BIO- INTENSIVE MANAGEMENT OF DAMPING – OFF OF TOMATO (*Solanum lycopersicum* L.)

Thesis

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CERTIFICATE – I

This is to certify that the thesis titled **"Bio-intensive management of damping-off of tomato (*Solanum lycopersicum* L.)"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) –173230 is a bonafide research work carried out by **Miss. Swadha Bhardwaj (H-2017-72-M)** daughter of Shri Deepak Kumar Bhardwaj under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis titled, **"Bio intensive management of damping-off of tomato (*Solanum lycopersicum* L.)"** submitted by **Miss. Swadha Bhardwaj (H-2017-72-M)** daughter of Shri Deepak Kumar Bhardwaj to Dr.Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173230, India in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place: Nauni, Solan

Date:…….. **(Swadha Bhardwaj)**

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important and remunerative vegetable crop all over the world, known as protective food because of its nutritive value and availability round the year. It belongs to family Solanaceae having chromosome number 2n =24. It is originated in Mexican and Peruvian region. It is considered as the second most significant vegetable crops in the world next to potato (Mohamed *et al*., 2010).

Tomato provides taste palatability as well as increases appetite. Large quantity of tomato is used to produce soup, juice, ketchup, puree, paste, pickles and powder. From the nutritional point of view, calorific value of tomato ranges from 20 to 40 calories per 100g. It is an excellent source of antioxidants including vitamins C, E, and K, lycopene, betacarotene, flavonoids and lutein (Dorais *et al.,* 2008) and contains high amounts of trace elements like Mn, Zn, Fe, and Cu (Ahmed *et al*., 2011). The ripe fruits have 94.5 per cent water, 3.9 per cent carbohydrate,1 per cent protein, 0.1 per cent mineral matter (0.02% phosphorus, 0.01% calcium, 0.0001% magnesium) and 0.1 per cent fat (Sheth, 2014).

Worldwide production of tomatoes reached 183.9 million tonnes in 2016 over an area of 7.6 million hectares (FAO, 2018). China is the major producer of tomato followed by India, USA, Turkey and Egypt (FAO, 2017). India ranks second in area as well as in production of tomato with about 7.97 lakh ha area with the production of 20.7 million tonnes (NHB, 2017). Madhya Pradesh is the leading state followed by Andhra Pradesh and Maharashtra in terms of area under tomato crop in India, however, in Himachal Pradesh, the annual production is 5.02 lakh tonnes from an area of 11070 ha (Department of Agriculture, 2019).

Tomato is generally grown as winter crop in plains of India but in Himachal Pradesh, it is cultivated as an off-season crop during April to October. It is prone to attack by various fungal, bacterial, viral and nematode diseases which may be seed borne, soil borne, wind borne or vector borne. Among soil borne diseases, damping-off, which may be pre-emergence or post-emergence, is serious at nursery stage. It is responsible not only for the poor seed germination but also for carryover of the pathogens to the field where transplanting

is done for raising the crop. Infected seedlings provide an ideal avenue for rapid and efficient spread of disease caused by soil and seed borne pathogens (Shyam, 1991).

In tomato nurseries, damping-off is mainly caused by *Pythium aphanidermatum* (Edson) Fitz. (Singh, 1985; Srivastava and Tiwari, 2003) and *Rhizoctonia solani* Kuhn (Katan *et al*., 1980; Khare *et al*., 2000). Singh (1987) reported the association of different fungi like *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotium, Phomopsis, Colletotrichum* etc. with damping-off in vegetable nurseries.

Prasad *et al*. (2017) reported soil borne fungal pathogens such as *Pythium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* infecting the tomato crop and causing damping-off disease. Species of *Fusarium*, *Rhizoctonia* and *Sclerotinia* fungi persist in soil because they produce resistant survival structures such as melanized hyphae, chlamydospores and sclerotia which make it hard to control.

Damping-off of tomato can be managed successfully by using cultural and biological methods. Among various cultural methods, soil solarization is most important. It is carried out by moist soil with polythene sheet during summer months for 4-6 weeks (Katan *et al*., 1976). Raj *et al*. (1997) first flood irrigated nursery bed soil and then covered with transparent polythene sheet (25 µm) for 40 days which resulted in killing of *Pythium* spp. and *Fusarium* spp. up to 30 cm soil depth. This lowered the incidence of pre-emergence damping-off and gave better seedling vigour. Higher temperature during soil solarization resulted either in killing of propagules or it inactivated the pathogen by various other mechanisms (Katan, 1981; Negi and Raj, 2013).

Antagonists of fungal or bacterial origin have been studied well for the management of plant diseases. *Trichoderma* is commonly found in soil and decaying organic matter as a saprophytic fungus. Species of *Trichoderma* have been extensively tested and used as biocontrol agent against wider range of plant pathogens *viz., Rhizoctonia, Fusarium, Sclerotium, Macrophomina, Sclerotinia, Pythium* spp. etc. (Papavizas, 1985; Chet, 1987; Hornby, 1990; Deacon, 1991). Similarly, use of *Brassica* plants as biofumigant, application of biocontrol agents like *Trichoderma* and *Pseudomonas* were found best in reducing damping-off incidence in tomato nursery (Thakur and Tripathi, 2015).

Environmental concern for management of soil-borne pathogens has lead to more interest in searching eco-friendly alternatives. Natural products isolated from plants causes minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). The use of plant extracts has been shown to be ecofriendly and effective against many plant pathogens (Saadabi, 2006; Gachomo and Kotchoni, 2008; Thobhunluepop, 2009; Duru and Onyedineke, 2010), most of these substances were evaluated in order to find a safe alternative control methods to human and environment.

Induced host resistance has also emerged as an important tool for disease management with minimal negative impact on the environment. Sticher *et al.* (1997) reported that induced systemic resistance or systemic acquired resistance (ISR or SAR) offers a viable alternative for eco - friendly management of plant diseases. Induction of systemic or localized resistance (ILR) has been successfully attempted in several hosts against different pathogens. Kessmann *et al.* (1994) and Hammerschmidt and Kuc (1995) reported that induced resistance can be triggered by certain chemicals, non-pathogens, plant growth promoting rhizobacteria (PGPR) and avirulent forms of pathogens.

Keeping in view, the importance of the crop and the damage caused by the pathogen(s), the present studies were envisaged with the following objectives:

Objectives:

1) To isolate and identify the associated pathogen(s)
2) To evaluate the efficacy of biological control agents, botanicals and resistance inducing chemicals against the pathogen
3) To devise suitable integrated disease management strategies against the disease by using soil solarization and different combinations of bio control agents, botanicals and resistance inducing chemicals

Chapter-2

REVIEW OF LITERATURE

The pertinent literature on various aspects of damping-off of tomato has been reviewed under the following headings:

2.1 GEOGRAPHICAL DISTRIBUTION

Damping-off of tomato is a widely distributed disease throughout the world. This disease in nurseries have been reported from Philippines (Ramos, 1926), Kent, Britain (Salmon and Ware, 1931) and Ohio, USA (Alexander *et al*., 1931), Nelspruit, South Africa (Wager, 1933), Germany (Chamberlain and Brien, 1937), Egypt (Matta, 1971) and Cameroon (Fontem, 1993).

In India, *Pythium aphanidermatum* causing damping-off was found to occur in a parasitic form on chilli seedlings (McRae, 1928). Singh and Shrivastava (1953) found that damping-off caused heavy damage to tomato seedlings in greenhouses and open fields at Kanpur and reported it as a serious problem in the tomato nurseries. Whipps and Lumsden (1991) reported that pre- and post-emergence damping-off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide. Similarly, Moulin *et al*. (1994) observed that damping-off is a serious problem for plant producers and has caused severe economic losses in commercial greenhouse vegetable production.

Among the various diseases in vegetables, *Sclerotium rolfsii, Fusarium oxysporum* and *Rhizoctonia solani* causing damping-off has been reported as the major constraints of vegetable cultivation in our country (Singh, 1984; Das *et al*., 2000). In tomato nurseries, damping-off is mainly caused by *Pythium aphanidermatum* (Edson) Fitz. (Singh, 1985; Srivastava and Tiwari, 2003) and *Rhizoctonia solani* Kuhn (Katan *et al*., 1980; Khare *et al*.,

2000). The genus *Pythium* includes a number of readily recognized species with wide distributions and host ranges (Singh, 1985; Srivastava and Tiwari, 2003) reported the association of different fungi like *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotium, Phomopsis*, *Colletotrichum* etc. with damping-off in vegetable nurseries. Lucas *et al.* (1997) also reported the association of various species of *Fusarium, Verticillium*, *Pythium* and *Rhizoctonia* responsible for the soil borne fungal diseases, wilt of flora and damping-off of seedlings.

Rhizoctonia solani (teleomorph: *Thanatephorus cucumeris*) can affect germination and emergence in tomatoes and causes basal rot of seedlings (Rivera *et al*., 2004). Jiskani *et al.* (2007) observed that *R. solani* Kuhn was isolated with highest frequency (60.0%) followed by *F. oxysporum* f.sp. *lycopersici* from root portion of infected tomato seedlings while the fungi with lowest frequency were *Macrophomina phaseolina*, *Alternaria solani* and *Verticillium albo-atrum.* Siddiqui *et al.* (2016) reported that *Fusarium oxysporum, Sclerotium rolfsii* and *Macrophomina phaseolina* are more common fungal pathogens among hundreds of soil-borne microorganisms which cause diseases in a large number of plant species.

2.2 SYMPTOMATOLOGY

Symptoms of the disease appear in two phases *viz*., pre-emergence and post-emergence. Pre-emergence damping-off causes seeds and young seedlings to rot before they emerge from the growing medium and results in patchy growth while ‘post-emergence’ damping-off kills newly emerged seedlings by causing a water-soaked, soft brown lesion at the stem base, near the soil line, that pinches off the stem causing the seedling to topple over and die. In the first case, seeds become soft, rotten, and fail to germinate. In the second case, stems of germinating seeds are affected with characteristic water-soaked lesions formed at or below the soil line (Cram, 2003; Landis, 2013). Post-emergence damping-off symptoms occur when seedlings decay, wilt and die after emergence. Seedling stems can become thin and tough (commonly known as “wirestem”), which often leads to reduced seedling vigor. These symptoms can also be accompanied by leaf spotting and a complete root rot may occur. Overall, the symptoms on the stem of the seedlings include water-soaked, sunken lesion at or slightly below the ground level and sometime also below ground line (i.e., on the roots) causing the plant to fall over (Wright, 1944; Filer and Peterson, 1975).

Anwar (1989) described diseases of seedlings such as damping-off, seedling blight, seed rot and hypocotyl rot. It is the complex syndrome including pre-germination decay of seed, post-emergence damping-off and seedling root rot. Different types of symptoms are associated with damping-off that bring about the death of at least some seedlings in any particular population. Symptoms start with round spots on seedlings and then having stem abrasions at surface level. Seedling vigor is reduced because stems may additionally grow to be skinny and hard. Occasionally, leaf spotting accompanies further symptoms, as ensured by a grey mildew boom on leaves and stems (Chana *et al.,* 1997). Mary (1998) reported that damping-off is caused by several different organisms and described symptoms of damping-off of the stems and roots of seedlings were black and rotted. Often entire root system was soft and the outer root sloughs off and stems often girdle or become water soaked and soft causing damping-off.

In symptoms of damping-off caused by *Rhizoctonia solani*, plants become stunted with typical discoloration at roots in early stage and form small lesions on roots near to soil level. The spots coalesce together and form large areas. In severe infection, the entire root was found rotted and plants were dried (Jiskani *et al.,* 2007). In mature plants, *Pythium* causes crown and root rot, where plants suddenly wilt when weather turns warm and sunny and when plants have their first heavy fruit load. In the root system, initial symptoms appear as brown to dark-brown. Radical and plumule of young seedlings undergo decay before emerging out of soil line and a water soaked zone near the soil surface is observed in soft succulent seedlings. They soon become necrotic and sunken which later topple down often before wilting (Crous, 2002; Akhtar *et al*., 2012; Horst, 2013).

2.3 CAUSAL ORGANISM

The damping- off disease of seedlings was first time investigated in Germany by Atkinson (1895) and *Pythium* spp. were considered as causal organism. The genus *Pythium* is cosmopolitan in its distribution with wide host range. The most of the species are soil dwellers and are found in aquatic habitats. Though ordinarily they are saprophytes in nature. Many become destructive pathogen inciting varied type of diseases viz. damping-off of seedlings, seed rots, seedling blight, root rots, collar rot and fruit rot, etc. both in the field and under storages during favourable weather conditions. Fourteen species of *Pythium* have been found to be associated with vegetable and fruit crops (Waterhouse, 1967).

Seed rot and damping-off of seedlings are incited mainly by *P. aphanidermatum* (Edson) Fitz., *P. adhaerens* Sparrow, *P. acanthicum* Drechsler, *P. angustatum* Sparrow, *P. irregulare* Buisman, *P. daroecandrum* Drechsler, *P. rostratum* Butler, *P. splendens* Braun, *P. vexons* de Barry, *P. ultimum* Trow (Hooker, 1953). Edson (1915) also described *P. aphanidermatum* as the causal organism of damping-off of sugar beets (*Beta vulgaris* L.) as well as radish (*Raphanus sativus*) in Wisconsin, USA. He was the earliest worker to report the causal organism of tomato seedlings as *Pythium* spp. Later on, Humbert (1918) and Berkley (1925) as well as Weber and Ramsay (1926) reported *P. debaryanum* to be the causal organism of damping-off in tomatoes. From India, Ayyer (1929) reported that *P. aphanidermatum* isolated from *Opuntia dellenii* can cause the blight in tomato seedlings. Similarly, Alexander *et al.* (1931) reported *P. ultimum* to cause severe damping-off of seedlings.

Damping-off caused by *Fusarium oxysporum,* is one of the most prevalent diseases of tomato (Ries *et al.,* 2005; Sudhamoy *et al.,* 2009). The pathogen occurs throughout most tomato growing areas worldwide causing a vascular wilt that can severely affect the crop (Moretti *et al*., 2008) and the disease is considered as one of the main soil borne systemic diseases (Schwarz and Grosch, 2003). Similarly, El-Mohamedy *et al*. (2014) reported that tomato plants are also infected by several soil-borne fungal pathogens like *Fusarium* spp.*, Rhizoctonia solani* and *Sclerotium rolfsii* which cause serious diseases such as wilting and rotting of the roots, the very thing that finally, reduces crop yield and quality. The occurrence of *Fusarium* is one of the problems that are the most limiting to the growth of seedlings in nurseries. This cosmopolitan pathogen is a native inhabitant of soil and is difficult to control. *Rhizoctonia solani* is an active mycelium in the soil and attacks more than 2000 species of plants. This fungus also causes considerable losses due to damping-off disease annually (Dawar *et al.,* 2007). With a wide host range, *Rhizoctonia* can cause a variety of diseases including stem rot, root rot, damping-off in seedlings and aerial blight of leaves. This pathogen can be found across all areas of the country. The severity of infection can vary from high to low resulting in major yield losses (from 25 to 100%). *R. solani* primarily attacks seeds of plants below the soil surface, but can also infect pods, roots, leaves, and stems. The most common symptom of *Rhizoctonia* is "damping off", or the failure of infected seeds to germinate. It may invade the seed before it has germinated to cause this pre-emergence damping-off, or it can kill very young seedlings soon after they emerge from the soil. Seeds

that do germinate before being killed by the fungus have reddish-brown lesions and cankers on stems and roots.

Sclerotium rolfsii is a widespread phytopathogenic fungus affecting a large number of agricultural plants (Bateman and Beer, 1965). Tomato plants are highly susceptible to it. It is a soil-borne pathogen that attacks over 500 different plant species. The fungus infects the lower stem near the soil surface and for some plants, the roots may be infected. Similarly, stem and root rot disease of tomatoes caused by *S. rolfsii,* are serious diseases as they greatly reduce the yield of the crop. This pathogen is a major problem in tomato crops in the warm, moist tropical regions of the world (Aycock, 1966), causing damping-off of nursery seedlings as well as stem rot, wilting and blight in adult plants (Flores-Moctezuma *et al*., 2006) with consistent loss of production (De Curtis *et al*., 2010). It was first observed by Peter Henry Rolfs in the year 1892 on tomato plants with 70 per cent losses (Dwivedi and Prasad, 2016).

2.4 MORPHOLOGICAL CHARACTERS

2.4.1 *Pythium ultimum*

The mycelium of *Pythium* consists of slender, coenocytic, hyaline hyphae which survive in the soil saprobically on dead organic matter or parasitically on the young seedlings of many susceptible species of different host. In host tissues, the hyphae are both intercellular and intracellular, no huastaria are produced. The sporangia of the organism constituting the asexual stages are globose to oval and one either terminal or intercalary on the somatic hyphae and germinate by producing generally zoospores. Germination is either by zoospores or rarely by germ tubes, production of zoospores preceded by the formation of bubbles like vesicles at the tip of a tube that issues from the sporangium. The sporangial protoplasts flows into the vesicle through the tube and differentiation of zoospores takes place in the vesicle which are later on liberated. Zoospores come to rest, encyst and germinate by germ tubes. Polyplanetism, however, has been reported in some members of the genus and zoospores of some species show a positive chemotoxic towards the root of their host. In *P. aphanidermatum* there is evidence that specific sugar residues are involved in recognition of root surface by zoospores (Longman and Callow, 1987).

2.4.2 *Fusarium oxysporum*

The colony colour of *Fusarium* varies from white to pink. The hyphae are septate and branched. *Fusarium* species features three types of conidia: microconidia, macroconidia and chlamydospores. The micro and macroconidia are formed externally on hypha-like conidiophore, which categorizes *Fusarium* as a Hyphomycete. Chlamydospores are formed from normal hypha which undergoes increased growth and thickening of their cell wall. Kelaniyangoda *et al.* (2011) reported white to pink colour colony of the culture and macroconidia which were hyaline and sickle shaped. Ferniah *et al.* (2014) reported morphological characters of *F. oxysporum* as, colony with white cottony aerial mycelium and purple on the reverse side with 4-5 cm diameter at 5 days incubation on potato dextrose agar medium. Conidia were grown from short phialid with a false head. Macroconida were straight fusiform, pedicellate basal cell, 27-46 x 3-4.5 µm in size, 3-5 septate. Microconidia were abundant, ellipsoid or fusiform without or with 1-2 septa, 5-15 x 2.2-3.5 µm in size. Chlamydospore was formed terminally or intercallary, single or in pairs.

2.4.3 *Rhizoctonia solani*

It was originally described by Julius Kühn which isolated it from potato in 1858. *R. solani* is a soil-borne Basidiomycete occurring world-wide, with complex biology (Carling and Sumner, 1992). Because of the lack of conidia and the scarcity of the sexual spores, *R.solani* exists as vegetative hyphae and sclerotia in nature. It is representative of order "Agonomycetales which is often referred to as the 'Mycelia sterilia'. It has light brown vegetative, septate hyphae of 5-14 µm wide and cell length over 100 µm. The branching occurs almost at right angles to the hyphal cell. Branched hyphae have a constriction at the point of branching. Fungus also produces sclerotia which are barrel shaped, comparatively smaller cells in groups, which are more than twice as wide as the vegetative cells ranging from 10-20 µm in length. The individual sclerotium is less than 2 µm in diameter, rounded and light brown coloured. Colonies are fast growing, initially dull white in colour and become brown coloured later on (Kodaru, 1998).

2.4.4 *Sclerotium rolfsii*

The basidial stage of this fungus falls in the species of *Athelia*. Initially, the mycelium of the fungus in pure culture is first silky white but gradually it loses its luster and becomes somewhat dull in appearance. The hyphae are hyaline, thin walled, sparsely septate when

young. The cells are 60- 350 µm long and 2-8 µm wide. Broader hyphae show clamp connections, which are absent in thin hyphae. Sclerotial initials are formed from hyphal strands that consist of 3-12 hyphae lying parallel. A spherical shape is soon assumed even if it is only a loose mass of hyphae. With further hardnening, differentiation takes place and the sclerotium shows the outer layer of polyhedral cells surrounding the compacted hyphae. Mature sclerotia are dark brown but variation from lighter brown to darker colour may be found. These are small about the size of radish seed, hard and usually round (Gupta and Thind, 2006).

The white mycelial growth forms over the infected tissue and often radiates over the soil surface. The fungus does not produce asexual spores and perpetuates as sclerotia on plant debris and in soil (Cilliers *et al*., 2000). If the plants lodge and heads touch the soil, seed infection is possible; otherwise seed transmission is negligible (Lakshmidevi *et al.,* 1991). Sclerotia disseminate by cultural practices (infected soil and contaminated tools), water (through irrigation), wind and contaminated seeds. The wide host range, prolific growth and ability to produce persistent sclerotia contribute to the large economic losses associated with this pathogen (Kokub *et al.,* 2007).

2.5 PATHOGENICITY

Fungi like *Pythium, Rhizoctonia, Sclerotium* and *Fusarium* can cause serious diseases on greenhouse vegetable crops resulting in significant crop losses. Their infection leads to damping-off in seedlings and crown and root rot of mature plants.

Pythium is a common soil borne pathogen that attacks tomato seedlings (Reddy *et al*., 2006). *Pythium* species are fungal-like organisms (Oomycetes), commonly referred to as water molds, which naturally exist in soil and water as saprophytes, feeding on organic matter. Sparrow (1932) gave the methods of conducting pathogenicity tests of various species of *Pythium*, including *P. dictyosporum, P. angustatum* and *P. adhaerens* and out of these, *P. adhaerens* was found highly virulent and also affected ripe and green tomato fruits. Similarly, Middleton (1943) reported that 10 different species of *Pythium* were found to infect more than 80 species of higher plants. Sowmini Rajagopalan (1961) conducted pathogenicity tests with 15 species of *Pythium* on 12 different hosts and found that tomato and chilli were infected by a number of isolates and 75 to 80 per cent damping-off was noticed when the soil was infected with *P. aphanidermatum.*

Tripathi and Grover (1976) found that all the three types of inoculum *viz.*, zoospore suspension, oatmeal: sand and homogenized mycelium was almost equally effective in causing damping-off in tomato. They further reported that post-emergence damping-off increased with increase in zoospore concentration, while increase in oat meal: sand concentration lead to increase in pre-emergence damping-off. Chamswrang and Cook (1985) proved the pathogenicity of ten different *Pythium* spp. including *Pythium ultimum* using medium containing cornmeal (5g), sand (485g) and water (120 ml).

Pathogenicity test of *Rhizoctonia solani* was conducted by artificially inoculating the steam sterilized soil. The maximum number of infected plants emerged from the infested soil after 30 days of sowing as compared to 15 days of sowing (Jiskani *et al*., 2007). Five pathogens *viz. Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii, Pythium aphanidermatum and Phytophthora megasperma* were inoculated to soil for pathogenicity test in cole crops seedlings and found *P. aphanidermatum* to be the most destructive as it caused more than 75 per cent seedlings death in cabbage, broccoli, knol-khol and cauliflower. Ismael and Mahmood (2016) isolated *Fusarium oxysporum* (Schlecht), *F. solani* (Mart.) Sacc., *F. acuminatum* (Ell. and Ev.), *F. equiseti* (Corda) Sacc., *F. compactum, Rhizoctonia solani* (Kuhn), *Macrophomina phaseolina* (Tassi), *Phoma lycopersici* (Kleb), and *Cephalosporium* sp. from the infected tomato plants and proved pathogenicity on tomato seeds (cv. Super Queen). *Fusarium oxysporum* and *R. solani* showed the highest level of seedling damping-off, with largest effect on decreasing the quality of seedling's vigor.

2.6 MANAGEMENT

Damping-off of tomato can be managed successfully by using cultural, biological and chemical methods.

2.6.1 Cultural methods

Cultural control involves practices and farming techniques that will help to increase the quality and quantity of the yield and also reduce the influence of pests and diseases. It involves manipulation of the environment in non-mechanical ways to control plants pests and diseases. It includes altering farming practices to make the environment unfavourable for the growth of disease pathogens and pests (Islam, 2001). Among various cultural methods, soil solarization is the most important. It is a physical disinfestation method of disease control which aims to eradicate or reduce the soil borne pathogens prior to sowing (Katan *et al.*,

1976). Soil solarization is a non-chemical approach for soil disinfestation and one of the most promising methods to control soil-borne pathogens. It is performed by placing polyethylene sheets over soil surface after sufficient irrigation. Soil-borne pathogens, pests and weeds become inactivated by high temperature and excessive moisture during the hot season in July and August (Al Karaghouli and Al-Kayssi, 2001). Under suitable climatic conditions, solarization can successfully control a wide range of soil-borne diseases and pests.

Chaube *et al.* (2002) reported that the polyethylene mulching increased daily soil temperature by 10 to15°C, while Mathur *et al*. (2002) recorded an increase of 15°C in solarized soils than that at the non-mulched surface when solarization was done for 20 days in the month of June. Akhtar *et al*. (2012) carried out a study regarding impact of soil solarization on some solanaceous crops nursery and reported eight weeks of solarization had better results in all the treatments compared to other durations of solarization. Higher temperature during soil solarization resulted either in killing of propagules or inactivating the pathogen by various other mechanisms (Negi and Raj, 2013). Soil solarization carried out as transparent polyethylene plastic placed on moist soil during the hot summer months increases soil temperatures to levels lethal to many soil-borne plant pathogens, weeds and nematodes (Abd El Kareem *et al*., 2004; Culman *et al*., 2006; Farrag and Fotouh, 2010; Saied-Nehal, 2011).

2.6.2 Biological control

Bio-control is widely practiced as an alternative disease management strategy to conventional fungicides especially when the latter are not effective or cause secondary problems such as seed phytotoxicity from fungicides (Burns and Benson, 2000). Thus, application of biocontrol agents/formulations is an important substitute to conventional fungicides with lower negative impacts. In recent decades, several microbial biocontrol agents have been reported for suppression of soil-borne pathogens.

Species of *Trichoderma* have been extensively tested and used as biocontrol agent against a wide range of plant pathogens *viz., Rhizoctonia. Fusarium, Sclerotium. Macrophomina, Sclerotinia, Pythium* spp. *etc*. (Papavizas, 1985; Chet, 1987; Hornby, 1990; Deacon, 1991).

The efficacy of *Gliocladium virens* and *Trichoderma longibrachiatum* as bioagents are economically important for soil-borne pathogens viz. *R. solani, S. rolfsii* and *P.*

aphanidermatum. The antagonistic potential of seven bioagents *viz*, *Trichoderma viride, T. harzianum, T. koningii, T. hamatum, Gliocladium virens, Bacilius subtilis* and *Pseudomonas fluorescens* against *P. ultimum* Trow. was evaluated *in vitro* by dual culture technique (Stack *et al*., 1986). Das *et al.* (2000) evaluated *T. harzianum, T. viride* and *T. koningii in vitro* against *S. rolfsii*, causing collar rot of tomato and *T. harzianum* was found the most effective biocontrol agent in inhibiting the mycelial growth (61.5%) and sclerotial production in dual cultures. El-Abbasi *et al*. (2003) reported that *Trichoderma harzianum* inhibited *Pythium* by 62.4 per cent, *Sclerotium rolfsii* by 54.3 per cent, *Macrophomina phaseolina* by 36.7 per cent and *Rhizoctonia solani* by 36.0 per cent. Similar *in vitro* efficacy of *Trichoderma* spp. against soil borne pathogens was reported by Kapoor (2008) who observed that maximum inhibition of mycelial growth of *Rhizoctonia solani, Pythium debaryanum, Sclerotinia rolfsii* and *Fusarium oxysporum* f. sp. *pisi* was shown by *T. harzianum.*

Rajput *et al*. (2010) evaluated fungal (*Trichoderma harzianum, T. viride*) and bacterial (*Pseudomonas fluorescens* and *Bacillus subtilis*) antagonists against chickpea wilt complex pathogens *viz.*, *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* and found that *T. harzianum* gave the maximum inhibition of radial growth of *F. oxysporum* f. sp. *ciceri* (65.23%), *R. bataticola* (89.67%) and *S. rolfsii* (86.00%) under dual culture tests. Kavitha and Nelson (2013) reported the antagonistic activity of *Trichoderma* spp. isolated from rhizosphere soil of sunflower wherein *Trichoderma viride* showed maximum growth inhibition of 72.20 per cent against *F. oxysporum* whereas, *Trichoderma koningii* effectively inhibited *P. debaryanum* (57.42%) in dual culture method. Theradimani *et al*. (2018) evaluated yeast, *Trichoderma viride, T. harzianum* and *Pseudomonas* spp. collected from tomato growing areas of Tamil Nadu, India against *F. oxysporum* f. sp. *lycopersici* (FOL) using a dual culture technique. Yeast 1 was found the best in inhibiting mycelial growth of FOL (69.59%) followed by *Trichoderma viride* 1 (68.50%).

Decurtis *et al.* (2010) studied the efficacy of bacterial isolates against *S. rolfsii* and *R. solani* causing damping-off of tomato. Among the 43 bacterial isolates, *Burkolderia cepacia* and *Pseudomonas* sp. were the most effective antagonists *in vitro* against *S. rolfsii* and *R. solani.* These bacterial isolates were further tested *in vivo* for their effect on disease and *B. cepacia* significantly inhibited damping-off caused *S. rolfsii*, reducing the disease index by 81 per cent compared to the untreated control (pathogen alone), whereas it did not yield a significant disease reduction against *R. solani. Pseudomonas* sp. was ineffective against *S.*

rolfsii, whereas, it significantly reduced the incidence o*f R. solani* by 47.45 per cent compared to the untreated control.

Biological control has become a promising and ecologically friendly alternative to chemical control in the management of soil-borne plant diseases and several biological control agents have been introduced as potential bio-fungicides. Dinakaran and Ramakrishnan (1996) conducted studies on the control of tomato damping-off with *T. viride* and reported that seed treatment with *T. viride* (4g/kg) significantly reduced disease incidence and increased seedling emergence of tomato. Seed treatment with the conidial suspension of *T. harzianum, T. viride* and *Gliocladium virens* was also found effective in protecting tomato seedlings from damping-off (Hazarika *et al*., 2000). Soil application of *T. viride* and *Pseudomonas fluorescens* effectively controlled the pre-emergence and post-emergence damping-off of tomato caused by *P. aphanidermatum* under pot culture experiments. Talc based formulation of antagonists significantly reduced the soil population of *Pythium* and increased the shoot length, root length and dry matter production of tomato seedlings.

Bio-priming of seeds with fungal antagonist *T. viride* and *T. harzianum* and bacterial antagonist *P. fluorescens* significantly improved the germination behavior of tomato seeds as compared to untreated control. *Bacillus pumilus* INR7, *Trichoderma harzianum* and *Rhizophagus intraradices* were used individually or in combination against *Rhizoctonia solani* root rot disease of common bean. Treatments containing *B. pumilus* INR7 were the best treatments for suppression of the disease in the simultaneous application method, where *B. pumilus* INR7 + *T. harzianum* reduced the disease up to 54 per cent, however, in pre-inoculation method, *T. harzianum* alone was the only treatment that reduced disease severity up to 49 per cent compared to the control (Hussein *et al*., 2018).

2.6.3 Plant extracts

Plant extract is decoction of different herbal, medicinal, aromatic and poisonous plants. The toxic substances in decoction react with biochemical and metabolic reagents of microorganisms after application and this toxic biochemical can be achieved by extraction processes such as like crushing, filtration, purification and maintaining the optimum concentration of such compounds. A number of medicinal plants found in India have been successfully used both for the therapeutic as well as curative purposes (Chopra *et al.,* 1965).

Earlier reports suggest suppressive properties of plant oils like those from cinnamon (*Cinnamomum zealanicum*), chaulmoogra (*Hydnocarpus kurzii*), karanja (*Pongamia glabra*), neem (*Azadirachta indica*), mohua (*Madhuca indica*)and citronella (*Cymbopogon nardus*) against soil borne pathogens like *Macrophomina phaseolina, S. roljsii, S. hydrophyllum, R. oryzae sativa* and *F. oxysporum* (Mukherjee, 1979).

The Plant extracts have assumed special significance in the present day strategy of developing ecologically safe method of plant disease management. The use of plant extracts has been shown to be eco-friendly and effective against many plant pathogens by various workers (Saadabi, 2006; Gachomo and Kotchoni, 2008; Thobhunluepop, 2009; Duru and Onyedineke, 2010) and most of these substances were evaluated in order to find a safe alternative control methods to the human and the environment.

Manian *et al.* (1988) screened ten leaf extracts from commonly available plants around Coimbatore for antifungal properties *in vitro* against three soil borne plant pathogens like *S. rolfsii, R. solani* and *P. aphanidermtum.* They reported that extracts from *Lantana camara* L. var. *aculeata* and *Cassia auricularia* inhibited the sclerotial production of S. *rolfsii,* though there was no significant suppression in mycelial growth. Similarly,, *Azadirachta indica* and *Piper betle* reduced the number of sclerotia of *R. solani,* whereas, there was no effect when these plant extracts were tested against *P. aphanidermatum.* Kumar *et al.* (1996) reported that the leaf extract of *Lantana camara* possesses six phenolic acids in substantial quality which on decomposition possess a potentially fungitoxic substances against *F. oxysporum* and *P. debaryanum.*

Jacob and Siva Prakasan (1994) and Arya *et al.* (1995) studied the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and reported inhibitory effect of extracts of garlic bulbs and *Bignonia* leaves on the mycelial growth of *Fusarium* sp. Leaf extract of *Piper betl*e (Alice, 1984) and *E. microthecia* was reported to control *Pythium* sp. when applied as soil drench. Various workers have tested the potential benefit of the plant extracts for the control of plant diseases in vegetables (Rodriguez and Montilla, 2002; Babu *et al*., 2000). Seventeen locally available plant extracts, three neem based commercial formulations, one botanical fungicide (Biotos), raw neem oil, neem cake extract, cow urine and cow dung ash were assessed for the management of damping-off of tomato under the mid hill conditions of North Western Himalayas. Among them, extracts of *Lantana camara*, neem cake extract, cow urine, *Urtica parviflora*, *Sapium* sp., *Ligustrum*

nepalensis, *Eucalyptus* sp., Azadirachtin (Achook ®) were found most promising for the management of pre-emergence damping-off and also simultaneously improved seedling emergence and seedling vigour (Hooda *et al*., 2011).

Pattnaik *et al*. (2012) reported that *A. marmelos, Tagetes patula, Piper nigrum* and *Ageratum conyzoides* showed high inhibition of mycelial growth of *Pythium debaryanum*. Similarly, bioefficacy of ten botanicals/ plant extracts *viz.,* mehandi (*Lawsonia innermis*), ginger (*Zingiber officinale*), datura (*Datura metal*), tulsi (*Oscimum sanctum*), parthenium (*Parthenium hysteriphorus*), neem (*Azardirachta indica*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), satawari (*Asparugus racemosus*) was evaluated against *Pythium ultimum* at 10, 15, and 20 per cent under *in vitro* conditions (Gholve *et al*., 2014).

Chandel and Sharma (2014) reported that disease can be controlled with application of different plant extracts (botanicals), biofumigant releasing crop residues and biocontrol agents both under *in vitro* and field conditions. Out of 11 botanicals tested including two commercial formulations of neem (neemgold, neemazal) revealed that seed extracts of *Melia azedarach* and leaf extract of *Adhatoda vasica* showed maximum inhibition in mycelial growth within the range of 44.38 to 44.25 per cent followed by *Murraya koenigii* (37.77%) and *Tagetes erecta* (37.62%). The commercial formulations of neem compared to other treatments were found statistically superior in inhibiting the mycelial growth of *R. solani*. Essential oil of *Artemisia herba alba* was found effective in inhibiting conidial germination, mycelial growth and sporulation of *F. oxysporum* (Bouzidi and Mederbal (2016). Similarly, fungicidal activity of *Lantana camara* against *P. aphanidermatum* was also observed.

Banakar *et al*. (2017) reported the efficacy of botanicals *viz*., neem leaf extract, eucalyptus leaf extract, jathropa leaf extract, tulsi leaf extract, garlic bulb extract, onion bulb and marigold leaf extract screened at three different concentrations (5, 10 and 15 %). Among the botanicals tested against *S. rolfsii* under *in vitro* conditions, onion bulb extract showed 100 per cent inhibition at all the three concentrations followed by garlic bulb extract (97.77 %, 98.88 % and 100% at 5, 10 and 15% concentration, respectively) while least mycelial inhibition was observed (22.55, 24.44 and 44.07 % inhibition at 5, 10 and 15% concentration, respectively) in jathropa leaf extract.

Sanaullah *et al.* (2018) evaluated the leaves extract of three medicinal plants, cinnamon (*Cinnamomum verum),* moringa (*Moringa oleifera*) and clove (*Syzygium*

aromaticum) against *R. solani* causing damping-off of tomato at three different concentrations (1%, 2% and 3%) and found that the maximum growth inhibition was observed at 3 per cent concentration followed by 2 and 1 per cent of each plant extracts. Clove leave extract showed highest antifungal activity giving complete mycelial inhibition. Efficacy of plants extracts was also examined on disease incidence as well as plant growth development in greenhouse and field experiment. Clove leave extract at 3 per cent concentration recorded highly significant in disease reduction and other plant growth parameters. Thus, clove leaves are the best choice for managing *R. solani* associated with damping-off disease of tomato.

Nasrin *et al.* (2018) screened nine plant extracts (*Calotropi sprocera*, *Curcuma domestica*, *Moringa oleifera*, *Tricosanthes dioica*, *Nigella sativa*, *Wedelia calendulacea*, *Andrographis paniculata*, *Trigonell afoenum-graceum* and *Momordica charantia*) and found that almost all plant extracts at 25 per cent concentration were effective in reducing the mycelial growth of *F. oxysporum* f. sp. *lycopersici.* The highest inhibition (87%) of mycelial growth of this pathogen was observed when treated with the plant extract of *Calotropis proceraon* 12 days after inoculation at 25 per cent concentration.

2.6.4 Resistance inducing chemicals

The excessive use of chemical fungicides to control plant diseases is a problem for today's plant production systems and research priorities call for novel protection methods which are compatible with sustainable agriculture, therefore, favours the use of alternative methods such as the applications of chemical inducers of resistance (Kuc, 2001). Chemical inducers of resistance usually have no direct antimicrobial activity and can be divided into three types: inorganic compounds, natural organic compounds and synthetic compounds. The benzithiadiazoles (BTH) are the best known representatives of synthetic compounds, which activate host resistance via salicylic acid (SA) mediated defense signaling pathways (Oostendorp *et al.,* 2001). A wide range of chemical compounds such as oligosaccharides (Yokoshiwa *et al*., 1993), glycoprotein and peptides (Benhamou, 1992) and salicylic acid (Yalpani *et al*., 1991) has been used for induction of systemic resistance in different plants.

The plants possess numerous defense mechanisms to protect themselves against pathogens attack; some of these mechanisms are inducible and become activated after

pathogen infection including synthesis of phytoalexins and production of anti-pathogen proteins (Mehdy, 1994; Jackson and Tylor, 1996; Mohammed *et al*., 2017). This resistance is referred to as systemic acquired resistance (SAR) (Rayals *et al.*, 1996). The resistance induced may be localized at the site of application or may be transmitted systemically to other plant tissues (Walters, 2007).

Some substances such as potassium- and sodium-2 benzothiazolylthioglycolate, 4-chloro-3,5-dimethyl-phenoxyethanol induced the resistance mechanism of tomato plants and reduced Fusarium wilt symptoms (Davis and Diamond, 1952; Diamond and Davis, 1953). Later, it was found that many different plant-growth regulating substances induced resistance of tomato plants against Fusarium wilt. Salicylic acid (SA), a phenolic compound, also regulates plant growth and confers resistance to plants against some viral, bacterial and fungal diseases (Raskin, 1992). Therefore, it provides multiple disease protection with only one application. In tobacco, SAR activation resulted in a significant reduction of disease symptoms caused by the fungi *Phytophthora parasitica, Cercospora nicotianae*, and *Peronospora tabacina*, the viruses tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV), and the bacteria *Pseudomonas syringae* pv *tabaci* and *Erwinia carotovora* (Vernooij *et al.*, 1995).

Benhamou and Belanger (1998) reported that benzo (1,2,3) thiadiazole-7-carbothioic acid ester (BTH, CGA 245704), a non-toxic, synthetic chemical, was applied as a foliar spray to cucumber S-methyl plants and to induce defense mechanisms against the soil borne pathogen *Pythium ultimum* Trow.Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), a synthetic chemical, was applied as a foliar spray to tomato (*Lycopersicon esculentum*) plants and was evaluated for its potential to confer increased resistance against the soil-borne pathogen *F. oxysporum* f. sp.*radicis-lycopersici*. In non treated tomato plants, all root tissues were massively colonized by pathogenic hyphae. Pathogen growth was restricted to the epidermis and the outer cortex, and fungal ingress was apparently halted by the formation of callose-enriched wall appositions at sites of fungal penetration (Benhamou and Belanger, 1999).

Karmakar *et al*. (2003) reported that soaking of rhizome seeds for one hr in 5 mM salicylic acid (SA), DL-β-aminobutyric acid (BABA) or 2,1,3 benzothiadiazole (BTH) significantly reduced the disease in ginger caused by *P. aphanidermatum*. Mandal *et al*.,

(2009)observed that application of salicylic acid at 200 mM, through root feeding and foliar spray, led to reduced vascular browning and leaf yellowing caused by *Fusarium oxysporum* f. sp. *lycopersici* on tomato plants. Akram and Anjum (2011) reported that salicylic acid (SA) is helpful in controlling fungal and bacterial diseases of tomato plants.

2.6.5 Integrated management

Damping-off can be successfully managed by integrating different eco-friendly management strategies including soil solarization, biological control agents, use of botanicals and SAR chemicals. Haritha (2003) evaluated *in vivo* different treatments *viz*., bioagents, organic amendments and soil solarization (alone and in combinations) for integrated management of *Pythium aphanidermatum*, causing damping-off of tobacco. Champawat and Sharma (2003) evaluated various treatments *viz.,* soil solarization, bioagents, organic amendments and cultural methods in brinjal, chilli, cabbage and onion. Bioagents used were *T. viride* and *T. harzianum* as a seed dresser and soil inoculation @ 4 g/kg seed and 500 g in 50 kg FYM/ha respectively. *Pseudomonas fluorescens* was applied @10 g/kg seed and 2.5 kg in 50 kg FYM/ha as soil inoculation, while *Azotobacter chrococcum* as seed treatment @15 g/kg seed and soil inoculation in 50 kg FYM/ha. Neem cake was applied @150 kg/ha as soil amendment. All treatments were found effective in brinjal and cabbage.

Panday *et al*. (2005) studied the integrated disease management against multiple diseases of tomato caused by *Pythium, Rhizoctonia, Sclerotium, Alternaria* and *Myrothecium.* For damping-off management, neem cake @ 50 g/m^2 area, *P. florescens* @ 5g/m^2 area and *T. viride* @10 g/m^2 area were applied just prior to seed sowing. They found that pre-emergence and post-emergence damping-off was reduced in integrated disease management schedule in comparison to control in both open pollinated (OP) and hybrids cultivars. Thus, they developed IDM module which was successful in reducing pre and post-emergence damping-off in OP and hybrid tomato during *Kharif* and winter season.

Jayaraj and Radhakrishnan (2008) reported that soil solarization combined with the soil inoculation of biocontrol agents like *P. fluorescens* and *T. harzianum* resulted in efficient control of damping-off of tomato seedlings. Similarly, seventeen locally available plant extracts, three neem based commercial formulations, one botanical fungicide (Biotos) and raw neem oil were assessed for management of damping-off of tomato under mid hill condition. Five biocontrol agents (*T. harzianum, T. hamatum, T. asperellum, Bacillus subtilis*

and *P. fluorescens*) and four botanicals (*Catharanthus roseus, Lantana camara, Eucalyptus globolus* and *Lawsonia intermis*) were tested against damping-off disease of chilly in the pot and field conditions caused by *P. aphanidermatum* and resulted in maximum disease reduction (Pandey *et al*., 2016). Mahato *et al.* (2017) conducted an experiment to develop a sustainable management strategy in tomato and found that the least disease incidence (4.17 %) and highest fruits yield (250.67 q/ha) was observed in treatment with mulching by transparent polythene + neem seed cake @ 150 kg/ha (soil application) + *T. harzianum* @ 2.5 kg/ha (soil application) followed by mulching + gypsum @ 4 tonne/ ha. + *T. harzianum* @ 2.5 kg/ha treated field with 5.56 per cent disease incidence in comparison to 27.78 per cent disease incidence and 200.67 q/ ha fruits yield in untreated control.

Chapter-3

MATERIAL AND METHODS

3.1 SYMPTOMATOLOGY

The symptoms of damping-off of tomato seedlings including above ground and below ground symptoms were studied on the diseased samples collected from tomato nurseries**.**

3.2 ISOLATION AND IDENTIFICATION

3.2.1 Isolation of the pathogen(s)

Repeated isolations were made from diseased seedlings collected from different locations on oat meal agar for *Pythium* spp and potato dextrose agar medium for species of *Fusarium, Rhizoctonia* and *Sclerotium*. A pinch of Streptocycline was added to the basal medium to avoid bacterial contamination. Small bits of 2-3 mm size were cut from the juncture of diseased and healthy tissues with the help of sterilized scalpel. The bits were surface sterilized by dipping in sodium hypochlorite 1% solution for 30 seconds and then washed thrice in sterilized distilled water. These bits were then placed on a sterilized filter paper to remove excess moisture. Finally, these bits were transferred to the slants and Petriplates containing oat meal agar (OMA) and potato dextrose agar (PDA) medium under aseptic conditions for *Pythium* spp, *Fusarium* spp, *Rhizoctonia* spp, *Sclerotium* spp respectively and incubated at 25±1°C. Cultures of the fungus so obtained were further purified by hyphal tip method and maintained at 4 ± 1°C. Stock cultures were sub-cultured at intervals of 20-25 days.

3.2.2 Identification on the basis of cultural and morphological characters of the pathogen(s)

The morphological characters of the pathogen were studied on the host, sections from diseased portions from infected seedlings as well as on culture of the associated fungi raised on the oat meal and potato dextrose agar medium by examination under microscope in the

laboratory. Septation and diameter of hyphae and shape and size of spores present, if any and sclerotia were recorded. The morphological characters of the isolated fungi viz. colony growth, its color, pigmentation, size, shape and septation of conidia, presence of chlamydospores and sclerotial formation were recorded using Olympus microscope and Promagnus software. These characters were then compared with the standard authentic descriptions and taxonomic keys (Waterhouse, 1967; Booth, 1971; Saccardo, 1911) and associated fungi were identified as *Pythium ultimum, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*.

3.3 PATHOGENICITY

Pathogenicity tests of the associated fungi were conducted under greenhouse conditions in two different sets to check that the isolated pathogens were responsible for both pre and post emergence damping-off of tomato.

3.3.1 Mass multiplication of pathogens

The mass culture of isolated fungi was prepared on corn: sand medium. The maize grains were boiled till they get softened. Excess water from grains was removed. Maize grains were air dried and mixed with sand (3 parts maize grains and 1 part sand) along with two per cent sucrose (Plate 3.1a). To avoid bacterial contamination, Streptocycline (100 ppm) was added in the mixture. The medium, thus, prepared was filled in autoclavable polypropylene bags (150 g per bag) or conical flasks (250 ml), plugged with non-absorbent cotton and was sterilized by autoclaving at 1.05 kg/cm^2 for one hour for three consecutive days. The sterilized medium was inoculated under aseptic conditions with seven days old culture of isolated pathogens i.e. *P. ultimum*, *F. oxysporum*, *R. solani* and *S. rolfsii*. Five to six bits (4 mm size) of the fungi were placed in different sides of each bag/ flasks. The inoculated flasks were incubated at 25±2^{o}C in BOD incubator for fifteen days. These flaks were shaken regularly after three days so that fungi grow uniformly (Plate 3.1b). Two weeks old mass culture of fungi was used for carrying various pot experiments.

3.3.2 Soil inoculation method/Preparation of the sick soil

Mass culture of isolated pathogens i.e. *P. ultimum*, *F. oxysporum, R. solani* and *S. rolfsii* was mixed @ 10g/pot in steam sterilized soil individually as well as in combination contained in pots 10 cm diameter upto 5 cm depth (Plate 3.1c). After inoculation, the soil was

Plate 3.1.Pictroial flow chart showing pathogenicity tests

Plate 3.1a.
Corn sand meal medium

Plate 3.1b.
Preparation of mass culture

Plate 3.1c.
Addition of inocula to the pots

Plate 3.1d.
Transplanting of seedlings to the sick pots

Plate 3.1e.
Symptoms of pre-emergence and post-emergence damping- off

sprayed with sterilized distilled water and allowed to establish under polythene cover for 6 days.

3.3.3 Sowing of seeds

After six days of inoculation, eight seeds of tomato cv. “Solan Lalima” were sown in pots. A treatment without inoculum served as control. In this set, pots were transferred to poly house after sowing the seeds and observed for pre-emergence damping-off symptoms, seed rotting and patchy germination. To confirm the identity, the re-isolated fungi were compared with the original.

3.3.4 Transplanting of seedlings

In another set for post- emergence damping off studies, ten healthy juvenile seedlings were transplanted in each pot containing sick soil (Plate 3.1d). Pots were kept in poly house conditions at 25°C. The plants were periodically observed for the appearance of post-emergence damping-off symptoms (Plate 3.1e).

3.4 DISEASE MANAGEMENT

3.4.1 Soil solarization

The effect of soil solarization was examined as pre-planting treatment for the management of damping-off of tomato in naturally infested soils at University Research Farm of Department of Plant Pathology at Nauni.

3.4.1.1 Thermal sensitivity of inocula of isolated pathogens *in vitro*

To study the thermal sensitivity of inocula of isolated pathogens i.e. *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii* under *in vitro* conditions, the mass culture of these pathogens grown on corn sand media (5g) (Plate 3.2a) was placed in a piece of muslin cloth which (Plate 3.2b) were then placed at three different temperatures 35^{o}C, 40^{o}C and 45^{o}C in the hot water bath for three different durations i.e. 5, 10 and 20 minutes (Plate 3.2c). After exposure, small bits of test pathogens were placed on sterilized PDA Petriplates under aseptic conditions and were incubated at 25±1°C for one week (Plate 3.2d). Observations on the growth of the fungi were recorded till the experiment was over.

3.4.1.2 Effect of soil solarization on soil temperature under field conditions

The experiment was carried out during May-June for two consecutive years i.e. 2018 and 2019 at experimental farm of the Department of Plant Pathology, UHF, Nauni. Raised beds (1 × 1 m) were prepared and covered with polyethylene sheet (25 μm) after irrigation up to the field capacity one day prior to mulching. Unsolarized beds were kept as control. The edges of the sheets were buried in the soil to make them airtight. The polythene sheet was removed after 45 days and the temperature at three depths (5, 10 and 20 cm) was measured in solarized and nonsolarized beds by dial soil thermometer at 2:00 PM daily.

3.4.1.3 Effect of soil solarization on viability of the pathogens under field conditions

To study the effect of soil solarization on the viability of the pathogens in soil, the mass culture of the pathogens i.e. *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii* grown on corn sand media and placed in nylon sieve bags (20g/bag) which were then placed at 5 cm depth in the plots prior to mulching with polyethylene sheets and also in unsolarized plots. One nylon bag each contained fungal culture which was retrieved after 10, 20 and 30 days. The viability of these test fungi was monitored by placing fungal cultures onto sterilized PDA petriplates under aseptic conditions and was incubated at 25±1°C.

3.4.2 Biological control

3.4.2.1 Procurement and maintenance of fungal and bacterial bio-control agents

Fungal (*Trichoderma viride, T. hamatum, T. harzianum and T. virens*) and bacterial (*Bacillus subtilis, Bacillus licheniformis, Bacillus* sp., *Pseudomonas fluorescens, Pseudomonas* sp.) bio-control agents (BCAs) were procured from Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni.

Fungal antagonists were grown on PDA slants while bacterial antagonists i.e. *Bacillus* spp. and *Pseudomonas* spp. were maintained on nutrient agar and King's B medium, respectively. These cultures were maintained at 4^{o}C in a refrigerator.

3.4.2.2 *In vitro* evaluation of BCAs

Effect of various fungal and bacterial biocontrol agents (*Trichoderma harzianum, T. viride, T. virens, T. hamatum, Bacillus subtilis, Bacillus licheniformis, Bacillus* sp., *Pseudomonas fluorescens, Pseudomonas* sp.) on mycelial growth of test fungi i.e. *P. ultimum,*

Plate 3.2 Thermal sensitivity of pathogens of damping-off under *in vitro* conditions

Plate 3.2a.
Mass culture of the associated pathogens

Plate 3.2b.
2-5 g mass culture of each pathogen kept in muslin cloth

Plate 3.2c.
Exposed to temperature i.e. 35, 40 and 45°C for 5, 10 and 15 min duration

Plate 3.2d.
Plating of inoculum on PDA plates and incubated at 25°C

F. oxysporum, R. solani and *S. rolfsii* was studied by dual culture method (Huang and Hoes, 1976) and streak plate methods, respectively, under *in vitro* conditions. The experiment was conducted in completely randomized design with three replications each.

Culture discs (5mm) each of fungal antagonist and the test pathogens were taken from the margins of the actively growing cultures and transferred to potato dextrose agar medium contained in Petri plates (90mm) on opposite sides approximately one cm away from the wall of the plate. Similarly, bacterial BCAs were streaked opposite to mycelial disc of the pathogens. A check having the test pathogens only, was also kept for comparison. The Petri plates were subsequently incubated at 25±1°C till the check plates were completely covered by colonies. Each treatment was replicated three times. Colony diameter of the test fungi as well as each antagonist up to the zone of inhibition was recorded and the per cent growth inhibition of the test pathogens over control was calculated according to Vincent (1947) as under:

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

Where,

I	=	Inhibition (%)
C	=	Linear growth in control (mm)
T	=	Linear growth in treatment (mm)

3.4.2.3 Evaluation of BCAs and natural bioresources under pot conditions

3.4.2.3.1 Preparation of mass culture of antagonists

The mass culture of fungal antagonists was prepared on wheat bran: saw dust: tap water (3:1:1 W/W/V) medium and autoclaved at 1.05 kg/cm^2 for 1h on two consecutive days. Flasks (250 ml) containing autoclaved medium were aseptically inoculated with mycelial discs (5 mm diameter) of fungal antagonists taken from their actively growing 3 days old cultures. Inoculated flasks were incubated at 25±1°C for 14 days. The bacterial antagonists were grown on nutrient broth and 48 h old bacterial culture was used for inoculation (Plate 3.3).

3.4.2.3.2 Inoculation and transplanting

Plastic pots (10 cm dia.) containing sterilized sandy loam soil were inoculated with wheat bran: saw dust culture @ 10g/pot of each fungal antagonist and after two days

inoculated with *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii* separately @ 10g/pot. After two days, pots were transplanted with ten tomato seedlings of cv. "Solan Lalima" and pots were kept in glasshouse. For bacterial antagonists, 10 seedlings were dipped in 48h old bacterial suspension (10^9cfu/ml) for 30 minutes, transplanted in the sick soil and kept in the glasshouse. Each treatment was replicated thrice and suitable control without culture of antagonists was maintained. For evaluating natural bioresources, the seeds were surface sterilized with 1.0 per cent sodium hypochlorite solution for five minutes, rinsed thrice with distilled water and dried under laminar air flow cabinet on blotting paper. The surface sterilized and dried seeds were soaked in beejamrit and jeevamrit (100 ml each) for 30 minutes and again dried under laminar air flow chamber for 2 hours by using filter paper. These seeds were, then, sown in the pots containing sick soil of test fungi separately and watered regularly. Data on number of plants infected were recorded after 10 days and incidence of the disease was recorded and per cent disease was calculated.

3.4.4 Evaluation of plant extracts

3.4.4.1 Preparation of extracts of botanicals

Different parts of botanicals including leaves and bulbs were first washed, surface sterilized (2% sodium hypochlorite), followed by 3 washings with sterile distilled water and kept in sterilized covered flasks and air dried. The plant materials weighed (100g), crushed with 100 ml of sterilized distilled water (1:1 w/v) in warring blender. The mixture was filtered through two fold muslin cloth and the filtrate was centrifuged at 5000 rpm for 15 minutes. Clear extract was diluted with sterilized distilled water to make volume of 1:1 (w/v). This was considered as 100 per cent concentration which was used for further experiments.

3.4.4.2 *In vitro* evaluation of botanicals

Water extracts of different plant species (Table 3.1) were evaluated at different concentrations (i.e. 2, 5, 10, 15%) under *in vitro* conditions against the test pathogen(s) i.e. *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii* by poisoned food technique (Falck, 1907) (Plate 3.4a).

The poisoned medium was poured in the sterilized Petri plates under aseptic conditions. The Petri plates were inoculated in centre with mycelial bit (3 mm dia) of fresh culture of the test pathogen. Separate control plates were maintained to evaluate the relative *in vitro* efficacy of botanicals. Each treatment was replicated three times and inoculated plates were incubated at 25±1^{o}C in BOD incubator. Inoculated plates were observed daily

Plate 3.3.Mass culture of fungal and bacterial biocontrol agents

T. viride

T harzianum

T. virens

T. hamatum

Bacillus subtilis

Bacillus licheniform

Pseudomonas fluorescence

Pseudomonas sp.

Bacillus sp

and the colony diameter of test pathogens was recorded till the control plates were full with mycelium of the test pathogen. The mycelial inhibition (%) was calculated by the formula given by Vincent (1947) as described earlier.

Table 3.1. List of the botanicals and their part used

Common name	Botanical name	Plant part used
Drake	*Melia azedarach*	Leaves
Bougainvillea	*Bougainvillea glabra*	Leaves
Lantana	*Lantana camara*	Leaves
Kaddu	*Vitex nigundo*	Leaves
Garlic	*Allium sativum*	Cloves
Ginger	*Zingiber officinale*	Rhizomes
Kaddipatta	*Murraya koenigii*	Leaves

3.4.4.3 Evaluation of botanicals on disease incidence under pot conditions

The water extracts of botanicals were further evaluated under pot conditions. The culture of the pathogens were added to the sterilized pot soil (10 g/pot) at the rate of 1:20 w/w ratio of the test pathogens separately and were allowed for multiplication for seven days. The seeds were sown in 6 earthen pots at the rate of 25 seeds per pot. Then these pots were kept in poly house conditions and watered regularly. When the seedlings came to two to four leaf stage, these were dipped in water extracts of botanicals namely, drake (*Melia azedarach*), bougainvillea (*Bougainvillea glabra*), lantana (*Lantana camara*), kaddu (*Vitex nigundo*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and kaddipatta (*Murraya koenigii*) for 30 mins (Plate 3.4b). Thereafter, these treated seedlings were transplanted in the sick soil. One set of control with pathogens separately was also maintained for comparison. Each treatment was replicated three times. Data on number of plants infected were recorded after 10 days and incidence of the disease was recorded and per cent disease control was calculated.

3.4.5 Management through systemic acquired resistance (SAR) inducers

3.4.5.1 Evaluation of systemic acquired resistance (SAR) inducers under pot culture conditions

To study the efficacy of systemic acquired resistance (SAR) or abiotic resistance inducers, namely, salicylic acid, oxalic acid, potassium dihydrogen phosphate, potassium chloride and potassium oxalate, against the four pathogens (*P. ultimum*, *F. oxysporum, R. solani* and *S. rolfsii*) causing damping-off of tomato under protected conditions. SAR inducers and their concentration are given below in the Table 3.2.

Table 3.2. Resistance inducing chemicals and their concentration

Sr. No.	Treatments	Concentration
1	Salicylic acid {$C_6H_4(OH)CO_2H$}	10mM
2	Oxalic acid ($H_2C_2O_4$)	20mM
3	Potassium dihydrogen phosphate (KH_2PO_4)	200mM
4	Potassium chloride (KCl)	300mM
5	Potassium Oxalate ($K_2C_2O_4$)	50mM
6	Control	-

The experiment was conducted in sick pots by inoculating with test pathogens as described earlier. The respective concentrations of SAR inducers were prepared in sterilized distilled water. Ten seedlings (15-25 days old) of tomato cv. 'Solan Lalima' were treated by dipping their roots in solutions of SAR or abiotic resistance inducers for each treatment for 20 minutes before transplanting. Treated seedlings were transplanted in sick pots of all pathogens separately. Experiment was conducted in a Completely Randomized Design (CRD) and each treatment was replicated thrice. The pots were incubated in plant growth chamber. The data on disease incidence was recorded after ten days of transplanting and per cent disease control was calucated.

3.4.6 Effect of soil solarization, bio-control agent, botanical extract and resistant inducing chemical on damping-off of tomato under the field conditions

To study the effect of soil solarization and its combination with most effective BCA (*T. harzianum*), botanical (*Lantana camara*) and SAR chemical (Potassium chloride), found best *in vitro,* on pre and post-emergence damping-off of tomato caused by *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii*, a field experiment was laid out at the experimental farm of Department of Plant Pathology, Nauni, during 2019 crop season. The experiment was conducted in randomized block design (RBD) and each treatment was replicated three times. First of all, nursery beds (1×1 m) were prepared and FYM @ 5kg/bed was applied. To make these beds sick, the culture of the above pathogens (25 g/bed,) was mixed and a light irrigation was applied. Soil solarization of these beds was done during the months of March-April while, BCA (*T. harzianum*) was raised on well decomposed FYM containing 20% moisture, maintained by mixing 2 lt of water containing 400 g molasses. Seeds of cultivar 'Solan Lalima' were sown in four lines in each bed. After the emergence of seedlings, these were drenched with the *Lantana camara* and Potassium chloride either alone or in the combinations. Beds without any treatment were sown with seeds and kept as control. Data

Plate 3.4. Evaluation of botanicals under pot conditions

Plate 3.4.a Water extracts of botanicals used under pot conditions

Plate 3.4.b Seedling dip treatment with botanicals before transplanting

was recorded as seedling mortality and per cent disease incidence was recorded and per cent disease control was calculated.

3.5 STATISTICAL ANALYSIS

The data recorded from various *in vitro* and field experiments were subjected to statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance at 5 per cent level using standard procedure as described by Gomez and Gomez (1984).

Chapter-4

RESULTS AND DISCUSSION

Results of present investigations are being described under the following headings:

4.1 Symptomatology

4.2 Isolation and Identification

4.3 Pathogenicity

4.4 Disease Management

4.1 SYMPTOMATOLOGY

Symptoms of the disease appeared in two phases *viz*., pre-emergence and post-emergence. Pre-emergence damping-off caused seeds and young seedlings to rot before they emerged from the growing medium and resulted in patchy growth (Plate 4.1a) while 'post-emergence' damping-off killed newly emerged seedlings by causing a water-soaked, soft brown lesion at the stem base, near the soil line, that pinched off the stem causing the seedling to topple over and die (Plate 4.2b). In the first case, seeds became soft, rotten and failed to germinate. In the second case, stems of germinating seeds were affected with characteristic water-soaked lesions formed at or below the soil line. Post-emergence damping-off symptoms occurred when seedlings decayed, wilted and died after emergence. Seedling stems became thin and tough (commonly known as "wirestem"), which often led to reduced seedling vigor. These symptoms were also accompanied by leaf spotting and ultimately a complete root rot. Overall, the symptoms on the stem of the seedlings included water-soaked, sunken lesions at or slightly below the ground level and sometimes also below ground line (i.e., on the roots) causing the plant to fall over.

The characteristic symptoms of damping-off as observed in pre-emergence and post emergence phases in the present investigations are in accordance with those described by earlier workers (Wright, 1944; Filer and Peterson, 1975; Cram, 2003; Landis, 2013).

4.2 ISOLATION AND IDENTIFICATION

4.2.1 Isolation of the pathogen(s)

Repeated isolations were made from diseased seedlings collected from different locations on oat meal agar for *Pythium* sp. and on potato dextrose agar medium for species of

Fusarium, Rhizoctonia and *Sclerotium*. Small bits after sterilization were transferred to the slants and Petri plates containing oat meal agar (OMA) for *Pythium* sp. and potato dextrose agar (PDA) medium for species of *Fusarium, Rhizoctonia* and *Sclerotium* under aseptic conditions and incubated at 25±1°C. Cultures of the fungi so obtained were further purified by hyphal tip method and maintained at 4 ± 1°C. Stock cultures were sub-cultured at intervals of 20-25 days.

In vegetable nurseries, damping-off is caused by different fungi like *Pythium, Phytophthora, Fusarium, Rhizoctonia, Sclerotinia, Phomopsis, Colletotrichum, Sclerotium* and *Phoma etc*. (Singh, 1987).

4.2.2 Identification on the basis of cultural and morphological characters of the pathogen(s)

The morphological characters of the associated pathogens were studied on the host, sections from diseased portions from infected seedlings as well as on culture of the associated fungi raised on the oat meal and potato dextrose agar medium by examination under microscope in the laboratory. The cultural and morphological characters of the isolated fungi viz. colony growth, its color, pigmentation, size, shape and septation of conidia, presence of chlamydospores and sclerotial formation were recorded using Olympus microscope and Promagnus software. These characters were then compared with the standard authentic descriptions and taxonomic keys and associated fungi were identified as *Pythium ultimum, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*. The cultural and morphological characters of the pathogens associated with damping-off of tomato have been presented in Table 4.1.

4.2.2.1 *Pythium ultimum*

Mycelial colonies of isolated fungus were white in colour with fluffy or cottony dense aerial growth on oat meal agar (Plate 4.2a). Fungus was fast growing and took 72-96 h to completely cover the Petri plate (90cm) with mycelium. Microscopic studies (400X magnification) revealed that the hyphae of the pathogen were hyaline and coenocytic (Plate 4.2b) but a few septa were also observed in old cultures. Hyphal diameter ranged from 4.65 to 8.67 μm with an average diameter of 6.19 μm. Sporangia were mostly round and somewhat ovoid in shape without any papilla (Plate 4.2c) and zoospore production was also observed. The average size of sporangium was 27.54 × 26.59 μm and it ranged from 23.08-34.87 ×

Plate 4.1. Symptoms of damping –off of tomato

Plate 4.1 a. Symptoms of pre-emergence damping –off of tomato

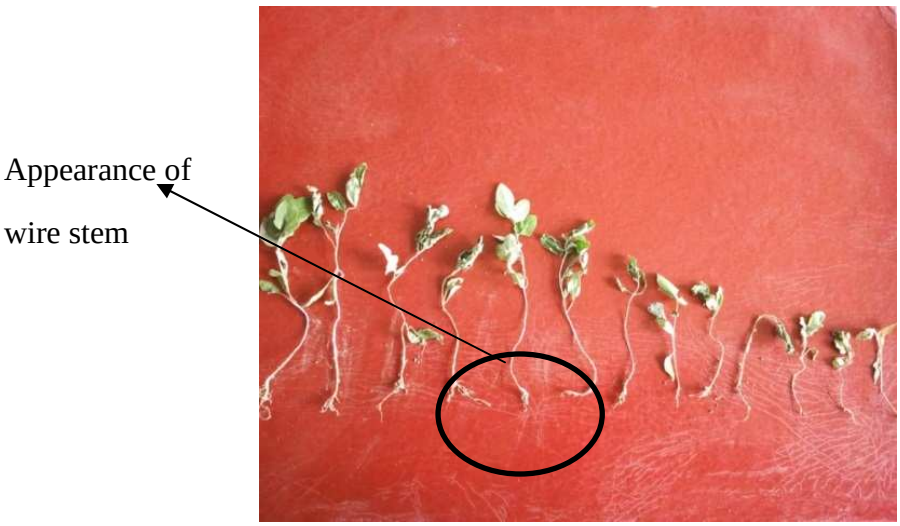


Plate 4.1 b. Symptoms of post-emergence damping –off of tomato

Plate 4.2 Cultural and morphological characters of *Pythium ultimum*

Plate 4.2a. Culture of *P. ultimum* on oat meal agar

Plate 4.2b. Coenocytic mycelium (400x magnification)

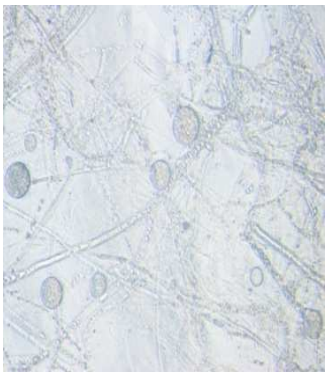


Plate 4.2c. Sporangia (400x magnification)

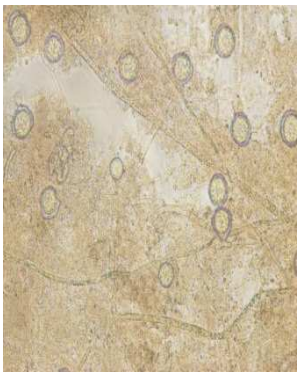


Plate 4.2d. Oospore (400x magnification)

22.3-33.56 µm. Oospores were round, smooth, thick walled, plerotic and hyaline (Plate 4.2d). Size of the oospores ranged from 18.58-26.95 × 18.36-26.78 µm with an average size of 23.09 × 22.91 µm. On the basis of coenocytic hyphae, absence of papillate sporangia, production of oospores and other morphological characteristics, this isolated pathogen was identified as *Pythium ultimum* which was confirmed on the basis of morphological characters documented in standard authentic descriptions and taxonomic keys (Waterhouse, 1967).

Table 4.1. Cultural and morphological characters of pathogenic fungi associated with damping-off of tomato

Cultural and morphological characters	***Pythium ultimum***	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***
Septation	Absent	present	Present	Present
Mycelium colour	white or cottony	hyaline at first becoming cream coloured with age	brownish on maturity	White
Size of mycelium (µm)	7 µm - 25 µm long and 5.5 - 22 µm wide	9.3-29.7 µm long and 2.7- 6.0 µm wide	6.19- 9.29 µm long and 5-14 µm wide	60- 350 µm long and 2-8 µm wide
Shape of sporangium	round and somewhat ovoid	-	-	-
Shape of oospores	round, smooth, thick walled	-	-	-
size of sporangium (µm)	27.54 × 26.59	-	-	-
Size of oospore (µm)	23.09 × 22.91 µm	-	-	-
Shape of macroconidia	-	hyaline, 3-5 septate, falcate having gradually pointed and curved ends	-	-
Shape of microconidia	-	ellipsoid, straight to the curved,	-	-
Size of macroconidia (µm)	-	27-46 x 3-4.5 µmin size if three septate or 35-60 x 3-5 µm if five septate	-	-
Size of microconidia(µm)	-	5-15 x 2.2-3.5 µm in size when one to two celled and hyaline	-	-
Shape of sclerotium	-	-	rounded	round or elongated
Size of sclerotium (mm)	-	-	1.0-3.0 mm	0.5- 2.0 mm

4.2.2.2 *Fusarium oxysporum*

The colony of *F. oxysporum* showed white cottony aerial mycelial growth and was with a purplish tinge on the reverse side having 4-5 cm diameter after 5 days of incubation on potato dextrose agar medium (Plate 4.3a.). The hypha of the fungus was septate (Plate 4.3b). The fungus produced macroconidia, microconidia and chlamydospores. Macroconida were the typical *Fusarium* spores, hyaline, 3-5 septate, falcate having gradually pointed and curved ends (Plate 4.3c) and appeared on sporodochia or without formation of such fruiting bodies and measured 27-46 x 3-4.5 µm in size, if three septate or 35-60 x 3-5 µm, if five septate . Microconidia were abundant, ellipsoid, straight to the curved, 5-15 x 2.2-3.5 µm in size when one to two celled and hyaline. Chlamydospores both smooth and rough walled characterized by thick walls formed terminally or intercallary, single or in pairs (Plate 4.3d). These characters were then compared with the standard descriptions given by Booth (1971) in key "The Genus Fusarium".

4.2.2.3 *Rhizoctonia solani*

The mycelium was septate, colour of the hyphae varied from hyaline when young to brownish on maturity (Plate 4.4a). The size of hyphae varied from 117.7-173.4 x 6.19- 9.29 µm, and cell length over 100 µm. The branching occurred almost at right angles to the hyphal cell Plate (Plate 4.2b). Branched hyphae have a constriction at the point of branching. Fungus also produced sclerotia which were barrel shaped, comparatively smaller cells in groups, brown to black and unlike other fungi sclerotia were undifferentiated into rind and medullae, ranging from 10-20 µm in length. The individual sclerotium was less than 3 mm in diameter, rounded and light brown coloured.

Mycelial branching at right angles and production of abundant brown sclerotia is a known feature for the identification of *R. solani* (Sneh *et al*., 1991).

4.2.2.4 *Sclerotium rolfsii*

The fungal mycelium was first silky white in color later turned to dull white with radial spreading giving fan like appearance (Plate 4.5a). The hyphae were hyaline, thin walled, sparsely septate when young (Plate 4.5b). The cells were 60- 350 µm long and 2-8 µm wide. Broader hyphae showed clamp connections, which were absent in thin hyphae. Sclerotia initially were formed from hyphal strands that consist of 3-12 hyphae lying parallel. Mature sclerotia were dark brown but variation from lighter brown to darker colour may be found. These were small about the size of radish seed, hard and usually round (Plate 4.5c).

Plate 4.3. Cultural and morphological characters of *Fusarium oxysporum*

Plate 4.3a. Culture of *F. oxysporum* on PDA

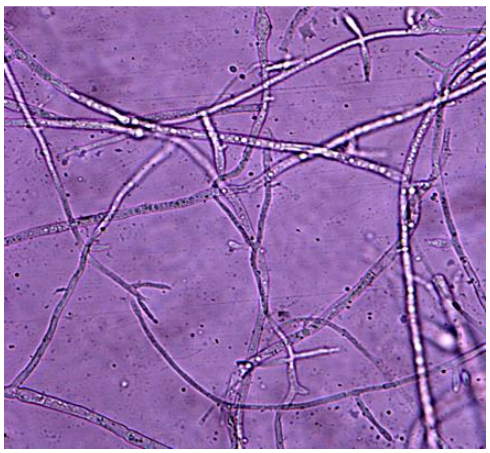


Plate 4.3b. Septate hyphae (400X magnification)

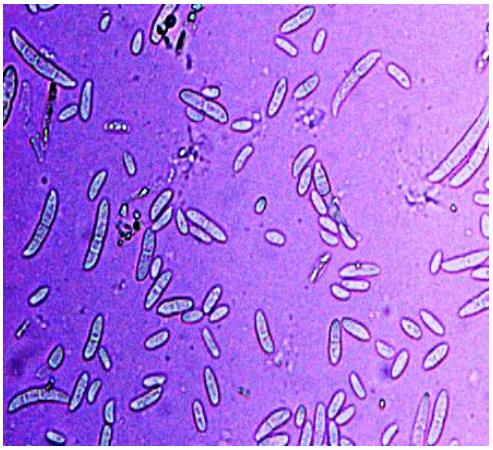


Plate 4.3c. Macroconidia and microconidia (400X magnification)

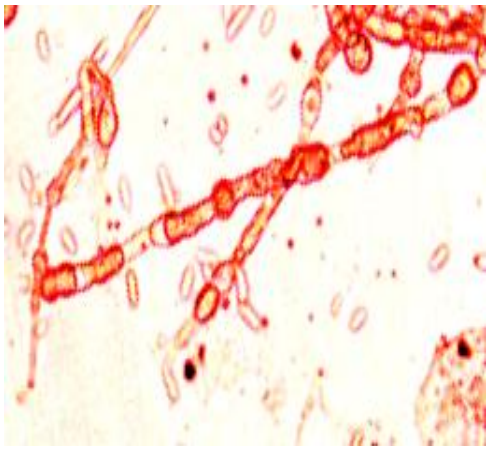


Plate 4.3d. Chlamydospores (400X magnification)

Plate 4.4. Cultural and morphological characters of *Rhizoctonia solani*

Plate 4.4a. Culture of *R. solani* on PDA

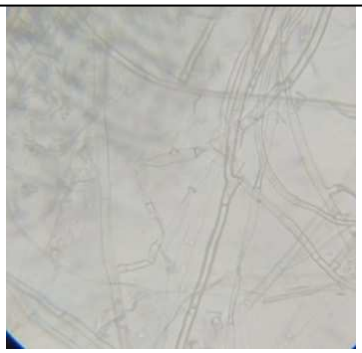


Plate 4.4b. Septate hyphae (100X magnification)

Plate 4.5. Cultural and morphological characters of *Sclerotium rolfsii*

Plate 4.5a. Culture of *S. rolfsii* on PDA

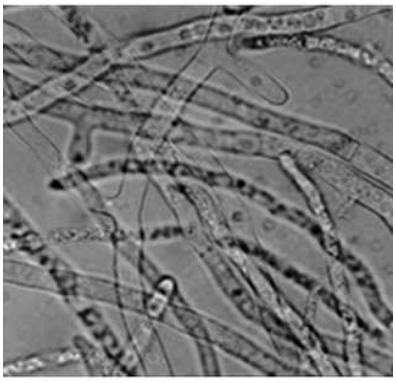


Plate 4.5b. Septate hyphae of *S. rolfsii* (400X magnification)

Plate 4.5c. Sclerotial formation in culture of *S. rolfsii*

Similar morphological characters of *S. rolfsii* were reported by Subramanian (1964), Barnett and Hunter (1972), Mahmood *et al*., (1976), Singh (1987), Mirza and Aslam (1993), Mohan *et al*. (2000).

4.3 PATHOGENICITY

In order to test the pathogenicity of the associated pathogens, seeds of tomato cv. "Solan Lalima" were sown in plastic pots (10 cm dia.) which were already inoculated with corn: sand culture of the test pathogens in separate pots six days prior to sowing of seeds for pre-emergence damping-off. Similarly, for post-emergence damping-off, seven to eight healthy seedlings were transplanted in each pot containing sick soil as above. Pots containing sterilized soil without any inoculum served as control. Each treatment was replicated thrice. After seed sowing and transplanting of seedlings, pots were kept at ambient temperature (25±1ºC). The observations on incubation period and disease incidence were recorded and have been presented in Table 4.2.

Table 4.2. Pathogenicity of pathogenic fungi associated with damping-off of tomato

Fungal pathogens	**Incubation period (h)**	**Disease incidence (%)**	
		Pre- emergence damping-off	**Post- emergence damping-off**
Pythium ultimum (P)	96	88.88 (73.94)	50.00 (45.00)
Fusarium oxysporum(F)	144	61.11 (51.49)	49.99 (45.00)
Sclerotium rolfsii(S)	120	33.33 (34.79)	11.10 (16.06)
Rhizoctonia solani(R)	144	50.00 (45.00)	38.89 (38.51)
S + R	120	50.00 (45.00)	16.66 (19.78)
R + P	96	88.88 (73.94)	22.21 (27.81)
P + F	96	50.00 (45.00)	27.77 (31.54)
F + S	120	61.11 (51.49)	27.77 (31.54)
P + F + S + R	96	94.44 (81.97)	22.21 (27.77)
Control	-	100.00 (90.00)	100.00 (90.00)
CD (0.05)	-	(17.34)	(15.33)

Figures in parentheses are arc sine transformed values

It is evident from the data (Table 4.2) that all the fungi were pathogenic in nature and caused damping-off disease either alone or in combination, however, out of these fungi, *P. ultimum* was the most pathogenic in nature developing disease earliest with incubation period of 96 hours either alone or in combination with other fungi. This was followed by *S. rolfsii* exhibiting an incubation period of 120 hours while it was the highest for *F. oxysporum* and *R. solani* showing 144 hours of incubation period. In combined application of inocula of these fungi, presence of *P. ultimum* hastened the disease development (96 h).

Data presented in Table 4.2 further revealed that similar trend was observed for disease incidence of pre-emergence damping-off and post-emergence damping-off and *P. ultimum* resulted in highest incidence of pre-emergence and post- emergence damping-off giving 88.88 and 50.00 per cent disease incidence, respectively.

P. ultimum, *F. oxysporum*, *R. solani* and *S. rolfsii* have been found to be pathogenic on tomato which caused pre-emergence and post-emergence damping-off. These results are in corroboration with the studies conducted by various workers such as Middleton (1943), Tripathi and Grover (1976), Sowmini Rajagopalan (1961), Jiskani *et al*., (2007), Ismael and Mahmood (2016) who reported that these fungi to be pathogenic in nature.

4.4 DISEASE MANAGEMENT

4.4.1 Soil solarization

4.4.1.1 Thermal sensitivity of inocula of isolated pathogens *in vitro*

Thermal sensitivity of inocula of four isolated pathogens was studied under *in vitro* conditions by exposing these fungi at three different temperatures *viz.*, 35^{o}C, 40^{o}C and 45^{o}C for 5, 10 and 15 minutes and data was recorded on mycelial growth and has been presented in Table 4.3 in terms of per cent mycelial growth inhibition.

Data (Table 4.3.) showed that with the increase in the temperature from 35^{o}C to 40^{o}C and 45^{o}C, there was a general increase in percent mycelial growth inhibition of all the four fungal pathogens, however, highest inhibition was observed at 45^{o}C temperature giving 6.59, 6.84, 5.70 and 4.03 per cent inhibition with *P. ultimum*, *F. oxysporum*, *R. solani* and *S. rolfsii*, respectively.

Table 4.3. ***In vitro*** **thermal sensitivity of inocula of four pathogens of damping-off of tomato on mycelial growth inhibition at different temperatures and exposure durations**

Temperature (°C)	Exposure time (min)															
	Mycelial growth inhibition of fungal pathogens (%)															
	Pythium ultimum				*Fusarium oxysporum*				*Rhizoctonia solani*				*Sclerotium rolfsii*			
	5	10	20	Mean	5	10	20	Mean	5	10	20	Mean	5	10	20	Mean
35	0.88	3.33	5.99	3.4	1.55	4.33	4.66	3.51	0.66	2.88	2.77	2.10	0.22	0.99	2.99	1.40
40	5.78	6.43	8.43	6.88	2.88	4.55	6.21	4.54	1.22	3.88	5.10	3.40	0.22	0.99	2.88	1.36
45	4.46	6.66	8.66	6.59	5.66	7.10	7.77	6.84	5.33	5.33	6.44	5.70	3.11	4.33	4.66	4.03
Mean	3.70	5.47	7.69	-	3.36	5.32	6.21	-	2.40	4.03	4.77	-	1.88	2.10	3.51	-
CD (0.05)																
Temperature (A)	1.58				1.55				0.81				0.65			
Exposure time (B)	1.37				1.34				0.82				0.56			
A x B	2.74				2.07				1.41				1.12			

Data further revealed that similar trend of growth inhibition was observed for exposure durations exhibiting an increase in growth inhibition with the increase in exposure time from 5 to 10 and 20 min. All the exposure durations were statistically different from each other for all the four pathogens.

Interaction studies showed that *F. oxysporum* exhibited highest growth inhibition at 45°C temperature for 20 minutes giving 7.77 per cent followed by exposure to 10 minutes, though statistically at par with each other. Heat sensitivity of artificially inoculated wheat seeds and naturally infected root segments exposed to different durations 1 to 4 hours showed the survival of the pathogen and it was directly proportional to the age of the inoculumn and inversely proportional to both temperature and duration of exposure. Thus, *Dematophora necatrix* was much more sensitive to heat above 40°C whereas temperature below 40°C can be lethal if maintained for longer periods. (Munnecke *et al.*, 1976, Pullman *et al*., 1976 and Cartia and Asero, 1994).

4.4.1.2 Effect of soil solarization on soil temperature under field conditions

Effect of soil solarization on soil temperature in mulched and unmulched beds at three depths (5, 10 and 20 cm) was recorded for two consecutive crop seasons i.e. 2018 and 2019 and data obtained are presented in Table 4.4a and 4.4b.

Table 4.4a. Effect of soil solarization on soil temperature during 2018 and 2019 in solarized nursery beds

Duration (week)	Temperature (ºC) in solarized nursery beds									
	5 cm			10 cm			20 cm			Overall mean
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	
1	46.14	29.78	**37.96**	41.71	26.71	**34.21**	37.64	23.85	**30.74**	**34.10**
2	43.92	31.42	**37.67**	40.07	27.92	**33.99**	34.50	25.07	**31.10**	**34.25**
3	40.71	33.78	**37.24**	38.50	31.21	**34.85**	33.64	28.57	**31.02**	**34.38**
4	41.14	34.00	**37.57**	37.57	31.9	**34.73**	34.55	27.50	**33.30**	**35.20**
5	42.00	37.21	**39.60**	38.21	34.00	**36.10**	35.35	31.25	**35.57**	**37.09**
6	46.07	39.14	**42.60**	42.35	36.07	**39.21**	37.50	33.65	**36.82**	**39.54**
7	47.11	39.75	**43.43**	43.20	37.25	**40.22**	38.78	34.87	**29.78**	**37.81**
Mean	**43.87**	**41.68**	**-**	**40.23**	**32.15**	**-**	**35.99**	**29.25**	**-**	**-**
Overall mean	**39.43**			**36.18**			**36.05**			**-**

Table 4.4b. Effect of soil solarization on soil temperature during 2018 and 2019 in non-solarised nursery beds

Duration (week)	Temperature (ºC) in non-solarized nursery beds									
	5 cm			10 cm			20 cm			Overall mean
	2018	2019	Mean	2018	2019	Mean	2018	2019	Mean	
1	41.35	27.14	**34.24**	37.78	24.21	**30.99**	34.42	21.07	**27.74**	**30.99**
2	34.50	28.78	**31.64**	32.50	24.78	**28.64**	31.00	21.57	**26.28**	**28.85**
3	38.07	30.92	**34.49**	35.68	28.21	**31.94**	32.35	25.28	**28.81**	**31.74**
4	38.64	34.14	**36.39**	35.71	26.42	**31.06**	33.78	23.71	**28.74**	**32.06**
5	39.87	35.00	**37.43**	34.85	30.21	**32.53**	31.71	26.28	**28.99**	**32.98**
6	40.07	36.28	**38.17**	37.28	32.92	**35.10**	34.45	29.42	**31.93**	**35.06**
7	41.71	36.75	**39.23**	38.71	32.25	**35.48**	35.50	30.00	**32.75**	**35.82**
Mean	**39.17**	**32.71**	**-**	**36.07**	**28.42**	**-**	**33.31**	**25.32**	**-**	**-**
Overall mean	**35.94**			**32.45**			**23.32**			**-**

Year	Temperature range (ºC)	
	Solarized nursery beds	Non solarized nursery beds
2018	34-47	31- 41
2019	23-39	21-36

The data (Table 4.4.) indicated that there was a significant increase in soil temperature in nursery beds covered with polythene mulch (25 μm) as compared to the unmulched beds at all depths. Maximum increase in the mean weekly temperature was recorded at 5 cm depth followed by 10 and 20 cm. During the year 2018, in case of mulched nursery beds highest average/ mean weekly temperature of (43.87ºC) was recorded at 5 cm depth while the lowest (35.99ºC) was at 20 cm depth. While in the year 2019, maximum increase in the mean weekly temperature of (41.68ºC) was recorded at 5 cm depth followed by 10 and 20 cm with the temperature of 32.15ºC and 29.25ºC, respectively (Table 4.4a). Nursery beds mulched with polythene sheet showed an increase in average/ mean weekly temperature. In general, with increasing soil depths, there is rise in temperature progressively. Maximum temperature of (37.81ºC) was recorded in the seventh week while the lowest (34.10ºC) was in the first week.

In the present investigations, maximum rise of 3.4ºC in mean soil temperature was attained in mulched beds at 5 cm depth over unmulched beds. The rise in soil temperature due to soil solarization is in consonance with various workers (Katan *et al*., 1976; Al Karaghouli and Al-Kayssi, 2001; Chaube *et al.,* 2002; Mathur *et al*., 2002; Akhtar *et al*., 2012; Negi and Raj, 2013; Farrag and Fotouh, 2010; and Saied-Nehal, 2011).

4.4.1.3 Effect of soil solarization on viability of the pathogens causing damping-off of tomato under field conditions

Effect of soil solarization was studied on viability of the pathogen by placing the mass culture of the pathogen at 5 cm depth and plating on PDA plates after 10, 20, 30 and 40 days in both solarized and non-solarized beds ((Plate 4.6.) and results are presented in Table 4.5.

Table 4.5. Effect of soil solarization on viability of the pathogens causing damping-off of tomato under field conditions

Duration (days)	Viability of the pathogens							
	Solarized nursery beds				Non solarized nursery beds			
	Pythium ultimum	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***	***Pythium ultimum***	***Fusarium oxysporum***	***Rhizoconia solani***	***Sclerotim rolfsii***
10	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+
30	+	-	-	+	+	+	+	+
40	-	-	-	-	+	+	+	+

+ denotes viable pathogen
- denotes non-viable pathogen

It is clear from the data (Table 4.5) that viability of the pathogens has an inverse relationship with duration of the soil solarization. With the increase in the duration of soil solarization, viability of the pathogen decreased. Soil solarization for 10 to 20 days resulted in the survival of all the pathogens. Soil solarization for 30 days resulted in killing of the pathogens *F. oxysporum* and *R. solani* while all the pathogen were found dead after 40 days of soil solarization. In case of non-solarized control, pathogens were viable at all the depths even after 40 days. Therefore, it was observed that soil solarization for 30 days was sufficient to reduce the viability of pathogens to some extent but 40 days were required for complete loss of viability.

Soil solarization for 30 days was sufficient to reduce the viability of *F. oxysporum* and *R. solani* but 40 days were required for complete loss of viability of all the pathogens. These results are in agreement with findings of various workers (Pullman *et al*., 1979; Raj *et al.,* 1997; Reddy *et al*, 2006) who have already reported the effectiveness of soil solarization against pathogens causing damping-off.

4.4.2 BIOLOGICAL CONTROL

4.4.2.1 *In vitro* evaluation of BCAs

Effect of various fungal and bacterial biocontrol agents (*Trichoderma harzianum, T. viride, T. virens, T. hamatum, Bacillus subtilis, Bacillus licheniformis, Bacillus* sp., *Pseudomonas fluorescens* and *Pseudomonas* sp.) on mycelial growth of test fungi i.e. *P. ultimum, F. oxysporum, R. solani* and *S. rolfsii* was studied by dual culture method (Huang and Hoes, 1976) and streak plate methods, respectively, under *in vitro* conditions. The experiment was conducted in completely randomized design with five replications each. Data on per cent mycelial inhibition was calculated and is presented in Table 4.6.

The data (Table 4.6.) revealed that all the fungal and bacterial antagonists were able to inhibit the mycelial growth of the tested pathogens (*P. ultimum, F. oxysporum, R. solani and S. rolfsii* to varying levels ranging from 28.84 to 63.93 per cent exhibiting minimum in *Pseudomonas fluorescence* while maximum in *T. harzianum,* respectively. Further, out of fungal antagonists, *T. harzianum* (63.93%) was statistically superior (Plate 4.7.) from other treatments followed by *T. viride* (59.76%). Among the bacterial antagonists, maximum

Plate 4.6. Effect of soil solarization on viability of the pathogens

Plate 4.6a.
Mass culture of the associated pathogens

Plate 4.6b.
3-5 g inoculum of each pathogen added to nylon mesh

Plate 4.6c.
Plating of inoculum on PDA plates and incubated at 25°C

mycelial growth inhibition was shown by *Bacillus licheniformis* (37.45%) while minimum mycelial was observed with *Pseudomonas fluorescence* (28.84 %) against the pathogens.

Table 4.6. *In vitro* evaluation of fungal and bacterial antagonists on growth inhibition of associated pathogens of damping-off of tomato

Treatments	Growth inhibition (%)				
	Associated fungal pathogens				
	Pythium ultimum	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***	**Mean**
Bacillus sp.	26.11 (30.42)	40.73 (39.66)	50.00 (45.00)	18.51 (25.39)	33.84 (35.12)
Bacillus subtilis	23.14 (28.67)	42.03 (40.41)	42.22 (40.52)	33.33 (35.07)	35.18 (36.17)
Pseudomonas fluorescence	18.54 (25.11)	42.40 (40.63)	44.25 (41.69)	10.18 (18.57)	28.84 (31.50)
Bacillus licheniformis	23.14 (28.67)	40.18 (39.34)	57.40 (49.31)	29.07 (32.62)	37.45 (37.48)
Pseudomonas sp.	15.36 (23.06)	41.66 (40.20)	62.96 (52.60)	17.59 (24.70)	34.39 (35.14)
T. harzianum	42.77 (40.83)	81.48 (64.61)	73.14 (58.87)	58.33 (49.83)	63.93 (53.53)
T. hamatum	34.63 (36.05)	52.96 (46.70)	77.22 (61.80)	66.66 (54.83)	57.87 (49.84)
T. viride	26.66 (30.80)	52.22 (46.28)	81.48 (64.56)	78.70 (62.59)	59.76 (51.06)
T. virens	29.07 (32.38)	66.85 (54.86)	67.59 (56.04)	59.25 (50.33)	55.69 (48.41)
Mean	26.60 (34.74)	51.17 (48.40)	61.81 (54.17)	41.29 (42.53)	-

Figures in the parentheses are arc sine transformed values

CD $_{(0.05)}$

Pathogens (P)	(2.08)
Concentration (C)	(3.29)
P x C	(6.59)

Data (Table 4.6) further showed that irrespective of the antagonists, *P. ultimum* exhibited minimum mycelial growth inhibition (26.60%) (Plate 4.7a) followed by *S. rolfsii* (41.29%) (Plate 4.7d), *F. oxysporum* (51.17%) (Plate 4.7b) and *R. solani* (61.81%) (Plate 4.7c).

Interaction studies have showed that *T. harzianum* resulted in highest inhibition of *F. oxysporum* giving 81.48 per cent inhibition followed by *T. hamatum* giving 77.22 per cent growth inhibition of *R. solani*, though statistically at par with each other.

Species of *Trichoderma* have been extensively tested and used as biocontrol agent against a wide range of plant pathogens *viz., Rhizoctonia, Fusarium, Sclerotium.*

Macrophomina, *Sclerotinia*, *Pythium* spp. *etc*. (Papavizas, 1985; Chet, 1987; Hornby, 1990; Deacon, 1991). El-Abbasi *et al*. (2003) reported that *T. harzianum* inhibited *Pythium* by 62.4 per cent, *Sclerotium rolfsii* by 54.3 per cent, *Macrophomina phaseolina* by 36.7 per cent and *Rhizoctonia solani* by 36.0 per cent. Similar *in vitro* efficacy of *Trichoderma* spp. against soil borne pathogens was reported by Kapoor (2008) who observed that maximum inhibition of mycelial growth of *Rhizoctonia solani*, *Pythium debaryanum*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *pisi* was shown by *T. harzianum.* Rajput *et al*. (2010) evaluated fungal (*Trichoderma harzianum*, *T. viride*) and bacterial (*Pseudomonas fluorescens* and *Bacillus subtilis*) antagonists against chickpea wilt complex pathogens *viz.*, *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Sclerotium rolfsii* and found that *T. harzianum* gave the maximum inhibition of radial growth of *F. oxysporum* f. sp. *ciceri* (65.23%), *R. bataticola* (89.67%) and *S. rolfsii* (86.00%) under dual culture tests.

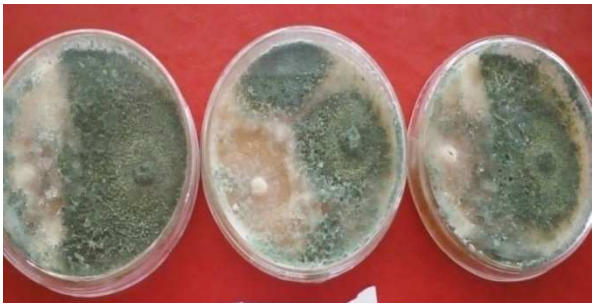
4.4.2.2 Evaluation of BCAs and natural bio resources under pot conditions

Fungal and bacterial antagonists evaluated under *in vitro* conditions and natural bio-resources namely, beejamrit and jeevamrit were further evaluated against the associated pathogens under pot conditions by mixing their cultures in the sterilized soil. Data on disease incidence were recorded and calculated per cent disease control has been presented in Table 4.7.

Data presented in Table 4.7 showed that all the fungal and bacterial antagonists reduced the incidence of post emergence damping-off irrespective of the associated fungal pathogen, however, minimum incidence of damping-off of tomato was recorded with soil application of mass culture of *T. harzianum* (15.83%) followed by jeevamrit application (18.33%), though statistically at par with each other. Data further showed that out of four associated fungal pathogens, mass culture of *P. ultimum* resulted in the highest incidence of post-emergence damping-off (40.83%) (Plate 4.8a) followed by *F. oxysporum* (35.83%) (Plate 4.8b), *R. solani* (35.55%) (Plate 4.8c) and *S. rolfsii* (35.27%) (Plate 4.8d), which were statistically different from *P. ulimum*.

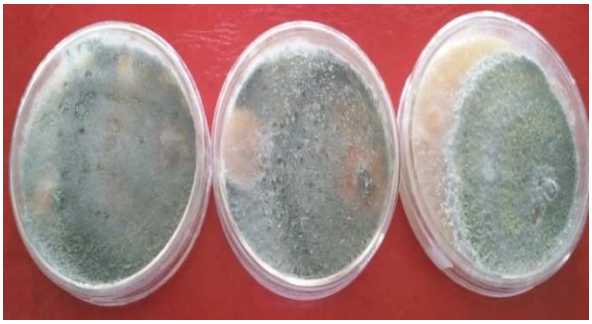
Interaction studies showed that *T. harzianum*, beejamrit and jeevarit gave minimum incidence of *F. oxysporum* and *T. viride* resulted in minimum incidence of *R. solani* and *S. rolfsii* under pot conditions.

Plate 4.7.Effect of *Trichoderma harzianum* on growth inhibition of the associated pathogens of damping off of tomato



4.7a. *Pythium ultimum*

4.7b. *Fusarium oxysporum*



4.7c. *Rhizoctonia solani*

4.7d. *Sclerotium rolfsii*

Plate 4.8. Effect of *Trichoderma harzianum* on the associated pathogens in pot conditions

4.8.a *Pythium ultimum*

4.8.b *Fusarium oxysporum*

4.8.c *Rhizoctonia solani*

4.9.d *Sclerotium rolfsii*

Data further revealed that the percentage disease control ranged from 46.67 to 84.17 percent exhibiting highest disease control with *T. harzianum* (84.17%) followed by jeevamrit and *T. viride* giving 81.67 and 79.17 per cent disease control.

Table 4.7. Evaluation of fungal and bacterial antagonists and natural bio-resources on post-emergence damping-off of tomato under pot conditions

Treatments	Incidence of post-emergence damping-off (%)					Percent disease control
	Associated fungal pathogens					
	Pythium ultimum	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***	**Mean**	
Bacillus sp.	30.00 (33.00)	43.33 (41.16)	43.33 (41.16)	43.33 (41.16)	40.00 (39.12)	60.00
Bacillus subtilis	40.00 (39.15)	30.00 (33.00)	30.00 (33.00)	30.00 (33.00)	32.50 (34.54)	67.50
Pseudomonas fluorescence	26.66 (31.00)	33.33 (35.01)	33.33 (35.01)	33.33 (35.01)	31.66 (34.01)	68.34
Bacillus licheniformis	50.00 (45.00)	46.66 (43.08)	46.66 (43.08)	43.33 (41.16)	46.66 (43.08)	53.34
Pseudomonas sp.	70.00 (57.00)	53.33 (46.92)	43.33 (41.16)	46.66 (43.08)	53.33 (47.04)	46.67
T. harianum	13.33 (21.15)	13.33 (21.15)	20.00 (26.07)	16.66 (23.86)	15.83 (23.05)	84.17
T. hamatum	36.66 (37.23)	20.00 (26.07)	23.33 (28.78)	20.00 (28.78)	25.00 (29.54)	75.00
T. viride	16.66 (23.86)	40.00 (39.15)	13.33 (21.15)	13.33 (21.15)	20.83 (26.32)	79.17
T. virens	43.33 (46.16)	23.33 (28.78)	30.00 (33.00)	30.00 (33.00)	31.66 (33.59)	68.34
Beejamrit	40.00 (39.15)	13.33 (21.15)	26.66 (31.00)	26.66 (31.00)	26.66 (30.57)	73.34
Jeevamrit	23.33 (28.78)	13.33 (21.15)	16.66 (23.86)	20.00 (26.07)	18.33 (24.96)	81.67
Control	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	-
Mean	40.83 (40.54)	35.83 (37.22)	35.55 (37.27)	35.27 (37.05)	-	-

Figures in the parentheses are arc sine transformed values

CD $_{(0.05)}$	
Antagonists (A)	(4.06)
Pathogens (P)	(2.34)
A x P	(9.50)

Krishnamoorthy and Bhaskaran (1990) evaluated different species of *Trichoderma* as seed treatment against *Pythium* spp. and reported that *T. viride* and *T. harzianum* gave good control of pre-emergence damping-off caused by *P. indicum* which supports the present findings pertaining to application of BCAs especially *T. harzianum* and *T. viride* for the management of damping off of tomato as seed treatment. Reduction of seedling diseases in

green house caused by various soil borne pathogens including *Pythium* spp. by *T. viride* and *B. subtilis* have already been reported (Yehia *et al*., 1981; Salman, 2010).

4.4.3 PLANT EXTRACTS

4.4.3.1 *In vitro* evaluation of botanicals

Aqueous extracts of seven botanicals namely, *Zingiber officinale*, *Vitex nigundu*, *Melia azedirach*, *Murraya koeningi*, *Lantana camara*, *Bougainvillea glabra* and *Allium sativum* were tested at different concentrations (2, 5, 10 and 15%) against the associated pathogens of damping-off of tomato under *in vitro* conditions. Data on mycelial growth inhibition was calculated and after statistical analysis is presented in Table 4.8a and 4.8b.

Table 4.8a. *In vitro* evaluation of botanical extracts on growth inhibition of associated pathogens of damping-off of tomato

Treatments	Growth inhibition of associated pathogens									
	Concentration (%)									
	Pythium ultimum					*Fusarium oxysporum*				
	2	5	10	15	Mean	2	5	10	15	Mean
Zingiber officinale	0.00	0.00	0.00	0.00	0.00	38.88 (38.55)	42.59 (40.73)	44.44 (41.80)	33.33 (35.21)	39.81 (39.07)
Vitex nigundu	0.00	0.00	0.00	0.00	0.00	57.40 (39.27)	49.99 (45.04)	64.81 (53.63)	61.11 (51.45)	57.33 (49.85)
Melia azedirach	0.00	0.00	0.00	0.00	0.00	48.15 (43.94)	35.18 (36.37)	46.29 (42.87)	57.40 (49.27)	46.75 (43.11)
Lantana camara	0.00	0.00	0.00	0.00	0.00	31.48 (34.11)	44.44 (41.80)	40.73 (39.65)	50.00 (45.00)	41.66 (40.14)
Murraya koeningii	0.00	0.00	0.00	0.00	0.00	5.55 (11.03)	9.25 (14.52)	18.52 (25.24)	50.00 (45.00)	20.83 (23.95)
Bougainvillea glabra	0.00	0.00	0.00	0.00	0.00	11.11 (19.47)	12.96 (21.01)	22.22 (28.01)	35.18 (36.32)	20.37 (26.20)
Allium sativum	0.00	0.00	0.00	0.00	0.00	14.81 (22.55)	22.22 (28.01)	33.33 (35.21)	42.59 (40.73)	28.23 (31.63)
Mean	0.00	0.00	0.00	0.00	0.00	25.92 (27.36)	27.08 (28.43)	33.79 (33.30)	41.20 (37.87)	-

Figures in the parentheses are arc sine transformed values

$CD_{(0.05)}$		
Treatment (T)	-	(3.32)
Concentration (C)	-	(2.34)
T x C	-	(6.65)

Plate 4.9.Effect of *Lantana camara* on associated pathogens

2% 5% 10% 15%

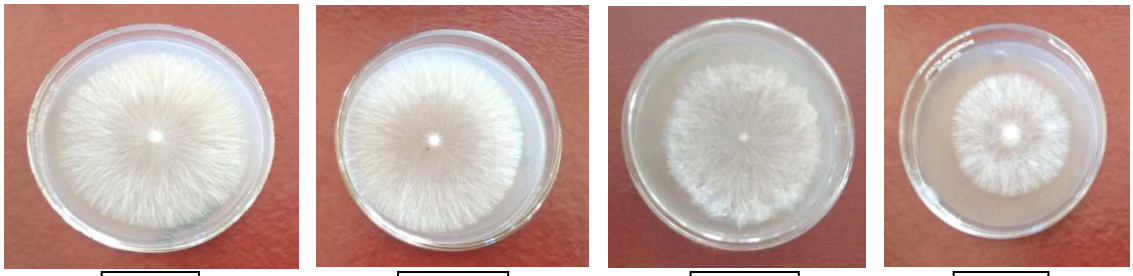
Plate 4.9.a Effect of *Lantana camara* on *Pythium ultimum*

2% 5% 10% 15%

Plate 4.9.b Effect of *Lantana camara* on *Fusarium oxysporumm*

2% 5% 10% 15%

Plate 4.9.c Effect of *Lantana camara* on *Rhizoctonia solani*



2% 5% 10% 15%

Plate 4.9.d Effect of *Lantana camara* on *Sclerotium rolfsii*

It is evident from data (Table 4.8a.) that there was no reduction in the growth of mycelium in *P. ultimum* with any of the extract of the botanicals while irrespective of concentration, (Plate 4.9.a) *Vitex nigundu* resulted in 57.33 per cent mycelial growth inhibition of *F. oxysporum* (Plate 4.9.b) which was followed by *Melia azedarach* (46.75%) and *Lantana camara* (41.66%). In case of *R. solani*, *Zingiber officinale* gave maximum mycelial growth inhibition (24.07%) (Plate 4.9.c) followed by *Melia azedarach* (20.82%) and *Lantana camara* (15.27%), whereas, in case of *Sclerotium rolfsii*, *Lantana camara* resulted in 25.00 per cent mycelial growth inhibition (Plate 4.9.d) followed by *Melia azedarach* and *Lantana camara* giving 20.82 and 15.27 per cent mycelial growth inhibition (Table 4.8b).

Table 4.8b. *In vitro* evaluation of botanical extracts on growth inhibition of associated pathogens of damping-off of tomato

Treatments	Growth inhibition (%)									
	Concentration (%)									
	Rhizoctonia solani					*Sclerotium rolfsii*				
	2	5	10	15	Mean	2	5	10	15	Mean
Zingiber officinale	20.37 (26.45)	12.96 (21.00)	33.33 (35.14)	29.62 32.83)	24.07 (28.86)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (0.00)	0.00 (0.00)
Vitex nigundu	7.40 (15.56)	3.70 (6.48)	9.25 (17.51)	7.40 15.57)	6.94 (13.79)	9.25 (2.89)	7.40 (2.65)	22.22 (4.79)	22.22 (28.01)	15.27 (20.88)
Melia azedarach	1.85 (4.54)	14.81 (22.34)	27.77 (31.71)	38.88 (38.55)	20.82 (24.29)	3.70 (2.04)	7.40 (2.86)	14.81 (3.86)	11.11 (19.47)	9.25 (16.52)
Lantana camara	1.85 (4.54)	3.70 (9.08)	24.07 (29.16)	31.48 (34.09)	15.27 (19.22)	3.70 (2.04)	14.81 (3.96)	24.07 (5.00)	57.41 (49.28)	25.00 (27.57)
Murraya koeningii	1.85 (4.54)	3.70 (9.08)	1.85 (4.54)	5.55 (13.63)	3.23 (7.94)	1.85 (1.52)	7.40 (2.86)	20.37 (4.61)	31.48 (34.11)	15.27 (20.25)
Bougainvillea glabra	0.00 (1.00)	0.00 (1.00)	3.70 (9.08)	7.40 (15.57)	2.77 (6.16)	0.00 (1.00)	0.00 (1.00)	5.55 (2.34)	11.11 (19.47)	4.16 (7.62)
Allium sativum	0.00 (1.00)	0.00 (1.00)	3.70 (6.49)	7.40 (15.57)	2.77 (5.51)	0.00 (1.00)	0.00 (1.00)	7.40 (2.86)	11.11 (19.47)	4.62 (8.76)
Mean	4.16 (6.95)	4.86 (8.50)	12.96 (16.71)	15.97 (20.73)	-	2.31 (4.65)	4.62 (8.33)	11.80 (16.59)	18.05 (21.23)	-
$CD_{(0.05)}$										
Treatment (T)		(4.41)					(3.89)			
Pathogen (P)		(3.11)					(2.75)			
T x P		(8.83)					(7.80)			

With regard to the concentration of the extract of the tested botanicals, data further revealed that concentration of the extracts was directly proportional to the mycelial growth

inhibition and therefore, with the increase in concentration, there was an increase in mycelial growth inhibition of the tested fungi such as in *F. oxysporum*, increasing the concentration from 2 to 15 per cent, mycelial growth inhibition was increased from 25.62 to 41.20 per cent, respectively. Similarly, in *R. solani*, 4.16 per cent growth inhibition was observed at 2 per cent concentration of botanicals while it was 15.97 per cent at 15 per cent concentration. Similar trend was observed in *S. rolfsii* wherein, increase in concentration from 2 to 15 per cent, there was a corresponding increase in mycelial growth inhibition giving 2.31 per cent at 2 per cent and 18.05 at 15 per cent concentration of the botanicals.

Interaction between concentration and growth inhibition of pathogens revealed that *V. nigundu* at 10 per cent concentration resulted in highest growth inhibition of *F. oxysporum* (64.80%) followed by 15 per cent concentration of *V. nigundu* inhibition *F. oxysporum* (61.11%), though statistically similar to each other.

Leaf extracts from commonly available plants (*in vitro*) against soil borne plant pathogens like *S. rolfsii, R. solani* and *P. aphanidermatum* have been found to be effective by earlier workers (Manian *et al.*, 1988; Kumar *et al.*, 1996; Jacob and Siva Prakasan, 1994; Arya *et al.*, 1995; Rodriguez and Montilla, 2002; Babu *et al*., 2000; Hooda *et al*., 2011; Pattnaik *et al*., 2012.

4.4.3.2 Evaluation of botanicals on disease incidence under pot conditions

Aqueous extracts of botanicals were further evaluated at 15 per cent concentration under pot conditions against the associated pathogens of damping-off of tomato. Plastic pots filled with sterilized soils were inoculated with mass culture of pathogens which were multiplied on corn: sand meal medium. In each pot, seven to eight seedlings (15 to 25 days old) treated by root dip treatment were transplanted. Data on disease incidence were recorded and is presented in Table 4.9.

Data presented in Table 4.9 showed that all the botanical extracts reduced the incidence of damping-off of tomato caused by all four tested pathogens, however, *L. camara* gave the minimum incidence (37.50%), (Plate 4.10.) irrespective of the pathogens, followed by *V. nigundu* (39.16%), though statistically at par with each other.

Data further revealed that out of four pathogens associated with damping-off of tomato, *F. oxysporum* and *S. rolfsii* were equally sensitive to the botanical extracts and exhibited 49.58 per cent disease incidence. This was followed by *R. solani* and *P. ultimum*

Plate 4.10. Effect of *Lantana camera* (15 %) on the associated pathogens in pot

Pythium ultimum

Fusarium oxysporum

Rhizoctonia solani

Sclerotium rolfsii

giving 57.08 and 76.25 per cent incidence, respectively, which were statistically different from each other.

Interaction studies showed that *L. camara* resulted in minimum incidence of *S. rolfsii* (20.00%) followed by *V. nigundu* giving minimum incidence of *S. rolfsii* (23.33%), though statistically similar to each other.

L. camara gave highest per cent disease control (62.50%) of damping-off to tomato under pot conditions followed by *V. nigundu* (60.84%), whereas, minimum disease control was achieved by soil application of extract of *B. glabra* (33.34%).

Table 4.9. Evaluation of botanical extracts on disease incidence and per cent disease control of damping-off of tomato under pot culture conditions

Treatment	Disease Incidence (%)					Per cent disease control
	Associated fungal pathogens					
	Pythium ultimum	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***	**Mean**	
Zingiber officinale	70.00 (57.00)	46.66 (43.08)	43.33 (41.16)	56.66 (48.85)	54.16 (47.52)	45.84
Vitex nigundu	66.66 (54.78)	30.00 (33.00)	36.66 (37.23)	23.33 (28.78)	39.16 (38.45)	60.84
Melia azedarach	66.66 (54.78)	46.66 (43.08)	30.00 (33.00)	50.00 (45.00)	48.33 (43.97)	51.67
Lantana camara	60.00 (50.85)	30.00 (33.00)	40.00 (39.15)	20.00 (26.07)	37.50 (37.27)	62.50
Murraya koenigii	80.00 (63.93)	40.00 (39.15)	76.66 (61.22)	30.00 (33.00)	56.66 (49.33)	43.34
Bougainvillea glabra	90.00 (75.00)	50.00 (45.00)	66.66 (54.78)	60.00 (50.85)	66.66 (56.41)	33.34
Allium sativum	76.66 (61.22)	53.33 (46.92)	63.33 (52.78)	56.66 (48.85)	62.50 (52.44)	37.50
Control	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	-
Mean	76.25 (63.45)	49.58 (46.66)	57.08 (51.16)	49.58 (46.43)	-	-

Figures in the parentheses are arc sine transformed values

$CD_{(0.05)}$

Treatments (T)	(4.25)
Concentration (C)	(3.01)
T x C	(12.79)

Various workers such as Chandel and Sharma (2014), Bouzidi and Mederbal (2016), Banakar *et al*. (2017), Sanaullah *et al.*(2018) and Nasrin *et al.* (2018) have reported that soil

borne diseases can be controlled with application of different plant extracts (botanicals), both under *in vitro* and field conditions.

4.4.4 MANAGEMENT THROUGH SYSTEMIC ACQUIRED RESISTANCE (SAR) INDUCERS

4.4.4.1 Evaluation of systemic acquired resistance (SAR) inducers under pot culture conditions

Five different systemic acquired resistance (SAR) or abiotic resistance inducers were evaluated under pot culture conditions against damping-off of tomato. Plastic pots filled with sterilized soils were inoculated with mass culture of pathogens which were multiplied on corn: sand meal medium. In each pot, seven to eight seedlings (15 to 25 days old) treated by root dip treatment were transplanted. Data on disease incidence and per cent disease control were recorded and after statistical analysis has been presented in Table 4.10.

Table 4.10. Evaluation of systemic acquired resistance (SAR) inducers on disease incidence and per cent disease control of damping-off of tomato under pot culture conditions

Treatments	Conc. (mM)	Disease Incidence (%)					Percent disease control
		Associated fungal pathogens					
		Pythium ultimum	***Fusarium oxysporum***	***Rhizoctonia solani***	***Sclerotium rolfsii***	**Mean**	
Oxalic acid	20	28.57 (31.80)	42.85 (38.09)	38.09 (38.03)	47.61 (43.63)	39.28 (38.56)	60.72
Potassium oxalate	50	42.85 (40.77)	28.56 (38.09)	38.09 (38.03)	66.66 (54.83)	44.04 (41.36)	55.96
Potassium chloride	300	4.76 (7.40)	9.52 (19.04)	19.04 (25.57)	33.33 (34.54)	16.66 (19.37)	83.34
Salicylic acid	10	85.71 (71.83)	85.71 (76.18)	76.18 (61.05)	85.71 (71.83)	83.32 (69.13)	16.68
Potassium dihydrogen phosphate	50	14.28 (18.17)	4.76 (61.90)	61.90 (52.60)	11.52 (21.04)	20.24 (19.54)	79.76
Control	-	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	-
Mean	-	35.23 (33.99)	34.28 (32.51)	46.66 (43.06)	46.66 (40.96)	-	-

Figures in the parentheses are arc sine transformed values

$CD_{(0.05)}$	
Treatment (T)	(8.68)
Pathogen (P)	(9.70)
T x P	(18.34)

Plate 4.11. Effect of potassium chloride on the associated pathogens in pot conditions

Pythium ultimum

Fusarium oxysporum

Rhizoctonia solani

Sclerotium rolfsii

It is evident from the data presented in Table 4.10 that all the SAR inducer treatments resulted in disease reduction, whereas, seedling dip treatment of potassium chloride (Plate 4.11) and potassium dihydrogen phosphate when applied at 300 and 200 mM concentration were found effective and resulted in 16.66 and 20.24 per cent disease incidence, respectively, though statistically at par with each other. Oxalic acid (20 mM) also reduced the disease incidence to 39.28 per cent while Salicylic acid 10 mM was least effective with maximum (83.32%) disease incidence.

Data (Table 4.10) also showed that out of four fungal pathogens tested, *F. oxysporum* gave minimum disease incidence followed by *P. ultimum* and were statistically similar to each other. Further, *R. solani* and *S. rolfsii* exhibited equal sensitivity to the SAR inducer chemicals by giving 46.66 per cent disease incidence.

Interaction studies showed that potassium chloride (300 mM) and potassium dihydrogen phosphate (50 mM) resulted in minimum disease incidence of *P. ultimum* and *F. oxysporum* giving 4.76 per cent followed by potassium chloride exhibiting 9.52 per cent incidence of *F. oxysporum*.

With regard to per cent disease control, it was found that potassium chloride resulted in highest disease control (83.34%) of damping-off of tomato followed by potassium dihydrogen phosphate (79.76%) and oxalic acid (60.72%).

SAR chemicals provide multiple disease protection with only one application as described by earlier workers (Raskin, 1992; Vernooij *et al.*, 1995) and Janjam *et al*., 1996). A wide range of chemical compounds such as oligosaccharides (Yokoshiwa *et al*.,1993), glycoprotein and peptides (Benhamou, 1992) and salicylic acid (Yalpani *et al*., 1991) has been used for induction of systemic resistance in different plants.

4.4.5 Integrated disease management under the field conditions

In order to study the effect of soil solarization and bio-control agent, botanical extract and resistant inducing chemical, found best under *in vitro* and *in vivo* conditions, on the incidence of damping-off of tomato, an experiment was laid out during 2019 crop season in randomized block design (RBD) and each treatment was replicated thrice times. The data on disease incidence of damping-off were recorded and calculated per cent disease control is presented in Table 4.11.

Data (Table 4.11) revealed that a significant reduction in incidence of damping-off was observed in all the treatments in comparison to control. The minimum disease incidence of damping-off was recorded in the combined treatment of soil solarization+ soil application of *T. harzianum*+ soil application of *Lantana camara* + soil application of potassium chloride which exhibited minimum disease incidence (13.11%) (Plate 4.12h) followed by treatment comprising of soil solarization + *L. camara* + potassium chloride with 30.27 per cent disease incidence Plate 4.12f), though statistically different from each other.

Combined application of soil solarization+ *T. harzianum*+ *Lantana camara* + potassium chloride also gave maximum disease control of 74.88 per cent followed by treatment including soil solarization + *L. camara* + potassium chloride giving 42.02 per cent disease control.

Table 4.11. Effect of soil solarization, bio-control agent, botanical extract and resistant inducing chemical on damping-off of tomato under the field conditions

Treatments	Disease incidence (%)	Percent disease control
Soil solarisation	45.55 (42.34)	12.75
Soil solarization + *T. harzianum* (TH)	35.82 (36.14)	31.39
Soil solarization + *Lantana camara* (LC)	40.55 (38.49)	22.33
Soil solarization + potassium chloride (PC)	41.38 (40.40)	20.74
Soil solarization + TH + LC	40.55 (38.49)	22.33
Soil solarization + LC + PC	30.27 (32.41)	42.02
Soil solarization + TH + PC	31.94 (46.09)	38.82
Soil solarization + TH + LC + PC	13.11 (21.39)	74.88
Control	52.21 (46.27)	-
$CD_{0.05}$	(15.12)	-

Figures in the parentheses are arc sine transformed values

It can be clearly seen that integration of different control measures had an additive effect in disease management over the individual treatment alone. These results are in

Plate 4.12. Integrated disease management under field conditions

4.12a.
Soil solarization

4.12b.
Soil solarization + biocontrol agent

4.12c.
Soil solazisation + botanical

4.12d.
Soil solarization + resistace inducing chemical

4.12e.
Soil solarization + biocontrol agent + botanical

4.12f.
Soil solarization + botanical + resistance inducing chemical

4.12g.
Soil solarization + biocontrol agent + resistance inducing chemical

4.12h.
Soil solarization + biocontrol agent+ botanical + resistance inducing chemical

(4.12i) Control

agreement with findings of various workers such as Haritha (2003), Champawat and Sharma (2003), Panday *et al*. (2005), Jayaraj and Radhakrishnan (2008), Jayaraj and Radhakrishnan (2008), (Pandey *et al*., 2016), Mahato *et al*. (2017).

Chapter-5

SUMMARY AND CONCLUSION

The present investigations on damping-off of tomato were undertaken in relation to the isolation and identification of the associated pathogens, symptomatology, morphological and cultural studies, pathogenicity and management of the disease through soil solarization, biological control, botanicals and resistance inducing chemicals alone and their integration. The results obtained are summarized as under:

The disease appeared in the two phases *viz*., pre-emergence and post-emergence. In pre- emergence, growth of the seedlings appeared in patches which act as characteristic symptom of the disease. Patchy growth among the seedlings in nursery beds were also observed. This was due to the infection of the seeds and germinating parts like plumule and radicle which resulted in rotting of the seeds before the emergence of the seedling. In case of post- emergence, small water soaked lesions appeared on the stem portion near the soil line which later enlarged in size and became brown to light brown in colour, resulted in constriction of the stem and toppling over of the seedling on ground due to the weight of the upper part of the young seedlings.

Identification of the isolated pathogens was done on the basis of various morphological and cultural characteristics. Morphological studies revealed that pathogens were fast growing on artificial medium with initially white, cottony and profusely growing mycelium attaining a colony diameter of 90 mm within 3-6 days. In case of *P. ultimum,* hyphae of the pathogen were hyaline and coenocytic, but septa were also observed in some old cultures. Pathogen produced sporangia which were mostly round in shape. In old cultures, production of oospore was also observed and they were round in shape. In *F. oxysporum,* the mycelium was hyaline at first becoming cream coloured with age and had branches and septations. The fungus produced macroconidia, microconidia and chlamydospores. Macroconida were hyaline, 3-5 septate, falcate having gradually pointed and curved ends and appeared on sporodochia or without formation of such fruiting bodies. Microconidia were abundant, ellipsoid, straight to curved and hyaline. Chlamydospores were both smooth and rough walled characterized by thick walls formed terminally or intercallary, single or in pairs. In *R. solani,* the mycelium was septate, colour of the hyphae varied from hyaline when young

to brownish on maturity. The branching occurred almost at right angles to the hyphal cell. Fungus also produced sclerotia which were barrel shaped, comparatively smaller cells in groups, brown to black and unlike other fungi, sclerotia were undifferentiated into rind and medullae. In *S. rolfsii* the hyphae was hyaline, thin walled, sparsely septate when young. Sclerotia initially were formed from hyphal strands that consist of 3-12 hyphae lying parallel. Mature sclerotia were dark brown but variation from lighter brown to darker colour was found. These were small about the size of radish seed, hard and usually round.

Pathogenicity of the associated pathogens with damping-off were proved on tomato cv. “Solan Lalima” for both pre and post emergence phase either alone or in the combination. It was done by sowing the seeds and transplanting of the seedlings in the pots containing sick soil, after seven days of inoculation of the pathogen. Incubation period was shorter i.e. 96 h in case of *P. ultimum* while it was maximum in case of *F. oxysporum* i.e. 144 h. Disease incidence was more in pre-emergence phase (94.44%) while it was 50.00 per cent in post-emergence phase.

During i*n vitro* thermal sensitivity of inocula of fungal pathogens of damping-off of tomato, it was observed that with the increase in the temperature and duration, mycelial growth inhibition of fungal pathogens also increased. Maximum mycelial growth inhibition was recorded in *P. ultimum* (5.56%) followed by *F. oxysporum* (4.95%) and *R. solani* (3.73%).

Soil solarization increased the mean soil temperature by 3.49ºC at 5 cm depth as compared to nonsolarized control at the same depth. Increment in temperature decreased with increase in soil depth in both solarized and non-solarized nursery beds but temperature recorded was comparatively high in solarized beds. Soil solarization for 10 to 20 days resulted in the survival of all the pathogens. Soil solarization for 30 days resulted in killing of the pathogens *F. oxysporum* and *R. solani* while all the pathogens were not viable after 40 days of soil solarization.

Among different fungal and bacterial antagonists evaluated against the pathogens, *T. harzianum* caused maximum mycelial growth inhibition (63.93%) followed by *T. viride* (59.76%). Among the bacterial antagonists, maximum mycelial growth inhibition was recorded by *Bacillus licheniformis* (37.45%) followed by *B subtilis* (35.18%) under *in vitro* conditions.

There was a significant reduction in the incidence of damping-off of tomato when fungal and bacterial antagonists were applied in the soil during the pot experiment. The antagonist. *T. harzianum* resulted in minimum disease incidence (15.83%) and was significantly superior from other treatments followed by Jeevamrit (18.33%). Among the bacterial antagonists, *Pseudomonas fluorescence* resulted in minimum disease incidence (31.66%) followed by *Bacillus subtilis* (32.50%).

Among the different botanicals used at different concentrations under *in vitro* conditions, there was no reduction in the growth of mycelium in *P. ultimum* with any of the botanical while in *F. oxysporum*, mycelial growth inhibition was 57.33 per cent by *Vitex nigundu* followed by *Melia azedarach* (46.75%) and *Lantana camera* (41.66%). In *R. solani*, maximum mycelial growth inhibition was (24.07%) by *Zingiber officinale* followed by *Melia azedarach* (20.82%) and *Lantana camara* (15.27%), whereas, in *S rolfsii*, maximum mycelial growth inhibition was 25.00 per cent by *Lantana camara* followed by *Melia azedarach* (20.82%) and *Vitex nigundu* (15.27%).

In the pot conditions, when botanicals were applied at 15 per cent concentration, there was a significant reduction in the disease incidence with *Lantana camara* (37.50%) followed by *Vitex nigundu* (39.16%).

Among the resistance inducing chemicals, all the chemical treatments resulted in disease reduction, whereas, seedling dip treatment of potassium chloride and potassium dihydrogen phosphate when applied at 300 and 200 mM concentration respectively, were found effective and resulted in 16.66 and 20.24 per cent disease incidence, respectively.

In integrated disease management study, significant reduction in incidence of damping-off was observed in all the treatments in comparison to control. The minimum disease incidence of damping-off was recorded in the combination of all the treatments (soil solarization + soil application of *T. harzianum* + soil application of *Lantana camara* + seedling treatment of potassium chloride) resulted in 13.11 per cent disease incidence followed by treatment (soil solarization + botanical+ resistance inducing chemicals) with 30.27 per cent disease incidence.

Therefore, the results of present investigations can be concluded as:

- *P. ultimum, R. solani, F. oxysporum* and *S. rolfsii* were found associated with damping-off of tomato.
- *T. harzianum, Lantana camara* and potassium chloride were found the best against all pathogens under pot conditions.
- For the management of damping-off of tomato under field conditions, soil solarization along with soil application of *T. harzianum, Lantana camara* and seedling treatment with potassium chloride were found effective

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APPENDIX-I

Composition of Media Used

1. Oat meal agar medium

Oat	:	40.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

2. Potato dextrose agar medium

Potato	:	250.0 g
Dextrose	:	20.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

3. Nutrient agar

Peptone	:	5.0 g
Yeast extract	:	2.0 g
Beef extract	:	3.0 g
NaCl	:	5.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml

4. Beejamrit (Per bigha)

Water	:	20 l
Cow urine	:	5 l
Cow dung	:	5 kg
Lime	:	50 g

5. Jeevamrit (Per acre)

Water	:	200 l
Cow dung	:	10 kg
Gram Flour	:	1 kg
Jaggery	:	1 kg
Cow urine	:	5 l

6. Wheat bran saw dust

Wheat bran	:	30 kg
Saw dust	:	10 kg
Sucrose	:	2%

7. Corn sand meal medium

Corn	:	30 kg
Sand	:	10 kg
Sucrose	:	2%

APPENDIX-II

ANOVA TABLES

ANOVA 1: Pathogenicity of pathogenic fungi associated with damping-off of tomato (4.2.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	9	25,169.350	2,796.594	20.831	0.00000
Factor B	1	14,519.049	14,519.049	108.146	0.00000
Intraction A X B	9	7,426.844	825.205	6.147	0.00002
Error	40	5,370.182	134.255		
Total	59	52,485.425			

ANOVA 2: *In vitro* thermal sensitivity of inocula of four pathogens of damping-off of tomato on mycelial growth inhibition at different temperatures and exposure durations (4.3.)

A. *Pythium ultimum*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	150.624	75.312	28.022	0.00000
Factor B	2	133.863	66.931	24.904	0.00000
Intraction A X B	4	15.542	3.885	1.446	0.23904
Error	36	96.752	2.688		
Total	44	396.780			

B. *Fusarium oxysporum*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	116.640	58.320	9.604	0.00045
Factor B	2	67.717	33.859	5.576	0.00777
Intraction A X B	4	3.284	0.821	0.135	0.96829
Error	36	218.601	6.072		
Total	44	406.242			

C. *Rhizoctonia solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	99.268	49.634	41.379	0.00000
Factor B	2	44.042	22.021	18.358	0.00000
Intraction A X B	4	15.210	3.803	3.170	0.02655
Error	32	38.384	1.200		
Total	44	203.881			

D. *Sclerotium rolfsii*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	70.182	35.091	33.830	0.00000
Factor B	2	41.344	20.672	19.929	0.00000
Intraction A X B	4	4.630	1.158	1.116	0.36415
Error	36	37.342	1.037		
Total	44	153.498			

ANOVA 3: *In vitro* evaluation of fungal and bacterial antagonists on growth inhibition of associated pathogens of damping-off of tomato (Table 4.6.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	8	18,087.515	2,260.939	51.494	0.00000
Factor B	3	18,158.027	6,052.676	137.854	0.00000
Intraction A X B	24	8,169.191	340.383	7.752	0.00000
Error	72	3,161.264	43.906		
Total	107	47,575.996			

ANOVA 4: Evaluation of fungal and bacterial antagonists and natural bio-resources on post-emergence damping-off of tomato under pot conditions (Table 4.7)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	11	68,685.417	6,244.129	108.332	0.00000
Factor B	3	757.639	252.546	4.382	0.00618
Intraction A X B	33	5,317.361	161.132	2.796	0.00005
Error	96	5,533.333	57.639		
Total	143	80,293.750			

ANOVA 5: *In vitro* evaluation of botanical extracts on growth inhibition of associated pathogens of damping-off of tomato (Table 4.8.)

A. *Fusarium oxysporum*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	6	14,320.480	2,386.747	83.275	0.00000
Factor B	3	4,086.603	1,362.201	47.528	0.00000
Intraction A X B	18	3,888.819	216.045	7.538	0.00000
Error	56	1,605.020	28.661		
Total	83	23,900.921			

B. *Rhizoctonia solani*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	6	5,970.198	995.033	43.689	0.00000
Factor B	3	2,847.111	949.037	41.669	0.00000
Intraction A X B	18	2,490.939	138.386	6.076	0.00000
Error	56	1,275.429	22.776		
Total	83	12,583.677			

C. *Sclerotium rolfsii*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	6	5,305.607	884.268	58.710	0.00000
Factor B	3	4,210.071	1,403.357	93.174	0.00000
Intraction A X B	18	3,529.019	196.057	13.017	0.00000
Error	56	843.453	15.062		
Total	83	13,888.151			

ANOVA 6: Evaluation of botanical extracts on disease incidence and per cent disease control of damping-off of tomato under pot culture conditions (4.9.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	7	32,929.167	4,704.167	80.643	0.00000
Factor B	3	11,412.500	3,804.167	65.214	0.00000
Intraction A X B	21	7,187.500	342.262	5.867	0.00000
Error	64	3,733.333	58.333		
Total	95	55,262.500			

ANOVA 7: Evaluation of systemic acquired resistance (SAR) inducers on disease incidence and per cent disease control of damping-off of tomato under pot culture conditions (Table 4.10.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	4	3.3922	8480.6	44.52	0.0000
Factor B	3	2132.1	710.71	3.73	0.0187
Intraction A X B	12	9720.3	810.02	4.25	0.0003
Error	40	7619.0	190.48		
Total	59	5.339			

ANOVA 8: Effect of soil solarization, bio-control agent, botanical extract and resistant inducing chemical on damping-off of tomato under the field conditions (Table 4.11.)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	8	4731.7	591.46	3.08	0.0264
Factor B	2	1002.9	501.45	2.61	0.1043
Intraction A X B	16	3072.0	192.00		
Total	26	8806.6			

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Title of Thesis	**:**	**Bio-intensive management of damping- off of tomato *Solanum lycopersicum* L.**
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Admission Number	**:**	H-2017-72-M
Degree Awarded	**:**	M.Sc. (Ag.) Plant Pathology
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ABSTRACT

In the present investigations on damping-off of tomato, characteristic symptoms of the disease first appeared in two phases i.e. pre- emergence and post- emergence. In the first phase, infected seeds failed to germinate while in second phase, small water soaked lesions on the stem appeared at soil line leading to toppling over of the seedling on ground level. Four fungal pathogens namely *P. ultimum*, *F. oxysporum*, *R. solani* and *S. rolfsii* were isolated from infected seedlings. Identification of the isolated pathogens was done on the basis of various morphological and cultural characteristics. Morphological studies revealed that pathogens were fast growing on artificial medium with initially white cottony and profusely growing mycelium attaining a colony diameter of 90 mm within 3-6 days days. In case of *P. ultimum,* hyphae were hyaline and coenocytic, produced sporangia and oospore. In *F. oxysporum*, the mycelium was hyaline and had branches and produced macroconidia, microconidia and chlamydospores. In *R. solani,* the mycelium was septate, colour of the hyphae was brown. The branching occurred almost at right angles to the hyphal cell and produced sclerotia. In *S. rolfsii,* the hyphae was hyaline, thin walled, sparsely septate when young. Sclerotia were dark brown but variation from lighter brown to darker colour was found. Pathogenicity test revealed that disease incidence was more 94.44 per cent in pre-emergence phase while it was 50.00 per cent in post-emergence phase. During i*n vitro* thermal sensitivity of inocula of fungal pathogens of damping-off of tomato maximum mycelial growth inhibition was recorded in *P. ultimum* (5.56%) followed by *F. oxysporum* (4.95%) and *R. solani* (3.73%). Soil solarization increased the mean soil temperature by 3.49 ºC at 5 cm depth as compared to nonsolarized control at the same depth. Soil solarization for 30 days resulted in killing of the pathogens *F. oxysporum* and *R. solani* while all the pathogen were not viable after 40 days of soil solarization. Among different antagonists evaluated, *T. harzianum* caused maximum mycelial inhibition under *in vitro* conditions and was also found effective under the pot conditions. There was significant reduction in the disease incidence with *Lantana camara* (37.50%) followed by *Vitex nigundu* (39.16%) in the pot conditions. Among SAR chemicals potassium chloride and potassium dihydrogen phosphate when applied at 300 and 200 mM concentration were found effective and resulted in (16.66%) and (20.24%) disease incidence, respectively. The minimum disease incidence of damping-off was recorded in the field conditions with the combination of all the treatments (soil solarization + soil application of *T. harzianum* + soil application of *Lantana camara* + seedling treatment of potassium chloride) resulted in 13.11 per cent disease incidence and 74.88 per cent disease control.

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