

STUDIES ON SEEDLING ROOT ROT OF *Cedrus deodara* (Roxb.) G. Don

Thesis

by

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CERTIFICATE-I

This is to certify that the thesis titled, “**Studies on Seedling Root Rot of *Cedrus deodara* (Roxb.) G. Don**” submitted in partial fulfilment of the requirements for the award of 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 **Ms. Rajneesh Thakur (H-2017-68-M)** D/o Sh. Ramesh Chand under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

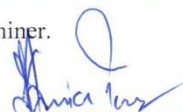
The assistance and help received during the course of investigations has been fully acknowledged.

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This is to certify that the thesis titled, “Studies on Seedling Root Rot of *Cedrus deodara* (Roxb.) G. Don” submitted by Ms. Rajneesh Thakur (H-2017-68-M) daughter of Sh. Ramesh Chand to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP)- 173230, India in partial fulfilment of the requirement for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY IN THE DISCIPLINE OF PLANT PROTECTION** has been approved by the Student’s Advisory Committee after an oral examination of the same in collaboration with the External Examiner.


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ABBREVIATIONS

<i>et al</i>	: co-worker
%	: per cent
/	: per
°C	: degree Celsius
µm	: micrometer
e.g.	: For example
cm	: centimeter
mg	: milligram
g	: Gram
m	: meter
ha	: Hectare
kg	: Kilogram
l	: Litre
ml	: Milliliter
BOD	: Biological Oxygen Demand
@	: at the rate
Fig.	: Figure
i.e	: that is
PDA	: Potato Dextrose Agar
CD	: Critical difference
etc.	: Et cetera
UHF	: University of Horticulture and Forestry
Cfu	: colony forming unit
viz.	: vide licet (namely)
BCA	: Biocontrol Agent

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Chapter-1

INTRODUCTION

Cedrus deodara (Roxb.) G. Don, also known Himalayan *Cedrus* is found in western Himalayas and slopes of Hindu Kush at 1100 to 3000 M elevation. It is high mountain tree which is found in variety of alpine forests. It is important tree of North West India (Himachal Pradesh, Uttarakhand, Kashmir), Afghanistan and Pakistan. Its strong and durable wood is commonly used for construction while other uses are general carpentry and furniture. It is a vital component of temperate forest, usually on North facing valley slopes where rainfall ranges from less than 1000mm up to 2500mm per year mostly in form of snow. In these forest *C. deodara* is associated with wide array of conifer and some broad leaf trees like Fir, Spruce, and Oaks etc. It grows with *Pinus wallichiana*, *Taxus baccata*, *Abies pindrow*, *Quercus* etc. This is state tree of Himachal Pradesh. The seedlings of young plants are often exposed to pathogen attack particularly those inciting root rot and wilt diseases at primary stage of plant establishing. The root rot fungi which pose serious threat to forest nurseries include the species of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Macrophomina* (Huang and Kuhlman, 1990; Asiegbu *et al.*, 1999; Wafaa and Haggag, 2002).

The perusal of literature reveals that some work has been done on the root rot disease in conifers and blue pine (Enebak *et al.*, 1990). Recently, Forest Department of Kandaghat and Solan blocks in Himachal Pradesh reported mortality in *Cedrus* seedlings. The preliminary investigations revealed that incidence in these two forest ranges was to the tune of 55 and 64 per cent, respectively. Upon further investigations, the seedlings of *Cedrus* were found to be weak, chlorotic with thin, black wiry tap root and few to none secondary roots. No work on the seedling root rot on *C. Deodara* has been reported so far. From India Meagre information about the causal pathogen responsible, symptomology and management of diseases is available for conifers (Kaushik *et al.*, 2002 and Bisht *et al.*, 2003). Due to Root rot disease in *C. Deodara* and the damage caused by it to the seedlings there was urgent need to identify and study the disease so as to develop proper management strategies.

Keeping in view the losses caused by the disease in seedlings, present study was proposed with the following objectives:

Objectives:

- i) To isolate and identify the causal organism(s) of root rot in *Cedrus deodara* seedlings.
- ii) To identify the cultural and morphological characteristics of the pathogens involved.
- iii) To evaluate efficacy of different fungi toxicants and native bio control agents against the causal pathogen(s).

Chapter-2

REVIEW OF LITERATURE

Cedrus deodara (Roxb.) G. Don. also known as Himalayan cedar is a high mountain tree, but it occurs in a wide range of habitats in the Himalayas. It grows at elevations between 1,700 m and 3,000 m msl in the western part of its range and between 1,300 m and 3,300 m in the eastern part, where the climate is less dry. Himalayan Cedar is a very important timber tree in North West India, Kashmir and Pakistan. Its strong and durable wood is mostly used for construction. Other uses are general carpentry and furniture. Fragrant oil can be distilled from wood chips and sawdust. *C. deodara*, the state tree of Himachal Pradesh is one of the most important conifers in the Himalayan moist temperate forests.

It extends from Afghanistan through the hilly regions of Pakistan and India to Kurnauli valley in Nepal occupying an elevation of 1200-3050 m. The total area of deodar forests in India is estimated to be around 203,263 hectares. Out of this, 69,872 hectares lie in Himachal Pradesh (Singh and Lakhanpal, 2000). Further, the elegant trees of *C. deodara* add to the scenic beauty of the Himalayas. Disease aspects of this valuable tree till date are subjected to only scant studies. A search into literature reveals that only a few diseases along with causal organisms were listed and described by Maheshwari and Biswas (1970) and Bakshi (1976). In Himachal Pradesh, Singh and Lakhanpal (2000) studied root rot disease of deodar (*C. deodara*) caused by *Phytophthora cinnamomi*. This disease resulted in widespread mortality of deodar trees near Chail and Shimla in Himachal Pradesh.

2.1 Economic Importance:

Cedrus is widely regarded as one of the strongest woods in India and is as important as Teak. It is easy to saw. It is unsuitable for polish or print work as the oil in the wood (especially near the knots) always oozes through when exposed to direct sunlight and discolours the coating. Seasoned heartwood of deodar is very durable and classified as durable class 1. The durability of deodar may be due to the presence of terpenes/resin acids present in the heartwood (Bakshi, 1976). Deodar forests play an important role in the economy of the country, as its wood is the strongest and most durable. It is commonly used in building houses in hilly regions of India and some parts of the world as it conserves heat (Bakshi, 1976).

2.2 Nursery diseases

The root rot fungi which pose serious threat to forest nurseries include the species of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Macrophomina* (Huang and Kuhlman, 1990; Asiegbu *et al.*, 1999; Wafaa and Haggag, 2002). These pathogens often invade the terminal unsubarized roots and young seedling and cause post-emergence damping-off or root rot/wilt, thereby killing the host. The fungus penetrates into root epidermal cell wall, grows intracellularly, decompose cell wall constituents and persist by metabolizing all contents. It is worldwide problem in pine and conifer seedlings and serious losses due to this disease have been reported from Ontario, Canada, USA, European countries. (Greifenghagen *et al.*, 1991; Lilja and Rikala, 2000). Vanterpool (1956) working in Saskatchewan, reported that *Rhizoctonia solani* (Kuhn) caused a damping off and seedling blight in Rape seed crop.

Forest trees are subjected to many nursery diseases. Root rots, wilts, damping off etc attack the growing seedlings and reduce its quality and quantity. *Fusarium* species have been found to be associated with root rots of various forest trees. Krugner *et al.* (1970) isolated number of species of *Fusarium* such as *F. solani*, *F. oxysporum* and *F. Semitectum* from diseased roots and soil of pine forests. Douglas fir (*Pseudotsuga menziesii*) seedlings were reported to be killed by root rot due to *F. oxysporum* (Bloomberg, 1976). Various *Fusarium* species have been isolated from *Lucaena leucocephala*, *Butea monosperma*, *Cassia tora*, *Murraya koengi*, *Cyprus rotundus* (Singh *et al.*, 1983), *Bauhinia* sp. *Indigofera* sp. and mango and pine seedlings (Madam, 1983; Kore and Patil, 1985). The nursery plants of forest trees are often exposed to pathogen attack particularly those inciting root rot and wilt diseases at primary stage of plant establishing. Besides *Fusarium* spp, *Rhizoctonia* and *Rhizoctonia* like species have been reported to be the cause of root rots and wilts in forest nurseries worldwide. Dar *et al.* (2011) reported *Fusarium* sp. and *R.solani* from the diseased seedlings of *Abies pindrow* and *Pinus wallichiana* from Kashmir. Similarly, Chakravarty and Mishra (2007) also reported *Rhizoctonia solani* as root rot pathogen in *Quercus* seedlings. Many *Rhizoctonia* spp are economically important plant pathogens in agriculture, horticulture and forestry. The early description of *Rhizoctonia* spp associated with forest tree species were mainly based on few anamorphic traits (Hietala and Sen, 1996).

2.3 Symptoms

Fusarium produces root rot and wilt symptoms in many agricultural crops and ornamental plants besides forests and fruit trees (Ciferri and Corte, 1927). Young leaves of

nursery plants turn light green and finally yellow in colour. In advanced stages, leaves become shrivelled dried, droop down and finally the root and collar portion show the development of brown to blackish colour. These symptoms have been observed in many forest nursery crops as well in vegetable crops (Krugner *et al.*, 1970; Goyal *et al.*, 1980; Kore and Patil, 1985). *Fusarium* root rot pathogens cause poor emergence, stunted seedlings, weak growth of plants and yield losses in soybean (Nelson, 1999).

Root rot caused by *Rhizoctonia* species is another common and wide spread soil borne disease that causes damping-off as well as lesions on hypocotyls and roots of many forest nursery crops. The roots of the nursery plants dry up giving the plant a stunted growth. Among the conifers root rot, this pathogen has mostly been encountered on pines followed by *Quercus* (Bhardwaj *et al.*, 1988). The root rot fungi posing serious threat to nursery reduce the dry matter production and particularly in affected plants (Aigbe and Remison, 2010).

The perusal of literature reveals that some work has been done on the management of root rot disease in conifers other than Blue pine (Enebak *et al.*, 1990). From India, meagre information about the causal pathogen responsible, symptomology and management of root rot/wilt diseases is available for conifer seedlings (Kaushik *et al.*, 2002 and Bisht *et al.*, 2003). In the field, the development of the fungus is favoured by the presence of host plants, abundant rainfall and high spring and summer temperatures. Poor soil structure also promotes its proliferation. The strain of *Rhizoctonia* which is responsible for *Rhizoctonia* root rot in various crops has been found to be serious pathogen in seedlings of many forest trees. Dar *et al.* (2011) while working on root rot of *Pinus wallichiana* in Kashmir found two pathogens namely *Fusarium oxysporum* and *Rhizoctonia solani* associated with disease. Pinto *et al.* (2006) also recorded the presence of *Fusarium oxysporum* and *Rhizoctonia solani* from *Pinus sylvestris*, *Picea* spp and *Abies pindrow* seedlings from Sweden. Lilza *et al.* (1995) and Stepniewska *et al.* (2006) frequently isolated binucleate *Rhizoctonia* from conifer roots, cone scales and seed of Scots pine from Florida and Poland Forest nurseries respectively. *Fusarium* and *Rhizoctonia* sp. reportedly are highly potential root rot pathogen in many tropical and temperate forest nurseries (Enebak *et al.*, 1990). Binucleate *Rhizoctonia* spp causing damping-off in conifer seedlings includes *R.callae*. (Saksena and Vaartaja, 1961), *R.endophytica* var. *endophytica* Saks and Vaar. (AG-A) (Ogoshi and Ui, 1983) and *Rhizoctonia* sp. AG-E (= CAG 6) (Huang and Kuhlman, 1990).

Rhizoctonia sp. primarily attacks below ground plant parts such as the seeds, hypocotyls, and roots, but is also capable of infecting above ground plant parts as well e.g. pods, fruits, leaves and stems. The most common symptom of disease caused by *Rhizoctonia* is referred to as "damping-off" characterized by non-germination of severely infected seed whereas infected seedlings can be killed either before or after they emerge from the soil. Infected seedlings not killed by the fungus often have cankers, which are reddish-brown lesions on stems and roots. In addition to attacking below ground plant parts, the fungus occasionally infects fruit and leaf tissue located near or on the soil surface. This type of disease often occurs because the mycelium and sclerotia of the fungus are close to plant root or splashed on the plant tissue (Adam, 1988).

Rhizoctonia sp. is soil borne and seed borne in nature. During second to third week of plants growth, it causes seedling mortality (Shailbala and Tripathi, 2007). Gupta and Chauhan, 2005 described the symptoms of root rot of soybean as sudden chlorosis and wilting of leaves followed by Black necrosis of leaf and stem. *Rhizoctonia* sp. can cause pre- and post-emergence damping off, root rot, and hypocotyl lesion on many seedlings. *Rhizoctonia* spp along with *Pythium* spp and *Phytophthora* spp were identified as the major causal agents in the soybean seedling disease in Iowa (Gupta and Chauhan, 2005). According to Rizvi and Yang (1996) *R. solani* caused pre-emergence and post emergence damping-off, root rot and aerial leaf blights on soybean around the world. Williamson *et al.* (2006) reported severe foliar blight due to *Rhizoctonia* sp. which started at base of the plant, then moved upward and downward, often causing defoliation. Similarly, Garibaldi *et al.* (2003) reported web blight caused by *R. solani* on *Lantana camara*. Needle blight of longleaf pine was found to be associated with binucleate *Rhizoctonia* AG3 (Barnard, 1979) Needles of four to five year old Norway spruce seedlings have been reported to be killed by binucleate *Rhizoctonia* sp. (Roll Hansen and Roll Hansen, 1968). Similarly binucleate *Rhizoctonia* AG-E (= CAG 6) was shown to be associated with loblolly pine in USA (Runion and Kelly, 1993).

Damping-off is a fungal disease that causes mortality during the first few weeks after germination. Pre-emergence damping-off is difficult to diagnose because the affected seeds are not visible. The soil- or seed borne fungi associated with the disease are not host-specific and they cause rapid decay and mortality of germinating seeds and emerged seedlings (Mikola, 1952; Vaartaja, 1952; Hanioja, 1969 and Lilja, 1979).

Seedling diseases by *Rhizoctonia* sp. initiated by seed decay, pre-emergence and post-emergence damping off primarily injure seedlings root or hypocotyl resulting in deformed, stunted, and poorly developed older plants (Windels and Jones, 1989). Post-emergence damping off is typically initiated by *Rhizoctonia* spp which infects the hypocotyl region below the soil line, and severely infected seedlings collapse and die (Whitney and Duffus, 1986). The first above ground symptoms of collar and root rot due to *Rhizoctonia* spp is sudden wilting and chlorosis of foliage and necrosis of petioles near the crown (Asher and Hanson, 2006). Wilted leaves subsequently die, forming a dry, brown or black rosette which persists throughout the growing season (Whitney and Duffus, 1986; Asher and Hanson, 2006). Isolates of *R.solani*, *R. endophytica* (=AG-A), *R.callae* and *R. repens* (binucleate) induced stunted shoot and root growth in *Pinus sylvestris*, *P.resinosa*, *Ulmus americana* and *Thuja occidentalis* (Saksena and Vaartaja, 1961).

2.4 Morphology

Different *Rhizoctonia* spp isolates have been reported to vary with respect to rate of growth, colony, hyphal and sclerotial characteristics (Parmeter and Whitney, 1970). Pigmentation of hyphae varied from creamish white to various shades of brown (Parmeter and Whitney, 1970; Thakur *et al.*, 1992). The isolates obtained from leguminous crops produced microsclerotia whereas those from okra, chillies, knolkhol and potato failed to do so (Thakur *et al.*, 1992). Singh *et al.* (1999) reported that rice, maize, soybean and moong bean isolates produced large, dark brown sclerotia in comparison to small, creamish white to greyish brown produced by cotton isolates. While working with five isolates of *R. solani*, Meena *et al.* (2001) observed that Madurai isolate produced deep dark brown mycelium.

The mycelium of *Rhizoctonia* species is septate and the colour of hyphae varies from hyaline when young to brown on maturity (Duggar, 1915; Peltier, 1916; Brown and Jenkins, 1928 and Walker, 1928). The dimensions of vegetative hyphae were found to vary with the age and type of media (Peltier, 1916). Dimensions of *Rhizoctonia* spp ranging between 3-17 cm have been recorded by various workers (Duggar, 1915; Matz, 1921; Valdez, 1955; Flentje and Saksena, 1957; Saksena, 1960 and Hansen, 1963). The compact mass of the chlamydospores forms the principal structure of the sclerotia. The basic colour of mature sclerotium is brown. However, numerous shades of brown have been employed to describe them (Duggar, 1915; Gratz, 1925; Matsumoto, 1934; Houston, 1945 and Flentje, 1956). Young sclerotia are initially white but later turn dark with maturity (Peltier, 1916; Matz,

1921; Matsumoto, 1934; Weber, 1939 and Valdez, 1955). Sclerotia also vary in their size ranging from pinhead to 0.5 inch (Rolfe, 1902; Peltier, 1916; Gratz, 1925 and Frederiksen *et al.*, 1938). The fungus *Rhizoctonia* spp produce usually long cells of septate mycelium which are hyaline when young and later turn yellowish brown. It produces large number of globose sclerotia which initially turn white, later turn brown to purplish brown. Sclerotia serve as a major source of primary inoculums (Paracer and Chahal, 1963). A range of *Rhizoctonia* species are reported to be associated with nurseries. However, there is still a clear need for further identification of putative *R.solani* anamorph. Burpee *et al.* (1980) stated that many of binucleate isolates in AG-E, F and R (= CAG 3, 4 and 5) were morphologically indistinguishable from isolates of *R.solani* yet, in majority of papers it was observed that anamorph was assigned to *R.solani*.

2.5 Pathogenicity

Several field-grown crops have been artificially inoculated with various *Rhizoctonia* spp. In field studies it was reported that inoculum grown on whole grain, or grain mixed with infested soil, can be mixed in and around plants or incorporated into soil before planting of potato tubers to raise the inoculums levels of *R. solani* pathogen to the maximum level while proving pathogenicity (LeClerc, 1941 and Houston, 1945). It is also reported that the inoculum concentration for hosts planted as seeds can range from 0.01 to 4% (v/v or w/v). In addition to that agar plugs from *Rhizoctonia* culture may also be placed in the soil before planting (Murray, 1981; Weller *et al.*, 1986 and MacNish *et al.*, 1995) or after emergence directly against stems, crowns, and other wounded or non-wounded plant parts of host plants (Spencer and Fox, 1978). For crown and stem diseases caused by *R. solani*, the inoculum source may be scattered over or around the base of plants and covered with a shallow layer of soil, sometime after plant emergence to prove the pathogenicity (Carling and Sumner, 1992). Lichtenzveig *et al.* (2006) used mycelial suspension of *R. solani* (diluted to the equivalent of 2.2 mg dry weight ml⁻¹) to inoculate vermiculite @ 5ml suspension per vermiculite cell. According to Mallesh *et al.* (2006) *Rhizoctonia* spp required higher inoculum level (eight to ten per cent) and took more time to cause 100 per cent root rot incidence in Sage (*Salvia officinalis* Linn.). Hall *et al.* (2008) used five mm mycelial plugs of *R. solani* from the margin of seven to ten day old cultures grown on potato dextrose agar (PDA) to inoculate on ten-week old cauliflower seedlings grown in seedling trays. The mycelial plugs were buried near the stem of each plant.

Naz *et al.*, 2008 studied the influence of inoculum levels (10, 15 and 20 g of *R. solani* AG-3) of the isolate CL-58 on Rhizoctonia disease by using two potato cultivars (Cardinal and Desiree) and three advanced breeding lines (SH-216-A, TPS-9813 and CIP-393594-61). They found significant ($P=0.05$) increase in all disease parameters measured. Moreover, 20g inoculum was ascertained as the most effective dose for green house screening. Amongst six inoculum sources of *R. solani* and different ages of rice seedlings evaluated to induce sheath blight in nursery, maximum disease incidence and severity was recorded in 30-40 days old seedlings in nursery with the inoculum raised on barley grains + Typha pieces closely followed by barley grains and rice grains (Singh *et al.*, 2010). Similarly, Mc Cormack *et al.* (2013) used 50gms of Rhizoctonia mass culture per one Kg soil to prove Koch's postulates.

Nik and Yapi (1979) studied biology, pathogenicity and control of *R. solani* Kuhn by using an isolate from infected French bean seed and proved pathogenicity by brushing the inoculum with a soft camel brush over the whole surface of the pod. The pathogenicity test of most predominant damping-off fungus *R. solani* on tomato was done by mixing the fresh inoculum of the fungus with 1kg of steam sterilized soil in plastic pots (Jiskani *et al.*, 2007). Minuto *et al.* (2008) isolated and described the fungus, *R. solani* from root stocks of the tomato in Italy first time and observed the symptoms like basal rot, wilt stunting and death of plant after 5 days to 6 weeks after transplanting of tomato seedlings. The pathogenecity was proved by applying the inoculum as small pieces of (1x1 cm) along with potato dextrose agar directly at the basal area of the 40 days old plant stem. Similarly, Pathogenicity of *R. solani* causing blight symptoms on stems was proved by stem inoculation technique on a tomato cultivar Arka vikas with 96.7 per cent of disease incidence by Pushpavathi *et al.* (2013).

2.6 Management

Management of diseases by combining different methods is modest approach in today's agriculture and forest farming systems. The plants are exposed to persistent pathogenic attacks, particularly those inciting root rot and wilt diseases at primary stages of plant. Root rot and wilt disease on conifers such as fir, pine and spruce at nursery stages has been a major problem throught out the world (Lilja *et al.*, 2010). The principal root rot fungi which pose serious threat to fir and other conifers nurseries include *Fusarium oxysporum* and different species of Rhizoctonia. The management practices generally employed for their control include chemical and biological methods.

Several approaches involving fungicide use and cultural measures have been adopted by the nursery growers to reduce the root rot incidence yet the disease continues to assume serious threat to conifers (Shah *et al.*, 1999). The effective use of antagonistic bacteria, actinomycetes and fungi against several soil-borne pathogens has been demonstrated in several field and horticultural crops (Yobo *et al.*, 2010). Dwivedi (1993) reported *Trichoderma* as a destructive parasite. Cultural filtrate of mycorrhiza, *Suillus collinitus*, *Hebeloma mesophaeum* and *Paxillus* sp. reportedly exhibited antagonistic effect on the mycelial growth and spore germination of *F. oxysporum* and *Pythium vexans* (Quarraqi *et al.*, 2005 and Yamaji *et al.*, 2005).

2.7 BIOCONTROL

Bio control agents play an important role in managing many soil borne pathogens. The native bio control agents have been found to successfully control the soil borne pathogens. Dubey and Patel (2001) evaluated three isolates of *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* against *Rhizoctonia solani* causing web blight of urd bean. Local isolates of *Trichoderma viride* and *Trichoderma virens* were found to be superior over other. Dutta and Kalha (2011) evaluated twelve isolates of residential bioagents viz., four isolates each of *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescence* were evaluated *in vitro* against *Rhizoctonia solani*. Bioagents showed fungistatic action and significantly inhibited mycelial growth of the test pathogen over untreated control. Similarly Pandey and Pundhir (2013) tested the eight isolates of *Trichoderma harzianum* and six isolates of *Pseudomonas fluorescence* *in vitro* for their antagonistic potential against *Rhizoctonia solani*. In dual culture both bio agents were found to arrest the growth of *R. solani* to significant levels.

Hadar *et al.* (1979) reported that an isolate of *Trichoderma harzianum* directly attacked the mycelium of *R. solani* when both fungi were grown in dual culture. Biological treatment of seed and soil against *Rhizoctonia* and *Fusarium* under glasshouse conditions with *T.viride* showed results similar to chemical control (Dumitras and Sesan, 1980). Elad *et al.* (1980) observed that in naturally infested soils; wheat bran preparations of *Trichoderma harzianum* significantly decreased the *Rhizoctonia* rot of beans. Antagonistic activity of *Trichoderma hamatum* against this pathogen has also been reported (Chet and Baker, 1981).

Haral and Konde (1986) reported that cultural filtrates of *Bacillus subtilis* reduced the mycelial weight of *R. solani* by an amount similar to the effects of Bavistin and Benlate. Similarly, good control of damping-off due to *Rhizoctonia* sp. was obtained when *Trichoderma viride* was applied in soil @ 5g/kg soil (El-Faranawany and Shama, 1996). A biocontrol formulation containing *B. subtilis* and metabolites, which were transformed into a wettable powder formulated with clay, surfactant and water, gave satisfactory control of *R. solani* on beans similar to that obtained with conventional fungicides (Lazzaretti and Bettiol, 1997). *In vitro* efficacy of *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma virens* against *R. solani* has been reported by Hazarika and Das (1998). They further observed that seed and soil application of *Trichoderma harzianum* and *Trichoderma viride* controlled root rot satisfactorily. Singh *et al.* (1997) reported that volatile and non-volatile compounds produced by *Trichoderma harzianum*, inhibited the growth of *Rhizoctonia solani* *in vitro* and *in vivo* conditions.

Singh *et al.* (1997) found that *Trichoderma viride* and *Trichoderma harzianum* effectively reduced the incidence of black scurf caused by *Rhizoctonia solani* in infested soil and gave significant increase in yield. Bazir and Srivastava (1999) noticed that *Trichoderma* isolates strongly inhibited the growth and colonized *Rhizoctonia solani* mycelium in dual culture on PDA. Hazarika *et al.* (1999) reported *T. viride*, *T. harzianum*, *T. koningii*, *Bacillus subtilis* and *Pseudomonas fluorescence* as potential biocontrol agents of black scurf caused by *Rhizoctonia solani*.

Krechel *et al.* (2002) identified fluorescent *Pseudomonas* strains useful for the suppression of *R. solani*. Bio- control agents as *T. harizianum*, *T. viride*, *B. subtilis* and *P. fluorescence* were found to inhibit the *in vitro* growth of *Rhizoctonia* spp. (Rajendra prasad *et al.*, 2017). *Trichoderma* spp are widely used as bio-fungicides in agriculture, horticulture and forestry. Induction of plant defense and mycoparasitism are considered to be most important mechanism of *Trichoderma* mediated biological control. Cell wall degrading enzymes such as chitinase, glucanase and proteases are thought to be closely related to mycoparasitism of *Trichoderma* strains (Saravana kumar *et al.*, 2016). Inhibitory volatile substances such as alkyl pyrrols also contribute to the biological activity of *Trichoderma* strains (Claydon *et al.*, 1987). The effective uses of antagonistic bacteria, fungi and actinomycetes have been demonstrated in many fields, horticultural and forest crops (Yobo *et al.*, 2010). The free living rhizosphere microorganisms such as *Trichoderma*, *Gliocladium*, *Penicillium*, *Bacillus*,

Azotobacter etc. favourably influence the plant growth directly or indirectly whereas mycotrophy benefits the host plants by enhancing the root capacity to absorb nutrients, extracting the nutrients beyond depletion zones in soil and protecting the root from pathogenic invasions (Eziashi *et al.*, 2006; Gacitua *et al.*, 2009 and Heydari and Pessarakli, 2010).

2.8 Chemical control

Chemical control measures have been tested and found effective in the control of many soil borne diseases (Ogundana and Denis, 1981; Plumbley, 1985). Different protectants and systemic fungicides have been reported to be used *in vitro* against the fungus *Fusarium* sp., *Rhizoctonia* sp., *Phytophthora* sp. and *Pythium* sp. etc. Fungicides may act on or interrupt the metabolic system of the pathogen (Bilgrami and Dube, 1976). The effectiveness of a fungicide depends on its innate toxicity and permeation. Certain protective fungicides although hazardous to environment are still used for the control of fungal diseases (Vaish and Sinha, 2003). Several approaches involving fungicide use and cultural measures have been adopted by the nursery growers to reduce the root rot incidence in *Pinus wallichiana* yet, the disease continued to assume serious threat (Shah *et al.*, 1999).

Oyekan (1979) studied the effect of chemicals as foliar spray for control of web blight caused by *Rhizoctonia solani* with Captafol @ 1.64 kg a/ ha and Oxycarboxin @ 0.16 kg/ ha. Mehrotra (1990) evaluated the fungicides *in vitro* amongst which Carbendazim gave complete inhibition of the mycelial growth of *Rhizoctonia solani* at all the concentrations tested (i.e., 25, 50, 100 and 500 ppm). Simultaneously, Meen and Chatopadhyay (2002) reported that carboxin, epoxiconazole, hexaconazole, propiconazole, and triadimefon completely arrested the growth of *Rhizoctonia solani* followed by ediphenphos, isoprothiolane, kitazin and tebuconazole respectively. Surulirajan and Kandhari (2003) found hexaconazole and propiconazole highly effective against *R. solani* *in vitro*.

Dutta and Kalha (2011) evaluated five fungicides viz., Carbendazim 50 WP, Hexaconazole 5 EC, Propiconazole 25 EC, Saaf 75 WP and Vitavax 75 WP @ 10, 25, 50, 100, 200 and 500 ppm each. Among the fungicides Saaf (carbendazim 12% + Mancozeb 63%) was the most effective fungicide which registered cent per cent inhibition of the mycelial growth of *R. solani* followed by Carbendazim (98.9%), Vitavax (98.2%), Propiconazole (74.8%) and Hexaconazole (72.9%) respectively. Effectiveness of Benomyl,

Carboxin, Thiabendazol and Captan against black scurf caused by *Rhizoctonia solani* under laboratory and field conditions was reported by Sudhakar *et al.* (2005). In a study, Shailbala and Tripathi (2007) tested hexaconazole, propiconazole, mancozeb and carbendazim for effective management of web blight caused by *Rhizoctonia solani* at the Crop Research Center (CRC) in Pantnagar, Uttar Pradesh, India, during 2000-02. They found hexaconazole, mancozeb and carbendazim as most effective fungicides under field condition.

Similarly, Mishra *et al.* (2005) reported that Hexaconazole was effective against *Rhizoctonia solani* *in vitro*. Thangasamy and Rangaswamy (1989) reported mancozeb and carbendazim as best chemicals for the control of sheath blight disease of rice caused by *Rhizoctonia*. Similarly, Chakrabarty (1993) reported that the captafol and mancozeb were effective against *R. solani* on cauliflower as soil drench. Tiwari *et al.* (2002) used various fungicides to control sheath blight of rice carbendazim + epoxiconazole (0.2%), hexaconazole (0.2%), epoxiconazole (0.24%) and propiconazole (0.2%) were significantly more effective in controlling disease severity than other fungicides. Ali *et al.* (2002) reported that the seed treatment with mancozeb, copper oxychloride, captan and hexaconazole reduced sheath blight incidence caused by *R. solani*.

In a study conducted by Mishra and Sinha (1999) eight fungicides were tested *in vitro* against *R. solani* in rice at different concentrations. Higher concentration of carbendazim ($5 \mu\text{gml}^{-1}$) and propiconazole ($10 \mu\text{l litre}^{-1}$) resulted in 100 percent inhibition of mycelial growth followed by hexaconazole, epoxiconazole and carboxin. However, non systemic fungicides mancozeb and thiram also inhibited growth of the fungus, among which thiram was the most effective.

Akter *et al.* (2001) reported that six fungicides namely carbendazim, hexaconazole, propiconazole and micronized sulfur and fertilizer, Muriate of Potash were tested against sheath blight of rice (cv. Swarna) caused by *R. Solani*. Of these hexaconazole appeared to be the best in reducing the percent relative lesion height, per cent disease index and tiller infection.

Ali *et al.* (2002) reported that the seed treatment with mancozeb, copper oxychloride, captan and hexaconazole reduced sheath blight incidence on rice. Three sprays of carbendazim (0.1%) and Mancozeb (0.1%) + seed treatment reduced disease incidence significantly. Tiwari *et al.* (2002) used seven fungicides to control sheath blight and reported

that carbendazim + epoxiconazole (0.2%), hexaconazole (0.2%), epoxiconazole (0.24%) and propiconazole (0.2%) were significantly more effective in controlling disease severity than other fungicides. Chahal *et al.* (2003) managed sheath blight of rice by using eight fungicides, of which Propiconazole (0.1%) minimized the disease to maximum extent followed by Edifenphos (0.1%), iprodione (0.3%) and carboxin (0.2%). *In vitro* evaluation of ten different fungicides viz., azoxystrobin, carbendazim, cyproconazole, fenpropimorph, fludioxonil, hexaconazole, iprodione, penicuron, propiconazole, tolclofos-methyl 500 for their effect on radial colony growth of six isolates of *R. solani* at concentrations ranging from 0.01 to 100 µg a.i. ml⁻¹ revealed that except azoxystrobin, all fungicides were highly effective. The highest inhibition of mycelial growth was obtained with penicuron followed by tolclofos-methyl, fludioxonil and hexaconazole respectively (Ali and Archer, 2003).

Sudhakar *et al.* (2005) investigated the effect of chemicals at the ratio of 0.2 and 0.3 per cent concentrations viz., hexaconazole, propiconazole, cyproconazole, tebuconazole, mancozeb, chlorothalonil, penicuron, iprodione, carbendazim and stated that the most effective fungicides in *in vitro* tests were carbendazim, hexaconazole and propiconazole respectively. However systemic fungicides were found more effective than non-systemic ones and mancozeb was the least effective among the treatments.

Bibi *et al.* (2005) studied the *in vitro* effects of mancozeb, COC and sancozeb at 500 ppm on the mycelial growth of *R. solani*. The lowest mean colony diameter (4.4 cm) was observed with Dithane M-45 (lower by 68 per cent than the control) and was found superior to sancozeb and COC in inhibiting the fungal growth of *Rhizoctonia* sp. Rai *et al.* (2007) reported the efficacy of twelve fungicides (each at 25, 50, 100 and 500 ppm), against *R. solani*, *in vitro* and found that Hexathir, Dhanustin, Rovral and Contaf showed almost complete inhibition of the test fungus at all the concentrations tested. Bag (2009) made an attempt to evaluate the efficacy of a new fungicide -a combination of two systemic fungicides viz., trifloxystrobin 25% (Strobilurin compound) and tebuconazole 50% (Triazole compound) along with two other commercially available fungicides hexaconazole and validamycin under controlled inoculation condition against *Rhizoctonia solani*, the incitant of sheath blight disease in rice. The new fungicide was most effective in decreasing disease severity (37.61% lower over control) and increasing grain yield (50% higher over control). The same fungicide also proved to be best or at par with leading triazole compound to manage the sheath blight disease of rice.

Hunjan *et al.* (2011) evaluated efficacy of some new fungicides, among those fungicides viz. trifloxystrobin + tebuconazole, tebuconazole, and propiconazole showed higher level of efficacy against *R. solani* under laboratory conditions. However, thifluzamide (spencer 24SC) was least effective against sheath blight (*Rhizoctonia solani*) under laboratory and field conditions with artificial inoculations during kharif seasons of 2008, 2009 and 2010. Karima and Nadia (2012) found that the highest reduction of mycelial growth of two pathogens *Fusarium solani* and *Rhizoctonia solani*, the causal organism of damping off and/or root rot diseases in tomato occurred with fungicide of Tachigaren, followed by Monceren, Aracur and Topsin M at different concentrations. Johnson *et al.* (2013) determined the toxicity of hexaconazole 5 SC against sheath blight disease of rice under field as well as glass house conditions. Hexaconazole 5 SC at different doses viz., 500, 1000, 1500 and 2000 ml ha⁻¹ effectively reduced the sheath blight disease incidence in all the three field trials, of which the highest dose 2000 ml ha⁻¹ was found superior and recorded lowest incidence of 3.0, 5.73 and 11.33 per cent in the first, second and third trials respectively. Kumar and Veerabhadraswamy (2014) screened various fungicides against sheath blight disease of rice and reported that thifluzamide 24% SC, RIL-068/F1 48 WG (kresoxim methyl 40% + hexaconazole 8% WG), propiconazole 25% EC, tricyclazole 75% (WP) and a new combination fungicide, fluxapyroxad 62.5 g/l + epoxiconazole 62.5 g/l EC (adexar w/v EC) were found effective under field trials.

2.9 Integrated Disease Management

Integration of seed treatment with bioagents and fungicides has been found to be more effective against soil borne pathogens than either of the two (Mukhopadhyay and Mukherjee, 1991; Mukhopadhyay *et al.*, 1992). Integration of chemical and biological agents provides opportunity for enhancement and greater efficiencies in suppressing damping-off induced by *R. solani* (Lifshitz *et al.*, 1985). The pot culture experiments conducted by Priyadarsini (2003) revealed that the *Trichoderma harzianum*, *Pseudomonas fluorescense*, turmeric powder-baking soda 10:1 combination were found to be effective in improving the plant growth and reducing the disease incidence in *Amaranthus* due to *Rhizoctonia* sp. Gour and Sharma (2010) also reported superior results when *Trichoderma viride* and *Trichoderma harzianum* were integrated with Metalaxyl and Cymoxanil 8 % + Mancozeb 64 % to control root rot due to *R. solani* in cotton.

Lewis and Papavizas (1980) found that integrated management by preventing colonization of plant residues, and combining *Trichoderma* with ploughing to a depth of 20-25 cm reduced the root rot of beans caused by *R. solani*. Malathi *et al.* (2002) conducted studies to test the efficacy of combined treatments of systemic fungicides viz., thiophanate methyl and carbendazim with fungal (*Trichoderma* sp.) and bacterial (*Pseudomonas fluorescens*) biocontrol agent over individual treatments and noticed the compatibility of systemic fungicides with fungal and bacterial biocontrol agents which were found effective against the pathogen *R. solani*. The study also revealed the efficacy of combined treatments over individual treatments. The treatment receiving combination of thiophanate methyl and *P. fluorescens* recorded the maximum plant survival of 81.6 percent, while other combined treatments had comparable effect.

Surilirajan and Kandhari (2003) conducted a study to assess the effect of *Trichoderma viride* on disease severity of *R. solani*, per cent disease incidence and yield parameters. They found that out of 16 treatments, *Trichoderma viride* + Carbendazim 50WP (0.1%) spray along with soil amendments (FYM 1% + saw dust 1%) showed maximum reduction in sheath blight severity, per cent disease incidence and higher grain yield of rice over the control. Kumar *et al.* (2011) undertook the studies to determine the comparative efficacy of treatments with native biocontrol agents viz., *Pseudomonas fluorescens*, *Trichoderma harzianum* isolate and *Trichoderma virens* isolate @ 5X10⁸ cfu/g talc; organic amendments viz., neem seed powder @ 250 mg/kg soil and farmyard manure @ 1500 mg/kg soil; chemicals viz., Carbofuran @ 33.4 mg/kg soil, Topsin-M @ 1.4 mg/kg soil and Bavistin @ 2.0 mg/kg soil against *R. solani* @ 1.5 g mycelial mass/kg soil on tomato cv. K-25 under pot culture conditions. Results revealed that all the treatments significantly increased the plant growth, fruit yield and decreased the root infection caused by the *R. solani* on tomato as compared to untreated inoculated plants. Simultaneously, antagonistic activity of *Trichoderma viride* against the wilt disease and its growth promoting ability on tomato plants was found to increase when it was applied in combination with Benomyl in the soil (Mushtaq Ahmed, 2013).

While working on root rot of *Hevea brasiliensis*, Ikerodah *et al.* (2012) observed that combined effect of *Trichoderma harzianum*, triadimefon or tridemorph resulted in decreased diseased incidence. While working on integrated disease management of root rot of *Abies pindrow* (*Fusarium oxysporum* f.sp. *pini*) in Kashmir, Dar and co-workers (2017) found that

among the various integrated treatments, combination of *Trichoderma harzianum*, + *L. laccata* + Carbendazim proved significantly superior to all the treatments with respect to reduction in disease intensity and increase in growth parameters and sturdiness quotient.

Gour and Sharma (2010) also reported superior results when *Trichoderma viride* or *Trichoderma harzianum* were integrated with Metalaxyl and Cymoxanil 8 per cent + Mancozeb 64 per cent to control root rot in cotton. Tariq *et al.*, 2012 studied that integration of Captan + Metalaxyl with *Trichoderma harzianum* and *Trichoderma virens* proved superior control of wilt compared to individual treatments in wilt incidence and yield of bell pepper, *Capsicum annum* var. California.

Singh *et al.* (2007) reported that treatment of tomato seeds with combination of *Trichoderma harzianum* and carbendazim resulted in highest disease control (84.05%) of *R. solani* at 60 days. Rehman and Lawrence (2010) used two fungal antagonists' viz., *Trichoderma viride* and *Trichoderma harzianum* along with fungicide carbendazim as seed treatment and soil drench and found significant inhibition in growth of *Rhizoctonia solani* in cabbage.

Chattopadhyay and Sen (1996) reported that the subsequent application of compatible fungicides has been found to support the growth of antagonists and also prevented the plant from the attack of soil borne pathogens. Integration of bio-control agents with fungicides gave significantly higher disease control in several crops than that obtained either by bio-control agent or by fungicide alone (Sawant and Mukhopadhyay, 1990; Dubey, 1997).

Andrabi *et al.* (2011) studied that *Trichoderma virens* with the application of Carbendazim recorded maximum yield in chickpea plants against *R. solani*. Poddar *et al.* (2004) studied the Integrated seed treatment with *Trichoderma harzianum* + Carbendazim which resulted in the maximum seed yield and lowest disease incidence against wilt in chickpea. De *et al.*, 1996 coated chickpea seeds with bio-control agents, *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis* and *Gliocladium virens* and chemical fungicides like carboxin and carbendazim which reduced wilt and increased the yield. Vyas (1994) controlled *R. bataticola* in soybean using *Trichoderma viride* or *Trichoderma harzianum* and carbendazim. Similarly, *Trichoderma harzianum* also controlled soil-borne plant pathogens when introduced in combination with carbendazim against *Jatropha* root rot caused by *Rhizoctonia bataticola* (Mehta *et al.*, 1993). *Trichoderma viride* in combination

with carbendazim (2 g kg⁻¹ seeds) reduced post-emergence mortality of blackgram caused by *R. bataticola* compared with individual application of both treatments (Suriachandraselvan and Seethuraman, 2002). Kumar *et al.* (2011) while working on *Jatropha* root rot caused by *Rhizoctonia bataticola* found that carbendazim (2 g kg⁻¹ seed) + neem extract (20%) was the most effective treatment with 67.3% reduction followed by *Trichoderma harzianum* + carbendazim and neem extract + *Trichoderma harzianum* in *Jatropha* root rot caused by *Rhizoctonia bataticola*.

English *et al.* (1986) while working on seedling blight of long leaf pine caused by binucleate *Rhizoctonia* concluded that soil microflora when tested against the pathogen along with systemic fungicide like carbendazim gave more than 80 per cent control of the disease over control. Similarly, Huang and Kuhlman (1991) while working on slash pine seedlings inhibited *Rhizoctonia* incited damping off by consortium of *Trichoderma viride* + *Trichoderma harzianum* and Benomyl. Hietala (1995) reported the management of Norway-spruce seedlings suffering from root die back caused by uni and binucleate *Rhizoctonia* spp. through soil amendements with native microflora and systemic fungicides. While studying root rot due to *Rhizoctonia solani* in forest nurseries, Chakravarty and Mishra (1982) reported the effectiveness of combination of native microflora and carbendazim followed by hexaconazole. Integration of biocontrol agents with chemicals have resulted in reduced disease incidence of root rot in many fruits, vegetables and forest nursery seedlings.

Chapter-3

MATERIALS AND METHODS

The present study was carried out in the Department of Plant Pathology Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan Himachal Pradesh, during 2018 -19.

3.1 Glassware used

Under *in vitro* experiments glassware of Borosil make viz., Petri plates (90 mm diameter), test tubes (10 and 20 ml), microscopic slides (24 x 7.5 x 1 mm), flasks (100, 250 and 500 ml), beakers (500 and 1000 ml) and pipettes (1, 5 and 10 ml) were used in the present course of investigations.

3.1.1 Equipments, Apparatus and other Materials used

Weighing of different chemicals used in the experiments was done on a single pan electrical balance (Narang Scientific Works Pvt. Ltd.) and culture of the root rot pathogen was incubated in Biological oxygen demand (BOD) incubator (REMI Instruments Ltd.). Forest nursery polybags were used for pot experiments.

3.1.2 Sterilization

The sterilization of the glass wares (wrapped in brown paper) was done by autoclaving at 1.05 kg/cm² pressure for 20 minutes. Autoclaved glassware was dried in hot air oven at 160°C for 60 minutes. Soil and Sand-wheat medium were sterilized by autoclaving for 1hr at 15 pounds per square inch pressure. Soil was sterilized by using 37% commercial formaldehyde (one part formalin was diluted in eight liters of water and covered for 7days). All *in vitro* experiments were carried out under aseptic conditions using laminar airflow.

3.2 DISEASE SURVEY

The Surveys were conducted in different forest nurseries of district Solan, Himachal Pradesh during June, July, and August. The nurseries surveyed were: Kandaghat range (Kiari, Karol, Bisha nurseries), Chail range (Chiunth and Gaura nurseries) and Solan range (Shilli and

Nauni nurseries). The diseased nursery plants were collected in paper bags, photographed and studied for symptom development. The disease incidence was calculated as per formula.

$$\text{Disease incidence \%} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

The diseased plants were brought to the laboratory for isolation and identification of the pathogen(s) involved. The diseased plants were stored in paper bags in refrigerator at 5°C for further studies.

3.3 ISOLATION FREQUENCY OF PATHOGENS

To ascertain the level of frequency of various pathogens associated with root rot wilt of *Cedrus deodar*, samples were collected from diseased nurseries of Solan district in Himachal Pradesh. In each sample 15 seedlings from each nursery showing any type of disease symptom (necrosis, wilting, drooping, damping off etc) were randomly collected. Laboratory analysis was made within 24hr after collection. Each plant was divided into roots and stems (collar portion). Roots and stems were cut into segments of 4-5mm length, pooled separately and eight of them were planted in each of the fifteen replication of each kind of plant tissue. Each treatment was replicated 15 times. Mean isolation frequency was determined as number of isolates of selected fungus from 15 sampled root and stem portion of diseased seedlings.

3.3.2 Isolation and maintenance of the pathogen

Potato-Dextrose-Agar medium having following composition was prepared by using method described by Johnston and Booth (1983).

Potato	-	200.0 g
Dextrose	-	20.0 g
Agar-agar	-	20.0 g
Water	-	1000 ml

The infected plant material was washed with running tap water to remove the surface soil. Small bits of 1 to 2 mm size were taken from the diseased and healthy portion of roots and collar region of the plant with the help of a sterilized blade under laminar air flow. These bits were surface sterilized with Sodium hypochlorite (0.05%) for 30 seconds and were

washed thrice with sterilized distilled water under aseptic conditions. The bits were then placed on the sterilized filter paper to remove the excess moisture and transferred to sterilized Petri plates containing potato dextrose agar (PDA) medium. The medium was supplemented with streptocyclin (10 mg/L) after sterilization in autoclave at 1.05 kg/cm² for 20 minutes. These Petri plates were later incubated at 27±1°C in BOD incubator and examined daily for mycelial growth, if any. The fungal growth developed in Petri plates was purified by hyphal tip technique. The culture slants were preserved at 5°C in refrigerator and subcultured fortnightly.

3.4 IDENTIFICATION OF PATHOGEN

The fungus was identified on the basis of morphological characters as documented by Barnett *et al.* (2003); Sneh *et al.* (1998) and Nelson *et al.* (1983). The culture slants were sent to National Centre of Fungal taxonomy, New Delhi for confirmation of the identification done in the laboratory. Hyphal diameter (µm), presence or absence of septa, pigmentation of substratum, colony type and diametric growth (mm) after 24, 48, 72, 96 and 120 hours were observed. The growth rate (mm\hrs) was calculated after 24, 48, 72, 96 and 120 hours till the petri plate became full by the formula has given under (Verma, 1997).

$$rg : \frac{cd_2 - cd_1}{t_2 - t_1}$$

Where,

rg	=	rate of mycelial growth
cd ₂	=	mean diameter of colony at time t ₂
cd ₁	=	mean diameter of colony at time t ₁
t ₂	=	time when mean diameter of colony was cd ₂
t ₁	=	time when mean diameter of colony was cd ₁

The hyphal diameter was measured in Olympus make Phase contrast compound microscope. The mycelium was stained with 3% KOH and Safranin O and examined under 400X using Phase contrast microscopy. For molecular confirmation, the internal transcribed spacer region was amplified using ITS1/ITS4 markers from Eurofins Genomics, USA and sequence was submitted to gene bank.

3.4.1 Pathogenicity test

The pathogenicity test of the causal organism was conducted by standard Koch's postulates under pot culture conditions. The earthen pots of 12 inches diameter were filled with sterilized soil. Thereafter, 50 g of mass cultured medium of pathogen was applied in these pots, a week ahead of planting of one month old rooted deodar seedlings. In all, three seedling of Deodar were grown in each pot at appropriate distance. Thirty pots were used in this experiment and control was maintained without addition of the causal fungus. The fungus was reisolated from the infected seedlings. The identity of the same was confirmed by Phase contrast stereoscopic microscope.

3.4.2 Multiplication in Potato dextrose broth (PDB)

Five mm discs of seven days old culture of binucleate *Rhizoctonia* were added to 100ml PDB in 250ml conical flasks. These flasks were incubated for 14 days on a rotary shaker at 80 - 100 rpm at $26 \pm 1^{\circ}\text{C}$ temperature.

3.4.2.1 Preparation of mycelial suspension

The mycelial mat of the pathogen formed on PDB was separated by using a sieve and washed repeatedly with sterilized distilled water. Excess water was drained by applying gentle pressure onto the mycelium. Five grams of this mycelial mat was added to one litre of sterilized distilled water and stirred constantly. The number of colony forming units were analyzed using Haemocytometre. The homogenized suspension thus prepared was used for inoculation immediately (Buttner *et al.*, 2004).

3.4.2.2 Preparation of mass culture of the root rot pathogen

The root rot pathogen was mass cultured on sand-wheat medium (1:2). The wheat grains were boiled just enough to make them soft. Excess of water was drained out and wheat grains were air-dried. Wheat grains were then mixed with sand (2 part wheat grain and 1part sand) along with 2 per cent sucrose. The medium thus prepared was filled in autoclavable polypropylene bags (150 g per bag), plugged with non-absorbent cotton and were sterilized twice by autoclaving for 1hr at 15 pounds per square inch pressure. The sterilized medium was inoculated under aseptic conditions with one week old culture of isolated pathogen. Five to six bits of 4 mm size of this fungus were placed in each bag. The bags were shaken regularly so that the culture grows uniformly. The inoculated bags were incubated at $27 \pm 1^{\circ}\text{C}$

in BOD incubator for 20 days and the mass culture was used for carrying out various experiments.

3.4.2.3 Soil infestation

To the polybags filled with sterilized soil 50 g of the binucleate *Rhizoctonia* mass multiplied on Wheat grain was applied uniformly. Immediately after inoculation the pots were sprinkled with water. These bags were left for seven days in a glasshouse for incubation. One month old *Cedrus* seedlings were planted seven days after soil inoculation. The treatment contained ten seedlings replicated thrice. The seedlings were observed daily for symptom expression. The typical symptoms of the disease were recorded. The data on total number of seedlings and number of affected seedlings were recorded to calculate per cent disease incidence (PDI).

PDI was calculated by adopting following formula:

$$\text{PDI} = \frac{\text{Number of plants affected}}{\text{Total number of plants}} \times 100$$

The pathogen was reisolated from the infected seedlings and the culture obtained was compared with the original culture.

3.4.2.4 Root dip inoculation

Seedlings of *Cedrus deodara* were raised in poly bags (15cm diameter) filled with sterilized soil. One month old seedlings were uprooted from the pots carefully, washed with sterilized distilled water to remove the excess soil present on the root surface and the distal one third of root system was trimmed. These trimmed seedlings were dipped in mycelial suspension of the pathogen for ten minutes and replanted in same poly bags. The mycelial suspension was poured over the root zone of the seedlings. The treatment contained ten seedlings replicated thrice. Transplanted seedlings were watered regularly and observed for symptom development.

The pathogen was reisolated from the infected seedlings and the culture obtained was compared with the original culture. The data on total number of seedlings and number of affected seedlings were recorded to calculate per cent disease incidence which was calculated by adopting the formula mentioned earlier.

3.4.2.5 Stem application

One month old Seedlings of *Cedrus deodara* were raised in poly bags (15cm diameter) filled with sterilized soil. The collar portion of the seedling was pricked with sterilized needle thrice. The mycelial suspension was applied with the help of camel hair brush and remaining mycelial suspension was added to the soil @ 5ml per pot. Immediately after inoculation, the inoculated stem portions were wrapped with blotter papers and the pots were watered regularly. Two days after inoculation the blotter paper wrappers were removed and the seedlings were observed daily for the presence of symptoms. The treatment contained ten seedlings replicated thrice. The pathogen was reisolated from the infected seedlings and the culture obtained was compared with the original culture. The data on total number of seedlings and number of affected seedlings were recorded to calculate per cent disease incidence. Control bags were kept as such without adding any pathogen inoculum and watered regularly.

3.5 DISEASE MANAGEMENT

3.5.1 Biological Methods

3.5.1.1 *In vitro* evaluation of rhizosphere microflora against root rot pathogen

An experiment was conducted to test the *in vitro* efficacy of rhizospheric fungal and bacterial antagonists against the test pathogen. The antagonists were isolated from rhizosphere of *Cedrus deodara* seedlings and trees. About 10 g of soil sample was taken in a 250 ml conical flask with 90 ml of sterile distilled water. The sample was agitated for 5 minutes and serial dilutions of soil suspensions were prepared. To isolate mycoflora and bacteria one ml of dilution was poured in to sterilized Petri plates containing PDA. The Petri plates were incubated at $25 \pm 2^{\circ}\text{C}$ temperature. The plates were examined daily. The individual fungal colonies were directly transferred onto the PDA slants with the help of a sterilized needle. The fungal cultures were further purified by hyphal tip method. Pure cultures of the fungal biocontrol agents were maintained on PDA at $25 \pm 2^{\circ}\text{C}$ by periodical transfers. Of these, the potential antagonistic fungi were identified based on their morphological and cultural characteristics (Barnett and Hunter, 2003). The effective bacterial microflora was cultured on nutrient agar. Fungal and bacterial antagonists were tested for their antagonistic activities against root rot by dual culture technique (Huang and Hoes, 1976). Culture discs (4 mm diameter) of each of antagonists and the pathogen were taken from margin of their vigorously growing culture and transferred aseptically to solidified

PDA(Potato Dextrose Agar) contained in Petri plates (90 mm) on the opposite sides. The Petriplates containing only culture of the pathogen served as control. The experiment was laid out in CRD and each treatment was replicated four times and the Petri plates were incubated at 25±1°C in BOD incubator. The colony diameter of test fungus was recorded after the control plates achieved full growth. The antagonistic activity of isolated bacteria against the root rot pathogen was observed by streak plate method (Utkhede and Rahe, 1983). The Petri plates containing sterilized PDA were streaked at the centre with 48 hours old colonies of bacteria with the help of bacterial loop. Mycelial bit (4 mm diameter) of the test pathogen was placed on opposite sides of the streak at a distance of 1 cm from the margin of the plate. Petri plates without bacterial streak served as control for comparison. Each treatment was replicated four times under Completely Randomized Design (CRD) and incubated at 25±1°C in BOD incubator. Per cent mycelial inhibition in the growth of test pathogen was calculated as per the formula:

$$\text{Percent inhibition over control (I)} = \frac{C-T}{C} \times 100$$

Where,

I = Inhibition percentage

T = Treatment

C = Control

3.6 CHEMICAL METHOD

3.6.1 *In vitro* evaluation of different fungicides against the root rot pathogen

Various fungicides were tested at different concentrations *in vitro* by poisoned food technique (Borum and Sinclair, 1968). The experiments were conducted in completely randomized design with ten treatments and three replications. The fungi toxicants were as under:

Sr.No.	Chemical	Concentrations
1.	Hexaconazole 5 EC	25ppm, 50 ppm, 100 ppm
2.	Thiophonate methyl 70 WP	25ppm, 50 ppm, 100 ppm
3.	Carbendazim, 50WP	25ppm, 50 ppm, 100 ppm
4.	Metalyxl 55% WS	500 ppm,1000 ppm, 1500ppm
5.	Pyraclostrobin + Metiram	500 ppm,1000 ppm, 1500ppm
6.	Copper oxychloride 50 WP	500 ppm, 1000 ppm, 1500ppm
7.	Mancozeb 75@% WP	500 ppm, 1000 ppm, 1500ppm
8.	Chlorothalonil 75 WP	500 ppm, 1000 ppm, 1500ppm
9.	Captan 50 WP	500 ppm, 1000 ppm, 1500ppm
10.	Tebuconazole +Trifloxystrobin	500ppm,1000ppm,1500ppm

Simultaneously, double concentrations of different fungicides were also prepared in sterilized distilled water so as to get desired concentration of fungicides after mixing the fungicide solutions in the double strength media. Fungicides solution were added separately to equal quantities of double strength PDA medium aseptically before pouring in Petri plates. These plates were then inoculated with the seven days old culture of root rot pathogen. A control treatment was also maintained in which only plain sterilized water was added to double strength medium. Each treatment was replicated thrice and the inoculated plates were incubated at 25±1°C in BOD incubator. The colony diameter of test pathogen was recorded till the control plates were fully covered with mycelium of the test pathogen. The per cent inhibition in the mycelia growth of the pathogen was calculated according to formula given by Vincent (1947).

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

Where,

I - Inhibition of mycelial growth (%)

C - Linear mycelial growth in control (mm) after 24 hrs

T - Linear mycelial growth in treatment (mm) after 24 hrs

The above experiment was carried out in Completely Randomized Design (CRD) and the data was recorded after every 24 hrs. until the control plates were fully covered with the mycelium.

Design	:	CRD
Replications	:	3
Treatments	:	31

3.6.2 Integration of best antagonists and chemicals against root rot of *C. deodara*

The best antagonists obtained from the previous experiment were integrated with the best fungi toxicants. The experiment was conducted in nursery bags in Completely Randomized design. Sixteen treatments replicated thrice were kept in glass house. The effect of three best chemicals individually and in combination with the three best antagonists was tested.

Treatments

C1	C2	C3
A1	A2	A3
C1A1	C1A2	C1A3
C2A1	C2A2	C2A3
C3A1	C3A2	C3A3
CONTROL	CONTROL	CONTROL

Where,

C – Best Chemical

A – Best Antagonist

Mass culture of best fungal and bacterial antagonist isolated from the rhizosphere was prepared by mixing wheat bran and saw dust in 1:1 ratio and 2 percent sucrose. Mixture was autoclaved for 1 hour at 15 pound pressure. Bits of isolated bio-control fungus were added to this medium. Autoclaveable propylene bags were used for this purpose. The bags were kept at 25⁰C in BOD incubator and observed regularly for fungal growth. The bacterial mass culture was prepared on nutrient agar media and one week old bits of bacterial culture were added to this media. The media was prepared in 2 liter borosil flask and incubated in rotary shaker till the turbid growth was achieved. The turbid growth was achieved in 5 days while fungal growth in bags was achieved after 15 days of inoculation. Three best chemicals from the *in vitro* experiment along with three best antagonists were tested individually as well as in combination against root rot of *C. deodara*. The mass culture of isolated fungal bio control agent was added to the sterilized soil in the ratio of 1:20 a week before sowing of seed. The bacterial biocontrol agent was added in the ratio of 1:30 in sterilized soil at the time of sowing. The mass culture of test fungus was applied a week before sowing @ 1:20. The soil was drenched with test chemicals at the recommended doses at the time of plantation. The control pots contained only the mass culture of isolated fungus. The experiment was laid in completely randomized design in the nursery bags with 16 treatments and three replication each. Three seed were sown per bag and data was recorded 25 days and 45 days after seed sowing. The disease incidence was calculated as per the formula.

$$\text{Disease incidence (\%)} : \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

Chapter-4

RESULTS AND DISCUSSION

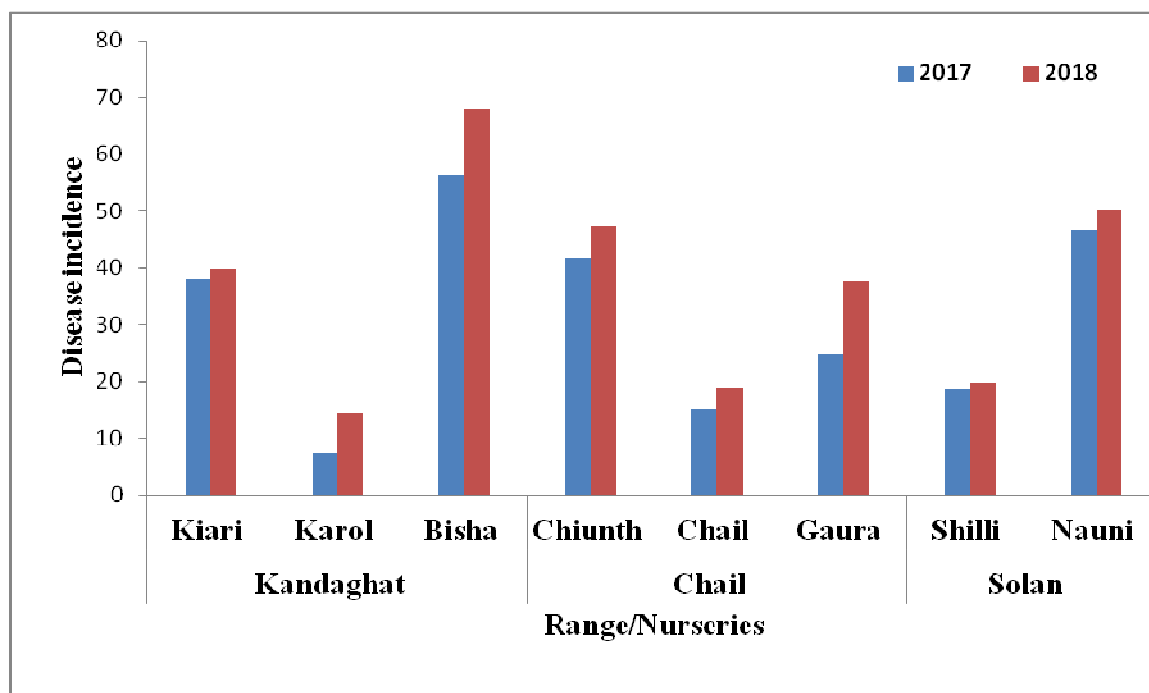
4.1 INCIDENCE OF ROOT ROT OF *Cedrus deodara* IN DIFFERENT PARTS OF DISTRICT SOLAN

Incidence of root rot of *Cedrus deodara* was recorded during the cropping season of 2017-2018 and 2018-2019 in different *Cedrus deodara* growing nurseries of Solan range (Table.1, Fig.1 and Plate.1). Incidence of root rot was found in all the locations surveyed. Maximum incidence of the root rot was recorded in Kandaghat range (37.35%) followed by Solan (33.85) and Chail range (30.93%), respectively. Amongst nurseries, Bisha nursery had maximum incidence (62.15%) followed by Nauni (48.46%) and Chiunth nurseries (44.65), respectively. The overall mean of different years suggested that disease was 5.56 per cent more in the year 2018 -19 than the previous year. Bisha, Nauni and Chiunth nurseries were worst affected with root rot disease. Over all, the incidence of disease ranged between 10.90 to 62.15 per cent in different locations that was detrimental for the production of healthy *Cedrus deodara* nurseries. Dar and coworkers (2017) while working on root rot of *Abies pindrow* in Kashmir valley recorded 29.3 and 32.2 per cent disease incidence for two consecutive years. Similarly, Dar and his associates while working on root rot of *Pinus wallichiana* during 2011 recorded 4 - 56.75 per cent disease incidence in various nurseries of Kashmir valley.

Table 1. Disease incidence of root rot in seedlings of *Cedrus deodara* in district Solan

Range	Root rot incidence (%) in the year			Pooled mean
	Nursery	2017	2018	
Kandaghat	Kiari	38.10	39.90	39.00
	Karol	7.50	14.30	10.90
	Bisha	56.30	68.00	62.15
Mean		33.96	40.73	37.35
Chail	Chiunth	41.80	47.50	44.65
	Chail	15.00	18.90	16.95
	Gaura	24.90	37.50	31.20
Mean		27.23	34.63	30.93
Solan	Shilli	18.70	19.8	19.25
	Nauni	46.50	50.43	48.46
Mean		32.60	35.11	33.85
Overall Mean		31.26	36.82	

Fig.1. Graphical representation of disease incidence of root rot in seedlings of *Cedrus deodara*



4.2 SYMPTOMATOLOGY, ISOLATION AND IDENTIFICATION OF THE PATHOGEN

4.2.1 Symptomatology

The symptoms of root rot in *Cedrus* seedlings varied from seed rot, hypocotyl rot and collar rot to root rot. The one month old seedlings showed collar rot and root rot. The roots turned black, thin and with little or no secondary root growth (Plate 2). In some nurseries the seeds failed to germinate due to complete seed rot. Some of the germinated seeds showed hypocotyl and radical rotting with brownish patches on the surface. On the seedlings symptoms were yellowing of the needles with poor growth and rapid root decay. Healthy seedlings had dark green needles and sturdy growth. The infected seedlings showed characteristic damping off with soft brown rotted tissue at collar portion. Similar symptoms were reported by Adam (1988) while working on *Rhizoctonia solani*. He reported that the most common symptom of disease caused by *Rhizoctonia* is referred to as "damping-off" characterized by non-germination of severely infected seed whereas infected seedlings can be killed either before or after they emerge from the soil. *Rhizoctonia* sp. primarily attacks below ground plant parts such as the seeds, hypocotyls and roots but is also capable of



**Nurseries showing root rot
(Bisha nursery)**



**Nurseries showing root rot
(Chiunth)**



**Nurseries showing root rot
(Shilli)**



Healthy nursery

Plate 1. Disease incidence of root rot in seedlings of *Cedrus deodara* in different nurseries



seedling rot and poor germination



Blackening of collar portion, no secondary roots and drying of seedling



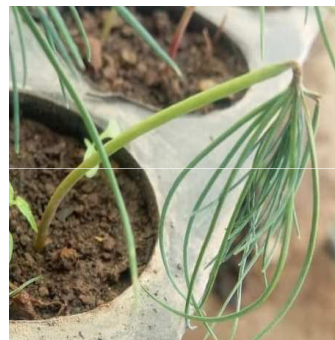
Completely dried *Cedrus* seedling at Chiunth nursery



Complete rotting of stem and drooping of seedling



Complete Rotting



Seedling showing drying and drooping down symptom at Bisha nursery.



Post emergence damping off



a) Initiation of browning of collar portion and roots b) few or none secondary root formation and drying, c) complete blackening of roots and necrosis

Plate 2. Symptoms on seedlings

infecting above ground plant parts as well e.g. pods, fruits, leaves and stems. The roots of the nursery plants dry up giving the plant a stunted growth (Bhardwaj *et al.*, 1988). According to Gupta and Chauhan, 2005 symptoms of root rot infected plant were sudden chlorosis and wilting of leaves followed by browning of leaf and stem. The present symptoms are in consonance with the works of various scientists who confirmed that *Rhizoctonia* sp. causes rapid decay and mortality of germinating seeds and emerged seedlings (Mikola, 1952; Vaartaja, 1952; Hanioja, 1969 and Lilja, 1979).

4.3 ISOLATION OF THE PATHOGEN

The pathogenic fungus was isolated in pure culture on potato dextrose agar medium from affected seedlings collected from different *Cedrus deodara* growing nurseries of the district Solan. Isolations of the pathogen associated with *Cedrus deodara* root rot were made from the diseased roots and collar region of the seedlings on the potato dextrose agar (PDA) medium using standard isolation techniques. The root rot pathogen was cultured on the PDA by taking the smaller bits of the host tissues from the peripheral regions of the affected plant parts. After culturing, in most of the bits cultured, the fungus protruded out of the infected tissues into the medium. The isolated fungus was then purified by hyphal tip method on the Petri plates containing PDA. Purity of the isolated fungus was regularly monitored and maintained by sub culturing after every 15 to 25 days.

4.4 IDENTIFICATION OF THE PATHOGEN

The fungus grew rapidly on potato dextrose agar medium at 25°C with fluffy white mycelium and attained growth in 3-4 days while after a week pale mycelium turned light brown in colour. The mycelium branched near the distal septum of the mother hyphal cells at right or acute angles and the branches were constricted at or near the point of origin of the septum (Plate 3). The size of the hyphae varied between 6.2-6.4µm (Table - 2). Sclerotia were formed within 55 days in culture and were brown to dark brown, large, round, rough and measured 0.75-3.0 mm in diameter. Monilioid cells arising from the sclerotia were brown and barrel shaped and measured 15-38 × 10-15µm in size. The fungus was identified on the basis of morphological characters as documented by Nelson *et al.*, 1983; Sneh *et al.*, 1998; Barnett and Hunter, 2003). The results are in conformity with the work done by Unal and coworkers (2014) who reported the width of hypha of binucleate *Rhizoctonia* AG-E isolate to be less than 7µm with two nuclei per cell and sclerotial diameter to be between 0.75 to 3.0

mm. Similarly, Olutoyosi and coworkers (2017) reported that morphologically binucleate *Rhizoctonia* AG-E on soya bean was pale to brown colour on PDA while the Moniliod cells were brown in colour and barrel shaped measuring 24x1415µm in size. The culture slants were sent to National Centre of Fungal taxonomy, New Delhi for confirmation of the identification done in the laboratory.

4.5 MOLECULAR IDENTIFICATION OF THE PATHOGEN

To ensure the identification of the isolated fungus, molecular sequencing was outsourced from Eurofins Genomics, Bengaluru. For molecular confirmation, the internal transcribed spacer region was direct sequenced using ITS1/ITS4 markers (Eurofins Genomics, USA) and sequence was submitted to gene bank. Fungal genomic DNA of isolate collected from Government forest nursery Bisha was named as Solan isolate. Amplification of ITS1-ITS4 region with primer pairs ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'- TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) yielded 706 bp amplicons (Plate 4, Fig.2) which were sequenced. The nucleotide sequence with GenBank Accession no. MK226512.1 showed 99.22, 99.19 and 99.18 per cent similarity to nucleotide sequences number EU591790.1, AY586174.1, AY586173.1, respectively which were identified as binucleate *Rhizoctonia* AG –E = *Ceratobasidium* sp. CAG 6. Many binucleate *Rhizoctonia* represent a diverse group of organisms that have been isolated from soils and plants throughout the world. Isolates of binucleate *Rhizoctonia* AG-E with *Ceratobasidium* teleomorphs have been reported by many workers throughout the world. A system developed in Japan (Ogoshi *et al.*, 1979; Ogoshi and Ui, 1983; Sneh *et al.*, 1998; Hyakumachi *et al.*, 2005) includes 21 anastomosis groups designated AG-A to AG-U, in which at present AG-J and AG-M still are in question as members of binucleate *Rhizoctonia*. Another system developed in the USA (Burpee *et al.*, 1980) includes 7 anastomosis groups designed as CAG-1 to CAG -7. CAG-1 corresponds to AG-D, CAG-2 to AG-A, CAG-3 and CAG-6 to AG-E, CAG-4 to AG-F, CAG-5 to AG-R, and CAG-7 to AG-S, respectively (Sneh *et al.*, 1998 and Ogoshi *et al.*, 1983). Gonzalez *et al.* (2001) found that certain binucleate *Rhizoctonia* belonging to CAG 4 and CAG -6 when clustered molecularly, were found to be close to *R. solani* CAG-4 isolates. Therefore, the identity of the present isolated pathogen is binucleate *Rhizoctonia* belonging to anastomosis group AG –E with a teleomorphic stage *Ceratobasidium* sp. group CAG 6.



Pure culture of *Rhizoctonia*



Sclerotia formation in culture



**Septate mycelium of *Rhizoctonia*
under 40X zoom.**



**Monilioid cells of *Rhizoctonia*
under compound microscope**



**Septate mycelium of *Rhizoctonia*
under phase contrast microscope**

Plate 3. Mycelium and sclerotia of binucleate *Rhizoctonia* AG-E

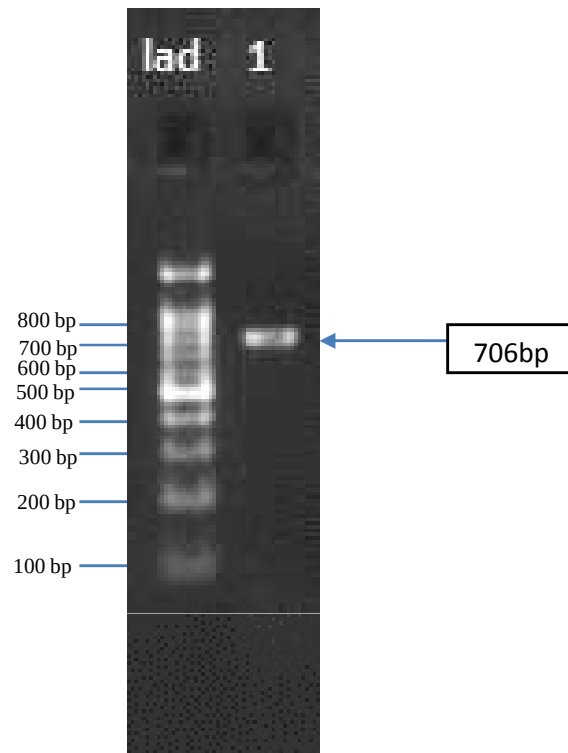


Plate 4. Agarose gel photograph of 706 bp amplicon of binucleate *Rhizoctonia* AG-E (Courtesy: Eurofins Genomics)

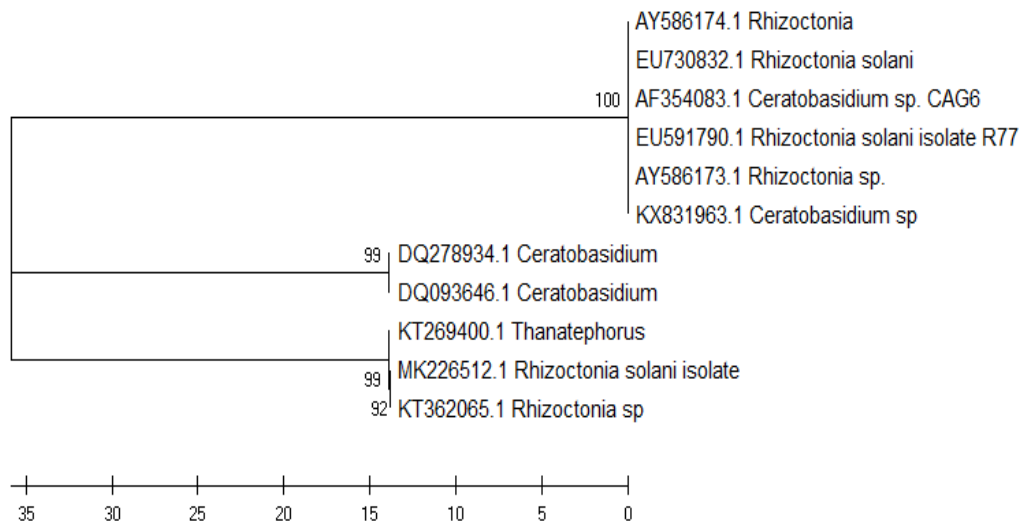


Fig.2 Phylogenetic tree based on ITS sequences drawn using the maximum-likelihood method showing relationship between the isolate of binucleate *Rhizoctonia* AG-E (MK226512.1) and similar other sequences deposited in the NCBI Gen Bank

Table 2: Cultural Characteristics of binucleate *Rhizoctonia* AG-E

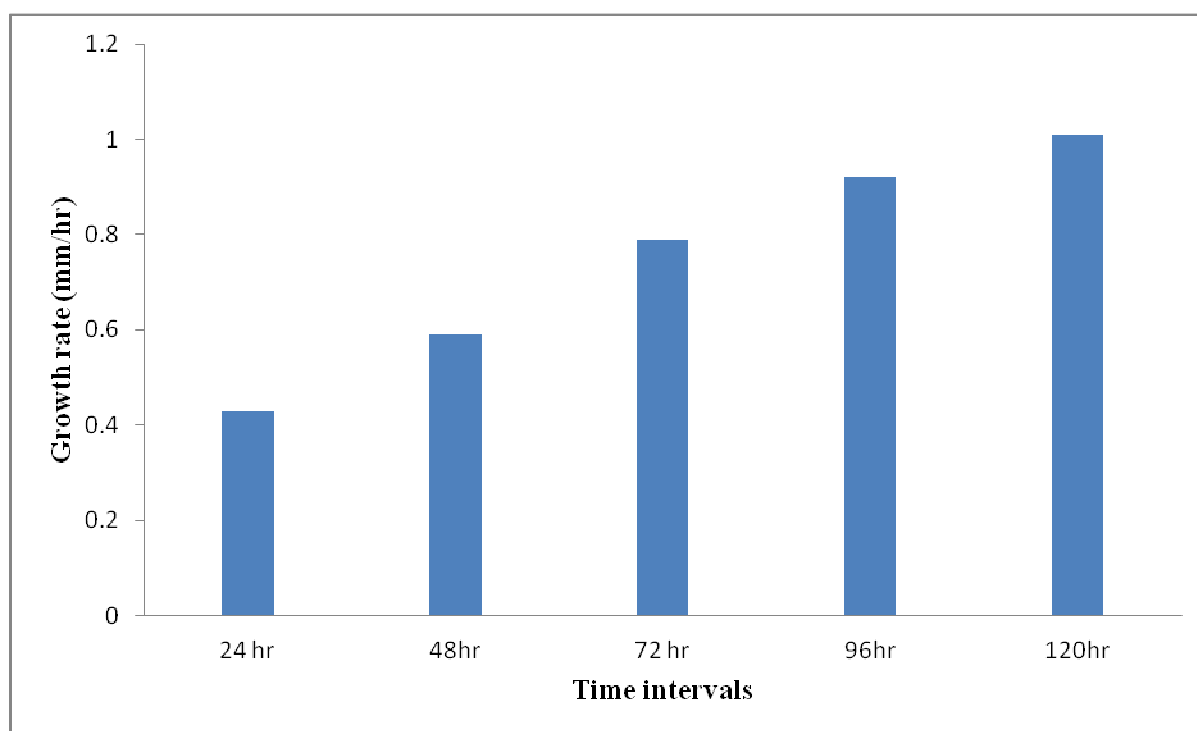
Fungal Character	Description
Hyphal diameter (µm)	6.2-6.4 (µm)
Shape and size of spores (µm)	No spore formation
Presence or absence of septa	Transverse septation present
Pigmentation of substratum	Mycelium thin, spreading, off-white to light brown
Colony type	Fluffy

The diametric growth rate of the fungus was studied after 24, 48, 72, 96 and 120 hours (Plate 5). The result presented in the Table 3 (Fig.3) revealed a progressive increase in the growth rate of fungus after every 24 hours. The growth attained a peak of 1.01mm/hr after 120 hours which was significantly different than growth at 96 and 72 hours respectively. Campbell and Altman (1977) found that linear growth of *R. solani* significantly increased after 24hr. Similarly, Tisserat *et al.*, 1977) found linear growth of *R. solani* increased after 48 hr on PDA. Gottlieb (1971) found that the growth rate of *R.solani* increased to a maximum after 5days. Sharma *et al.*, 2013) revealed that radial growth of different isolates of *R. solani* significantly varied when recorded after 24hrs. for 5 days.

Table 3: Diametric growth rate of binucleate *Rhizoctonia* AG-E at different time intervals

Time (hr)	Growth rate(mm/hr)
24 hr	0.43a
48hr	0.59b
72 hr	0.79c
96hr	0.92d
120hr	1.01e
CD _{0.05}	0.01

Fig.3. Graphical representation of diametric growth of binucleate *Rhizoctonia* AG-E at different time intervals

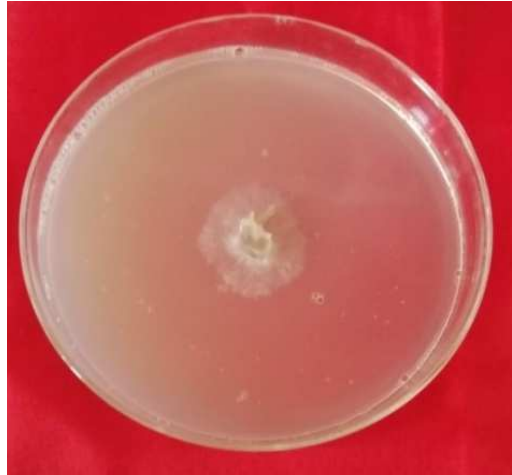


4.6 ISOLATION FREQUENCY

To ascertain the level of frequency of various pathogens associated with root rot of *Cedrus deodara*, samples were collected from diseased nurseries of Solan district in Himachal Pradesh. Mean isolation frequency was determined as number of isolates of selected fungus from 15 sampled root and collar portion of diseased seedlings. The results in Table4 indicated that out of the three ranges surveyed, many pathogens were isolated viz., *Fusarium oxysporum*, *Rhizoctonia* spp, *Phoma* spp, *Phytophthora* spp, *Mucor* spp, and *Trichoderma* spp. However, the maximum frequency was found to be of binucleate *Rhizoctonia* AG-E, *Fusarium oxysporum* and *Phoma exigua* (Plate 6). The identity of three



24 hours



48 hours



72 hours



96 hours

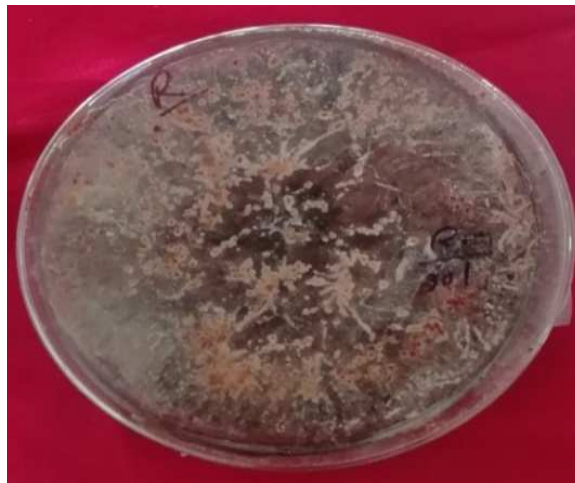


120 hours

Plate 5. Diametric growth rate of binucleate *Rhizoctonia* AG-E at different time intervals



Phoma exigua



Binucleate *Rhizoctonia* AG-E



Fusarium oxysporum

Plate 6. Frequently isolated pathogens from different *Cedrus* growing area

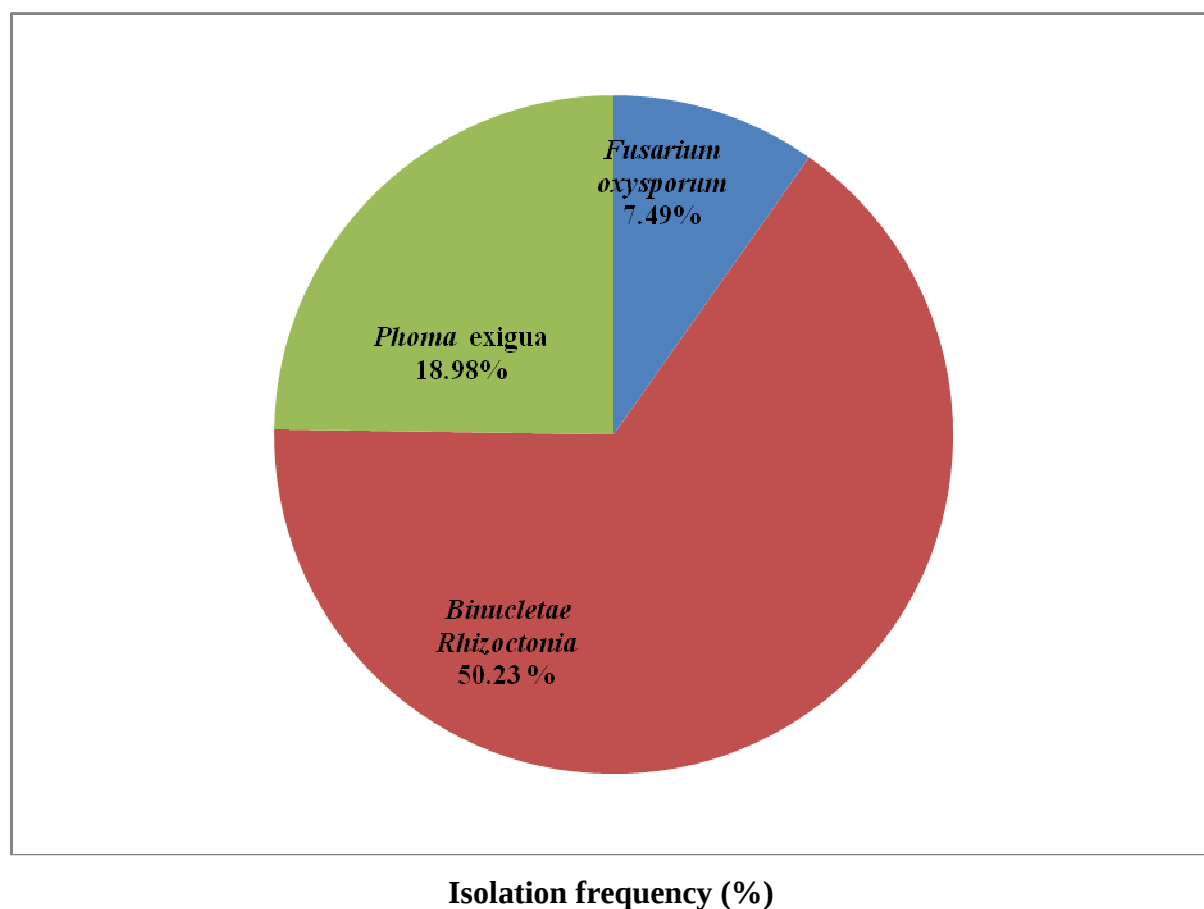
pathogens was confirmed from National Centre of Fungal taxonomy, New Delhi. binucleate *Rhizoctonia* AG-E was isolated from all the nurseries whereas *Fusarium oxysporum* and *Phoma exigua* were absent in some of them. The results presented in Table 4 (Fig.4) indicated that out of the three ranges Chail range comprising of two nurseries showed the maximum isolation frequency of 37.71 per cent followed by Kandaghat (34.42%) and Solan (19.37%), respectively. Amongst the three major pathogens isolated binucleate *Rhizoctonia* AG-E was most frequently isolated with a maximum frequency of 50.23 per cent followed by *Phoma exigua* (18.98%) and *Fusarium oxysporum* (7.49%), respectively. Isolation frequency of *Phoma exigua* and *Fusarium oxysporum* were significantly lesser as compared to binucleate *Rhizoctonia* AG-E. The results also revealed that population of *Rhizoctonia* was equally higher in root as well as collar portion whereas other two pathogens were isolated more from the root portions. In his studies on isolation of fungus from roots of *Abies pindrow*, Dar *et al.* (2017) also revealed the presence of various pathogens viz., *Fusarium oxysporum* f.sp. *pini*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp. Menkis *et al.* (2006) recorded 44.6 per cent isolation frequency of *F. oxysporum* from colonized roots of *Pinus sylvestris* seedlings and 0.3 per cent isolation frequency of *R. solani* from the diseased roots of *Picea* and *Abies* seedlings from Uppsala Sweden. Ahanger *et al.* (2011) were of the opinion that *F. oxysporum* was the most abundant pathogenic fungus in diseased roots of blue pine seedlings with isolation frequency of 38.6% where as *Rhizoctonia solani* and *Macrophomina phaseolina* showed isolation frequencies of 11.0 and 3.3 per cent, respectively which is contrary to the results presented here as isolation frequency of *Rhizoctonia* sp. was more as compared to *Fusarium* sp. However, Work done by Lilja *et al.* (2010) is in consonance with the present study where population of *Rhizoctonia solani* in many forest nurseries was more than other soil borne pathogen.

Table 4. Isolation frequency (%) of main pathogens associated with root rot of *Cedrus deodara*

Range/ Nursery		Isolation frequency (%)												Mean	Pooled mean
		<i>Fusarium</i> spp.				<i>Rhizoctonia</i> spp.				<i>Phoma</i> spp.					
		2017		2018		2017		2018		2017		2018			
		Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root		
Solan	Nauni	6.67 (14.23)	12.50 (20.43)	5.83 (13.47)	10.83 (19.03)	45.83 (42.57)	75.83 (61.65)	8.33 (16.73)	5.00 (10.59)	4.17 (11.35)	24.17 (29.35)	1.67 (4.30)	30 (33.14)	19.23 (23.07)	19.37 (22.62)
	Shilli	5.83 (13.90)	8.33 (15.92)	4.17 (11.64)	9.17 (16.76)	70.00 (57.14)	80.00 (63.63)	2.50 (7.33)	4.16 (9.60)	0.83 (3.03)	14.17 (21.97)	4.17 (11.64)	30.83 (33.60)	19.51 (22.18)	
Kandaghat	Kiari	6.67 (14.89)	6.66 14.75)	4.17 (9.60)	5.83 (13.62)	42.50 (40.60)	42.50 (40.60)	58.33 (49.78)	74.17 (59.50)	4.17 (9.60)	21.67 (27.43)	1.67 (4.30)	25.83 (29.79)	24.51 (26.20)	34.42 (32.81)
	Karol	4.17 (9.60)	3.33 (10.36)	2.50 (7.33)	6.67 (14.89)	25.83 (30.45)	25.83 (30.45)	15.83 (23.42)	21.67 (27.63)	2.50 (5.29)	4.17 (11.35)	1.67 (4.30)	6.67 (14.89)	10.06 (15.83)	
	Bisha	10.00 (18.42)	8.33 (15.92)	4.17 (9.60)	9.17 (17.19)	75.00 (60.09)	75.00 (60.09)	77.50 (62.53)	83.33 (66.17)	1.67 (4.30)	6.67 (14.47)	3.33 (8.61)	9.17 (17.34)	30.27 (29.58)	
Chail	Chiunth	6.67 (14.75)	11.66 (19.78)	5.83 (13.47)	6.67 (14.89)	49.17 (44.50)	49.17 (44.50)	61.67 (52.11)	75.83 (61.17)	4.17 (9.17)	7.50 (15.74)	1.67 (4.30)	25.83 (29.79)	25.48 (27.08)	37.71 (35.45)
	Gaura	5.00 (12.63)	19.16 (25.94)	1.67 (4.30)	18.33 (25.26)	55.00 (47.85)	55.00 (47.85)	68.33 (56.08)	83.33 (66.53)	55.00 (47.85)	86.67 (68.91)	68.33 (56.08)	83.33 (66.53)	49.93 (43.82)	
Mean		6.42 (14.06)	10.00 (17.58)	4.04 (9.92)	9.52 (17.38)	51.90 (46.17)	57.61 (49.82)	41.78 (38.28)	49.64 (43.06)	10.35 (12.94)	23.57 (27.03)	11.78 (13.36)	30.23 (32.15)		
Overall Mean		7.49 (14.73)				50.23 (44.33)				18.98 (21.36)					
C.D _{0.05}		Nurseries = 2.80, Pathogen = 4.66, Nurseries x Pathogen = 9.70													

Figures in parentheses are angular transformed value

Fig.4. Graphical representation of isolation frequency (%) of main pathogens associated with root rot of *Cedrus deodara*



4.7 PATHOGENICITY TEST

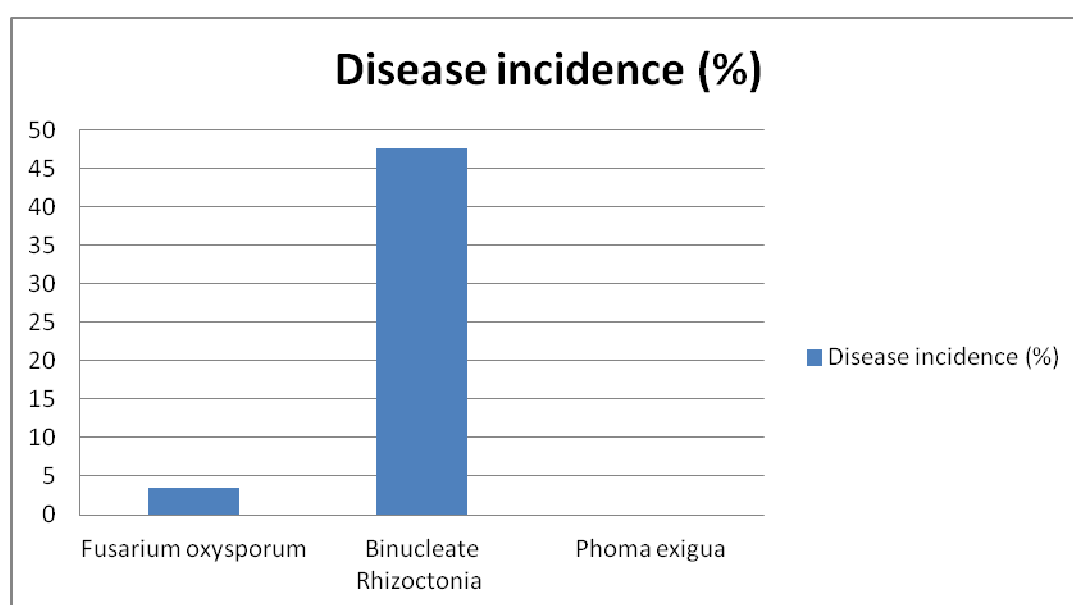
The pathogenicity test of the isolated fungus was conducted on one month old rooted cuttings of *Cedrus deodara*. The pathogenicity test of the causal organism was conducted by standard Koch's postulates in nursery bags. Most frequently isolated three main fungi viz., binucleate *Rhizoctonia* AG-E, *Fusarium oxysporum* and *Phoma exigua* were put to pathogenicity test to ascertain the causal organism of root rot in seedlings of *Cedrus deodara*. The results present in Table 5 (Fig.5) revealed that maximum incidence (47.53%) was recorded in seedlings inoculated with binucleate *Rhizoctonia* AG-E followed by *Fusarium oxysporum* (3.55%) and *Phoma exigua* (0.00%), respectively. Moreover, the root rot symptoms were conspicuous in the seedlings inoculated with binucleate *Rhizoctonia* within 25 days of inoculation whereas root rot symptoms were negligible in case of *Fusarium oxysporum* and totally absent in case of *Phoma exigua*. *Fusarium oxysporum* produced wilting and needle chlorosis along with slight vascular discoloration after 35 days of inoculation (Plate 7) while *Phoma exigua* exhibited no root rot symptoms at all. Root rot

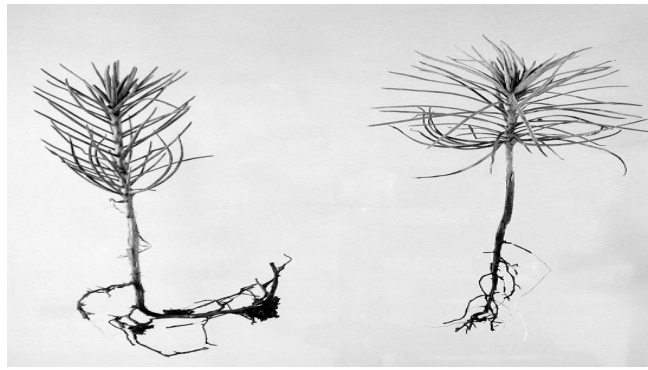
symptoms like thinning and blackening of roots, rotting at collar porting, damping off were recorded on seedlings inoculated with binucleate *Rhizoctonia* AG-E. All the pathogens were re isolated from the inoculated seedlings and hence due to root rot symptom production like extensive root decay, absence or lack of lateral roots, cortical tissue decay, stunted growth and needle discolouration, binucleate *Rhizoctonia* AG-E was used for further studies as the main pathogen of root rot of *Cedrus deodara*. While working on stem blight of *Catharanthus rosea* (*V. rosea*, *Vinca minor*) and *Vinca major* (big-leaf or variegated) caused by the fungus *Phoma exigua*, Scott and Broadhurst (2011) reported rapidly expanding, dark brown to black, girdling lesions on stems at the soil line which resulted in dieback. Similar symptoms of root rot by *Rhizoctonia solani* were reported by various workers while working on root rot of *Pinus wallichiana*, *Abies pindrow* and Urd bean (Rana and Tripathi, 1983; Ahangar *et al.*, 2011; Dar *et al.*, 2013).

Table 5. Per cent Disease Incidence of root rot of *Cedrus deodara* by three frequently isolated fungi under *in vitro* conditions

Fungi	Disease Incidence (%)
<i>Fusarium oxysporum</i>	3.55
Binucleate <i>Rhizoctonia</i>	47.53
<i>Phoma exigua</i>	0.00
CD0.05	1.21

Fig.5. Graphical representation of Per cent Disease Incidence of root rot of *Cedrus deodara* by three frequently isolated fungi under *in vitro* conditions





Fusarium oxysporum



Binucleate *Rhizoctonia* AG-E



Phoma exigua

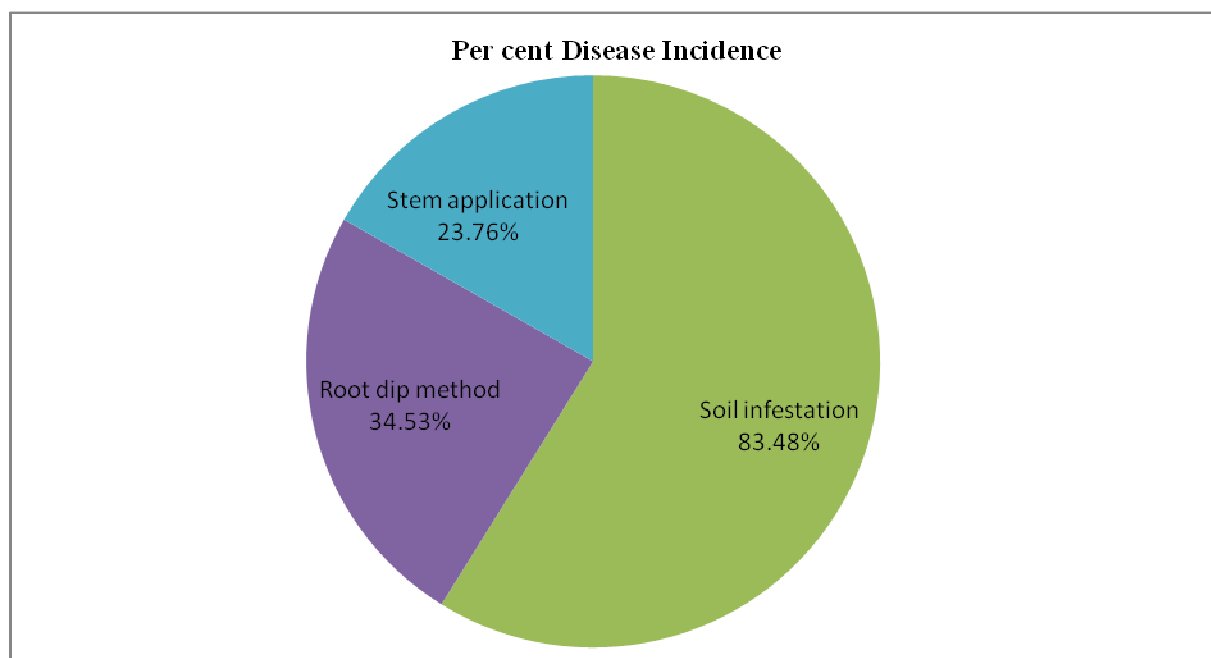
Plate 7. Symptoms produced by three frequently isolated pathogens

Pathogenicity of binucleate *Rhizoctonia* was proven by three different methods to test their efficacy in producing root rot symptoms. The data presented in Table 6 (Fig.6) clearly depicted that all the inoculation methods were found to be effective in producing root rot symptoms. However, the diseases incidence was maximum (83.48%) in soil infestation inoculation method followed by root dip (34.53%) and stem application method (23.76%) respectively. Soil infestation method resulted in pre- damping off symptoms. The typical symptoms were observed as rotting of emerging seedlings in soil and development of necrotic lesions at collar region of the seedlings. These symptoms lead to death of seedlings due to collapse of such seedlings. The pathogen was reisolated from the infected seedlings and the culture obtained was found to be similar with the original culture in all respects. The soil infestation method resulted in higher disease incidence due to proper establishment of culture in soil which lead to the vigorous symptom production and death of seedlings within 15 days of inoculation (Plate 8). In case of root dip and stem application method symptoms appeared after 28 and 33 days of inoculation, respectively and delayed the death and collapse of seedlings. The control plants showed no sign of root rot at all as no pathogen was inoculated in the soil. The pathogen was reisolated from the infected seedlings and the culture obtained was found to be similar with the original culture in all respects. The results are in agreement with studies of Jiskani *et al.* (2007) who confirmed *R. solani* as the predominant damping off causing fungus while conducting an experiment to prove its pathogenicity on tomato through soil infestation method. These findings are also supported by the observations made by Gordon *et al.*, 2005 who reported the superiority of the soil infestation method over root dip inoculation method while working with a soil borne fungal pathogens.

Table 6. Performance of inoculation methods in proving pathogenicity of binucleate *Rhizoctonia* AG-E

Method of inoculation	Per cent Disease Incidence (%)
Soil infestation	83.48
Root dip method	34.53
Stem application	23.76
Control	0.00
CD 0.05	5.68

Fig.6. Graphical representation of Performance of inoculation methods in proving pathogenicity of binucleate *Rhizoctonia* AG-E



4.8 DISEASE MANAGEMENT

4.8.1 *In vitro* evaluation of biological control agents against the binucleate *Rhizoctonia* AG-E

Antagonistic activities of the native fungal and bacterial microorganisms were determined against root rot pathogen (binucleate *Rhizoctonia* AG-E) so that the most effective antagonists could be evaluated under pot conditions for the management of the disease. The data presented in Table 7 (Fig.7) depicted that all the microbial antagonists evaluated under *in vitro* conditions, inhibited the growth of the root rot pathogen (Plate 9). Out of four species of fungal antagonists *Trichoderma viride* was found to be most effective and showed significant superiority amongst all the antagonists tested that resulted in 75.02 per cent inhibition of the mycelial growth of pathogen followed by *T. harzianum* with 70.41 per cent inhibition. Out of two bacterial antagonists, *Bacillus subtilis* was found better than *Pseudomonas fluorescens* which inhibited the mycelial growth up to 66.11 per cent as compared to *P. fluorescens* with 40.75 per cent mycelial inhibition. Dubey and Patel (2001) evaluated three isolates of *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* against *Rhizoctonia solani* and found the superiority of *Trichoderma viride* over other two biological control agents. Similarly, Dutta and Kalha (2011) evaluated twelve isolates of residential bio agents viz., four isolates each of *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Rhizoctonia solani* and found that *Trichoderma viride*



Diseased seedlings



Healthy seedlings

Plate 8. Pathogenicity of binucleate *Rhizoctonia* AG-E Causing root rot of *cedrus deodara*



Trichoderma viride



Trichoderma harzianum



Trichoderma virens



Trichoderma hamatum



Aspergillus sp.



Bacillus subtilis



Pseudomonas fluorescences



Control

Plate 9. Mycelial growth inhibition of binucleate *Rhizoctonia* AG-E by biocontrol agents

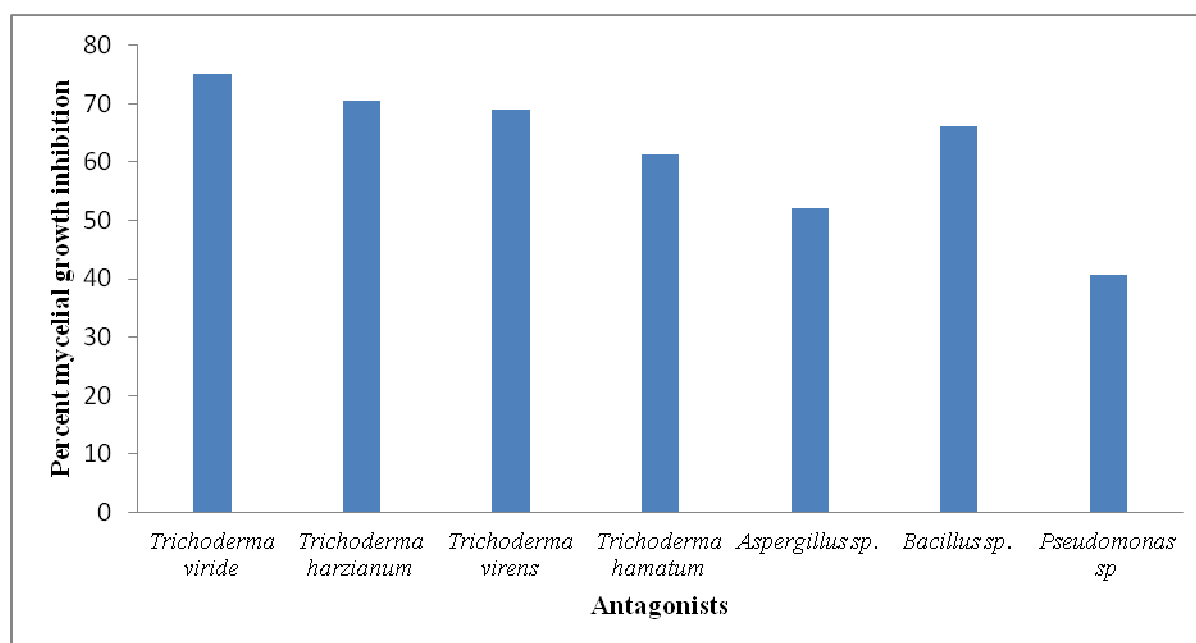
was most effective under *in vitro* conditions while *Pseudomonas fluorescens* was least effective in mycelial inhibition. The present results are also in consonance with the work done by various workers (Obiegilo, 1992; Kreb *et al.*, 1993; Utkhede, 1993; Moon *et al.*, 1995; Pandey and Upadhyay, 2000; Sharma, 2001; Ramesh and Korikanthimath, 2001; Khan and Khan, 2002; Sharma and Tripathi, 2002; Hatvani *et al.*, 2006; Sharma and Kumar, 2009; Agrawal and Kotasthana, 2010; Seema and Devaki, 2012; Pandey and Pundhir, 2013; Chandel and Sharma, 2014; Rajendra prasad *et al.*, 2017) who reported the effectiveness of *Trichoderma viride* and *Trichoderma harzianum* isolates in inhibiting mycelial growth of *Rhizoctonia* sp. under *in vitro* conditions.

Table 7. *In vitro* Mycelial growth inhibition of binucleate *Rhizoctonia* AG-E by biocontrol agents

Antagonists	Per cent inhibition in mycelial growth
<i>Trichoderma viride</i>	75.02 (59.99)a
<i>Trichoderma harzianum</i>	70.41 (57.02)b
<i>Trichoderma virens</i>	69.06 (56.17)b
<i>Trichoderma hamatum</i>	61.22 (51.46)c
<i>Aspergillus</i> sp.	52.00 (46.12) d
<i>Bacillus</i> sp.	66.11 (54.37)e
<i>Pseudomonas</i> sp.	40.75 (39.64)f
CD(0.05)	0.92

*Figures in parentheses are angular transformed value

Fig .7 Graphical representation of per cent inhibition in mycelial growth of binucleate *Rhizoctonia* AG-E by biocontrol agents



4.8.2 Chemical method

In vitro screening of fungicides against binucleate *Rhizoctonia* AG-E

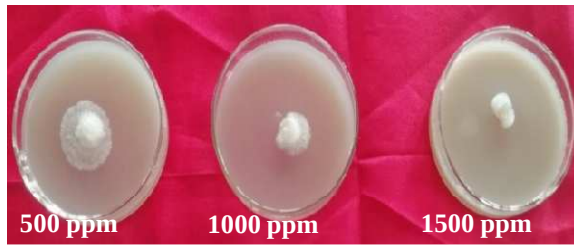
The efficacy of different fungicides viz., pyraclostrobin + metiram, copper oxychloride, captan, mancozeb, tebuconazole + trifloxystrobin, chlorothalonil75WP, metalayxl, thiophonate methyl 70 WP, carbendazim 50 WP and hexaconazole5EC was evaluated by Poisoned Food Technique (Falck, 1907) against binucleate *Rhizoctonia* AG-E. The data presented in Table 8 (Fig.8, Plate 10) depicted that all the fungicides significantly inhibited the mycelial growth of the root rot pathogen in comparison to control. Tebuconazole + trifloxystrobin was found most effective and showed significant superiority among all the chemical tested that resulted in 95.18 per cent inhibition of the root rot pathogen followed by Carbendazim 50WP (92.88 %) and hexaconazole 5EC (85.47 %). While metalayxl (29.80%) and copper oxychloride (26.50%) wereleast effective in mycelial inhibition. It was also noticed that as the concentrations of the fungicide increased, there was corresponding increase in per cent mycelial inhibition of the pathogen. At lowest concentration of 500ppm, trifloxystrobin + tebuconazole was found to be effective with 87.52 per cent mycelia inhibition which was significantly superior over other fungicides tested at this concentration.

Table 8. *In vitro* percent mycelial inhibition of binucleate *Rhizoctonia* AG-E by different fungicides

Treatments	Per cent inhibition in mycelial growth at concentrations (%)			
	500ppm	1000ppm	1500ppm	Mean
Pyraclostrobin + Metiram	63.37 (52.73)	81.24 (27.48)	86.28 (60.71)	76.96 (46.97)a
Copper oxychloride	21.31 (60.17)	26.31 (69.30)	31.88 (51.47)	26.50 (60.31)b
Captan	76.10 (29.59)	82.57 (64.31)	84.05 (30.84)	80.90 (41.58)c
Mancozeb	75.29 (65.33)	81.88 (64.79)	87.04 (74.63)	81.40 (68.25)c
Tebuconazole +trifloxystrobin	87.52 (62.24)	100.00 (90.01)	100.00 (90.01)	95.18 (80.75)d
Chlorothalonil75WP	61.23 (34.36)	72.33 (66.45)	77.31 (68.90)	70.29 (56.57)e
Metalayxl	24.41 (85.34)	27.39 (61.53)	37.60 (37.80)	29.80 (61.56)f
Mean	58.46 (55.11)	66.38 (63.40)	71.89 (59.19)	
CD_{0.05} Fungicides = 0.65, Concentrations =0.42 , Fungicides X Concentrations = 1.13				

Treatment	25ppm	50ppm	100ppm	Mean
Thiophonate methyl 70WP	37.10 (37.51)	62.77 (52.38)	86.46 (67.90)	62.11 (52.59)a
Carbendazim 50WP	87.05 (68.90)	93.38 (75.94)	100.00 (90.01)	92.88 (78.28) b
Hexaconazole 5EC	82.39 (64.73)	83.72 (66.18)	90.31 (71.38)	85.47 (67.43) c
Mean	68.85 (57.05)	78.96 (63.50)	89.66 (70.97)	
CD_{0.05} Fungicides = 0.59, Concentrations =0.61 , Fungicides X Concentrations = 1.03				

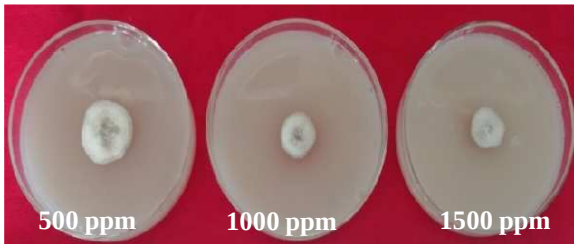
Figures in parentheses are angular transformed values



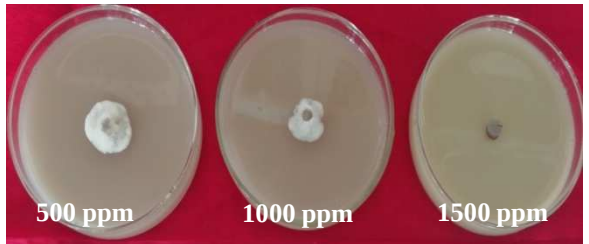
Pyraclostrobin + Metiram



Copper oxychloride



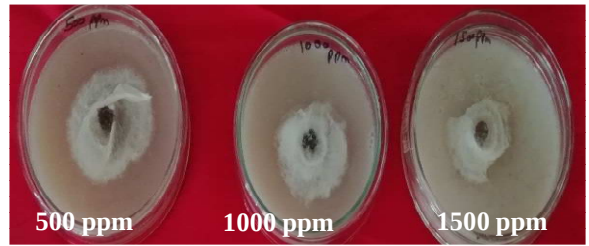
Captan



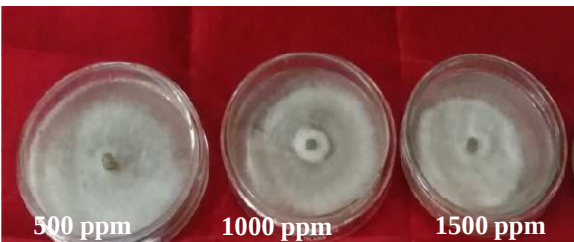
Mancozeb



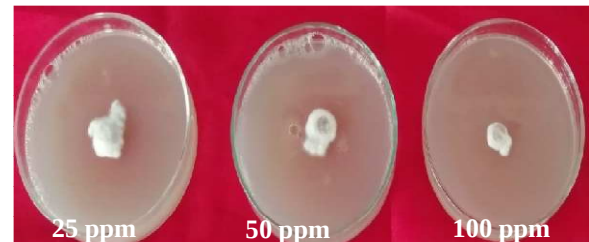
Tebuconazole + Trifloxystrobin



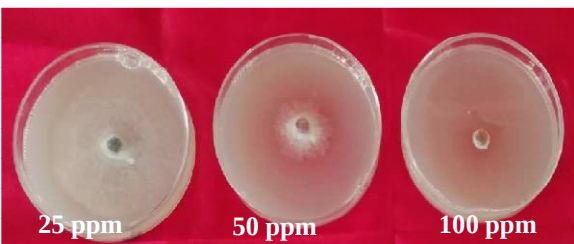
Chlorothalonil 75WP



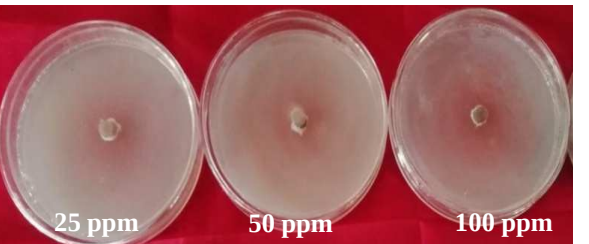
Metalyxl



Hexaconazole 5EC



Thiophonate methyl 70WP



Carbendazim 50WP

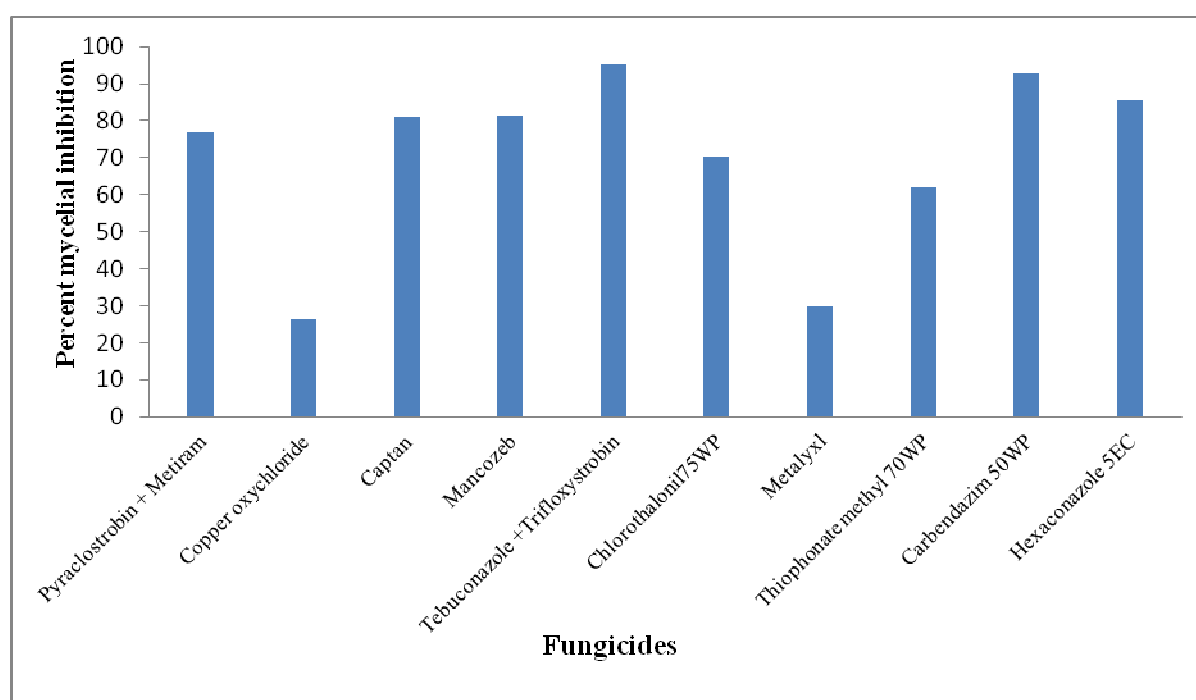


Control

Plate 10. *In vitro* per cent mycelial inhibition of binucleate *Rhizoctonia* AG-E by different fungicides

The results are in conformity with Hunjan *et al.* (2011) and Bag (2009) who evaluated the efficacy of some new fungicides, among these fungicides. trifloxystrobin + tebuconazole showed higher level of efficacy against *R. solani* under *in vitro* conditions. Similarly, Sudhakar *et al.* (2005) found tebuconazole, Carbendazim and hexaconazole most effective against *R. solani* under *in vitro* condition while mancozeb was found to be least effective in mycelial inhibition. The present results are in consonance with Rajendra prasad *et al.*, 2017 who found that tebuconazole + trifloxystrobin and carbendazim were superior over other fungicides. Meena *et al.*, 2018 reported that Tebuconazole 50% + Trifloxystrobin 25% WG was most effective fungicide *in vitro* as compared to carbendazim 12% + mancozeb 63% WP against *R.solani*. In the same fashion, Balaganur (2016) found carbendazim and combi product of tebuconazole 50% + trifloxystrobin 25% WG effective at all the concentrations tested against *R. solani* under *in vitro* conditions.

Fig .8 Graphical representation of *In vitro* percent mycelial inhibition of binucleate *Rhizoctonia* AG-E by different fungicides



4. 8.3 Integrated disease management

Effect of integration of bio-control agents and chemicals on disease incidence of root rot of *Cedrus deodara*

An experiment was carried out in Complete Block Design during 2018 to assess the effect of integration of bio-control agents and chemicals on incidence of root rot of *Cedrus*

deodara. It is evident from the data presented in Table 9 (Fig.9, Plate 11) that all the treatments significantly reduced the root rot incidence over control. Integration of *Trichoderma viride* with tebuconazole + trifloxystrobin gave maximum reduction in disease incidence of root rot with 11.05 per cent disease incidence followed by *Trichoderma harzianum* + tebuconazole + trifloxystrobin (12.55%) and *Bacillus subtilis* + tebuconazole + trifloxystrobin (14.55%) respectively. Individual application of tebuconazole + trifloxystrobin showed 16.85 percent disease incidence which was found to be significant superior over carbendazim (25.5%) and hexaconazole (60.94%) respectively. The disease incidence was found to be significantly more after 45 days (48.20%) as compared to 25 days (38.89%) of sowing. The data revealed that combination of fungicides with biocontrol was effective in reducing root rot in *Cedrus deodara* seedlings as compared to individual biocontrol and fungicides. Suneeta *et al.*, 2017 reported that combination of tebuconazole + trifloxystrobin with *Trichoderma viride* was best treatment module in reducing collar rot incidence and increasing growth and yield parameters of *Gerbera*. This was followed by tebuconazole which was significantly effective against collar rot disease of *Gerbera*. Similarly, Dwivedi and Prasad (2016) reported that the combination of tebuconazole and *Trichoderma viride* (local strain) was effective against *Sclerotium rolfsii* causing collar rot of tomato. Krishnamoorthy *et al.*, 2017 reported that Combined application of *Trichoderma viride* and tebuconazole + trifloxystrobin was found to be highly effective against *R.solani* causing root rot with the lowest disease incidence of 8.67 per cent indicating 78.55 per cent reduction over control followed by tebuconazole + trifloxystrobin alone with disease incidence of 8.74 per cent and 78.37 per cent reduction over control. Kumar *et al.* (2011) reported that carbendazim alone could reduce the root rot disease of *Jatropha* caused by *R. bataticola* up to 36.2 per cent but its combination with *T. harzianum* was found to be more effective with 54 per cent reduction in disease incidence. Vyas (1994) reported the effective control of *R. bataticola* in soybean using combination of *Trichoderma viride* or *Trichoderma harzianum* and carbendazim. Similarly, *Trichoderma harzianum* was found to control soil-borne plant pathogens (*Rhizoctonia* sp. and *Fusarium* sp.) when introduced in combination with carbendazim (Mehta *et al.*, 1993). *Trichoderma viride* in combination with carbendazim (2 g kg⁻¹ seeds) reduced postemergence mortality of blackgram caused by *R. bataticola* compared with individual treatments (Suriachandraselvan and Seethuraman, 2002). Sriraj *et al.* (2014) reported that the basal soil application of mahua cake and *Trichoderma viride* in the ratio of 1:2 (1g of mahua cake mixed with 2g *Trichoderma viride* per kg pot soil)



Seed rot



Pre emergence damping off and seed rot



Rotting of seedlings and no Secondary root formation



Post emergence damping off



Healthy seedling

Plate 11. Symptoms of root rot of *Cedrus deodara* caused by binucleate *Rhizoctonia* AG-E

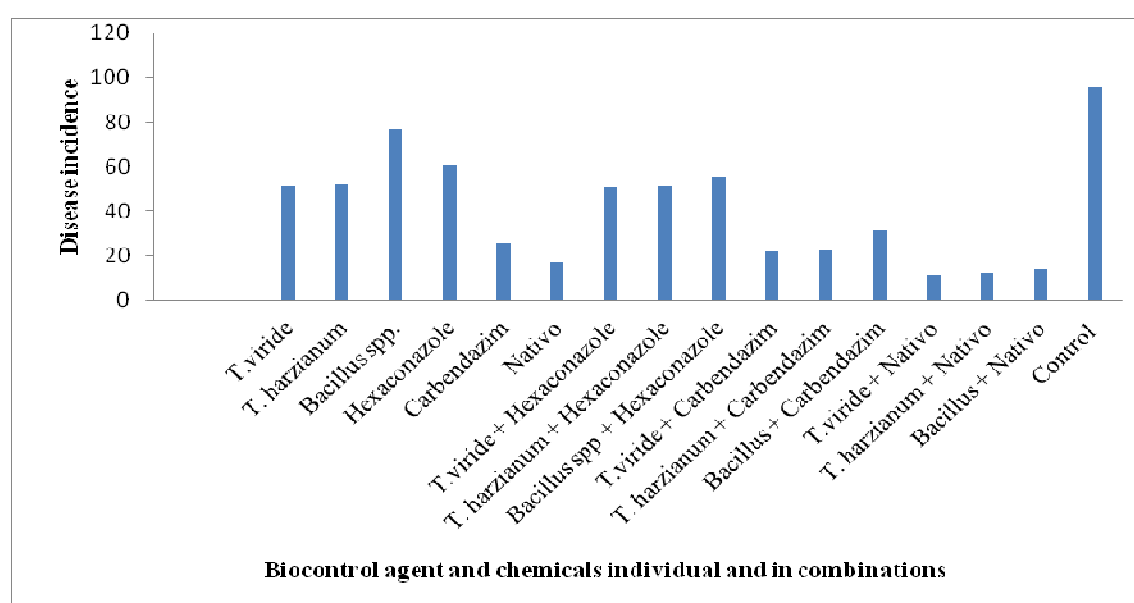
followed by foliar spray of tebuconazole + trifloxystrobin @ 0.5% significantly reduced the percent disease incidence of *R.solani* causing leaf blight of turmeric over other treatments.

Table. 9 Integration management of root rot of *Cedrus deodara* through biocontrol agents and chemicals

Treatments	Disease incidence (%)		Mean
	25 Days	45 Days	
<i>T. viride</i>	44.44 (41.79)	58.50 (49.87)	51.49 (45.84)a
<i>T. harzianum</i>	44.44 (41.79)	59.50 (50.45)	51.97(46.12)a
<i>Bacillus</i> sp.	66.73 (54.75)	86.73 (68.61)	76.73 (61.68)b
Hexaconazole	55.66 (48.23)	66.22 (54.4)	60.94 (51.33)c
Carbendazim	22.48 (28.29)	28.54 (32.28)	25.51 (30.28)d
Tebuconazole +Trifloxystrobin	11.11 (19.46)	22.59 (28.36)	16.85(23.91)e
<i>T.viride</i> + hexaconazole	45.55(42.43)	55.55(48.16)	50.55(45.29)f
<i>T.harzianum</i> + hexaconazole	46.55 (43.01)	56.55 (48.74)	51.55 (45.87)a
<i>Bacillus</i> sp. + hexaconazole	48.55 (44.15)	61.55 (51.69)	55.05 (47.90)g
<i>T.viride</i> + carbendazim	15.55 (23.20)	28.55 (32.49)	22.05 (27.79)h
<i>T. harzianum</i> + carbendazim	11.11 (19.45)	33.73 (35.49)	22.42 (27.47)h
<i>Bacillus</i> sp. + carbendazim	18.55 (25.49)	44.26 (41.68)	31.40 (33.59)i
<i>T.viride</i> + tebuconazole + trifloxystrobin	5.55 (13.58)	16.55 (23.75)	11.05 (18.78)j
<i>T. harzianum</i> + tebuconazole + trifloxystrobin	7.55 (15.92)	17.55 (24.22)	12.55 (20.33) k
<i>Bacillus</i> sp. + tebuconazole + trifloxystrobin	9.55 (17.97)	19.55 (26.22)	14.55 (22.10) l
Control	88.88 (70.50)	100.00(90.00)	95.43 (80.10)m
Mean	33.89 (34.37)	48.20 (45.11)	
C.D _{0.05} Treatments = 0.81, Days = 0.28, Treatments X Days = 1.15			

Figures in parentheses are angular transformed values

Fig.9 Graphical representation of integrated management of root rot of *Cedrus deodara* through biocontrol agents and chemicals



Chapter-5

SUMMARY AND CONCLUSION

Cedrus deodara (Roxb.) G. Don. also known as Himalayan Cedar is a high mountain tree. It grows at elevations between 1,700 m and 3,000 m msl in the western part of its range and between 1,300 m and 3,300 m in the eastern part, where the climate is less dry. Himalayan Cedar is a very important timber tree in North West India, Kashmir and Pakistan. The total area of deodar forests in India is estimated to be around 203,263 hectares. Out of this, 69,872 hectares lie in Himachal Pradesh (Singh and Lakhanpal, 2000). The root rot fungi which pose serious threat to forest nurseries include the species of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Macrophomina*. Root rot of *Cedrus deodara* is a soil borne disease and such diseases are difficult to control due to various factors working in tandem in the soil ecosystem. Therefore, the present investigations were undertaken with the objectives to record the incidence of root rot of *Cedrus deodara* in forest nurseries of district Solan, to isolate and identify the pathogen causing root rot and to devise a disease management strategy for its effective management in nurseries.

A field survey was conducted in different *Cedrus* growing areas of district Solan in Himachal Pradesh. Incidence of root rot was found in all the locations surveyed. Maximum incidence of the root rot was recorded Kandaghat (37.35%) followed by Solan range (33.85%) and Chail range (30.93%). Amongst nurseries surveyed, Bisha nursery had maximum incidence (62.15%) followed by Nauni (48.46%) and Chiunth nurseries (44.65). The overall mean of different years suggested that disease was 5.59 per cent more in the year 2018 than the previous year (2017). Bisha, Nauni and Chiunth nurseries were worst affected with root rot disease. Over all, the incidence of disease ranged between 10.90 to 62.15 per cent in different locations that was detrimental for the production of healthy *Cedrus deodara* nurseries.

The symptoms of root rot in *Cedrus* seedlings varied from seed rot, hypocotyl rot and collar rot to root rot. The one month old seedlings showed collar rot and root rot. The roots turned black, thin and with little or no secondary root growth. In some nurseries the seeds failed to germinate due to complete seed rot. Some of the germinated seeds showed hypocotyls and radical rotting with brownish patches on the surface. On the seedlings

symptoms were yellowing of the needles with poor growth and rapid root decay. Healthy seedlings had dark green needles and sturdy growth. The infected seedlings showed characteristic damping off with soft brown rotted tissue at collar portion.

The fungus associated with root rot pathogen was isolated in pure culture and was identified as *Rhizoctonia solani* on the basis of morphological characters i.e. hyphal diameter, no spore formation, transverse septation, thin, spreading, off-white to light brown mycelium, colony type, growth rate of pathogen and sclerotia formation. The mycelium branched near the septum of the hyphal mother cells at right acute angles and the branches were constricted at or near the origin of septum. While small sclerotia formed within 25 to 45 days possessing brown to dark brown colour with rough surfaces. Morphological characters were identified under compound microscope and under phase contrast microscope. The cultures were sent to National Centre of fungal taxonomy, New Delhi for the confirmation of the identity.

To ensure the identity of the fungus, molecular sequencing was outsourced from Eurofin Genomics, Bengaluru. The ITS region was amplified by using ITS1 and ITS4 primers and sequenced (White *et al.*, 1990) the sequence of a representative isolate was deposited in GenBank (Accession No. MK226512.1). Comparison of the ITS sequences obtained showed 99.22, 99.19 and 99.18% similarity with other sequences of binucleate *Rhizoctonia* viz., EU591790.1, AY586174.1 and AY586173.1, respectively which were identified as binucleate *Rhizoctonia* AG-E = *Ceratobasidium* sp. CAG 6

To ascertain the level of frequency of various pathogens associated with root rot and wilt of *Cedrus deodara*, samples were collected from diseased nurseries of Solan district in Himachal Pradesh. In each sample 15 seedlings from each nursery showing any type of disease symptom (necrosis, wilting, drooping, damping off etc.) were randomly collected along with their nursery bags. The crown and root portion cultured on PDA media and incubated in BOD at 25°C. Different pathogens like *Phytophthora* sp., *Pythium* sp., *Aspergillus* sp., *Mucor* sp., *Yeast*, *Bacillus* sp., *Pseudomonas* sp., *Trichoderma* sp., *Rhizoctonia* sp., *Fusarium* sp., *Penicillium* sp., *Phoma* sp. were isolated from the diseased portions. But the isolation frequency of binucleate *Rhizoctonia* AG-E., *Fusarium oxysporum*, *Phoma exigua* recorded highest as compared to other pathogens. The pathogenicity test of these three pathogens revealed that only binucleate *Rhizoctonia* produced root rot symptoms similar to the ones encountered during surveys. *Fusarium oxysporum* produced slight wilting but no root rot while *Phoma exigua* exhibited no root rot at all. Therefore, binucleate

Rhizoctonia AG-E was used in further experiments. The pathogenicity of this pathogen was proved by three different methods viz., root dip, soil infestation and stem application method. Out of the three methods, soil infestation method produced maximum disease incidence (83.48 %) therefore, this method was used to infest the soil with the inoculum of binucleate *Rhizoctonia* in the further experiments.

Different biocontrol agents were tested *in vitro* for evaluating their efficacy against root rot pathogen binucleate *Rhizoctonia* AG –E. Among the fungal antagonists *Trichoderma viride* was found most effective and showed significant superiority among all the antagonists tested that resulted in 75.02 per cent inhibition of the root rot pathogen followed by *Trichoderma harzianum* with 70.41 per cent inhibition. Out of two bacterial antagonists, *Bacillus subtilis* was found better than *Pseudomonas fluorescens* which inhibited the mycelial growth up to 66.11 per cent while and *P. fluorescens* were found least effective with 40.75 per cent inhibition.

Amongst ten fungicides evaluated *in vitro* against binucleate *Rhizoctonia* AG –E, tebuconazole + trifloxystrobin was found most effective and showed significant superiority among all the chemicals tested that resulted in 95.18 per cent inhibition of the pathogen followed by carbendazim 50WP (92.88 %) and hexaconazole 5EC (85.47 %). While metalyxl (29.80%) and copper oxychloride (26.50%) were least effective in inhibiting the mycelial growth of the test fungus. It was evident from the studies that with the increase in concentrations of the fungicide, there was a corresponding increase in per cent mycelial inhibition of the pathogen.

The Three best performing antagonists and chemicals under *in vitro* studies were used singly and in different combinations for testing their efficacy against root rot of *Cedrus deodara* under *in vivo* conditions. *Trichoderma viride*, *Trichoderma harzianum* and *Bacillus subtilis* were used as bioagents while hexaconazole, carbendazim and tebuconazole + trifloxystrobin were used as chemicals individually as well as in different possible combinations. Integration of *Trichoderma viride* with tebuconazole + trifloxystrobin gave maximum reduction in disease incidence (11.05%) of root rot followed by combination of *Trichoderma harzianum* with tebuconazole + trifloxystrobin with 12.55 per cent and *Bacillus subtilis* + tebuconazole + trifloxystrobin with 14.55 per cent disease incidence of root rot, respectively. It was evident from the data that individual application of *Trichoderma*

viride and *Trichoderma harzianum* showed 51.49 per cent and 51.97 per cent incidence as compared to *Bacillus subtilis* (76.73%). In case of chemicals, individual application of tebuconazole + trifloxystrobin recorded 16.85 per cent disease incidence followed by carbendazim (25.51%) which were found to be effective in root rot management. Integration of select BCA's with chemicals gave a satisfactory control of the disease as compared to control.

The root rot of *Cedrus deodara* in district Solan nurseries was found to be caused by binucleate *Rhizoctonia* AG-E. The disease was prevalent in all the nurseries surveyed. Out of the isolated BCA, *Trichoderma viride* and *Trichoderma harzianum* significantly arrested the mycelial development *in vitro*. Amongst the chemicals tested *in vitro* trifloxystrobin + tebuconazole and carbendazim gave maximum per cent mycelial inhibition. Out of the combination of three best chemicals and BCA'S tested in nursery, combination of *T.viride* + tebuconazole + trifloxystrobin and *T.harzianum* + tebuconazole + trifloxystrobin were effective in managing root rot of *Cedrus deodara*.

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APPENDIX – I

Culture media used for growing micro-organisms

1) Potato Dextrose agar medium

Pealed potato	:	250 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

2) Nutrient agar medium

Beef extract	:	3 g
Peptone	:	5 g
Agar	:	15 g
Distilled water	:	1000 ml

APPENDIX- II

ANOVA TABLES

ANOVA 1: Isolation frequency (%) of main pathogens associated with root rot of *Cedrus deodara*

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Nurseries	6	16,318.98	2,719.83	75.16	0.00
Pathogen	11	48,807.14	4,437.01	122.61	0.00
Nurseries X Pathogen	66	33,343.44	505.20	13.96	0.00
Error	168	6,079.45	36.18		
Total	251	104,549.02			

ANOVA 2: Diametric growth of binucleate *Rhizoctonia* at different time intervals

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Treatment	5	1.01	0.20	124.63	0.00
Error	18	0.02	0.01		
Total	23	1.03			

ANOVA 3: Mycelial growth inhibition of binucleated *Rhizoctonia* by biocontrol agents

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Treatment	6	1,197.75	199.62	508.70	0.00
Error	21	8.24	0.39		
Total	27	1,205.99			

Anova 4: *In vitro* percent mycelial inhibition of binucleated *Rhizoctonia* by non-systemic different fungicides

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Fungicides	6	4,434.48	739.08	1,580.12	0.00
Concentration	2	15.83	7.91	16.92	0.00
Fungicides X Concentration	12	12,591.57	1,049.29	2,243.35	0.00
Error	42	19.64	0.46		
Total	62	17,061.53			

Anova 5: *In vitro* percent mycelial inhibition of binucleated *Rhizoctonia* by systemic different fungicides

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Fungicides	2	2,146.93	1,073.46	1,161.77	0.00
Concentration	2	4,189.60	2,094.80	2,267.12	0.00
Fungicides X Concentration	4	1,476.43	369.10	399.47	0.00
Error	54	49.89	0.92		
Total	62	7,862.87			

Anova 6. Integrated Management of root rot of *Cedrus deodara* through chemicals and biocontrol agents

Source of Variation	DF	Sum of Squares	Mean Squares	F-CAL	F-TAB
Treatments	15	28,642.89	1,909.52	4,018.23	0.00
Days	1	2,508.12	2,508.12	5,277.86	0.00
Treatments X Days	15	636.43	42.42	89.28	0.00
Error	64	30.41	0.47		
Total	95	31,817.87			

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ABSTRACT

The present investigation entitled “**Studies on Seedling Root Rot of *Cedrus deodara* (Roxb.) G. Don**” was conducted in the laboratories and nursery of the Department of Plant Pathology. During the survey of the nurseries of district Solan, 10.90 to 62.15 per cent incidence of root rot was recorded. A field survey was conducted in different *Cedrus* growing areas of district Solan in Himachal Pradesh. Maximum incidence of the root rot was recorded Kandaghat (37.35%) followed by Solan range (33.85%) and Chail range (30.93%), respectively. Amongst nurseries surveyed, Bisha nursery had maximum incidence (62.15%) followed by Nauni (48.46%) and Chiunth nurseries (44.65), respectively. The pathogen was molecularly identified as binucleate *Rhizoctonia* AG-E. Among the fungal antagonists *Trichoderma viride* was found most effective and showed significant superiority among all the antagonists tested that resulted in 75.02 per cent mycelial inhibition *in vitro* of the root rot pathogen followed by *T. harzianum* with 70.41 per cent inhibition. Out of two bacterial antagonists *Bacillus subtilis* was found better than *Pseudomonas fluorescens* which inhibited the mycelial growth up to 66.11 per cent while and *P. fluorescens* were found least effective with 40.75 per cent inhibition. Amongst ten fungicides evaluated *in vitro* against binucleate *Rhizoctonia* AG –E, tebuconazole + trifloxystrobin was found most effective and showed significant superiority among all the chemicals tested that resulted in 95.18 per cent inhibition of the pathogen followed by carbendazim 50WP (92.88%) and hexaconazole5EC (85.47%), respectively. The Three best performing antagonists and chemicals under *in vitro* studies were used for testing their efficacy against root rot of *Cedrus deodara* under *in vivo* conditions. *T. viride*, *T. harzianum* and *B. subtilis* were used as bioagents while hexaconazole, carbendazim and tebuconazole + trifloxystrobin were used as chemicals individually as well as in different possible combinations. Integration of *Trichoderma viride* with tebuconazole + trifloxystrobin gave maximum reduction in disease incidence (11.05%) of root rot followed by combination of *T. harzianum* with tebuconazole + trifloxystrobin with 12.55 per cent and *Bacillus subtilis* + tebuconazole + trifloxystrobin with 14.55 disease incidence of root rot, respectively. Integration of select BCA's with chemicals gave a satisfactory control of the disease as compared to control.

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