MANAGEMENT OF SOUTHERN BLIGHT OF BELL PEPPER (CAPSICUM ANNUM L.) BY NATURAL ANTIFUNGAL COMPOUNDS OF DATURA METEL L.

BY

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MANAGEMENT OF SOUTHERN BLIGHT OF BELL PEPPER
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COMPOUNDS OF DATURA METEL L.

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By

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DECLARATION

I hereby declare that the research work reported in the thesis entitled “Management of Southern Blight of Bell Pepper (Capsicum annum L.) by Natural Antifungal Compounds of Datura metel L.” is my own work and submitted as research work under the supervision of Dr. Arshad Javaid and Dr. Ejaz Ahmed. I also certify that I have written this thesis independently and used no other aids and resources than those indicated.

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CERTIFICATE

It is certified that the research work contained in this thesis titled “Management of Southern Blight of Bell Pepper (Capsicum annum L.) by Natural Antifungal Compounds of Datura metel L.” has been carried out and completed by Ms. Nadia Jabeen under Roll No.09 is an original work of the author and has been carried out under our direct supervision. We have personally gone through all the data, results, materials reported in the manuscript and certify their correctness and authenticity. We further certify that the material included in this thesis has not been used in part or full in manuscript already submitted or in the process of submission in partial or complete fulfillment of the award of any degree from any institution. We also certify that the thesis has been prepared under our supervision according to the prescribed format and we endorse its evaluation for the award of Ph. D. degree through the official procedure of the University of the Punjab, Lahore, Pakistan.

Here thesis is in pure academic language and it is free from typos and grammatical errors.

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To Allah Almighty for giving me strength to carry this research to the end inspite of all hurdles

To my husband and my beloved sons and daughter.

I'm highly indebted to you for your struggle and prayers.
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SUMMARY

Bell pepper (*Capsicum annum* L.), family Solanaceae, is an important vegetable having nutritive and medicinal values. It contains vitamin A, C and E, carotenoids, phenolics, flavonoids, minerals and numerous secondary metabolites. It has antioxidant, antispasmodic and antiseptic properties. Southern blight caused by *Sclerotium rolfsii* Sacc. is one of the most destructive soil-born diseases of bell pepper causes significant yield losses. *S. rolfsii* can be controlled by different disease management strategies but the most extensively used is chemical control by with fungicides. However, chemical fungicides are responsible for environmental pollution, health hazards, pest resurgence, development of resistance in pathogens, destruction of non target species and deterioration of natural habitats. Due to these negative effects of synthetic fungicides, the present study was conducted for management of *S. rolfsii* and southern blight disease of bell pepper by extracts and soil amendment with dried plant biomass of *Datura metel* L., a medicinal plant of family Solanaceae.

In laboratory screening bioassays, antifungal activity of methanolic leaf, stem, root and fruit extracts of *D. metel* was investigated against *S. rolfsii*. Different concentrations viz. 0.5, 1.0, 1.5, …, 4% of methanolic extracts were prepared in malt extract broth and their antifungal activity was studied against the target fungus in 100-mL volume conical flasks each containing 20 mL of growth medium. All concentrations of stem, leaf and fruit extracts significantly decrease the fungal biomass over control while the effect of root extract was insignificant. Different concentrations of leaf stem and fruit extracts reduced fungal biomass by 29-88%, 69-86%, 69-94% and 9-32% over control, respectively.

Methanolic extracts of leaf, stem and fruit were further fractionated by using four organic solvents viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Seven concentrations viz. 3.125, 6.25, 12.50, 25, 50, 100 and 200 mg mL\(^{-1}\) of each sub-fraction of methanolic extracts were prepared and their antifungal activity was assessed against the *S. rolfsii* in malt extract broth medium. In general, different sub-fractions of methanolic extracts of all the three plant parts showed variable antifungal activities. However, chloroform sub-fraction of leaf, stem and fruit showed the highest antifungal
activity and reduced the fungal biomass up to 43%, 50% and 62%, respectively. Gas chromatography-mass spectrometry (GC-MS) analysis of chloroform sub-fractions was carried out to determine the possible antifungal constituents. Chloroform sub-fraction of leaf, stem and fruit contained 18, 6 and 12 compounds, respectively. These compounds belong to ester, alkene, alcohol, carboxylic acid and arene groups. Among the isolated compounds, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; 1-hexadecene; pentadecene, 1-eicosanol; 7-tetradecene and 1,3(15),10-bisabolatriene are likely to have antifungal activity.

In pot trials, soil was made sick with S. rolfsii and dried powder biomass of above ground parts of D metel was mixed in the pot soil at 0.5%, 1%, 1.5%, 2%, 2.5% and 3% (w/w). The highest disease incidence (100%) was recorded in positive control treatment where S rolfsii was inoculated without dry biomass of D. metel. All the doses of dry biomass of D. metel as soil amendment significantly alleviated biotic stress of S. rolfsii and enhanced plant growth and yield in bell pepper. A 2% doses of D. metel dry biomass as soil amendment completely controlled southern blight disease and enhanced bell pepper yield by 136% over positive and 30% over negative control, respectively.

Physiological tests of leaf and root samples of different treatments in pot experiment were carried out for the estimation of chlorophyll, protein, phenolic contents, and peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities. In general, protein content and activities of all the defence related enzymes viz. PO, PPO and PAL were significantly increased by S. rolfsii inoculation over negative control. The effect was generally more pronounced in roots than in leaves. In S. rolfsii inoculated soil, application of different doses of dry biomass of D. metel insignificantly reduced protein content as well as activities of PO, PPO and PAL probably because of reduction of the pathogen inoculum in the soil.

The present study concludes that soil amendment with 2% dry biomass of D. metel completely controls southern blight disease of bell pepper. However, the highest fruit yield of bell paper in S. rolfsii inoculated soil can be obtained by with 2.5% amendment of D. metel biomass.
Chapter 1

INTRODUCTION

1.1. Importance of bell pepper

Bell pepper (*Capsicum annuum* L.), family Solanaceae, is the second most consumed vegetable of the world (Mateos *et al*., 2013). Sensory and nutritional values of bell pepper have made it an important vegetable of the world (Troncoso *et al*., 2005). It is considered the first spice that has been used by humans with its fossils found as early as 6,000 years ago (Perry *et al*., 2007). Bell pepper has been named as “the Christmas ornaments of the vegetable world” because of its beautiful shape and diverse colours. It can be in many colours like green, red, yellow and orange. It is an inhabitant to Central America and Mexico (Kumar *et al*., 2009). Asia is the largest producer of bell pepper. The yield of bell pepper in the world is 472,526 thousand tons (FAO, 2013). Total cultivated area of bell pepper in Pakistan was about 62.5 thousand hectares and production was 145.1 thousand tons during 2013-2014 (Agricultural Statistics of Pakistan, 2014). Bell pepper has a variety of uses and has both nutritional and medicinal values (Vengaiah and pandey, 2006).

Nutritional value of bell pepper is very high. Bell pepper is consumed as fresh or cooked with fish, meat and other vegetables. It can be used as immature, mature, fresh, cooked, canned and also as a spice. It is also an important ingredient in ready to eat food. Dehydrated bell pepper can be used in frozen pizzas, salad dressings, instant soups, and in a large range of sauces. It is also used as pickles (Berke, 2002; Anonymous, 2003; Horvitz and Cantalejo, 2013). It is a source of vitamin A and C, carbohydrates, potassium, provitamin A, carotenoids, vitamin B6, folic acid, tocopherols, phenolics and flavonoids. Bell pepper also contain trace elements such as sodium, calcium, dietary fibres and phosphorus (Niizu and Rodriguez-Amaya, 2005; Kidmose *et al*., 2006; Chatterjee *et al*., 2007; Vora *et al*., 2014).

Bell pepper is also used for its medicinal values since ancient time. It acts as appetite stimulant, carminative, astringent, antispasmodic, antiseptic, condiment, accentuator and anti rheumatic. All coloured capsicums help to prevent cancers of the bladder, cervix, pancreas and prostate by having phytonutrients and antioxidants (Kritchevsky, 1992). It reduces the risk of arthritis and cardiovascular disease,
triggers the brain to release endorphins, and helps to neutralize the mouth cavity-causing acids. It is taken for the treatment of varicose veins, cold stage of fevers, debility in convalescence or old age, cold, cough and heart pains. It also stimulates blood circulation, helps in gastro intestinal detoxification and aid in the removal of waste products. It is used in the treatment of unbroken chilblains, sprains, pleurisy, bubonic plague and neuralgia (Nadeem et al., 2011; Olawuyi et al., 2014). It also has hypocholesterolemic, antioxidant, immunosuppressive and anti-mutagenesis properties (El-Ghoraba et al., 2013). It contains a wide array of beneficial antioxidant components like flavonoids, carotenoids, and vitamins, which help to minimize the risk of degenerative diseases (Zhang and Hamauzu, 2003; Shotorbani et al., 2012; Chandra et al., 2014). Harmful oxidation reactions in human body can be minimized by compounds like phenolics and flavonoids, β-carotene, capsanthin and violaxanthin, which could contribute to antioxidant activity (Deepa et al., 2007; O’Connell et al., 2007; Sun et al., 2007). Carotenoids are required for human epithelial cellular differentiation. Numerous potential health benefits have been associated with increased carotenoids consumption present in bell pepper. Carotenoids not only serve as precursors of vitamin A but also perform as antioxidants in cell protection and prevent degenerative diseases (Stahl and Sies, 2003; O’Connell et al., 2007). Antioxidant property of bell pepper significantly reduces cholesterol. It oxidizes low density lipids in our blood. It also retards the hardening of arteries and lowers blood pressure. It is also effective against aging and protects skin from free radical damage (Guine et al., 2007). Bell pepper is a rich source of vitamin C that is helpful in wound healing. It builds up the lining of mucous membrane of nose to prevent nose bleeding. Leutolin present in bell pepper may help to prevent breast cancer by reducing excess estrogen formation (Durucasu and Tokusoglu, 2007). It helps to burn calories by increasing metabolisim. It gives relief in headache and migraines (Olawuyi et al., 2014). Marin et al. (2004) analyzed the bell paper fruit and identified the antioxidant constituent like O-glycosides of quercetin, hydroxycinnamic derivatives, luteolin, and chrysoeriol, and a large number of C-glycosyl flavones.

1.2. Southern blight of bell pepper

Southern blight of bell pepper is caused by *Sclerotium rolfsii* Sacc. Southern blight is also identified as southern wilt, southern stem rot, collar rot and crown rot.
The pathogen has attained great economic importance because it causes significant yield losses in different crops (Kalmesh and Gurjar, 2001; Javaid and Iqbal, 2014). S. rolfsii is a well known polyphagous, ubiquitous and a non-target soil-borne plant pathogen worldwide that causes stem rot, root rot, foot rot and wilt diseases in more than 500 plant species, on almost all horticultural and agricultural crops including dicotyledonous and monocotyledonous species (Cilliers et al., 2000; Ciancio and Mukerji, 2007; Kotaosthane et al., 2015). The fungus was placed in the genus Sclerotium by Saccardo (Saccardo, 1911), as it forms differentiated sclerotia and sterile mycelia. Punja (1985) exposed the telemorph state, confirming that the fungus belongs to Basidiomycetes. The first report of the fungus pathogenicity dates back to 1892 in connection with tomato blight in Florida (Weber, 1931). S. rolfsii commonly occurs in the Central and South America, southern United States, southern European countries near the Mediterranean border, the West Indies, Africa, Hawaii, Japan, India and Philippines (Punja, 1985).

Southern blight has wide geographical distribution in warm climates. High soil temperature, good soil moisture and low organic matter in the soil favor disease development. Maximum mycelial intensification of S. rolfsii was observed at temperatures 30–35 °C, pH 5 to 6 and relative humidity 77% (Dalvi and Raut, 1986; Tripathi and Khare, 2006). It is common where high temperature exists during the rainy season. The pathogen S. rolfsii, cannot withstand low temperate for long time. Hence, it is not important in temperate regions (Khanna and Sharma, 1993). Major sources of dissemination of southern blight are water, infected soil, infected seedlings, agricultural machines and farming tools (Beute and Rodriguez-Kabana, 1981). S. rolfsii infects the root (Harinath, 2000; Gaur et al., 2005), stem (Kajal and Chitreswar Sen, 2000; Deepthi and Reddy, 2013), leaves and fruit (Gupta and Sharma, 2004) and collar region (Ansari and Agnihotri, 2000; Rao et al., 2004) of the host plant. The most distinctive effect of this pathogen is rottening of affected tissues that are directly attacked by the fungus. Mycelium mass of the fungus secretes oxalic acid as well as pectinolytic, cellulolytic and some other enzymes which kill and disintegrates tissues before it actually penetrates the host. Once established in the plants, the fungus progress and generates both mycelium and sclerotia quite rapidly, especially at high moisture and high temperature i.e. between 30–35 °C (Agrios, 2005). Amino acids like methionine, phenylalanine and leucine were abundantly present in culture filtrates of this fungus. Phenolic acids such as gallic, ferulic, chlorogenic and cinnamic acids,
and other biochemical constituents like carbohydrate and nitrogen fractions are common both in mycelium and culture filtrates of *S. rolfsii* (Maddu and Ravuri, 2015). Initial disease symptoms include gradually yellowing and wilting of leaves and dark brown lesion at collar and lower stem part which is in contact with soil. The pathogen forms a thick white cottony thread like mycelial mat or layer on collar, lower stem and even on soil surrounding the collar under suitable soil moisture conditions. Mature plants of chili from standing crop were collapsed and dried down suddenly. Sclerotia are spherical small (1–2 mm in diameter) and tan to brown. Sclerotia formed abundantly on and in this white mycelial growth. Sclerotia have an outer differentiated, pigmented rind, which can grow up the stem of host plant and also spread out across the soil to infect other plants (Kalmesh and Gurjar, 2001; Steven *et al*., 2007; Koike, 2014; Madhuri and Gayathri, 2014). Large numbers of sclerotia are produced by *S. rolfsii* which act as a major over-wintering tool as well as primary inoculum for disease (Anahosur, 2001). Biochemical studies revealed that reducing and nonreducing sugars, proteins, free amino acids, tannins and phenols are present in sclerotia in different composition at different stages of sclerotial growth (Karnataka, 2014). Sclerotia are made by three layers, an outer rind, a middle cortex and an inner medulla (Anahosur, 2001). Sclerotia remain alive up to 15 cm depth for 45 days, and present in soil either freely or associated with plant debris (Gurjar *et al*., 2004). Sclerotia which are buried deep in the soil survive for a year while those present at the soil surface may germinate due to release of alcohols and other volatile compounds from decomposed plant sources (Akram *et al*., 2007).

### 1.3. Management of *Sclerotium rolfsii*

Management of the southern blight caused by soil-borne pathogen *S. rolfsii* is not easily possible because of the polyphagus nature, extensive host range, fastidious pathogen growth and its potential of producing large number of sclerotia that may persist in soil for many years (Punja, 1985; Vivek *et al*., 2014; Mahato and Mondal, 2014). Control of the *S. rolfsii* depends upon detailed knowledge of the host plant, the pathogen, and the suitable environmental conditions for infection. Assessments of disease incidence, disease severity, and potential crop losses are key factors when considering the disease management strategies. Disease management strategy must be economical and time of control measures is also critical. Management approach
should safe and simple, economical to apply, and adequately effective to reduce disease to an acceptable level. Multiple management options can be used as beneficial cultural practices, use of resistant varieties, biological practices and natural compounds from plant sources (Divya and Sudini, 2013; Veena et al., 2014).

1.3.1. Management by cultural practices

Cultural practices involve all the activities carried out on the farm before, during and after planting of crops. These are grouped into pre-planting, planting and post-planting operations. A variety of management practices have been used to manage southern blight disease. Tillage practices, burning of the crops, mulching, crop rotation, good soil drainage and proper fertilizer application may help to reduce disease incidence (Thiessen and Woodward, 2012; Veena et al., 2014). Deep ploughing before sowing helps to reduce the pathogen by deep burying of inoculum (Divya and Sudini, 2013; Veena et al., 2014).

Among various cultural practices, soil solarization is generally used to control soil-borne plant pathogens by rising the soil temperature through transparent polyethylene sheets on a moist soil surface. It reduces the population of many soil-borne pathogens including nematodes, bacteria and fungi as well as weeds (Verma et al., 2005). Mulching is an alternative to expensive fungicides. The effect of mulching was more profound on upper soil surface since higher temperatures in mulched soils were recorded at 5 cm depth as compared to 10 cm depth. Maximum sclerotial mortality occurred at 5 cm followed by 10 cm in mulched soils. Similarly, minimum disease infection and better plant growth was recorded in plants grown in soil taken from 5 cm depth in mulched plots. Maximum sclerotial mortality and minimum pathogen infection was noted after 15 days of mulching (Yaqub and Shahzad, 2009; Veena et al., 2014). Mulching has been used to control a number of fungal pathogens including Phytophthora spp., Fusarium spp., Rhizoctonia solani J.G. Kuhn and Pythium spp. (Raoof and Rao, 1997; Ashrafi et al., 2010; Saremi et al., 2011). Yaqub and Shahzad (2009) found mortality of sclerotia of S. rolfsii in mungbean [Vigna radiata (L.) Wilczek] and sunflower (Helianthus annuus L.) by polythene mulching. Likewise, Steven et al. (2003) observed that southern blight of tomatoes (Solanum lycopersicum L.) caused by sclerotia of S. rolfsii can be effectively controlled by
mulching. Mulching limitation include development of heat tolerant pathogens, ineffective in control of some diseases, and polythene pollution.

Crop rotation is a traditional and effective cultural method to reduce inoculum of soil-borne pathogens. Crop rotation is successive planting of different crops in the same area. If the host is not present for particular number of years then the amount of inoculum will be reduced (Ellouze et al., 2014). It supports to develop different microflora in soil. Rotation with non-susceptible crops can break the disease cycle and helps to reduce the pathogen. Crop rotation was found helpful to reduce the number of pathogen propagules in the soil and subsequent disease incidence in susceptible crops. Rhizoctonia sp., Verticillium sp., Streptomyces sp. and Phytophthora sp. (Kheyrodin, 2011; Thiessen and Woodward, 2012). Rodfrguez-K-Bana et al. (1991) managed the root knot nematode caused by Meloidogyne arenaria and southern blight caused by S. rolfsii by rotation of cotton (Gossypium hirsutum L.) in rotation with peanut (Arachis hypogaea L.). However, generally crop rotation is not very effective in controlling southern blight caused by S. rolfsii because it has wide host range and seclerotia survival in the soil. Crop rotation with non-susceptible crops viz, corn or wheat, may lower the initial inoculum and decrease the disease incidence in following years (Mullen, 2001).

Management of irrigation will help to minimize the dispersal of soil-borne pathogens and control the spread of pathogens to other areas. Maintaining adequate moisture without overwatering and good soil drainage reduces the number and activity of certain oomycetes like Pythium spp. and nematodes. Flooding practice for long periods or dry fallowing may also reduce Fusarium spp., Sclerotinia sclerotiorum (Lib.) de Bary and nematodes. Flooding can cause lack of oxygen and increases carbon dioxide that is toxic to pathogens. Long span of high soil moisture promotes the disease (Katan, 2010). Irrigation also helps to reduce the diseases caused by Macrophomina phaseolina (Tassi) Goid., S. rolfsii, R. solani, Verticillium dahliae Kleb. (Thiessen and Woodward, 2012; Divya and Sudini, 2013; Veena et al., 2014). Davis et al. (1996) evaluated the effect of irrigation on stem rot disease of peanut caused by S. rolfsii and found that yield was significantly influenced by irrigation in area under the disease progress curve. However, generally literature regarding control of southern blight by irrigation management is scarce.

Application of fertilizers not only improves the overall plant health but also reduces the impact of severity of diseases. Southern blight disease influenced by
nutrition of the host and effect of the nutrients on *S. rolfsii* and related soil microflora. Nitrogen availability is incredibly important for plant health. Nitrogen applications as ammonium may directly inhibit the germination and limit the mycelial growth of *S. rolfsii*. Application of ammonium bicarbonate, calcium and gypsum can reduce the viability of sclerotial bodies of *S. rolfsii* by improving cell wall (Agrios, 2005; Veena *et al.*, 2014). Application of phosphatic fertilizers also influences the host resistance by increasing the production of phytoalexins. Ammonium fertilizers release ammonia that inhibits the mycelia growth and sclerotial germination of *S. rolfsii* (Punja, 1985).

Application of organic amendments such as cotton, mustard, castor, neem and groundnut oils, vermicompost, and farmyard manure is effective against southern blight of chilies caused by *S. rolfsii*. Least disease incidence of 19% was recorded by application of neem oil (Gurjar *et al.*, 2004). Use of neem cake with and without oil was found effective in reducing the wilt disease incidence in potato (*Solanum tuberosum* L.) caused by *S. rolfsii* (Baswaraj, 2005). Timing of solarization (with clear plastic mulch) in relation to the planting of pepper and the timing of soil amendment with a bran prill formulation of *Gliocladium virens* Corda were found effective for the control of southern blight and the survival of sclerotia of *S. rolfsii* in bell pepper fields (Ristaino and Lumsden, 1996). Ashlesha and Paul (2014) studied the effect of five organic inputs viz. panchgavya, vermiwash, biosol, cow urine and butter milk against major pathogens of bell pepper including *S. rolfsii* and found fermented cow urine best in inhibition in the fungal growth (up to 99%) of different test pathogens. Anahosur (2001) found that Sclerotium wilt of potato can be reduced by integrated disease management (IDM) having the components crop with rabi sorghum varieties for 2 years + application of farmyard manure to the soil + tuber treatment prior to planting @ 4 g kg⁻¹ with *Trichoderma viride* Pers or *T. harzianum* Rifai. Vanitha and Suresh (2002) noticed that collar rot of brinjal (*Solanum melongena* L.) caused by *S. rolfsii* can be reduced by organic amendments such as farmyard manure plus dry adathoda (*Justicia adhatoda* L.) leaf powder. Pawar *et al.* (2014) reported that soil application of organic matter with and without fungicidal seed treatment significantly reduced pre and post mortality of soybean by *S. rolfsii* and also increased the number of pods.
1.3.2. Use of resistant varieties

Use of resistant varieties is a preferred, safest and economical method for disease management (Infantino et al., 2006). However, it is difficult to find a source of resistance against targeted pathogen like *S. rolfsii* that has wide host range. Host plant resistance not only reduces the crop losses but also reduces the expenditure incurred on disease management as well as reduces the pollution hazards. The selection of plant genotypes resistant to pathogens is an important progress in phytoprotection (Sillero et al., 2010). *Capsicum chinensis* var. PI-224428, *C. frutescens* cultivar Green Leaf Tabasco, and *C. annum* cultivars Golden California Wonder and Santaka have shown resistance against *S. rolfsii* (Duke and Fery, 1984). There is not enough literature on sources of resistance in bell pepper against *S. rolfsii*. It was observed that Golden California Wonder cultivar of bell pepper gave resistance against southern blight. The level of resistance accustomed in Golden California Wonder by single recessive gene now ready to use in pepper breeding programs (Fery and Dukes, 2005). There is a need of team of experts to carry and monitor the cultivar for resistance. Many R genes may lack the durability due to loss of mutation in the corresponding avirulence gene. Resistant cultivars fail to provide resistance after a certain period of time because of development of new races of the pathogen (Thakur, 2007).

1.3.3. Chemical control

Chemical fungicides are used to protect the plant from diseases and to eradicate the pathogens. In order to control soil-borne diseases such as crown and root rots, seedling blights, damping-off and others, soil is generally treated with fungicides in the form of dusts, granules or liquid drenches. The areas where irrigation is possible, fungicides are mixed with the irrigation water. Fungicides used for soil treatments contain pentachloronitrobenzene (PCNB), metalaxyl, chloroneb, diazoben, and captan. Fungicides can create toxic barrier between the host surface and pathogens. Fungicides like metalaxyl, propamocarb and prothiocarb are helpful for the control of oomycetes pathogens. Fosetyl-Al is a foliar spray fungicide that controls the soil-borne pathogens (Sultana and Ghaffar, 2010; Divya and Sudani, 2014).
Sensitivity of various isolates of *S. rolfsii* has been tested against different chemical fungicides. Chloropyriphos showed maximum inhibition (57%) followed by pendimethalin (46%) and hiram (44%) (Palaiah, 2002). Mancozeb, dithane M-45, carboxin, thiram, carbendazim, sulfur dust, streptocycline blue copper, ziram and thiophanate methyl were effective at 2500 ppm in controlling collar rot in lentil (*Lens culinaris* Medik.) caused by *S. rolfsii*. Mancozeb, thiram and carboxin gave 100% control of the pathogen (Singh *et al*., 2005). Madhavi and Bhattiprolu (2011) found that vermicompost and seedling dip with carbendazim+mancozeb and *Trichoderma harzianum* was effective for control of dry root rot of *C. annum* caused by *S. rolfsii*. Rather *et al*. (2012) managed *S. rolfsii* in bell pepper with fungicides namely captan, carbendazim, metalaxyl and carboxin by application as seed and seedling treatment. Das *et al*. (2014) evaluated *in vitro* efficacy of six systemic fungicides namely propiconazole, hexaconazole, myclobutanil, thiophanate methyl, tebuconazole and carbendazim; three non-systemic fungicides namely captaf, mancozeb and copper oxychloride, against growth of *S. rolfsii* by using poisoned food technique. Hexaconazole followed by tebuconazole showed maximum inhibition in radial growth of the fungus at all the concentrations. Likewise, Madhuri and Narayan (2013) evaluated efficacy of eight fungicides *in vitro* against *S. rolfsii* using poisoned food technique and found that oxyflourfen, alachlor and quizalofop-p-ethyl completely inhibited the fungal growth.

Although certain chemical fungicides are very effective to control *S. rolfsii*, however, different kind of environmental pollution can be caused by indiscriminate use of synthetic fungicides, biodiversity losses and deterioration of natural habitats (Sheikh *et al*., 2011; Enyiukwu *et al*., 2014). Pesticides not only pollute the soils and water but also cause toxicological hazards by becoming the part of the food chain, enter the body system, and poison blood and body organs. There are also reports of development of resistance against pesticides, killing of non-target species and outbreak of secondary pests (Sattler *et al*., 2007).

### 1.3.4. Biological control

Biological control of soil-borne diseases is a popular and challenging goal and has been a focus for research since many years. Biological control is attractive in an environmental and economic sense because it offers safe and cost effective alternative
to fungicides (Zaher et al., 2013; Abada and Eid, 2014). Biological control is the use of beneficial organisms to control the pathogens and diseases e.g. agrobacterium Smith and Townsend, Bacillus subtilis Ehrenberg, Septomyces lydicus Waksman and Henrici, Septomyces griseoviridis Waksman and Henrici, Ampelomyces quisqualis Ces, Candida oleophila Montrocher, Gliocladium catenulatum Gilman and Abbot Trichoderma sp. (Saxena et al., 2014; Moorman, 2015). The main mechanisms involved in the antagonism of biocontrol agents are, competition for space and nutrients, mycoparasitism, stimulation of the plant’s defensive capacity, and secretion of bioactive compounds such as cell wall degrading enzymes and antibiotics (Bakker et al., 2007; Van der Ent et al., 2008). There are many reports where biocontrol agents significantly controlled S. rolfsii. Rao et al. (2004) reported that among the various biocontrol agents used for management of Sclerotium wilt in potato, Trichoderma harzianum inoculated treatment showed the least disease incidence of 10% followed by T. viride inoculated treatment with 12% disease incidence. Similarly, Baswaraj (2005) observed that T. harzianum and T. viride found the best in controlling Sclerotium wilt in potato. Likewise, Anahosur (2001) found that T. harzianum inoculated treatments showed the least Sclerotium wilt incidence in potato (10%) followed by T. viride inoculation (14%). Pattana-pipitpaisal and Kamlandharn (2012) isolated 283 chitinolytic actinomycetes strains screened for chitinolytic activity. Among the 68 selected chitinolytic isoates, 13 showed remarkable fungal growth inhibition potential against S. rolfsii. These actinomycetes produced chitinase which catalyzed the degradation of chitin, resulting in inhibition of S. rolfsii growth.

Chowdary (2000) worked out an integrated management strategy against southern blight disease in bell pepper cultivar California Wonder under glasshouse conditions in pot culture. Out of the eight treatments, combined application of Trichoderma viride at 15 g kg\(^{-1}\) soil, cheshnut compound at 25 mL kg\(^{-1}\) soil and neem cake at 100 g kg\(^{-1}\) soil was found significant to control the disease followed by combined application of T. viride and neem cake. Madhavi and Bhattiprolu (2011) tested T. harzianum, T. viride, T. hamatum and Pseudomonas fluorescens Flügge against S. rolfsii and recorded 57.5%, 55.8%, 44.46% and 40.7% reduction in mycelial growth of S. rolfsii, respectively.
1.4. Natural compounds as fungicides

Due to the negative effects of synthetic fungicides, scientists are in search of natural substitutes of synthetic agrochemicals from plants and microbes for disease management (Rauf and Javaid, 2013). In recent years, research has been focused at utilization of higher plant products as chemotherapeutants for plant defense (Farooq et al., 2011). Plants contain phenolics, coumarins, alkaloids, terpenoids, steroids, tannins and quinines in roots and aerial parts. Phenolic compounds e.g. carvacrol, eugenol, and thymol exhibit antimicrobial activity against pathogenic microorganisms (Xuan et al., 2005; Das et al., 2010). Demand of biopesticides and pesticides based on natural compounds is increasing day by day and are being used universally as green pesticides.

1.4.1. Antifungal activity of crude extracts of plants

Antagonistic, curative and protective potential of plants against a number of phytopathogens has been reported by large number of scientists (Zahid et al., 2012; Amin and Javaid, 2013; Sab et al., 2014). Parveen et al. (2014) explored the potential of five plants namely Rumex obtusifolius L., Artemisia absinthium L., Malva sylvestris L., Plantago lanceolata L. and Taraxacum officinale Weber ex Wiggers as natural fungicide against the fungal rot diseases of fruits and vegetables caused by Mucor priformis Scop. Penicillium expansum Link ex Thom. and Alternaria alternata (Fr.) Keissler. A. absinthium leaf extract significantly inhibited the fungal growth of all the tested fungal species. Amin and Javaid (2013) found that different concentrations of methanolic fruit extract of Syzygium cumini (L.) Skeels suppressed the biomass of Ascochyta rabiei (Pass.) Lab. by 44–66%. Likewise, different organic fractions of methanolic fruit extract viz. n-hexane, ethyl acetate, chloroform and n-butanol suppressed the fungal biomass by 58%, 66%, 66% and 67%, respectively. Antifungal activity of aqueous and n-hexane extracts of root, stem and leaf of Datura alba Nees. was tested against charcoal rot of sunflower (Helianthus annuus L.) caused by M. phaseolina (Tassi) Goid. All the concentrations of aqueous as well as n-hexane extracts significantly suppressed the fungal biomass. Among the various aqueous extracts, root extract significantly suppress the fungal biomass followed by stem and leaf extracts, resulting in 85–92%, 72–83% and 60–65%, respectively (Javaid et al.,...
Islam and Faruq (2012) reported that seed treatment of some winter vegetables with extracts of neem (Azadirachta indica A. Juss) leaves and garlic (Allium sativum L.) cloves significantly reduced damping off disease of tomato, chili and egg plant (Solanum melongena L.) caused by R. solani. In the recent years a lot of research work has done on this topic. Zeng et al. (2015) screened 18 Chinese medicinal plants against Embellisia astragalii Li and Nan, the cause of yellow dwarf and root-rot disease in Astragalus adsurgens Pall. Among the tested medicinal plants, ethanolic extracts of Juglans regia L., Allium sativum L. and Saposhnikovia divaricata (Turcz.) Schischk completely inhibited mycelial growth of the target pathogen. Javaid et al. (2015) found that methanolic extracts of Imperata cylindrica (L.) Beauv. significantly reduced in vitro growth of M. phaseolina. In another study, Javaid and Akhtar (2015) reported that methanolic root extract of a medicinal plant Withania somnifera (L.) Dunal had pronounced antifungal activity against Fusarium oxysporum f. sp. cepae, causing basal plate rot in onion (Allium cepa L.). Likewise, methanolic extracts of various parts of two Asteraceous weeds namely Eclipta alba (L.) Hassk and Launea nudicaulis Cass. showed variable antifungal activities against M. phaseolina (Banaras and Javaid, 2015; Banaras et al., 2015). Kakad et al. (2015) studied antifungal activity of methanolic extracts of twenty-five plants against Aspergillus niger Tiegh., Candida albicans (Robin) Berkhout and Daedalea flavida Lév. The maximum antifungal activity was found by methanolic extracts of Datura stramonium L., Acacia nilotica (L.) Delile, Eucalyptus globules Labill. and Terminalia bellerica (Gaertn.) Roxb. which significantly suppressed growth of all the fungal species. Mehrara et al. (2015) investigated the antifungal and antimicrobial activity of methanolic extract of Glaucium vitellinum Boiss. against Trichophyton mentagrophytes Priestley, T. rubrum (Castell.) Sabour., A. flavus, Epidermophyton flucossum (Harz) Langeron and Miloch., Microsporum canis Gruby, Candida albicans (Robin) Berkhout. Musman et al. (2015) identified the phytofungitoxic agents from methanolic extracts of Euphorbia hirta L., D. metel, Barringtona racemosa L. and Hydnophytum formicarum Jack against Saprolegnia sp.

Aqueous and organic solvent extracts of some plant species have been evaluated against S. rolfsii and very encouraging results were obtained. Farooq et al. (2010) studied the inhibitory effect of aqueous extract of twenty plant species in vitro on mycelial growth of S. rolfsii causing southern Sclerotium rot in sugar beet. Generally, all the plant species inhibited mycelial growth of the pathogen to variable
extent. However, maximum inhibition was recorded due to extracts of *Azadirachta indica* L. (74%) followed by *Cassia fistula* L. (73%) and *Cannabis sativa* L. (67%). Vivek *et al.* (2014) studied the inhibitory efficacy of extracts of 18 plants against *S. rolfsii*. All plants gave promising results and act as alternative to synthetic fungicides.

### 1.4.2. Effect of soil amendments

Soil amendments with plant material not only improve the soil fertility but also reduce the fungal population in the soil by releasing antifungal compounds during decomposition. Riaz *et al.* (2007) studied the effect of five allelopathic plant leaf residues viz. *Momordica charantia* L., *Parthenium hysterophorus* L., *Calotropis procera* Br., *A. indica* and *Ageratum conyzoides* L. against corm rot diseases of gladiolus (*Gladiolus grandiflorus* L.) caused by *Fusarium oxysporum* f. sp. *gladioli* and found that various soil amendments reduced the disease incidence by 60–80% and increased the plant survival to 100%. Likewise, Riaz *et al.* (2010) investigated the effect of mixing of *Coronopus didymus* (L.) Sm. aerial parts in the soil and observed reduced incidence of corm rot disease of gladiolus. Similar effects of soil amendment with *C. didymus* have also been reported in the control of basal rot of onion caused by *F. oxysporum* f. sp. *cepae* (Niaz, 2013). Recently, Javaid and Iqbal (2014) mixed the dried powdered shoots of *C. didymus* at the rate of 1%, 2% and 3% (w/w) in soil inoculated with *S. rolfsii* and recorded significantly suppress in collar rot of bell pepper due to 3% soil amendment. The effect of leaf powders of candle bush [*Cassia alata* (L.) Roxb.] and *Dennetia tripetala* Baker as soil amendment were observed against cocoyam [*Xanthosoma sagittifolium* (L.) Schott] corm rot caused by *S. rolfsi* and very encouraging results were obtained (Nwachukwu and Osuji, 2008). Javaid and Saddique (2011) noticed 80% reduction in mungbean [*Vigna radiate* (L.) R. WSilczek] mortality by *M. phaseolina* by application of leaf powder of *D. metel* in the soil at 1.5% w/w. Núñez-Zofío *et al.* (2011) recorded up to 93% reduction in incidence of pepper crown and root rot diseases caused by *Phytophthora capsici* Leonian by soil amendment with fresh green manure of Ethiopian mustard (*Brassica carinata* Braun) and pellets of fresh green *Sinapis alba* L. Sintayehu *et al.* (2014) evaluated the effect of cabbage (*Brassica oleracea* L.), garden cress (*Lepidium sativum* L.), *B. carinata* and rapeseed (*Brassica napus* L.) green manure as soil amendment for the management of Fusarium basal rot in onion caused by *F.*
oxysporum f. sp. cepae. Rapeseed and Ethiopian mustard significantly reduced disease incidence and disease severity by 21% to 30% and 23% to 29%, respectively. Mokhtar et al. (2014) assessed the antifungal properties of botanical plant powders of cabbage leaves, chili pods (Capsicum annuum L.) and Eucalyptus leaves (Eucalyptus obliqua L'Her.) mixed in soil individually or combined with compost before sowing. All the treatments significantly inhibited the root rot disease of beans (Phaseolus vulgaris L.) caused by Fusarium solani and R. solani as compared to untreated control. Recently, Javaid and Rauf (2015) found that dried leaf biomass of Chenopodium album L. leaves (3% w/w) can be used as alternative to chemical fungicides against basal rot of onion. Afzal (2013) found a significant reduction in charcoal rot of mungbean caused by M. phaseolina due to soil amendment with 1% dry biomass of a Brassicaceous weed Sisymbrium irio L. in combination with T. harzianum.

1.4.3. Natural antifungal compounds

Many pure natural compounds with antifungal activity have been isolated from plants. Momilactone A and B, the two pronounced allelochemicals produced by rice (Kato-Noguchi, 2011), are known to have antifungal activity against the rice blast pathogen Magnaporthe oryzae (Herbet) Barr (Kato et al., 1993). Prats et al. (2007) isolated 3-acetyl-4-acetoxyacetophenone from sunflower showing antifungal properties against Sclerotinia sclerotiorum. Many natural compounds with α-pyrone (α,β-unsaturated-δ-lactone or 6-substituted 5,6-dihydro 2H-pyran-2-one) structural units are known to possess antifungal activity (Das et al., 2009). One such compound is (6S)-5,6-dihydro-6-[(2R)-2-hydroxy-6-phenyl hexyl]-2H-pyran-2-one identified from Ravensara crossifolia (Bak.) Danguy, exhibited potent antifungal activity against a plant pathogenic fungal species Cladosporium cucumerinum (Raoelison et al., 2001), as well as against other fungal species namely Fusarium solani (Mart.) Sacc, F. oxysporum, Colletrotichum gloeosporides (Stoneman) Spauld. and Schrenk and Botrytis cinerae (Fukuta et al., 2007). Bioassays guided fractionation of methanolic leaf extract of Melia azedarach L. revealed the presence of antifungal compounds namely ursolic acid, β-amyrin; β-sitosterol; 3,5 dimethoxybenzoic acid and maesol in chloroform sub-fraction of the extract which were very effective against Ascochyta rabiei (Pass.) Lab., the cause of chickpea (Cicer arietinum L.)
Blight (Jabeen et al., 2011). Kanwal et al. (2010) identified five flavonoides namely (-)-epicatechin (2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol; 6-(p-phenoxbenzyl) taxifolin-7-O-β-D-glucoside (tricuspid); (-)-epicatechin-3-O-β-glucopyranoside; quercitin-3-O-α-glucopyranosyl-(1→2)-β-D-glucopyranoside and 5-hydroxy-3-(4-hydroxylphenyl) pyrano[3,2-g] chromene-4(8H)-one from leaves of mango (Mangifera indica L.) and reported that these compounds reduced growth of various fungal species by 56–98% over control. Likewise, Kanwal et al. (2011) identified two flavonoides namely genistein 7-O-glicoside and (-)-epicatechin which reduced in vitro growth of two plant pathogenic fungal species namely Macrophomina phaseolina (Tassi) Goid. and Alternaria alternata (Fr.) Keissler by 82–99%. Zabka and Paveela (2013) evaluated 21 phenolic components of essential oils against species of the Aspergillus, Fusarium and Penicillium. Carvacrol and thymol were found the most effective components. The MIC values for thymol and carvacrol ranged from 76 to 255 μg mL⁻¹ and 131 to 262 μg mL⁻¹, respectively. Recently, Ramos et al. (2015) purified an osmotin/thaumatin-like protein CpOsm from latex of Calotropis procera (Aiton) Ation which showed antifungal activity against F. solani by causing dramatic morphological changes to spores of the test fungal species. A novel compound Heptane, diethyl ether, acetone has been identified from a lichen Cladonia uncialis exhibited strong antifungal activity against Candida albicans (Robin) Berkhout (Studzińska-Sroka et al., 2015). Monoterpenes rich essential oil of Citrus aurantium L. showed antifungal activity against various fungal species namely F. solani, F. oxysporum, F. avenaceum Cook, Bipolaris sorokiniana (Ito and Kurib) Drehler ex Dastur and B. cinerea (Metoui et al., 2015).

1.5. Datura metel L.

D. metel (family Solanaceae) is a perennial herbaceous plant grows in warmer regions of the world. All plant parts viz. leaf, stem and fruit have extensively been used as medicine for asthma, cough, spasm and mental illness. It is also well known for its sedative, anodyne, antiasthmatic, antispasmodic, antitussive, bronchodilator, hallucinogenic insecticidal, herbicidal, antifungal, anti-bacterial, anti-cancer, anaesthetic, hypnotic, mydriatic, anti-inflammatory and anti-rheumatoid activities (Jyothi and Taskeen, 2013; Mandal and Shah, 2013). It can be used to treat epilepsy, hysteria catarrh, skin diseases, wounds, diarrhea, insanity, rheumatic pains,
hemorrhoids, painful menstruation, burns, skin ulcers and to calm cough (Chopra et al., 1986; Ali and Shuab, 1996; Dabur et al., 2004). Pan et al. (2007) identified the withanolides in D. metel by means of spectroscopy exhibited cytotoxic activity against cancer cells. Meena et al. (2013) identified the antibacterial compound withametalin B using 1H-NMR, 13C-NMR and mass spectra techniques from the D. metel for the management of Xanthomonas oryzae pv. oryzae causes bacterial blight in rice. A number of phytochemicals have been reported in D. metel. These phytoconstituents are saponins, alkaloids, phenols, flavonoids, tannins, sterols, scopolamines and hyoscyamine. Hyoscyamine is the most common alkaloid of family Solanaceae showed in vitro antifungal activities and having different quantities of hyoscine and in rare cases traces of atropine are also present. A steroidal constituent datura-sterol is present in its leaves as a major constitute of essential oil with antifungal property (Ali and Shuaib 1996; Dabur et al., 2004; Mishra, 2011; Sayyed and Shah, 2014). Khanzada et al. (2006) studied the effect of leaf aqueous extract of D. metel, on the mycelial growth of Sclerotium rolfsii Sacc. and found promising results. Bajwa et al. (2008) managed the chickpea blight caused by Ascochyta rabiei by aqueous and methanolic extracts of D. metel. Its shoot methanol and aqueous extracts showed 20–40% and 21–34% inhibition in growth of A. rabiei. Likewise, root methanol and aqueous extracts inhibited the fungal growth by 11–29% and 15–25%, respectively. Lakshmeesha, et al. (2013) observed that D. metel methanolic leaf extract inhibited the growth of Macrophomina phaseolina. Similarly, Rinez et al. (2013) assessed the antifungal activity of methanolic, petroleum ether, chloroform and aqueous extract of D. metel leaves and flowers against Fusarium oxysporum f. sp. melonis, F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. tuberosum, T. harzianum and T. viride. Leaf extracts showed higher antifungal activity than the flower extracts. Sakthi et al. (2011) explored the antifungal activity of ethanol and ethyl acetate extracts of leaves of D. metel against six fungal pathogens viz. Penicillium chrysogenum Thom, A. flavus, A. niger, A. fumigates, Candida glabrata and C. albicans. The extracts significantly inhibited the fungal pathogens to variable extents. Lakshmeesha et al. (2013) found the maximum antifungal activity of D. metel out of ten botanicals against soybean [Glycine max (L.) Merr.] seed-born fungus M. phaseolina. Sarkar et al. (2014) analyzed the phytochemical constituent of D. metel root and found the flavonoids in Ethanolic extract and triterpinoids, alkaloids, fixed oils, fats, carbohydrates and flavonoids in aqueous extract. However, studies regarding the
management of southern blight disease of bell pepper by extracts and dry biomass as soil amendment are lacking. Therefore, the present study was carried out to manage the disease by exploiting antifungal properties of *D. metel*.

### 1.6. Objectives

The main objective of the proposed study is to seek nature friendly alternatives for the management of southern blight disease of bell pepper. To achieve this goal, the present study aims at:

- Screening of methanolic extracts of different parts of *Datura metel* for their antifungal activity against *S. rolfsii*.
- Isolation of the potential antifungal constituents from *D. metel*.
- Structural elucidation of the active antifungal constituents of *D. metel*.
- Use of dry leaf materials of *D. metel* as soil amendment of the management of disease.
Chapter 2

MATERIALS AND METHODS

2.1. Isolation, Purification and Identification of the Pathogen

Bell pepper plants showing symptoms of southern blight were collected from Vegetable Farm, University of the Punjab, Lahore, Pakistan. Diseased specimens were collected in polythene bags and brought to Biofertilizers and Biopesticide Lab., Institute of Agriculture Sciences, University of the Punjab Lahore. Diseased plant portions were cut into 0.5 cm pieces and surface sterilized with 1% sodium hypochlorite solution for 1 minute. Thereafter, root pieces were rinsed with sterilized water several times. Malt extract agar medium (2%) was prepared by autoclaving at 121 °C and 103 kPa pressure for 30 min. Afterward medium was poured in 9-cm Petri plates and allowed to cool at room temperature. Surface sterilized infected root pieces were placed on malt extract agar medium plates under aseptic condition in a laminar flow. Petri plates were incubated at 27 °C for one week. There were white, radiant coarse mycelia appeared in Petri plates. A large number of sclerotia, measured 0.5–2 mm in diameter, were appeared on mycelial mat. Initially sclerotia were smooth and white, which later on changed colour to light tan to brown (Plate 2.1). The fungus was identified as Sclerotium rolfsii on the bases of morphological characters following Somani and Chuhan (1996).

Plate 2.1. Pure culture of Sclerotium rolfsii. A: Young colony; B: Mature colony
2.2. Collection of Plant Materials

*Datura metel* was selected for the management of southern blight of bell pepper (Plate 2.2). Plants of *D. metel* were collected from undistributed areas of University of the Punjab, Quaid-e-Azam, Campus, Lahore and surrounding areas. Roots, stems, leaves and fruit of *D. metel* were separated, thoroughly washed with tap water, dry under the sun and stored in polythene bags.

![Plate 2.2. Mature plant of *D. metel*](image)

2.3. Laboratory Bioassay with Methanolic Extracts

2.3.1 Preparation of methanolic extract

Dried and powdered materials of roots, stems, leaves and fruits (200 g each) of *D. metel* were soaked in methanol (1000 mL) in closed containers for 14 days. Thereafter, materials were passed through double layered cheese cloth. Filtrates were collected and residues were again soaked in 500 mL methanol for one week and passed through cheese cloth to get filtrates. Methanolic extracts from these filtration phases were combined and passed through Whatman No. 1 filter papers to remove traces of residues. Filtrates of each of the four plant parts were separately evaporated under vacuum on a rotary evaporator at 45 °C to yield 19.90 g, 17.88 g, 18.64 g, and 16.41 g of crude methanolic extract of leaves, stems, roots and fruits, respectively (Plate 2.3).
2.3.2. Bioassay with methanolic extracts

Methanolic extract (14.4 g) of different parts of *Datura metel* was dissolved in 5 mL dimethyl sulfoxide (DMSO) followed by addition of sterilized distilled water to prepare 18 mL of each stock solution. In a similar way, for preparation of 72 mL of control solution, 20 mL DMSO were mixed with 52 mL distilled water. Control solution was later on used to maintain concentration of DMSO constant in all the treatments. Two percent malt extract broth (76 mL) was autoclaved in 250-mL volume flasks by autoclaving at 121 °C at 103 kPa pressure for 30 minutes and cooled at room temperature. Nine concentrations were prepared by adding a mixture of stock and control solutions in different proportions to 76 mL malt extract broth in each flask to prepare different concentrations of each extract as shown in Table 2.1.

Eighty millilitres of growth medium in each 250-mL flask was divided into four equal portions and poured in each 100-mL volume flask. Mycelial discs were cut from actively growing parts of 7 days old pure culture of *S. rolfsii* by a sterilized cork borer of 5 mm diameter and transferred one plug in each 100-mL flask containing 20 mL growth medium. All the treatments were replicated 4 times. Flasks were kept in an incubator for 7 days at 26 °C. Thereafter, materials from each flask were filtered on a pre-weighed filter paper. Biomass of *S. rolfsii* on filter papers from different treatments was dried at
60 °C till complete dryness and weighed. Weight of filter paper was subtracted from the total weight to get the weight of *S. rolfii*.

**Table 2.1:** Different quantities of stock and control solutions added to malt extract broth (76 mL) to prepare different concentrations of methanolic extracts in growth medium.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stock solution (mL)</th>
<th>Control solution (mL)</th>
<th>Final concentration of extract in medium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>4.0</td>
<td>0.0</td>
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<tr>
<td>2</td>
<td>0.5</td>
<td>3.5</td>
<td>0.5</td>
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<tr>
<td>3</td>
<td>1.0</td>
<td>3.0</td>
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<tr>
<td>4</td>
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<td>3.0</td>
<td>1.0</td>
<td>3.0</td>
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<tr>
<td>8</td>
<td>3.5</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>0.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**2.4. Fractionation of Effective Methanolic Extracts**

Methanolic extracts of stems, leaves and fruits were found highly effective in suppressing growth of *S. rolfii*. Therefore, these extracts were selected for further fractionation using organic solvents of different polarity natures namely n-hexane, chloroform, ethyl acetate and n-butanol.

**2.4.1. Preparation of methanolic extracts**

Five kilograms of each dried and powdered stems, leaves and fruits of *D. metel* were extracted with 15 L methanol at room temperature for two weeks. Following filtration, the methanolic extracts of all the three selected parts of the plant were evaporated in rotary evaporator flask at 45 °C. Thereafter, complete evaporation of methanol was carried out in an oven at 45 °C to obtain 814 g of leaves, 397 g crude extract of stems and 348 g of fruits.

**2.4.2. Fractionation of methanolic extracts**

Five hundred millilitres of distilled water were added to crude methanolic stem extract and shaken well until whole methanolic extract was completely mixed in water to
form a single phase. To this mixture, 500 mL n-hexane was added, thoroughly mixed and transferred to separating funnel set on a stand. The two phases viz. aqueous and n-hexane were allowed to separate for about 3 h. Thereafter, n-hexane phase was collected and stored in a closed bottle. Aqueous phase was again mixed with n-hexane and process was repeated several times until all the n-hexane soluble compounds were separated from the aqueous phase. n-Hexane fraction was evaporated on a rotary flask and finally 107 g of dried material of this fraction was obtained. Aqueous fraction was successively extracted with different organic solvents namely chloroform (2 × 500 mL), ethyl acetate (2 × 500 mL) and n-butanol (2 × 500 mL) (Plate 2.4). Organic solvents were evaporated on a rotary evaporator to yield 1.809 g of chloroform, 5.379 g ethyl acetate and 11.338 g n-butanol fraction. The remaining aqueous extract was also evaporated on rotary flask to obtained 58.15 g of this fraction.

Methanolic leaf extract of *D. metel* was mixed with 500 mL distilled water and fractionation was performed in a separating funnel using different organic solvents similar to fractionation of methanolic stem extract. All the sub-fractions were evaporated on a rotary evaporator and finally 147 g of *n*-hexane, 318 g of chloroform, 34.78 g of ethyle acetate, 49.44 g of *n*-butanol and 137 g of aqueous sub-fraction were obtained. In a similar way, 58 g of *n*-hexane, 20 g of chloroform, 6 g of ethyl acetate, 15.4 g of *n*-butanol and 125.7 g aqueous sub-fraction of methanolic extract of *D. metel* were obtained after fractionation in separating funnel followed by evaporating on a rotary evaporator.

### 2.5. Bioassay with Fractions of Methanolic Extracts

For investigation of antifungal activity of various fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous) of *D. metel* methanolic leaf, stem and fruit extracts were examined *in vitro* against *rolfsii*. Weighed amount (1.2 g) of each methanolic extracts were dissolve in 0.5 mL of DMSO and 5.5 mL malt extract broth was added to get 6.0 mL of each stock solution of 200 mg mL\(^{-1}\) concentration. Three milliliters of each stock solution was used in antifungal bioassays while rest quantity was serially double diluted by adding malt extract broth to obtain lower concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg mL\(^{-1}\). A series of control treatments was made so as to have a control treatment for each extract concentration with same DMSO concentration both in control and experimental. To achieve this, 0.5 mL DMSO were
mixed with 5.5 mL malt extract broth and serially double diluted by adding sterilized growth medium. Bioassay were performed with three replication in glass test tubes of 10-mL volume each contained 1 mL of medium. Suspension of *S. rolfsii* inoculum (50 µL) was added under aseptic conditions using a micropipette. Test tubes were incubated at 25 °C for one week. Fungal biomass of each test tube was filtered on pre-weighed filter papers. Fungal biomass was recorded after drying at 60 °C.
2.6. GC-MS Analysis

GC-MS analysis of chloroform fractions of methanolic extracts of all the three parts was performed using the GC 2010. Compounds were separated on 30 × 0.25 mm × 0.25 µmdf capillary column. Samples injection split ratio was 10:1 and a flow rate of helium was 1 mL min\(^{-1}\). Column flow was 1.33 mL min\(^{-1}\) and the pressure was 100 kPa. Mass detector used was Turbo mass 5.2 gold Perkin Elmer. Sample amount injected was 2 µL. Other conditions viz. oven temperature was up to 100 °C for 3 min and raised the temperature at the rate of 10 °C min\(^{-1}\) hold up to 325 °C. Other conditions like oven temperature program was set at 100 °C and held isothermal for 3 min and finally raised to 325 °C at the rate of 10 °C min\(^{-1}\) Injector temperature was maintained at 200 °C. Total GC running time was 25 min. Computer library NIST 02 was attached to the GC-MS instrument and documented the compounds after comparison with those available in the library (Kiruthika and Sornaraj, 2011).

2.6.1. Identification of the compounds

The compounds in chloroform extract of the leaf, stem and fruit of *D. metel* were identified on the base of GC retention time in the 30 × 0.25 mm × 0.25 µmdf capillary column. The chromatograms were interpreted by using NIST library. The compound name, molecular weight, molecular formula and compound structure were ascertained.

2.7. Pot Trial

In pot trial, the effect of soil amendment with dry biomass of above ground parts of *D. metel* was investigated for the management of southern blight disease of bell pepper caused by *S. rolfsii*.

2.7.1. Mass multiplication of *S. rolfsii* inoculum

Two kilograms seeds of pearl millet [*Pennisetum glaucum* (L.) R. Br.] were boiled and excess moisture was dried under a fan. These boiled seeds were packed in transparent plastic bags (400 g bag\(^{-1}\)) and autoclaved at 121 °C and 103 kPa pressure for 30 minutes. After cooling at room temperature, autoclaved seeds were incubated by 7 days old culture of *S. rolfsii* and incubated at 27 °C for one week.
2.7.2. Preparation of pots

Experiment was conducted in earthen pots of 28 cm diameter and 30 cm deep with 5 kg soil per pot. Soil used in this experiment was loam in texture with pH 7.8. Chemical analysis of the soil revealed that it contained 0.69% organic matter, 14.3 mg kg\(^{-1}\) available phosphorus and 302 mg kg\(^{-1}\) available potassium. Fifty grams pearl millet seeds based \textit{S. rolfsii} inoculum was completely mixed in the soil of respective pots and watered all the pots. For establishment of inoculum in soil, pots were left for one week under natural environmental conditions. Thereafter, dried and powdered biomass of above ground parts of \textit{D. metel} including leaves, stems and fruit was mixed in soil of respective pots at 0.5%, 1%, 1.5%, 2%, 2.5% and 3% (w/w). Pots of positive control were only inoculated with the pathogen inoculums whereas pots of negative control were without any fungal inoculums and dry powdered biomass of \textit{D. metel}.

2.7.3. Treatments and experimental design

There were following 14 treatments in the pot experiment which were arranged in a completely randomized design with six replications:

\begin{tabular}{ll}
\textbf{T\textsubscript{1}} & Negative Control \\
\textbf{T\textsubscript{2}} & Positive Control [\textit{Sclerotium rolfsii} (SR) inoculation only] \\
\textbf{T\textsubscript{3}} & 0.5\% amendment of dry biomass of \textit{D. metel}. \\
\textbf{T\textsubscript{4}} & 0.5\% amendment of dry biomass of \textit{D. metel} + SR \\
\textbf{T\textsubscript{5}} & 1.0\% amendment of dry biomass of \textit{D. metel}. \\
\textbf{T\textsubscript{6}} & 1.0\% amendment of dry biomass of \textit{D. metel} + SR \\
\textbf{T\textsubscript{7}} & 1.5\% amendment of dry biomass of \textit{D. metel}. \\
\textbf{T\textsubscript{8}} & 1.5\% amendment of dry biomass of \textit{D. metel} + SR \\
\textbf{T\textsubscript{9}} & 2.0\% amendment of dry biomass of \textit{D. metel}. \\
\textbf{T\textsubscript{10}} & 2.0\% amendment of dry biomass of \textit{D. metel} + SR \\
\textbf{T\textsubscript{11}} & 2.5\% amendment of dry biomass of \textit{D. metel} \\
\textbf{T\textsubscript{12}} & 2.5\% amendment of dry biomass of \textit{D. metel} + SR \\
\textbf{T\textsubscript{13}} & 3.0\% amendment of dry biomass of \textit{D. metel}. \\
\textbf{T\textsubscript{14}} & 3.0\% amendment of dry biomass of \textit{D. metel} + SR \\
\end{tabular}
2.7.4. Sowing of seeds

Seeds of bell pepper were surface sterilized with 1% sodium hypochlorite solution for 5 min followed by through washing with sterilized water. For raising of nursery, surface sterilized seeds were sown in earthen pots each containing 3 kg of fumigated sandy loam soil. Pots were irrigated with sterilized water and left for 6 weeks. Thereafter, seedlings were transplanted in treatment pots at 6 plants per pot, watered and put in a green netting house under natural environmental conditions. Pots were regularly irrigated with tap water whenever required.

2.7.5. Harvesting and data collection

Plants were harvested at maturity. Data regarding plant height, fresh and dry weights of root, shoot and fruit, number of fruits, disease incident, disease severity and plant morality were recorded. Various physiological test of fresh roots and leaves samples viz. estimation of chlorophyll, total protein and phenolic contents, as well as evaluation of peroxydase, polyphenol oxidase and phenyl alanine ammonia lyase activity.

Disease incidence was calculated by following formula:

\[
\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \times 100
\]

Disease severity was measured according to disease rating scale of Horsfall and Barratt (1945) as follows:

0  Healthy plant, no wilting.
1  Yellowing appeared on 25% plant parts.
2  About 50% plant diseased with yellowing and browning.
3  More than 50% plants wilted or died.

2.8. Physiological Tests

Different physiological tests were carried out at maturity. For all physiological parameters fresh leaves and roots were taken from pots grown bell pepper plants and tests were performed immediately.
2.8.1. Estimation of chlorophyll contents

For the estimation of chlorophyll content, 0.5 g leaf tissue from each treatment was macerated with liquid nitrogen and 1mL of 80% ethanol was added and materials were centrifuge at 8000 rpm for 15 min. Supernatant was collected and chlorophyll was quantified by using a spectrophotometer (UT2100UV) at 645 and 663 nm (Lichtenthaler and wellburn, 1983).

2.8.2. Estimation of protein contents

To determine protein content, 0.5 g fresh leaf and root samples of bell pepper were grinded in liquid nitrogen and thoroughly mixed in 1 mL of 20% trichloroacetic acid and centrifuged for 10 min at 3000 rpm. Supernatants were removed and pellets were re-extracted with 5 mL of 0.1 N NaOH. Took 1 mL of the extract and 5 mL protein reagent in a test tube and mixture was kept in dark for 10 min. After that 0.5 mL of folin phenol reagent was added and mixture was again kept in dark for 30 min. Protein contents were noted at 660 nm in a spectrophotometer UT2100UV (Bradford, 1976).

2.8.3. Estimation of phenolics

For estimation of phenolics, 0.5 g fresh leaf and root samples of each treatment were grinded in liquid nitrogen pre-chilled pestle and mortar and poured into an eppendorf. To this 1 mL of 80% ethanol and centrifuged the mixture at 10,000 rpm for 15 min and supernatant 1 was transferred to a reaction mixture tube. One milliliter of 80% ethanol was added again in a residue and centrifuged at 10,000 rpm for 10 min and supernatant 2 was obtained. Supernatant 1 and 2 were combined in a reaction mixture tube. Took 1 mL supernatant in reaction mixture tube and added 0.5 mL folin phenol reagent and 1 mL Na2CO3. Reaction mixture was boiled in a water bath for 10 min, 5 mL distilled water was added and absorbance of developed blue colour was observed at 660 nm on a spectrophotometer UT2100UV (Bray and Thorpe, 1954).

2.8.4. Estimation of peroxidase (PO) activity

For estimation of peroxidase (PO), after grinding 0.5 g of fresh leaf and root samples of each treatment in liquid nitrogen, materials were homogenized in 1 mL of 0.1 M sodium phosphate buffer. Centrifuged the mixture at 10,000 rpm for 15 min and take
0.5 mL supernatant in a separate tube and add 1 mL of 0.05 M pyrogallol and 1 mL of phosphate buffer. Mixture was incubate at 25 °C for 15 min and added 1-mL of 2.5 N H₂SO₄. Change in absorbance was recorded at 595 nm over 1 min in spectrophotometer UT2100UV. Change in absorbance of 0.01 for 1 g fresh weight per minute known as one unit enzyme activity (George and Irvine, 1955).

2.8.5. Estimation of polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) activity was determined by colour change intensity of catechol oxidation products by taking 0.5 g of fresh leaf and root sample of each treatment were grinded in liquid nitrogen pre-chilled pestle and mortar and poured into an eppendorf. To this 1 mL 0.1M sodium phosphate buffer centrifuge the homogenate at 10,000rpm for 15 min. After centrifugation the reaction mixture consists of 100µL enzyme extract and 1.5mL of 0.1M sodium phosphate buffer. The reaction was started by the addition of 200µL of 0.01M catechol. The colour change intensity of catechol oxidation products was measured spectrophotometrically at 495nm under spectrophotometer UT2100UV (Mayer et al., 1965).

2.8.6. Estimation of phenylalanine ammonia lyase (PAL) activity

Phenylalanine ammonia lyase activity was based on the production of trans-cinnamic acid by taking 0.5 g of fresh leaf and root sample of each treatment were grinded in liquid nitrogen pre-chilled pestle and mortar and poured into an appendorf. To this 1 mL 0.1M sodium phosphate buffer centrifuge the homogenate at 10,000 rpm for 15 minutes. Enzyme extract of 0.4 mL contained by reaction mixture and made its volume up to 1.5 mL by the addition of 0.1M sodium borate buffer and 0.5 mL of 12mM L-phenylalanine. Mixture was incubated at 25°C for one hour. Formation of trans-cinnamic acid formed from L-phenylalanine calculated spectrophotometrically at 290 nm in spectrophotometer UT2100UV (Dickerson et al., 1984).

2.9. Statistical Analysis of Data

In laboratory bioassays, estimation of fungal biomass reduction percentage of Sclerotium rolfsii due to different concentrations of the extracts over control was calculated by applying the formula:
Fungal biomass reduction (%) = \frac{\text{Biomass of control - Biomass of extract treatment}}{\text{Biomass of control}} \times 100

Standard errors of mean in both laboratory and pots bioassays trials were calculated using computer software Microsoft Excel. All the data were analyzed by analysis of variance (ANOVA) followed by Tukey's HSD Test at 5% level of significance using computer software Statistics 8.1.
Chapter 3

RESULTS

3.1. Antifungal Activity of Methanolic Extracts of *D. metel* against *S. rolfsii*

Analysis of variance (ANOVA) for the effect of different concentrations of methanolic extract of different parts of *D. metel* viz. leaf, stem, root and fruit on biomass of *S. rolfsii* is presented in Table 3.1. The effect of plant parts (P), extract concentration (C) and interactive effect of PxC was significant (P≤0.001) for the fungal biomass.

3.1.1. Antifungal activity of leaf extract

Data regarding the effect of different concentrations of methanolic leaf extract of *D. metel* on biomass of *S. rolfsii* is presented in Fig. 3.1 and Plate 3.1. The highest fungal biomass (0.352 g) was recorded in control. All the concentrations of methanolic extracts significantly (P≤0.05) reduced fungal biomass over control. The effect of 0.5% and 1.0% concentrations was low where 29% and 32% reduction in fungal biomass was recorded over control, respectively. The effect of higher concentrations (1.5% to 4.0%) of the extract was much more pronounced where 80–99% reduction in fungal biomass was recorded due to various concentrations (Fig. 3.5). There was an inverse and linear relation between extract concentration and fungal biomass with $R^2 = 0.7439$ (Fig. 3.6 A).

3.1.2. Antifungal activity of stem extract

Data concerning the effect of different concentrations of methanolic stem extract of *D. metel* on biomass of *S. rolfsii* is shown in Fig. 3.2 and Plate 3.2. There was a gradual decrease in fungal biomass with an increase in extract concentration. All the extract concentrations exhibited a significant adverse effect on fungal growth resulting in 69–86% reduction in fungal biomass as compared to control (Fig. 3.5). Relationship between extract concentration and fungal biomass was non-linear with $R^2 = 0.8909$ (Fig. 3.6 B).
3.1.3. Effect of root extract

Data about the effect of different concentrations of methanolic fruit extract of *D. metel* on biomass of *S. rolfsii* is illustrated in Fig. 3.4 and Plate 3.4. Root extract exhibited the least antifungal activity against the target fungal pathogen causing 9–32% reduction in fungal biomass over control (Fig. 3.5). The effect of all the extract concentration on fungal growth was statistically insignificant. Relationship between extract concentration and fungal biomass was linear with $R^2 = 0.0644$ (Fig. 3.6 C).

3.1.4. Effect of fruit extract

Data pertaining to the effect of various concentrations of methanolic root extract of *D. metel* on biomass of *S. rolfsii* is expressed in Fig. 3.3 and Plate 3.3. Among all types of methanolic extracts, fruit extract was found to be the most effective against *S. rolfsii*. All the concentrations of the extract significantly reduced fungal biomass by 69 –94% over control (Fig. 3.5). There was a non linear relationship between extract concentration and fungal biomass with $R^2 = 0.9503$ (Fig. 3.6 D).
**Fig. 3.1:** Effect of methanolic leaf extract of *Datura metel* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.1:** Effect of different concentrations of methanolic leaf extract of *D. metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.2:** Effect of methanolic stem extracts of *Datura metel* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.2:** Effect of different concentrations of methanolic stem extract of *D. metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.3: Effect of methanolic root extracts of *Datura metel* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

Plate 3.3: Effect of different concentrations of methanolic root extract of *D. metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.4: Effect of methanolic fruit extracts of *Datura metel* on biomass of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by tiple Tukey's HSD Test.

Plate 3.4: Effect of different concentrations of methanolic fruit extract of *D. metel* on growth of *Sclerotium rolfsii*. 
Table 3.1: Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem, root and fruit extracts of *Datura metel* on biomass of *Sclerotium rolfsii*.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant parts (P)</td>
<td>3</td>
<td>0.839</td>
<td>0.280</td>
<td>95</td>
</tr>
<tr>
<td>Concentration (C)</td>
<td>8</td>
<td>0.845</td>
<td>0.106</td>
<td>253*</td>
</tr>
<tr>
<td>P × C</td>
<td>24</td>
<td>0.270</td>
<td>0.011</td>
<td>10*</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.119</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>2.074</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, Significant at P≤0.001.

Fig. 3.5: Percentage decrease in biomass of *Sclerotium rolfsii* due to different concentrations of methanolic leaf, stem, root and fruit extracts of *Datura metel* over control.
Fig. 3.6: Regression analysis for the effect of different concentrations of methanolic leaf, stem, root and fruit extracts of *Datura metel* on biomass of *Sclerotium rolfsii*.
3.2. Antifungal Activity of Fractions of Methanolic Leaf Extract

A series of control treatments were used in these experiments using different concentrations of DMSO corresponding to different concentrations of this compound used in different concentrations of experimental treatments. Higher concentrations of DMSO adversely affected the fungal growth. In general, there was a gradual reduction in fungal biomass with the increase in DMSO concentration in the growth medium Fig. 3.7. There was a linear relationship between DMSO concentration and fungal biomass with $R^2 = 0.9887$ (Fig. 3.12).

Different fractions of methanolic leaf extract of *D. metel* exhibited variable antifungal activities against the target fungal pathogen. Chloroform fraction exhibited the best antifungal activity where different concentrations of the extract reduced fungal biomass by 36–47% over corresponding control treatments (Fig. 3.8, Plate 3.6). Ethyl acetate fraction was found comparatively less effective than chloroform fraction where there was 5–43% suppression in fungal biomass due to various concentrations used in the experiment (Fig. 3.9, Plate 3.7). The effect of *n*-hexane fraction on fungal biomass was not much pronounced. Different concentrations of this fraction caused 11–29% decline in fungal biomass as compared to corresponding control treatments (Fig. 3.7, Plate 3.5). The two lower most concentrations (3.125 & 6.25 mg mL$^{-1}$) of *n*-butanol and aqueous fractions stimulated the fungal growth to variable extents resulting in 11–40% increase in fungal biomass over control. In contrast, higher concentrations (12.50 to 200 mg mL$^{-1}$) of both *n*-butanol and aqueous fractions exhibited adverse effects on fungal growth resulting in 17–36% and 0–36% reduction in fungal biomass over control, respectively (Fig. 3.11 & 3.11, Plate 3.8 & 3.9). There was a linear relationship between fungal biomass, and concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction with $R^2 = 0.9669, 0.9792, 0.9258, 0.8223$ and $0.7848$, respectively (Fig. 3.12).
**Fig. 3.7:** Effect of different concentrations of \( n \)-hexane fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (\( P \leq 0.05 \)) as determined by Tukey's HSD Test.
**Fig. 3.8:** Effect of different concentrations of chloroform fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.6:** Effect of different concentrations of chloroform fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.9: Effect of different concentrations of ethyl acetate fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

Plate 3.7: Effect of different concentrations of ethyl acetate fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*.
**Fig. 3.10:** Effect of different concentrations of n-butanol fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.8:** Effect of different concentrations of n-butanol fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*.
**Fig. 3.11:** Effect of different concentrations of aqueous fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.9:** Effect of different concentrations of aqueous fraction of methanolic leaf extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.12: Relationship between concentrations of different fraction of methanolic leaf extract of *Datura metel* and biomass of *Sclerotium rolfsii*. 

- Control: $y = -7.3804x + 75.709, R^2 = 0.9887$
- n-Hexane Fraction: $y = -6.4289x + 64.284, R^2 = 0.9669$
- Chloroform Fraction: $y = -5.4039x + 50.089, R^2 = 0.9792$
- Ethyl Acetate Fraction: $y = -7.6186x + 63.804, R^2 = 0.9258$
- n-Butanol Fraction: $y = -11.309x + 88.567, R^2 = 0.8223$
- Aqueous Fraction: $y = -10.834x + 87.14, R^2 = 0.7848$
3.3. Antifungal Activity of Fractions of Methanolic Stem Extract

Among the five fractions of methanolic stem extract of *D. metel*, chloroform fraction showed the best antifungal activity. Different concentrations of this extract reduced fungal biomass by 43–62%. The effect of lower concentrations (3.125 to 25 mg mL\(^{-1}\)) was statistically significant at 5% level of significance (Fig. 3.14, Plate 3.11). There was a linear relationship between fungal biomass and various concentrations of chloroform fraction with \(R^2 = 9474\) (Fig. 3.18).

The effect of different fractions of methanolic stem extract except chloroform fraction was generally insignificant. Among these ethyl acetate and \(n\)-butanol fractions showed comparatively better antifungal activity than the other two fractions resulting in 9–50% and 0–42% reduction in fungal biomass over corresponding control treatments, respectively (Fig. 3.15 & 3.16, Plate 3.12 & 3.13). Lower concentrations (3.125 to 25 mg mL\(^{-1}\)) of \(n\)-hexane fraction stimulated fungal growth while higher concentrations of this fraction (50 to 200 mg mL\(^{-1}\)) reduced fungal biomass by 3–30% as compared to control (Fig. 3.13, Plate 3.10). Aqueous fraction did not show any antifungal activity (Fig. 3.17, Plate 3.14). Relationship of fungal biomass with different concentrations of \(n\)-hexane, ethyl acetate, \(n\)-butanol and aqueous fractions was linear with \(R^2 = 0.9666, 0.9575, 0.9155\) and 0.9865, respectively (Fig. 3.18).
**Fig. 3.13:** Effect of different concentrations of *n*-hexane fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.10:** Effect of different concentrations of *n*-hexane fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.14:** Effect of different concentrations of chloroform fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.11:** Effect of different concentrations of chloroform fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.15:** Effect of different concentrations of ethyl acetate fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.12:** Effect of different concentrations of ethyl acetate fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.16: Effect of different concentrations of *n*-butanol fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
**Fig. 3.17:** Effect of different concentrations of aqueous fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.14:** Effect of different concentrations of aqueous fraction of methanolic stem extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.18: Relationship between concentrations of different fraction of methanolic stem extract of *Datura metel* and biomass of *Sclerotium rolfsii*. 
3.4. Antifungal Activity of Fractions of Methanolic Fruit Extract

Data regarding the effect of different fractions of methanolic fruit extract on growth of *S. rolfsii* is presented in Fig. 3.19 to 3.23 and Plate 3.15 to 3.19. Chloroform fraction showed the most pronounced inhibitory effect on fungal growth resulting in 36–50% decline in biomass of the target fungal pathogen (Fig. 3.20, Plate 3.16). Ethyl acetate fraction showed relatively lower inhibitory effect than chloroform fraction on fungal growth. There was up to 17% reduction in fungal biomass due to this fraction over corresponding control treatments. However, the effect was insignificant statistically (Fig. 3.21, Plate 3.17). The other fractions of methanolic fruit extract viz. *n*-hexane, *n*-butanol and aqueous fractions either had insignificant effect or significantly stimulated fungal growth to variable extents (Fig. 3.19, 3.22, 3.23; Plate 3.15, 3.18, 3.19). There was linear relationship between fungal biomass and different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction with $R^2 = 0.9154, 0.9091, 0.5957, 0.8210$ and 0.8627, respectively (Fig. 3.24).
Fig. 3.19: Effect of different concentrations of n-hexane fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

Plate 3.15: Effect of different concentrations of *n*-hexane fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*.
**Fig. 3.20:** Effect of different concentrations of chloroform fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.15:** Effect of different concentrations of chloroform fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.21:** Effect of different concentrations of ethyl acetate fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.17:** Effect of different concentrations of ethyl acetate fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.22:** Effect of different concentrations of \( n \)-butanol fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (\( P \leq 0.05 \)) as determined by Tukey's HSD Test.

**Plate 3.18:** Effect of different concentrations of \( n \)-butanol fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
**Fig. 3.23:** Effect of different concentrations of aqueous fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

**Plate 3.19:** Effect of different concentrations of aqueous fraction of methanolic fruit extract of *Datura metel* on growth of *Sclerotium rolfsii*. 
Fig. 3.24: Relationship between concentrations of different fraction of methanolic fruit extract of *Datura metel* and biomass of *Sclerotium rolfsii*. 
3.5. GC-MS Analysis of the Most Effective Fractions of Methanolic Extracts

3.5.1. Compounds from chloroform fraction of methanolic leaf extract

Eighteen compounds were detected in chloroform fraction of methanolic leaf extract of *D. metel*. Active principles with their retention time, molecular weight, molecular formulae and peak areas are given in Table 3.2. Among these, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester was found in the highest percentage (26.06%) followed by 1-hexacosanol (10.31%), 1-octadecene (8.57%) and 2-methyl-3-phenyl-2-propenal (6.99%). Other compounds included 1,6,10-farnesatrien-3-ol (5.11%), 1,3(15),10-bisabolatriene (5.11%), 1-docosene (0.76%), 1-octadecanol (5.32%), 1-heptadecene (5.32%), 1-hexadecene (1.90%), 1-tetradecanol (1.90%), methyl palmitate (1.64%), 1-pentadecanol (3.90%), octadecanoic acid, methyl ester (2.98%), 3-hexadecene, (Z)- (3.90%), 7-tetradecene (2.06%), 1-eicosanol (5.44%) and 1-pentadecene (1.90%). Structures of these compounds are presented in Fig. 3.25.

3.5.2. Compounds from chloroform fraction of methanolic stem extract

Six compounds were identified in chloroform fraction of methanolic stem extract of *D. metel*. Molecular weight and molecular formulae of these compounds along with their retention time and peak areas are shown in Table 3.3. Oleic and hexadecanoic acids were the predominantly occurring compounds each with 25.55% peak area. Other compounds were 1-octadecanol (17.94%), 1-heptadecene (11.13%), 1,6,10-farnesatrien-3-ol (10.70%) and 1-pentadecanol (8.18%). Structures of these compounds are illustrated in Fig. 3.26.

3.5.3. Compounds from chloroform fraction of methanolic fruit extract

Twelve compounds were recorded in chloroform fraction of methanolic fruit extract of *D. metel*. Molecular weight, molecular formulae and peak areas of these compounds are illustrated in Table 3.4. The most predominant compound was 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester (31.32%) followed by 1-hexacosanol (12.16%), 1-octadecanol (10.09%), 1-octadecene (10.09%), 1-eicosanol (6.41%), 1-heptadecene (6.26%), 1,3(15),10-bisabolatriene (6.02%), 1,6,10-farnesatrien-3-ol
(6.02%), 1-pentadecanol (4.60%), 1-pentadecene (2.24%), 1-tetradecanol (2.25%) and 1-dococene (0.89%). Structures of these compounds are presented in Fig. 3.27.

Table 3.2: Compounds identified from chloroform fraction of methanolic leaf extract of *D. metel* through GC-MS analysis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Names of compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area (%)</th>
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</thead>
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<tr>
<td>1</td>
<td>2-Methyl- 3 phenyl- 2- propenal</td>
<td>C_{16}H_{10}O</td>
<td>146</td>
<td>6.99</td>
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<tr>
<td>2</td>
<td>1,6,10- Farnesatrien-3-ol</td>
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<td>222</td>
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<tr>
<td>3</td>
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<td>5</td>
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<tr>
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<td>1-Hexadecene</td>
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<td>1.90</td>
</tr>
<tr>
<td>9</td>
<td>1-Tetradecanol</td>
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<td>10</td>
<td>1,2-Benzene dicarboxylic acid, bis</td>
<td>C_{16}H_{22}O_{4}</td>
<td>278</td>
<td>26.60</td>
</tr>
<tr>
<td></td>
<td>(2-methylpropyl) ester</td>
<td></td>
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</tr>
<tr>
<td>11</td>
<td>Methyl palmitate</td>
<td>C_{17}H_{34}O_{2}</td>
<td>270</td>
<td>1.64</td>
</tr>
<tr>
<td>12</td>
<td>1-Pentadecanol</td>
<td>C_{15}H_{32}O</td>
<td>228</td>
<td>3.90</td>
</tr>
<tr>
<td>13</td>
<td>Octadecanoic acid, methyl ester</td>
<td>C_{19}H_{38}O_{2}</td>
<td>298</td>
<td>2.98</td>
</tr>
<tr>
<td>14</td>
<td>3-Hexadecene, (Z)-</td>
<td>C_{16}H_{32}</td>
<td>224</td>
<td>3.90</td>
</tr>
<tr>
<td>15</td>
<td>7-Tetradecene</td>
<td>C_{14}H_{28}</td>
<td>196</td>
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</tr>
<tr>
<td>16</td>
<td>1-Eicosanol</td>
<td>C_{20}H_{42}O</td>
<td>298</td>
<td>5.44</td>
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<tr>
<td>17</td>
<td>1-Hexacosanol</td>
<td>C_{20}H_{54}O</td>
<td>382</td>
<td>10.31</td>
</tr>
<tr>
<td>18</td>
<td>1-Pentadecene</td>
<td>C_{15}H_{30}</td>
<td>210</td>
<td>1.90</td>
</tr>
</tbody>
</table>
Fig. 3.25: Structures of compounds isolated from chloroform fraction of methanolic leaf extract of *D. metel.*
Table 3.3: Compounds identified from chloroform fraction of methanolic stem extract of *D. metel* through GC-MS analysis.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Names of compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oleic acid</td>
<td>C_{18}H_{34}O_2</td>
<td>282</td>
<td>25.55</td>
</tr>
<tr>
<td>2</td>
<td>1-Pentadecanol</td>
<td>C_{15}H_{32}O</td>
<td>228</td>
<td>8.18</td>
</tr>
<tr>
<td>3</td>
<td>1-Octadecanol</td>
<td>C_{18}H_{36}</td>
<td>252</td>
<td>17.94</td>
</tr>
<tr>
<td>4</td>
<td>1-Heptadecene</td>
<td>C_{17}H_{34}</td>
<td>238</td>
<td>11.13</td>
</tr>
<tr>
<td>5</td>
<td>1,6,10- Farnesatrien-3-ol</td>
<td>C_{15}H_{26}O</td>
<td>222</td>
<td>10.70</td>
</tr>
<tr>
<td>6</td>
<td>Hexadecanoic acid</td>
<td>C_{16}H_{32}O_2</td>
<td>256</td>
<td>25.55</td>
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</tbody>
</table>
Fig. 3.26: Structures of compounds isolated from chloroform fraction of methanolic stem extract of *D. metel*.
Table 3.4: Compounds identified from chloroform fraction of methanolic fruit extract of *D. metel* through GC-MS analysis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Names of compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-Pentadecanol</td>
<td>C_{15}H_{32}O</td>
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</tr>
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<td>1-Dococene</td>
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<td>1-Heptadecene</td>
<td>C_{17}H_{34}</td>
<td>238</td>
<td>6.26</td>
</tr>
<tr>
<td>4</td>
<td>1-Octadecanol</td>
<td>C_{18}H_{36}</td>
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<td>10.09</td>
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<tr>
<td>5</td>
<td>1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester</td>
<td>C_{16}H_{22}O_{4}</td>
<td>278</td>
<td>31.32</td>
</tr>
<tr>
<td>6</td>
<td>1-Pentadecene</td>
<td>C_{15}H_{30}</td>
<td>210</td>
<td>2.24</td>
</tr>
<tr>
<td>7</td>
<td>1-Tetradecanol</td>
<td>C_{14}H_{30}O</td>
<td>214</td>
<td>2.25</td>
</tr>
<tr>
<td>8</td>
<td>1,3(15),10-Bisabolatriene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
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</tr>
<tr>
<td>9</td>
<td>1-Octadecene</td>
<td>C_{18}H_{36}</td>
<td>252</td>
<td>10.09</td>
</tr>
<tr>
<td>10</td>
<td>1-Eicosanol</td>
<td>C_{20}H_{42}O</td>
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<td>11</td>
<td>1-Hexacosanol</td>
<td>C_{26}H_{54}O</td>
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<td>12</td>
<td>1,6,10- Farnesatrien-3-ol</td>
<td>C_{18}H_{36}O</td>
<td>222</td>
<td>6.02</td>
</tr>
</tbody>
</table>
Fig. 3.27: Structures of compounds isolated from chloroform fraction of methanolic fruit extract of *D. metel*.
3.5. Pot Trial

3.5.1. Effect of soil amendment and *S. rolfsii* inoculation on incidence and severity of disease

Data regarding the effect of soil amendment with dry biomass of *D. metel* and *S. rolfsii* on incidence and severity of southern blight disease is presented in Table 3.5 and Plate 3.20. In negative control as well as in treatments where pot soil was amended with different doses of dry biomass of *D. metel*, southern blight disease did not appear. In positive control treatment where soil was inoculated with *S. rolfsii*, all the plants were found infected with disease showing 100% disease incidence. According to disease rating scale, disease severity was the highest in positive control i.e. 3. Soil amendment with different doses of dry biomass of *D. metel* showed pronounced effect on incidence and severity of southern blight disease. In treatments with 0.5%, 1.0% and 1.5% soil amendment, there was 33%, 33% and 10% disease incidence with disease severity ranging from 0–1. Further increase in quantity of soil amendment to 2% and higher completely controlled the disease.

3.5.1. Effect of soil amendment and *S. rolfsii* inoculation on shoot growth

Shoot length in negative control was 38 cm. *S. rolfsii* inoculation (positive control) significantly reduced shoot length by 42% over negative control. All the doses of soil amendment significantly improved shoot length under biotic stress of *S. rolfsii*. Different doses of dry biomass of *D. metel* in combination with *S. rolfsii* enhanced shoot length by 0–31% and 77–124% over negative and positive control treatments, respectively. Shoot length in treatments with dry biomass of *D. metel* were generally higher than shoot length in treatments with dry biomass plus *S. rolfsii* (Fig. 3.28A).

Shoot fresh biomass in negative control was 74 g pot\(^{-1}\) that was reduced to 10.4 g pot\(^{-1}\) in positive control due to *S. rolfsii* inoculation resulting in 86% decrease in shoot biomass of bell pepper. All the doses of soil amendment except 3% significantly reduced *S. rolfsii* stress and enhanced shoot biomass by 374–988% over positive control (Fig. 3.28B). The effect of *S. rolfsii* and various soil amendment doses on dry biomass of bell pepper plants was generally similar to the effect on fresh biomass. *S. rolfsii* inoculation reduced shoot dry biomass by 79% over negative control. Different doses of dry biomass of *D. metel* as soil amendment significantly alleviated biotic stress of *S. rolfsii* and enhanced shoot dry biomass by 71–456% over positive control. Shoot biomass was
significantly higher in 1.0–2.5% amendments alone as compared to treatments where these amendments were used in combination with *S. rolfsii* inoculation (Fig. 3.28C).

### 3.5.1. Effect of soil amendment and *S. rolfsii* inoculation on root growth

Root fresh and dry biomass in control was 14.34 g and 2.1 g, respectively, in negative control. *S. rolfsii* inoculation alone reduced root fresh and dry biomass to 1.0 g and 0.2 g in plants of positive control treatment that were significantly lower than biomass in negative control by 93% and 90%, respectively. Soil amendment with different doses of dry biomass of *D. metel* generally stimulated root growth both in the presence and absence of *S. rolfsii* inoculation. In the absence of *S. rolfsii*, root fresh and dry biomass was gradually increased by increasing the dose of dry biomass of *D. metel* up to 2% and declined thereafter. The highest root fresh (13.08 g) and dry biomass (64.8 g) was recorded in 2% soil amendment treatment without *S. rolfsii* inoculation that were 78% and 84% higher than biomass in negative control treatment, respectively (Fig. 3.29 A&B). All the doses of dry biomass of *D. metel* alleviated biotic stress of *S. rolfsii* and significantly enhanced root fresh and dry biomass over positive control. The highest stimulatory effect on root biomass against *S. rolfsii* stress was recorded due to 2.0% dry biomass dose of *D. metel* (Fig. 3.29 A&B).

### 3.5.1. Effect of soil amendment and *S. rolfsii* inoculation on fruit yield

In negative control, average number of fruits per pot was 4.3 which was reduced to 3.2 per pot in positive control. Soil amendment with 0.5 to 2.5% dry biomass of *D. metel* without *S. rolfsii* inoculation had 5 to 6 number of fruits per pot. The highest dose of dry biomass of *D. metel* adversely affected the fruiting and reduced number of fruits to 3.8 per pot. In the presence of *S. rolfsii* inoculation, number of fruits in different doses of dry biomass of *D. metel* was 3.3–5.5 per pot (Fig. 3.30 A, Plate 3.21).

In negative control, fruit fresh biomass was 127.5 g pot$^{-1}$. *S. rolfsii* inoculation significantly reduced fruit fresh biomass to 81 g pot$^{-1}$ that was 36% lower as compared to negative control. Soil amendment with different doses of dry biomass of *D. metel* increased fruit biomass both in *S. rolfsii* inoculated and non-inoculated treatments. In general, there was a gradual increase in fruit biomass with an increase in dose of soil amendment up to 2.0% and a decrease thereafter. The effect of soil amendments was more pronounced in non-inoculated than in *S. rolfsii* inoculated treatments. There was 7–66%
and 68–162% increase in fruit fresh biomass over negative and positive control treatments, respectively, due to soil amendment with different doses of *D. metel* without *S. rolfsii* inoculation. Likewise, soil amendment with *D. metel* dry biomass in *S. rolfsii* inoculated treatments increased fruit fresh biomass by 0.3–46% and 37–130% over negative and positive control treatments, respectively (Fig. 3.30 B).

The effect of *S. rolfsii* inoculation and soil amendment with different doses of *D. metel* on fruit dry biomass was generally similar to that of effect on fruit fresh biomass. In the presence of *S. rolfsii*, different doses of dry biomass of *D. metel* (0.5–2.5%) increased dry biomass of bell pepper fruit by 12–42% and 103–157% over negative and positive controls, respectively. A 3% dry biomass dose in combination with *S. rolfsii* showed lower fruit dry biomass than negative control but enhanced this parameter by 42% over positive control (Fig. 3.30 C).
Table 3.5: Effect of *Sclerotium rolfsii* (SR) inoculation, and soil amendment with dry biomass of *Datura metel* (DBD) on incidence and severity of collar rot disease on bell pepper.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease incidence (%)</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; – Control</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; + Control (SR)</td>
<td>100±0</td>
<td>3</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; 0.5% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; 0.5% DBD + SR</td>
<td>33±10</td>
<td>0 – 1</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; 1% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt; 1% DBD + SR</td>
<td>33±10</td>
<td>0 – 1</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt; 1.5% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt; 1.5% DBD + SR</td>
<td>10±4</td>
<td>0 – 1</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt; 2% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;10&lt;/sub&gt; 2% DBD + SR</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;11&lt;/sub&gt; 2.5% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;12&lt;/sub&gt; 2.5% DBD + SR</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;13&lt;/sub&gt; 3% DBD</td>
<td>0±0</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;14&lt;/sub&gt; 3% DBD + SR</td>
<td>0±0</td>
<td>0</td>
</tr>
</tbody>
</table>

± shows standard errors of means of six replicates.
**Fig. 3.28:** Effect of *Sclerotium rolfsii* (SR) inoculation, and soil amendment with dry biomass of *Datura metel* (DBD) on shoot growth of bell pepper. Vertical bars show standard errors of means of six replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
**Fig. 3.29:** Effect of *Sclerotium rolfsii* (SR) inoculation, and soil amendment with dry biomass of *Datura metel* (DBD) on root growth of bell pepper. Vertical bars show standard errors of means of six replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Fig. 3.30: Effect of Sclerotium rolfsii (SR) inoculation, and soil amendment with dry biomass of Datura metel (DBD) on number and biomass of fruits of bell pepper. Vertical bars show standard errors of means of six replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey’s HSD Test.
Plate 3.20: Effect of *Sclerotium rolfsii* inoculation and soil amendment with dry biomass of *Datura metel* on growth and yield of bell pepper.

<table>
<thead>
<tr>
<th>Plate</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T_1</strong></td>
<td>Negative Control</td>
</tr>
<tr>
<td><strong>T_2</strong></td>
<td>Positive Control [<em>Sclerotium rolfsii</em> (SR) inoculation only]</td>
</tr>
<tr>
<td><strong>T_3</strong></td>
<td>0.5% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_4</strong></td>
<td>0.5% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
<tr>
<td><strong>T_5</strong></td>
<td>1.0% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_6</strong></td>
<td>1.0% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
<tr>
<td><strong>T_7</strong></td>
<td>1.5% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_8</strong></td>
<td>1.5% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
<tr>
<td><strong>T_9</strong></td>
<td>2.0% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_10</strong></td>
<td>2.0% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
<tr>
<td><strong>T_11</strong></td>
<td>2.5% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_12</strong></td>
<td>2.5% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
<tr>
<td><strong>T_13</strong></td>
<td>3.0% amendment of dry biomass of <em>D. metel</em></td>
</tr>
<tr>
<td><strong>T_14</strong></td>
<td>3.0% amendment of dry biomass of <em>D. metel</em> + SR</td>
</tr>
</tbody>
</table>
Plate 3.21: Effect of *Sclerotium rolfsii* (S) inoculation and soil amendment with dry biomass of *Datura metel* (DBD) on fruit yield of bell pepper in different replicates (R). For details of treatments 1–14, see Plate 3.20.
3.6. Physiological Tests

3.6.1. Effect of soil amendments on chlorophyll content

The highest chlorophyll content (0.205 mg g\(^{-1}\)) in leaves of bell pepper was recorded in treatment where soil was amended with 0.5% dry biomass of *D. metel*. In general, chlorophyll was higher in pathogen non-inoculated than in corresponding pathogen inoculated treatments. There was 0.152–0.205 mg g\(^{-1}\) and 0.119–0.164 mg g\(^{-1}\) chlorophyll content in non-pathogenic and pathogen inoculated treatments, respectively. However, difference in chlorophyll content among the treatments was statistically non-significant (Fig. 3.31).

3.6.2. Effect of soil amendments on protein content

The highest protein content (0.291 mg g\(^{-1}\) on fresh weight basis) was recorded in leaves of positive control treatment. There was 0.206 mg g\(^{-1}\) protein in leaves of negative control plants that was 29% lower than positive control treatment. Protein contents in all treatments where soil was amended with different doses of dry biomass of *D. metel*, either with or without *S. rolfsii* inoculation, were similar to that of protein content in negative control and were in the range of 0.176–0.230 mg g\(^{-1}\) (Fig. 3.32 A).

Pattern of protein content in roots of bell pepper in different treatments was different from that of leaves. In general, protein contents were significantly higher in treatments where *S. rolfsii* inoculum was added as compared to non-inoculated treatments. Protein content in negative control and treatments with different doses of dry biomass of *D. metel* without *S. rolfsii* inoculation was in the range of 0.27–0.49 mg g\(^{-1}\). In contrast, protein content in positive control and treatments where *S. rolfsii* was inoculated in combination with different doses of dry biomass of *D. metel* was in the range of 0.67–0.97 mg g\(^{-1}\) (Fig. 3.32 B).

3.6.3. Effect of soil amendments on phenolic content

Leaf and root phenolic contents in negative control were 61 and 34 μg g\(^{-1}\) which were significantly increased to 320 and 280 μg g\(^{-1}\) in positive control, respectively. In all treatments where soil was amended with dry biomass of *D. metel* with or without *S. rolfsii* inoculation, leaf and root phenolic content were comparable to phenolic contents of
corresponding control treatments and were in the range of 48–60 μg g\(^{-1}\) and 32–60 μg g\(^{-1}\), respectively (Fig. 3.33).

### 3.6.4. Effect of soil amendments on peroxidase activity

Leaf peroxidase activity in negative control was 0.82 min\(^{-1}\) mg\(^{-1}\) protein that was enhanced to 1.0 min\(^{-1}\) mg\(^{-1}\) protein in positive control. In all *D. metel* dry biomass soil amendment treatments either with or without *S. rolfsii* inoculation, peroxidase activity was in the range of 0.76–0.97 min\(^{-1}\) mg\(^{-1}\) protein and was insignificantly different from negative control (Fig. 3.34 A).

Root peroxidase activity in negative control was 3.73 min\(^{-1}\) mg\(^{-1}\) protein that was significantly increased to 5.0 min\(^{-1}\) mg\(^{-1}\) protein due to *S. rolfsii* inoculation in positive control. Addition of *D. metel* dry biomass with or without *S. rolfsii* inoculation exhibited an insignificant effect on peroxidase activity with respect to negative control but significantly reduced the studied parameter by 21–28% as compared to positive control (Fig. 3.34 B).

### 3.6.5. Effect of soil amendments on polyphenol oxidase activity

Polyphenol oxidase activity (PPO) in leaf and root showed similar trends in different soil amendment treatments. In negative control, polyphenol oxidase activity in leaf and root was 0.33 and 0.473 min\(^{-1}\) mg\(^{-1}\) protein that was increased to its maximum level i.e. 0.903 and 1.243 min\(^{-1}\) mg\(^{-1}\) protein, respectively, in positive control (174% and 163% increase over negative control, respectively). In different soil amendment treatments, generally, polyphenol oxidase activity was significantly higher (66–141% in leaf and 64–156% in root) where both *D. metel* dry biomass and *S. rolfsii* were added to the soil as compared to treatments where *D. metel* dry biomass alone was added to the soil (3.35 A&B).

### 3.6.6. Effect of soil amendments on phenylalanine ammonia lyase activity

Phenylalanine ammonia lyase (PAL) activity was significantly increased by 15% and 192% in leaf and root, respectively, due to *S. rolfsii* inoculation in positive control as compared to negative control. Likewise, PAL activity was generally significantly higher in treatments where soil was amended with both dry biomass of *D. metel* and *S. rolfsii* as
compared to dry biomass of *D. metel* alone. The effect was more pronounced in roots than in leaves. However, PAL activity in *D. metel* dry biomass plus *S. rolfsii* treatments was significantly lower than positive control (*S. rolfsii* alone) by 8–14% and 22–51% in leaves and roots, respectively (3.36 A&B).
**Fig. 3.31:** Effect of *Sclerotium rolfsii* inoculation and soil amendment with dry biomass of *Datura metel* on chlorophyll content of bell pepper leaves. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Fig. 3.32: Effect of Sclerotium rolfsii inoculation and soil amendment with dry biomass of Datura metel on protein content (mg g$^{-1}$ fresh weight) of leaves (A) and roots (B) of bell pepper. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Fig. 3.33: Effect of *Sclerotium rolfsii* inoculation and soil amendment with dry biomass of *Datura metel* on phenolic content (μg g⁻¹ of fresh weight) of leaves (A) and roots (B) of bell pepper. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey’s HSD Test.
Fig. 3.34: Effect of *Sclerotium rolfsii* inoculation and soil amendment with dry biomass of *Datura metel* on peroxidase (PO) activity of leaves (A) and roots (B) of bell pepper. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Fig. 3.35: Effect of *Sclerotium rolfsii* inoculation and soil amendment with dry biomass of *Datura metel* on polyphenol oxidase (PPO) activity of leaves (A) and roots (B) of bell pepper. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Fig. 3.36: Effect of *Sclerotium rolsii* inoculation and soil amendment with dry biomass of *Datura metel* on phenylalanine ammonia lyase (PAL) activity of leaves (A) and roots (B) of bell pepper. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.
Chapter 4

DISCUSSION

Southern blight of bell pepper caused by *S. rolfsii* is one of the most important diseases which affect the quality and yield of bell pepper. Application of synthetic fungicides such as mancozeb, metalachlor, fluchloralin, alachlor, pendimethalin, captan, copper oxychloride, tridemorph and carboxin is a common practice for control of this pathogen (Singh *et al*., 2005; Madhuri and Gayathri, 2014). However, this practice poses negative attributes, such as simultaneous killing of beneficial microorganisms, potential atmospheric ozone depletion, and health and environmental risks (Akhtar *et al*., 2009; Sheikh *et al*., 2011; Chiejina and Ukeh, 2012). Scientists are in search of eco-friendly alternatives based on naturally occurring compounds to control fungal diseases. Application of botanical products can be a very attractive and environmentally safe method for controlling fungal diseases. Antifungal activity of botanicals has also been shown in many recent studied studies (Amin and Javaid 2013; Javaid and Akhtar, 2015; Javaid and Rauf, 2015). The present study was designed to investigate the antifungal potential of *D. metel* for the management of southern blight of bell pepper. Both laboratory bioassays and pot trials were carried out and very encouraging results were obtained regarding management of the pathogen and the disease by application of extracts and dry biomass of *D. metel*, respectively.

In initial *in vitro* screening bioassays, antifungal activity of methanolic extracts of different parts of *D. metel* was evaluated against *S. rolfsii*. Methanol is generally used for extraction of phytochemicals because most of the compounds from plants are soluble in this solvent. Furthermore, methanolic extracts are preferred over aqueous extracts because of no chance of contamination during extraction (Javaid and Samad, 2012; Rauf and Javaid, 2013). Methanolic extracts of all the parts of *D. metel* showed variable antifungal activities and significantly reduced biomass of *S. rolfsii*. There are numerous examples in literature where plant methanolic extracts controlled fungal growth (Rinez *et al*., 2013; Banaris *et al*., 2015; Javaid *et al*., 2015). Plants have various compounds causig disruption of the cell wall and cell membrane integrity and also cause lesions in the plasma membrane of pathogens (Ansari *et al*., 2013). Phyto constituents also inhibit ergosterol biosynthetic pathway of pathogens by disruption of the cell membrane integrity and loss of the intracellular content. Kumar *et al*. (2011) and Sivakumar (2014) found different
compounds like lipids, active fatty acids, hydrocarbons and active metabolites exhibiting antimicrobial activity. Lipids cause disruption of the cellular membrane. They penetrate into the extensive meshwork of peptidoglycan of the cell wall without visible changes and leads to membrane disintegration. Cowan, (1999) found that alkaloids compounds intercalate with fungal cell wall and DNA. In the present study, variability in antifungal activity among the methanolic extracts of different parts of D. metel was evident. Among the methanolic extracts of all the four plant parts used, fruit extract exhibited the best antifungal activity followed by stem and leaf extracts resulting in 69-94%, 69-86% and 29-88% reduction in fungal biomass over control, respectively. Root extract showed the least antifungal activity resulting in 9-32% fungal growth inhibition. Methanolic extract of Datura fruits contain high amount of phenolics and flavonoids (Hossain et al., 2014), which may be responsible for its antifungal activity (Kanwal et al., 2010). Rinez et al. (2013) reported that methanolic leaf extract of D. metel acts as natural fungicides against Fusarium oxysporum f. sp. melonis, F. oxysporum f. sp. lycopersici and F. oxysporum f. sp. tuberosi. In the present study, variation in antifungal activity in different plant parts may be attributed to the presence of different types of compounds in different parts of the test plant species (Amin and Javaid, 2013). Similar variable antifungal effects of methanolic extracts of different parts of allelopathic plants like C. album, C. didymus, S. cumini, W. somnifera and M. azedarach have also been reported against fungal plant pathogens namely S. rolfsii, Ascochyta rabiei Lib. and M. phaseolina (Javaid and Amin, 2009; Iqbal and Javaid, 2012; Javaid and Saddique, 2011; Khan and Javaid, 2013; Javaid et al., 2015). In the present study, generally there was a gradual reduction in fungal biomass with increasing extract concentration. This concentration dependent activity of the plant extracts is in agreement with the finding of earlier workers, where higher concentrations of methanolic extracts of different parts of allelopathic plants like C. album, C. didymus and Eclipta alba (L.) Hassk exhibited greater antifungal activity than the lower ones (Iqbal and Javaid, 2012; Rauf and Javaid, 2013; Banaris et al., 2015). However, by contrast there are also reports where extract concentrations were negatively correlated with the fungal growth (Naqvi et al., 2012).

Methanolic leaf, stem and fruit extracts were selected for further fractionation because of their pronounced antifungal activity in screening bioassays. Various organic solvent fractions viz. n-hexane, chloroform, ethyl acetate and n-butanol were partitioned from the crude methanolic extracts. This technique is very useful for separation of compounds on the base of their polarity as these organic solvents have different polarities.
When these are successively employed in an increasing order of their polarity, organic compounds of methanolic extracts having different polarities are dissolved in different solvents and become separated (Javaid et al., 2014). Different concentrations ranging from 3.125 to 200 mg mL\(^{-1}\) of these organic fractions of methanolic extracts were tested in vitro for their antifungal activity against *S. rolfsii*. A marked variation in antifungal activities among different organic fractions was noticed. Chloroform fractions of methanolic leaf, stem and fruit extracts showed highly pronounced activity resulting in 30-44%, 50-71% and 36-50% reduction in fungal biomass over corresponding control treatments, respectively. Similar variable antifungal potential of different organic solvent fractions of methanolic extracts of *W. somnifera, S. cumini, Imperata cylindrica* and *M. azedarach* has also been reported against other fungal species (Javaid and Munir, 2012; Amin and Javaid, 2013; Khan and Javaid, 2013; Javaid et al., 2015). Variation in antifungal activities of different organic solvents fractions might be attributed to presence of different chemical nature compounds in different fractions. Earlier, Monira and Munan (2012) and Sharma (2002) found the highest antifungal activity of chloroform fraction of *D. metel* against *Aspergillus fumigates* Fresenius, *Aspergillus flavus* Link and *Aspergillus niger* Van Tieghem. Likewise, Rehman et al. (2015) reported the antifungal activity of chloroform extract of *Oxalis corniculata* L. against *Aspergillus flexner* Micheli, *A. flavus* and *F. solani*, and also identified the compounds 5,7,4′-trihydroxy-6,8-dimethoxyflavone and 5-hydroxy-6,7,8,4′-tetramethoxyflavone as an antifungal agent from this fraction.

Chloroform fractions of methanolic leaf, stem and fruit extracts of *D. metel* exhibited the best antifungal activities and thus selected for further analysis by GC-MS. GC-MS spectra of chloroform soluble fractions of methanolic leaf, stem and fruit extracts of *D. metel* revealed 18, 6 and 12 compounds, respectively. Eighteen compounds identified in chloroform fraction of methanolic leaf extract comprised of alkenes group (24%), alcohol (32%), aldehyde (7%), arenes (5%) and esters (32%). Among the 12 different compounds identified in chloroform fraction of methanolic fruit extract, alcohols, alkenes, arenes and esters were 42%, 20%, 6% and 32%, respectively. Likewise, in chloroform fraction of methanolic stem extract identified compounds belonged to carboxylic acid (51%), alcohols (38%) and alkenes (11%). Earlier, Tuney et al. (2006) reported that antimicrobial activity may be related to volatile compounds such as terpenoid, volatile fatty acids compounds, phenols, phytol (acyclic diterpene alcohol), alkenes and esters. Lee et al. (2008) and Kim and Park (2012) found significant antifungal activity of alcohols (citronellol and geraniol) and aldehydes (neral and geranial) isolated
from *Acorus gramineus* (Sol.) Aiton against *Phytophthora cactorum* (Libert and Cohn) J. Schort., *Cryphonectria parasitica* (Murrill) Barr, *Fusarium cincinatum* Nirenberg and Donnell and three *Aspergillus* species. There were significant differences in the antifungal activities among compounds having the same functional group. It has been noticed that the position of the hydroxyl group is related to the antifungal activity. Antifungal activity of primary alcohols (citronellol and geraniol) was greater than those of secondary (isopulegol) and tertiary alcohols (linalool). Manohar (2015) identified 21 bioactive components from *Geodorum densiflorum* Lam. through GC-MS where alcohols and esters had antimicrobial activity. Kolawole *et al.* (2015) identified 78 organic compounds in *Senna alata* (L.) Roxb. through GC-MS and found that aldehydes, alkanes, alkenes, fatty alcohol, acetic acid, ketones and ester had good antifungal activity. Pohl *et al.* (2011) reported that alkene and fatty acids have antifungal activity. They disrupt the cell membrane of the cells have less sterol content. Alkenes inhibited the myrisoylation of proteins of cell membrane. They also inhibited b-oxidation, triacylglycerol and sphingolipid synthesis and topoisomerase activity. Kiruthika and Sornaraj, (2011) identified the alkenes in the *D. metel* flower through GC-MS having antimicrobial activity. Ganie *et al.* (2015) studied the antimicrobial activity of *Holoptelea integrifolia* Roxb. seeds against *Staphylococcus aureus* Rosenbach, *Pseudomonas aeruginosa* Schrotter, *Klebsiella pneumoniae* Schroeter and *Salmonella typhimurium* Lignieres. The extracts of compounds showed C=O and -OH as the major functional groups, which indicated the presence of ester, phenolic and alcoholic compounds. Frelesleben and Jäger (2014) observed the nitrogen containing amines and aldehydes has antifungal mechanisms of action is mainly by disruption of cell membrane, by inhibition of ergosterol biosynthesis, or by formation of complex with ergosterols. These also interfere with the biosynthesis of chitin because chitin is a part of the fungal cell wall. These compounds expressed antifungal mechanisms of action through some interference with the cell wall and cell membrane. McDonnell and Russell (1999) studied the antifungal and microbial activity of alcoholic group like, ethanol, alcohol, isopropanol, and *n*-propanol. Alcoholic compounds cause cell membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis. Kubo *et al.* (2012) observed the aldehydes and alcohols antifungal mechanisms. Aldehyde and alcohol act as nonionic surfactants and disrupt the hydrogen bonding and denature the functional confirmation of lipid-protein, integral proteins and transport proteins.
High peak percentage area of different compounds in leaf of *D. metel* were 1,2-benzenedicarboxylic acid (24.86%), 1-hexacosanol (9.64%), 1-octadecene (8.01%), 1-eicosanol (5.09%), 1-octadecanol (4.97%), and 1-heptadecene (4.97%), 2-methyl-3-phenyl-2-propenal (4.78%) and 1,6,10-farnesatrien-3-ol (4.78%). In stem, compounds peak percentage area were 11.41% each of oleic acid and hexadecanoic acid (palmitic acid), 8.01% of 1-octadecanol, 4.97% of 1-heptadecene. In *D. metel* fruit compounds peak percentage area were 24.86% of 1,2-benzenedicarboxylic acid, 9.64% of 1-hexacosanol, 8.01% of 1-octadecanol and 1-octadecene, 5.09% of 1-eicosanol and 4.78% of 1,6,10-farnesatrien-3-ol. Many of these identified compounds are known to have antifungal activities against other fungal species. Agoramoorthy et al. (2007) determined the antifungal properties of fatty acids methyl esters such as oleic acid and palmitic acid isolated from *Excoecaria agallocha* L. against *Candida Parapsilosis* Berkh., *Candida albicans* (Robin) Berkhout and *Candida krusei* (Castellani) Berkhout. Vila et al. (2013) isolated hexadecanoic acid from dichloromethane bark extract of *Calycophyllum spruceanum* (Griseb.) Chodat & Hassl and found its antifungal activity against *Tricophyton mentagrophytes* and *Microsporum gypseum*. Mallaiah and Muthamilan (2015) identified the antifungal activity of 1,2-benzenedicarboxylic acid, octodecanoic acid and hexadecanoic acid isolated from of crossandra (*Crossandra infundibuliformis* (L.) Nees) against *Fusarium incarnatum* (Desm.) Sacc. Similarly, Devi et al. (2015) identified antifungal activity of hexadecanoic acid from petroleum ether fraction of leaves of *Zanthoxylum acanthopodium* DC. against *A. niger*, *A. flavus*, *A. fumigates*, *Candida krusei* and *C. albicans*. Unnithan et al. (2014) identified the antifungal long chain alcoholic compounds such as tetra-decanol and do-decanol from leaf extracts of *Eupatorium odoratum* L. against *C. albicans*. Al-Shammari et al. (2012) found the antimicrobial activity of 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; 1-octadecene; 1-hexadecene and 1-octadecanol isolated from *Carduus pycnocephalus* L. Panda and Bandyopadhyay (2013) determined the antimicrobial activity of methanol, chloroform, acetone and benzene extracts of *Andrographis paniculata* (Burm.) Wall. and *D. metel*. They found 1,2-benzenedicarboxylic acid and bis (2-methylpropyl) ester in *D. metel* having antimicrobial activity. Sombie et al. (2013) found 1-octadecene and 1-hexadecene from *Guitera senegalensis* J.F.Gmel as antifungal agents against different kinds of fungi in vitro. Devi et al. (2015) identified 43 compounds from *Zanthoxylum acanthopodium* DC. leaves by GC-MS and found antifungal property of hexadecanoic acid against *Candida albicans* and *C. krusei*. 
In pot trial, different doses of dried powdered biomass of *D. metel* were mixed in *S. rolfsii* inoculated soil to manage southern blight disease. *D. metel* markedly reduced the biotic stress of *S. rolfsii* and enhanced plant growth and fruit yield of bell pepper. The highest disease incidence (100%) and severity was recorded in positive control where soil was inoculated with *S. rolfsii* without any amendment. Soil amendment with 0.5% to 2% *D. metel* dry biomass gradually and significantly alleviated the biotic stress with increasing dose of soil amendment. All the *D. metel* biomass amended treatments variably reduced the pathogen effect and enhanced growth and yield of bell pepper. Soil treatment with 1.0 to 2.5% *D. metel* biomass gave significant results in various aspects of bell pepper growth and yield. Different withanolide and other antifungal compounds present in leaves of *D. metel* released in the rhizospheric soil by decomposition of residues or by extraction in the irrigation water and caused suppression of fungal growth (Javaid and Saddique, 2011). Earlier, there are reports that soil amendment with dry biomass of various plant species can reduce incidence and severity of soil-borne fungal diseases (Javaid and Saddique, 2011; Javaid and Iqbal, 2014). Troncoso et al. (2005) found that disease incidence and severity by *Alternaria alternata* can be significantly reduced by incorporation of Brassicaceous plant materials in the soil. Ikram and Dawar (2013) used dried plant parts of *Prospis juliflora* (Sw.) DC. for the control of root rot diseases in *Vigna unguiculata* L. and *Vigna radiata* (L.) Wilczek caused by *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*. Javaid and Saddique (2011) controlled root rot of mungbean caused by *Macrophomina phaseolina* by using *D. metel* dried leaf manure as soil amendment. They found that 1.5% dose of soil amendment significantly suppressed disease severity and enhanced root and shoot growth as compared to positive control. Javaid and Rauf (2015) managed the basal rot of onion caused by the fungus *Fusarium oxysporum* by soil amendment with dry leaf biomass of *Chenopodium album*. Dried powder of botanicals are not only effective in disease suppression but bestow bonus remuneration over composts like reduced bulk density, increased shelf-life, consistent composition and also reduce the pathogen’s inoculums for future crops. Botanicals may be added to the soil a few weeks before sowing of crop so that they initially release antimicrobial toxins, kill the pathogen and later lose toxicity by decomposition & provide good source of organic biomass for the crop which would be sown few weeks later. Quick loss of toxicity of organics is environment friendly and in sharp contrast to chemicals used for soil sterilization which are soil pollutants, causing devastating effect by biomagnification (Javaid and Saddique, 2011; Singh and Garampalli, 2013; Javaid and Rauf,
Amendments not only change physical and chemical properties of soil but also support a wide variety of antagonistic microorganisms (Timm et al., 2001). The addition of soil amendments results in a considerable increase in the liberation of CO$_2$ which enhances the activities of soil saprophytes, which can suppress the activities of disease-causing agents. Due to rapid multiplication of micro-organisms within the soil, soil nitrogen, which is often scarce, is quickly consumed resulting in nitrogen deficiency and its deficiency greatly reduces the growth of pathogens (Hussain et al., 2011). Volatile compounds released by decomposition of plant dried materials act as fungistatic or fungicidal by reducing the hyphal growth and by releasing chitin which suppress the sclerotial germination. The mechanisms of plant soil amendment include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Blum and Rodríguez-Kában, 2004; Sowley et al., 2014). Soil amendment with dried toxic plants suppresses plant pathogens directly by releasing toxic substances like phenols. (Blazevic et al., 2010; Hussain et al., 2011; Ikram and Dawar, 2013).

Results of the present study showed that *S. rolfsii* induced considerable alteration in physiology and biochemistry of the bell pepper tissues (leaf and root), whereas stress on these metabolic activities was alleviated due to soil amendment with dry leaf biomass of *D. metel*. Total chlorophyll contents were decreased in pathogen inoculated treatments than in corresponding pathogen non-inoculated treatments. Contribution of photosynthetic process in growth, development and yield of plants is well-known (Zhu et al., 2012). Negative effect of *S. rolfsii* on chlorophyll content could be attributed to degeneration of chloroplast due to production of oxalic acid by the fungus which probably resulted in rupturing of chloroplast membrane (Tariq and Jeffries, 1985; Perveen et al., 2010). Likewise, decline in chlorophyll content due to fungal infection was found to be linked with decrease in protein nitrogen content (Pinto et al., 2000). Improvement in chlorophyll content due to soil mixing with different doses of dry biomass of *D. metel* could be due to allelopathic effects of a variety of phytochemicals in *D. metel* against *S. rolfsii*. Thus an increase in chlorophyll likely enhanced photosynthetic rate with an increase in plant growth, biomass and yield as was recorded (Abassi et al. 2010).

Total protein content of leaf of bell pepper was increased due to inoculation with *S. rolfsii*. Protein content in all treatments where soil was amended with different doses of dry biomass of *D. metel*, either with or without pathogen inoculant were similar to that of protein content in negative control. However, in root protein content in positive control
and treatments where *S. rolfsii* was inoculated in combination with different doses of dry biomass of *D. metel* was significantly higher as compared to negative control and treatments with different doses of dry biomass of *D. metel*. Enhancement in content of protein after pathogen infection might be obvious sign of hypersensitive response (HR) probably due to synthesis of stress related protein in host (Ashry and Mohamed, 2012). Thus variation in total protein content of leaf and root could be attributed to differential demand of substrate necessary to the production of plant defense mechanisms in roots, which was the immediate site of contact with pathogen in soil. Decrease in protein content of leaf and root after soil amendment with dry biomass of *D. metel* might be linked with suppression of reactive oxygen species by antioxidant enzymes that could plays a key role in the development of efficient defense responses in plants by enhancing pathogen-induced HR-mediated cell death (Tahani *et al.*, 2010).

Phenolic contents of leaf and root were significantly increased due to inoculation with *S. rolfsii*, however, in rest of treatments it was declined. Accumulation of phenolic compounds from surrounding healthy tissue might be the cause of increase in its content during stress conditions (Patel *et al.*, 2015). However, phenolic contents were reported to increase with increase in aging, wounding and infection by the fungal pathogen (Ndoumou *et al.* 1996). Phenolics act generally as substrate for synthesis of compounds like pterocarpan, phytoalexins and hydroxycinnamic acid esters (Dixon and Lamb, 1990), which possibly work against the pathogen. Insignificant alteration in the phenolic contents of leaf and roots after soil mixing with plant biomass could directed the effective disease management potential of *D. metal* through its secondary metabolites by different mode of actions.

Like phenolic contents, activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) of both leaf and root were increased in treatments inoculated with *S. rolfsii*, and in rest of the treatments these were insignificantly different as compared to negative control. Enhancement in activities of all the three enzymes after pathogen inoculation could be due to stress caused by pathogen infection. All the three enzymes are known to be activated during stress conditions to provide resistance against biotic and abiotic stresses. The current investigations were in accordance with Gurjur *et al.* (2015), who reported increase in PO activity in both susceptible and resistant cultivars of grapes due to fungal infection. Presently, PO likely worked as signal of general host defense response rather function as defense mechanism (Asada, 1992). Interference in H$_2$O$_2$ concentration and other metabolites by augmented
enzyme activity could be linked with higher activity of *S. rolfsii* in plant tissue. PPO and PAL accumulate during pathogen attack and functions to catalyze oxidation of phenols and these were reported to produce in greater amount in resistance variety than the susceptible one (Moeen *et al*., 2014). However, plant cell membrane disruption occurrence might initiate formation of quinines following an increase in accessibility of PPO to its substrate. Enhancement in activity of PPO has been found in cucumber leaf nearby lesion caused by pathogen (Avdiushko *et al*., 1993). This might reveal that induction in PPO enzyme could be defense responsive against *S. rolfsii* infection at the early stage in bell pepper (Zheng *et al*., 2005). Increase in activity of PAL has been noticed during both incompatible and compatible interactions between plants and pathogens (Harllen *et al*., 2004). Like PPO, early induction of PAL by *S. rolfsii* infection probably resulted in activation of plant defenses, but subsequently pathogen caused collar rot and mortality, that might cause blockage of the activation of defense related enzymes (Zheng *et al*., 2005). Besides, dried biomass of *D. metel* could be able to induce resistance against *S. rolfsii* without considerable accumulation of PO, PPO and PAL in bell pepper tissues could be due to inhibition in growth of *S. rolfsii*. That accumulation was not correlated with soil amendment but well-lined in treatments when pathogen was inoculated. It could be concluded that soil amendment with dried plant biomass of *D. metel* could manage stress of collar rot disease without causing change in content of phenolics and activities of PAL and PPO.
Conclusion

- Chloroform fractions of methanolic extracts of different parts of *D. metel* were found highly antifungal against *S. rolfsii*.
- Among the isolated compounds, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; 1-hexadecene; pentadecene,1-eicosanol; 7-tetradecene and 1,3(15),10-bisabolatriene are likely to be responsible for antifungal activity.
- Soil amendment with 2% dry biomass of *D. metel* can completely control southern blight of bell papper.

Future Prospects

- Structures of the major antifungal compounds can be used as analogues for preparation natural product based fungicides for the management of *S. rolfsii*.
- Disease management potential of 2% dry biomass of *D. metel* as soil amendment can be assessed under field conditions.


Hossain, M.H., K.M. Al Sabari, A.M. Weli and Q. Al-Riyami. 2014. Gas chromatography-mass spectrometry analysis and total phenolic contents of various


