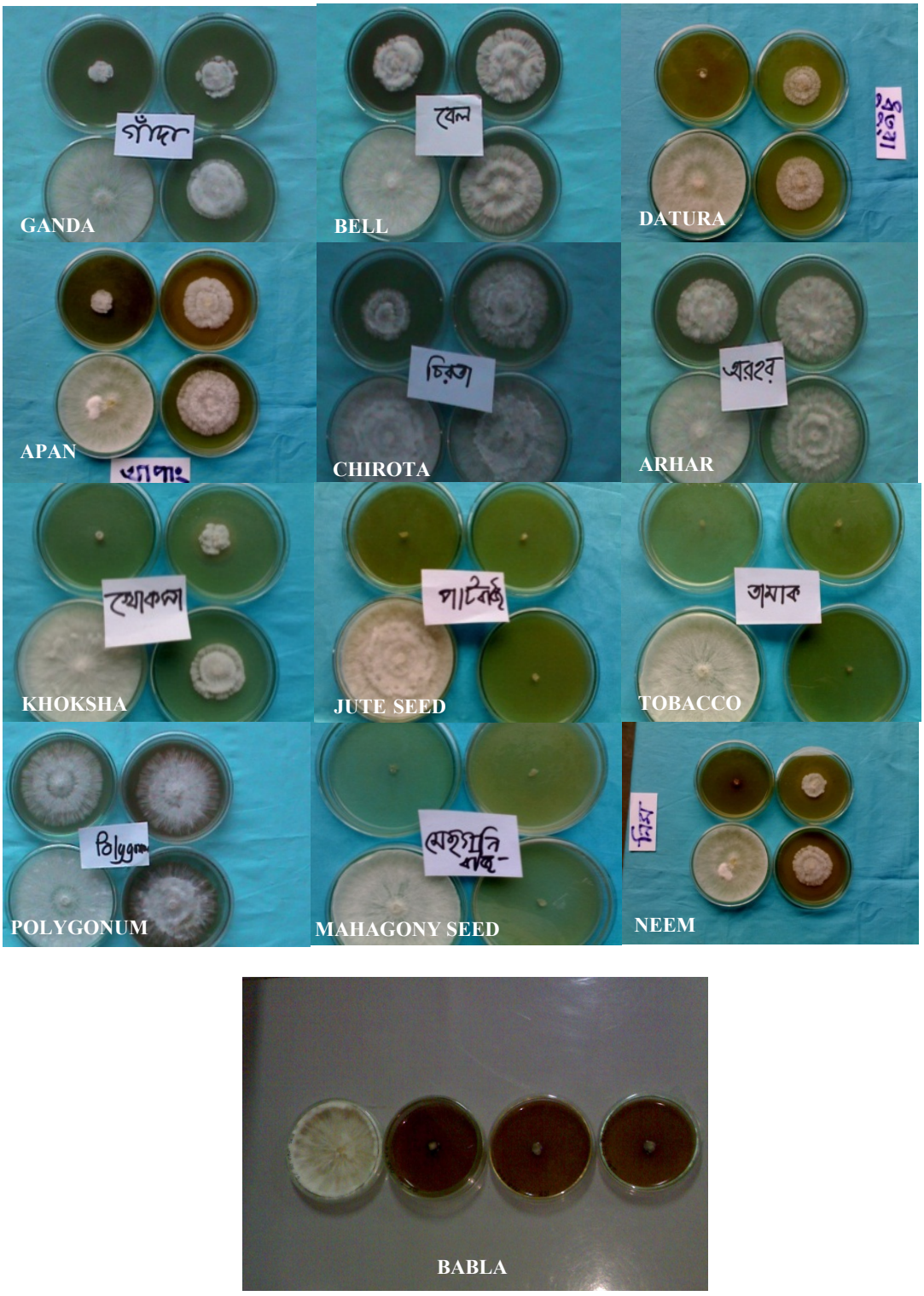

Plate 2.2 Effect of plant extracts on *Sclerotium rolfsii* in PDA media

2.4 Conclusion

The use of natural fungicides of plant origin is one of the best alternatives for synthetic chemicals which have detrimental effect to human and environment. In the present study, 26 plant extracts exhibited inhibitory activity to varied extent against foot rot causing fungus *S. rolfsii*. These plants can be exploited as natural fungicides against foot rot. Further field experiments are required to ascertain the possible role of these botanical extracts for the management of foot rot disease of brinjal.

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Chapter - 3

Effect of Botanical Extracts on Germination of Plant Pathogenic Sclerotia *(Sclerotium rolfsii)*

Abstract

This study was conducted in Plant Pathology Lab, Bangladesh Agriculture Research Institute (BARI), Joydebpur during 5th February- 23rd May, 2013 to find out the effect of botanical extract on sclerotial germination of plant pathogenic fungi *(Sclerotium rolfsii).* Aqueous extracts of 13 plants (datura, lantana, babla, tulsi, akondo, garlic, jute seed, mahogany seed, tobacco, telachucha, alamonda, hazarmony and khoksha) were tested in sterile soil and PDA media against the sclerotial germination. Out of 13 extracts, *Acacia nilotica* (babla), *Datura stramonium* (datura) and *Swietenia macrophylla* (mahagony) were found to inhibit *in vitro* germination of sclerotia of *Sclerotium rolfsii* compare to control. Twenty percent concentration of *Acacia nilotica* (babla) extract showed a high inhibition against sclerotial germination in both PDA and sterilized soil. A total inhibition of sclerotial germination was observed when combination of *Acacia nilotica* with *Swietenia macrophylla* (20%) and *Acacia nilotica* with *Datura stramonium* (20%) were used.

Key words: Plant extracts, sclerotia, inhibition, germination.

3.1 Introduction

The fungus is characterized by white fluffy, branched, septate mycelium, and spherical or irregular shaped brown sclerotia, which range from 0.5-2.0 mm in diameter and at maturity, resemble mustard seed. The mycelium of *S. rolfsii* survives best in sandy soils, whereas, the sclerotia survive best in moist, aerobic conditions found at the soil surface (Punja, 1985; Arunasri, *et al.,* 2011). Sclerotia having a internally differentiated rind, cortex and medulla (Punja and Rahe 1992). Mycelia developed from sclerotia initiate infection causing pre or post emergence damping off (Bedendo, 1995). *Sclerotium rolfsii,* the causal agent of foot rot or collar rot of many crops (Aycock, 1966) having a wider host range (Talukdar, 1974; Bhattacharrya *et al.,* 1977) attracted the attention of plant pathologist and professional researcher throughout the world. The pathogen is known to cause diseases of cereals, pulses, oil crops, potatoes, vegetables, ornamentals and nursery seedlings of fruits and forest trees.

In Bangladesh, diseases caused by *Sclerotium rolfsii* in different crops have been reported among many others by Miah (1973), Talukdar (1974) and Meah and Khan (1987). *S. rolfsii* causes serious damping off to seedling resulting plant stand percentage is very low (Bertus, 1929). In every year, 60% seedling death caused by this disease, especially in eggplant seed plot. Collar rot of eggplant also caused by *S. rolfsii* occurs at any growth stage of the plant (Begum *et al.* 1985). Existence and occurrence of this disease has been reported from South Eastern Nigeria by Nwufo and Onysagba (1989) and from India by Sugha *et al.* (1990). About 40 percent of the seedlings are rejected on account of this disease in registered apple nurseries (Sonali and Gupta, 2004). There is no resistant rootstock against this disease and fungicides such as thiram & aureofungin are also not much effective (Bhardwaj, 1977). The diseases of plants caused by soil borne pathogens are in general difficult to control .It has, however, been reported from various countries that fungicidal treatment of soil has some effects on the control of *S. rolfsii* and other soil borne pathogens (Backmam and Kabana, 1975; Fellman *et al.* 1983; Sharma and Verma,1985). Therefore, this study was carried out to assess the role of different plant extracts on the inhibition of sclerotial germination of *S. rolfsii.*

3.2 Materials and Methods

3.2.1 Experimental Period

This experiment was conducted in Plant Pathology Lab, BARI, Joydebpur during 5 February, 2013 to 23rd May, 2013.

3.2.2 Preparation of Botanical Extract

Fresh leaves and seeds of 13 plants were collected from different areas of Rangpur and Rajshahi. Plant names both local and scientific and plant parts used in this experiment are shown in Table 3.1. Before cutting and grinding, the plant materials were dried up in lab for 25-30 days. After grinding, three different concentrations (10%, 15% and 20%, w/v) of aqueous extract of each botanical were prepared to evaluate the inhibition effect on sclerotial germination of *S. rolfsii.*

Table 3.1 Plant parts used against the germination of sclerotia *(Sclerotium rolfsii)*

Local name of plant	Scientific name	Plant parts used
Datura	*Datura stramonium*	Leave
Lantana	*Lantana camara*	Leave
Babla	*Acacia nilotica*	Leave
Tulsi	*Ocimum sanctum*	Leave
Akondo	*Calotropis procera*	Leave
Garlic	*Allium sativum*	Bulb
Jute	*Corchorus capsularis*	Seeds
Mahogany	*Swietenia macrophylla*	Seeds
Tobacco	*Nicotiana tabacum*	Leaves
Telachucha	*Coccinia cordifolia*	Leaves
Alamonda	*Allamanda cathartica*	Leaves
Hazarmony	*Phyllanthus niruri*	Leaves
Khoksha	*Ficus hispida*	Leaves

3.2.3 Plant Pathogen Isolation, Culture and Sclerotial Germination

Plant pathogen, *Sclerotium rolfsii* was isolated from infected eggplant seed-plot during seedling stage and cultured on PDA media supplemented with streptomycin (25 ppm). Sclerotia were collected from 15-day-old culture of the pathogen grown on potato dextrose at medium. Afterwards, sclerotial surface was sterilized with 10% Clorox for 2 minutes. Sclerotia were repeatedly washed with sterilized distilled water and kept in dry sterilized blotter paper (9cm) to remove excess moisture. Sclerotia were stored in sterilized petri plate and sterilized test tube under dry conditions (Plate 3.1). Sclerotia were soaking in extract about one hour (Plate 3.2). About nine to twenty five sclerotia were placed in sterile soil and PDA media having aqueous plant extracts in petridish. After then the petri plates were incubated at 28 + 1°C for 72 hours. Combination treatment was prepared by mixing 1:1 (V/V) of individual concentration of plant materials. A standard chemical fungicide bavistin was also used for comparison of inhibition effect. Tests of all treatments were carried out in vitro in Plant Pathology Lab of BARI, Joydebpur. A control (distilled water) was also maintained without any extract. Each treatment consisted of three replications and sclerotial germination was observed after 72 hours incubation period.

Formation of young sclerotia Mature sclerotia Sclerotia

Plate 3.1 Collection of sclerotia from 10 days old PDA culture plate

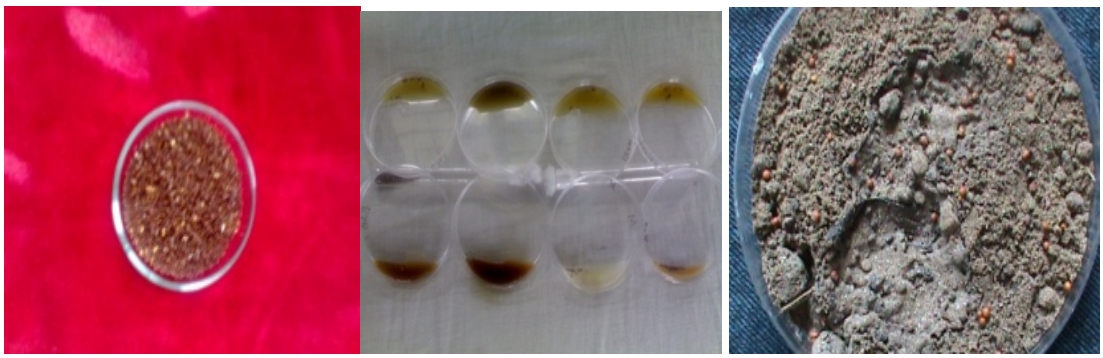

Plate 3.2 Sclerotia soaked in extracts and sowing in sterilized soil

Plate 3.3 Germination of sclerotia in petridish on soil

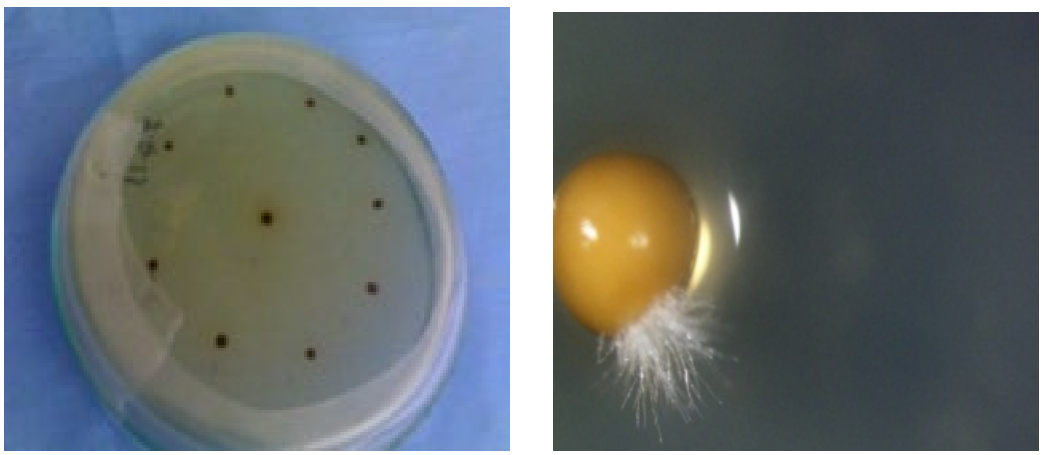

Plate 3.4 Germination of sclerotia on PDA

3.2.4 Data Analysis

The data was first input in Excel program and finally analyzed by ANOVA and the significant level was tested by Duncan's Multiple Range Test (Duncan, 1951).

3.3 Results and Discussion

3.3.1 Effect of Individual Botanical Extract on Sclerotial Germination

To study the effect of plant extract on sclerotial germination, three different concentrations (10%, 15% and 20%, w/v) of each botanical were prepared and the per cent inhibition of germination was recorded after 72 hrs and the results are shown in Table 3.2 (Fig.3.1). The sclerotial germination in PDA media with different plant extracts (20%) of datura, lantana, babla, akondo, garlic, jute seed, mahogany seed, tobacco, allamonda, hazarmony and khoksha was 24.00%, 90.64%, 5.32%, 94.12%, 62.64%, 50.64%, 30.64%, 64.00%, 52.00%, 90.64 and 69.32%, respectively (Table 3.2 and Fig. 3.3).

Table 3.2 Effect of individual plant extract on sclerotial germination of *Sclerotium rolfsii* in soil and PDA media

Treatments	Botanical Extract Concentration (%)					
	Sclerotial germination in PDA (%)			Sclerotial germination in soil (%)		
	10%	15%	20%	10%	15%	20%
Datura	57.32b	26.64c	24.00c	36.00 b	17.32c	14.64c
Lantana	96.00a	96.00a	90.64a	94.64a	86.4a	96a
Babla	33.32b	26.64b	5.32c	33.32b	20.00c	0.0d
Tulshi	100a	100a	100a	100a	94.64b	96.00ab
Akondo	97.32a	97.32a	94.12a	96.00a	96.00a	93.32a
Garlic	81.32a	62.64b	62.64b	74.64ab	68ab	62.64b
Jute seed	'70.64b	50.64c	50.64c	58.64b	36.00c	38.64c
Mahagoni seed	70.64b	45.32c	30.64d	65.32b	37.32c	34.64c
Tobacco	77.32b	60.00c	64.00c	77.32b	54.64c	42.64d
Telachucha	100a	100a	100a	92.00b	94.64a	94.64a
Alamonda	74.64b	52.00c	52.00c	68.00b	49.32c	46.64c
Hazarmony	100a	85.32b	90.64ab	100a	85.32bc	81.32c
Khoksha	78.64b	72.00b	69.32b	62.80b	52.00c	49.32c
Control (Distilled water)	90.64a	90.64a	90.64a	89.32a	89.32a	89.32a

Same letters are not significantly different according to Duncan multiple range test (p=0.05)

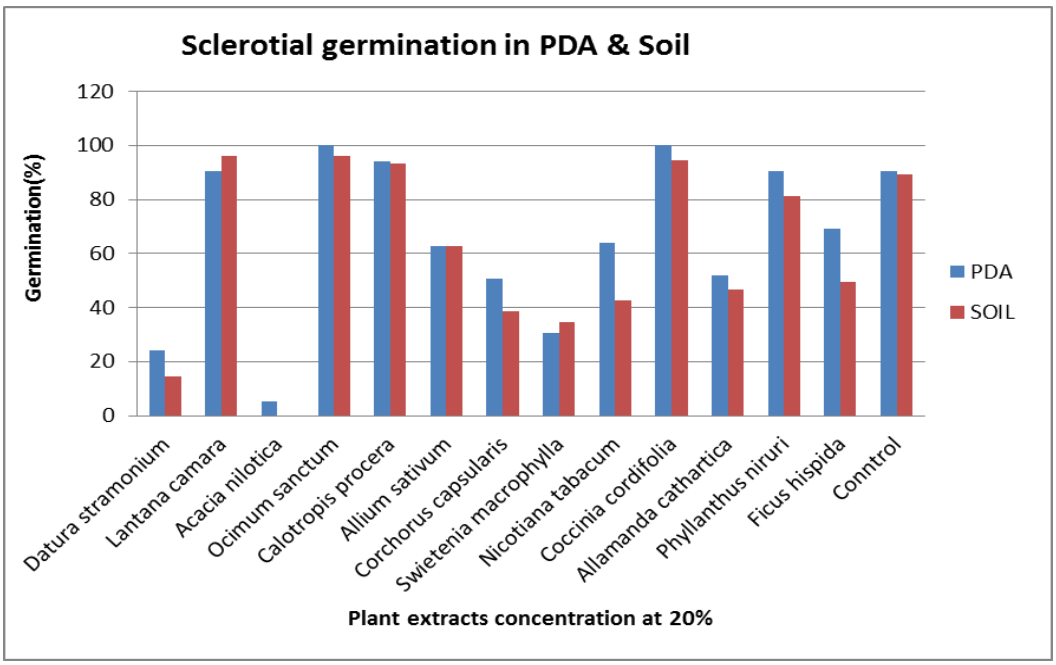

Fig. 3.1 Effect of plant extracts on sclerotial germination of *S. rolfsii*

During this study, extract of *Acacia nilotica* (20%) showed significant activity against sclerotial germination of *Sclerotium rolfsii* in both soil and PDA media. A total inhibition of germination of sclerotia was observed in *Acacia nilotica* treatment in soil but 5.32% germination was occurred in PDA media. *Datura stramonium* and *Swietenia macrophylla* caused 14.64% and 34.64% sclerotial germination in sterile soil whereas 24.00% and 30.64% germination were noticed in PDA plate. Over all sclerotial germination was found poor in soil but high in PDA media (Table 3.2 and Plate3.4). The aqueous extracts of *Cocdnia cordifolia, Phyllanthus niruri, Calotropis procera, Ocimum sanctum* and *Lantana camara* enhanced the sclerotial germination up to 100% in PDA media. Likewise tulsi, lantana, telachucha and akando were also resulted more than 90% sclerotial germination in soil (Table3.2 and Fig.3.2 and Plate3.3).

3.3.2 Effect of Combinations of Two Botanical Extracts on Sclerotial Germination

The effect of combinations of two botanical extracts on germination of sclerotia was monitored after 72 hrs for getting the maximum growth. Combinations of two

different plant extracts were found more effective against sclerotial germination (Table-3.3.) during this study. Combination of *Acacia nilotica* with *Swietenia macrophylla* and *Acacia nilotica* with *Datura stramonium* resulted a total inhibition of sclerotial germination in sterile soil at 28+l°C. But, combination of *Acacia nilotica* with *Ocimum sanctum* resulted enhancement of germination of sclerotia compare to control (Table3. 3).

Table 3.3 Efficacy of combined plant extracts on sclerotial germination of *Sclerotial rolfsii* in sterile soil

Treatments	Sclerotial Germination (%)	Inhibition of Germination (%)
Babla-Mahogany (15%)	10.64e	87.72
Babla- Mahogany (20%)	0.0	100
Babla-Datura(15%)	17.32e	80.01
Babla- Datura (20%)	0.0	100
Babla-Tobacco(15%)	54.64c	36.94
Babla- Tobacco (20%)	50.44c	41.79
Babla- Allamonda (15%)	62.64b	27.71
Babla- Allamonda (20%)	66.64b	23.10
Babla-Jute(15%)	30.64d	64.64
Babla- Jute (20%)	33.33d	61.53
Babla-Tulsi(l5%)	90.64a	0.00
Babla- Tulshi (20%)	86.64a	0.02
Control	86.66a	-
Bavistin (2g/L)	9.32e	89.24

Same letters are not significantly different according to Duncan multiple range test (p=0.05)

Commercial fungicide bavistin (2g/L) caused 9.32% sclerotial germination. However, combined plant extracts of *Acacia nilotica* with *Swietenia macrophylla* showed 100% inhibition of sclerotial germination and exhibited as strong fungicide than commercial bavistin (Table 3.3).

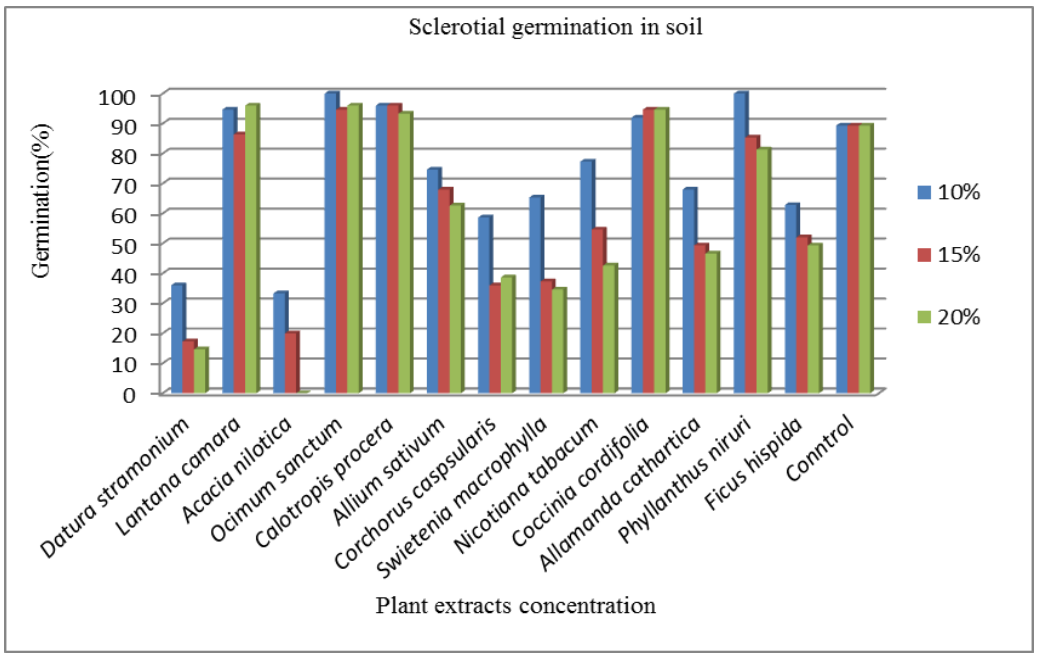

Fig. 3.2 Effect of different concentrated plant extracts on sclerotial germination of *S. rolfsii*

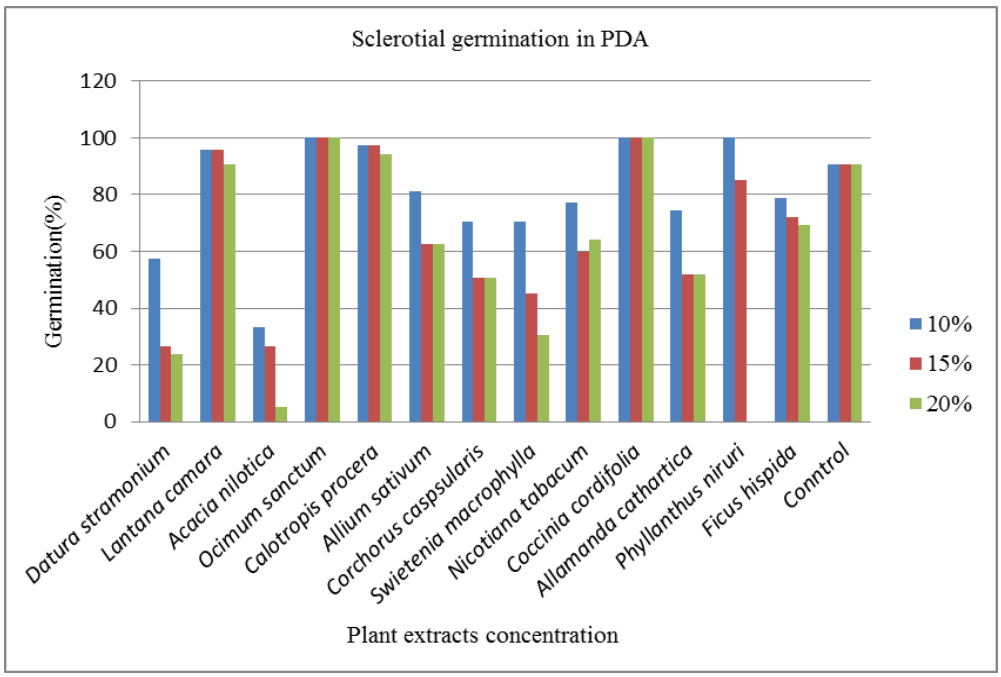

Fig. 3.3 Effect of plant extracts on sclerotial germination of *S. rolfsii*

Sonali and Gupta (2004) reported that different plant extracts viz., neem cake (1%), deodar needles (3%), mustard cake (5%), neem oil (3%) and pine needles (5%) reduced sclerotial germination whereas aqueous extract of mustard cake (1, 2 and 4% concentration) enhanced germination of sclerotia up to 100%. Combination of mustard cake (5%) with neem oil (3%) resulted a total inhibition of sclerotial germination and combination of mustard cake (5%), neem cake (1%) & deodar needles (3%) resulted in enhanced germination of sclerotia. Gamliel and Stapleton (1993) characterized antifungal volatile compounds from soil amended with organic residues and reported reduced propagulla number and viability of *Sclerotium rolfsii* in amended soil. Sharma and Gupta (1995) observed reduced root rot *(Dematophora necatrix)* disease incidence in apple by using of organic amendments of neem cake, deodar needles and Daphne leaves. Neem products viz., neem cake and neem oil were also highly effective against many soil borne plant pathogens (Karthikeyan and karunanidhi, 1996; Sharma and Gupta, 1995). The effect of organic amendments on the activity of pathogenic fungi was attributed to CO_2 accumulation, non-availability to nitrogen and volatile compounds present in oil cakes (Stover, 1962). Volatile fraction of two medicinal plants; *Azadirachta indica* and *Eucalyptus globules* were more effective in suppressing the sclerotial germination of *Macrophomina phaseolina* than non-volatile fractions (Dubey & Kishore,1990). Shahzad and Ghaffar (1988) reported that *Paecilomyces lilacinnus* (a fungal parasite) was effective to inhibit growth of sclerotial fungi i.e. *Macrophomina phaseolina, Rhizocotinia solani* and *Sclerotium oryzae*, causing root rot in many plants. Banerjee *et al.* (1989) observed that Neem, Citronella and Karanja oil strongly inhibited sclerotial germination of *Sclerotium rolfsii* infecting rice under *in vitro* condition whereas, Mahua oil was non-effective. Composted onion waste reduced viability of sclerotia of *S. cepivorum* Bark. In greenhouse pot tests (Coventry *et al.*, 2002). Coley-Smith & Parfitt (1986) stated that onion volatile thiols and sulphides activate sclerotia germination.

An enhance germination of sclerotia of *Sclerotium rolfsii* is reported when mustard cake used alone. Mustard cake with neem cake and deodar needles could be exploited

to kill the pathogen by promoting the conditions for enhanced germination and its subsequent killing by antagonistic micro-organisms (Muthusamy and Marriappan, 1992). Therefore, addition of plant extracts to infested soil may be helpful in controlling the fungal diseases partially or completely.

3.4 Conclusion

The present results suggest that babla *(Acacia nilotica)* and mahagony *(Swietenia macrophylla)* extracts have good potential as toxicant agent against the sclerotial germination of plant pathogenic fungi *(Sclerotium rolfsii).* Combination of babla *(Acacia nilotica)* with mahogany*(Swietenia macrophylla)*, and babla *(Acacia nilotica)* with datura (*Datura stramonium)* also showed good efficacy on the inhibition of sclerotial germination. Whereas tulshi *(Ocimum sanctum)*, akondo *(Calotropis procera)*, hazarmony (*Phyllanthus niruri)*. Lantana (*Lantana camara)* and telachucha *(Coccinia cordifolia)* leaves extract did not show any activity against sclerotial germination rather enhanced the germination rate. Therefore, farmers of Bangladesh can use the botanicals of babla, mahogany and datura for controlling of pathogenic fungi *(Sclerotium rolfsii)* in brinjal vegetable field.

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Chapter - 4

Antifungal Activity of Some Plant Extracts against *Fusarium oxysporum* Causing Foot Rot Disease in Brinjal

Abstract

In Bangladesh, eggplant/brinjal suffers from 12 diseases but most serious disease is foot/collar rot. The antifungal effect of crude aqueous and methanol extracts of 26 medicinal plant was studied *in vitro* following Poisoned Food Technique. It was found that all the plant extracts at 15% concentration were effective in reducing the mycelial growth of *Fusarium oxysporum*. Among the 26 plant extracts prepared in water, a higher inhibition was noticed for 5 plants extracts namely *Acacia nilotica, Allium sativum, Swietenia macrophylla, Datura stramonium* and *A. indica.* Out of the 13 plant extracts in methanol solvents at 15% concentration, a complete inhibition was noticed in 3 plants extracts namely *Acacia nilotica, Nicotiana tabacum* and *Swietenia macrophylla*. These plants were selected further for different concentrations of 5%, 10% and 15%. *Acacia nilotica* at all concentration showed 100% inhibition. *Acacia nilotica* showed maximum inhibition in water and methanol after 7 days of incubation. Combination between *Acacia nilotica* with *Corchorus capsularis* and *Nicotiana tabacum* with *Corchorus capsularis* revealed excellent inhibition compare to control at 20% concentration against *Fusarium oxysporum.* At the low concentration of 5% in methanol and water, *Acacia nilotica* had showed more inhibitory effect (100% and 69.09%, respectively), while methanolic extract of *Clerodendrum viscosum and Ocimum sanctum* showed very low inhibition (1.59 and 5.75%). Aqueous extracts of *Tagetes patula* (ganda), *Cleome viscosum* (holde hur hurae), *Carum roxburghianum* (soj) and *Coccinia cordifolia* (telachucha) and methanolic extract of *Ficus hispida* (khoksha) at 5% concentration did not show any inhibion to fungus.

Key words: Plant extracts, Antifungal effect, *Fusarium oxysporum*, eggplant.

4.1 Introduction

Brinjal (*Solanum melongena* L. fam. Solanaceae) or eggplant or aubergine in an important widely consumed nutritious vegetable crop in India or cultivated commercially throughout the tropical and subtropical region of the world. The name brinjal is popular in India subcontinents and is derived from Arabic and Sanskrit. It is considered a native of India or major Asia where the major domestication of large fruited cultivars occurred. It has been cultivated in India for the last 4,000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable. Brinjal is important in the warm areas of Far East, being grown intensively in India, Bangladesh, Pakistan, China and Philippines. It is also popular in Egypt, France, Italy and US. In India, it is popular and principal vegetable crops grown throughout the country except higher altitudes. It is a perennial but grown commercially as an annual crop. Many fungi have been identified by various workers as causal organisms of various plant diseases. Brinjal plant (*Solanum melongena* L.) is affected by various diseases. Brinjal is grown as an important vegetable crop in all over world mostly in Indian subcontinent and Southeast Asia. It is grown in India over an area of 0.4 million hectares with an annual production of 7.8 million tons (Datar, 1999). Among the different diseases that attack brinjal crop, wilt has become a major disease causing significant reduction in yield. *Fusarium oxysporum*, is a plant pathogenic fungi responsible for wilt diseases in more than hundred species of vascular plants such as pigeon pea, gram, guava, brinjal, tomato, potato, sugarcane, cowpea, musa, pea, ginger, etc. it colonizes the xylem of the host plant, and as a result, blockage and breakdown of the xylem leads to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant (Agrios, 1997). The wilt of brinjal is characterized by yellowing of foliage drooping of apical shoot to ultimate death of whole plant. The pathogen is a soil inhabiting fungus and forms in the senescing tissues of the diseased plant and may survive in the soil for many years. Throughout the world lots of approaches had been taken to combat this disease. In the past decades the use of fungicides to control crop disease has contributed to increased production of food worldwide. Nevertheless, the massive use of synthetic fungicides in crop defense had severe environmental impact. Synthetic fungicides also pose serious health risks within our food chain and have been linked to increased

occurrence of several types of cancer. The increasing number of fungal population with enhanced resistance is common fungicides.

Management of *F. oxysporum* is required, as this pathogen and its many special forms affect a wide variety of hosts of economic value. Control of *Fusarium* wilt disease has been accomplished primarily by the application of chemical fungicides, long crop rotations, pasteurizing seedbeds with steam or fumigants (Spletze *et al*., 1999). Nevertheless, the massive use of synthetic fungicides in crop defense had severe environmental impact. The inappropriate use of agrochemicals especially fungicides were found to possess adverse effects on ecosystems and a possible carcinogenic risk than insecticides and herbicides together (Osman *et al*., 2003). Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective (Zhonghua *et al.,* 2005). Due to the aforementioned considerations, there may be a need to develop new management systems to reduce the dependence on the synthetic agrochemicals. In this respect, plant extract may represent an ideal solution to the problem be easily tested *in vitro*. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens (Nwachukwu *et al*., 2001). Medicinal plants are part and parcel of human society to combat disease. The antimicrobial activity of medicinal plant oils and extracts has been recognized for many years. Aqueous extracts of 46 plants against *Fusarium* spp. revealed that 12 plants have recorded significant antifungal activity and that these plants could be exploited for eco-friendly management (Satish *et al.,* 2009). The results of a study on the inhibitory activity of extracts from Kenyan medicinal plants against three soil pathogens (Rugutt *et al*., 2006), showed that *Warburia ugandensis*, *Azadirachta indica*, *Tagetes minuta* and *Urtica massaica* were active against the fungus *Fusarium oxysporum*, *Alternaria passiflorae* and *Aspergillus niger*. However, in Bangladesh few investigations have conducted to find antimicrobial activity of oils and extracts of traditional medicinal plants against plant pathogenic microbes.

Considering the vast potentiality of medicinal plant as sources for antimicrobial agents, the present study was designed to evaluate the *in vitro* antifungal activity of some plant extracts: *Datura stramonium L., Allamanda cathartica L., Acacia nilotica L., Phyllanthus niruri L., Cocciniacor difolia L. Allium sativum L., Calotropis procera*

L., Ocimum sanctum L., Lantana camara L., Swietenia macrophylla L., Corchorus caspsularis L., Ficus hispida L., Nicotiana tabacum L., Swertia chirata L., Vitex negundo L., Carum roxburghianum L., Polygonum orientale L., Lawsonia inermis L., Annona squamosa L., Tagetes patula L., Cleome viscosa K., Aegle marmelos L., Cajanus cajan L., Azadirachta indica L., Achyranthes aspera L., Clerodendrum viscosum L.

Therefore, the current experiment was carried out with the following objectives.

- To survey of different brinjal host plant,
- To identify the fungal disease in brinjal,
- Isolation, purification, identification and characterization of pathogenic fungi from brinjal,
- To identify the herbs, shrubs and trees of Bangladesh having fungicidal activities and
- To evaluate the efficacy of selective fungicides plant extracts against *F. oxysporum*.

4.2 Materials and Methods

4.2.1 Experimental site

All the experiments were conducted during July 2011 to June 2014 in IES-Laboratory, Rajshahi University and Plant Pathology Laboratory, Botany, Department, Rajshahi University and Plant Pathology Laboratory and pot house in Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

4.2.2 Collection of isolates

The *Fusarium oxysporum* isolates used in this study were collected from field from different brinjal growing areas of Rajshahi. These isolates were collected during the month of January to June, 2012 from the infected brinjal field. Then the specimens were taken to the Plant Pathology Laboratory, BARI and were subjected to the process of isolation.

4.2.3 Origin of Isolates

Fusarium oxysporum were obtained from the diseased plants of brinjal *(Solanum melongena* L.).

4.2.4 Method of Sterilization and Incubation

After collection the diseased plant materials were brought to the laboratory as soon as possible so that stems are still fresh during the isolation of the pathogen. In the laboratory the advancing or water soaked portion of the lesion were cut into small sections (approximately 5x5 mm per section) and surface-sterilize by soaking for 15 seconds in 5% commercial Clorox (commercial Clorox) usually consists of 5.25% sodium hypochlorite; by weight. This is done to kill microorganisms or pathogens on the surface of the stem tissues that may complete with the growth of *F. oxysporum.* 5.25% sodium hypochlorite solution contain in a beaker, surface sterilization of materials was continued for 15 seconds 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 materials was removed by gently pressing them between to 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. In the early practices, the sterilized materials were placed over the moist filter paper. 2-3 glass bars (90 mm x 2 mm x 2 mm) were placed on the moist filter paper of the humid chamber, which prevented them from coming in contact with the moisture of the filter papers. The materials under this condition when incubated were found to maintain their good health for longer period and support profuse mycelia of the fungus.

4.2.5 Preparation of Humid Chamber

The humid chamber used for the incubation of the diseased plant parts consisted of a Petri plate (90 mm dia.) with its lids. Before being used the Petri dishes were surface sterilized by flaming. The linear walls of the dish and its lid were lined previously sterilized filter paper, which were then moistened with sterilized distilled water. The

excess water was poured out and the plate with its lid was used as humid chamber. Desired number of diseased materials from each of the samples lots collected from the field was incubated using a separate humid chamber.

4.2.6 Isolation of the *Fusarium oxysporum*

Isolation of *Fusarium oxysporum* from diseased brinjal plants noted above was made following either (i) by direct method or (ii) by plant method.

4.2.6.1 Direct Method of Isolation

The method consisted of sterilization and incubation of the diseased plant parts in humid chamber following the technique described before. The affected parts were incubated for 15 days at 28±2°C. During this period, the fungus was found to grow mycelia on the necrotic areas. The mycelia were collected on a very small agar block (about 2 mm in length), mounted at the tip of a flamed spearheaded tungsten needle. This was then transferred to a freshly prepared PDA plate. On incubation fungal colonies were found to develop and free from contamination.

4.2.6.2 Plating Method of Isolation

In this period of isolation, diseased parts were cut into small pieces, about 0.5 cm in length, in such a manner so as to include both healthy and necrotic tissues in each piece. The pieces were then washed in running tap water, sterilized in 5.25% sodium hypochlorite solution and washed repeatedly for several times in sterilized distilled water to remove sodium hypochlorite solution. Three pieces were taken out using flamed forceps and dried between filter papers and finally transferred to a PDA plate. Plates were incubated at 28+2°C for 15 days, during which period the fungal colonies appeared on the PDA plates. Often the colonies were found to become contaminated due to unwanted growth of bacteria and other fungi. No doubt *Fusarium* species was obtained in pure form but not so as it was with the direct method. Moreover, this method was found to be time consuming and it required further transfer to get pure culture. Whereas, in the direct method of pure colony of *Fusarium* could be obtained

by single transfer. Otherwise stated direct method has been used throughout this work for the isolation of *Fusarium* species.

4.2.7 Identification of Isolated Fungus

The fungus isolated from infected tissues of stem on brinjal plant 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 Subramanian (1971).

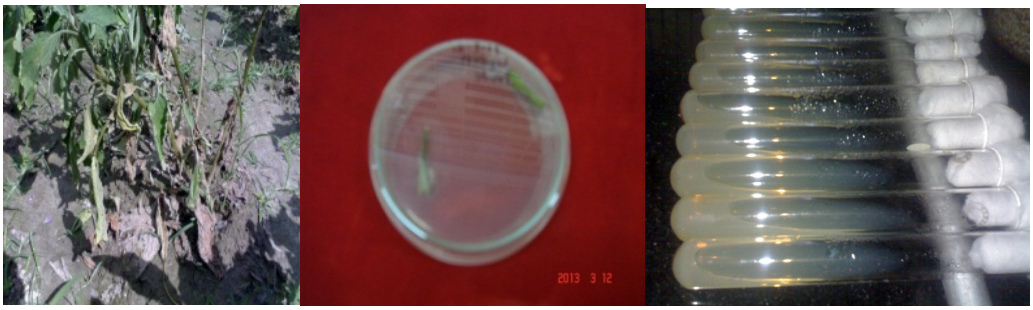

Plate 4.1 Collection, isolation, identification and purification of *F. oxysporum*

4.2.8 Pathogenicity Tests

4.2.8.1 Raising of Host Plants

For pathogenicity test, the host plants are grown, in earthen pot (9 inches) containing loam soils. Five to ten seedlings were raised in each pot from treated seed of a particular verity of eggplant. The hosts of *Solanum melongena L.* were inoculated with a dense mycelial suspension of *Fusarium oxysporum* its stem by artificial inoculation.

4.2.8.2 Preparation of Spore Suspension and Counting spores of *F. oxysporum*

About 10 days old culture of *F. oxysporum* was used.10 ml sterile water was added to each petridish. Then the surface of the fungal growth was separated gently with a glass slide to disperse the spores. The suspension was decanted into a conical flask and sieved to separate the hyphal fragments from the suspension. Finally, the number of spores per ml suspension was counted by Haemacytometer method and it was 1.25 x 10^7spores/ml.

4.2.8.3 Method of Inoculation

Seedlings of most plants (in eggplant) were inoculated by spraying a spore suspension with an atomizer. The inoculated plant was covered with polythene bags and incubated at 28±2°C for two days. Plant was then transferred to the pothouse. 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 host plant had developed characteristic rot and compared with the maturity-developed symptoms recorded before.

4.2.8.4 Re-isolation of Pathogens

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

4.2.9 Preparation of Slides

A suitable portion of culture of *Fusarium oxysporum* on from PDA plate was selected under a stereoscopic binocular microscope and was taken out with the help of forceps and needles and put in 1 or 2 drops of lactophenol on clean slide. It was then gently waned 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.

4.2.10 Preparation of Clearing Reagent

Throughout the present investigation corning glassware's and chemicals supplied by Merck, Germany 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 ml

4.2.11 Preparation of 0.1% Mercuric Chloride ($HgCl_2$) Solution

Mercuric chloride ($HgCl_2$) solution is used for surface sterilization of the diseased sample.

Mercuric chloride	l00.00mg
Distilled water	100.00ml

Shaken well till it was dissolved

4.2.12 Preparation of Different Culture Media

The following culture media were used for laboratory.

(a)Potato Dextrose Agar (PDA) medium:

Peeled and sliced potatoes	200.00g
Dextrose	I5.00g
Agar	20.00g
Distilled water	1000ml

The liquid media were solidified with the addition of 2% agar, wherever required. All solid media were autoclaved at 15 Ib. /inch^2 pressure for 20 minutes. In case the medium contained any substance liable to decomposition or denaturation ion, 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 inoculum was taken from 7-10 days old culture and size of the inoculum was the same in all cases. After inoculation the culture were incubated at 25±2°C for 15 days for mycelial growth and spore formation.

4.2.13 Control Measure

In order to find out the control of mycelial growth of *Fusarium oxysporum* different plant extracts and fungicides were used in *in vitro.*

4.2.13.1 Method of studying *in vitro* Fungicdal effect

The effect of fined amount (2g/100 ml) of two fungicides namely; Bavstin and Provax on mycelial growth of *F. oxysporum* was tested in this experiment. The medium was sterilized at 15 Ib./ins in 121°C. The experiment was setup in laboratory. 20 ml of media were poured after sterilization under specific condition in sterilized 90 mm Petri dishes and inoculated as described earlier. The measurements of radial growth of the colony were taken at intervals of 24 hours were recorded and expressed in mm.

4.2.14 Collection of Plant Materials

The plant materials of twenty six species (table1) were collected from different places in Rajshahi University campus and Rangpur district. The collected plants were identified and authenticated by a botanist in the Department of Botany, University of Rajshahi, Bangladesh. Then washed with tap water; surface sterilized with 2% sodium hypo chlorite for 5 min and washed thoroughly 2-3 times with sterile distilled water.

4.2.15 Method of Studying Plant Extracts Preparation

4.2.15.1 Preparation of powder from leaves and seeds

The selected parts (leaves and seeds) of different medicinal plants were cut into small pieces and shade dried at room temperature for fifteen days. Dried leaves and seeds were grinded to produce powder using an electric grinder. The powders were put in plastic containers with tightly fitted lids, which were kept in the laboratory before use. The powders were used, within one week of preparation.

4.2.15.2 Preparation of Plant Extracts

4.2.15.2.1 Aqueous extract

The powdered leaves extracted with sterile distilled water at room temperature at different level of concentrations (5, 10, 15 and 20%) i.e. 5 g in 100 ml, 10 g in 100 ml, 15 g in 100 ml and 20 g in 100 ml. Then extracts were filtered through double layered muslin cloth and finally through Whatman filter paper No.1. in 250 ml Pyrex flasks. The flasks were tightly wrapped with aluminum foil. The extracts were used

for screening their antifungal activity. Extracts were stored at 4°C in pre-sterilized flasks until use.

4.2.15.2.2 Methanol extract

Finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using sox let apparatus. For extraction, 50 gm. leave and seed powder of each of the selected plants was transferred into separate conical flasks containing 200 ml of methanol and mixed well. The flasks were left for two days with occasional stirring. The extracts were then filtered (Whatman No.1 filter paper),concentrated in vacuum under reduced pressure and dried in the desiccator (Manasa, *et al.*, 2013).This crude extract were kept under low temperature (4°C) in the refrigerator until use.

4.2.15.2.3 Dose used in the experiment

Four types of concentration viz. 5%, 10%, 15% and 20% of water and methanol extracts were prepared during this study by dissolving 5mg of botanical extract in 100 ml PDA media. Dry methanol extract was dissolved in DMSO before adding to PDA media.

4.2.16 Mycelial Growth Inhibition Test /Antifungal Activity of Selected Plants

4.2.16.1 *In vitro* tests with water leaf extracts

In order to determine the inhibitory potential of extracts against mycelial growth of *F. oxysporum*, we employed Poisoned food technique (Mishra and Tiwari, 1992, Nene and Thapliyal, 1993, Basher *et al.,* 1991). Here, Potato dextrose agar (Hi Media, Mumbai) was prepared, poisoned with extracts. To obtain 5 percent plant extracts medium, 95 ml PDA was poured in 100 ml sterilized conical flask and 5 ml of plant extract was poured in each flask with the help of sterilized pipette. Desired concentrations of individual plant extract were 5%, 10% and 15 % (v/v). After thorough mixing with plant extracts the medium was autoclaved and approximately 20 ml of melted PDA mixed with extracts was poured into each 90 mm petridish. The pH of the medium was adjusted to 6.5 by using pH meter with the help of 1N HCl or 1N NaOH. After solidification, the plates were inoculated by placing 5 mm mycelial

disc of *Fusarium oxysporum* from the periphery of 7 days old culture and sclerotia from one month culture. Sterile Potato dextrose agar (HiMedia, Mumbai) medium containing streptomycin is able to prevent bacterial contamination. The inoculums (5 mm mycelial disc of *F. oxysporum*) were placed upside down on agar media at the Centre of the petriplates and incubated at 25±1ºC. Control plates maintain without plant extract. The plates were arranged in Completely Randomized Design (CRD) with three replications. Mycelial Growth of the fungus was measured by taking the diameter in two directions and the average was recorded at an interval of 24 hours. Final growth reading was recorded when the growth of the fungus in control plate was full. Then these plates were kept for 30 days for sclerotia formation. Inhibition of radial growth was computed based on colony diameter on control plate using the specified formula (Sultana, J.N. *et al*., 2012):

$$\% \text{ Inhibition} = \frac{x - y}{y} \times 100$$

Where,

X = Average growth (mm) of *F. oxysporum* in control petridishes.

Y = Average growth (mm) of *F. oxysporum* in each plant extract and fungicides treated petridishes.

Here a petridish was maintained as control to compare it with others. Average radial growth of the fungus (cm) after 3days, 5days and 7days was computed from the data recorded and per cent inhibition of radial growth over control was calculated using the average value. The data were subjected to statistical analysis following CRD.

4.2.16.2 *In vitro* tests with methanol extracts

The methanolic extracts of thirteen different plant extracts were screened for antifungal activity by "Poisoned food technique" at three different concentrations(5mg/100ml, 10mg/100ml and 15mg/100ml of medium) with three replicates were inoculated with fresh 7 days old culture of *Fusarium oxysporum*. 5 mm agar disc of fungal culture was prepared with the help of sterile cork borer and kept upside down on agar plates, these plates were incubated at 25 ± 1ºC. Plates with DMSO served as control and standard (Bavistin 5µg/ml) was maintained in agar plate. The colony diameter in mutual perpendicular directions was measured using a ruler.

Antifungal activity of extracts (in terms of inhibition of mycelial growth) was calculated using the formula:

Mycelial growth inhibition (%) = (x-y/x) × 100, where 'x' is average colony diameter (CD) in control plate and 'y' is average colony diameter in poisoned plates (Kambar, *et al.,* 2014). The experiment was repeated two times and the result was presented as Mean ± Standard deviation.

4.3 Result and Discussion

4.3.1 Efficacy of botanicals on mycelial growth of *Fusarium oxysporum*

4.3.1.1 Effect of Aqueous Extract

Antifungal activity of twenty six botanical extracts was assayed and data on effect of plant extracts on the growth of *Fusarium oxysporum* presented in Table 4.1 and 4.2.The data revealed that significant reduction in growth of *Fusarium oxysporum* was observed with extracts of twenty six medicinal plants and the extracts showed significant differences in their efficacy. Among all the twenty six plant aqueous extracts, 61% plants showed inhibition of mycelial growth compare to over control.

Table 4.1 Antifungal activity of the plant extracts against *Fusarium oxysporum*.

Botanicals		**Effect of aqueous extract on Mycelial Growth (cm) of *F. oxysporum***			
Local Name	Scientific name	Control	5%	10%	15%
Datura	*Datura stramonium*	5.88±.04d	5.02±.22c	5.62±.09b	6.45±.05a
Alamanda	*Allamanda cathartica*	4.61±.12b	3.92±.04c	4.47±.24b	5.28±.13a
Babla	*Acacia nilotica*	4.56±.13a	2.01±.11b	1.21±.10c	.87±.01d
Hazarmony	*Phyllanthus niruri*	4.63±.14e	8.1±.01a	7.12±.16b	6.11±.14c
Telachcha	*Coccinia cordifolia*	6.12±.06c	6.70±.07b	6.92±.02ab	6.73±.13b
Garlic	*Allium sativum*	8.28±.22a	7.92±.22a	6.78±.24b	4.43±.21c
Akando	*Calotropis procera*	3.95±.08c	7. 03±.17b	8.82±.05a	8.90±.10a
Tulsi	*Ocimum sanctum*	4.43±.18c	6.22±.32b	7.27±.43a	7.65±.06a
Lantana	*Lantana camara*	5.95±.10ab	5.28±.24c	5.61±.21bc	6.20±.12a
Mahagony (Seed)	*Swietenia macrophylla*	6.88±.04a	2.80±.16b	2.76±.13b	2.56±.06b
Jute (Seed)	*Corchorus caspsularis*	7.37±.18a	6.60±.07ab	5.87±.21b	4.65±.28c
Khoksha	*Ficus hispida*	7.26±.15a	7.62±.23a	7.12±.50a	7.85±.20a
Tobacco	*Nicotiana tabacum*	4.61±.12c	6.05±.30b	6.82±.18a	6.13±.22ab

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (p=0.05).

Table 4.2 Antifungal activity of plant extracts against *Fusarium oxysporum*

Botanicals		**Effect of aqueous extract on Mycelial Growth (cm) of *F. oxysporum***			
Local name	*Scientific name*	Control	5%	10%	15%
Chirota	*Swertia chirata*	4.20±.29c	6.75±.12b	7.33±.33b	8.40±.20a
Nishinda	*Vitex negundo*	4.03±.03c	6.83±.16b	7.91±.20a	8.43±.17a
Soj	*Carum roxburghianum*	7.95±.22a	8.11±.01a	6.88±.11b	6.15±.20c
Polygonum	*Polygonum orientale*	7.20±.15a	6.70±.10b	6.50±.15b	5.23±.03c
Mehedi	*Lawsonia inermis*	8.08±.14a	8.05±.10a	6.98±.17b	5.88±.09c
Ata	*Annona squamosa*	5.91±.16b	5.30±.35b	7.28±.06a	7.50±.05a
Ganda	*Tagetes patula*	6.00±.17b	5.28±.21c	6.45±.16ab	6.83±.16a
Holdahur	*Cleome viscosa*	6.16±. 06b	6.73±.06a	6.93±.03a	6.73±.17a
Bell	*Aegle marmelos*	5.58±.21a	5.43±.08ab	5.00±.11bc	4.83±.21c
Arhar	*Cajanus cajan*	4.26±.06d	6.73±.13c	7.25±.12b	7.65±.07a
Neem	*Azadirachta indica*	6.63±.14a	5.63±.23b	5.33±.24bc	4.83±.12c
Apang	*Achyranthes aspera*	5.45±.18a	4.93±.12b	5.26±.17ab	4.80±.10b
Vat	*Clerodendrum viscosum*	6.16±.08b	6.73±.01a	6.50±. 11a	6.10±.05b

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (p=0.05).

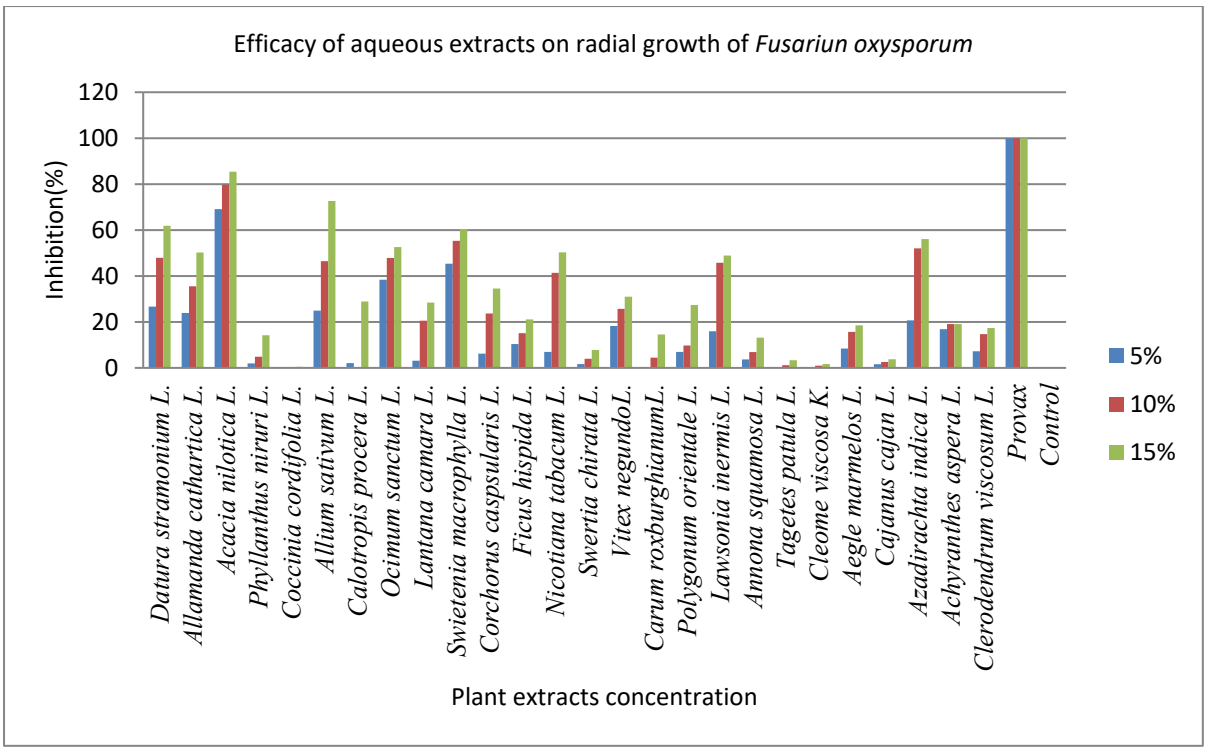

Fig.4.1 Effect of plant extracts on radial growth of *Fusarium oxysporum*

Efficacy of botanicals on mycelial growth of varied with different botanicals at different concentration viz., 5%, 10% and 15% (Table 4.1 and Fig .4.1). At 15 percent concentrations *Acacia nilotica* recorded maximum inhibition(.87cm) in mycelial growth, followed by *Swietenia macrophylla*(2.56cm), *Allium sativum*(4.43cm), *A. indica*(4.83cm) and *Corchorus capsularis*(4.65cm). At 10 percent and 5 percent concentrations *Acacia nilotica*(1.21 and 2.01) showed maximum inhibition in mycelial growth, followed by *Swietenia macrophylla*(2.76 and 2.80). As a whole, extract of *Acacia nilotica* at all the concentration were highly effective in inhibiting the mycelial growth of the test fungus (Table 4.3 - Appendices and Fig.4.1).

4.3.1.2 Effect of Combinations

At 20 percent concentrations of combination(Table 4.4) between *Nicotiana tabacum* and *Corchorus capsularis* (2.60cm) recorded maximum inhibition in mycelial

growth, followed by combination of *Nicotiana tabacum* and *Swietenia macrophylla* (3.21cm), *Nicotiana tabacum* and *Datura stramonium* (3.86cm).

At 20 percent concentrations of combination(Table 4.5) between *Acacia nilotica* and *Corchorus capsularis* (1.30) recorded maximum inhibition in mycelial growth, followed by combination of *Acacia nilotica* and *Swietenia macrophylla* (1.66cm), *Acacia nilotica* and *Nicotiana tabacum* (2.41cm), *Acacia nilotica* and *Datura stramonium* (2.86cm). *Acacia nilotica* and *Allamanda cathartica* (3.16cm) showed significant efficacy compared to control (6.22cm).

Table 4.4 Effect of combinations between Tobacco extract and several fungistatic extracts at different concentrations on *Fusarium oxysporum*.

Extracts combination and concentration (%)	Mycelial growth
Tobacco/Mahogany(20)	3.21 ± .09 cd
Tobacco/Mahogany (15)	4.21 ± .31 b
Tobacco/Jute (20)	2.60 ± .15 d
Tobacco/Jute (15)	3.85 ± .10 bc
Tobacco/Lantana (20)	5.16 ± .18 a
Tobacco/Lantana (15)	5.93 ± .47 a
Tobacco/Alamonda (20)	4.21 ± .17 b
Tobacco/Alamonda (15)	4.75 ± .16 b
Tobacco/Datura (20)	3.86 ± .06 bc
Tobacco/Datura (15)	4.63 ± .24 b
Provax (2g/L)	.00 ± .00 e
Control	6.20 ± 1.35 a

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (p=0.05).

Table 4.5 Effect of combinations between Babla extract and several fungistatic extracts at different concentrations on *Fusarium oxysporum.*

Extracts combination and concentration (%)	**Mycelial growth**
Babla/Mahogani (20)	1.66 ± .41 fg
Babla/Mahogani (15)	2.18 ± .14 ef
Babla/Jute (20)	1.30 ± .30 g
Babla/Jute (15)	1.58 ± .36 fg
Babla/Tobacco (20)	2.41 ± .01 e
Babla/Tobacco (15)	2.63 ± .09 de
Babla/Datura (20)	2.86 ± .03 dc
Babla/Datura (15)	3.73 ± .13 c
Babla/Alamonda (20)	3.16 ± .16 cd
Babla/Alamonda (15)	3.30 ± .36 cd
Provax (2g/L)	.00 ± .00 h
Control	6.22 ± .18 a

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

At 15 percent concentrations of combination of *Acacia nilotica* and *Corchorus capsularis* (1.58) showed maximum inhibition in mycelial growth, followed by combination of *Acacia nilotica* and *Swietenia macrophylla* (2.18), *Acacia nilotica* and *Nicotiana tabacum* (2.63), *Acacia nilotica* and *Allamanda cathartica* (3.30), *Acacia nilotica* and *Datura stramonium* (3.73) compared to control (Table 4.5). At 15 percent concentrations of Combination (Table 4.4) between *N. tabacum* with different plant extracts recorded moderate to low inhibition in mycelial growth followed by control and provax as standard fungicide. As a whole, combinations of plant extract between *A. nilotica* and *Corchorus capsularis* at all the concentrations (15%, 20%) were highly effective in inhibiting the mycelial growth of the test fungus.

Table 4.6 Efficacy of methanolic plant extracts on radial growth of *Fusarium oxysporum*

S. No	Botanicals (Scientific name)	Control	5 % Mycelial growth (cm)	Percent inhibition over control	10 % Mycelial growth (cm)	Percent inhibition over control	15 % Mycelial growth (cm)	Percent inhibition over control
1	*Datura stramonium* L.	6.26±.12a	4.96±.03b	20.76	4.73±.06b	24.44	4.26±.03c	31.94
2	*Allamanda cathartica* L.	6.26±.12a	4.83±.03b	22.84	4.36±.20c	30.35	3.86±.08d	38.33
3	*Acacia nilotica* L.	6.26±.12a	.00±.00b	100	.00±.00b	100	.00±.00b	100
4	*Allium sativum* L.	6.26±.12a	3.43±.12b	45.20	2.93±.06c	53.19	1.43±.08d	77.15
5	*Ocimum sanctum* L.	6.26±.12a	5.90±.10ab	5.75	5.83±.16ab	6.86	5.66±.13b	9.58
6	*Lantana camara* L.	6.26±.12a	4.93±.06b	21.24	4.63±.08b	26.03	3.70±.10c	40.80
7	*Swietenia macrophylla* L.	6.26±.12a	1.90±.10b	69.64	1.80±.05b	71.24	.00±.00c	100
8	*Corchorus caspsularis* L.	6.26±.12a	5.30±.14b	14.37	4.93±.06c	21.24	4.56±.08d	27.15
9	*Ficus hispida* L.	6.26±.12ab	6.36±.03a	00	5.66±.08bc	9.58	5.20±.35c	16.93
10	*Nicotiana tabacum* L.	6.26±.12a	4.96±.03b	20.76	4.36±.14c	30.35	.00±.00d	100
11	*Lawsonia inermis* L.	6.26±.12a	4.20±.10b	32.90	3.93±.03b	37.22	3.20±.05c	48.88
12	*Azadirachta indica* L.	6.26±.12a	4.16±.03b	33.54	3.90±.05c	37.69	3.76±.03c	39.93
13	*Clerodendrum viscosum* L.	6.26±.12a	6.16±.03ab	1.59	6.06±.03ab	3.19	6.00±.00b	4.15
14	Provax	6.26±.12a	.00±.00b	100	.00±.00b	100	.00±.00b	100

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

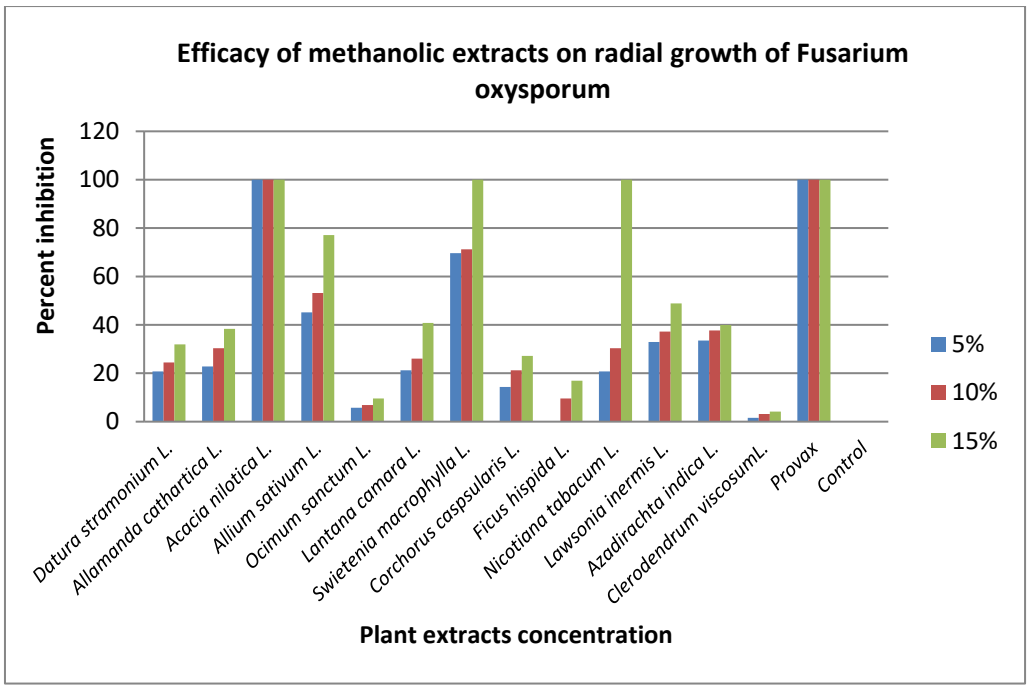

Fig. 4.2 Effect of methanolic extracts against *F. oxysporum*

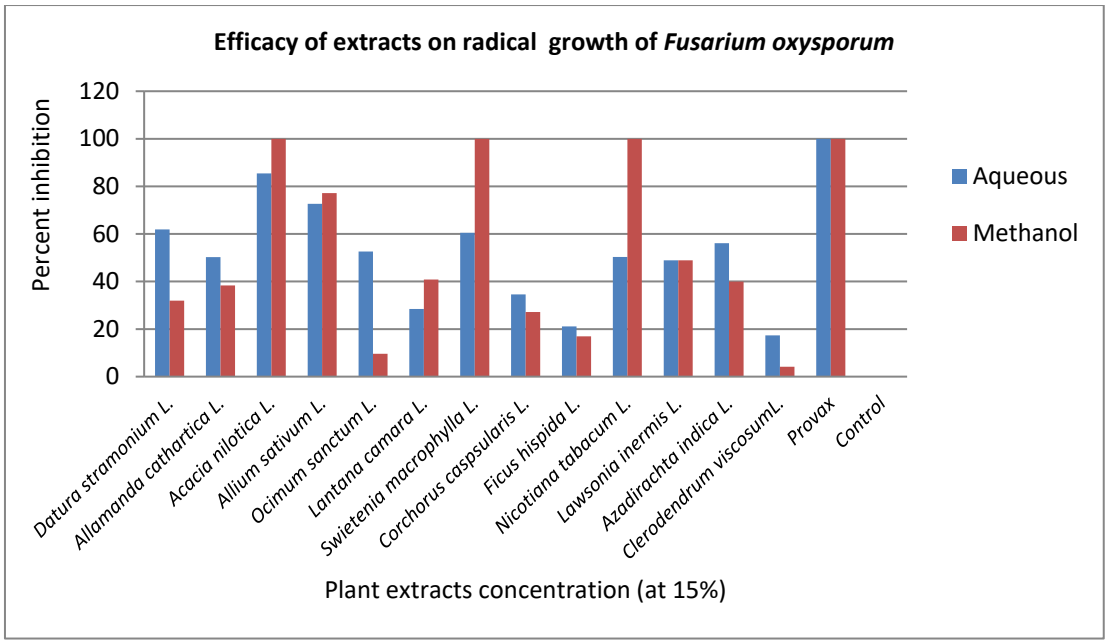

Fig. 4.3 Aqueous and Methanolic extract against *Fusarium oxysporum*

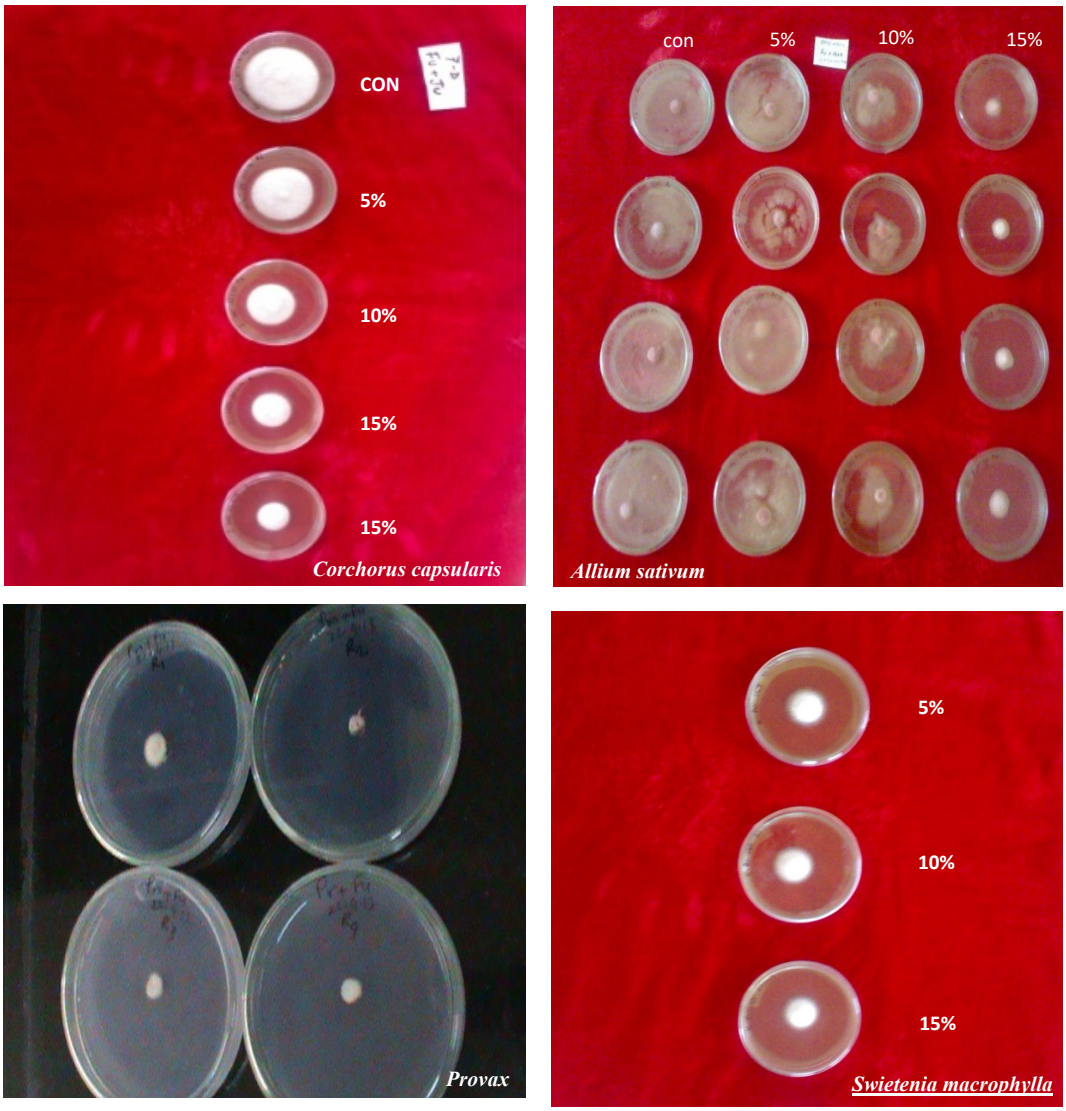

Plate 4.2 Effect of plant extracts against plant pathogenic-*Fusarium oxysporum* on PDA

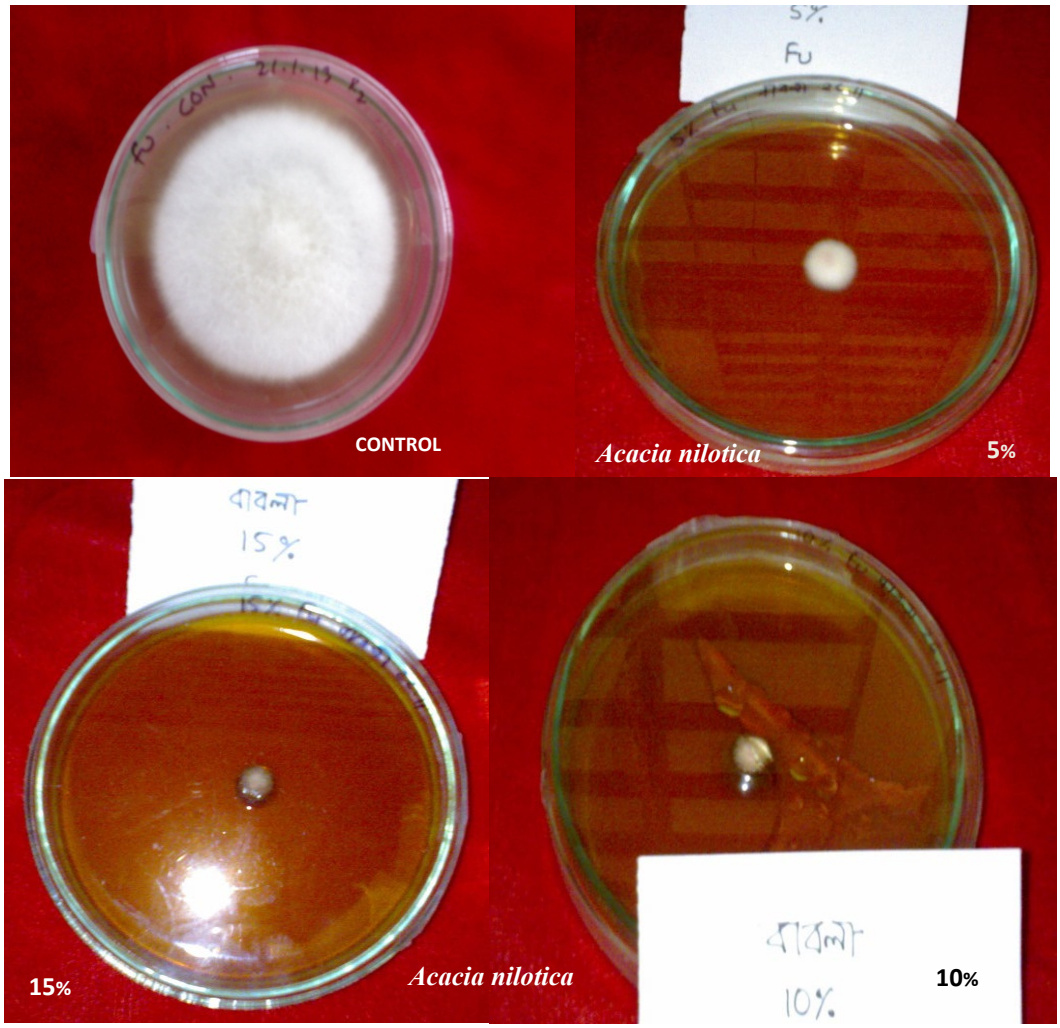

Plate 4.3 Effect of *Acacia nilotica* extracts against plant pathogenic-*Fusarium oxysporum* on PDA

4.3.1.3 Effect of Methanolic extract

All the methanolic plant extract of *Acacia nilotica, Lawsonia inermis, A. indica, Allamanda cathartica, Lantana camera Corchorus capsularis, Nicotiana tabacum, Datura stramonium ,Swietenia macrophylla, Ficus hispida, Ocimum sanctum, Allium sativum* and *Clerodendrum viscosum* at 5, 10, 15% concentration inhibited mycelial growth to different degrees (Table 4.6 and Fig. 4.2). At 05% concentration *Acacia nilotica* showed complete inhibition of mycelium growth, *Swietenia macrophylla* showed prominent, *Allium sativum* showed moderate and *Nicotiana tabacum, Lantana camera, Corchorus capsularis, Datura stramonium, Allamanda cathartica, Lawsonia inermis and A. indica recorded* low inhibition of mycelial growth till 7 days of incubation while *Ficus hispida, Ocimum sanctum* and *Clerodendrum viscosum* did not show efficacy in comparison of control. At 10% concentration *Acacia nilotica* reported total inhibition of mycelial growth, *Swietenia macrophylla* showed prominent, *Allium sativum*, *A. indica* and *Lawsonia inermis* reported moderate inhibition of mycelial growth. Rest of the plants were less effective in reducing the mycelial growth of *Fusrium oxysporum* in compared to control while *Clerodendrum viscosum* as similar as control. At 15 per cent concentration *Acacia nilotica*, *Nicotiana tabacum* and *Swietenia macrophylla* showed complete inhibition of mycelial growth, *Allium sativum* showed prominent, *Allamanda cathartica, A. indica, Lawsonia inermis and Lantana camera* showed moderate, *Clerodendrum viscosum* did not show any activity. Rests of the species were less effective in reducing the mycelial growth of *Fusarium oxysporum*. Maximum inhibition recorded at 15% concentration. The extract of plant *Acacia nilotica* showed maximum activity even at lower concentrations. The control plate representing DMSO did not exhibit inhibition on the tested fungi where as standard antifungal drug Provax have antifungal activity even at 2mg/L.

In particular, the authors may recommend that the methanolic extract of *Acacia nilotica* to be used as potent biocide to treat diseases in plants caused by *Fusarium oxysporum* as it showed maximum activity even at lower concentrations nearly equal to the standard antifungal agent. It was revealed in this study, that the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. It also supports the earlier investigation (Banso & Adeyemo, 2007) that the tannins

isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and may assume pharmacological importance. Extensive bioprocess parameter studies should be undertaken for the methanolic extract of *Acacia nilotica* as a strong antifungal agent against *Fusarium oxysporum* causing plant diseases.

The ethanolic extracts of *A. sativum* and A. *hirtifolium* revealed considerable antifungal activity against *F. oxysporum*. Okigbo *et al*. (2009) investigated the fungi toxic effects of *A. sativum* against six plant pathogenic fungi such as *F. oxysporum*, *F. solani*, *Botryodiplodia theobromae*, *M. phaseolina*, *Penicillium oxalicum* and *Aspergillus niger*. The obtained results revealed *A. sativum* had effective inhibition (25.2% - 86.9%) on the mycelial growth of all tested fungi. Jacob and Sivaprakasan (1994) and Arya *et al.* (1995) evaluated the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and the results stated extracts of garlic bulbs and Bignonia leaves inhibited the mycelial growth of *Fusarium pallidoroseum*. Effectiveness of garlic and neem as bio-fungicides has already been reported by many against different fungi. Garlic extract at 15% inhibited the growth of the pathogen (88.26%) (AnkitaShukala *et al*., 2012). Pradeep Kumar Singh *et al.,* (2010) also reported highest inhibition of radial growth of *F. udum* by *A. indica* (67.8%) at 5%. Effectiveness of garlic and neem extract in different fungi is also reported by many workers**(** N. Siva, *et al.,* 2008; R. R. Sharma, *et al*., 2005).

Mohana and Raveesha, (2007) also investigation the antifungal potential of both aqueous extract and petroleum ether solvent extract has been demonstrated that 50% concentration of aqueous extract totally inhibited both storage and field fungi. None of the earlier reports (Phadke *et al*., 1994; George *et al*., 1999a; George *et al*., 1999b; Thangadurai *et al*., 2002; Elizabeth *et al*., 2005) have demonstrated the antifungal activity against the wide range of phytopathogenic field and storageknown to cause a variety of diseases in sorghum, maize and rice. Nashwa S.M.A. *et al.,* (2012) indicated that all tested plant extracts, *Ocimum basilicum*, *Azadirachta indica*, *Eucalyptus chamadulonsis*, *Datura stramonium*, *Nerium oleander* and *Allium sativum*, caused a significant reduction in the linear growth of *A. solani*. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Similar effects of various other plant products effective against

Alternaria spp. were reported by several authors (Latha *et al.* 2009; Goussous *et al*. 2010). Similar studies have been carried out by Misra and Dixit (1976) on the antifungal activity of *Allium sativum* against eighteen different fungi including *Fusarium* spp. and they reported that crude leaf extract of *A. sativum* completely checked the mycelia growth of all the test fungi. Jacob and Sivaprakasan (1994) and Arya *et al.* (1995) studied the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and reported inhibitory effect of extracts of garlic bulbs and Bignonia leaves on the mycelial growth of *Fusarium pallidoroseum*. Karade and Sawant (1999), Datar (1999), and Anwar and Khan (2001) observed the same results with the plant extracts of other plants. Our findings are also in agreement with those of Bashir (2001) and Bhat (2002).

Similar result was recorded at 15 percent concentration. *Acacia nilotica, Swietenia macrophylla*(seed) and *Nicotiana tabacum* recorded complete inhibition(100%) in mycelial growth, followed by *Allium sativum* (77.15) and *Lawsonia inermis* (48.88%). The effect of plant extracts of different parts of plants on various species of *Fusarium* were studied extensively by different workers employing different angiospermic plant species.

Mycelial growth of various species of *Fusarium* was inhibited by the plant extracts of *Convolvulus alsinoides* and *C. Pluricutis*(Furgalwegrazyeka, 1984); *Allium cepa* (El. Sharmi *et al., 1986) Adhatoda vasica, Azadirachta indica, Cinnamomum camphora* and *Ocimum sanctum* (Prasad and Ojha, 1986); *Agave americana, Cassia nodosa* (Reddy and Reddy, 1987); *Azadirachta indica*(Eswaramoorthy *et al*; 1989); *Allium cepa*(Patel, 1989); *Avicennia marina, Aegicer ascorculatum, Kandelia candel, Excoecaria agallocha* and *Acanthus ilicifolius*(So, 1990); *Agave Americana* (Pandey *et al.,* 1992); *Allium sativum* and *Sapindus trifoliate* (Gohil and Vala, 1996); Neem seed extract (Gour and Sharmaik, 1998); *Azadirachta indica, Atropha belladona, Calotropis procera, Ocimum basilicum, Eucalyptus amygdalina, Ailanthus excels* and *Lantana camera* (Bansal and Gupta, 2000). In accordance with the above reports, in the present study, 100% inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *Melongenae* by water and methanol leaf extracts of *L. camara, S. macrophylla, N. tabacum and A. nilotica* and 60% to 98% of mycelial growth inhibition were recorded

in the plant extracts of 19 different species of angiosperms . Wilt is an important disease of brinjal plant causing significant reduction in yield. In present study the *in vitro* bio-efficacy of plant extracts of *Azardiachta indica, Allium sativum, Acacia nilotica, Lawsonia innermis* were tested to control wilt pathogen. Result of wilt diseases of brinjal indicated that there was a significant reduction (41.34%) in treated plants with neem extract. Similar finding were obtained against Fusarium wilt of Carnation (Chandel and Tomar, 2008). Thirteen plant extracts were testes for antifungal assay showing 58-99% mycelia growth inhibition (Minz *et al.,* 2012). The bio-efficacy of neem extract over pathogens can be attributed to the fact that neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and azadirone which are antifungal, antibactrial and anti-insecticidal in nature (Bohra *et al.,* 2006).

4.4 Conclusion

The main purpose of using plant extracts was to study their antifungal activity against the pathogen for eco-friendly disease management as most of the plant extracts are readily available, environmentally safe, less risky for developing resistance in pests, and pest resurgence, has less adverse effect on plant growth, less harmful to seed viability and quality, and above all, less expensive. The present study explores the possibilities of controlling *F. oxysporum*, by using extracts of *Acacia nilotica. Allium sativum, Nicotiana tabacum, Swietenia macrophylla* and *Datura stramonium.*The fungitoxic effects of the phyto-extracts indicate the potentials of selected plant species as a source of natural fungicidal material. Antifungal activity was confirmed by all of the selected plant species and the results revealed *Acacia nilotica*, *Swietenia macrophylla* and *Allium sativum* are the most effective inhibitor for the mycelia growth of *F. oxysporum*. The finding of the present investigation could be an important step towards the possibilities of using natural plant products as bio-pesticides in the control of plant diseases caused by *F. oxysporum*. Further purification, extraction and phytochemical analysis of the active compounds of those plants would give a strong antifungal activity comparable to synthetic fungicides.

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Chapter- 5

Inhibitory Effect of Plant Extract on Conidial Germination of the Phytopathogenic Fungus *Fusarium oxysporum*

Abstract

In an approach towards the development of eco-friendly antifungal control strategy, plant extracts of ten plants were tested for antifungal activity on pathogenic fungi *Fusarium oxysporum* caused diseases in brinjal. Different concentrations of extracts obtained from leaves, seeds and bulbs of different plants. These extracts were evaluated for efficacy on the spore germination of *Fusarium oxysporum.* Among ten tested plant species, two plants did not show any inhibition rather enhanced germination, one showed low inhibition, four had an intermediate level of antifungal activity, and only three inhibited (64.91-95.14%) fungal germination. The extract of *A. sativum* at highest concentration proved highly effective in reducing the spore germination of *Fusarium oxysporum* followed by the extract of *A. nilotica* and *Lawsonia innermis,* respectively in ethanol extract and the least efficiency was recorded in *Lantana camara* in all solvents. Other doses of concentrations of leaf, seeds and bulb extracts also caused inhibition in spore germination but to a lesser extent than highest concentrations. The inhibition in spore germination increases with increasing in the extract concentration. These findings suggest that some botanic extracts possess antifungal activities against *Fusarium oxysporum* and could be used as potential antifungal agents for the control of fungal plant diseases of brinjal.

Keywords: Plant Extract, *Fusarium oxysporum*, Anti-spore Activity, spore germination, Natural fungicide.

5.1 Introduction

In the past decades the use of fungicides to control crop disease has contributed to increased production of food worldwide. Nevertheless, the massive use of synthetic fungicides in crop defense had severe environmental impact. Synthetic fungicides also pose serious health risks within our food chain and have been linked to increased occurrence of several types of cancer. The increasing number of fungal population with enhanced resistance to common fungicides, on the other hand, urges the development for new formulations which are both effective and environmentally friendly. In this respect, plant extract may represent an ideal solution to the problem and can be easily tested *in vitro*, using a micro-titer plate assay to rapidly evaluate plant extracts (Wilson *et al.,* 1997) .This study showed that, when tested against *Botritys cinerea*, high levels of antifungal activity were found in 13 of the 345 plant species, mainly among the families of *Allium* and *Capsicum*. Furthermore (H. Khair and G. Nadia, 2011) a strong antifungal activity detected in 22 different plant extracts against two soil pathogens, *Fusarium solani* and *Rhizoctonia solani*, and it hypothesized that their effect on the fungal mycelium comes both from the inhibition of enzymes within the fungi and the a concomitant induction of endogenous plant proteins associated with defense against biotic stress. Aqueous extracts of 46 plants against *Fusarium* spp. (Satish *et al.,* 2009) revealed that 12 plants have recorded significant antifungal activity and that these plants could be exploited for eco-friendly management. The results of a study on the inhibitory activity of extracts from Kenyan medicinal plants against three soil pathogens (Rugutt *et al*., 2006) showed that *Warburia ugandensis*, *Azadirachta indica*, *Tagetes minuta* and *Urtica massaica* were active against the fungus *Fusarium oxysporum*, *Alternaria passiflorae* and *Aspergillus niger*.

In recent years the *Brassicacea* family has drawn special interest due to its high levels of glucosinolates. The glycosidic compounds are have no biocidal activity in their native form but are converted via enzymatic hydrolysis to the active degradation products (mainly isothiocyanates), whose cytotoxic activity has been extensively documented. Many authors (Daugovish, *et al.,* 2004; Manici, *et al*., 1997; Manici, 1999; Marciano, 2004; Villeneuve, 2004) have studied *in vivo* activity of seed flour of

Brassicaceae species against soil which had been inoculated with fungal spores; however its use in bio fumigation to control soil fungi has generated mixed results. For example, plant residues of *Brassicaceae* in the soil can enhance the growth of the fungus *Pythium* spp. **(**Walker and Morey, 1999). Glucosinolates from seed meal of *Brassica napus* (Cohen and Mazzola, 2006), strongly alter the soil micro biome (both pathogenic and useful microorganisms). A comprehensive study (Samuel *et al.,* 2008) showed that the use of plant residues of *Brassica* spp. to control the soil fungi *Pythium* and *Fusarium* brought no substantial benefit in controlling the disease in melon crops. Two years of experiments showed that soil treated with *B. napus* and *Brassica juncea* suffered from increased infestation by *Fusarium oxysporum* and *Pythium* spp. when compared to untreated soil. In this study, we have developed an *in vitro* multiwall assay to determine the biocidal activity of botanic extracts against the phytopathogenic fungus *F. oxysporum* and applied the method on a wide panel of plant extracts and powders. This work allowed us to select the best candidate extracts for future field trials, with the ultimate aim of developing a green alternative to synthetic fungicides.

Fusarium oxysporum is one of the most important soil pathogen that presents in the rhizosphere and stem of plants. Most *F. oxysporum* strains live saprophytically on organic substrates in soil. Some of soil-born strains are plant pathogens and causing plant disease. Some of them are bio control agent. The species is very complex group and based on the pathogenicity toward particular plant species take place into physiological races and form specialist (Armstrong and Armstrong, 1981).There are several ways for controlling of cucumber damping-off including biological control (Kim and Jee, 1988., Mohamed Gehad, 1996) chemical control (EL-Shami Mona *et at*., 1988) and plant extracts (Shalaby and Atia, 1996. Ehteshamul *et al*., 1998. Tohamy *et al*., 2002). Chemical fungicide traditionally been used against fungal plant pathogen. Frequency use of fungicide leaded to the development of resistant population of the pathogen against various chemical fungicide groups. The other side, the toxic properties of fungicides limited the use of these compounds (Lin , 1981).Some plant species were assayed for pharmacological and biological activity such as antibacterial and antifungal activity (Al-Mughrabi, 2003. Ismail *et al.,* 2003. Hoffman *et al.,*2004). Fungicidal properties of plant species depended on various plant products including oil, gums,

resin, saponin, organic acid and alkaloids (Sofowora, 1984. Asthana *et al.,* 1989. Chaturvedi, R.., Dikshit and Dixit, 1987. Daoud, Qasim and Al-Mallah, 1990. Cowan, 1999. Al-Mughrabi *et al.,* 2001).

Khalil and Dababneh (2007) investigated the inhibitory effect of four medicinal plant extracts against *Rhizoctonia solani*, *F. oxysporum*, *Verticillium* sp. and *Penicillium* sp. *Vartemia iphionodes* showed maximum inhibitory for *Verticillium* sp. (44.8%), followed by *R. solani* (42.9%), *F. oxysporum* (42.7%) and *Penicillium* sp. (18.2%) at 1000 ppm. The effect of *Zataria multiflora* and *Satureja hortensis* essential oil were evaluated on the growth rate and ability of mycotoxin production by *F. graminearum* in PDA and PDB. The results indicated the essential oils had inhibitory activity on this fungus and mycotoxin production (Lahooji, Mirabolfathy and Karami-Osboo, 2010). Asalem *et al.,* (2010) studied antifungal activity of five plant species against three important pathogens including *Altrenaria solani*, *R. solani* and *Macrophomina phaseolina*. *Adhatoda zylanica* showed maximum inhibition against *M. phaseolina* whereas, *Dodonaea viscosa* had the most effective on *A. solani* and *R. solani*. Dababneh and Khalili (2007) evaluated five Jordanian medicinal plants against five pathogenic fungi. The highest growth inhibition of all fungi was observed with *Achillea santolina* at 1000 ppm. The growth inhibition of *F. oxysporum* and *R. solani* was 42.2 and 42.0%, respectively.

Morsy, *et al.* (2009) investigated the growth rate of five plant pathogen fungi such as *F. oxysporum, F. solani, Sclerotium rolfsii, R. solani* and *M. phaseolina*. The results stated plant extracts of onion and garlic reduced the growth rate of *F. oxysporum*, *F. solani* and *S. rolfsii*. The highest inhibition effect on *F. oxysporum* was observed when garlic extract was applied at 1.5ml/plate. The inhibitory effect of *Thymus vulgaris*, *Satureja hortensis*, *Anthum grareolens* and *Mentha sativa* were investigated against *F. graminearum.* The result stated the essential oils reduced the growth rate and mycotoxim production (Hoseiniyeh Farahani *et al*., 2012). Efficacy of Mancozeb and garlic extract were evaluated against *Alternaria alternate*, *F. oxysporum*, *Aspergillus* sp., *Rhizopus* sp. and *colletoterichum graminicola* on sorghum and groundnut seed. The results showed that garlic extraction was effective on seed germination and mycelia weight of the fungi (Syed, 2012).

The objectives of this study were to determine the effect of *Mentha piperita* L., *Cinnamomum zeylanicum*, *Allium hirtifolium* and *Allium sativum* L. on the growth rate and spore germination of *F. oxysporum*. The fungal rots are world-wide in occurrence and have been reported almost in all parts of the world (Soki, 1994; Snowndon, 1990; Ali *et al*., 2005; Fontema *et al.,* 1996; Mitsovek *et al.*, 2007), resulting in huge economic losses to the plants (Jones *et al*., 2001). Several management strategies such as cultural, physical, chemical, biological and regulatory methods have been used for the control of pathogenic fungi but each of these have one or other limitations. The continuous uses of fungicides develop resistance in these fungi and are toxic to the environment. Therefore, there is need for developing novel plant protectant that interferes with the fungal pathogenicity factors. Use of natural plant products for the control of fungal disease is considered as alternative to synthetic fungicides due to their slower negative effect on the environment. The plant extracts being harmless and non-phytotoxic proved effective on the germination and viability of fungal spores. Several plants and their products have been used for the control of plant disease and have proved to be harmless and non-toxic unlike that of chemical fungicides (Khalil, 2001; Abu-Jawdah *et al*., 2002; Bovers and Locke, 2002; Sharma and Kumar, 2009; Satish *et al*., 2009). Keeping in view the non-phytotoxic effect of plant extracts, ten *plants, Lawsonia inermis, Ficus hispida, Acacia nilotica, Azadirachta indica, Lantana camara, Nicotiana tabacum, Allium sativum, Corchorus capsularis, Datura stramonium* and *Allamanda cathartica* were evaluated for their efficacy on the spore germination of *Fusarium oxysporum* responsible for wilt disease of brinjal.

5.2 Materials and Methods

5.2.1 Experimental site

All the experiments were conducted during July 2011 to June 2014 in IES-Laboratory, RU and Plant Pathology Laboratory, Dept. of Botany RU, Pot house experiment was conducted in research field of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur.

5.2.2 Plant materials

Plant materials of twenty six species (Table1) were collected from different places in Rajshahi University campus and Rangpur district. The collected plants were identified and authenticated by a botanist in the Department of Botany, University of Rajshahi, Bangladesh. Plants were washed with tap water; surface sterilized with 2% sodium hypo chlorite for 5 min and washed thoroughly 2-3 times with sterile distilled water.

5.2.3 Preparation of extracts

5.2.3.1 Aqueous extract

The powdered leaves extracted with sterile distilled water at room temperature at different level of concentrations (5, 10, 15 and 20%) i.e. 5 g in 100 ml, 10 g in 100 ml, 15 g in 100 ml and 20 g in 100 ml. Then extracts were filtered through double layered muslin cloth and finally through whatman filter paper No.1. in 250 ml Pyrex flasks. The flasks were tightly wrapped with aluminum foil. The extracts were used for screening their antifungal activity. Extracts were stored at 4°C in pre-sterilized flasks until use.

5.2.3.2 Methanolic and ethanolic extracts

Finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using soxhlet apparatus. For extraction, 50 gm. leave and seed powder of each of the selected plants was transferred into separate conical flasks containing 200 ml of methanol and mixed well. The flasks were left for two days with occasional stirring. The extracts were then filtered (Whatman No.1 filter paper), concentrated in rotary evaporator under reduced pressure and dried in the desiccator (Manasa *et al.*, 2013).This crude extract were kept under low temperature (4°C) in the refrigerator until use.

5.2.4 Preparation of Spore Suspension and Counting spores of *F. oxysporum*

About 10 days old culture of *F. oxysporum* was used.10 ml of sterile water was added to each petridish. Then the surface of the fungal growth was separated gently with a glass slide to disperse the spores. The suspension was decanted into a conical flask

and sieved to separate the hyphal fragments from the suspension. Finally, the number of spores per ml suspension was counted by Haemacytometer method and it was 1.0 X 10^4 spores/ml.

5.2.5 Anti-fungal activity assay

5.2.5.1 Determination of percent inhibition of spore germination by cavity slide Method

Antifungal activity of ethanolic, methanolic and aqueous extract of the test plants on the spore germination of *F. oxysporum was* assayed by cavity slide method **(**Reddick and Wallance, 1910 and Maji *et al*., 2005). Conidial suspension of *F. oxysporum* was prepared in sterile distilled water using dextrose 0**.**039g/ml and spore concentrations which adjusted to 1.0 X 10^4 spores/ml was prepared from 10 days old fungal culture. 90 µl of conidial suspension then placed in a cavity slide. The setup was placed in large Petri dishes laden with moist blotter paper to maintain moisture level. 10 µl each of ten plant ethanolic, methanolic and aqueous extracts were placed in separate conidial suspended cavity slide and mixed well. 10µl of distilled water and DMSO placed in 90 ml of conidia suspended media served as separate control. All treatments were maintained in triplicates. The setup was incubated for 12, 24 and 36 hours at 25 ±1°C. Germination of spore was counted under compound microscope (Singh and Tripathi, 1999). The percent of inhibition was calculated using the formula given by Vincent in1947.

Plate 5.1 Preparation of plant extract

Plate 5.2 Preparation of spore suspension

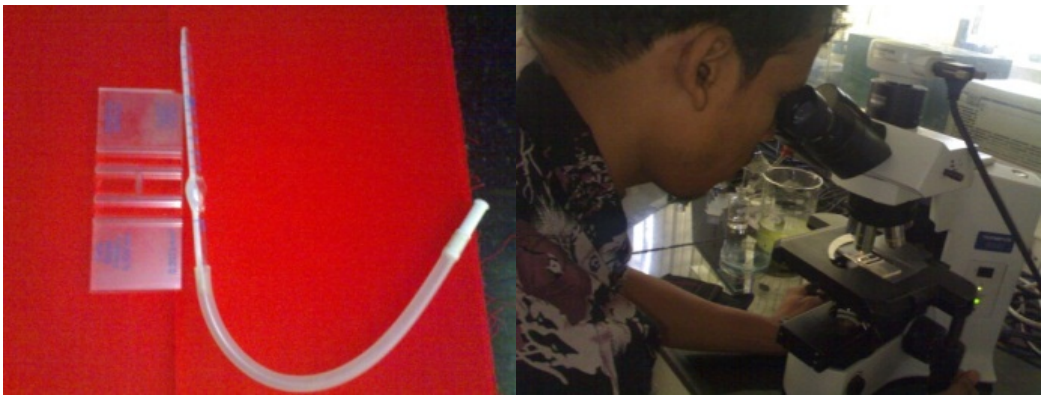


Plate 5.3 Determination of spore germination

Percent inhibition = Gc– Gt / Gc X 100

Where,

Gc = Number of spores germinated in control (Average of 10 microscopic field)

Gt= Number of spores germinated in treated (Average of 10 microscopic field). Data were subjected to statistical analysis and compared with Duncan.

5.3 Results

All tested botanical extracts tested varied degree of inhibition over control in the conidial/spore germination (Tables-5.1, 5.2, 5.3 in Appendices and Fig.5.1, 5.2, 5.3) of the pathogen *F. oxysporum* at 15% concentration after 36 hours incubation. Result revealed that highly significant percent inhibition of spore germination of *F.*

oxysporum was observed in *Allium sativum* (95.14) followed by *A. nilotica* (91.51) at 15% concentration after 36 hours of incubation in ethanolic extract. The results are similar to chemical fungicide-provax (Table5.1 & Plate5.1). The ethanolic extracts of *Lowsonia inermis* (69.97), *A. indica*(64.35), *Nicotiana tabacum* (52.38), *Datura stramonium* (48.51) and *Allamonda cathertica* (55.06) showed moderate significant percentage inhibition over control, whereas ethanolic extracts of *Ficus hispida and Corchorus capsularis* did not show any inhibitory activities, in addition enhanced conidial germination**.** In other words *Lantana camara,* showed least inhibition percent of germination compare to control (Plates 5.5, 5.6, 5.7).

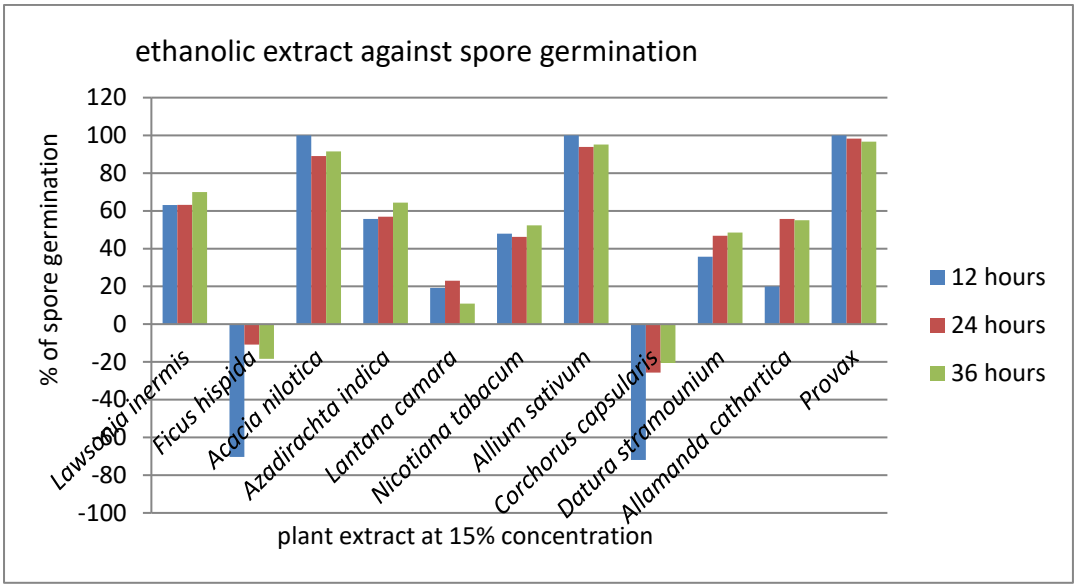


Fig. 5.1 Effect of ethanolic extract on spore germination at 15% concentration

The results on the effect of the leaf, bulb and seed extract of the plant species on spore germination of *F. oxysporum* are presented in Table-5.2 Appendices. The methanolic extracts of the eight plant species (*A. indica, Lantana camara, Nicotiana tabacum, Lawsonia inermis, Datura stramonium, Allamonda cathertica, Allium sativum* and *A. nilotica*) inhibited spore germination of the fungus. The maximum inhibition in spore germination was found in *A. nilotica* (93.76) followed by *Allium sativum* (92.88) at 15% concentration after 36 hours incubation, whereas methanolic extracts of *Ficus hispida* and *Corchorus capsularis* did not show any inhibitory activities, rather enhanced germination compare to control (Table 5.2 & Fig.5.2).

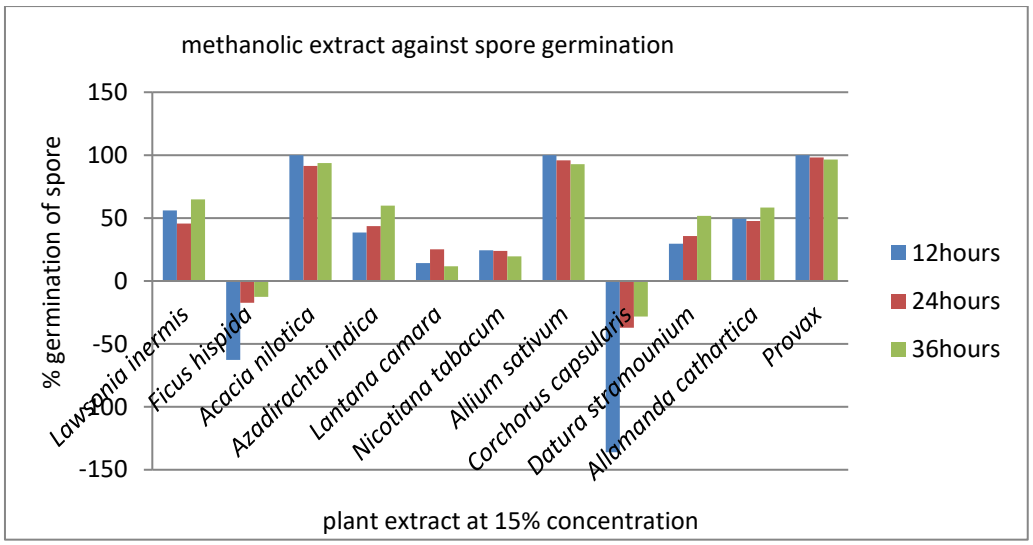
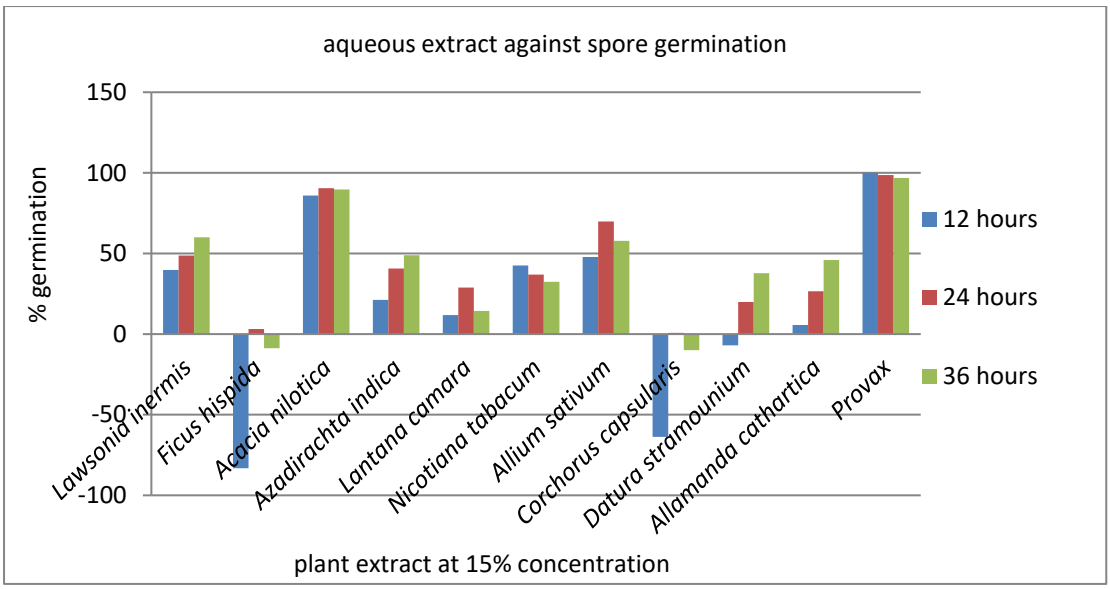


Fig. 5.2 Effect of methanolic extract on spore germination at 15% concentration

The methanolic extracts of *Lowsonia inermis*(64.91), *A. indica* (59.86), *Nicotiana tabacum*(19.63), *Datura stramonium* (51.75) and *Allamonda cathertica* (58.33) showed significant percentage inhibition over control. In other words *Lantana camara,* showed least inhibition percent of germination compare to control (Table 5.2 & Fig.5.2).

Result presented in Table- 5.3 in Appendices showed that highest inhibition of spore germination was observed in *A. nilotica* (89.73) followed by *Lawsonia inermis*(60.06) and *Allium sativum* (57.85) compare to control. The aqueous extract of *Allamonda cathertica* (45.96) and *A .indica* (48.92) showed intermediate inhibitory effect. *Datura stramonium* (37.80) and *Nicotiana tabacum* (32.45) exhibited less inhibitory effect at 15% concentration after 36 hours . The other tested plants showed no inhibitory activities. Furthermore, *Lantana camara,* showed least inhibition percent of germination compare to control (Table 03), as well as *Ficus hispida* and *Corchorus capsularis* enhanced germination compare to control (Table 5.3 in Appendices & Fig. 5.3).



. **Fig. 5.3 Effect of aqueous extract on spore germination at 15% concentration**

On the other hand, the experiments revealed that highly significant percent inhibition of spore germination of *F. oxysporum* was observed in *Allium sativum* (96.63) followed by *A. nilotica* (93.25) at 20% concentration after 36 hours of incubation in ethanolic extract. The results are similar to chemical fungicide (provax). The ethanolic extracts of *Lowsonia inermis* (77.46), *A. indica* (73.00), *Nicotiana tabaccum* (61.21), *Datura stramonium* (48.51) and *Allamonda cathertica* (55.06) showed moderate significant percentage inhibition over control, whereas ethanolic extracts of *Ficus hispida and Corchorus capsularis* did not show any inhibitory activities, in addition enhanced conidial germination**.** In other words *Lantana camara,* showed least inhibition percent of germination compare to control (Table-5.4 in Appendices & Fig. 5.4).

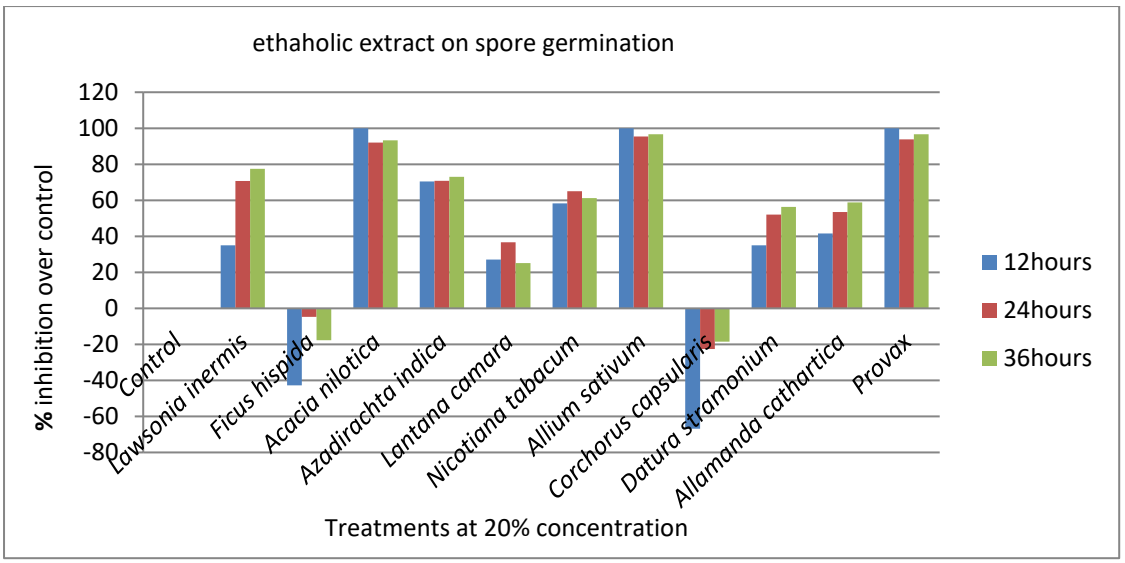


FIG. 5.4 Effect of ethanolic extract on spore germination at 20% concentration

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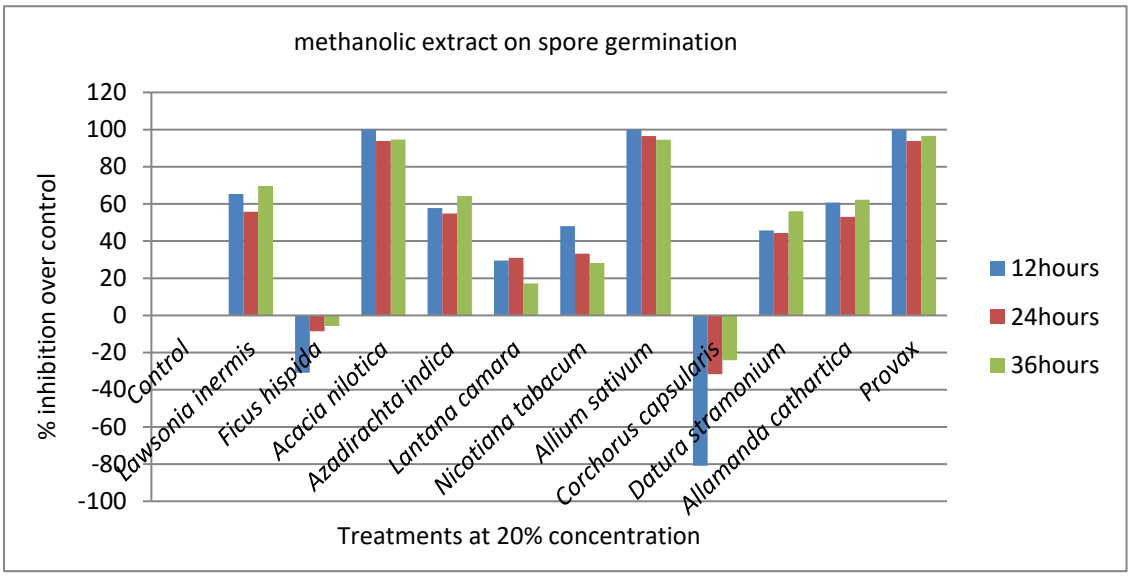

Fig. 5.5 Effect of methanolic extract on spore germination at 20% concentration

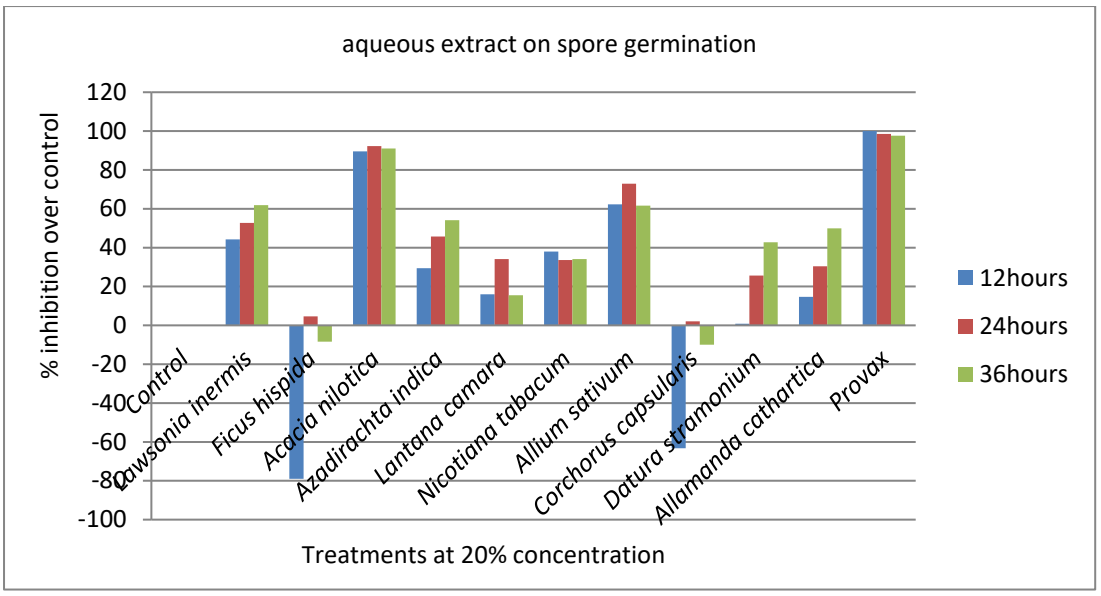

Fig. 5.6 Effect of aqueous extract on spore germination at 20% concentration

The methanolic extracts of the eight plant species (*A. indica, Lantana camara, Nicotiana tabacum, Lawsonia inermis, Datura stramonium, Allamonda cathertica, Allium sativum* and *A. nilotica*) inhibited spore germination of the fungus. The maximum inhibition in spore germination was found in *A. nilotica* (94.66) followed by *Allium sativum* (94.54) at 20% concentration after 36 hours incubation. The results are similar to fungicide(provax), whereas methanolic extracts of *Ficus hispida* and *Corchorus capsularis* did not show any inhibitory activities, rather enhanced germination compare to control (Table 5.5 in Appendices and Fig. 5.5).

The methanolic extracts of *Lowsonia inermis* (69.67), *A. indica* (64.26), *Nicotiana tabacum* (28.26), *Datura stramonium* (56.05) and *Allamonda cathertica* (62.27) showed significant percentage inhibition over control. In other words *Lantana camara,* showed least inhibition percent of germination compare to control (Table 5.5 in Appendices and Fig. 5.5)

Result presented in Table- 5.6 in Appendices showed that highest inhibition of spore germination was observed in *A. nilotica* (91.08) followed by *Lawsonia inermis*(61.84) and *Allium sativum* (61.62)compare to control. The aqueous extract of *Allamonda cathertica*(49.96) and *A.indica*(54.16) showed intermediate inhibitory effect. *Datura stramonium*(42.78) and *Nicotiana tabacum* (34.14) exhibited less inhibitory effect at 20 % concentration after 36 hours. The other tested plants showed no inhibitory activities. Furthermore, *Lantana camara,* showed least inhibition percent of germination compare to control (Table 5.6), as well as *Ficus hispida* and *Corchorus capsularis* enhanced germination compare to control (Table 5.6 in Appendices and Fig. 5.6).

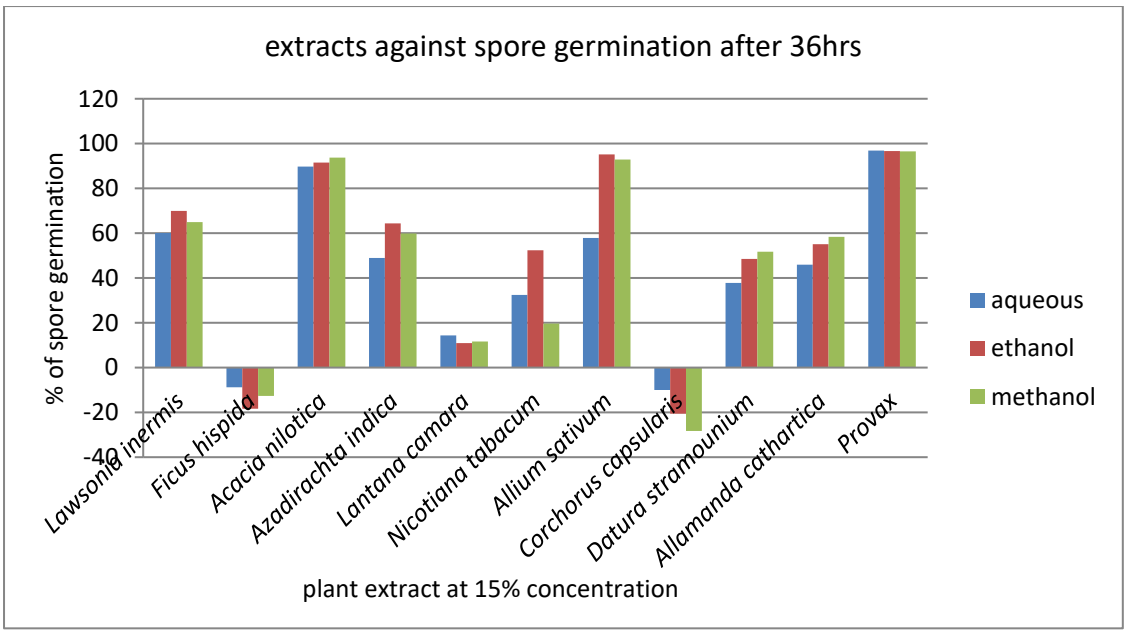

Fig. 5.7 Effect of extract in different solvents on spore germination at 15% concentration

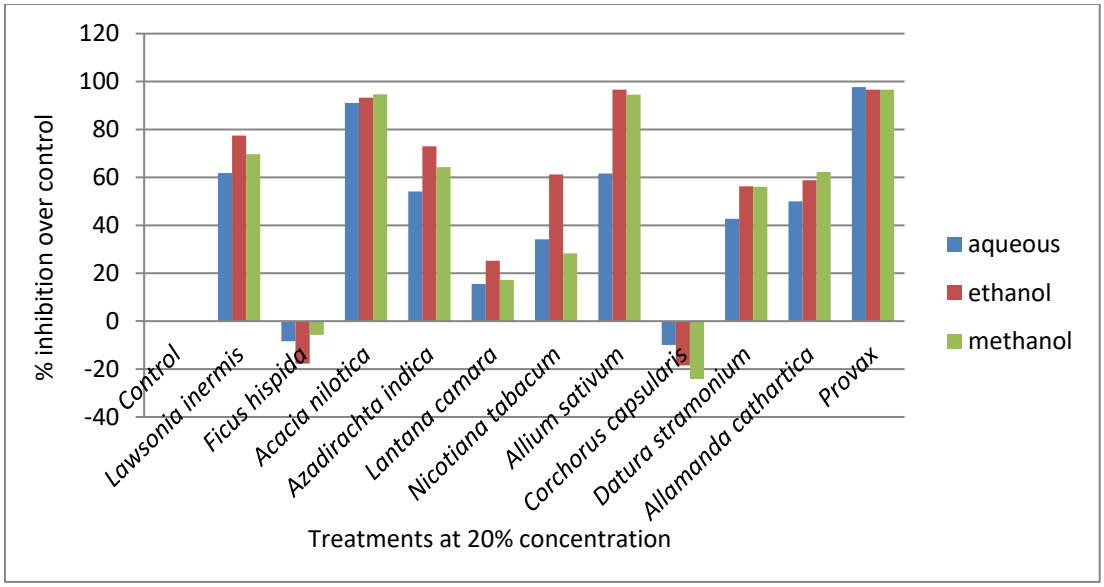

Fig. 5.8 Effect of extract in different solvents on spore germination at 20% concentration

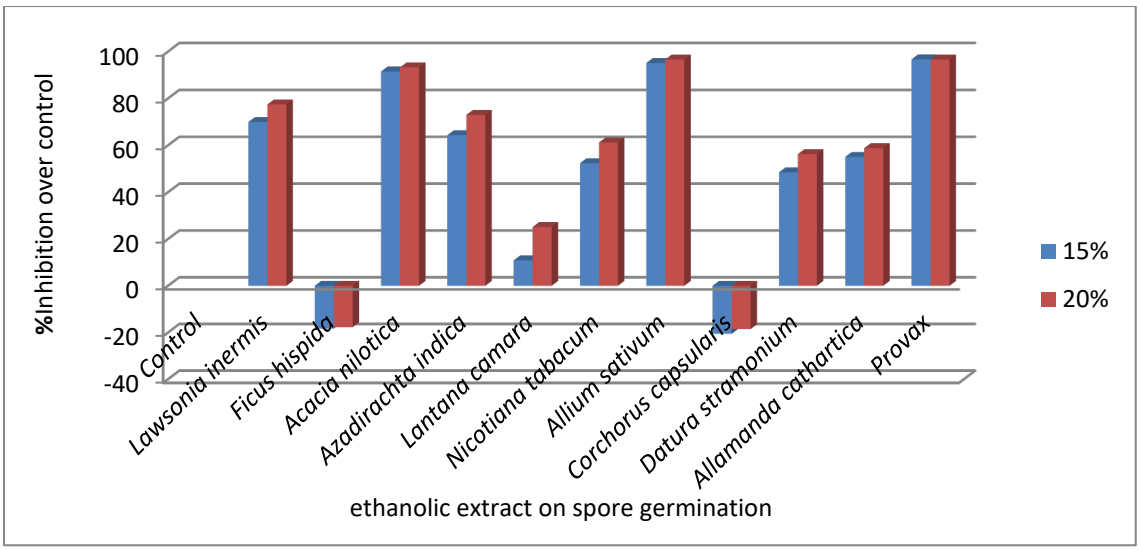

Fig. 5.9 Effect of ethanolic extract on spore germination(at 15% & 20% concen.)

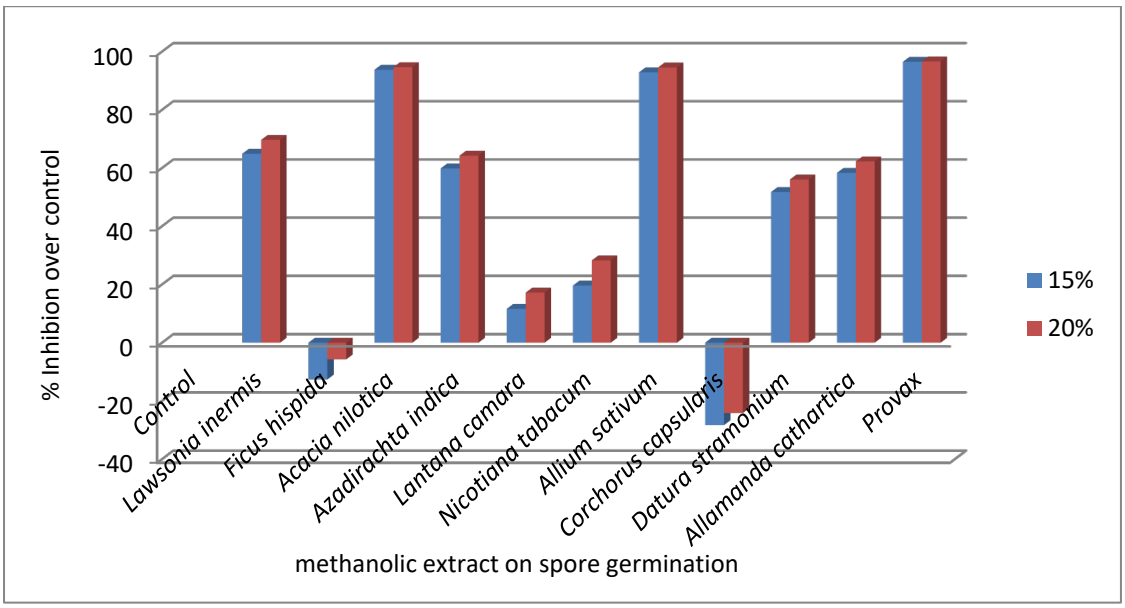

Fig.5.10 Effect of methanolic extract on spore germination(at 15% & 20% concen.)

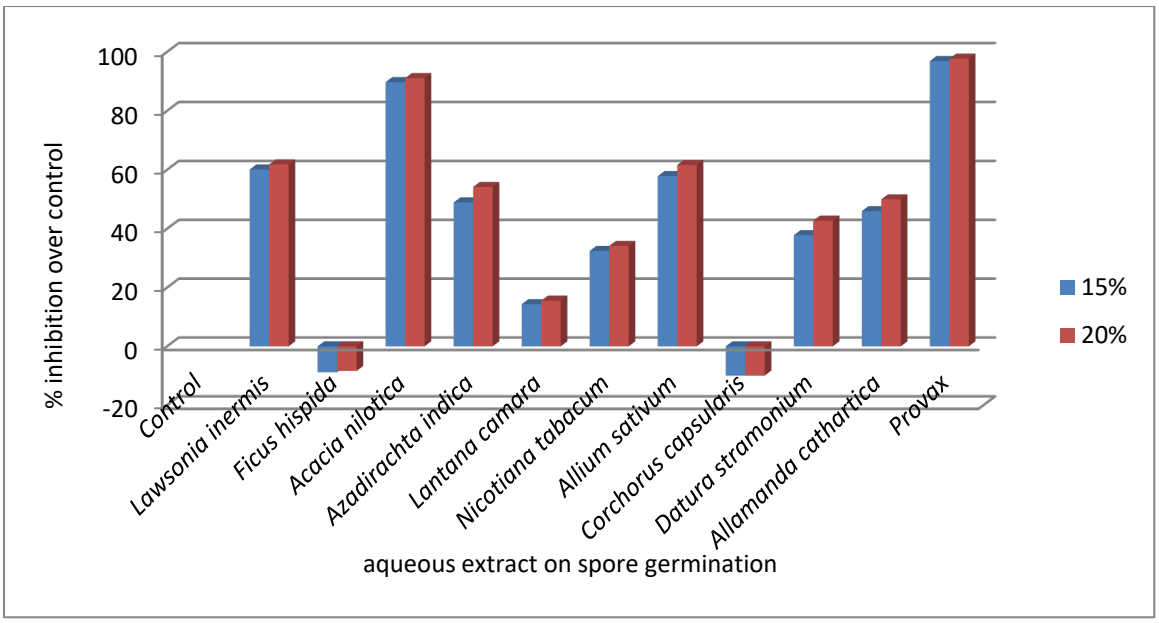

Fig.5.11 Effect of aqueous extract on spore germination(at 15% & 20% concen.)

It was observed that, in most of the treatments there was significant interaction with respect to the concentrations. With the increase in the concentration of the extract, there was corresponding increase in the inhibition of the pathogen (Fig. 5.8, 5.9, 5.10).

5.4 Discussion

According to some authors, the uses of plant materials for the control of phytopathogens exceed 4% of the pesticide market within a few years. The substitution of synthetic fungicides with ecofriendly products is holds great potential because the use of pesticides will be increasingly restricted by European regulation for their undesired side effects such as recalcitrance to degradation, accumulation in the food chain and interference with soil microbiology. Finally, there is the economic aspect, the possibility of using plant molecules in crop protection, in fact, even as carriers or dispersants used as such or for the production of coformulants, requires large quantities with positive effects on agriculture

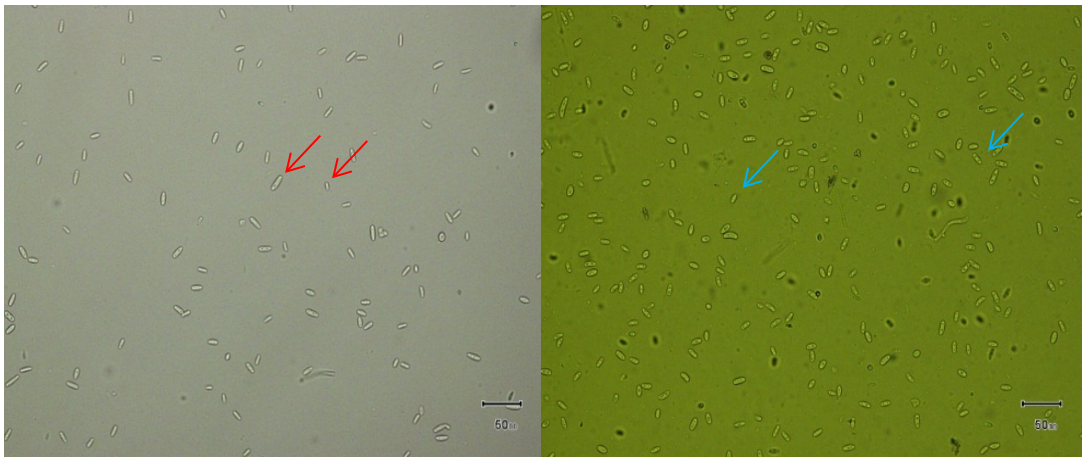

Plate 5.4 Macro and micro spore of *Fusarium*

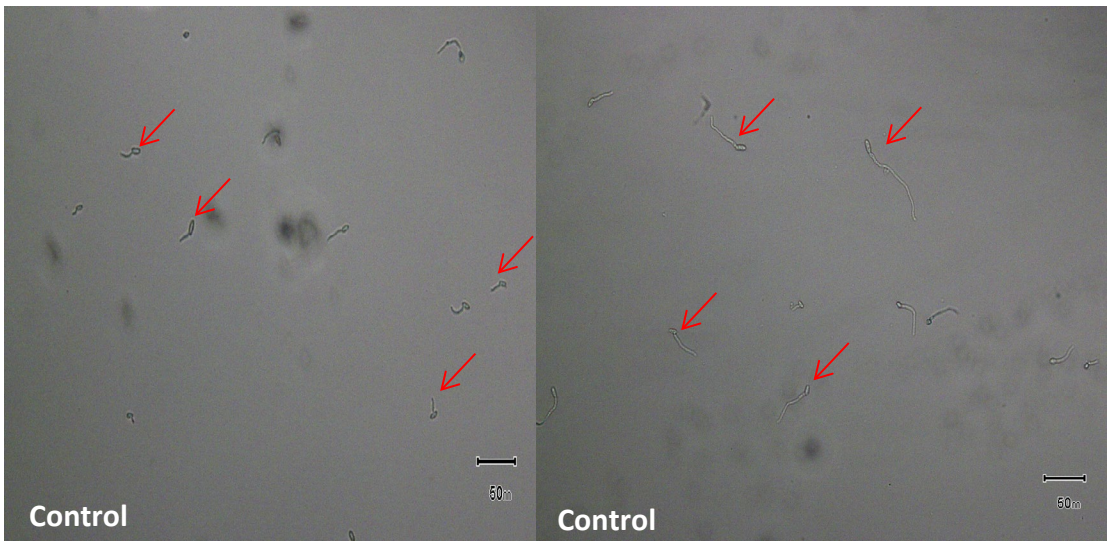

Plate 5.5 Effect of Tap water (with DMSO) on spore germination

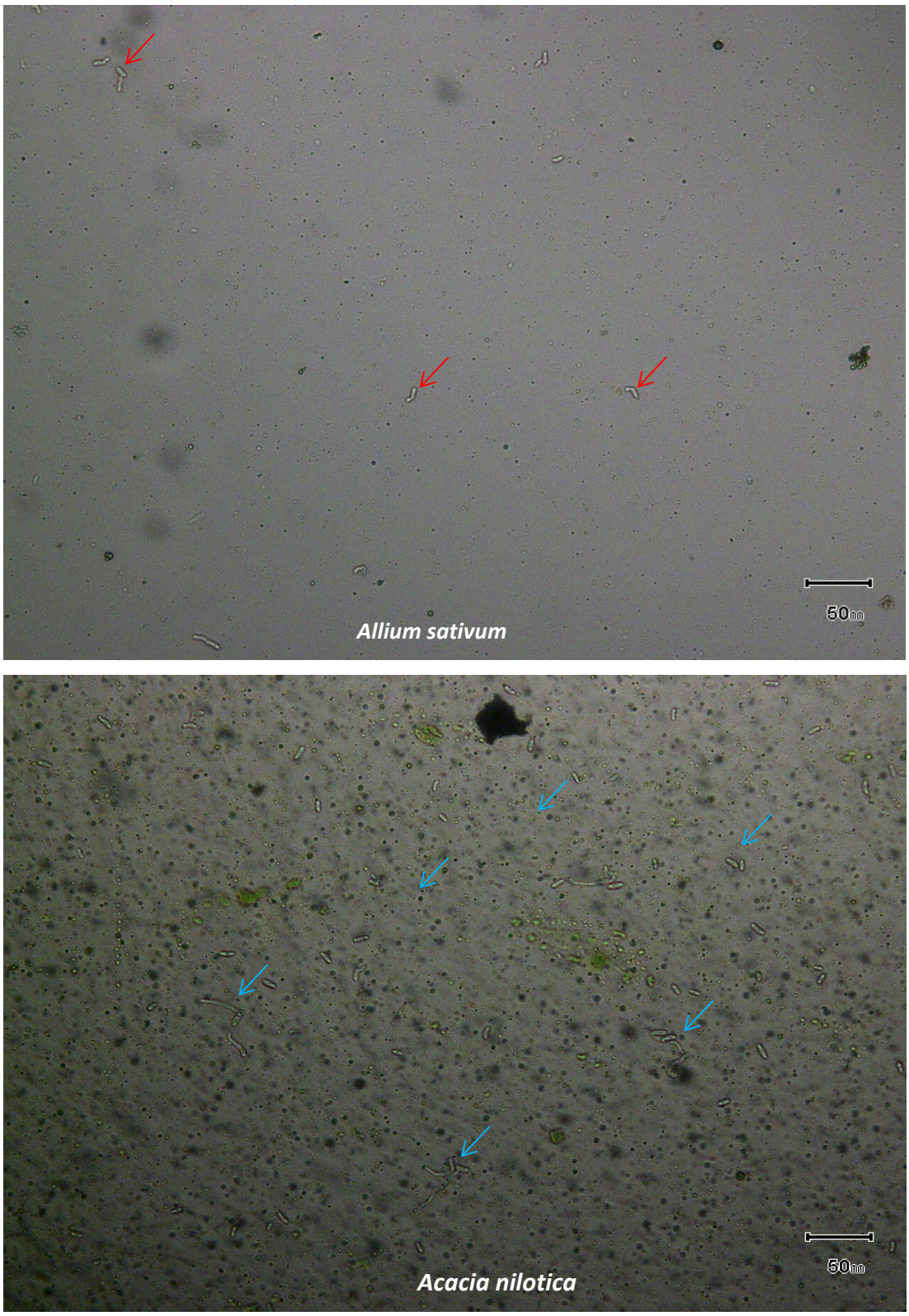

Plate 5. 6 Effect of different plant extracts on spore germination

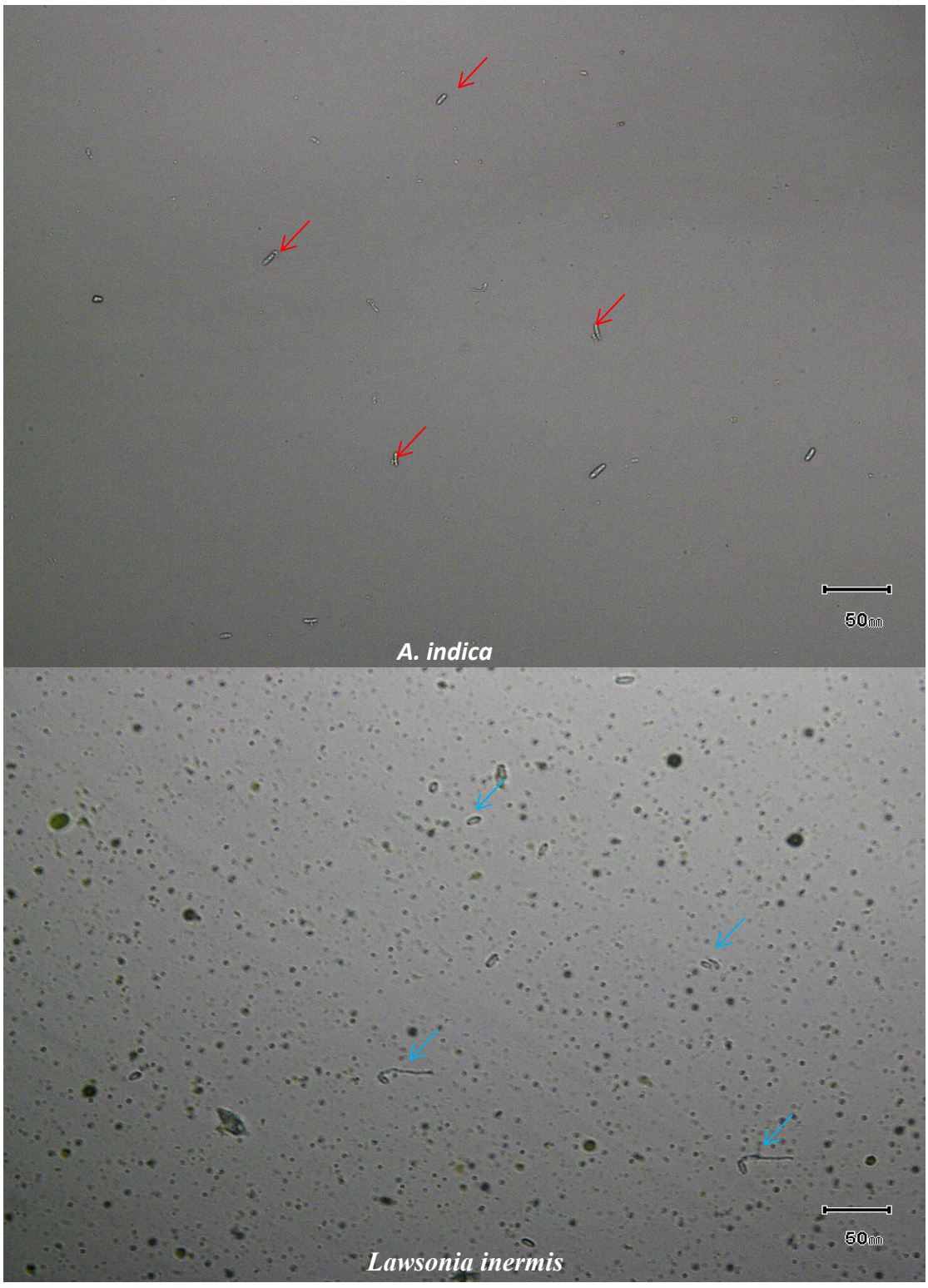

Plate 5. 7 Effect of different plant extracts on spore germination

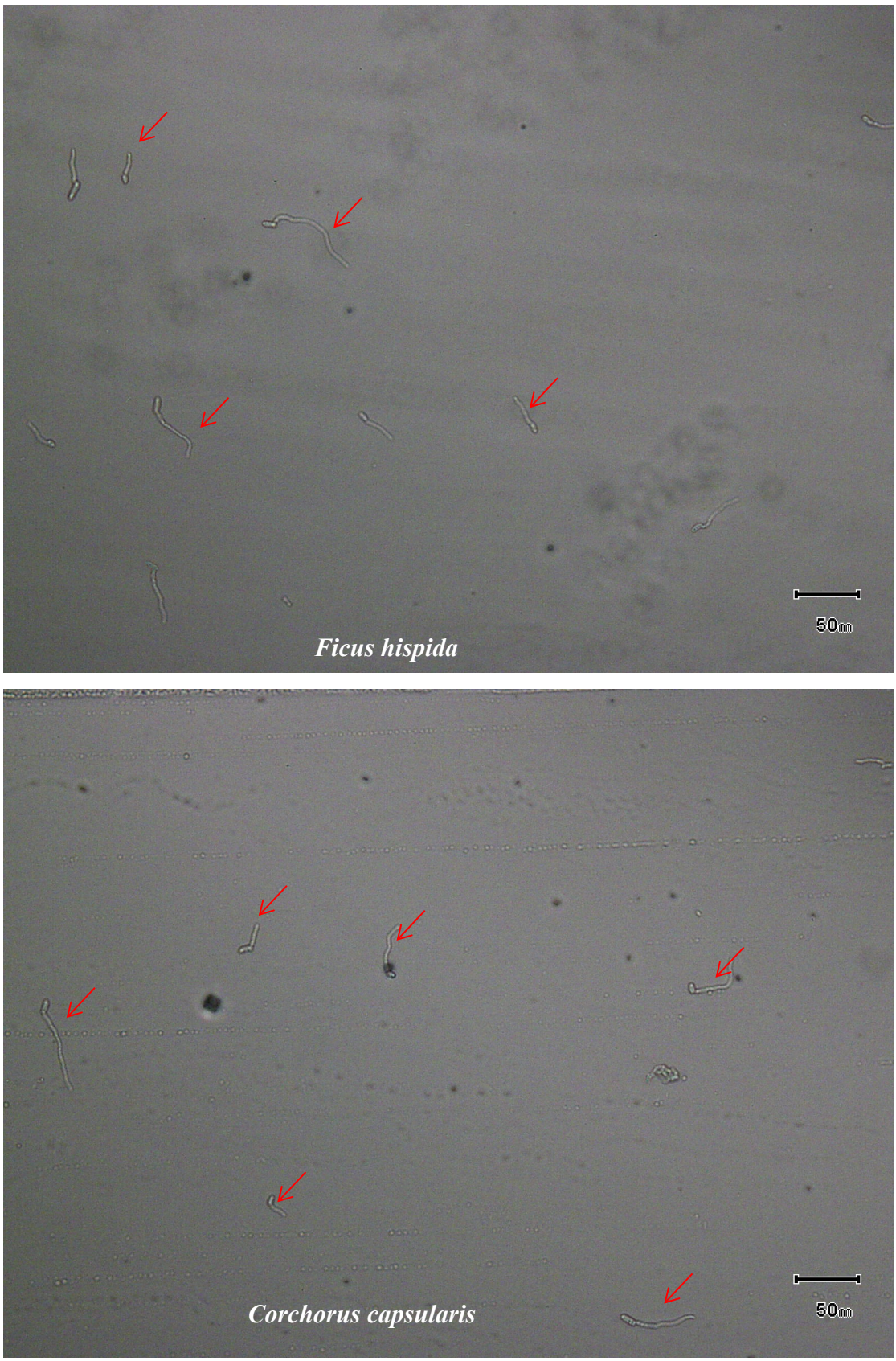

Plate 5. 8 Effect of different plant extracts on spore germination

In our research, we have used the soil pathogen *F. oxysporum*, causal agent of a disease known as "wilts". This disease occurs in many plant species, including grasses, legumes and horticultural crops. It is very difficult the crop protection against those soil pathogens, especially now that is no more admitted the use of Methyl bromide as a fumigant.

Bangladesh, being a country with an intensive agricultural, the control of the soil borne infections has always represented a serious problem. The genus *Fusarium* is one of the major fungal genera responsible for diseases on several crops. There are many alternative strategies to reduce the use of fungicides, including the use of resistant plant varieties, health certificated seed, phytosanitary measures, good cultural practices, water management, bio fumigations, soil solarisation, biological control, the use of natural fungicides obtained from plants. This last aspect would seem the most effective to reduce the use of synthetic fungicides **(**Satish *et al*., 2009; Singh and Verma**,** 2001**).**

The aim of this research was to identify the best plant with a high level of antifungal activity against *Fusarium* spp. The plants with high or intermediate fungal activity have been found. Among those plants, this research has been able to detect 8 plant extracts capable to inhibit conidial germination of *F. oxysporum*. The results obtained are in conformity with the Nidhi Sharma and Trivedi (2002) who have reported that the leaf extract of *L. inermis* was effective against mycelial growth of cumin wilt caused by *Fusarium oxysporum* f. sp. cumini. They have also screened *C. procera* (Ait.) R. Br. against the pathogen and found effective. Maji *et al.,* (2005) have screened *C. gigantea*, *Cassia tora* and *Cassia sophera* against some mulberry foliar pathogens by conidial germination and poison food technique and obtained good results. Naik (2006) has screened *C. limon, T. erecta* and *L. inermis* against *F. solani*, another root rot pathogen of mulberry and obtained significant results with *L. inermis* in poison food technique. The present study reveals that, aqueous, ethanolic or methanolic extract of *Acacia nilotica, Allium sativum* and *L. inermis* gives significant results with maximum inhibition of spore germination at 15% concentrations after 12hr, 24hr and 36 hours of incubation.

This study revealed that the leaf extracts of *Acacia nilotica* bulb extract of *Allium sativum* and leaf extract of *Lawsonia inermis* at 15% concentration had considerable effect on growth and germination of *F. oxysporum*. It is clear from the results, that

concentration of the leaf extracts of the three plant species showed maximum inhibition in growth and spore germination on isolates as compared to the control. At highest (15%) concentration all the leaf extracts proved highly effective in inhibiting growth and germination of the fungal isolates. The results also indicate that the extract of *A. nilotica* was highly effective as compared with the extracts of *Allium sativum* and *Lawsonia inermis*. This is similar to the findings of (Okigbo *et al*., 2009) who reported that *A. indica* is more active in inhibiting the growth of *A. niger, B. theobromae, F. oxysporum* and *P. oxalicum* associated with postharvest rot of cassava tubers. B.M. Soma and V. Belewa, (2011) observed that extract of *A. indica* and *Chromolalena odonata* inhibited the growth of *A. niger, F. oxysporum, R. stolonifer* and *Geotrichum candidum*. The work of Anukworji *et al*., 2012, indicated that extracts of *Allium sativum* and *A. indica* inhibit the growth of *Sclerotia rolfsii, B. theobromae, R. solani* and *A. niger*. The ability of the leaf extract of *A. indica*, *R. comunis* and *M. indica* to inhibit growth and spore germination of *R. stolonifer* and *F. oxysporum* could be due to presence of fungitoxic compounds in the extracts of the three plant species (Anukworji, *et al*.2012). The extract of *A. indica* proved highly effective in inhibiting the growth and spore germination of *R. stolonifer*. Similar results were also obtained in growth and spore germination of *F. oxysporum* (Ibrahim *et al.,* 2014). Mahshid *et al*., (2013) studied Extract of *Cinnamomum zeylanicum*, *Menthapiperita*, *Allium hirtifolium* and *Allium sativum* showed maximum inhibition on the spore germination of *Fusarium oxysporum* schlecht. Domenico R.. *et al.,* (2012) reveled that among 500 plant species tested, about 84% did not exert significant inhibition, 7.6% showed low inhibition, 5.2% had an intermediate level of antifungal activity, and only 3% inhibited fungal germination completely against *Fusarium oxysporum,* f. sp. *lycopersici.* The study of Manmohan, M. S. and Govindaiah (2012) reveals that, aqueous extract of *P. guajava* and *L. inermis* gives significant results with maximum inhibition of spore germination and mycelial growth in all the concentrations. Ghorbany *et al.,* (2010) showed that seed extracts of *Trachyspermum copticum*, leaf extracts of *Lavandula angustifolia* and flower extracts of *Rheum ribes* effectively inhibited the radial growth and spore germination. Similar studies have been carried out by different researcher on antifungal activity of plant extract. The effect of plant extracts and essential oils from *Rosmarinus officinalis*, *Thymus vulgaris*, *C. zeylanicum* and *Syzigium aromaticum* was evaluated on the mycelial growth and spore germination of *F. oxysporum*f. sp. *cubense in vitro*.

The results indicated that plant extracts and essential oils of *C. zeylanicum* and *S. aromaticum* were effective at 500 ppm against the mycelial growth (Fernando *et al.,* 2013). Boniface *et al.* (2012) investigated the antifungal activity of *C. zeylanicum* essential oil. The results proved the oil had fungicidal properties against *F. oxysporum* and *Penicilium digitatum*.

Bowers and Locke (2000) studied antifungal activity of various plant extract against *Fusarium solani* f. sp. *melongenae* and the results showed that the extract of *Allium sativum* exhabited maximum inhibition in spore germination followed by *Datura stramonium*, *Artemisia* spp. *Mentha spicata* and *Juglans regia*. Efficacy of fungal activity of various plant extracts were studied against the spore germination of *Alternaria brassicae* causing *Alternaria* blight in rapeseed and mustard. The results indicated that garlic demonstrated maximum inhibition in spore germination (Khurana *et al*., 2005). Tongbram and Chherty (2012) also reported *A. sativum* extract completely inhibited mycelial growth and spore germination. *A. indica* also inhibited the growth of the pathogen above 50% against *Fusarum udum*, the causal agent of *Fusarium* wilt of pigeonpea. Mohana and Raveesha (2007) revealed that highly significant percent inhibition of spore germination of *F. solani* and *A. flavus* was observed in *D. hamiltonii* (95.3 and 38.3%). The aqueous extracts of *C. coriaria*, *E. tirucalli* and *L. aspera* did not show significant activities, whereas aqueous extracts of *A. mexicana, P. amarus, T. cordifolia* and *T. terrestris* did not show any inhibitory activities. Vijayan (1989) reported that the bulb extract of *A. sativum*, leaf extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*

Nisa *et al*., (2010) revealed that all the concentrations of plant extracts ((*Allium cepa* L, *Allium sativum* L and *Mentha arvensis* L.) brought about significant inhibition in the spore germination of *A. alternata* and *R. stolonife*r. Bowers and Locke (2000) indicates that the maximum inhibition in spore germination of *Fusarium solani* f. sp. *melongenae* was exhibited by the extract of *Allium sativum* followed by *Datura stramonium*, *Artemisia* spp. *Mentha spicata* and *Juglans regia*. Thirumala and Sitaramaiah (2000), Lolpuri, (2002) and Teqida Menesens *et al*., (2002) reported antifungal activity of some wild plants against *Penicillium* sp. Various plant extracts of neem, mint, mehendi, safeda and garlic were used for their effect on the inhibition control of spore germination of *Alternaria brassicae* causing *Alternaria* blight in

rapeseed and mustard. All the plant extracts showed inhibitory effect but garlic proved most effective in inhibiting spore germination (Khurana *et al.*, 2005). Several studies with plant extract of onion, ginger and other plants also indicates their inhibitory effect on the spore germination and mycelial growth of several pathogenic fungi (Moubasher *et al*., 1970; Singh *et al.*, 1990; Hassan *et al*., 2005; Tagoe *et al*., 2011). Ethanol, methanol, and aqueous extracts of *L. inermis* leaves were involved also in defensive mechanism against spore germination of *Drechslera oryzae* (Natarajan and Lalitha, 1987).

5.5 Conclusion

It was revealed from the study that the bulb extract of *A. sativum* and leaf extracts of *A. nilotica and Lawsonia inermis* caused inhibition in growth and spore germination of *F. oxysporum*. This demonstrated fungitoxic potential of the bulb and leaf extracts of these three plant species against the pathogenic fungi (*F. oxysporum*). Therefore, use of the leaf and bulb extracts of these plants provide better alternative to synthetic chemicals which are expensive and pose potential danger to the farmers, marketers, consumers and environment. The plant extracts can be used as bio-pesticides for the control of foot rot, root rot and wilt diseases of brinjal.

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Chapter - 6

Collection, Isolation, Purification, Morphological Characterization and Efficacy of the Isolates of *Trichoderma*

Abstract

This experiment was carried out in the laboratory and pot house of Plant Pathology Division, BARI, Gazipur to find out the effectiveness of *Trichoderma* spp. as a bio-agent for controlling of foot and root rot and wilt diseases of brinjal caused by *Sclerotium rolfsii* and *Fusarium oxysporum.* A total of 6 fungal isolates were collected from rhizosphere of different crops and other sources and identified on the basis of their morphological and cultural characteristics. The collected fungal isolate of *Trichoderma harzianum* was evaluated. The radial mycelial growth of six isolates namely TRU-5, TRU-14, TB-23, TR-37, TD-44 and TTG-50 of *Trichoderma* spp. showed significant differences during 24 hours, 48 hours and 72 hours incubation period. The *Trichoderma* isolate, TB-23 caused the maximum growth on PDA. Antagonistic activity of *Trichoderma harzianum* was conducted on PDA following dual culture plate technique against *S. rolfsii* and *F. oxysporum.*

Key words: *Trichoderma harzianum,* dual culture, antagonistic activity, *S. rolfsii* and *F. oxysporum.*

6.1 Introduction

6.1.1 The fungus *Trichoderma*

History of *Trichoderma*

The fungus *Trichoderma* was described as early as 1794 AD by the mycologist Person. After more than one and a quarter century, the potential for use of *Trichoderma* as a biocontrol agent was suggested by Weindling (1932), who was the first to demonstrate the parasitic activity of the members of this fungus genus to pathogens such as soil borne plant pathogenic fungi e.g., *Rhizoctonia solani.* However, with the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, Dennis and Webster (1971b) described the antagonistic properties of *Trichoderma* in terms of antibiotic production and hyphal interactions in the control of *S. rolfsii.* Several *Trichoderma* species were formulated commercially for the protection and growth enhancement of a number of crops in several countries such as the United States (Mcspadden and Fravel, 2002). Mausam *et al.,* (2007) documented the information on the classification of the genus *Trichoderma,* mechanisms of antagonism and their role as biocontrol agents, owing to their capabilities of ameliorating crop-yields by multiple activities, such as bio-pesticide, bio-herbicides and plant growth promoter.

6.1.2 Biology and Nomenclature

The genus *Trichoderma* belongs to the sub-division Deuteromycotina. It is classified as an imperfect fungus having no known sexual reproductive stage (Gams and Bisset, 1998). Rifai (1969) differentiated nine species primarily by conidiophoral branching patterns and conidial morphology as *Trichoderma aureoviride. T. haniatuni, T. harzianum, T. koningii, T. longibrachiatum,T. piluliferum, T. polysporum, T. pxeudokaningii,* and *T.viride.*

A sectional classification was proposed for *Trichoderma* recognizing the following five sections; *Trichoderma, Longibrachiatum, Saturnisporum, Pachybasium* and *Hypocreanum* (Bissett, 199la). Twenty species were assigned to section *Trichoderma.* They were described and differentiated on the basis of conidiophore and conidial

morphology (Bissett, I991b). In the section *Trichoderma,* Persoon (1794) characterized species by narrow and flexuous conidiophores with branches and phialides uncrowded, frequently paired, and seldom in verticals of more than three. The taxonomy of this fungal genus is continually being revised, and many new species are being described (Komon-Zelazowska *et al.,* 2007; Kubicek *et al.,* 2008; Overton *et al.,* 2006; Samuels, 2006). Druzhinina and Kubicek (2005) have extensively reviewed species concepts and biodiversity in *Trichoderma* fungi. The authors have mentioned that *Trichoderma* fungi are difficult to distinct morphologically, however, the phylogenetic classification has rapidly reached 100 (Druzhinina *et al.,* 2006), and it is expected to increase consistently. In this context, the advancements as well as limitations of modern methods like genealogical concordance phylogenetic species recognition (GCPSR) and DNA-barcode system for safe identification of *Trichoderma* spp. warrant future investigations. The GCPSR requires the analysis of trees of several unlinked genes, whereas, DNA-barcode system is based on the defined nucleotide sequence differences of different *Trichoderma* spp.

6.1.3 Characteristics of the Genus *Trichoderma*

The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or repeatedly branched conidiophore with lageniform phialides and hyaline or green conidia borne in slimy heads (Bissett, 1984). The primary branches of conidiophore produce smaller secondary branches that also may produce tertiary branch, and so on. The final branches are very simply constructed, majority with a single phialide (Rifai, 1969). Conidiophore may end up in sterile appendages with the phialides borne on lateral branches in some species. Conidia are hyaline or, more usually, green, smooth walled or roughened. Hyaline chlamydospores are usually present in the mycelia of older cultures (Domsch *el al.,* 1980).

Phialides are ampulliform to lageniform, usually constricted at the base, more or less swollen near the middle, and abruptly near the apex into short sub cylindric neck. They are disposed in verticals terminally on branches of the conidiophore, or less

frequently singly or in whorls directly beneath septa along the conidiophore and its branches (Bissett, 199l).

6.1.4 Growth Medium and Sporulation

Trichoderma species are saprophytic fungi and use a wide range of compounds as carbon and nitrogen sources. The carbon and energy requirements of *Trichoderma* can be satisfied by monosaccharides and disaccharides (Papavizas, 1985). Danielson and Davey (I973a) suggested ammonium as the most readily utilized source of nitrogen of *Trichoderma* spp. in buffered media, and also, they suggested other sources of nitrogen, such as amino acids; urea and nitrate support vegetative growth. Elad *el al.* (1981) found special *Trichoderma* selective media (TSM) that had been recommended for the quantitative isolation of *Trichoderma* from soil. Selectivity was obtained by using chloramphenicol as a bacterial inhibitor and pentachloronitrobenzene (PCNB), and rose bengal as fungal inhibitors. The colony of *Trichoderma* grows rapidly and matures in 5 days on potato dextrose agar at 25°C, and the colony is wooly and become compact. The colony color is white; as the conidia are formed, scattered blue-green or yellow-green patches become visible. These patches may sometimes form concentric rings. They are more readily visible on potato dextrose agar compared to other media. Reverse is pale, tan, or yellowish (Stitton *et al.,* 1998 and De Hoog *et al.,* 2000). Most species of *Trichoderma* are photosensitive, sporulating on many natural and artificial substrates. Exposure of agar cultures for 20-30 seconds to light of 85-90 lux intensity is usually sufficient to induce some sporulation (Papavizas, 1985). The best photoinduction of phialoconidiogenesis has been obtained with exposure to daylight for three minutes or to near UV radiation (366 nm) for 10-30 seconds (Betina and Spisiakova, 1976). Acidic pH levels *in vitro* enhanced the growth of *T. harzianum* and stimulated its conidiophore formation and conidial germination (Chet and Baker, 1980). There has been also considerable interest in CO_2 effects on *Trichoderma* growth *in vitro.* The effect of CO_2 on growth depends on its concentration and medium pH (Danielson and Davey, I973b). Pugh and Van (1969) found that *Trichoderma* growth was rapid at high concentration of CO_2. *Trichoderma* species are rarely reported to occur on living plants and have not been found as endophyte of living plants (Petrini, 1986).

6.1.5 Ecology

Trichoderma species are ubiquitous in the environment, especially in soils. They have been used or encountered in many human activities, including commercial applications in production of enzymes besides biological control of plant disease (Samuels, 2006). *Trichoderma* species are widely distributed all over the world (Domsch *et al.,* 1980), and found in all soils including forest humus layer (Wardle *et al.,* 1993) as well as in agricultural orchard soils (Roiger *et al.,* 1991) and in natural habitats, especially those containing or consisting of organic matter (Papavizas, 1985). They are also found on root surfaces of various plants and on decaying bark, especially when it is degraded by other fungi; and on sclerotia or propagules of other fungi (Papavizas, 1985). Caldwell (1958) was the first to observe that chlamydospores survive in soil better than conidia. *Trichoderma* species can produce chlamydospores on natural substrates, such as oat kernels placed in sterile and natural soils. These structures may play an important role in the survival of the genus in the soil (Hem's and Papavizas, 1983). Hypha also survives in soil (Papavizas *et al.,* 1984). Papavizas *et al.* (1982) found that the conidia of *Trichoderma harzianum* added to soil without nutrient supplying amendments survived between 110-130 days depending on the isolate. Davit (1979) determined that, conidia added to soil decreased in number initially, and then was stabilized for two years to about one-tenth the original number added. Lewis and Papavizas (1984) demonstrated the potential of various *Trichoderma* species aggregates to form chlamydospores readily and in great numbers in natural soil or in fragments of organic matter after the introduction of the fungus to the soil as conidia. Propagation of *Trichoderma* enhanced by the low pH, in the former study indicating that acidification of soil could induce suppressiveness by *Trichoderma* (Chet and Baker, 1980). Soil moisture enhances the *Trichoderma* conidia to survive longer than in dry soil (Lui and Baker, 1980). Several compounds also affect growth of *Trichoderma* spp. (Backman and Rodriguez-Kabana, 1975; Curl *et al.,* 1968; Rodriguez-Kabana *et al.,* 1968). The importance of these pesticides in suppressing disease, directly or indirectly, under field conditions has not been determined. *Trichoderma* can tolerate fungicides, such as methyl bromide, captan and maneb (Ruppl *et al.,* 1983). Lewis and papavizas (1984) demonstrated, however, that the number of colony forming units of *T. harzianum* and *T. viride* introduced four days before, or at the time of fumigation with sub lethal rate of sodium methane, was significantly less than

those in non-fumigated soil, and the number of colony forming units of strains introduced four days after fumigation was similar to that in non-fumigated soil. Papavizas (1981) reported that *T. harzianum* did not survive well in the rhizophere of bean and pea seedling when seeds were coated with conidia of the fungus. In addition, he found that the number of colony forming units recovered per gram of rhizosphere soil including the roots and decaying seed coats and cotyledons was less than the number of conidia added per individual seed. The minimal effective amount of *Trichoderma* was found by Chet and Baker (1980) to be around $1xl0^6$ CFU/g soil.

6.1.6 Soil Fungistasis

Fungistasis is a natural mechanism of inhibition of growth of fungi imposed by soil microbes and overcome by the nutrient-rich rhizosphere of a host plant, allowing soil borne pathogens to infect the plant (Papavizas and Lumsden, 1980).*Trichoderma* and other potential biocontrol fungi proliferate abundantly in various natural soils when added as young mycelium in intimate contact with food base, but not as conidia with or without bran (Lewis and Papavizas 1984). *Trichoderma* conidia have been found to be either very sensitive to fungistasis (Lockwood, 1977) or to be relatively insensitive, and these are more sensitive than chlamydospores and hyphae are less sensitive than conidia (Mitchell and Dix, 1975). Danielson and Davey (1973) found that the sensitivity to fungistasis was higher in neutral or alkaline than in acid soils.

6.1.7 *Trichoderma* Modes of Action

No single mode of action for *Trichoderma* species against fungal plant parasites function alone. There are several mechanisms of action suggested for *Trichoderma* spp. mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients, and inactivation of the pathogens enzymes (Samuels, 2006). The first three were the ones by which these fungi have always been considered to function; other mechanisms are suggested but not yet been confirmed (Harman, 2000). The colonization of the root system by rhizosphere competent strains of *Trichoderma* results in increased development of root and or aerial systems and

crop yields (Chacon *et al.,* 2007; Yedidia *et al.,* 2001; Harman and Kubicek, 1998). Some strains of *Trichoderma* have also been reported to be aggressive bio degraders in their saprophytic phases (Wardle *et al.,* 1993), in addition to acting as competitors to fungal pathogens, particularly when nutrients are a limiting factor in the environment (Simon and Sivasithamparam, 1989). These facts strongly suggest that in the plant root environment *Trichoderma* actively interacts with the components in the soil community, the plant, bacteria, fungi, other organisms, such as nematodes or insects that share the same ecological niche.

6.1.8 Mycoparasitism

Mycoparasitism is considered as an important mechanism of biological control and probably depends on the production of lytic enzymes including β-l, 3-gluconase, and proteases (Haran *et al.,* 1996a). Several chitinolytic enzymes have been reported in *T, harzianum* including endochitinases, exochitinases and 1,4-β-N-acetylglucosaminidases which are induced during growth in liquid medium containing chitin as carbon source (Haran *et al.,* I996b). Mycoparasitism is a complex process including several steps. The initial interaction shows that the hypha of the mycoparasites grows directly towards its host (Chet *et al.,* 1981). When the mycoparasite reaches the host, its hypha coils it or attaches to it by forming a hook-like structure. Following these interactions hypha sometimes penetrates the host mycelium, apparently, by partially degrading its cell wall (Elad *et al.,* 1983). The control of *Rhizoctonia solani* and *Pythium ultimum* by *Trichoderma* species, including *T. harzianum,* may be affected through direct penetration of host hyphae (Dennis and Webster, 1971; Benhamou and Chet, 1993). They grow toward hyphae of other fungi, coil about them in a lectinmediated reaction, and degrade cell walls of the target fungi by the activity of enzymes, which may be associated with physical penetration of the cell wall (Chet, 1987). Recently, the role of extracellular enzymes has been well documented by several researchers *e.g.,* proteolytic enzymes (Kredics *et al.* 2005; Pozo *et al,* 2004), 1, 3- glucanolytic system (Kubicek *et al.,* 2001: Vazquez-Garciduenas *et al.,* 1998), chitinase (Hoell *et al.,* 2005). The complex groups of extracellular enzymes have been reported to be a key factor in pathogen cell wall lysis during mycoparasitism.

6.1.9 Antibiosis

Antibiosis is the process of secretion of anti-microbial compounds by antagonist fungi to suppress and/or kill pathogenic fungi in the vicinity of its growth area (Schirmbock *et al.,* 1994). The importance of antibiotics for biocontrol activity was demonstrated in several studies. Dennis and Webster (1971) found that many *Trichoderma* strains produced volatile and nonvolatile antibiotics. Howell and Stipanovic (1983) isolated and described a new antibiotic, gliovirin, from *T. virens* that was strongly inhibitory to *Pylhium ultimum* and a *Phytophthora* species. Lumsden *et al.* (1992) found that suppressive activity of *T. virens* to damping off of zinnias, incited by both *R. solani* and *P. ultimum,* was correlated with production of the antibiotic gliotoxin by the biocontrol agent. Wilhite *et al.* (1994) used mutation to demonstrate that loss of the antibiotic gliotoxin production in *T. virens* mutants had an adverse effect on biocontrol activity. *Trichoderma* spp. produces 43 substances that have antibiotic activity which do not include enzymes (Sivasithamparam and Ghisalbetri, 1998). Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, dikcyopiperazines, sesquiterpenes, and steroids have been associated with biocontrol activity of some species and strains of *Trichoderma* (Howell, 1998).

6.1.10 Competition and Rhizosphere Competence

Competition for space or nutrients has long been considered one of the classical mechanisms of biocontrol by *Trichoderma* spp. (Elad *et al.,* 1999). The competition for nutrients, primarily carbon, nitrogen, and iron is one of the methods of the biological control of soil borne plant pathogens (Scher *et al.,* 1984). *Trichoderma* species are generally considered to be aggressive competitors and the ability of *Trichoderma to* compete is species dependent (Wardle *et al.,* 1993). However, the excess nutrient in granules containing *T. harzianum* can be used rapidly by *Phytophthora cinnamomi* when the growth of the antagonist was suppressed by lack of oxygen, and the pathogen increased rather than the antagonist (Kelly, 1976). Competition through rhizosphere competence is a mechanism that has gained adherents in recent years (Howell, 2003). It is an important mechanism because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. Lo *et al.* (1996) found that a strain of *T. harziamtm* (T-22) was strongly

rhizosphere competent and able to control several plant pathogenic fungi including *R. solani* causing brown patch, and it reduced the initial disease severity by as much as 71% on a variety of crops. *Trichoderma* spp. could compete and sequester ions of iron (the ions are essential for the plant pathogen, *Serpula lacrymam* as part of a non-enzymatic complex) by releasing compounds known as siderophores (Srinivasan *et al.,* 1992). Thus, the cited examples confirmed that significance of competition for nutrients between *Trichoderma* and pathogenic fungi. Several authors have been highlighted the significance of lytic enzymes in BCA activity and studied isolates of *Trichoderma* spp. with cellulose and chitin degradation characteristics (Brewer and Larkin, 2005; Roberts *et al.,* 2005; De Marco *et al.,* 2003). Hutchinson (1999) and Hanson and Howell (2002) have reported the significance of secondary metabolites (antibiotic activity) in antagonistic action of *Trichoderma* spp. against pathogenic fungi *Pythium ultimum* and *Rhizoctonia solani.* However, there seems to be a general consent on the combined synergistic effect of the two factors (enzymes and antibiotic compounds) (Liu and Yang, 2005; Howell and Puckhaber, 2005).

6.1.11 Induced Resistance

Induction of resistance in host plant by *Trichoderma* species is another mechanism in biological control (Howell, 2003). Specific strains of fungi in the genus *Trichodenmi* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which leads to induced systemic resistance (ISR) in the entire plant (De Meyer *et al.,* 1998). Yedidia *et al.* (1999) showed that inoculating roots of 7-days-old cucumber seedlings in a hydroponic system with *T. harzianum* (T-203) spores to concentration of 105 per ml initiated plant defense responses in both roots and leaves of treated plants. Also they demonstrated that hyphae of the biocontrol fungus penetrated the epidermis and upper cortex of the cucumber root. The plant response was marked by an increase in peroxidase activity (often associated with the production of fungitoxic compounds), an increase in chitinase activity, and the deposition of callose-enriched wall appositions on the inner surface of cell walls (Howell, 2003). Yedidia *et al.* (2000) showed that inoculation of cucumber roots with *T. harzianum* (T-203) induced an array of pathogenesis-related proteins, including a number of hydrolytic

enzymes which were similar to plants treated with a chemical inducer (2,6-dichloroisonicotinic acid) of disease resistance displayed defense responses. Resistance elicitation in plants by *Trichoderma* is becoming a more active field of research. Xylanase and other elicitors were produced by *Trichoderma* spp. and proved to induce resistance (Anderson *et al.,* 1993). Some *Trichoderma* strains were inducers of systemic acquired resistance-like responses (SAR) (De Meyer *et al.,* 1998). Cucumber plants were larger in the presence of *Trichoderma,* and the cell wall of roots were strengthened in the area of *Trichoderma* penetration, and both chitinase and peroxidase activities in both root and leaf tissues of treated seedlings were evident (Howell *et al.,* 1999). Also, they reported that *Trichoderma virens* mutants that lacked both mycoparasitic ability and the capacity to produce antibiotics were more effective than the parental strains in biocontrol of *Rhizoctonia solani.* This high level of protection was associated with significantly enhanced levels of the various terpenoid phytalexins known to be involved in disease resistance in cotton. Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are also very important. These fungi colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling off of the *Trichoderm* thallus. At the same time, the transcriptome and the proteome of plants are substantially altered. As a consequence, in addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake occur. The studies on mycoparasitism demonstrated that these fungi produce a rich mixture of antifungal enzymes, including chitinases and β-l, 3glucanases.These enzymes are synergistic with each other, with other antifungal enzymes, and with other materials. The genes encoding the enzymes appear useful for producing transgenic plants resistant to diseases and the enzymes themselves are beneficial for biological control and other processes (Harman, 2006).

6.1.12 Stabilization and Sequestration of Inorganic Plant Nutrients

Plant nutrients undergo sometimes transitions in soil from soluble to insoluble forms that influence their accessibility and absorption by roots. These transitions may be influenced by microorganisms (Altomare *et al.,* 1999). Iron and manganese have been investigated with regard to both microbial solubilization of oxidized forms of these

elements and their influence on plant disease (Graham and Webb, 1991). In vitro, strain of *Trichoderma harzianum* produces a large number of chemicals to solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+} and increase iron availability and enhance iron uptake (Altomarc *et al.,* 1999). A direct role for the nutrient solubilization and chelating abilities of *Trichoderma* has not been demonstrated, but circumstantial evidence of its ability to solubilize iron and make it usable to plants is available (Harman, 2000).

6.1.13 Inactivation of the Pathogen Enzymes

Enzymes such as chitinases and or glucanases produced by the biocontrol agent are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and β-glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity (Howell, 2003). Elad and Kapat (1999) suggested that *T. harzianum* (T39) produced proteases that are capable of degrading the pathogen's plant cell wall degrading enzymes, and thereby reducing the ability of the pathogen to infect the plant. They also showed that protease may be directly toxic to germination of the pathogen and also may inactivate its enzymes.

6.1.14 Growth and Formulation of *Trichoderma* for Application as a Biocontrol Agent

Backman and Rodrigucx-Kabana (1975) used molasses-enriched clay granules as a food base for growing the antagonist and the pathogen *(Trichoderma harzianum* and *Sclerotium rolfsii)* and a carrier to facilitate dispersal in the field. They observed a significant decrease in *S. rolfsii* damage to peanuts. Hadar *et al.,* (1979) found that agricultural wastes, such as manure, sawdust, and wheat bran proved to be the best media for the growth and speculation of *T. harzianum.* They also suggested that the fungal preparation contained $2.9x10^9$ conidia/g dry weight; 80% of *T. harzianum* conidia were viable even after storage for 6 months at either 4°C or at room temperature (24-30°C).

Elad *et al.* (1982) found new coating technique, in which seeds were treated with either Pelgel (Nitragin, USA) solution (10% w/v) containing $5xl0^8$ conidia/ml as an

adhesive, or ethylmercury chloride (350g/ton seed) applied as seed treatment with *Trichoderma* for controlling pathogens in naturally infested soil. The wheat bran preparation of *T. harzianum* increased bean plant growth in non-infested soil and controlled *S. rolfsii* more efficiently than conidial suspension and reduced significantly bean diseases caused by *S. rolfsii and R. solani* in pathogen infested soil (Chet, 1987).

6.2 Materials and Methods

6.2.1 Experimental Period:

This experiment was conducted in Plant Pathology Lab, BARI, Joydebpur during 1st March, 2014 to 10th April, 2014.

6.2.2 Collection of soil samples

For isolation of *Trichoderma* species rhizosphere soil of healthy plants were collected from different vegetable fields from five different districts of Bangladesh (Table 6.1). From a selected field several healthy plants were pulled out gently with soil. Then the roots and stubbles were removed from soil and all the soils collected from different plants were mixed to make a composite sample. The composite sample was divided into 4 components over a plastic sheet. Two component samples from any two corners were discarded and again the left 2 samples were mixed. The process continued until the volume of the sample reaches to about 400-500 g which was treated as working sample and was taken into a plastic bag with proper labeling and stored in a refrigerator (4°C) in the laboratory.

Plate 6.1 Collection of soil samples

6.2.3 Isolation, purification and preservation of *Trichoderma* isolates

Trichoderma were isolated from soil following dilution plate technique (Subba, 2003). One gram of working soil sample was taken in a test tube containing 9 ml of sterilized water to make 1:10 dilution. Similarly a series of dilution process were continued until the sample was diluted to 1:10000. All working samples were diluted in the same process. A number of PDA plates were prepared in aseptic condition in the laboratory. 1 ml of each sample soil suspension was placed in each petri-plate. The soil suspension was thoroughly mixed with the medium using a glass spreader. The petri-plates were incubated for 5 days at room temperature (25±2 ^{0} C).

After 3 days of incubation, plates were observed for *Trichoderma* colony. The growing margin of *Trichoderma* colony was cut into *5* mm blocks with the help of a cork borer. The blocks were carefully placed in PDA plates and incubated as before. Hyphal tip/mycelial block of *Trichoderma* were transferred to PDA for purification. The well-developed pure cultures of *Trichoderma* were sub-cultured to PDA plates and slants for preservation. The slants were prepared by tilting individual test tube or whole basket of test tubes containing sterilized PDA at an angle sufficient to make a sloped surface of 0.5" to 3.5" from the bottom of the tube. The tubes were left in this position until solidified. The fully-grown *Trichoderma* in slants were preserved in the refrigerator at 4°C for further use (Plate 6.2). The isolates were identified following the key of Kubieek and Harman (1998).

Table 6.1 List of *Trichoderma* isolates collected from different hosts and locations of Bangladesh

Sl. No.	Districts	Upazillas	Number of samples	Host plant/ Location	No. of *Trichoderma* isolates
1	Rajshahi	Rajshahi Sardar	TRU1,TRU2,TRU3,TRU4,TRU5, TRU6,TRU7,TRU8,TRU9,TRU10, TRU11,TRU12,TRK13,TRK14, TRK15,TRK16	Marigold/3rd building-RU Dhoncha/ east-end	01(TRU-5) 01(TRU-14)
2	Bogra	Shibgonj	TB17,TB18,TB19,TB20,TB21, TB22,TB23,TB24,TB25,TB26	Eggplant/ highway	01(TB-23)
3	Gybanda	Palashbari	TG27,TG28,TG29,TG30,TG31	Chili/kumor-hat	00
4	Rangpur	Mithapukur	TR32,TR33,TR34,TR35,TR36, TR37,TR38,TR39,TR40,TR41, TR42,TR43	Potato-land/ zygir-hat	01(TR-37)
5	Dinajpur	Birgong	TD44,TD45,TD46,TD47,TD48,	Eggplant/highway	01(TD-44)
6	Thakurgaon	Thakurgaon Sadar	TTG49,TTG50,TTG51,TTG52, TTG53,TTG54	Maize/ lilarhat	01(TTG-50)
Total			**54**		**06**

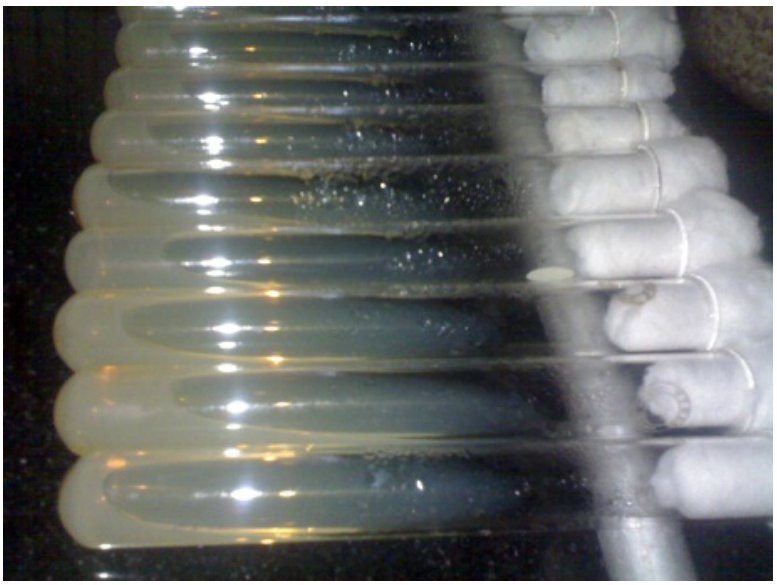

Plate 6.2 Stock culture in slants

6.2.4 Morphological characters of different *Trichoderma* isolates

The isolates of *Trichoderma* spp. were characterized morphologically. In all, 06 isolates were purified through mycelial block culture and were maintained on Acidified Potato Dextrose Agar (APDA) medium at 25±1°C (incubation) for studying cultural characteristics (Singh and Singh, 2004).

Plate 6.3 Mass culture of *Trichoderma* isolstes

6.2.5 Preparation of Lactophenol

Lactophenol solution was used as a mounting medium consisted of the following composition (Aniworth, 1963)

Phenol crystals	20gm
Glycerol	40ml
Lactic acid	20ml
Distilled water	20ml.

6.2.5.1 Preparation of Lactophenol cotton Blue stain

One gram of cotton blue was added to 100ml lactophenol and shaken well until cotton blue was dissolved. The solution of lactophenol and lactophenol cotton blue was stored in an amber colored bottle and it was placed in a cool dark place.

6.2.5.2 Preparation of slide

A suitable portion of pure culture of different fungi colony from PDA was selected. It was taken out with the help of forceps and needle on clean slide. Then, two drops of

lactophenol were added on that portion. It was then gently warmed by heating and cooling over a low flame of spirit lamp for 6-8 times. But it was never allowed to boil. Whenever in need, the material was stained with adding a small quantity of cotton blue. A clean cover glass was then placed over material and excess fluid then removed by tapping on blotting paper and examined under a stereoscopic binocular compound microscope.

6.2.5.3 Study on growth and conidia production of different *Trichoderma* isolates:

Radial mycelial growth and spore production of the isolates of *Trichoderma* spp. were studied following the method of Sultana *et al.,* (2001) A 6 mm block of each *Trichoderma* isolate of seven days old was placed at the center of Petri plate containing PDA. Four replications were maintained. The fungal growth after 24 hours of inoculation was measured by recording colony diameter. Growth of *Trichoderma* was very fast and within 5-6 days it produced abundant spores throughout the plate (Plate 6.4). After 24, 48 and 72 hours of inoculation, the radial mycelial growth was measured as the mean of two perpendicular diameters and mean of four replications were recorded for each isolate. Colony characters were noted in respect of:

- Shape
- Color
- Growth habit
- Compactness
- Spore density /ml

In order to determine the conidia production, 30 ml water was poured into each PDA plate of 15 days old culture of *Trichoderma* spp. The conidial suspension was made by scraping the spore masses on the medium. The conidial suspension was then taken in a beaker containing 400 ml water and one drop of Tween-20 was added and stirred for 15 minutes (Plate 6.5). Then the number of conidia per plate was determined with the help of Haemocytometer following the procedure of Ashrafuzzaman (1976).

Plate 6.4 Spore suspension of *Trichoderma* isolate

6.2.6 Laboratory bioassay of the isolates of *Trichoderma* spp.

6.2.6.1 Inhibition zone technique

An *in vitro* test was conducted to find out the antagonistic effect of the selected *Trichoderma* isolates against *S. rolfsii* and *F. oxysporum* on PDA by dual culture technique (Dhingra and Sinclair, 1985). One mycelial disc of 5 mm size picked up by sterilized block cutter from7 days old culture of individual isolates of *Trichoderma* and one disc (same size and age) of a *S. rolfsii and F. oxysporum* were placed simultaneously on the edge of the each PDA Petri plate at opposite direction. Three plates (replications) were used. The plates were arranged on the laboratory desks following completely randomized design (CRD). The plates which received only discs of *S. rolfsii /F. oxysporum* treated as control. The plates were incubated in the laboratory having ambient temperature of 25±2°C. Inhibition zone was measured on the day when control plate was full (Nene and Thapliyal, 1993). Thereafter percentages inhibition of *S. rolfsii / F. oxysporum* were calculated based on the growth of the pathogen on PDA plates following the formula as suggested by Sundar *et al.,* (1995).

$$\% \text{ growth inhibition} = \frac{x - y}{x} \times 100$$

Where,

X = Mycelial growth of pathogen in absence of *Trichoderma*

Y = Mycelial growth of pathogen in presence of *Trichoderma*

6.2.6.2 Determination of the effect of fungitoxic metabolite produced by *T. harzianum* against *S. rolfsii and F. oxysporum*

The ability of *Trichoderma* isolates to inhibit the mycelial growth of *S. rolfsii* and *F. oxysporum* through production of fungitoxic metabolites was tested according to the method mentioned by Dennis and Webster (197la and 197lb). Fifty (50) ml of potato dextrose broth (PDB) of p^H 6 in 250 ml media bottle was inoculated with 7mm agar disk from 7 day old PDA cultures of promising *Trichoderma* isolates at 25°C without shaking. After 10 days of incubation, the cultures were filtrated through Millipore membrane filter (0.45u.m) and were autoclaved at 121°C for 15 minutes. The culture filtrate (2ml) was placed in Petri dishes (90 mm diameter) and approximately 10ml of PDA was added and mixed with the filtrate (20% v/v). The filtrate amended PDA plates were then centrally inoculated with 6 mm mycelial plugs of *S. rolfsii and F. oxysporum,* separately.

Unamend PDA plates served as control. Plates were incubated at 25±2°C with three replication. The linear growth rate of *S. rolfsii* and *F. oxysporum* were measured after every 48 hours until the control plates was full and percent inhibition was calculated.

6.3 Results and Discussion

6.3.1 Collection, isolation, purification, identification and morphological characterization of isolates of *Trichoderma* spp.

In all 06 isolates collected from 06 different fields of five different locations of the country were *Trichoderma harzianum* according to the key of Kubicek and Harman (1998).

6.3.2 Morphological characterization of *Trichoderma* isolates

Different isolates of *Trichoderma* spp. distinctly differed on their cultural and morphological properties like mycelial growth, colony color and colony consistency and sporulation rate. Morphological characteristics of 06 isolates of *Trichoderma harzianum* on PDA plates were recorded at 24 hours interval up to 168 hours (7 days).

Radial mycelial growth of different isolates of *Trichoderma harzianum* in PDA

Mycelial growth rate of different isolates varied considerably up to 72 hours (3 days). Mycelia of most isolates showed growth along the surface of the medium that was

lying to the base and appeared as compact while a few showed less compact appearance. The whole plate for each isolate was covered with mycelium within 3 days. Mycelial growth of 06 isolates collected from five agro-ecological zones of Bangladesh grown on PDA medium was found to vary. Isolate-TRU-5 showed maximum radial mycelial growth both at 1 and 2 days after inoculation (DAI). At 3 DAI the maximum radial mycelial growth (90 mm) and average mycelial growth rate (30 mm/day) was observed in isolate-TB-23, isolate-TRU-5 and isolate-TR-37.After three DAI the average linear mycelial growth rate varied significantly among the isolates (Table 6.2 and Fig. 6.1). The minimum mycelial growth rate (15.33mm/day) was observed-in the isoIatc-TTG-50 followed by the isolalc-TD-44(26.88 mm/day) and isolate-TRU-14 (27.55 mm/day). The maximum mycelium growth rate (30 mm/day) was found in the isolate- TB-23.

Table 6.2 Radial mycelial growth of different isolates of *Trichoderma harzianum* in PDA

***Trichoderma* isolates**	**Average liner mycelial growth(mm)**			**ALMGR(mm/day)**
	1DAI	**2DAI**	**3DAI**	**3DAI**
TRU-5 (1)	34.33±.33a	72.66±.33a	88.66±.66a	29.55a
TRU-14 (2)	34.33±.88a	69.66±.88b	82.66±.66b	27.55b
TB-23 (3)	20.66±.33b	66.33±.33c	90.00±.00a	30.00a
TR-37 (4)	22.66±.88b	65.66±.88c	87.66±1.33a	29.22a
TD-44 (5)	14.33±.88c	50.66±.66d	80.66±.33b	26.88b
TTG-50 (6)	10.66±.88d	20.33±1.45e	46.00±2.00c	15.33c

ALMGR= Average Linear Mycelial Growth Rare, DAI = Day After Incubation

T = *Trichoderma*, RU = Rajshahi University, B= Bogra, R = Rangpur, D = Dinajpur,

TG = Thakurgaon

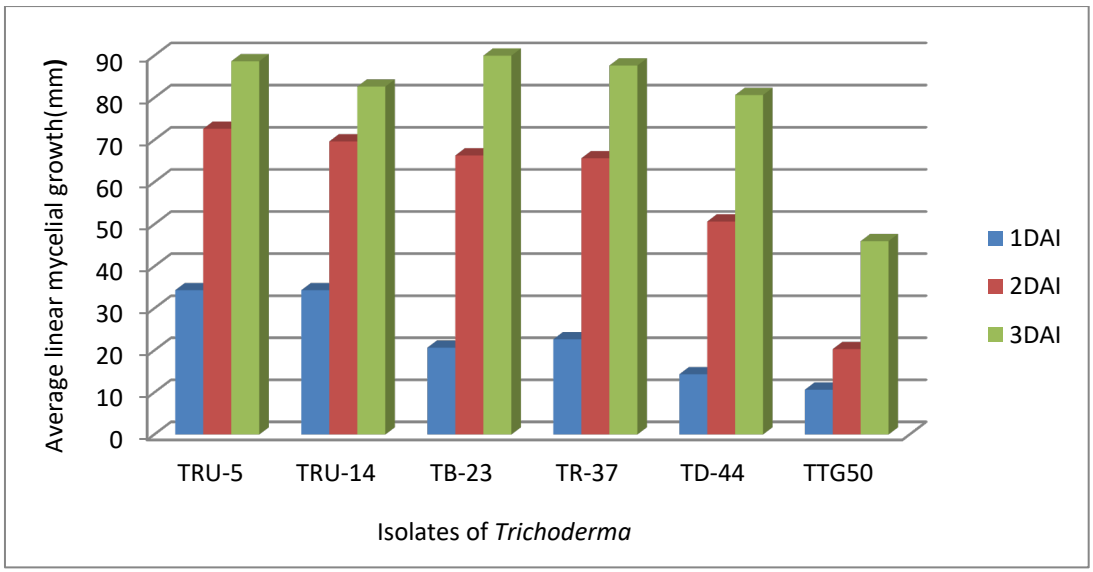

Fig. 6.1 Radial mycelial growth of different isolates of *T. harzianum* in PDA

Colony and conidial characters of different isolates of *T. harzlanum* in PDA

Colony and conidial characters of 06 isolates of *T. harzianum* are presented in Table 6.3.

Colony shape

All the 06 isolates of *Trichoderma harzianum* produced regular shaped colony (Table 6.3)

Colony colour

Trichoderma isolates produced three different colony colors viz., dark green, green, and whitish green (Table 6.3). Isolates-TB-23 and isolates-TRU-5 produced dark green colony, TRU-14, TR-37 isolates produced green colony and TTG-50, TD-44 isolates produced whitish green colony (Plate 6.5).

green dark green whitish green

Plate 6.5 Isolates of different colour

Growth habit

Three types of growth habit were observed in *Trichoderma* isolates - fast, medium and slow. Out of 06 isolates, 03 were fast growing, 02 were moderately fast growing and TTG-50 isolate was slow growing type.

Table 6.3 Colony and conidial characters of different isolates of *Trichoderma harzianum i*n PDA

***Trichoderma* isolates**	**Colony characters**				**Conidial characters**	
	Shape	**Colour**	**Growth habit**	**Compactness**	**Conidial shape**	**No. of spore ($x10^6$)/plate**
TRU-5 (1)	Regular	Dark green	Fast grower	Compact	Subglobose	81.33ab
TRU-14 (2)	Regular	green	Moderate grower	Compact	Subglobose	56.33c
TB-23 (3)	Regular	Dark green	Fast grower	Compact	Subglobose	82.33a
TR-37 (4)	Regular	green	Fast grower	Compact	Subglobose	78.66b
TD-44 (5)	Regular	Whitish green	Moderate grower	Compact	Subglobose	51.33d
TTG-50 (6)	Regular	Whitish green	Slower grower	Compact	Subglobose	39.33e

Colony consistency

All isolates produced compact colony consistency.

Sporulation capacity

Highest spore density was obtained from the isolates having dark green colour, whereas, the lowest was found in whitish green colour colony (plate 6.5).

Altogether 06 isolates were collected from rhizosphere soils of different field of five different districts of Bangladesh. Sawangsri *et al.,* (2007) studied four hundred and sixty two strains of *Trichoderma* spp. were obtained. *Trichoderma* spp. was most abundant in the field of Yaha district. Askew and Laing (1994) reported that *Trichoderma* spp. from different environmental sites possessed different degrees of

aggressiveness against *R. solani.* It was reported that cultivated soils were a better source of potential antagonistic *Trichoderma* spp. Howell (2003) easily isolated several species of *Trichoderma* from soil, decaying wood and other forms of plant organic matter. The isolates were purified through hyphal tip culture and all the isolates were identified as *Trichoderma harzianum* according to the key of Kubieek and Harman (1998). Isolates distinctly differed on their cultural and morphological properties like mycelial growth, colony color, colony consistency and sporulation rate. This was supported by Wang *et al*., (1999). The isolates producing dark green colony was very fast growing and green colony was slow growing fungus. In between these two categories some isolates were moderately growing type contained green and whitish-green in color. A similar study was conducted in Hebron University, Palestine by Radwan *et al*., (2006) who studied forty seven isolates of *Trichoderma* and measured mean radial mycelial growth above 8 cm and the present result agrees with this report. Finally six strains of *Trichoderma harzianum* were identified in this study to test of effectiveness and potentiality as a bio-control agent brinjal field.

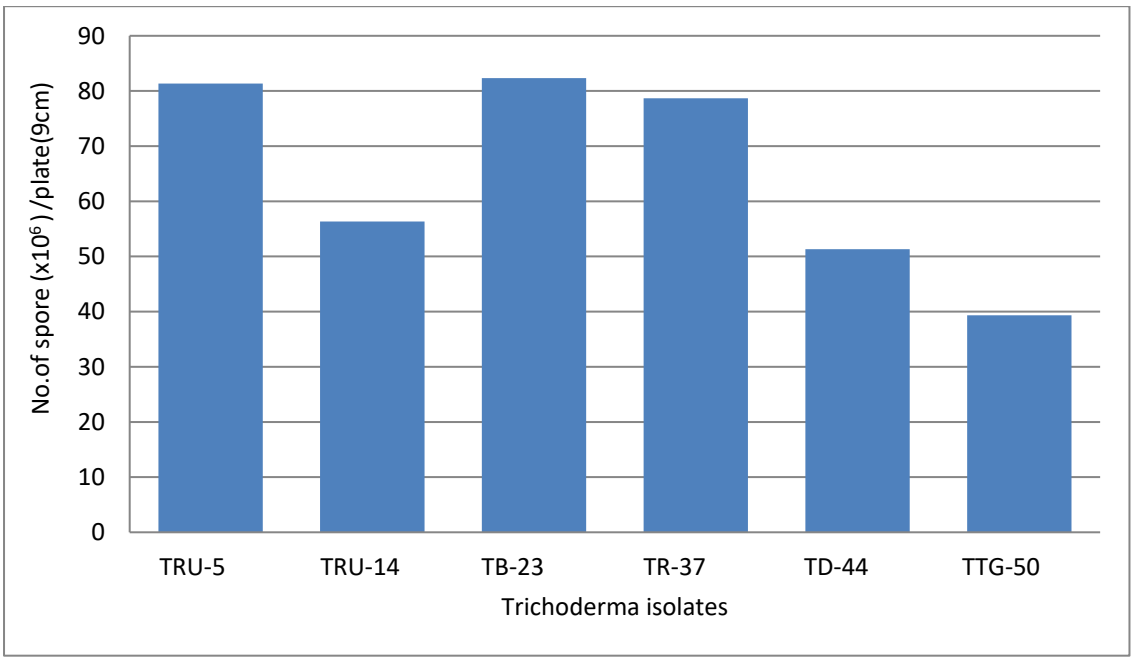

Fig. 6.2 Number of spore/plate produced by different isolates of *T. harzianum* in PDA

6.3.3 Laboratory bioassay of the isolates of *Trichoderma* spp.

6.3.3.1 Zone inhibition technique

The isolate *T. harzianum* was evaluated against *S. rolfsii* and *F. oxysporum* in dual culture method. After 5/7 days of inoculation when the control plates were full the bio agents produced significantly different inhibition zone against *S. rolfsii* and *F. oxysporum* respectively in dual culture method (Table 6.4 & 6.5). The isolate showed significant/considerably percentage of inhibition against (63.30% to 57.81%) both pathogens (Plates 6.6 & 6.7).

Table 6.4 Percent inhibition of isolates of *T. harzianum* against *S. rolfsii* following dual culture method *in vitro*

Accession name	Average radial growth of *S. rolfsii* at 48 hrs.(cm)		Average radial growth of *S. rolfsii* at 72 hrs.(cm)	
	At dual culture	Growth suppression (%)	At dual culture	Growth suppression (%)
TRU12	3.346	41.710	3.46	47.96
TRU7	4.156	27.574	4.20	36.84
TRM1	3.35	41.535	3.33	49.92
TTG6	3.076	46.422	3.30	50.37
TB23	2.25	60.73	2.44	63.30
S. rolfsii	5.73		6.65	

Table 6.5 Percent inhibition of isolates of *T. harzianum* against *F. oxysporum* following dual culture method *in vitro*

Accession name	Average radial growth of *F. oxysporum* at 48 hrs.(cm)		Average radial growth of *F. oxysporum* at 72 hrs.(cm)	
	At dual culture	Growth suppression (%)	At dual culture	Growth suppression (%)
TRU12	1.696	40.11	2.183	45.868
TRU7	2.016	28.82	2.816	30.166
TRM1	1.67	41.05	2.516	37.604
TTG6	1.25	55.88	1.816	54.952
TB23	1.653	41.64	1.70	57.81
F. oxysporum	2.833		4.033	

6.3.3.2 Effect of fungitoxic metabolite produced by *T. harzianum* against *S. rolfsii* and *F. oxysporum*

The selected isolate-TB 23 which had better performance in zone inhibition technique was further tested for their effect of fungitoxic metabolite inhibitory to the growth of *S. rolfsii* and *F. oxysporum*. The growth rate of *S. rolfsii* and *F. oxysporum* was reduced significantly at 2 DAI, 4 DAI and 6 DAI. Two days after inoculation isolate-TB-23 showed the highest growth inhibition (16.66% & 20.81%). Similar trend of growth inhibition was observed four days after inoculation. After six days when the control plates were full the maximum growth inhibition was found in isolate (52.54% & 48.88%) which was significantly similar with the growth inhibition of the isolate-TB-23(Table 6.6).

Table 6.6 Determination of the effect of fungitoxic metabolite produced by *T. harzianum* against *S. rolfsii* and *F. oxysporum*

Pathogenic fungi	***Trichoderma***	**% inhibition**		
S. rolfsii		2 DAI	4 DAI	6 DAI
	TB-23	16.66(9.33±.88c)	30.94(21.66±.88b)	52.54(44.66±1.20a)
F. oxysporum	TB-23	20.81(6.66±.33c)	25(11.00±.57b)	48.88(29.33±.66a)

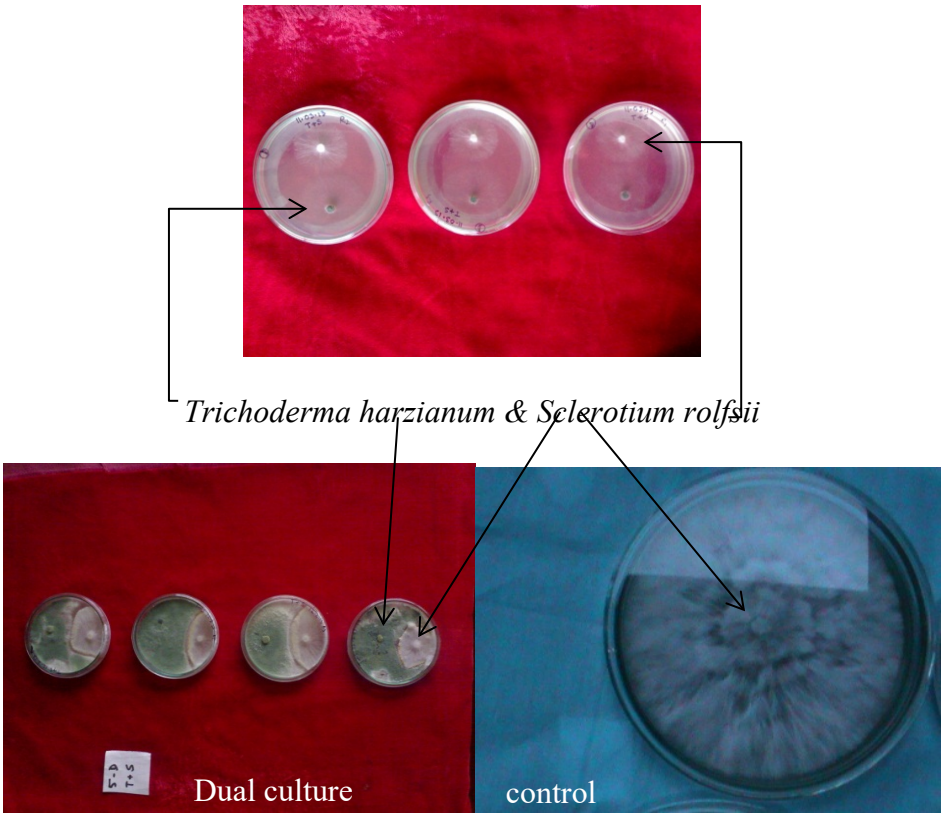

Plate 6.6 Effect of *T. harzianum* on *S. rolfsii* in dual culture

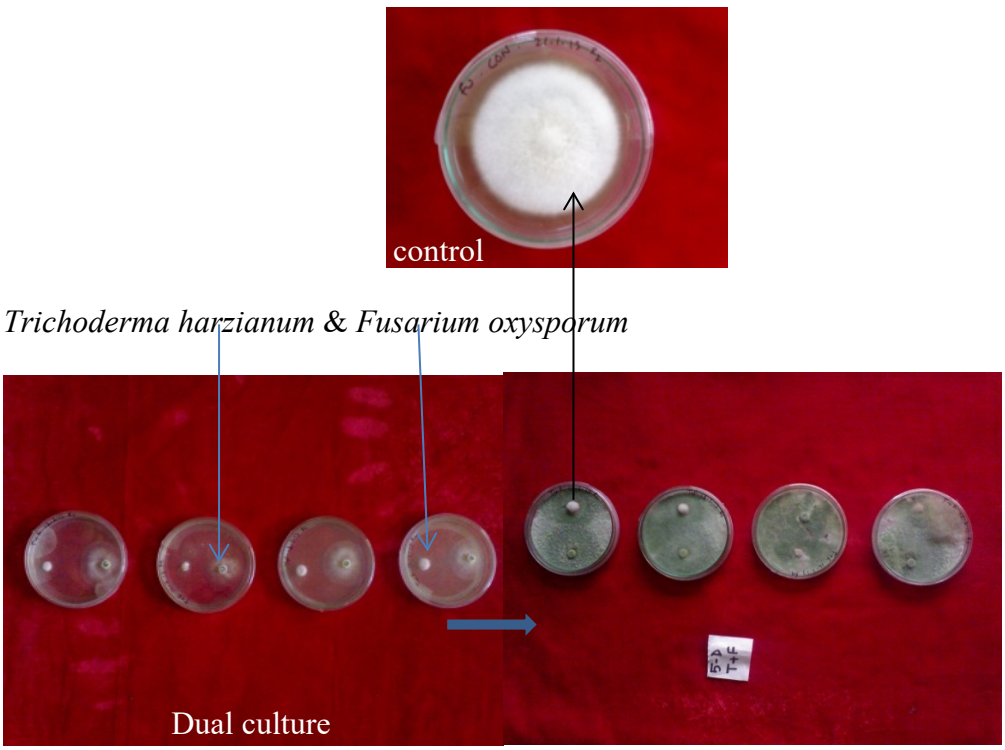

Plate 6.7 Effect of *T. harzianum* on *F. oxysporum* in dual culture

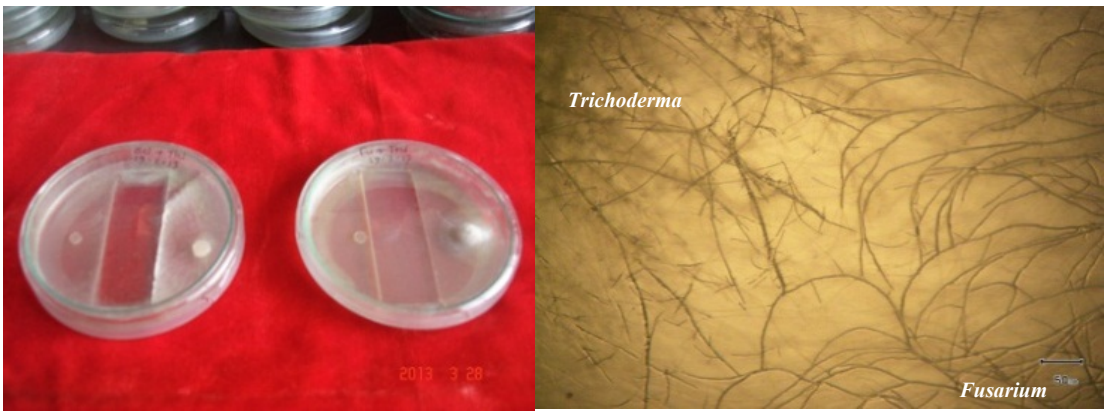

Plate 6.8 Hyphal interaction between *Trichoderma* with *Fusarium oxysporum*

Laboratory bioassay of *Trichoderma* isolates

Antagonistic potential of *T. harzianum* against the virulent isolate of *S. rolfsii* (brinjal isolate) and *F. oxysporum* has been proved (63.30 &57.81% inhibition, respectively) which agree with Prasad *et. al.* (1999) who found the similar findings and reported 61.4% inhibition of *S. rolfsii* with *T. harzianum,* while Yogendra and Singh (200**2**) found maximum of 64.44% inhibition of *S. rolfsii* by *T. harzianum* at 4 DAT. Al-Chabbi and Matrod(2002) achieved 77% growth inhibition of *Fusarium oxysporum.* Sundar *et al*., (1995) and Deshmukh and Raut(1992) reported over grew colonies of *Fusarium oxysporum.* Xu *et al., (*1993) also observed the hyphal growth inhibition of *Fusarium oxysporum* by two isolates of *Trichoderma harzianum* T82 and NF9. Pranab *et al.,* (2002) recorded 61.5% inhibition of *S. rolfsii* by *T. harzianum.* Faruk *et al.,* (2002) found that *T. harzianum* significantly reduced the radial colony growth of *S. rolfsii* in dual culture on PDA. Similar findings were also obtained by Sultana and Hossain (2000). The findings of the present study clearly screened the isolate TB-23 as potential biocontrol agent for *S. rolfsii* and *F. oxysporum*.

In contrast to the interaction in dual culture, results revealed that the growth rate of *S. rolfsii and F. oxysporum* were reduced due to the production of fungitoxic metabolites produced by *Trichoderma* isolates. The growth rate of *S. rolfsii* and *F. oxysporum* was reduced significantly at 2 DAI, 4 DAI and 6 DAI. Similar results were observed by Prasun and Kanthadai (1997) who reported that *Trichoderma* (isolate Td-1) produced higher concentration of fungitoxic metabolities at higher temperatures and it effectively suppressed the growth of *S. rolfsii* at or below 33 ^{0}C.

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Chapter - 7
Bio-efficacy of Selected Botanicals, Bio-agents and Fungicides on Growth Parameters and Control of Diseases of Foot and Root Rot and Wilt Diseases of Brinjal

Abstract

In present study, aqueous extract of ten plants, one antagonist and two fungicides were tested for the vegetative growth and disease control of vegetable plant of brinjal (*Solanum melongena* L.). Botanicals were found to increase shoot height, root height, shoot weight, root weight and number of leaves of brinjal plant over control. Numbers of brinjal seed germination and seedling mortality were calculated as percentage and diseases per plant. The percentage of reduction of disease of infestation was calculated after the spray of extracts. Four botanicals (*Acacia nilotica, Allium sativum, Datura stramonium* extracts and *Trichoderma* formulation) were found most effective agent in controlling foot rot, root rot, and wilt disease in comparison to control under field conditions. The result showed that plant extracts and *Trichoderma* formulation act as potential source for management of several diseases of brinjal. In addition, botanical extracts and *Trichoderma* were found to increase the growth of brinjal plants as positive effect on growth parameters.

Key words: Antifungal effect, Plant extracts, *Trichoderma*, brinjal, foot and root rot, wilt, growth parameters.

7.1 Introduction

Brinjal (*Solanum melongena*) of *Solanaceae* family is a most important vegetable worldwide. As it is a relatively short duration crop and gives a high yield, it is economically attractive; therefore the area under cultivation is increasing daily. Brinjal plant can reach a height of over two meters. The first harvest is possible 45-55 days after flowering, or 90-120 days after sowing. The shape of the fruit differs per cultivar. The color ranges from white to black. The temperature lower within the crop and the fruits grow in the shade of the leaves. Because the fruits are covered, the sun does not damage it. The main root produces dense lateral and adventitious roots. Growth habit ranges between erect and prostrate. The stem is solid and coarse. Flowers are bisexual, regular and grow opposite or between leaves. Ovary is superior and with 2-9 compartments. Fertilization of brinjal is mostly self but partly also cross-pollinated. Bees and bumblebees are the most important pollinators. Fruits are fleshy berry, globular to oblate in shape. It covers about 60 thousand 65 hectors of land and produces about 3 lack and 58 thousand 370 tons (BBS, 2006). But the yield is quite low. Diseases are important factor for causing low yield.

Some bacterial/fungal diseases are commonly found in brinjal. These are wilt, foot and root rot, leaf spot and fruit spot diseases. Fusarial wilt, foot and root rot are economically important brinjal diseases these occur worldwide**.** Fusarial wilt is a fungal disease of brinjal. About 10%-30% in general and 80% - 100% in severe case of wilting of eggplant happen due to the *Fusarium oxysporum*. *Fusarium oxysporum* f. sp. is a soil and seed borne pathogen colonizing the xylem vessels and blocking them completely to cause wilting. Wilt (*Fusarium oxysporum* f. sp.) is one of the serious diseases of different vegetables and crops belong to brinjal. Fusarial wilt disease of Chickpea, causing heavy loss up to 10-100% depending on fungal inoculum and environmental condition (Sugha *et al*., 1995; Sumitha *et al*.,1995, 1998b).

Brinjal plant (*Solanum melongena* L.) is affected by various diseases, which in turn produce heavy loss to the crop. The diseases include wilt, blight, little leaf, etc. Among them wilting of eggplants is one of the important diseases causing great reduction in the field. The fungus *Fusarium oxysporum* causes wilt disease. The main

symptoms of the disease induce wilting of seedling and adult plants. The plant infected with the fungus that produces wilt has older leaves that droop and afterwards turn yellow. Leaf yellowing can occur on one side of the plant and gradually most leaves form yellow and wilt. In order to prevent the plant diseases and to protect the crop plants against pathogens chemical control methods were in practice.

Dutta and Das (2002) observed that *Sclerotium rolfsii* Sacc. had a host range of more than 500 species of cultivated and wild plants in tropical region. The pathogen causes pre-and post-emergence root/collar rot and wilt of the seedlings. The disease results from infection by germinating sclerotia produced by the pathogen which are resistant survival structures and control of the pathogen through host resistance or fungicides is difficult. Chowdhury and Ahmed (1985) tested the reaction of twenty-two different crop plants maize, wheat, gram, khesari, lentil, mashkalai, mungbean, soybean, sunflower, sesame, brinjal, bitter gourd, bottle gourd, cowpea, cucumber, okra, radish, tomato, chilli, coriander, garlic and onion to *Sclerotium rolfsii* in the pot-house. Before sowing the seeds, sterilized soil in pots was inoculated with a culture of *S. rolfsii* grown in oats. Records of pre-emergence and post-emergence mortality, up to 45 days after germination, were taken. All the crops were found to be susceptible under experimental conditions. The rate of pre-emergence mortality varied from 5.6% in garlic to 85.0% in gram. Soil drenching with fungicides are generally used to control of this disease, however, frequent and indiscriminant use of it leads to ill effects on environment causing soil and water pollution and development of new strain with more virulence.

In view of the high cost of chemical pesticides and their hazardous consequence use of biodegradable different material like fresh plant extracts from parts gained importance during last three decades from plant disease control (Fowcett and Spenser, 1970; Mitra *et al*., 1984; Grainge and Ahamed, 1988; Jespers and Ward, 1993). Several workers studied the control of *Fusarium* species on various plants extract Furga Wegrzycka, (1984); Shami *et al*., (1986); Reddy and Reddy, (1989); Eswaramoorthy *et al*.,(1989) Patel (1989); So(1990); Tariq and Magee (1990); Pandey *et al*.,(1992); Gohil and Vala (1996); Gour and Sharmaik, (1998); Bansal and

Gupta, (2000). This study was conducted to find out the effective bio-agents and selected plant extracts for the control of pathogens *Sclerotium rolfsii* and *Fusarium oxysporum* f .sp. *melongenae* causing wilt, foot and root rot disease in Brinjal plant.

7.2 Materials and Methods

7.2.1 Experimental site and Period

All the experiments were conducted during July 2011 to June 2014 in IES-Laboratory, RU and Plant Pathology Laboratory of BARI. The pot house and field experiments were conducted out in Bangladesh Agricultural Research Institute, Joydebpur, Gazipur

The experiments were performed during January 2014 to June2014

7.2.2 Field Experiment

7.2.2.1 Eggplant Variety and Source

The variety of BARI-1 was used in the experiment. Seeds of eggplant were collected from Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur Bangladesh.

7.2.2.2 Preparation Earthen pot

Soil for the raising of seedlings in earthen pot was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1. The prepared soil was heaped like a square block. Formalin solution (4%) @ 200 ml/cft soil were mixed with the soil heap and the soil was covered by a polythene sheet for 48 hours and then exposed for aeration, after it was heated about thirty minutes in iron bowl by gas. The sterilized soil was poured in the sterilized earthen pot @ 5 kg soil/ tray after 7 days (Dash gupta, 1988). The pathogenic fungi *S. rolfsii* and *F. oxysporum* were multiplied in the autoclaved wheat bran, khesari bran and rice husk based substrates. The colonized wheat bran, khesari bran and rice husk with MOC substrate were air dried at room temperature. Pot soil was inoculated with mentioned substrates colonized with *S. rolfsii* and *F. oxysporum* at 20g/kg soil. Inoculated soil was incubated for 10

days with proper soil moisture. The *S. rolfsii* and *F. oxysporum* inoculated soil was treated with formulated *Trichoderma* 20 g/kg soil and was incubated for 7 days with proper soil moisture. Ten seeds of brinjal var. BARI- 1 were sown in one pot after 7 days of addition of formulated *Trichoderma* without soak in plant extracts. The *S. rolfsii* and *F. oxysporum* inoculated pot soil was treated with each plant extracts after germination of brinjal seeds.

7.2.2.3 Sowing Seeds in Earthen pot and Care of Seedlings

Seedlings were raised in earthen pot in the pot house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur with proper care and management to ensure disease free healthy seedlings production (Islam, 2006). Ten seeds were sown in each pot. Before sowing the seeds were soaking six hours in individual plant extracts. Seeds were sown in a circle and labeled by a permanent marker. Watering was done to maintain the soil moisture. Shade was provided to save the young and delicate seedlings from heavy showering and scorching sunlight (Islam, 2006).

7.2.3 Collection of isolates (*Sclerotium rolfsii* and *Fusarium oxysporum*)

The *Sclerotium rolfsii* and *Fusarium oxysporum* isolates used in this study were obtained from different major brinjal growing areas of Rajshahi. These isolates were collected during the month of March, 2014 from the infected brinjal field. Then the specimens were taken to the Plant Pathology Laboratory, BARI and were subjected to the process of isolation.

Origin of isolates

Sclerotium rolfsii and *Fusarium oxysporum* were obtained from the diseased plants of brinjal *(Solanum melongena* L.).

Soil amendment with inoculum

Humidity formation by watering

Inoculated soil ready for sowing *S. rolfsii* *F. oxysporum*

Plate7.1 Inoculation of Pot soil with mentioned substrates colonized with *S. rolfsii* and *F. oxysporum*

Seeds soaking in different extracts

Treated seeds for sowing

Sowing

S. rolfsii and *F.oxysporum* inoculated pot

S.rolfsii inoculated pot with seeds

F.oxysporum inoculated pot with seeds

Plate7.2 Sowing Seeds in Earthen pot

7.2.4 Method of Sterilization and Incubation

After collection the diseased plant materials were brought to the laboratory as soon as possible so that stems are still fresh during the isolation of the pathogen. In the laboratory the advancing or water soaked portion of the lesion were cut into small sections (approximately 5x5 mm per section) and surface-sterilize by soaking for 15 seconds in 5% commercial Clorox (commercial Clorox usually consists of 5.25% sodium hypochlorite; by weight). This is done to kill microorganisms or pathogens on the surface of the stem tissues that may compete with the growth of *S. rolfsii.* About *5.25*% sodium hypochlorite solution contains in a beaker, surface sterilization of materials was continued for 15 seconds 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 materials was removed by gently pressing them between to 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. In the early practices, the sterilized materials were placed over the moist filter paper. 2-3 glass bars (90 mm x2 mm x 2 mm) were placed on the moist filter paper of the humid chamber, which prevented them from coming in contact with the moisture of the filter papers. 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.

7.2.4.1 Preparation of Humid Chamber

The humid chamber used for the incubation of the diseased plant parts consisted of a Petri plate (90 mm dia.) with its lids. Before being used the Petri dishes were surface sterilized by flaming. The linear walls of the dish and its lid were lined previously sterilized filter paper, which were then moistened with sterilized distilled water. The excess water was poured out and the plate with its lid was used as humid chamber. Desired number of diseased materials from each of the samples lots collected from the field was incubated using a separate humid chamber.

7.2.5 Isolation of The Pathogen (*Sclerotium rolfsii* and *Fusarium oxysporum)*

Isolation of *Sclerotium rolfsii / Fusarium oxysporum* from diseased brinjal plants noted above was made following either (i) by direct method or (ii) by plant method.

7.2.5.1 Direct Method of Isolation

The method consisted of sterilization and incubation of the diseased plant parts in humid chamber following the technique described before. The affected parts were incubated for 15 days at 28±2°C. During this period, the fungus was found to grow mycelia, sclerotia and conidia on the necrotic areas. The structure of sclerotia / conidia could be seen easily under stereoscopic binocular microscope and from the mycelia and sclerotia were collected on a very small agar block (about 2 mm in length), mounted at the tip of a flamed spear-headed tungsten needle. Sclerotia were collected by touching them with the agar block and could be seen lying attached to transparent surface of agar, which was then transferred to a freshly prepared PDA plate. On incubation fungal colonies were found to develop and free from contamination.

7.2.5.2 Plating Method of Isolation

In this period of isolation, diseased plant parts were cut into small pieces, about 0.5 cm in length, in such a manner so as to include both healthy and necrotic tissues in each piece. The pieces were then washed in running tap water, sterilized in 5.25% sodium hypochlorite solution and washed repeatedly for several times in sterilized distilled water to remove sodium hypochlorite solution. Three pieces were taken out using flamed forceps and dried between filter papers and finally transferred to a PDA plate. Plates were incubated at 28±2°C for 15 days, during which period the fungal colonies appeared on the PDA plates. Often the colonies were found to become contaminated due to unwanted growth of bacteria and other fungi. No doubt *Sclerotium / Fusarium* species was obtained in pure form but not so as it was with the direct method. Moreover, this method was found to be time consuming and it required further transfer to get pure culture. Whereas, in the direct method pure colony of *Sclerotium / Fusarium* had could be obtained by single transfer. Unless otherwise

stated direct method has been used throughout this work for the isolation of *Sclerotium / Fusarium* species.

7.2.5.3 Identification of Isolated Fungus

The fungus isolated from infected tissues of stem on brinjal plant describes 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 Subramanian (1971).

7.2.6 Collection of plant materials

The plant materials of twenty six species (Table1) were collected from different places campus and Rangpur district. The collected plants were identified and authenticated by a botanist in the Department of Botany, University of Rajshahi, Bangladesh.

7.2.6.1 Preparation of Aqueous Plant Extracts

Fresh leaves of selected botanicals were cleaned with tap water to remove the dust particle. Aqueous plant extracts was prepared by crushing the leaves in the mortar and pestle with sterile distilled water (1:1, w/v). The macerated extract was filtered through three-folded muslin cloth to remove fibrous and suspended material, this extracts was used as crude aqueous extract for experimental works (Bambode *et al*., 1973).Then filtrate was kept under normal room temperature. 20 ml of this crude aqueous extract added with 80 ml of distilled water to make 20% concentration before spray on brinjal plants twice a week from 7^{th} day onward.

7.2.7 Growth, yield and disease parameters

Morphological measurements of *Solanum melongena* L. were taken 10-90 days with 3/7 days intervals after transplantation till harvest time. The following parameters were measured as follows: seed germination, mortality, shoot height, root height, shoot weight, root weight and number of leaves were investigated after 2 weeks of sowing and 4 weeks of transplantation. Numbers of diseases were calculated as

percentage and disease per plant. The percentages of reduction of diseases were also calculated after the spray of plant extracts.

Data were collected on percentage seed germination, seedling mortality, plant growth parameters such as number of leaves, shoot height, shoot weight, root length and root weight. The experiment was followed completely Randomized Design (CRD) with 3 replications.

Data was analyzed by the computing SPSS program and following the standard statistical procedures. Treatment effects were compared to each other by computing Duncan's Multiple Range Test (DMRT).

7.2.8 Preparation of formulated *Trichoderma*

7.2.8.1 Collection, Isolation and culture of Bio-Agent

Culture of bio-agent *Trichoderma harzianum* was collected from six districts belongs to Rajshahi and Rangpur divisions followed by serial dilution methods in plant Pathology laboratory, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. The fungal antagonist *(Trichoderma harzianum*) was cultured in Potato Dextrose Agar medium (Ashrafuzzaman, 1976).

7.2.8.2 Collection of Substrates

Four different substrates viz. wheat bran, khesari bran, and rice husk were collected from shops of Joydebpur Bazar, Gazipur. The collected materials were kept in brown paper packets at 4^{O}C until use.

Substrate and their combination

Four substrates in combination with different grain bran viz. wheat bran, khesari bran, MOC and rice husk and water in different ratios (1:1:1:1:2) were tested for their potentials for multiplication and formulation of *Trichoderma harzianum*

7.2.8.3 Preparation, Sterilization and Inoculation of Substrates

The requisite amount of materials for each substrates (wheat bran: khesari bran: rice husk: MOC: water = 1: 1:1: 1:2) were thoroughly mixed in a 1000 ml Erlenmeyer

flask and autoclaved at 121°C for 20 minutes at 15 Ib. psi pressure for sterilization. The sterilized substrate allowed to cool down and then inoculated with 5 mm dia mycelial disc of 7 days old *Trichoderma* culture under aseptic condition. 10 discs for each flask/container were used for inoculation. Inoculated flasks were then incubated at room temperature (25°C ±2) for 7 to 10 days. Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of the fungus. The substrates turn whitish-green due to mycelial growth of the test fungus. These mass inoculums were spread in the experimental sick plot before one weeks of sowing.

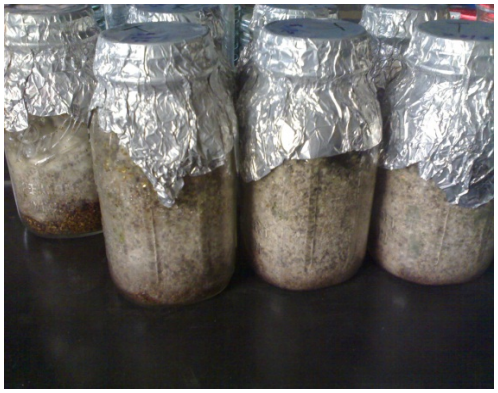
7.2.8.4 Preparation of mass inoculum of Pathogen(*Fusarium oxysporum* f. sp. and *Sclerotium rolfsii)*

The requisite amount of materials for each substrates (wheat bran: khesari bran: rice husk: MOC: water = 1: 1:1:.1:2) were thoroughly mixed in a 1000 ml Erlenmeyer flask and autoclaved at 121°C for 20 minutes at 15 Ib. psi pressure for sterilization.

The sterilized substrate allowed to cool down and then inoculated with 5 mm diamycelial disc of 7 days old *Fusarium oxysporum* f. sp. and *Sclerotium rolfsii* culture under aseptic condition. 10 discs for each flask/container were used for inoculation. Inoculated flasks were then incubated at room temperature (25°C ±2) for 7 to 10 days. Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of the fungus. The substrates turn whitish due to mycelial growth of the test fungus. The colonized wheat bran + rice husk+ khesari bran + potato chips substrates were air dried at room temperature. These mass inoculums were spread in the experimental sick pot and plot before one weeks of sowing.

Substrates preparation

Substrates inoculation



Multiplication of *Trichoderma harzianum*

Multiplication of *Sclerotium rolfsii*

Multiplication of *Fusarium oxysporum*

Preparationfor soil inoculation

Plate7.3 Preparation of substrates and multiplication of *T. harzianum* and mass inoculum of Pathogen (*S. rolfsii* and *F. oxysporum* f. sp.)

7.2.9 Seed treatments

Fungicides treatment was given @ 2 gm. /L of distilled water for soaking of seeds. For plant extracts treatment, seeds were soaked for six hour in the desired concentration of aqueous plant extracts and untreated seed served as control. The experiment was carried out in three replication and periodic observations for disease incidence were recorded. The bio-agent, fungicides and botanicals were evaluated *in vivo* by Randomized Block Design and data were statistically analyzed.

7.3 Result and Discussion

In total, 10 plants extract, a bio-agent/antagonist and two fungicides were studied against *Fusarium oxysporum* causing brinjal wilt and *Sclerotium rolfsii* causing brinjal foot rot. Among 10 plants, 7 were prepared from fresh leaves, two from seeds and one from bulb. The details regarding the name of the plants and parts used are enumerated in Table (1). Effect of medicinal plants on growth parameter is *Solanum melongena.* The data presented in Table (1) and Figure (Graph1) in shoot height, weight, germination (%), mortality (%), root height, weight and number of leaves were noticed. *In vivo* pot culture experiment employing water extracts at 15% concentration maximum plant species showed an increase in the root and shoot length and fresh and dry weight of root and shoot and number of leaves with the consequent reduction in the disease symptoms of the eggplant.

7.3.1 Effect of Plant Extract and *Trichoderma* formulation on vegetative growth

Effect of Plant Extracts and *Trichoderma* formulation on Number of Leaves

In present study, effect of ten botanical extracts, one bio-agents and one fungicide was tested against *Sclerotium rolfsii* and *Fusarium oxysporum* in experiment brinjal field. Treatments were found to have profound effect on the physiology of plants. *A. indica* treated plant showed highest leaves number (10.66) followed by *Trichoderma harzianum* formulation (10.33), fungicides-bavistin (9.33), *Lawsonia innermis* and *Lantana camara* extracts in comparison to control. In the *Corchorus capsularis* treated plants recorded lowest leaves number (3.00) of brinjal against *Sclerotium rolfisii* inoculated field. *Lantana camara, Ficus hispida* and *Datura stramonium*

treated plant showed similar leaves number, respectively against *Sclerotium rolfsii* inoculated field (Table 7.1 Fig. 7.3). The plants treated with *A. indica* extract showed highest leaves number (12.33) followed by bio-agents *Trichoderma harzianum* (9.66), *Lantana camara* (9.66) and *Ficus hispida* (9.00) in comparison to control (5.00). *Corchorus capsularis* (0.00) extract treated showed nil number of leaves whereas *Acacia nilotica* showed lowest leaves number (1.66) against *Fusarium oxysporum* inoculated field of brinjal plant (Table 7.2 Fig. 7.6).

Effect of Plant Extract and *Trichoderma* formulation on Root Length in Brinjal Plants

The root length was more (8.0 cm) in *Trichoderma harzianum,* Bavistin and *Lantana camara* extract treated plants. *Nicotiana tabacum* and *Allium sativum*t reated plants recorded same root length as per as control (7.00 cm). There was variation found in the *A. indica, Datura stramonium Ficus hispida, Corchorus capsularis, and Swietenia macroplylla* treated plants. Very low root length (3.0 cm) was observed in *A. nilotica* treated plants against *Sclerotium rolfsii* inoculated brinjal field/pot (Table7.1). The root length was more (8.3 cm) in *A. indica, Trichoderma harzianum* and *Datura stramonium* extract treated plants. *Nicotiana tabacum, Lantana camara* and *Ficus hispida* treatments produced same root length as per as control (7.33). There was variation found in the *Swietenia macrophylla, Allium sativum* and Provax treated plants. Very low root length (1.33 cm) was observed in *Acacia nilotica* treated plants. In case of *Corchorus capsularis* treated plants showed no visible root length against *Fusarium oxysporum* inoculated field (Table7.2).

Effect of Plant Extracts and *Trichoderma* formulation on Shoot Length in Brinjal Plants

The shoot length was high measuring 16.0 cm in *Trichoderma* treated plants compare to bavistin (14.0 cm). Three plant extracts, viz. *Lantana camara, Datura, Stramonium* and *Ficus hispida* showed nearly the same range of length. Four plant extract, viz. *Allium sativum, A. indica, Lawsonia inermis* and *Nicotiana tabacum* showed nearly the same range of length of as per as control. Less than 1 cm difference was noticed between *Swietenia macrophylla* and *Corchorus capsularis.* Very low shoot length

(4.00 cm) was observed in *A. nilotica* treated plants against *Sclerotium rolfsii* inoculated field (Table7.1, Fig.7.2,).

The shoot length was high measuring 14.0 cm in *Lantana camara* treated plants. Five plant extracts viz. *Swietenia macrophylla, Ficus hispida, Datura stramonium. A. indica, Lawsonia inermis* and bio-agents *Trichoderma harzianum* showed nearly the same range of length as per as control. Rests of the treatment were found variation. In case of *Corchorus capsularis* treated plants did not show visible growth of shoot height against *Fusarium oxysporum* inoculated field/soil/pot. Very low shoot height (1.66 cm) was observed in *Acacia nilotica* treated plants (Table 7.2 Fig.7.5).

Effects of Plant Extracts and *Trichoderma* formulationon Fresh and Dry Weight of Root

Trichoderma harzianum treated plants showed maximum fresh and dry weight root. The fresh weight was more in *Trichoderma harzianum* followed by bavistin, *Lantana camara, Ficus hispida* and *Lawsonia inermis. Ficus hispida* treated plants recorded more dry weight followed by *Allium sativum* and *Corchorus capsularis.* Fresh weight of three plant, viz. *Nicotiana tabacum, Lantana camara* and *Datura stramonium* showed nearly the same range. In the *Corchorus capsularis* treated plants less fresh and dry weight was observed against *Sclerotium rolfsij* inoculated brinjal field (Table 7.2). *Trichoderma harzianum* treated plants showed maximum fresh and dry weight followed by *Lantana camara, Datura stramonium, A. indica* and *Lawsonia innermis.* In case of *Corchorus capsularis* showed nil. *Acacia nilotica* and *Allium sativum* treated plants showed lowest fresh and dry weight. *Nicotiana tabacum* and *Swietenia macrophylla* treated plant showed nearly the same as per as control against *Fusanium oxysporum* inoculated field (Table7.2).

Effect of Plant Extracts and *Trichoderma* formulation on Fresh and Dry Weight of Shoot

The plants treated with Bavistin showed maximum fresh and dry weight for shoot followed by *Trichoderma harzianum.* Three plants extract viz. *Nicotiana tabacum, Ficus hispida* and *Datura stramonium* showed nearly the same range of dry and fresh weight compare to control. The plant treated with *Allium sativum*,*A. indica* and

Lawsonia innermis and without treated plants showed statistically similar fresh and dry weight for shoot. *Acacia nilotica* and *Swietenia macrophylla* showed similar weight whereas *Corchorus capsularis* seed extract treated plants showed lowest fresh and dry weight for shoot against *Sclerotium rolfsii* inoculated brinjal field (Table7.1).

The plants treated with *Datura stramonium* showed maximum fresh and dry weight for shoot followed by *Lantana camara, Trichoderma harzianum A. indica* and *Lawsonia innermis* compare to control. Provax, *Acacia nilotica* and *Allium sativum* treated plants recorded less fresh and dry weight was observed whereas *Corchorus capsularis* treated plants recorded nil against *Fusarium oxysporum* inoculated brinjal field (Table7.2).

7.3.2 Effect of Plant Extracts and *Trichoderma* formulation on Germination and Seeding Mortality

Effects of *Trichoderma* and plant extracts against soil borne pathogens of brinjal are presented in table 7.1 & 7.2. The substrate formulated with *Trichoderma harzianum* and *A. nilotica* extract significantly increased seed germination and decreased foot and root rot and wilt diseases of brinjal caused by *S. rolfsii* and *F. oxysporum.* Bavistin, *Nicotiana tabacum, Corchorus capsularis, Lantana camara*, *Ficus hispida, Datura stramonium, Allium sativum* and *A. indica treated* seeds recorded same significant seed germination. Among them Bavistin, *Nicotiana tabacum, Swietenia macrophylla* and *Allium sativum* significantly decreased foot and root rot disease of brinjal caused by *S. rolfsii* comparison to control. *Corchorus capsularis* treated plants increased seeding mortality (41.66 & 45.00) compare to control (36.66 & 40.00) caused by *S. rolfsii* and *F. oxysporum. Nicotiana tabacum, Datura stramonium, Allium sativum* treated plants recorded increased seed germination and decreased foot and root rot and wilt diseases of brinjal caused by *F. oxysporum.* Rests of the plants were less effective in reducing the mortality and increased the germination percentage (Fig.7.1 & Fig.7.4). The highest seeding mortality 41.66% and 45.00% was recorded in *Corchorus capsularis* against foot and root rot and will disease caused by *S. rolfsii* and *F. oxysporum*. Significantly lower seeding mortality 11.66% and 10.00% was recorded in soil amendments with substrate formulation with *Trichoderma harzianum* followed by seed treatments with bavistin and provax (13.33% and 11.66%) seeding mortality, respectively.

Table 7.1: Effect of treatments against *Sclerotium rolfsii* of brinjal in pot

Treatment	Germination (%)	Seedling mortality (%)	Shoot height	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weigt	Number of leaves
Trichoderma	98.33±1.66a	11.66±1.66g	16.00±1.00a	32.19±2.59ab	3.50±.46ab	8.66±.72a	1.84±.15a	.53±.03a	10.33±.88a
Bavistin	88.33±6.66ab	13.33±3.33g	14.00±.57a	34.95±.86a	3.92±.09a	8.83±1.92a	1.48±.09ab	.41±.01ab	9.33±.33ab
Babla	96.66±1.66a	15.00±2.88g	4.00±2.08c	3.08±2.59dc	.32±.27d	3.00±1.52b	.10±.09e	.02±.02e	4.33±.88ef
Tobacco	86.66±3.33ab	13.33±1.66g	8.66±.88bc	21.04±9.28abc	2.11±.86bc	7.83±1.09ab	.92±.36bcd	.24±.08bcd	6.33±.33cd
Mahogany	63.33±19.22c	21.66±1.66efg	5.00±2.51c	3.78±2.11dc	.42±.23d	3.66±2.02ab	.22±.13e	.05±.02e	4.66±.66def
Jute seed	88.33±7.26ab	41.66±3.33a	4.83±2.45c	.82±.50e	.07±.07d	5.00±2.88ab	.04±.02e	.02±.01e	3.00±.57f
Lantana	86.66±4.40ab	31.66±3.33bcd	11.66±1.20ab	18.33±9.07bcd	2.31±.65bc	8.00±1.52ab	1.08±.41bcd	.27±.11bcd	8.33±.33bc
Khoksha	85.00±.00ab	30.00±2.88cde	12.00±.57ab	23.32±4.29abc	2.54±.47abc	6.66±.88ab	1.24±.10abc	.31±.03bc	8.00±.57b
Datura	90.00±5.00ab	26.66±1.66de	11.66±.66ab	21.16±5.49abc	2.29±.52bc	6.33±.6ab	.90±.19bcd	.25±.02bcd	7.66±.88bc
Garlic	95.00±2.88ab	16.66±1.66fg	7.66±2.33bc	10.13±6.33cde	1.17±.65cd	7.00±1.52ab	.44±.21de	.11±.06de	5.66±.33de
Neem	83.33±1.66abc	25.00±2.88def	8.33±1.33bc	14.42±4.46cde	1.53±.43cd	6.66±.88ab	.57±.21cde	.13±.03de	10.66.33a
Henna	78.33±7.26abc	21.66±1.66efg	8.00±2.57bc	13.44±.49cde	1.53±.44cd	6.33±.66ab	1.24±.15abc	.27±.11bcd	8.33±.33b
Control	80.00±5.00abc	36.66±6.00abc	7.33±.88bc	9.55±3.05cde	1.08±.37cd	7.00±1.52ab	.48±.13de	.15±.04cde	5.66±.33de

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (p=0.05).

Table7.2: effect of treatments against *Fusarium oxysporum* of brinjal in pot

Treatment	Germinatin (%)	Seedling mortality (%)	Shoot height	Shoot fresh weight	Shoot dry weight	Root length	Root fresh weight	Root dry weight	Number of leaves
Trichoderma	93.33±1.66ab	10.00±2.88g	12.00±1.52a	45.66±12.83a	4.38±1.67a	9.33±.33ab	2.34±.59a	.53±.12a	9.66±.33b
Provax	98.33±1.666a	11.66±1.66g	13.00±1.52a	4.23±2.50bc	.42±.23bc	5.00±.57cd	.233±.15bc	.04±.02bc	6.66±.88de
Babla	95.00±2.88ab	13.33±1.66fg	1.66±1.66d	3.16±2.58bc	.32±.25bc	1.33±1.33e	.15±.12c	.02±.02c	1.66±.33h
Tobacco	90.00±2.88abc	15.00±.00efg	10.00±1.00ab	28.96±13.89ab	3.03±1.38ab	7.00±.00abc	1.16±.64abc	.29±.14ab	6.33±.66def
Mahogany	83.00±4.40bcd	25.00±2.88cdef	6.33±.66bc	25.02±7.88abc	2.48±.82bc	6.00±1.52bc	1.17±.33abc	.26±.07abc	3.66±.33g
Jute seed	85.00±5.00abcd	45.00±2.88a	.00±.00d	.00±.00c	.00±.00c	.00±.00e	.00±.00c	.00±.00c	.00±.00i
Lantana	90.00±5.00abc	35.00±5.77abc	14.00±1.15a	42.87±.71a	4.28±.06a	7.66±.33abc	2.21±.27a	.48±.04a	9.66±.88b
Khoksha	86.66±3.33abcd	31.66±1.66bcd	12.66±.33a	36.76±2.45a	3.63±.26a	7.66±.33abc	1.94±.11a	.44±.01a	9.00±.57bc
Datura	90.66±2.88abc	20.16±10.24defg	12.33±1.33a	46.55±12.79a	4.52±1.40a	9.66±.33a	2.20±.38a	.51±.11a	7.66±.33cd
Garlic	86.66±8.81abcd	20.00±.00defg	3.00±3.00cd	3.34±2.45bc	.63±.33bc	2.66±2.66de	.17±.15c	.06±.03bc	5.33±.33ef
Neem	85.00±2.88abcd	20.00±.00defg	12.33±.88a	41.26±11.49a	3.88±.98a	8.33±.33abc	2.10±.59a	.45±.11a	12.33±.33a
Henna	81.66±4.49bcd	26.66±3.33cde	12.33±1.33a	36.76±2.45a	3.66±.36a	7.00±.00abc	1.94±.11a	.46±.01a	7.16±.33cd
Control	76.66±1.66cde	40.00±2.88ab	11.00±1.20a	28.78±8.51ab	2.95.72ab	7.33±.88abc	1.42±.403ab	.36.09a	5.00±.57fg

Values with the same letter in the same column are not significantly different according to the least significant difference (LSD) test 5%

In vivo pot culture experiment employing water extract of six plant species (*Adhatoda vasica*, *Sapinduse marginatus, Azadirachta indica*, *Jatropha curcas, Ocimum sanctum* and *Vitex negundo*) showed an increase in the root and shoot length and fresh and dry weight of root and shoot with the consequent reduction in the disease symptoms of the eggplant (N. Siva *et al*., 2008). Similar result was carried out by Nahak and Sahu (2014) who showed that neem based formulations in all the treatments were found to be superior over control not only on shoot height, number of leaves, number of buds, number of flowers, number of fruits but in respect of diseases control also such as wilt and leaf spot under field conditions.

Growth stimulating effect often medicinal plant extracts (P. *pinatta, A. marmelos, A .indica, B. campestris, P. nigrum, E. tirucalli, V. negundu, A. conyzoides, T. patula* and *Z. jujube)* on *Lycopersicum esculentum* have been observed (Pattnaik *et al.,* 2012). Similar experiment was carried out by Okunlola and Ofuya (2013) who showed the effect of *Azadirachta indica* and *Piper guineense* on the growth and yield of jute under sole and mixed cropping. All growth parameters increased in comparison to control this may be due to reduction in insect pests or the percentage infestation by the pests or both. In another experiment ethanolic extracts of *Melia azedarch, Eucalyptus robosta, Sapium sebiferum* had no significant influence on growth and devolopment of Soybean seedlings (Wan *et al.,* 2012). Effect of tea seed extracts on growth of beet, mustard, oat and barley were studied. Different concentrations of these extracts increased the growth, yield and biomass of the crops. The growth stimulating effect is not exclusively by its adverse effect on pathogen or by an increase in nutrient uptake. However substances with hormone like properties can stimulate of effect biomass allocation in plants. In addition to hormones, medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on growth and yield of the plant (Andresen and Cedergreen, 2010). In particular, extracts of *Azadirachta indica* substantially reduced the number of infected leaves and number of lesions on foliage and curtailed disease development, which in turn, protected flowers and capsules from infection (Enikuomehin, 2005). Reduction of leaf spot diseases up to 40% *A. indica* extract has been reported in *Lycopersicum esculentum* (Pattnaik *et al.,* 2012). It proves that the plant extracts can serve as alternatives to the synthetic pesticides for controlling of bacterial leaf spot disease of *Solanum* without any adverse effect on crop yield and

yield parameters. The efficacy of different plant extracts belonging to different species other than the tested botanical extracts against the *A. solani* either under laboratory or greenhouse conditions have been reported (Tegegne *et al*.,2008; Anyanwu and Dawat, 2005; Chutia M, 2009; Anand, *et al.,* 2009; Zaker and Mosallanejad, 2010). Similarly *A .indica was both* for Early blight and leaf spot and *A. marmelos, P. pinatta and A. conyzoide* for Fruitspot, Blossom end rot and Sunscold. Literatures are avalible on effect of plant extracts on various diseases of tomato **(**Kharde, 2010; Suleiman, 2011**).** Similar reports on plant products containing fungi-toxic constituents that have the potential to control plant diseases **(**Tewari and Nayak, 1991; Enikuomehin *et al*., 2002**)** are available in recent literature. *Vitex nigudo* showed maximum fugal activity against *Colletotrichum falcatum* which causes red rot diseases **(**Prince and Prabakaran, 2011**)**. Similarly some medicinal plants are like *Pandanus, Sedrus, Capparis, Mirabilis, Eicchornia, Nymphaea* etc inhibited *Setophaeria rostrata* causing seedling blight disease in sugar cane (Mahalingam *et al*., 2011) and *Pythium debaryanum* was inhibited by some medicinal plants namely *Lawsonia, Phyllanthus, Vinca, Tephrosia* and *Mimosa* (Ambikapathy *et al*. 2011). Kamdi *et al.,(*2012) revealed that *Trichoderma viride, Bacillus subtilis* and aqueous plant extracts of *Azadirachta indica* and *Lantana camara* seed treatments were also significant in reducing wilt incidence and more yield as compared to control**.**

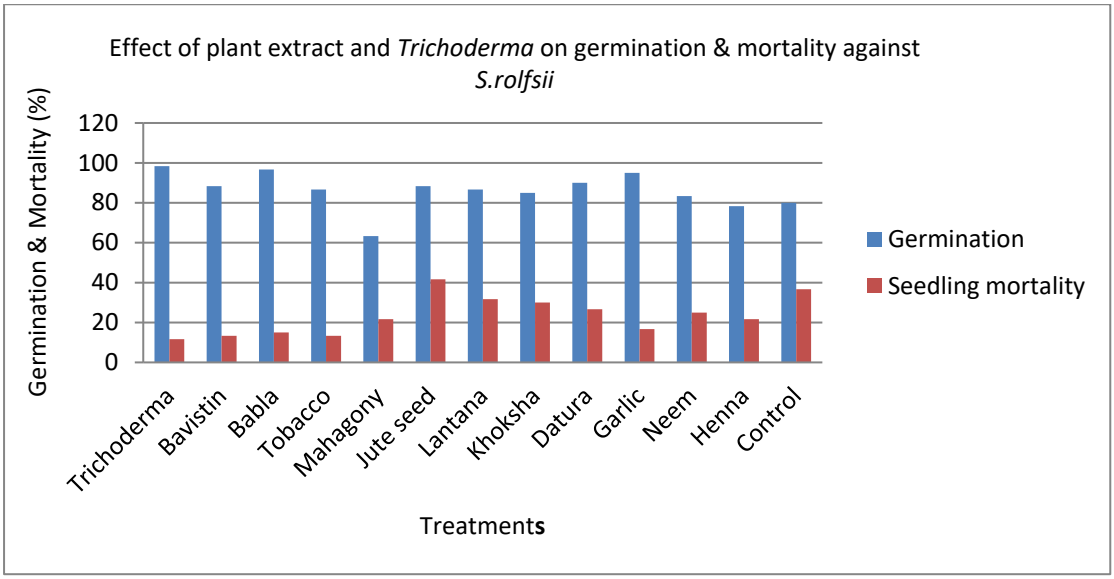


Fig. 7.1 Effect of plant extract and *Trichoderma* on germination & mortality of *Solanum melongena*

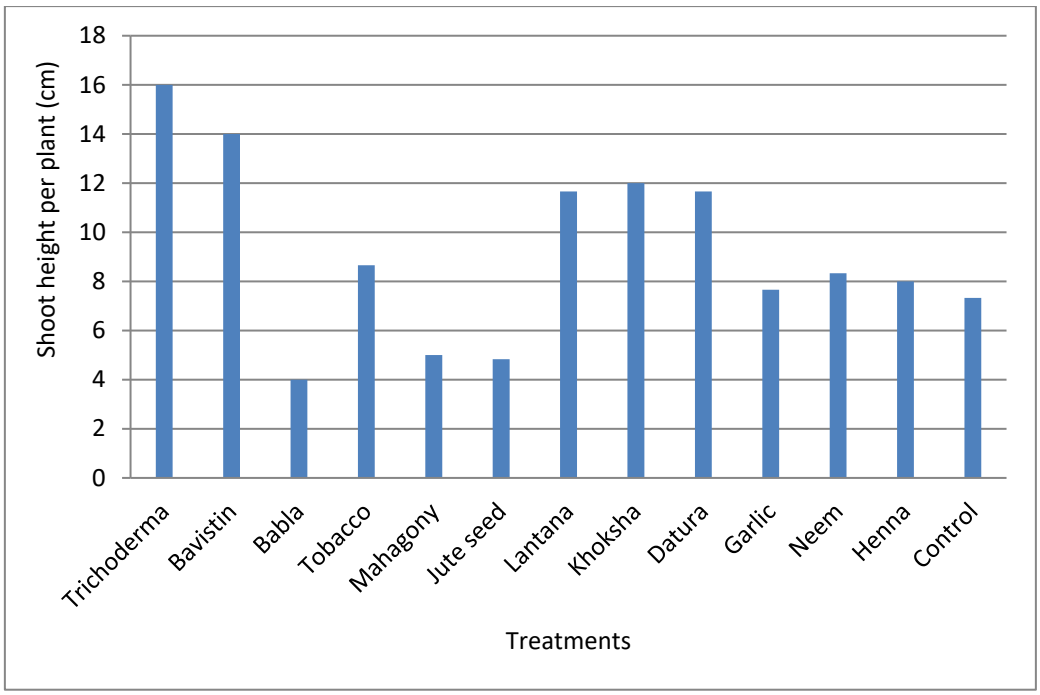


Fig. 7.2 Effect of plant extract and *Trichoderma* on plant height of *Solanum melongena* against *S. rolfsii*

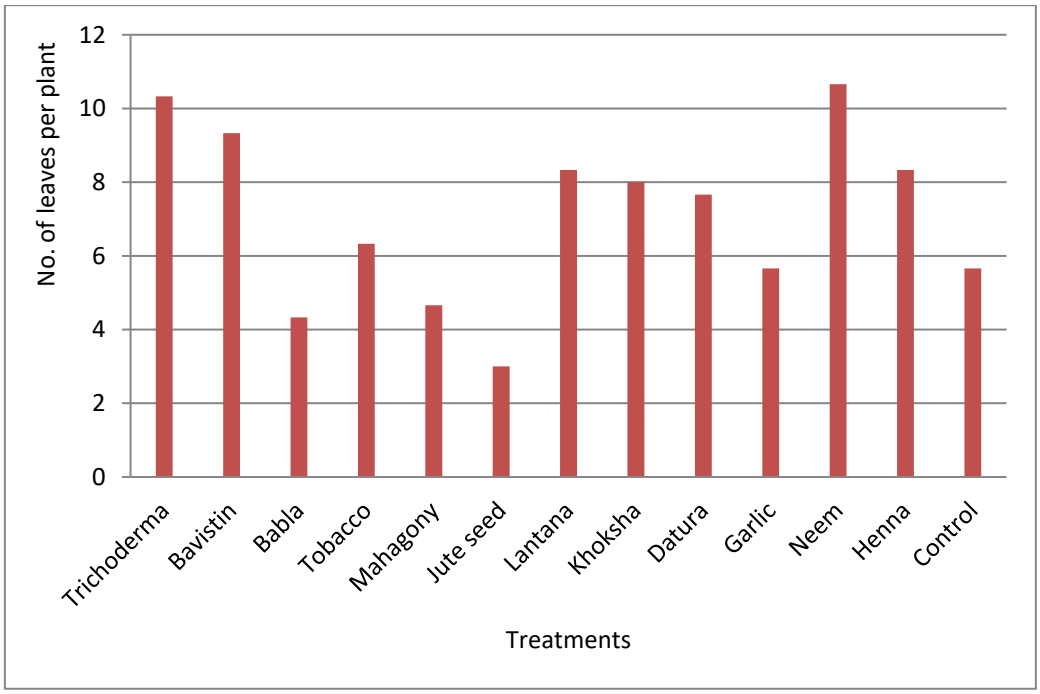


Fig. 7.3 Effect of plant extract and *Trichoderma* on No. of leaves of *Solanum melongena* against *S. rolfsii*

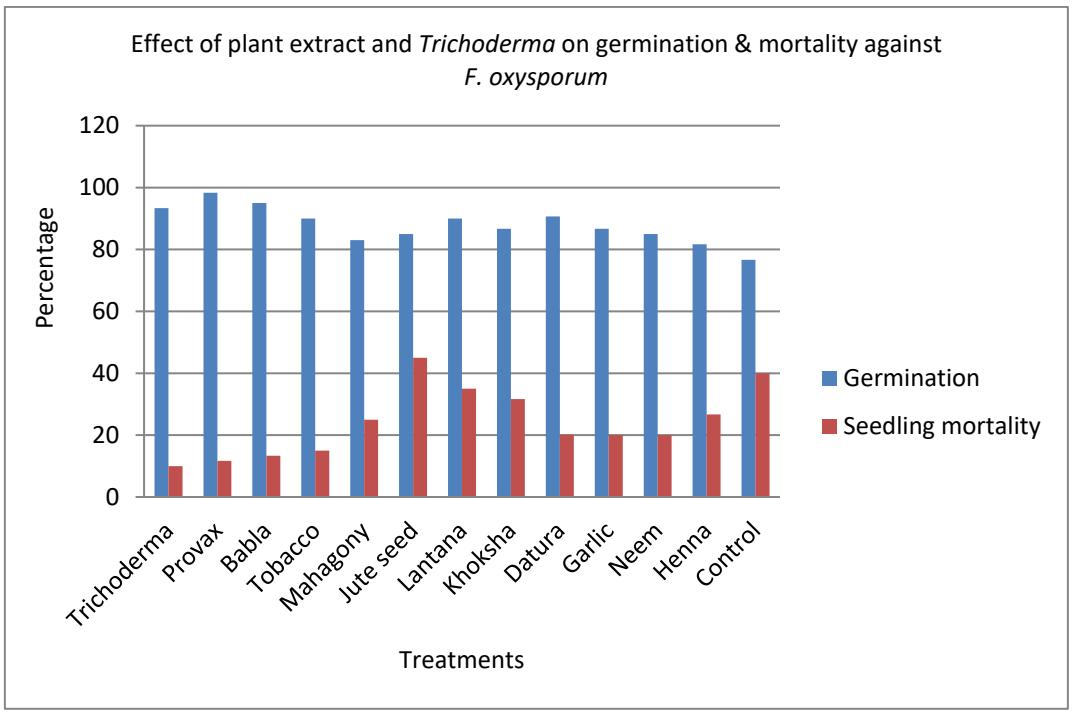


Fig. 7.4 Effect of plant extract and *Trichoderma* on germination & mortality of *Solanum melongena*

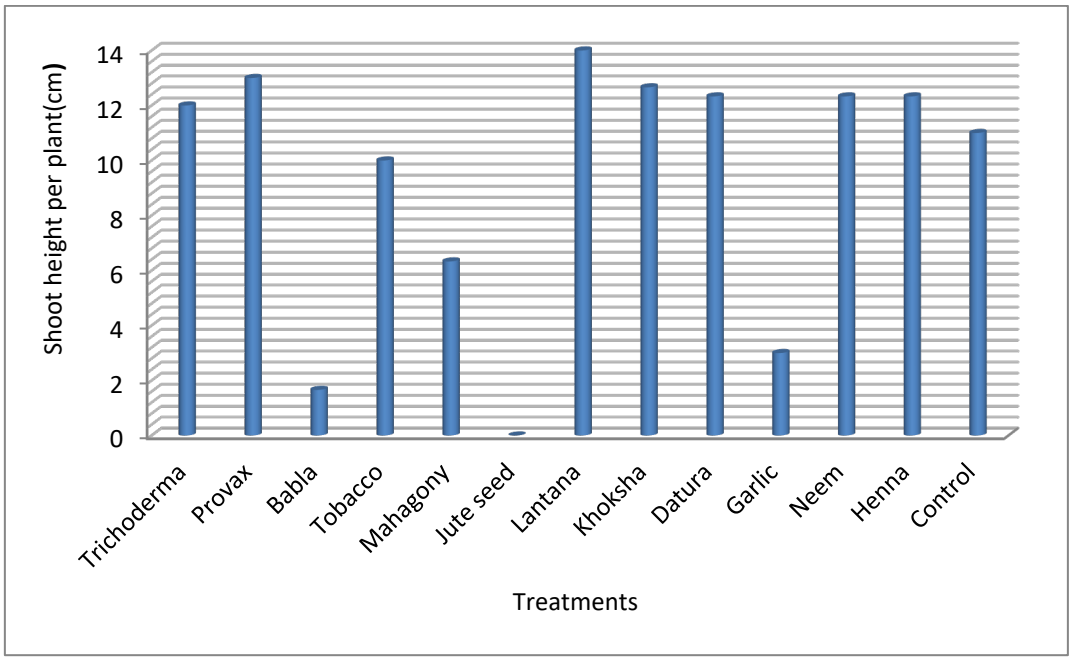


Fig. 7.5 Effect of plant extract and *Trichoderma* on plant height of *Solanum melongena* against *F. oxysporum*

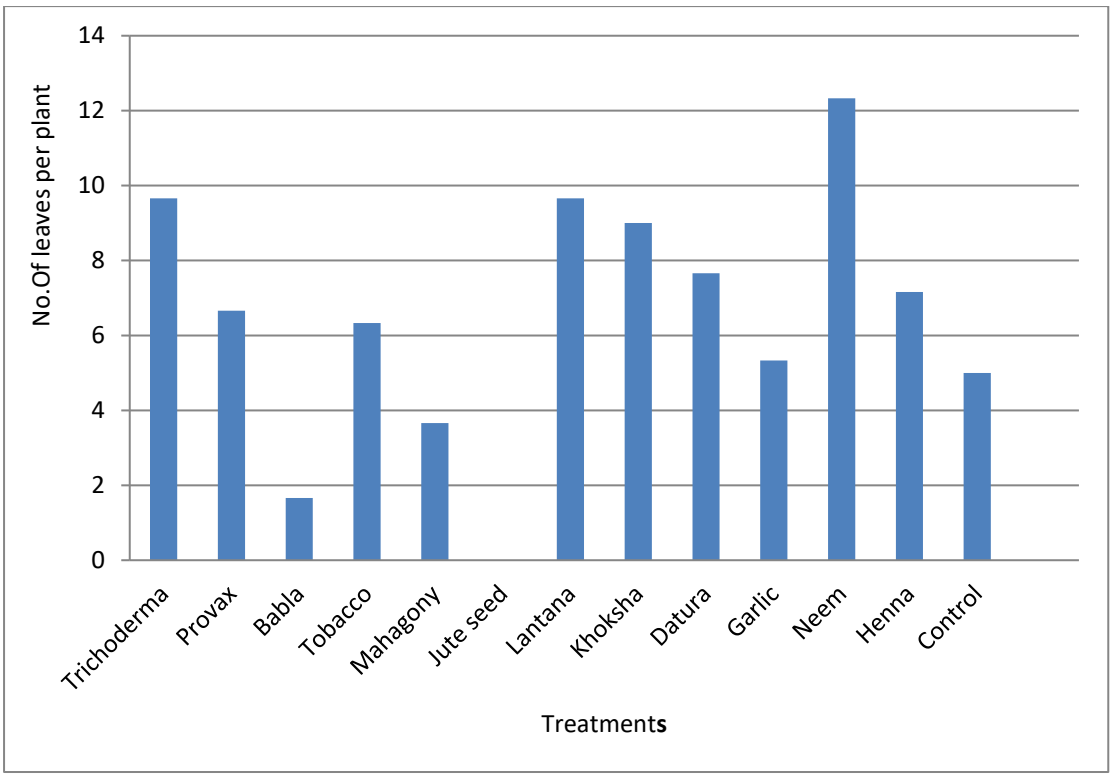


Fig. 7.6 Effect of plant extract and *Trichoderma* on No. of leaves of *Solanum melongena* against *F. oxysporum*

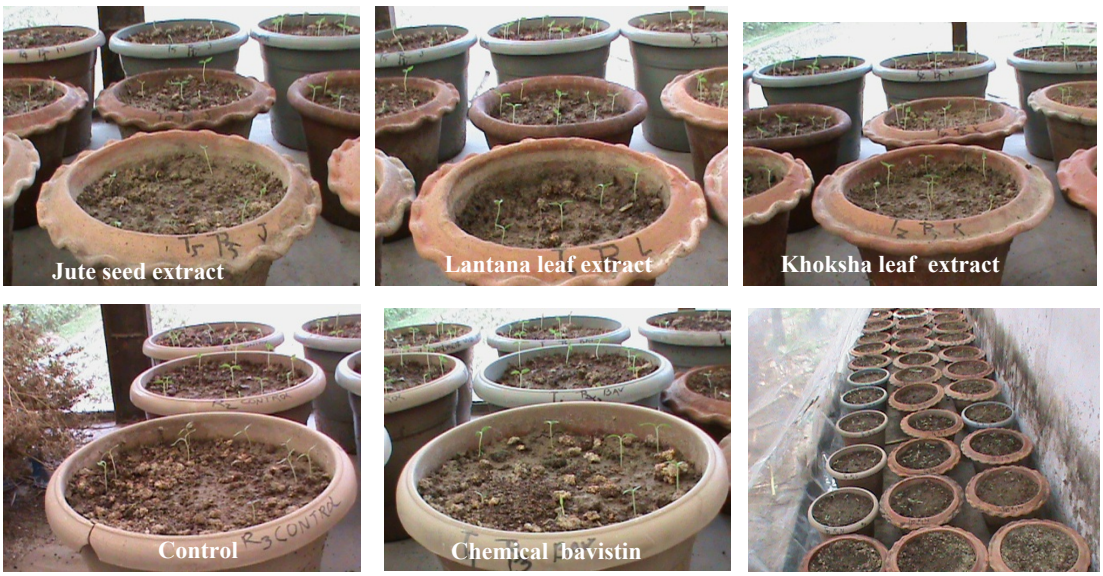


Plate 7.4 Seedling of treated seeds on soil borne pathogen (*S. rolfsii*) inoculated soil

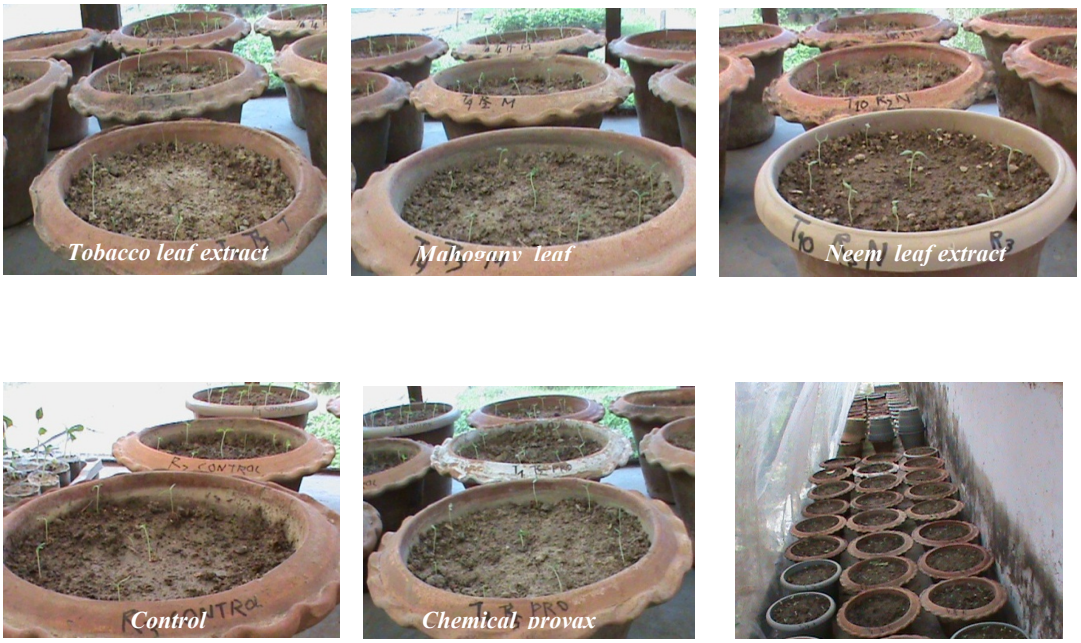


Plate 7.5 Seedling of treated seeds on soil borne pathogen (*F. oxysporum)* inoculated soil

Plate 7.6 Foot & Root rot and Wilt diseases at seed bed of Brinjal caused by

A-foot & root rot by *S. rolfsii*, **B-** foot & root rot by *F. oxysporum* , **C-**wilting causes *F. oxysporum*

Plate 7.7 Data collection of vegetative growth

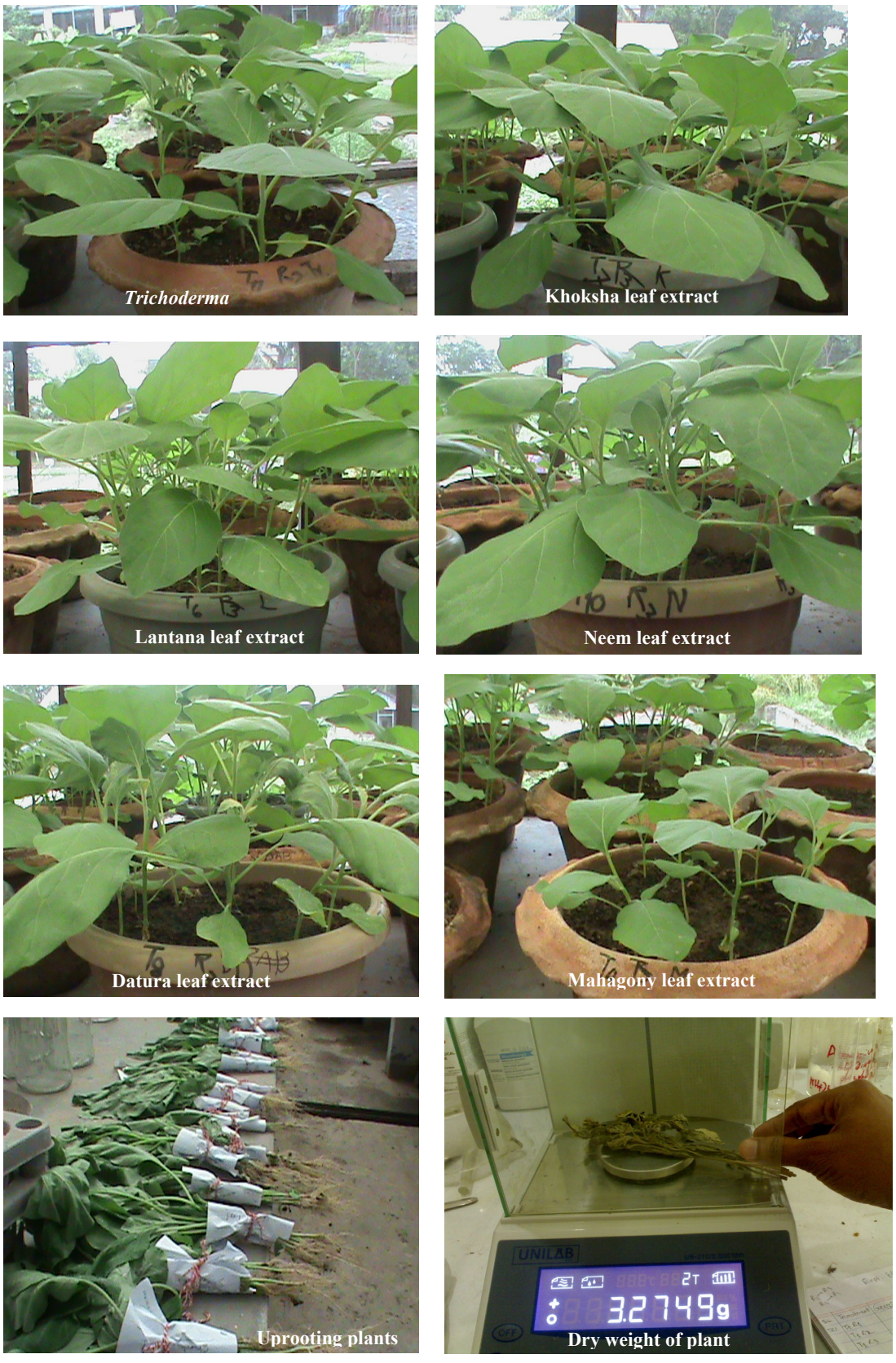


Plate 7.8 Data collection of vegetative weight

7.4 Conclusion

The differences in the inhibitory effect of various plant extracts may be due to qualitative and quantitative differences in the antifungal principles/compounds present in them. This was also confirmed by *in vivo* pot culture experiment employing water extract of *Acacia nilotica, Allium sativum, Datura stramonium*, *Nicotiana tabacum and Trichoderma* where there was an increase in the shoot/root length and number of leaves, fresh and dry weight of shoot/root with the consequent reduction in the disease symptoms of the eggplant was observed. As the disease is soil borne and persist for long years in soil, management of disease by single approaches will be difficult and uneconomical. The results of present finding will help to tackle the disease by integration of bio-agents and botanical fungicides for efficient and ecofriendly way.

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Chapter - 8

Integration of Carrier Materials for Effective Formulation of *Trichoderma harzianum* and *Rhizobium* sp. Against Foot and Root Rot and Wilt Diseases of Brinjal in Pot Experiment

Abstract

Three different carrier materials such as Khesari bran, Wheat bran and Rice husk alone and their integration with Mustard Oil Cake (MOC) and Potato Chips (PC) were used for effective formulation of *Trichoderma harzianum* and *Rhizobium* sp. as a bio-fungicide. The formulated *T. harzianum* and *Rhizobium* bio-fungicide was tested against the soil-borne pathogens of brinjal like *Sclerotium rolfsii* and *Fusarium oxysporum* causing foot and root rot and wilts diseases. All the substrates formulated with *Trichoderma* had significant effect in seed germination and decreasing seedling mortality as well as increasing plant growth over control. Among the carrier materials, integration of khesari bran, PCD and wheat bran or integration of rice husk with PCD and khesari bran or integration of rice husk with PCD and wheat bran or integration of rice husk, khesari bran and wheat bran with MOC or integration of rice husk, khesari bran and wheat bran with PCD (Potato Chips Dust) or wheat bran or khesari bran were found to be better carrier for effective formulation of *T. harzianum* against foot and root rot and wilt diseases of brinjal.

Key words: Carrier Materials, *Trichoderma harzianum, Rhizobium,* formulation, soil-borne pathogen, brinjal.

8.1 Introduction

Brinjal are rich in and cheaper source of vitamins and minerals and also a good source of carbohydrates and play an important role in balanced diet of human beings. Vegetable yield in Bangladesh is low compared to other countries. The low yield is due to environment, and pest and diseases. Among the diseases, foot rot/collar rot, root rot and wilt caused by soil borne plant pathogen is one of the major diseases. It significantly reduces the vegetable yield. It attacks the plants at any growth stage but more devastating at seedling stage (Bag and Sinha, 1997). Soil-borne plant pathogens such as *Sclerotium rolfsii, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* are the major obliges for crop production especially vegetable crop production all over the world. Among the plant pathogens, *S. rolfsii, F. oxysporum* and *R. solani* are the major soil-borne pathogen which causes germination failure and damping off of vegetable seedlings (Harman *et al.,* 1991). It is very difficult to control the soil borne pathogens through conventional method such as application of fungicides, cultural methods, etc. On the other hand the resistant cultivar against the disease is not always available and also not well acceptable due to grower's choice and preferable horticultural traits.

Farmers try to overcome this problem through different cultural practices and use of chemical fungicides. But the control of soil borne pathogens with chemicals is very expensive and is almost impractical in Bangladesh. In addition, unwise use of chemicals in agriculture causes environment pollution and health hazards, destroying the natural balance and beneficial micro-flora of the soil. Moreover, consumers are becoming increasingly concerned about chemical pollution of the environment and pesticide residues in food, and farmers more often face with development of pathogen's resistance to chemical fungicides. Therefore, there is a need for development of efficient alternative measures to combat the disease and inoculums buildup in soil.

Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. A considerable role in limiting the populations of these pathogenic fungi inhabiting the above ground parts of plants is played by antagonistic microorganisms. Such

properties are first of all exposed by the fungi *Trichoderma* and *Gliocladium*. (Kaewchai and Soytong, 2010) mentioned that application of different bio-agents to the soil under greenhouse conditions, to control the root rotting fungi revealed that the plant irrigated with water, containing *B. subtilis* (2.5 g/L) decreased the disease severity over control. *Trichoderma* is recognized as a successful saprophytic fungus, besides being reported as a parasite on other fungi. Dhahira and Qadri (2010) found that the conventional chemical control measures are unable to provide total control. Hence, antagonistic microorganisms were evaluated individually and in combinations for their bio-control potential against plant pathogenic fungi.

As alternative of chemical method, biological fungicides are included in the concept of biological control. Therefore, biological control can be an alternative method to combat the melody. The antagonistic nature of *Trichoderma* was demonstrated more than seven decades ago (Weindling 1934), in this control, living microorganisms act as antagonist, parasites and predators (Kwok *et al*., 1987). The antagonism of a biological agent can reduce pathogen's ability to produce inoculum. In the absence of other sources of inoculum, future disease levels will eventually be reduced by such biological control measures (Fokkema, 1995). *Trichoderma* spp. have played a considerable role as biocontrol agent (Papavizas, 1985) and is recognized as an effective biocontrol agent of soil-borne plant pathogenic fungi such as *Fusarium, Sclerotium, Rhizoclonia* etc. (Diet and Inbur, 1994). *Trichoderma* significantly destroys the sclerotia of *S. rolfsii* (Susceelendra and Schlosser, 1999) and it is antagonistic to *S. rolfsii,* overlaps the pathogen and suppresses its growth (Iqbal *et al*., 1995). Biological seed treatment in tomato, potato, chickpea, lentil and peanut with *Trichoderma harzianum* and *Gliocladium virens* resulted in excellent protection against a wide range of pathogens including *S. rolfsii* and the treatments were consistently as effective as or better than fungicidal seed treatment (Mukhopadhyay, 1989).

Since then many potential strains of *Trichoderma* have been isolated and characterized from different natural habitat and used in biological control experiments against several plant pathogenic fungi in many countries. Management of soil-borne pathogen by *Trichoderma* based bio-fungicides is considered as eco-friendly and cost effective alternative to chemical pesticides. The major limitation is the lack of

appropriate technique for effective formulation of *Trichoderma* and absence of suitable carrier (Harman *et al.,* 1991). *T. harzianum* has been formulated in various carrier materials including wheat bran, rice bran, maize bran, sawdust (Das *et al.,* 1997); rice straw, chickpea bran, grass pea bran, rice course powder, black gram bran (Shamsuzzaman *et al.,* 2003); cow dung, poultry manure, ground nut shell, black ash, coir waste, spent straw from mushroom bed, talc, vermiculite (Rettinassababady and Ramadoss, 2000). Although all these carrier materials are available in Bangladesh, but there integration for effective formulation of *T. harzianum* have not yet been explored. Therefore, the present study was undertaken to find out the most cost-effective formulation of *T. harzianum* as bio-fungicides and its effectiveness against *S. rolfsii* and *F. oxysporum.*

8.2 Materials and Methods

8.2.1 Collection, isolation, purification and morphological characterization of isolates of *Trichoderma* spp.

8.2.1.1 Collection of soil samples

For isolation of *Trichoderma* species rhizosphere soil of healthy plants were collected from different vegetable fields from five different districts of Bangladesh (Table 8.1). From a selected field several healthy plants were pulled out gently with soil. Then the roots and stubbles were removed from soil and all the soils collected from different plants were mixed to make a composite sample. The composite sample was divided into 4 components over a plastic sheet. Two component samples from any two corners were discarded and again the left 2 samples were mixed. The process continued until the volume of the sample reaches to about 400-500 g which was treated as working sample and was taken into a plastic bag with proper labeling and stored in a refrigerator (4°C) in the laboratory (Plate 8.1).

Plate 8.1 Collection of soil sample

8.2.1.2 Isolation, purification and preservation of *Trichoderma* isolate

Trichoderma were isolated from soil following dilution plate technique (Subba, 2003). One gram of working soil sample was taken in a test tube containing 9 ml of sterilized water to make 1:10 dilution. Similarly a series of dilution process were continued until the sample was diluted to 1:10000. All working samples were diluted in the same process. A number of PDA plates were prepared in aseptic condition in the laboratory. 1 ml of each sample soil suspension was placed in each petri-plate. The soil suspension was thoroughly mixed with the medium using a glass spreader. The petri-plates were incubated for 5 days at room temperature (25±2º C).

After 3 days of incubation, plates were observed for *Trichoderma* colony. The growing margin of *Trichoderma* colony was cut into *5* mm blocks with the help of a cork borer. The blocks were carefully placed in PDA plates and incubated as before. Hyphaltip/mycelial block of *Trichoderma* were transferred to PDA for purification. The well-developed pure cultures of *Trichoderma* were sub-cultured to PDA plates and slants for preservation. The slants were prepared by tilting individual test tube or whole basket of test tubes containing sterilized PDA at an angle sufficient to make a sloped surface of 0.5" to 3.5" front the bottom of the tube. The tubes were left in this position until solidified. The fully-grown *Trichoderma* in slants were preserved in the refrigerator at 4°C for further use (Plate 8.**2**). The isolates were identified following the key of Kiibicek and Harman (1998).

Plate 8.2 Serial dilution, Isolation, purification and preservation of *Trichoderma* isolate

Table 8.1 Collection and isolation of *Trichoderma harzianum* from different locations in northern Bangladesh

Sl. No.	Districts	Upazillas	Number of samples	No. of *Trichoderma* isolates
1	Rajshahi	RajshahiSardar	TRU1, TRU2, TRU3, TRU4, TRU5, TRU6, TRU7, TRU8, TRU9, TRU10, TRU11, TRU12, TRK13, TRK14, TRK15, TRK16	02
2	Bogra	Shibgonj	TB17, TB18, TB19, TB20, TB21, TB22, TB23, TB24, TB25, TB26	01
3	Gybanda	Palashbari	TG27, TG28, TG29, TG30, TG31	00
4	Rangpur	Mithapukur	TR32, TR33, TR34, TR35, TR36, TR37, TR38, TR39, TR40, TR41, TR42, TR43	01
5	Dinajpur	Birgong	TD44, TD45, TD46, TD47, TD48	01
6	Thakurgaon	Thakurgaon Sadar	TTG49, TTG50, TTG51, TTG52, TTG53, TTG54	01
Total			**54**	**06**

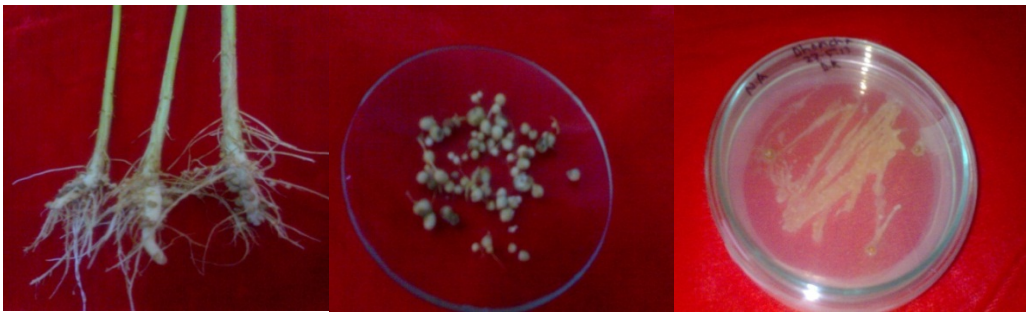
8.2.2 Collection, isolation, purification and morphological characterization of isolates of *Rhizobium* spp.

8.2.2.1 Collection of soil samples

For isolation of *Rhizobium* species healthy Dhaincha (*Sesbania rostata*) plants were collected from different fields from three different districts of Bangladesh (Table 8.2). From a selected field several healthy plants were pulled out gently with soil. Then the roots and stubbles were removed from soil and all the Nodules collected from different plants after then washed by distilled water and then Clorox.

8.2.2.2 Isolation, purification and preservation of *Rhizobium* isolate

A number of PDA plates were prepared in aseptic condition in the laboratory. The transverse section of Nodules were carefully placed in Nutrient agar media plates and incubated for 2 days at temperature (28±2º C). After 2 days of incubation, plates were observed for *Rhizobium* colony. The well-developed pure cultures of *Rhizobium* spp. were sub-cultured to PDA and NA plates and slants for preservation (Plate 8.3).



Sesbania rostata with Nodules Isolated Nodules *Rhizobium* colony

Plate 8.3 Isolation, purification and preservation of *Rhizobium* isolate

Table 8.2 List of *Rhizobium* sp. isolates collected from different hosts and locations of northern Bangladesh

Location	Number of plant	Number of nodule
BARI, Wheat research field (RBA)	10	50
Rajshahi University, Eastern part (RRU)	10	50
Rangpur, Mithapukur (RRM)	10	50

8.2.2.3 Morphological characters of different *Trichoderma* isolates

The isolates of *Trichoderma* spp. were characterized morphologically. In all, 06 isolates were purified through mycelial block culture and were maintained on Acidified Potato Dextrose Agar (APDA) medium at 25±1°C (incubation) for studying cultural characteristics (Singh and Singh, 2004).

8.2.2.4 Study on growth and conidia production of *Trichoderma* isolates:

Radial mycelial growth and spore production of the isolates of *Trichoderma* spp. *were* studied following the method of Sultana *et al.* (2001) A 6 mm block of each *Trichoderma* isolate of seven days old was placed at the Centre of Petri plate containing PDA. Three replications were maintained. The fungal growth after 24 hours of inoculation was measured by recording colony diameter. Growth of *Trichoderma* was very fast and within 5-6 days it produced abundant spores throughout the plate (Plate 8.4). After 24, 48 and 72 hours of inoculation, the radial mycelial growth was measured as the mean of two perpendicular diameters and mean of four replications were recorded for each isolate. Colony characters were noted in respect of:

- Shape
- Color
- Growth habit
- Compactness
- Spore density /ml

In order to determine the conidia production, 30 ml water was poured into each PDA plate of 15 days old culture of *Trichoderma* spp. The conidial suspension was made by scraping the spore masses on the medium. The conidial suspension was then taken in a beaker containing 400 ml water and one drop of Tween-20 was added and stirred for 15 minutes (Plate 8.4). Then the number of conidia per plate was determined with the help of Haemocytometer following the procedure of Ashrafuzzaman (1976).

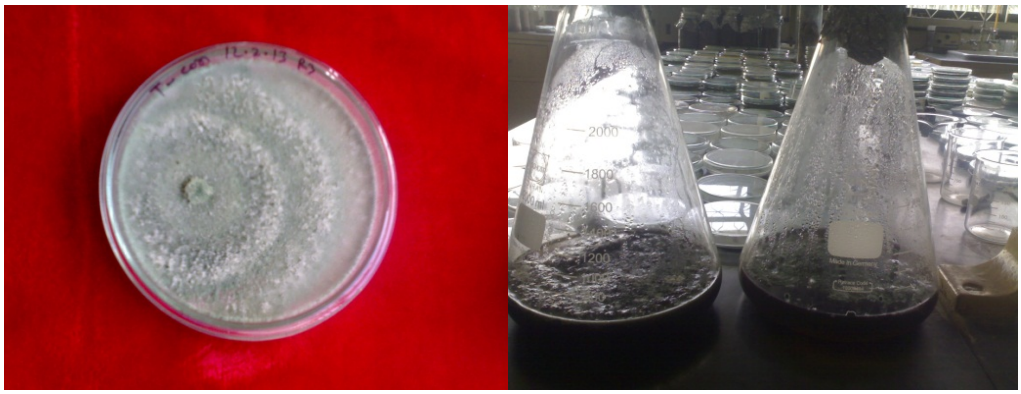


Plate 8.4 Mass culture and Spore suspension of *Trichoderma harzianum*

8.2.3 Collection, isolation, purification and morphological characterization of isolates of *S. rolfsii and F. oxysporum*

8.2.3.1 Collection, Isolation, and purification of *S. rolfsii* and *F. oxysporum*

S. rolfsii and *F, oxysporum* were isolated from diseased plant samples collected from different locations (Table 8.3 & 8.4). The samples with typical root and collar rot symptoms were brought to the Plant Pathology Lab and washed initially with tap water to remove sand and soil particles. Then specimens were cut into small pieces (1.0 cm) along with healthy and dead tissues (inocula). The surface of pieces were sterilized with 1% Clorox, washed thrice in sterilized water and placed on filter paper to remove excess water adhering with the pieces. Thereafter, 5 pieces were placed in PDA plates aseptically maintaining equal distances (Begum *et al.,* 1999). The plates were incubated at 28±1ºC for 7 days and observed regularly to see the growth of fungi from the inocula. The fungus was purified by hyphal tip culture technique (Tuite 1969). The isolated fungi were identified following the keys outlined by Aycock (1966) and Barnett (1960). The pure cultures (Plate 8.5) of the isolates of *S. rolfsii* and *F. oxysporum* were preserved in PDA plates at 4±1°C in refrigerator as stock culture for future use (Begum, 1997)

Plate 8.5 The pure cultures of the isolates of *S. rolfsii* and *F. oxysporum*

Table 8.3 Isolates of *S. rolfsii* collected from different hosts and locations

Isolates of *Sclerotium rolfsii*	Host plant	Location-Thana
1.S.Mohanpur ($S_{1)}$	Eggplants	Mohanpur, Rajshahi
2.S.Charghat (S_2)	Eggplants	Charghat, Rajshahi
3.S.Boalia (S_3)	Eggplants	Boalia, Rajshahi
4.S.Poba (S_4)	Eggplants	Poba, Rajshahi
5.S.Horian (S_5)	Eggplants	Horian, Rajshahi
6.S.Durgapur (S_6)	Eggplants	Durgapur, Rajshahi

Table 8.4 Isolates of *F. oxysporum* collected from different hosts and locations

Isolates of *Fusarium oxysporum*	Host plant	Location-Thana
1.F.Mohanpur ($S_{1)}$	Eggplants	Mohanpur, Rajshahi
2.F.Charghat (S_2)	Eggplants	Charghat, Rajshahi
3.F.Boalia (S_3)	Eggplants	Boalia, Rajshahi
4.F.Poba (S_4)	Eggplants	Poba, Rajshahi
5.F.Horian (S_5)	Eggplants	Horian, Rajshahi
6.F.Durgapur (S_6)	Eggplants	Durgapur, Rajshahi

8.2.4 Preparation of stock culture:

To prepare stock culture, same procedure was followed as of *Trichoderma* isolates described earlier. Eight sterilized test tubes were taken. After preparation of stock culture each tube was inoculated using one tube for one isolate of *F. oxysporum* and *S. rolfsii* and kept in incubator at 25± 2°C for optimum growth. After one week when the growth of the pathogens was maximum, tubes were preserved in freeze at 4±1°C wrapped with brown paper for future use (Plate 8.6).

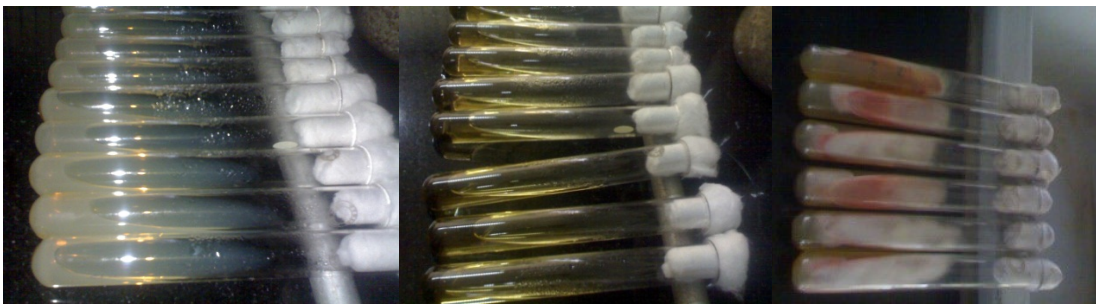


Plate 8.6 Stock culture of different isolates

8.2.5 Conserving the isolates

The isolates, after confirmation, were maintained in petri dishes on PDA media and transferred to new petri plate once a month (Plate 8.6). To store for long periods, the isolates were maintained on PDA slants in test tubes and kept at 4°C.

8.2.6 Preparation of inocula of *S. rolfsii* and *F.oxysporum*

Khesari bran, rice husk and wheat bran were soaked in sterilized water. The water was drained off, water soaked the substrates were taken into bottle and autoclaved at 121°C with 15 psi for 20 minutes (Yaqub and Shahzad, 2005). The bottle containing autoclaved substrates were brought out and allowed to cool at room temperature for two hours. Then 10 mycelial discs (5mm dia) cut from the edge of 3 days old culture of pathogen (*S.rolfsii, F. oxysporum* and *Trichoderma harzianum*) were added to each bottle/container and incubated at 25°C for 20 days. For even colonization, the bottle/container containing inoculated substrates were shaken thoroughly by hand at every 3 days interval (Plates 8.7, 8.8, 8.9). The colonized substrates were air dried for 1 day and stored at 4°C temperature for further use.

Different substrates used for multiplication of fungi substrates inoculation

Plate 8.7 Preparation of carrier materials/substrates and inoculation

Plate 8.8 Multiplication of *Trichoderma harzianum* and *Sclerotium rolfsii* in substrate

Plate 8.9 Multiplication of *Fusarium oxysporum* in substrate

8.2.7 Determination of the efficacy of different substrates in the formulation of *Trichoderma* / *Rhizobium*

8.2.7.1 Substrate and their combination

Khesari bran, rice husk and wheat bran based substrates in combination with potato chips, mastered oil cake and water in different ratios were tested for their potentials for multiplication and formulation of effective isolate of *T. harzianum* and *Rhizobium* sp. against *F. oxysporum* and *S. rolfsii.* The substrates used in this experiment are listed (Table 8.5).

8.2.7.2 Preparation, sterilization and inoculation of substrates

The requisite amount of materials for each substrate were thoroughly mixed in a 1000 ml Erlenmeyer flask and autoclaved at 121°C for 15 minutes for sterilization. The sterilized substrates were allowed to cool down and then inoculated with 5mm diameter mycelial disc of 7 days old *Trichoderma* culture. 10 discs for each flask were used for inoculation. Three flasks for each substrate for *Trichoderma sp.* were used. Inoculated flasks were then incubated at room temperature (25 ± 2°C). The sterilized substrates were inoculated with disc of 2 days old *Rhizobium* culture.

8.2.7.3 Formulation and measurement of CFU/g substrate

After incubation for 25 days, the substrates were taken out from the flasks, shade dried in laminar airflow cabinet and ground in a Moulinex blender. The ground materials were. kept in polythene bag with labeling and treated as formulated *Trichoderma/ Rhizobium.* The CFU/g formulated products were measured by Dilution plate technique (Alam, 2003).

Prepared inoculum Soil inoculation Watering to inoculated soil

Covering for humidity Mycelial growth of pathogen in pot soil

Mycelial growth in soil

Plate 8.10 Preparation of inoculum and inoculation of pot soil with *S. rolfsii*, *F. oxysporum*, *T. harzianum* and *Rhizobium* sp.

Table 8.5 List of substrates for multiplication of bioagent (*Trichoderma*) with composition.

SL. No.	Substrates ingredients	Ratio
1	Wheat bran :Water	4: 1 (W/ V)
2	Khesari bran: Water	4: 1 (W/ V)
3	Rice husk: Water	4: 1 (W/ V)
4	(Wheat bran + Rice husk+PCD): Water	2: 2:1:1(W/W/W/V)
5	(Khesari bran + Rice husk+PCD:) Water	2: 2:1:1(W/W/W/V)
6	(Wheat bran + Khesari bran + PCD): Water	2: 2:1:1 (W/W/W/V)
7	(Wheat bran + Khesari bran+ Rice husk +PCD): Water	2:2:.5:.5:1(W/W/W/W/V)
8	(Wheat bran + Khesari bran+ Rice husk +MOC): Water	2:2:.5:.5:1(W/W/W/W/V)

8.2.8 Pothouse assay of effective *Trichoderma* and *Rhizobium* isolate against *F. oxysporum* and *S. rolfsii*

8.2.8.1 Determination of formulated *Trichoderma* and *Rhizobium* on seed germination and seedling diseases of eggplant in pot soil

Six different formulated *Trichoderma* and three different formulated *Rhizobium* grown on pot soil were tested against *S. rolfsii* and *F. oxysporum* causing foot rot, root rot and wilt diseases of eggplant.

8.2.9 Preparation of trays/pots and sterilization of soil

pot substratum was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1 and sterilized with 5 ml formalin (40%) diluted with 20 ml water for 4 kg soil (Dashgupta, 1988) and the prepared soil was heaped in square block. Soil heap was covered by a polyethylene sheet for 48 hrs. After 7 days of treatment, surface sterilized earthen pot was filled up with the sterilized soil.

8.2.9.1 Experimental design and treatments

The experiment was laid out in CRD with three replications.

8.2.10 Preparation of inoculum and inoculation of pot soil with *S. rolfsii* and *F. oxysporum*

Preparation of inoculum was same as described in 2.3.4.1. pot soil was inoculated with substrate culture of *F. oxysporum* and *S. rolfsii*@10g/kg of soil. The inocula were thoroughly mixed with soil and covered with polythene sheet to maintain soil moisture for proper growth of pathogen (Plate 8.10).

8.2.11 Treatment of pot soil with formulated *Trichoderma* and *Rhizobium*

After 7 days of inoculation different formulated *Trichoderma* and *Rhizobium* (20g/kg soil) were thoroughly mixed with soil.

8.2.12 Sowing of Seeds

10 seeds of each eggplant were sown in each pot just after mixing with formulated *Trichoderma* and *Rhizobium* in a circle and labeled by a permanent marker. Watering was done to maintain the soil moisture. Shade was provided to save the young and delicate seedlings from heavy showering and scorching sunlight (Islam, 2006).

8.2.13 Laboratory bioassay of the isolates of *Rhizobium* spp.

8.2.13.1 Inhibition zone technique

An *in vitro* test was conducted to find out the antagonistic effect of the selected *Rhizobium* isolates against *S. rolfsii* and *F. oxysporum* on PDA by dual culture technique (Dhingra and Sinclair, 1985). One bacterial colony picked up by sterilized loop from two days old culture of individual isolates of *Rhizobium* and one disc (6 mm in size) of a *S. rolfsii and F. oxysporum* were placed simultaneously on the edge of the each PDA Petri plate at opposite direction. Four plates (replications) were used. The plates were arranged on the laboratory desks following completely randomized design (CRD). The plates which received only discs of *S. rolfsii / F. oxysporum* treated as

control. The plates were incubated in the laboratory having ambient temperature of 28±1°C. Inhibition zone was measured on the 3rd day compare to control plate (Nene and Thapliyal, 1993). Thereafter percentages inhibition of *S. rolfsii / F. oxysporum* were calculated based on the growth of the pathogen on PDA plates following the formula as suggested by Sundar *et al.,* (1995).

$$\% \text{ growth inhibition} = \frac{x - y}{x} \times 100$$

Where,
X = Mycelial growth of pathogen in absence of *Rhizobium*
Y = Mycelial growth of pathogen in presence of *Rhizobium*

8.2.14 Recording Data

Disease incidence was observed regularly and recorded at 7, 14 and 30 days after sowing to estimate the effect of *S. rolfsii* and *F.oxysporum* on the following parameters.

Germination (%)
Mortality (%)
Foot and root rot (%)
shoot and root height
shoot and root weight

8.3 Results and Discussion

8.3.1 Effect of carrier in the formulation of *T. harzianum* bio-fungicide on germination and seedling mortality.

Different substrates and its integration showed significant potentiality on seed germination and seedling mortality caused by *S. rolfsii* for eggplant (Table 8.6). Seed germination was higher in all the treatments compared to control against *S. rolfsii*. All the integration treatment more or less enhanced germination and decreased seedling mortality of brinjal seed compared to control. Among the substrates, khesari bran significantly increased seed germination and decreased mortality compared to chemical seed treatment with Bavistin. The second highest potentials went to the rice husk formulated with wheat bran and potato chips showed significantly increased germination (Fig.8.1). Among the carrier materials, integration of Khesari bran,

Table 8.6 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on suppression of seedling disease caused by *S. rolfsii* and vegetative growth of brinjal

Name of substrates	Germination (%)	Mortality (%)	Shoot length (cm)	Shoot weight (g/plant)	Root length (cm)	Root weight (g/plant)
Wheat bran	69.66 (56.58c)	11.33 (19.65cd)	6.93±.32cd	5.27±.11d	5.86±.07c	.42±.01bc
Khesari bran	75.66 (60.45a)	10.66 (19.04d)	8.30±.49bc	5.16±.03d	6.43±.07b	.46±.01a
Rice husk	69.33 (56.38c)	14.33 (22.23b)	6.23±.49d	4.80±.07e	5.36±.03d	.39±.00cd
Wheat bran + Rice husk+PCD	73.66 (59.13ab)	12.66 (20.83bcd)	6.63±.29d	4.78±.01e	6.48±.15b	.42±.00bc
Khesari bran + Rice husk+PCD	72.66 (58.48abc)	12.33 (20.53bcd)	8.60±.83b	6.11±.07c	6.56±.12b	.42±.00bc
Wheat bran + Khesari bran + PCD	73.00 (58.69abc)	11.66 (19.94cd)	8.50±1.03bc	6.56±.06b	6.51±.06b	.45±.01ab
Wheat bran + Khesari bran+ Rice husk +PCD	70.33 (57.01bc)	13.33 (21.40bc)	10.20±.47a	6.85±.02a	6.85±.02a	.43±.00ab
Wheat bran + Khesari bran+ Rice husk +MOC	71.66 (57.84bc)	13.66 (21.67bc)	10.70±.70a	6.90±.05a	6.91±.04a	.44±.00ab
Seed treatment with Provax	71.66 (57.84bc)	12.66 (20.84bcd)	4.50±.40e	4.16±.06f	4.36±.06e	.37±.01d
Control	53.66 (47.09d)	35.33 (36.46a)	3.90±.30e	3.23±.12g	3.71±.14f	.34±.01e

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the .parenthesis is the Arcsine Transformed value.

Potato chips, wheat bran and rice husk or integration of wheat barn, khesari bran with MOC and rice husk or Bavistin showed statistically similar followed by carrier materials of rice husk. Besides that integration of khesari bran with potato chips and rice husk or wheat bran, khesari bran and potato chips showed statistically similar followed by carrier materials wheat bran and rice husk. In controlling seedling mortality of eggplant due to *S. rolfsii* all the integrating treatments significantly reduced it over control but they were statistically similar between integration of wheat bran, khesari bran with potato chips and the wheat bran. In other words integration of Khesari bran, wheat bran, rice husk with potato chips and integration of khesari bran,

wheat bran, rice husk with MOC or integration of khesari bran, rice husk, potato chips, integration of rice husk, wheat bran with potato chips or bavistin showed similar results for mortality caused by *S. rolfsii.*

Table 8.7 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on suppression of seedling disease caused by *F. oxysporum* and vegetative growth of brinjal

Name of substrates	Germination (%)	Mortality (%)	Shoot length (cm)	Shoot weight (g/plant)	Root length (cm)	Root weight (g/plant)
Wheat bran	85.33 (67.54bc)	14.00 (21.97b)	8.90±.35bc	6.48±.06c	6.65±.07d	.41±.00de
Khesari bran	86.00 (68.09bc)	12.66 (20.84bcd)	9.40±.30bc	6.56±.06c	6.75±.10d	.43±.00cde
Rice husk	83.66 (66.16cd)	11.33 (19.66d)	7.90±.20cd	6.16±.08d	6.63±.07e	.40±.00ef
Wheat bran + Rice husk+PCD	83.33 (65.91cd)	13.66 (21.69bc)	8.16±.54cd	6.51±.06c	6.77±.04d	.44±.02bcde
Khesari bran + Rice husk+PCD	81.00 (64.16d)	11.66 (19.96cd)	9.40±.85bc	8.46±.14b	8.13±.06b	.46±.01abcd
Wheat bran + Khesari bran + PCD	80.33 (63.68d)	14.33 (22.20b)	9.83±.58b	8.50±.02b	7.70±.05c	.5±1.03a
Wheat bran + Khesari bran+ Rice husk +PCD	88.33 (70.07ab)	12.66 (20.83bcd)	10.40±.03ab	8.68±.01ab	8.66±.08a	.47±.00abc
Wheat bran + Khesari bran+ Rice husk +MOC	89.66 (71.31a)	13.33 (21.40bcd)	11.53±.33a	8.80±.08a	8.75±.01a	.48±.00ab
Seed treatment with Provax	83.66 (66.97bcd)	11.66 (19.94cd)	6.90±.35de	5.13±.03e	5.20±.02f	.39±.00ef
Control	74.00 (55.15e)	33.66 (35.45a)	6.03±.63e	3.84±.04f	4.25±.02g	.36±.02f

Values in a column having same letter did not differ significantly (P=0.05) by LSD; values within the .parenthesis is the Arcsine Transformed value.

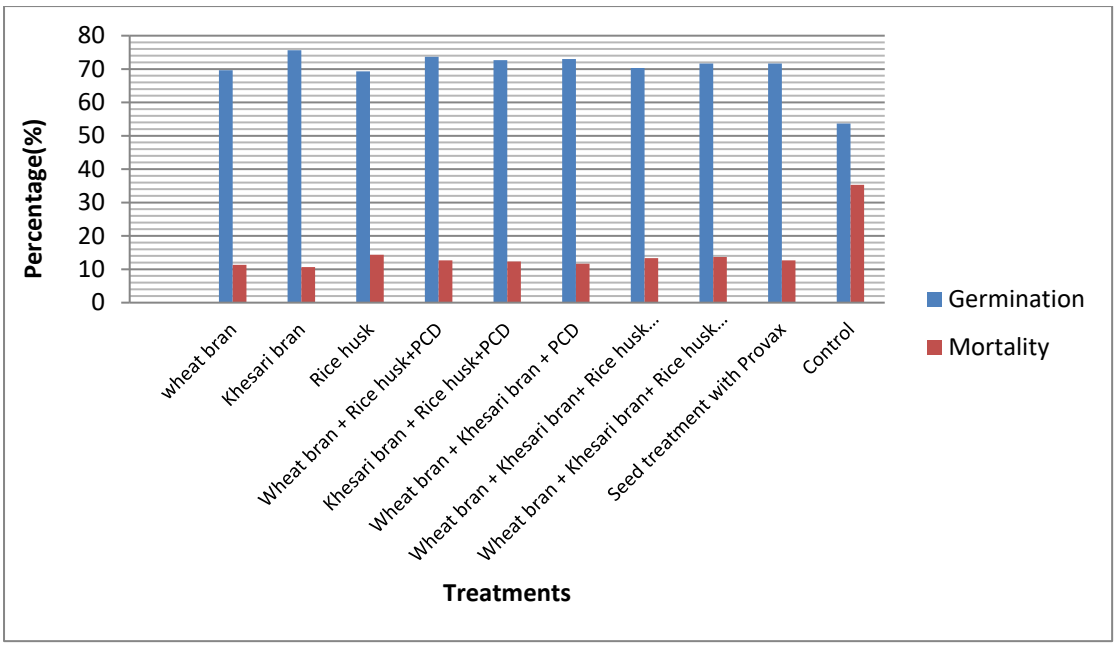


Fig.8.1 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on suppression of seedling disease caused by *S. rolfsii*

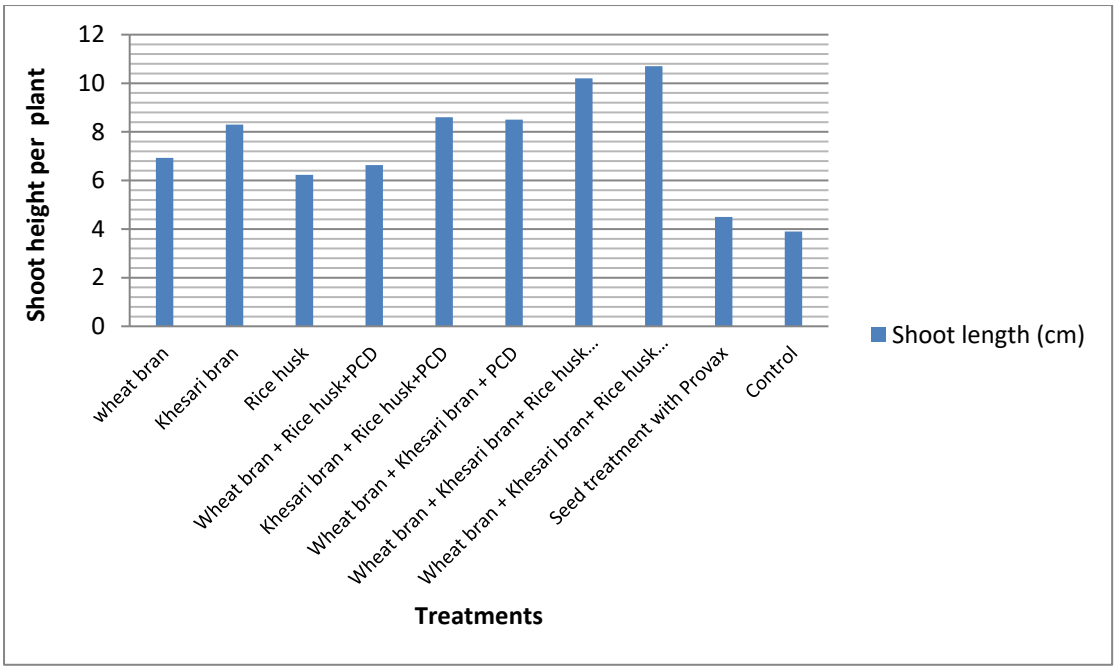


Fig.8.2 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on vegetative growth

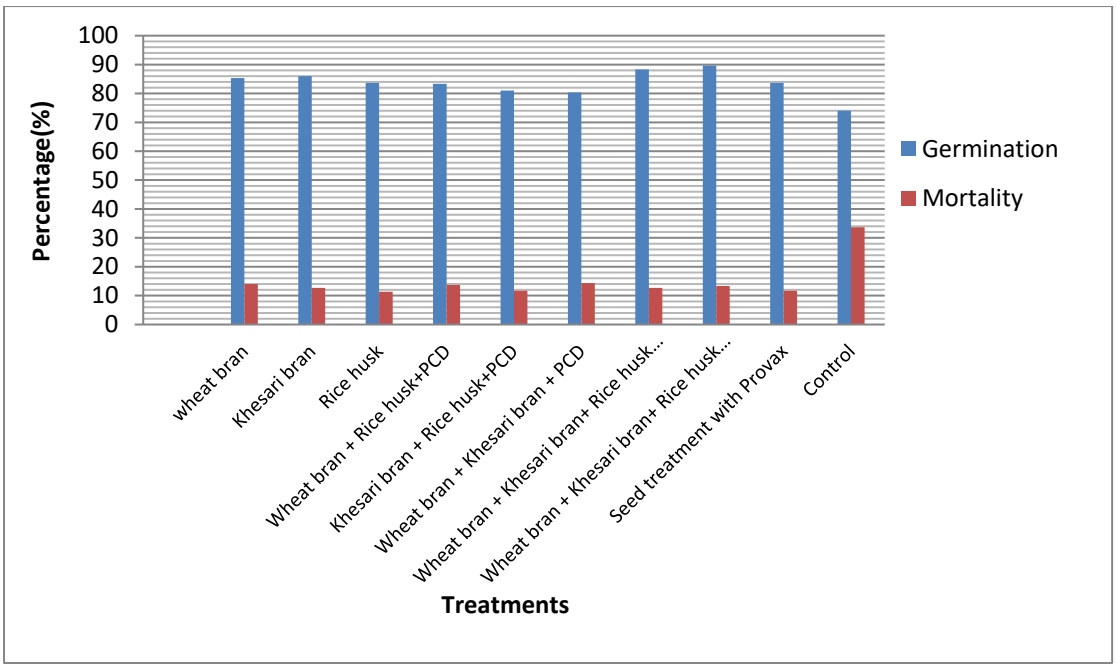


Fig.8.3 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on suppression of seedling disease caused by *Fusarium oxysporum*

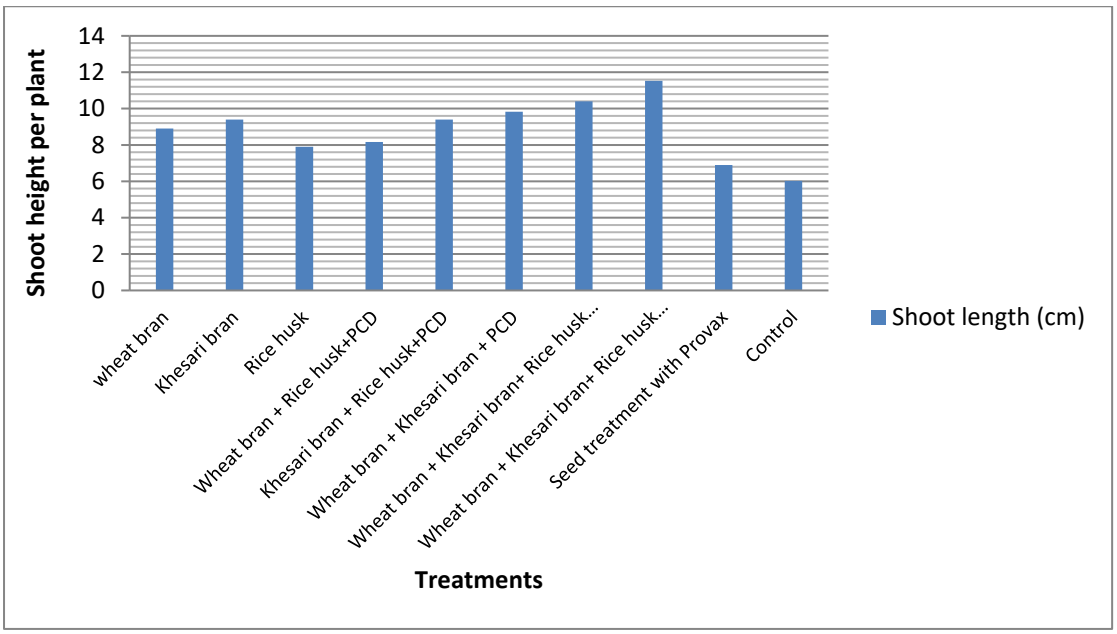


Fig.8.4 Effect of carrier in the formulation of *Trichoderma* based bio-pesticide on vegetative growth

8.3.2 Effect of carrier in the formulation of *T. harzianum* bio-fungicide on short, root length and shoot, root weight

Significant effect of formulated *T. harzianum* in different carrier materials on the shoot length, shoot weight, root length and root weight of eggplant. Among them, the carrier materials, integration of khesari bran, wheat bran, rice husk and potato chips or integration of wheat bran, khesari bran, rice husk with mustard oil cake showed significantly higher performance on the vegetative growth of eggplant against *S. rolfsii* inoculate soil compared to control and treatment with chemical fungicide Bavistin (Table 8.6 Fig. 8.2).

Among the carrier materials, integration of wheat bran, khesari bran with potato chips or integration of khesari bran and rice husk with potato chips or khesari bran were found moderate carrier for effective formulation of *T. harzianum* against *S. rolfsii* of eggplant vegetative growth viz. shoot length, shoot weight, root length and root weight. Rest of the treatments showed very low activity. But the root weight was high measuring in khesari bran treated plants compared all formulation. Significantly difference was found for tested individual substrates during formulation of *Trichoderma* against Pathogen inoculated soil.

8.3.3 Effect of carrier in the formulation of *Trichoderma* bio-fungicide on germination and seedling mortality.

Different substrates and its integration showed significantly potentiality on seed germination and seedling mortality caused by *F. oxysporum* for eggplant (Table 8.7). Seed germination was higher in all the treatments compared to control against *F. oxysporum*. All the integration treatments more or less enhanced germination and decreased seedling mortality of brinjal seed compared to control. Among the substrates integration of khesari barn, wheat barn, rice husk with mustard oil cake significantly increased seed germination compared to integration of khesari bran, wheat bran and rice husk with potato chips. The carrier materials of khesari bran or wheat bran were statistically similar for germination. Rests of the treatments were found less effective formulation of *Trichoderma harzianum* against *Fusarium oxysporum* of eggplants seed germination (Fig.8.3).

In other words the carrier materials, rice husk showed minimum seedling mortality followed by integration of khesari bran, rice husk with potato chips or seed treatment with provax. Among the carrier materials, integration of khesari bran, wheat bran, rice husk with potato chips or integration of rice husk with potato chips or integration of rice husk, wheat bran and khesari bran with mustard oil cake or khesari bran were found better carrier for effective formulation of *T. harzianum* against *F. oxysporum* of eggplant. Furthermore, rests of the formulation of *T. harzianum* were less effective against pathogen.

8.3.4 Effect of carrier in the formulation of *T. harzianum* bio-fungicide on Shoot-Root length and Shot-Root weight

Significant effect of formulated *T. harzianum* in different carrier materials on vegetative growth viz. shoot-root length and shoot-rhoot weight of eggplant. Among the carrier materials, integration of khesari bran, wheat bran and rice husk with mustard oil cake or integration of khesari bran, wheat bran, rice husk with potato chips showed maximum performance on the vegetative growth of eggplant against *F. oxysporum* inoculated soil comparison to control and chemical fungicide-provax. In other words, the carrier materials, integration of wheat bran and khesari bran with potato chips or integration of khesari bran and rice husk with potato chips were found moderate carrier for effective formulation of *T. harzianum* against *F. oxysporum* of eggplant vegetative growth (Table 8.7 Fig.8.4). While integration of wheat bran, khesari bran with potato chips showed maximum weight of root comparison to all treatments. Vegetative growth of eggplant the carrier materials khesari bran, wheat bran or rice husk showed nearly the same range. Besides that integration of rice husk, wheat bran with potato chips showed least activity. Significantly difference was found for tested individual substrates during formulation of *Trichoderma* against pathogen inoculated soil.

8.3.5 Laboratory bioassay of the isolates of *Rhizobium* spp.

8.3.5.1 Zone inhibition technique

The isolate *Rhizobium* was evaluated against *S. rolfsii* and *F. oxysporum* in dual culture method. After 3 days of inoculation compare to the control plates were the

bio- agents produced significantly different inhibition zone against *S. rolfsii* and *F. oxysporum* respectively in dual culture method (Tables 8.8 & 8.9). The isolate showed significant/considerably percentage of inhibition against (77.39% to 57.07%) both pathogens (Plate 8.11).

Table 8.8: In vitro suppression of *Sclerotium rolfsii* by *Rhizobium sp*. Isolates

Accession name	Average radial growth of *S. rolfsii* at 24 hrs(cm)		Average radial growth of *S. rolfsii* at 48 hrs(cm)		Average radial growth of *S. rolfsii* at 72hrs(cm)	
	At dual culure	Growth suppression (%)	At dual culure	Growth suppression (%)	At dual culure	Growth suppression (%)
RBA	1.7	34.10	1.7	60.46	1.7	70.43
RRU	1.3	49.61	1.3	69.76	1.3	77.39
RRM	1.4	45.73	1.4	67.44	1.5	73.91
S. rolfsii	2.58		4.3		5.75	

Table 8.9: In vitro suppression of *Fusarium oxysporum* by *Rhizobium sp*. Isolates

Accession name	Average radial growth of *F. oxysporum* at 24 hrs(cm)		Average radial growth of *F. oxysporum* at 48 hrs(cm)		Average radial growth of *F. oxysporum* at 72hrs(cm)	
	At dual culure	Growth suppression (%)	At dual culure	Growth suppression (%)	At dual culure	Growth suppression (%)
RBA	1.2	22.58	2.0	26.73	2.0	49.49
RRU	1.1	29.03	1.5	45.05	1.7	57.07
RRM	1.3	16.12	1.5	45.05	1.9	52.02
F.oxysporum	1.55		2.73		3.96	

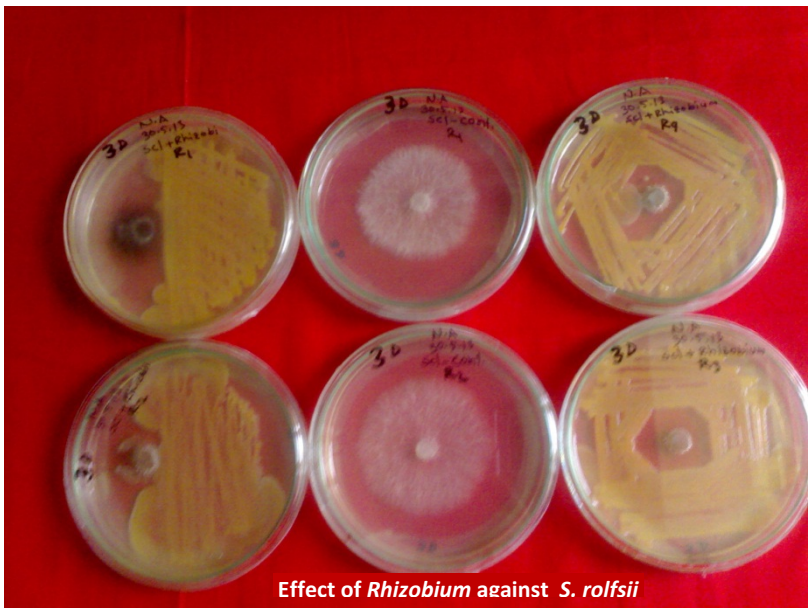


Plate 8.11 Effect of *Rhizobium* against *S. rolfsii* and *F. oxysporum*

8.3.6 Effect of carrier in the formulation of *Rhizobium* sp. bio-fungicide on germination and seedling mortality

Different substrates and its integration showed significant potentiality on seed germination and seedling mortality caused by *S. rolfsii* and *F. oxysporum* of eggplant (Table 8.8 & 8.9). Seed germination was higher in all the treatments compared to control against soil borne pathogen both *S. rolfsii* and *F. oxysporum*. All the integration treatments more or less enhanced germination and decreased seedling mortality of eggplant compared to control. Among the substrates, khesari bran significantly increase seed germination, decreased mortality against *S. rolfsii* comparison to control and seed treated with bavistin(Table 8.8 Fig. 8.5). Not only that integration of khasari bran, wheat bran with rice husk have showed highest seed germination followed by khesari bran against *F. oxysporum.* While integration of khesari bran with wheat bran or khesari bran decreased mortality compared to control and seed treated with provax against *Fusarium oxysporum (*Table 8.9 Fig.8.5). Integration of khesari bran with rice husk or integration of khesari bran with wheat bran showed similar results/ activities for germination of eggplant seed against *S. rolfsii.* In other words integration of khesari bran, wheat bran, rice husk and seed treated with bavistin showed statistically equal on germination and seedling mortality. Seed treatment with provax and integration of khesari bran with wheat bran showed better carrier for effective formulation of *Rhizobium* on germination and seedling mortality of eggplant against *F. oxysporum.* On the other hand rest of the formulation showed less activity on germination or seedling mortality.

Table 8.10 Effect of carrier in the formulation of *Rhizobium* based bio-pesticide on suppression of seedling disease caused by *S. rolfsii* and vegetative growth of brinjal

Treatments	Germination (%)	Seedling mortality (%)	Shoot height	Shoot weight	Root length	Root weight
K+RRU	97.33±1.45a	10.66±1.33b	17.66±1.20a	32.69±2.80a	9.50±.86a	2.38±.072ab
K+R+RBA	96.66±1.33a	9.66±.33b	16.66±1.20a	33.56±3.38a	10.66±.66a	2.73±.14a
K+W+RRM	92.66±1.76a	10.66±.66b	15.66±1.66a	26.70±1.10a	8.33±.33a	2.13±.18b
CON	80.33±4.17b	39.00±4.04a	10.00±1.52b	13.10±3.47b	9.00±1.00a	1.03±.10c

Values in a column having same letter did not differ significantly (P=0.05)

Table 8.11 Effect of carrier in the formulation of *Rhizobium* based bio-pesticide on suppression of seedling disease caused by *F. oxysporum* and vegetative growth of brinjal

Treatments	Germination (%)	Seedling mortality (%)	Shoot height	Shoot weight	Root length	Root weight
K+RRU	97.00±1.52a	11.33±1.33b	13.66±2.18a	67.72±7.78a	10.33±.88ab	3.20±.65a
K+R+RBA	92.33±3.84ab	11.00±.57b	14.33±.66a	68.33±6.38a	11.33±.66a	3.86±.68a
K+W+RRM	94.33±2.33a	10.33±.33b	13.66±1.20a	68.36±6.02a	7.66±.33c	3.20±.61a
CON	82.66±4.37b	39.00±1.52a	13.00±1.73a	35.16±5.69b	8.33±.66bc	1.92±.18a

Values in a column having same letter did not differ significantly (P=0.05)

(K= khesari bran, W = wheat bran and R = rice husk)

(Isolates of *Rhizobium* sp. = RRU, RBA, RRM)

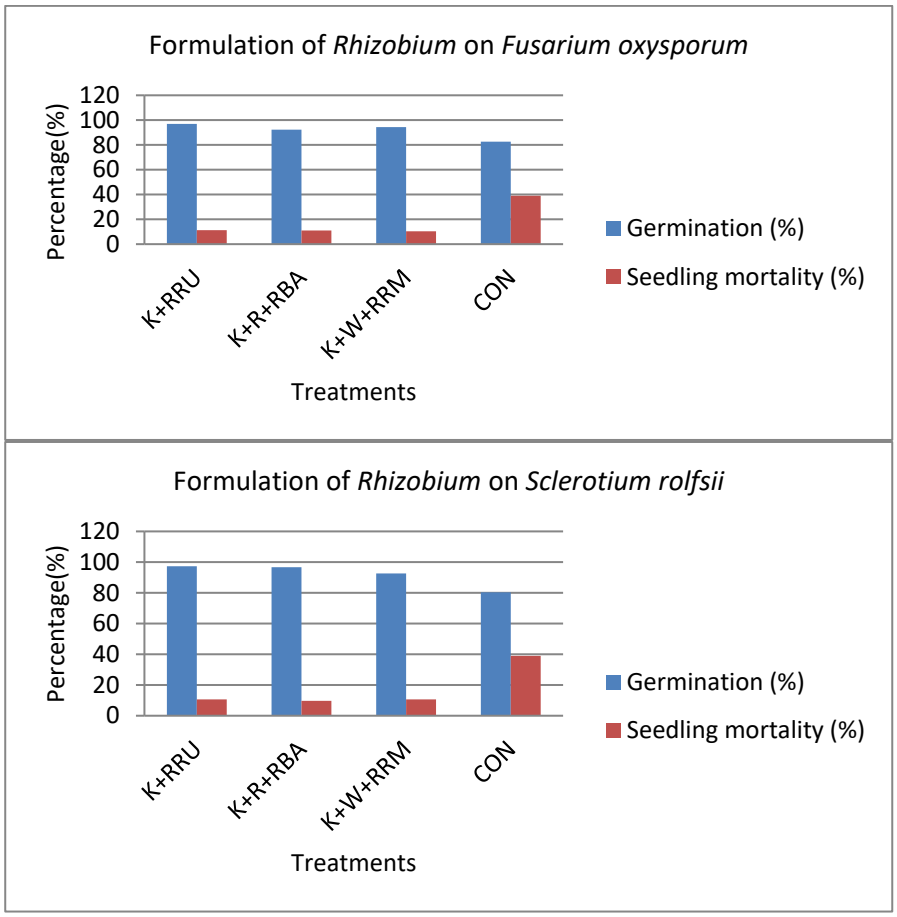


FIG.8.5 Effect of formulation of *Rhizobium* on germination and seedling mortality.

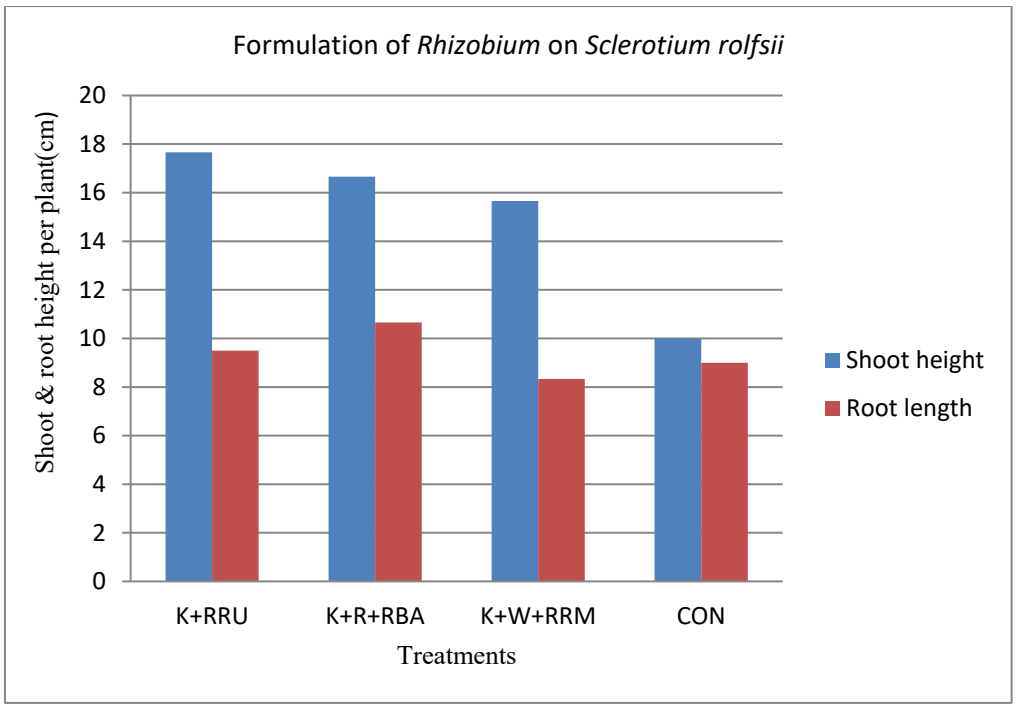


Fig. 8.6 Effect of formulated *Rhizobium* on shoot and root height.

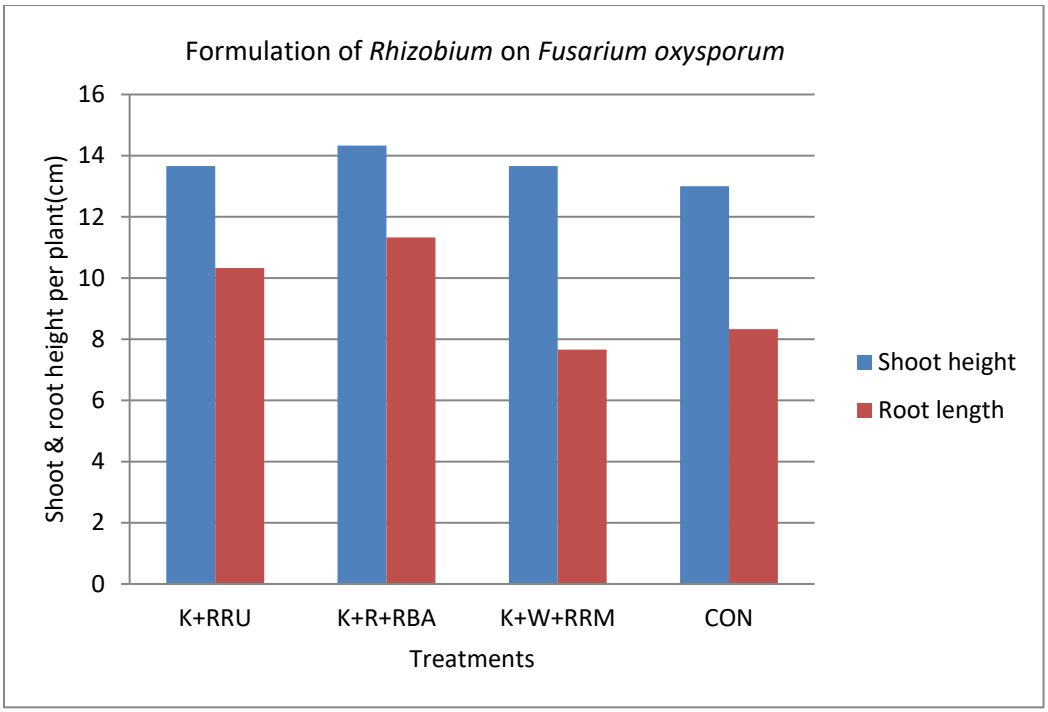


Fig. 8.7 Effect of formulated *Rhizobium* on shoot and root height.

Wheat bran + Khesari bran+
Rice husk +MOC

Seed treatment with Provax

Control

Wheat bran + Rice husk+ PCD

Wheat bran + Khesari bran + PCD

Wheat bran + Khesari

Plate 8.12 Seedling of treated seeds on soil borne pathogen (*S. rolfsii*) inoculated soil

Wheat bran

Khesari bran

Khesari bran + Rice husk+ PCD

Rice husk

Plate 8.13 Seedling of treated seeds on soil borne pathogen (*S. rolfsii*) inoculated soil

Control

Wheat bran + Khesari bran+ Rice husk +MOC

Wheat bran + Khesari bran+ Rice husk +PCD

Khesari bran

Wheat bran + Rice husk+ PCD

Khesari bran + Rice husk+ PCD

Plate 8.14 Seedling of treated seeds on soil borne pathogen (*F. oxysporum*) inoculated soil

Wheat bran

Seed treatment with Provax

Rice husk

Wheat bran + Khesari bran + PCD

Plate 8.15 Seedling of treated seeds on soil borne pathogen (*F. oxysporum*) inoculated soil

Trichoderma against *S. rolfsii*

Trichoderma against F. oxysporum

Plate 8.16 Formulated *Trichoderma* on *S. rolfsii* and *F. oxysporum*

S. rolfsii inoculated soil

Rhizobium & *S. rolfsii* inoculated soil

F. oxysporum inoculated soil

Rhizobium & *F. oxysporum* inoculated soil

Plate 8.17 Formulated *Rhizobium* on *S. rolfsii* and on *F. oxysporum*

8.3.7 Effect of carrier in the formulation of *Rhizobium* sp. bio-fungicide on vegetative growth of eggplant

Significant effect of formulated *Rhizobium* sp. in different carrier materials on the shoot length, root length, shoot weight and root weight of eggplant. Among them, the integration of khesari bran with wheat bran showed best performance on vegetative growth of eggplant against *S. rolfsii* follwed by integration of khasari bran, wheat bran with rice husk compared to control. Integration of khasari bran with rice husk were found better carrier for effective formulation of *Rhizobium* sp. against *S. rolfsii.* Rest of the formulation showed least activity (Fig. 8.6).

On the contary, among the substrates, integration of khesari bran, wheat bran with rice husk showed significantly highest performance on vegetative growth of eggplant against *F. oxyporum* followed by integration of khasari bran with rice husk. Integration of khasari bran with wheat bran showed highest performance on root weight. Rest of the formulation showed least performance on vegetative growth against *F. oxysporum* (Fig. 8.7).

Biocontrol of soil-borne pathogens has been more successful under controlled environmental conditions using simplified potting mixtures presumably low in microbial diversity (Fravel, 1999; Copping, 2001). Biological control agents may use a variety of inhibitory and suppressive mechanisms: (1) competition for resources and space, (2) antibiotic production, (3) removal of pathogenicity factors produced by the pathogen, (4) production of degrading enzymes that target the pathogen and (5) the induction of resistance in the host plant (Whipps, 2001). Biological activity of antagonist fungi and bacteria may partially be associated with production of antibiotic (Etebarian *et al.,* 2000). The production of antibiotics were; Trichodermin (Godtfredsen and Vangedal, 1964), ergokonin (Kumeda *et al.,* 1994). Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. *Trichoderma harzianum* and *Bacillus sublitis*, *Rhizobium* sp. which are common saprophytic or symbiotic fungi found in almost any soil and rhizosphere micro flora in all governorates under study, have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic

fungi, particularly many common soil borne pathogens, these findings were in agreement with (El-Katatny *et al*., 2006; Dubey *et al.,* 2007).

Currently, the role of biological control agents is a well-established fact and has become increasingly crucial, and in several cases, complementary or even replacing the chemical counterparts where antagonistic fungi play an important role (Whipps and Lumsden, 2001; Chet, 1993). Biotic agent's, *viz*. *Trichoderma harzianum* and *B. subtilis* were the most effective to reduce disease incidence and severity (Baraka *et al*.,2011).Samiro-Moni and Bhattacharyya (2008) reported that five isolates of *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* have reduced root rot of sesame significantly. In this context, *Trichoderma* spp. have been the cynosure of many researchers who have been contributing to biological control pursuit through use of fungi (Heraux *et al*., 2005a; Heraux *et al*., 2005b; Ortiz and Orduz, 2001).Furthermore, *Trichoderma* spp. share almost 50% of fungal Biological Control Agents market, mostly as soil/growth enhancers and this makes them interesting candidates to investigate (Whipps and Lumsden, 2001).

In these studies, all the treatments significantly proved their bio-control potentiality against *S. rolfsii* and *F. oxysporum* over control. Several investigators have reported ability of *T. harzianum* isolates against soil-borne pathogens such as *S. rolfsii* (Elad *et al.,* 1983; Kohl and Schosser, 1998; Sreenivasa Prasad and Manibhushanarao, 1993). Increases of plant height, fresh and dry weight of several crops have been reported by Hyakumachi (1994). It was reported that *T. harzianum* treated vegetable seedlings were much developed and vigorous with high chlorophyll content (Inbar *et al,* 1994). Different workers reported the antagonistic activity of different *Trichoderma* isoletes against different phytopathogenic fungi such as *R. solani, F. oxysporum* and *S. rolfsii* (Deshmukh and Raut, 1992; Xu *et al.*, 1993; Askew and Laing, 1994; Linderman, 1989; Shahid *et al.,* 1990). Rini and Sulochana (2007) reported that locally available organic media viz., coir pith, cow dung and neem cake are excellent sources of nutrition for antagonistic fungi like *T. harzianum* and *T. viride*. KAU (2002) reported that cow dung and neem cake mixture is a recommended practice for field multiplication of *Trichoderma.* Bulluck and Ristaino(2002) reported that organic soil amendments are effective against soil bone pathogen *S. rolfsii* which also enhanced the

yield of the crop. Some researchers have been reported that poultry refuse and mustard oilcake are effective in controlling root-not nematode and enhancing plant growth and yield of brinjal (Bari *et al*., 2004; Ahmad *et al*., 1987). Islam *et al*. (2002) evaluated nine organic substances for their suitability for mass culture of an isolate of *Trichoderma harzianum* and found that maize meal was the best substrate colony forming diameter, mycelial growth and spore production of bio-control agent. Thangavelu *et al*., (2004) tested five different organic substrates (rice bran, rice chaffy grain farmyard manure, banana pseudo stem and dried banana leaf) found that banana leaf was the best carrier materials to support *T. harzianum* growth which produced high density of propagates ($4.6x10^{32}$CFU/g of leaf).

Hossain and Naznin (2005) found satisfactory control of seedling diseases of summer vegetables when *Trichoderma* based biofungicide was applied prior planting . Pranab *et al*., (2002) observed that in the field conditions, soil application of *Trichoderma* spp. inoculum at the time of transplanting reduced disease incidence caused by *S. rolfsii* and increased dry mass of roots and shoots and yield. Cuevas *et al*. (2001) found that control of pathogenic activity of *S. rolfsii* in respect of percent seed germination and percent survival of seedlings in plots was most effective when *Trichoderma* was applied as seed treatment before seed sowing. These reports are in agreement with the results of the present study. Hossain *et al*., (2009) found that soil application of *Trichoderma* based biofungicide significantly reduces the seedling diseases of blackgram, mungbean and lentil. Prasad *et al*., (2002) showed that soil application of *T. harzianum* and *T. viride* one week before sowing was more effective in reducing root rot. Hassan *et al*., (2002) expressed that root rot and wilt caused by *Fusarium sp.* was lowest when *T. harzianum* was applied 5 days before soil infestation. Mukherjee *et al*., (1995) observed that *Trichoderma harzianum* was effective in suppressing *Sclerotium rolfsii* and *Rhizoctonia solani*. Mukhopadhyay (1995) used *T. harzianum* for treating various crop seeds like chickpea and lentil for protection against a wide range of soil borne pathogen viz. *S. rolfsii, F. oxysporum and Rhizoctonia solani.*

8.4 Conclusion

From this study, it is concluded that integration of khesari bran, PCD and wheat bran or integration of Rice husk with PCD and khesari bran or integration of Rice husk with PCD and wheat bran or integration of Rice husk, Khesari bran and Wheat bran with MOC or integration of Rice husk, Khesari bran and Wheat bran with PCD or wheat bran or khesari bran were found better carrier for effective formulation of *Trichoderma harzianum / Rhozobium* against *S. rolfsii* and *F. oxysporum* causing foot & root rot and wilt diseases of brinjal which also significantly increased the vegetative growth of the plant.

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APPENDICES

APPENDICES

Table 2.3 Efficacy of aqueous plant extracts on radial growth of *Sclerotium rolfsii*

S. No	Botanicals (Scientific name)	Control	5 % Mycelial growth(cm)	Percent inhibition over control	10 % Mycelial growth(cm)	Percent inhibition over control	15 % Mycelial growth(cm)	Percent inhibition over control
1	*Datura stramonium* L.	8.83±.08a	4.60±.25b	47.90	3.63±.23c	58.89	1.00±.00d	88.67
2	*Allamanda cathartica* L.	8.83±.16a	4.00±.57b	54.69	2.90±.20c	67.15	1.00±.00d	88.59
3	*Acacia nilotica* L.	9.00±.00a	9.00±.00a	00	2.80±1.19b	68.88	.97±01c	89.22
4	*Phyllanthus niruri* L.	9.00±.00a	9.00±.00a	00	9.00±.00a	00	9.00±.00a	00
5	*Coccinia cordifolia* L.	9.00±.00a	9.00±.00	00	6.56±.12b	27.11	4.97±.08c	44.77
6	*Allium sativum* L.	9.00±.00a	9.00±.00a	00	8.45±.48a	6.11	.97± .10c	89.22
7	*Calotropis procera* L.	9.00±.00a	9.00±.00a	00	7.41±.29b	17.66	6.15±.15c	31.66
8	*Annona squamosa* L.	9.00±.00a	9.00±.00a	00	9.00±.00a	00	9.00±.00a	00
9	*Lantana camara* L.	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100
10	*Swietenia macrophylla* L.	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100
11	*Corchorus caspsularis* L.	9.00±.00a	.00±.00b	100	.00±.00b	100	.00± .00b	100
12	*Ficus hispida* L.	8.75±.25a	6.97±.20b	19.84	4.42±.21c	49.48	.00±.00d	100
13	*Nicotiana tabacum* L.	9.00±.00a	.00±.00b	100	.00±.00b	100	.00 ±.00b	100
14	*Swertia chirata* L.	9.00±.00a	8.73±.06b	3	6.93±.06c	23	4.06±.06d	54.88
15	*Vitex negundo* L.	9.00±.00a	8.83±.16a	1.88	8.70±.10ab	3.33	8.43±12b	6.33
16	*Carum roxburghianum* L.	9.00±.00a	8.53±.03b	5.22	7.56±.03c	16	6.83±16d	24.11
17	*Polygonum orientale* L.	9.00±.00a	9.00±.00a	00	8.66±.08b	3.77	8.46±14b	6
18	*Lawsonia inermis* L.	9.00±.00a	4.96±.03b	44.88	2.83±.16c	68.55	1.00±.00d	88.88
19	*Ocimum sanctum* L.	9.00±.00a	7.86±.13b	12.66	7.63±.06b	15.22	5.30±.17c	41.11
20	*Tagetes patula* L.	9.00±.00a	8.26±.14b	8.22	7.20±.25c	20	4.83±.24	46.33
21	*Cleome viscosa* K.	9.00±.00a	8.63±.06b	4.11	7.63±.08c	15.55	5.20±.15d	42.22
22	*Aegle marmelos* L.	9.00±.00a	8.93±.06a	.77	8.90±.05b	1.11	8.10±.15b	10
23	*Cajanus cajan* L.	9.00±.00a	8.93±.06a	.77	8.76±.033a	2.66	7.80±.15b	13.33
24	*Azadirachta indica* L.	9.00±.00a	8.76±.14a	2.66	8.43±.06b	6.33	1.53±.03c	83
25	*Achyranthes aspera* L.	8.93±.06a	5.66±.12b	36.61	4.63±.23c	48.15	2.06±.03d	76.93
26	*Clerodendrum viscosum* L.	9.00±.00a	9.00±.00a	00	8.66±.08b	3.77	8.56±.06b	5.55
27	Bavistin	9.00±.00a	.00±.00b	100	.00±.00b	100	.00±.00b	100

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (P = 0.05)

Table 4.3 Efficacy of aqueous plant extracts on radial growth of *Fusarium oxysporum*

S. No	Botanicals **(Scientific name)**	control	5 % Mycelial growth (cm)	Percent inhibition over control	10 % Mycelial growth (cm)	Percent inhibition over control	15 % Mycelial growth (cm)	Percent inhibition over control
1	*Datura stramonium* L.	6.45±.15a	4.73±.17b	26.66	3.36±.18c	47.90	2.46±.26d	61.86
2	*Allamanda cathartica* L.	6.45±.15a	4.91±.08b	23.87	4.16±.14	35.50	3.21±.11d	50.23
3	*Acacia nilotica* L.	6.18±.13a	1.91±.04b	69.09	1.25±.10c	79.77	.90±.02d	85.43
4	*Phyllanthus niruri* L.	6.18±.13a	6.06±.06ab	1.94	5.88±.07b	4.85	5.30±.05c	14.23
5	*Coccinia cordifolia* L.	6.13±.08a	6.13±.13a	00	6.10±.05a	0.07	6.10±.10a	0.49
6	*Allium sativum* L.	6.26±.08a	4.70±.17b	24.92	3.35±.12c	46.48	1.90±.05d	72.66
7	*Calotropis procera* L.	6.26±.08a	6.13±.13a	2.07	4.50±.00b	28.11	4.45±.16b	28.91
8	*Ocimum sanctum* L.	6.33±.06a	3.90±.10b	38.38	3.30±.15c	47.86	3.00±.11c	52.60
9	*Lantana camara* L.	6.33±.06a	6.13±.13a	3.15	5.03±.06b	20.53	4.53±.06c	28.43
10	*Swietenia macrophylla* L.	6.33±.06a	3.46±.16b	45.33	2.83±.08c	55.29	2.50 ±.02d	60.50
11	*Corchorus caspsularis* L.	6.16±.06a	5.78±.27a	6.16	4.70 ±.10b	23.70	4.03±.01c	34.57
12	*Ficus hispida* L.	6.16±.06a	5.96±.01a	10.38	5.23±.23b	15.09	4.86±.06b	21.10
13	*Nicotiana tabacum* L.	6.16±.06a	5.73 ±.26a	6.98	3.61 ±.19b	41.39	3.06±.03c	50.32
14	*Swertia chirata* L.	6.00±.00a	5.90±.10a	1.66	5.76±.23a	4	5.53±.14a	7.83
15	*Vitex negundo*L.	6.03±.03a	4.93±.06b	18.24	4.48±.08c	25.70	4.16±.16c	31.01
16	*Carum roxburghianum* L.	7.20±.05a	7.20±.02a	00	6.88±.06b	4.44	6.15±.00c	14.58
17	*Polygonum orientale* L.	7.20±.15a	6.70±.10b	6.94	6.50 ±.15b	9.72	5.23±.03c	27.36
18	*Lawsonia inermis* L.	6.38±.04a	5.36 ±.08	15.98	3.46±.06c	45.76	3.26 ±.08c	48.90
19	*Annona squamosa* L.	6.23±.08a	6.00 ±.00ab	3.69	5.80±.20b	6.90	5.41±.08c	13.16
20	*Tagetes patula* L.	6.00±.00a	6.00±.00a	00	5.93±.06ab	1.16	5.83 ±.03b	3.33
21	*Cleome viscosa* K.	6.06±.03a	6.06 ±.03a	00	6.00±.00a	0.99	5.96±.03a	1.65
22	*Aegle marmelos* L.	5.93±.03a	5.43 ±.08ab	8.43	5.00±.11bc	15.68	4.83±.21c	18.54
23	*Cajanus cajan* L.	6.16±.03a	6.06±.03ab	1.62	6.00 ±.00b	2.59	5.93±.06b	3.73
24	*Azadirachta indica* L.	6.38±.04a	5.06±.03b	20.68	3.06±.03c	52.03	2.80 ±.05d	56.11
25	*Achyranthes aspera* L.	5.93±.03a	4.93±12b	16.86	4.80±.10b	19.05	4.80±.10b	19.05
26	*Clerodendrumviscosum* L.	5.93±.03a	5.50±.00b	7.25	5.06 ±.06c	14.67	4.91±.01d	17.36
27	Provax	6.18±.13a	.00±.00b	100	.00±.00b	100	.00±.00b	100

In each column, numbers followed by the same letter are not significantly different according to Duncan multiple range test (p=0.05).

Table 5.1 Effect of ethanolic plant extracts on the conidial germination of *Fusarium oxysporum* at 15% concentration.

	Spore germination			Percentage inhibition over control		
Treatments	**12 hours**	**24 hours**	**36 hours**	**12 hours**	**24 hours**	**36 hours**
Control	9.03±2.41 (31.65a)	38.40±3.17 (42.48ab)	78.47±5.72 (69.73b)			
Lawsonia inermis	3.33±.88b (11.48d)	14.13±2.37 (24.36c)	23.56±1.33 (32.19c)	63.12	63.20	69.97
Ficus hispida	15.38±1.61 (25.57ab)	42.56±1.88 (45.23ab)	92.87±2.42 (83.25a)	-70.32	-10.83	-18.35
Acacia nilotica	.00±.00 (.00e)	4.20±4.20 (7.70d)	6.66±6.66 (9.83d)	100	89.06	91.51
Azadirachta indica	4.00±1.52 (12.37d)	16.53±6.04 (25.97c)	27.97±9.34 (34.86c)	55.70	56.95	64.35
Lantana camara	7.30±2.04 (17.01bcd)	29.57±1.29 (36.55bc)	69.91±3.30 (63.07b)	19.15	22.99	10.90
Nicotiana tabacum	4.70±1.15 (13.72cd)	20.65±1.91 (29.96c)	37.36±4.95 (41.76c)	47.95	46.22	52.38
Allium sativum	.00±.00 (.00e)	2.36±1.55 (7.78d)	3.81±2.31 (10.01d)	100	93.85	95.14
Corchorus capsularis	15.53±4.38 (25.32abc)	48.24±10.84 (48.89a)	94.60±1.33 (85.27a)	-71.98	-25.62	-20.55
Datura stramounium	5.80±.94 (15.37bcd)	20.43±.80 (29.84c)	40.40±2.42 (43.83c)	35.76	46.79	48.51
Allamanda cathartica	7.23±1.93 (16.98bcd)	17.00±2.51 (26.91c)	35.26±1.12 (40.47c)	19.93	55.72	55.06
Provax	.00±.00 (.00e)	.66±.66 (3.00d)	2.66±1.20 (9.91d)	100	98.28	96.68

The value means of three replicates ± standard error. Values with different letters are statistically different (P≤0.05). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value

Table 5.2 *In vitro* screening of methanolic plant extracts against spore germination of *Fusarium oxysporum* at 15% concentration

	Spore germination			Percentage inhibition over control		
Treatments	**12 hours**	**24 hours**	**36hours**	**12 hours**	**24 hours**	**36 hours**
Control	7.58±1.67 (17.51b)	36.29±3.27b (41.07abc)	75.99±5.68 (67.75bc)			
Lawsonia inermis	3.33±.33 (11.65c)	19.73±4.94 (28.86d)	26.66±1.76 (34.51e)	56.06	45.63	64.91
Ficus hispida	12.33±1.33 (22.78a)	42.58±2.48 (45.21ab)	85.58±1.42 (75.21b)	-62.66	-17.33	-12.62
Acacia nilotica	.00±.00 (.00d)	3.10±.58 (11.15e)	4.74±1.11 (13.75f)	100	91.45	93.76
Azadirachta indica	4.66±.88 (13.73bc)	20.48±7.48 (29.17d)	30.50±9.75 (36.71e)	38.52	43.56	59.86
Lantana camara	6.50±2.00 (15.92bc)	27.15±1.18 (34.84bcd)	67.15±2.98 (61.15cd)	14.24	25.18	11.63
Nicotiana tabacum	5.73±.83 (15.31bc)	27.61±2.08 (35.17bcd)	61.07±3.70 (57.11d)	24.40	23.91	19.63
Allium sativum	.00±.00 (.00d)	1.51±1.05 (6.11e)	5.41±2.94 (13.67f)	100	95.83	92.88
Corchorus capsularis	17.90±5.05 (27.33a)	49.78±11.08 (49.94a)	97.50±1.33 (91.71a)	-136.14	-37.17	-28.30
Datura stramonium	5.33±.33 (14.81bc)	23.33±1.66 (32.05cd)	36.66±2.40 (41.38e)	29.68	35.71	51.75
Allamanda cathartica	3.83±.44 (12.49bc)	19.00±.57 (28.70d)	31.66±2.96 (37.99e)	49.47	47.64	58.33
Provax	.00±.00 (.00d)	.66±.66 (3.00e)	2.66±1.20 (9.91f)	100	98.18	96.49

The value means of three replicates ± standard error. Values with different letters are statistically different (P≤0.05). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value.

Table 5.3 *In vitro* screening of aqueous plant extracts against spore germination of *Fusarium oxysporum at* 15% concentration.

	Spore germination			Percentage inhibition over control		
Treatments	12 hours	24 hours	36 hours	12 hours	24 hours	36 hours
Control	9.68±1.47 (20.01bc)	46.13±11.23 (47.26a)	85.80±3.29 (75.68ab)			
Lawsonia inermis	5.83±.8 4(15.44c)	23.66±2.18 (32.29bc)	34.26±2.61 (39.77f)	39.77	48.71	60.06
Ficus hispida	17.73±1.61 (27.61a)	44.66±3.27 (46.58ab)	93.35±2.85 (84.08a)	-83.16	3.18	-8.79
Acacia nilotica	1.36±.68(6.09d)	4.40±1.41 (13.00de)	8.81±1.58 (18.96g)	85.95	90.46	89.73
Azadirachta indica	7.63±2.10 (17.42c)	27.36±7.40 (34.56abc)	43.82±10.21 (45.76def)	21.17	40.68	48.92
Lantana camara	8.54±2.88 (18.25bc)	32.81±3.87 (38.73ab)	73.50±5.56 (65.82bc)	11.77	28.87	14.33
Nicotiana tabacum	5.56±1.08 (15.00c)	31.11±6.42 (37.39abc)	57.95±8.55 (55.21cd)	42.56	36.89	32.45
Allium sativum	5.05±1.42 (14.07c)	13.90±2.93 (24.04cd)	36.16±7.44 (40.76ef)	47.83	69.86	57.85
Corchorus capsularis	15.85±4.65 (25.60ab)	45.78±9.60 (47.25a)	94.37±2.08 (85.17a)	-63.73	0.75	-9.98
Datura stramounium	10.36±1.42 (20.77abc)	36.93±6.85 (41.35ab)	53.36±2.94 (52.14de)	-7.02	19.94	37.80
Allamanda cathartica	9.13±1.67 (19.38bc)	33.86±7.43 (39.18ab)	46.36±3.54 (47.66def)	5.68	26.59	45.96
Provax	.00 ±.00(.00d)	.66±.66(3.01e)	2.66±1.20f(9.91g)	100	98.59	96.89

The value means of three replicates ± standard error. Values with different letters are statistically different (P≤0.05). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value

Table 5.4 Effect of ethanolic plant extracts against spore germination of *Fusarium oxysporum* at 20% concentration.

	Spore germination			Percentage inhibition over control		
Treatments	12 hours	24 hours	36hours	12 hours	24 hours	36 hours
Control	9.01±2.09ab	38.50±3.13a	79.00±5.34b			
Lawsonia inermis	5.86±.90bcd	11.26±1. 51cd	17.80±2.35e	34.96	70.75	77.46
Ficus hispida	12.86±1.48a	40.33±1.45a	93.00±2.64a	-42.73	-4.75	-17.72
Acacia nilotica	.00±.00d	3.06±3.03d	5.33±4.37f	100	92.05	93.25
Azadirachta indica	2.66±.33cd	11.23±4.04cd	21.33±5.23e	70.47	70.83	73
Lantana camara	6.56±2.02bc	24.36±2.08b	59.10±2.82c	27.08	36.72	25.18
Nicotiana tabacum	3.76±.86bcd	13.45±2.63bcd	30.64±2.15d	58.26	65.06	61.21
Allium sativum	.00±.00d	1.76±.95d	2.66±1.45f	100	95.42	96.63
Corchorus capsularis	15.03±4.47a	47.21±10.58a	93.63±1.05a	-66.81	-22.62	-18.51
Datura stramonium	5.86±.90bcd	18.46±.26bc	34.53±1.61d	34.96	52.05	56.29
Allamanda cathartica	5.26±.67bcd	17.90±1.33bc	32.53±1.61d	41.62	53.50	58.82
Provax	.00±.00d	2.39±1.85d	2.66±.88f	100	93.79	96.63

The value means of three replicates ± standard error. Values with different letters are statistically different ($P \leq 0.05$). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value.

Table 5.5 Effect of methanolic plant extracts against spore germination of *Fusarium oxysporum* at 20% concentration.

	Spore germination			Percentage inhibition over control		
Treatments	12 hours	24 hours	36hours	12 hours	24 hours	36 hours
Control	8.66±2.02bc	38.00±3.60b	79.33±5.48b			
Lawsonia inermis	3.00±.00de	16.80±3.24c	24.06±1.06d	65.35	55.78	69.67
Ficus hispida	11.33±2.33ab	41.23±2.19ab	83.87±2.23b	-30.83	-8.5	-5.72
Acacia nilotica	.00±.00e	2.33±.88d	4.23±3.51e	100	93.86	94.66
Azadirachta indica	3.66±.33de	17.16±5.59c	28.35±8.04d	57.73	54.84	64.26
Lantana camara	6.10±1.44cd	26.23±.97c	65.68±2.76c	29.56	30.97	17.20
Nicotiana tabacum	4.50±.87cde	25.38±1.99c	56.91±2.95c	48.03	33.21	28.26
Allium sativum	.00±.00e	1.33±.66d	4.33±2.40e	100	96.5	94.54
Corchorus capsularis	15.66±3.84a	50.03±9.88a	98.50±.76a	-80.83	-31.65	-24.16
Datura stramounium	4.70±.35cde	21.16±.60c	34.86±2.43d	45.72	44.31	56.05
Allamanda cathartica	3.40±.40de	17.86±.59c	29.93±2.31d	60.73	53	62.27
Provax	.00±.00e	2.33±1.85d	2.66±.88e	100	93.86	96.64

The value means of three replicates ± standard error. Values with different letters are statistically different (P≤0.05). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value

Table 5.6: Effect of aqueous plant extracts against spore germination of *Fusarium oxysporum* at 20% concentration.

	Spore germination			Percentage inhibition over control		
Treatments	**12 hours**	**24 hours**	**36hours**	**12 hours**	**24 hours**	**36 hours**
Control	9.68±1.47b	46.13±11.23a	85.80±3.29ab			
Lawsonia inermis	5.40±1.23bcd	21.80±2.21cd	32.74±2.42e	44.21	52.74	61.84
Ficus hispida	17.33±1.66a	44.00±3.21ab	93.00±3.21a	-79.02	4.61	-8.39
Acacia nilotica	1.00±.57cd	3.56±1. 82e	7.65±1.76f	89.58	92.28	91.08
Azadirachta indica	6.83±1.69bc	25.03±7.11bcd	39.33±9.36de	29.44	45.74	54.16
Lantana camara	8.13±2.94b	30.40±3.23abcd	72.46±5.47b	16.01	34.09	15.54
Nicotiana tabacum	6.00±.57bcd	30.60±5.63abcd	56.50±69.08c	38.01	33.66	34.14
Allium sativum	3.65±.82bcd	12.50±2.17de	32.93±6.96e	62.29	72.90	61.62
Corchorus capsularis	15.75±4.78a	45.16±9.45a	94.33±2.18a	-63.22	2.10	-9.94
Datura stramonium	9.60±.98b	34.30±6.51abc	49.13±2.91cd	0.82	25.64	42.73
Allamanda cathartica	8.26±1.59b	32.10±5. 61abc	42.93±3.45cde	14.66	30.41	49.96
Provax	.00±.00d	.66±.33e	2.00±.57f	100	98.56	97.66

The value means of three replicates ± standard error. Values with different letters are statistically different (P≤0.05). Colum by Colum analysis; values within the parenthesis is the Arcsine Transformed value