STUDIES ON DISEASES OF NAGA KING CHILLI (*Capsicum chinense* Jacq.)

Thesis

submitted to

NAGALAND UNIVERSITY

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of

Doctor of Philosophy

in

Plant Pathology

by

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DECLARATION

I, Pezangulie Chakruno, hereby declare that, the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

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CERTIFICATE – I

This is to certify that, the thesis entitled "***Studies on diseases of Naga King Chilli (Capsicum chinense* Jacq.)"** submitted to Nagaland University in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy (Agriculture) in Plant Pathology is the record of research work carried out by Mr. Pezangulie Chakruno Registration No. 595/2014 under my personal supervision and guidance.

The result of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

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This is to certify that the thesis entitled **"Studies on diseases of Naga King Chilli (*Capsicum chinense* Jacq.)"** submitted by Pezangulie Chakruno Admission No. Ph-153/13 Registration No. 595/2014 to the NAGALAND UNIVERSITY in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Plant Pathology has been examined by the Advisory Board and External examiner on 03/04/2018.

The performance of the student has been found **Satisfactory/Unsatisfactory.**

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ACRONYMS & ABBREVATION

°C	-	Degree celcius
%	-	Percentage
µ	-	Micron
CRD	-	Complete Randomised Design
CD	-	Critical difference
DAI	-	Days after inoculation
et al.	-	and others
etc.	-	etcetera
F	-	Field
Fig	-	Figure
g	-	Gram
ICAR	-	Indian Council of Agricultural Research
i.e.	-	That is
Max	-	Maximum
MSL	-	Mean Sea Level
ml	-	Millilitre
mm	-	millimeter
Min	-	Minimum
NA	-	Nutrient Agar
No.	-	Number
PDI	-	Per cent disease Index
PI	-	Percent Inhibition
PDA	-	Potato Dextrose Agar
pH	-	Puissance de Hydrogen
psi	-	Pounds per square inch
SASRD	-	School of Agricultural Science and Rural Development
spp.	-	Species
SEm	-	Standard Error Mean
T	-	Treatment
Viz.	-	Namely

STUDIES ON DISEASES OF NAGA KING CHILLI (*Capsicum chinense* Jacq.)

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ABSTRACT

Capsicum chinense Jacq. locally known as Naga king chilli is one of the important spices of the Nagaland as well pride for the state. The Naga King Chilli does not grow well in all areas and like any other cultivated crops, it does suffer from plant pathogenic diseases. Keeping in mind the above context, disease survey was carried out during the kharif season of 2014 and 2015 in three Naga King Chilli growing districts of Nagaland *viz.* Dimapur, Kohima and Peren. During this investigation seven plant pathogenic diseases were recorded *viz.* Anthracnose (*Colletotrichum capsici*), Cercospora leaf spot (*Cercospora capsici*), damping-off (*Rhizoctonia solani*), Fusarium wilt (*Fusarium oxysporum*), stem rot (*Sclerotium rolfsii*), bacterial wilt (*Ralstonia solanacearum*) and leaf veinal mottle (*Chilli veinal mottle virus*). Dimapur district was recorded with highest disease incidence of anthracnose (44.39%), Cercospora leaf spot (9.97%), damping-off (7.08%), Fusarium wilt (3.53%), stem rot (2.11%) and bacterial wilt (9.17%). While Peren district was recorded with highest disease incidence for leaf veinal mottle (65.82%). Among seven bio-control agents, *Trichoderma harzianum* was recorded with highest mycelial growth inhibition against *Colletotrichum capsici* (79.61%), *T. koningii* and *T. viride* against *Fusarium oxysporum* (100%), *T. harzianum* against *Sclerotium rolfsii* (85.07%) and *T. koningii*, *T. viride*, *T. harzianum* and *T. virens* were recorded with highest mycelial growth inhibition against *Rhizoctonia solani* (92.20%).

Keywords: Naga king chilli, disease incidence, bio-control

CHAPTER I

INTRODUCTION

INTRODUCTION

Capsicum chinense Jacq. locally known as Raja Mircha or Naga King Chilli is one of the important crops of Nagaland as well as pride for the state. It is native to Nagaland and parts of North-Eastern states. In Nagaland, Naga King Chilli was cultivated in an area of 1,000ha with the total production of 6,000 metric tons (Statistical Handbook of Nagaland, 2014). It is a self-pollinated species belonging to the genus *Capsicum* and family Solanaceae. The pod is used as the edible part; it is commonly used in curry and chutney preparation. Naga King Chilli is used as an everyday food item by Nagas. The product is highly prized and accordingly, consumption is also the highest in Naga society. It is used in many forms; fresh, dried, powdered and in pickled form.

C. chinense is a very pungent chilli measuring 10,01,304 Scoville Heat Units (SHU). This chilli pepper is called by various names such as Naga Jolokia, Naga Morich, Raja Mircha, Bhut Jolokia, Bih Jolokia etc. Whatever the names by which it is called, they seem to be only different cultivars of the same chilli. Besides Nagaland, Naga King Chilli is also found to be cultivated in Assam, Manipur, and other North Eastern part of the country. In Nagaland, it is traditionally cultivated in hilly jhum fields along with rice and in backyard kitchen garden. Generally, it is sown in the month of February and March; one to two weeks old seedlings are transplanted in the main field or direct seeding is also practiced in case of jhum paddy fields and it is harvested usually in the month of August/September. In Nagaland, it has been reported to be cultivated in the foot hills of Dimapur, Mon, Wokha, Peren and Mokokchung (Bhagowati & Changkija, 2009). Nagaland is believed to be the original home of this chilli pepper and Nagaland Government has obtained the GI rights for this crop in

2008 (Bhagowati & Changkija, 2009). The Nagaland government had in 1999 passed the Nagaland Geographical Indication of Goods (Registration and Protection) Act, to provide some safety net to Naga farmers in the cultivation of the King Chilli. In recent years, the Naga King Chilli is gaining importance in the scientific community due to its extra-ordinary pungency level; oleoresin powder which is extracted from Naga King Chilli is predicted to dominate the world market in coming years as the mainstay for riot control (Ritesh *et al*., 2000).

Capsicum species suffers from several numbers of viral, fungal, bacterial, nematode and phytoplasma diseases. *Colletotrichum capsici* was reported to cause anthracnose, die-back and fruit rot of chilli (*Capsicum annum* L.) from various parts of Odhisa (Singh *et al*., 2013). Anthracnose and powdery mildew disease of chilli were estimated to yield losses upto 59.47% (Hingole & Kurundkar, 2011). Among bacteria *Ralstonia solanacearum* is reported to cause wilt in chilli (Kumar *et al*., 2013) and viruses are also known to cause different symptoms like leaf curling, mosaic, ring spot, yellowing etc., on chilli and these pathogens result in heavy losses. The chilli leaf curl virus (ChiLCV) disease on chilli was first reported in Pakistan by Shih *et al.* (2003). Earlier some workers have conducted studies on viral diseases on chilli considering their destructive nature, the extent of yield losses due to leaf curl complex ranged from 25 to 80 per cent (Gowda, 1979; Bidari, 1982; Ilyas & Khan, 1996).

The Naga King Chilli, despite its reputation, is actually a very sensitive and vulnerable crop; it does not grow well in all areas and like any other cultivated crops it does suffer from several diseases. *Colletotrichum gloeosporioides* and *Rhizoctonia solani* have been reported to cause diseases on Naga King Chilli (Ngullie *et al.*, 2010; Ngullie & Daiho, 2013). Five fungal

diseases *viz.* anthracnose or fruit rot caused by *Colletotrichum capsici*, die-back caused by *Colletotrichum gloeosporioides*, stem rot and wilt caused by *Sclerotinia sclerotiorum*, collar rot caused by *Rhizoctonia solani* and leaf spot caused by *Corynespora cassiicola* were identified and the only bacterial disease, bacterial wilt caused by *Ralstonia solanacearum* was observed in chilli variety Bhut Jolokia (*Capsicum chinense* Jacq.) in Assam districts (Talukdar *et al*., 2012). Though Nagaland is native to this chilli species, extensive study on occurrence of various diseases in the Naga King Chilli growing areas has not been carried out. This necessitated a detail investigation of various diseases causing damage to *Capsicum chinense* Jacq. in Nagaland. Keeping in mind the above context, this research programme was carried out with the following objectives.

1. Survey of Naga King Chilli diseases in selected districts of Nagaland.
2. Isolation and identification of disease causing agents.
3. Testing effectiveness of bio-control agents *in vitro* against important pathogens of Naga King Chilli.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Literatures relevant to the different aspects of the proposed investigation entitled "Studies on diseases of Naga King Chilli (*Capsicum chinense* Jacq.)" have been reviewed in this chapter under the following heads.

2.1 Anthracnose

2.1.1 Causal organism

The word anthracnose is a Greek word meaning 'coal'. It is commonly used for plant diseases which are characterized by dark sunken lesions having spores (Isaac, 1992). Anthracnose disease is one of major constraints that restrict profitable production of chilli. It is caused by *Colletotrichum* species. Genus *Glomerella* belongs to Kingdom-Fungi, Phylum- Ascomycota, Class-Sordariomycetes, Order- Phyllochorales and Family- Phyllochoraceae (Sahitya *et al.,* 2014). *Colletotrichum* is a large genus of Ascomycete fungi, containing species that cause anthracnose disease on wide range crops of economic value (Sahitya *et al.,* 2014). Several *Colletotrichum* species have been reported as causal agents of chilli anthracnose disease worldwide (Than *et al*., 2008).

2.1.2 Survey

Ekbote (2002) conducted a survey of the prevalent diseases of chilli (*Capsicum annuum*) in 6 taluks (Byadagi, Hirekerur, Haveri, Ranebennur, Savanur and Shiggo) in the Haveri district of Karnataka, India from 1998-2000. Fruit rot caused by *Colletotrichum capsici* was the most prevalent disease (36.4%) of chilli, followed by the Murda complex (34.56%), powdery mildew caused by *Leveillula taurica* (17.54%) and leaf spot caused by *Cercospora capsici* (12.11%).

Talukdar *et al*. (2012) carried out field surveys in three Bhut jolokia growing districts of Assam (Sivasagar, Jorhat and Golaghat). The results revealed more fungal diseases (2.75%) than the bacterial wilt disease (0.67%) in the farmer's field. Highest fungal diseases were recorded from Jorhat districts with an average disease incidence of 4.25%, while Golaghat district was free from fungal diseases. However, highest bacterial wilt disease was recorded from Golaghat districts (1.5%) with no disease incidence recorded from Jorhat district. Five fungal diseases, anthracnose or fruit rot caused by *Colletotrichum capsici*, die-back caused by *Colletotrichum gloeosporoides*, stem rot and wilt caused by *Sclerotinia sclerotiorum*, collar rot caused by *Rhizoctonia solani*, and leaf spot caused by *Corynespora cassicola* were identified.

Bediako *et al.* (2015) made a disease survey to assess the incidence and severity of viral and fungal diseases infecting pepper in some major producing areas in Ghana and to identify farmers' agronomic practices that influence disease incidence and severity. It was reported that there were high incidences (up to 86.3%) and severities (11.8-32.1%) of pepper mosaic disease, leaf anthracnose, anthracnose fruit rot, phytophthora blight and cercospora leaf spot in all the fields surveyed.

Onyeani and Amusa **(**2015**)** carried out a systematic field survey of mango fruit anthracnose in four locations (Agege, Ayetoro, Ibadan and Ogbomosho) in Southwest Nigeria. The results showed that 60% of mango trees surveyed were found to be infected with anthracnose and over 34% of fruits produced on those trees were severely infected. They concluded that anthracnose disease, especially at the postharvest stage, is a threat to production and marketing of fresh mango fruits in South West Nigeria.

Darge *et al.* (2016) conducted field surveys in North West Ethiopia, Pawi district during 2011 and 2012 cropping seasons to determine the distribution of anthracnose and the association of disease parameters (incidence and severity) with climatic variables and crop management practices. Nineteen mango fields were surveyed and all were infected by anthracnose. The fields were surveyed at two growth stages, flowering (2011) and fruiting (2012). It was recorded that no statistically significant difference was observed for the incidence of anthracnose between the two seasons with mean disease incidence of 65.7% in 2011 and 66.5% in 2012. However, severity of anthracnose was significantly different in 2012 cropping season (81.2%) than in the 2011 cropping season (59.8%).

Nasehi *et al.* (2016) reported that anthracnose fruit rot has emerged as a significant threat to pepper (*Capsicum annuum*) production in the Cameron Highlands, Pahang State, Malaysia. The disease incidence was up to 60% in severely infected pepper glasshouses.

Prasad (2016) conducted a survey to assess the per cent disease incidence of anthracnose of chilli in five locations in Bulileka area of Fiji. The percentage incidence of anthracnose affected fruits under field conditions were more in green fruits which ranged from 65.5% to 78.5%. Therefore, the percent disease index (PDI) revealed that the predominant presence of anthracnose is a major constraint to profitable cultivation of chilli in Bulileka area, Fiji.

2.1.3 Symptomatology

Anthracnose symptoms consist of necrotic spots on the leaves, twigs and branches on mango trees. Dark brown to black lesions coalesce forming large patches on the infected leaves later that lead to apical and marginal scorching. (Ismail *et al*., 2015). Typical anthracnose symptoms on chilli fruits

include sunken necrotic tissues, with concentric rings of acervuli, often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions (Than *et al*., 2008; Oo & Oh, 2016). In die-back phase, the disease causes necrosis of tender twigs from the tip backwards. The entire branch or the entire top of the plant may wither away. Dead twigs become grayish white to straw coloured in advanced stages of the disease; a large number of black dots (acervuli) are found scattered all over the necrotic surface of the affected twig (Singh, 2005).

2.1.4 Morphology

Sangdee *et al.* (2011) made comparison of ten isolates of *Colletotrichum capsici* causing chilli anthracnose collected from 10 provinces in the northeast of Thailand. The colony diameter of different groups ranged from 65 to 80 mm after 7 days incubation. Group CC-I to CC-V produced zigzag cottony colonies whereas the isolate in group CCVI possessed circular colonies. The conidial shape of the different groups was fusiform with both their ends pointed. Average length and width of conidia varied between 23.5 to 35.0 μm and 2.5 to 3.75 μm, respectively. Similarly, Ghosh *et al.* (2016) made morphological comparison of *Colletotrichum* isolate (CI) associated with leaf spot disease of soybean with 2 isolates of *Colletotrichum capsici* (CII and CIII) and one isolate of *Colletotrichum gloeosporoides* (CIV) from chilli. On the basis of morphological and cultural characteristics the pathogen was identified as *C. capsici*. The CI, CII and CIII isolates showed dense white to greyish brown mycelia with ring like zonations in solid oat meal medium. *C. gloeosporoides* (CIV) produced pinkish white colony with cylindrical conidia. The conidia of *Colletotrichum* isolate from soybean and chilli (CI, CII and CIII) was falcate, fusiform with acute apices. The size ranges from 19.65–21.00 μm in length and 3.0–3.5 μm in breadth. In *C. gloeosporiodes* (CIV)

conidia were hyaline, one celled, cylindrical. The size ranged from 8–12 μm in length and 4–6 μm in width.

2.2 Cercospora leaf spot

2.2.1 Causal organism

The fungus *Cercospora capsici* was first isolated from bell pepper by Heald and Wolf (1911). Infection, sporulation and spore germination of *Cercospora capsici*, causal agent of leaf spot on *Capsicum annuum* L. was investigated by Padmavathi *et al*. (1993).

Meon (1990) reported that *Cercospora capsici* was found to be consistently associated with leaf spot of chilli, reducing appreciably the photosynthetic activity of infected plants leading to losses in yield. Mallappa (2007) isolated *Cercospora nicotianae* Ell. and Eve. from tobacco by tissue isolation technique and proved pathogenicity.

Silva *et al.* (2016) made first report of Cercospora leaf spot Caused by *Cercospora* cf. *alchemillicola* in *Toona ciliata* in Brazil.

2.2.2 Survey

Rathore (2006) reported the maximum disease incidence (35%) and low yield in Cercospora leaf spot infected greengram fields in Rajastan during 2006 and 2008 (Rathore, 2009).

Survey for prevalence of frog eye leaf spot disease was conducted in seven bell pepper growing locations of Kashmir *viz*. Bugham, Gangbugh, Kanihama, Dal-area, Noorbagh, Shalimar and Shalteng at fruit set and fruit harvest during 2003. The overall incidence and intensity of the disease at fruit set stage ranged from 20.06 to 37.20 per cent and 7.46 to 18.33 per cent,

respectively. During fruit harvest stage, the incidence and intensity of the disease ranged from 54.63 to 68.92 per cent and 32.46 to 44.03 per cent, respectively. Disease incidence and intensity was recorded significantly higher at Noorbagh and minimum at Kanihama locations (Farooq *et al.*, 2008).

Five different varieties of winter mungbean viz., Binamoog 1, Mut 2a(5)B, MC38, MC89, MB63 were used to determine incidence and disease severities. The incidence and severity of Cercospora leaf spot recorded 45 days after sprouting was found to be consistently increasing with the increasing maturity of the crop growth in field and varied among the strains (Hossain *et al.*, 2011).

Swamy *et al*. (2012) reported the incidence of Cercospora leaf spot caused by *Cercospora capsici* on chilli (*Capsicum annuum*) in Koppal, Raichur, Gulbarga and Bellary districts of Karnataka during 2009-10. The mean per cent disease index for this period was 35.9. The average disease severity in Koppal, Raichur, Gulbarga and Bellary districts reached 23.2, 40.9, 37.0 and 42.5%, respectively. Sowing on 1st July resulted in the lowest disease incidence (15.5%), whereas sowing on 15th August resulted in the highest disease incidence (33.9%).

2.2.3 Symptomatology

Munjal *et al.* (1960) described the symptoms of *Cercospora canescens* on *Vigna radiata* as fungus produced spots on leaves, which were first brown, later turning grey or dirty grey with narrow reddish brown margins bearing fructification on both the surfaces.

The typical disease symptoms of frog eye leaf spot of pepper caused by *Cercospora capsici* were observed on leaves, stem and peduncles. On leaves,

the spots appeared as necrotic, circular to sub-circular with greyish white centre, surrounded by brown to greyish brown area and marginated by definite darker zone. The spots enlarged up to a mean diameter of 9.8 mm, coalesced frequently and led to defoliation with or without yellowing. The spots become raised and resembled frog eye. The lesions on stem, peduncle and petioles, however, were longer rather than round (Bhat *et al*., 2008).

Patel *et al.* (2001) described the symptoms of frog eye leaf spot of tobacco caused by *Cercospora nicotianae*. The spots were brown with ash grey centre, often the centre may turn white and dry up. The typical spot had a white centre, surrounded by black margin resembling the eye of frog; several spots may coalesce towards the leaf tip and margin causing the leaf to dry up from the margin.

2.2.4 Morphology

Conidia and conidiophores of *Cercospora* were 4-5 X 70 -250 µm and 4.5 X 30-60 µm (Heald & Wolf, 1911). Vasudeva (1963) described the morphological characters of *Cercospora capsici* as conidiophores in clusters, tufted, amphigenous, brown, 10-15 facsciculae, septate, 30-60 X 4.5-5.5 µm. Conidia straight, clavate, dilute brown in colour and septate.

The mycelium of *Cercospora capsici* was irregularly branched, septate, light brown, 3.9 to 4.8 µm in diameter. Conidiophores were light brown, un-branched, continuous, 1 to 5 septate, straight to sub-straight and borne on stromata in fascicles of 7 to 13. Conidia were acicular, continuous, 1 to 13 septate, hyaline and borne solitary on conidiophores. The conidia and conidiophore measured 3.5 - 5.2 X 25 - 86 µm and 3.5 - 5.5 X 30-75 µm respectively (Bhat *et al.*, 2008).

2.3 Damping-off

2.3.1 Causal organism

Gayed *et al.* (1978) reported *Rhizoctonia solani* and *Pythium ultimum* to cause damping-off on flue-cured tobacco (*Nicotiana tabacum* L.) in Ontario. Post emergence damping-off was differentiated into: a) seedling rot initiated early where infection starts on the young leaves in touch with the organic soil, b) typical damping-off resulting from the infection of the stem of erect seedlings either directly from the soil or indirectly from already infected leaves.

Polizzi *et al*. (2011) first reported damping-off caused by *Rhizoctonia solani* AG-4 on Pink Ipê (*Tabebuia impetiginosa*) in Italy. The seedlings were being watered with overhead irrigation. More than 5% of the seedlings showed disease symptoms. Initial symptoms were black lesions at the seedling crown that expanded rapidly to girdle the stem. On infected seedlings, leaves turned black and gradually died. Black extended stem lesions were followed by death of the entire seedling in a few days. A fungus with mycelial and morphological characteristics of *Rhizoctonia solani* Kühn was consistently isolated from crown and stem lesions when plated on potato dextrose agar (PDA) amended with streptomycin sulfate at 100 μg/ml.

2.3.2 Survey

Jiskani *et al.* (2007) conducted survey of tomato fields of Hyderabad district to estimate the incidence of damping-off disease. Maximum disease incidence was recorded at village Darya Khan Nahiyoun (65.0%) followed by Khatian Station (60.0%) and the minimum were at Khesano Mori (35.0%). *Rhizoctonia solani* Kuhn was isolated as the predominant damping-off fungus with highest frequency (60.0%) from the overall tomato fields followed by

Fusarium oxysporum f. sp. *lycopersici, Macrophomina phaseolina, Alternaria solani* and *Verticillium albo-atrum*.

Koike *et al.* (2013) made a survey of diseased fields in the central coast to determine which pathogens are involved in damping-off and root rot pathogens of spinach in California. *Pythium* was the soil organism most commonly isolated from diseased spinach roots. *Fusarium* was recovered with the second highest incidence and was associated with larger spinach plants that had dark brown to black taproot tips. *Rhizoctonia* was found third most frequently and was also linked to darkened taproot tips.

Rao *et al*. (2016) conducted survey to observe the disease incidence and severity on the major vegetables like tomato and chilli, cultivated in sub zoba Hamelmalo of Eritrea state during two different seasons i.e. autumn and winter. The crops were reported to be affected by different diseases such as early blight, late blight, powdery mildew, wilt, blossom end rot and leaf curl in tomato and damping-off, leaf curl, bacterial leaf spot in chilies.

2.3.3 Symptomatology

Pre-emergence damping-off occurs when fungi infect developing radicals and kill seedlings while shoot tissues are still below ground (Filer & Peterson, 1975). Post-emergence damping-off occurs when fungi infect the succulent tissue of germinants with aboveground shoots, causing decay, wilting, and mortality (Boyce, 1961). Seedlings infected with *Rhizoctonia solani* have reddish brown to black lesions on the stem and roots. Stems are often girdled or become water-soaked and soft, causing the plant to fall over. Infections of small seedlings are often difficult to distinguish from Pythium rot without laboratory confirmation (Olsen, 1988). Damping-off is also a disease of germinating and newly emerged conifer and hardwood seedlings that causes decay of succulent tissue, wilting, and seedling mortality. Many species of

pathogenic fungi can cause damping-off. Some of the factors influencing damping-off include pathogen populations, host susceptibility, and soil temperature, moisture, and pH. The severity of damping-off can vary from field to field and year to year depending on these factors (Cram, 2003).

2.3.4 Morphology

Goswami *et al* (2010) described multiple isolates of *Rhizoctonia solani* collected from soil of different agro-ecological zones of Bangladesh and also from infected plant parts of different crops and grasses. Growth rate (cm/24 hrs.) ranging from 2.84-3.50, zonation of colonies (No.) 0.89-3.67, width of hyphae (μm) 5.83-7.38, duration for sclerotia initiation (days) 2.96-14.00 and number of sclerotia /cm^2 ranging from 3.53-79.00.

Eighteen isolates of *Rhizoctonia solani* were collected from infected rice plants of four different locations of Bangladesh, these isolates were studied by their morphological characters and by using molecular markers. It was reported that all isolates produced superficial sclerotia, some of which produced dark brown runner hyphae, exuded droplet on sclerotial surface. The quality, size, shape and distribution of sclerotia within the colonies showed high variablity. Such as some of the isolates produced sclerotia near the inoculum (GA3), peripheral region (CO2), scattered isolate (GO3), near margin (GO5), near inoculums scattered (TA5) and also abundant sclerotia (TA3) (Moni *et al.*, 2016).

2.4 Fusarium wilt

2.4.1 Causal organism

Mushtaq and Hashmi (1997) reported *Fusarium oxysporum, F. moniliforme , F. solani, F. anthophlium, Alternaria alternate, Rhizoctonia solani, Cephalosprium acremonium, Pythium aphanidermatum* and

Macrophomina phaseolina to be found predominantly in *Capsicum annum* plants showing symptoms of wilting in Mirour Khas District, Sindh, Pakistan. Similarly, Wani *et al.* (2014) also reported that fusarium wilt disease caused by *Fusarium solani* was recorded from nursery during transplantation and was found maximum during flowering/fruiting stage.

White Dendrobium (*Dendrobium candidum* Wall. ex Lindl.) a traditional Chinese medicinal herb that is used raw or processed for health care products in China was reported to suffer from wilt disease caused by *Fusarium oxysporum* (Xiao *et al.,* 2012). Bastidas *et al.* (2014) first reported *Fusarium oxysporum* f. sp. *cubense* tropical race 4 associated with panama disease of banana outside Southeast Asia.

2.4.2 Survey

Manikandan and Raguchander (2014) made a survey on the incidence and severity of Fusarium wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* in ten districts of Tamil Nadu namely Dindugal, Karur, Tirupur, Coimbatore, Erode, Namakkal, Salem, Dharmapuri, Krishnagiri and Vellore districts at different growth stages in 2012. The result of the survey revealed that wide range of infection and severity of wilt disease occurred in the major tomato growing areas. The occurrence of wilt disease incidence ranged from 19 % to 45%.

Pepper wilt disease intensity was assessed on-farm in two districts *viz*. Bako Tibbe and Nonno districts of West Shewa Zone, Ethiopia during the main cropping season of October 2012. It revealed that the overall per cent prevalence and incidence of wilt disease was 96.7 and 86.4%, respectively. Identification and pathogenicity tests revealed that *Ralstonia solanacearum* and four fungal wilt pathogens (*Rhizoctonia solani*, *Fusarium* spp.,

Phytophthora spp. and *Verticillium* spp.) were detected in the surveyed fields. The percentage of occurrence of *Rhizoctonia solani*, *Fusarium* spp., *Phytophthora* spp. and *Verticillium* spp. were 45.0, 17.48, 12.59 and 11.89%, respectively; whereas, the frequency of *R. solanacearum* was 100% (Assefa *et al.* 2015).

Kutama *et al.* (2016) conducted a survey of Fusarium wilt of Garden Egg (*Solanum melongena)* at Imawa village of Kura local government area, Nigeria. It resulted in isolation of 4 isolates of *Fusarium oxysporum* with 50% abundance followed by *Rhizopus stolonifer* and *Aspergillus niger* each with 2 isolates conforming to 25% abundance each.

Rao *et al.* (2016) made a survey to observe the disease incidence and severity on the major vegetables like tomato and chilli, cultivated in sub zoba Hamelmalo during two different seasons i.e. autumn and winter. The crops were affected by different diseases such as early blight, late blight, powdery mildew, wilt, blossom end rot and leaf curl in tomato and damping-off, leaf curl, bacterial leaf spot in chillies. In tomato and chillies, the percentage of disease incidence showed more than 77% in all villages during winter season but the severity was observed between 20 and 60%.

2.4.3 Symptomatology

Fusarium wilt symptoms on lettuce depicted vascular tissues of affected seedlings appearing red or brown. Affected plants appeared stunted and developed yellow leaves and brown or black streaks in the vascular system. The vascular streaks in the yellow leaves extended from the crown and were continuous with a red-brown discoloration in the vascular system of the crown and upper taproot. Symptoms were typically not visible on the outside of the crowns or roots (Garibaldi *et al.*, 2002).The symptoms of Fusarium wilt

included leaf chlorosis, vascular discoloration, and wilting of chilli plants. High temperature and high moisture were conducive to symptom development of wilt (Joshi *et al.*, 2012). Affected leaves of chilli become yellow so visit the field daily to find and uproot these plants. Other disease symptoms include leaf necrosis, wilting and internal brown discoloration of the stem which help to distinguish it from other causes of yellowing, such as nematodes and viruses. The leaves, stem and fruit are all affected by the disease (Shahzad, 2014).

2.4.4 Morphology

Morphological characters of *Fusarium oxysporum* includes three to five septate sickle-shaped macroconidia, with a foot-shaped basal cell, ellipsoid microconidia borne in false heads on short monophialides, and chlamydospores in culture. A typical cream-coloured colony developed on PDA, with purple pigmentation on the reverse side (Booth, 1971). Mycelia of the *Fusarium* isolates were delicate, white to creamy and pink or purple tinge, margins slightly lobed or smooth on PDA. Microconidia formed singly, oval to reniform and without any septation. The size of microconidia ranged from 7.50 - 16.25 and 2.50 - 4.50 µm. Macroconidia were falcate to almost straight, usually 3-septate, rarely four to 5-septate, thin walled, both ends almost pointed, notched basal cell, apical cell short and in some cases slightly curved. Macroconidia were produced in sporodochia as well as on normal hyphae and slimy conidial masses were also observed on water soaked wheat bran. The size of the macroconidia ranged from 20.27 - 40.50 µm and 5.00 - 6.75µm (Hussain *et al.*, 2012).

2.5 Stem rot

2.5.1 Causal organism

The fungus *Sclerotium rolfsii* Sacc., is a soilborne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions

of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops (Aycock, 1966; Farr *et al.,* 1990). Root rot of chilli caused by *S. rolfsii* was first time reported from Rajasthan near Jaipur chilli growing areas, where the severe mortality of chilli plants during March-April was observed (Kalmesh & Gurjar, 2001). Deepthi and Reddy (2013) reported *Sclerotium rolfsii* an incitant of stem rot disease of groundnut (*Arachis hypogaea* L.).

2.5.2 Survey

Singh *et al.* (1987) proposed that, the disease incidence varied with soil moisture and soil temperature, while a maximum of 72 per cent disease incidence was recorded at high relative humidity and moderate temperature in case of collar rot of pigeon pea incited by *Sclerotium rolfsii.*

Muthukumar and Venkatesh (2013) conducted roving survey during 2011-2012 around Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni districts to assess the incidence of collar rot disease of peppermint caused by *Sclerotium rolfsii*. It was reported that maximum incidence of 32.33% was recorded in Therkupalayam village of Coimbatore district. The level of pathogenicity of these isolates revealed that *S. rolfsii* (I1) from Therkupalayam in Coimbatore district was the most virulent and caused higher levels of collar rot incidence (93.66%). An inoculum load of *S. rolfsii* at 5% to 1 kg of soil registered the maximum incidence of 92.66% collar rot of peppermint.

Rani *et al.* (2016) conducted a survey in major groundnut growing areas of Andhra Pradesh during kharif 2012 and in Telangana during rabi 2012-13 respectively to assess the distribution and the incidence of collar rot caused by *Sclerotium rolfsii* and stem rot caused by *Aspergillus niger*. Groundnut cultivar

Kadiri-6 (K-6) was the prominent cultivar in all the districts surveyed. The highest incidences of stem rot and collar rot were observed in Chittoor district of Andhra Pradesh. Whereas, lowest incidences of stem rot and collar rot were observed in Mahaboobnagar and Warangal districts respectively.

2.5.3 Symptomatology

Wilson (1953) described the symptoms of stem rot as, mycelium covering the plant stem near the soil surface and produced organic acids, which were toxic to living plant tissue. This followed the necrosis of plant cells. The mycelium invaded the stem, gynophores and also pods causing rotting of the tissues. The production of abundant white mycelium, and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease. Mehrotra and Aneja (1990) noticed the cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debris. The mycelial mat may extend several centimetres up to the stem above the soil line. Kalmesh and Gurjar (2001) described the symptoms of root rot of chilli caused by *Sclerotium rolfsii*. A severe mortality of chilli plants was observed during March-April in chilli growing area. Mature plants of chilli from standing crop collapsed and dried down suddenly. Close examination of the diseased plants showed deep cracks near collar region. Roots were shredded and unhealthy with white mycelial growth on the surface of freshly infected area.

2.5.4 Morphology

Sclerotium rolfsii Sacc. isolated from groundnut was identified based on mycological characters. The fungal mycelium was first silky white in color later turned to dull white with radial spreading giving fan like appearance. Microscopic examination of *Sclerotium rolfsi* revealed aerial hyaline, thin walled, septate hyphae with profusely branched mycelium showing clamp

connections. When fungus attained maturity small mycelial knots were formed which later turned to mustard seed like sclerotia which were deep brown or brownish black, shiny, hard and spherical to irregular in shape (Kumar *et al.*, 2014). In *Sclerotium rolfsii* Sacc. on PDA medium, white mycelial threads along with small, uniformly sized, globoid sclerotia in larger number were observed. Sclerotia measured 1–3 mm in diameter and they were initially white and turned dark brown at maturation (Mahadevakumar *et al.*, 2016).

2.6 Bacterial wilt

2.6.1 Causal organism

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi, is responsible for significant reduction in production of chilli (Aslam *et al*., 2015). Bacterial wilt incited by *Ralstonia solanacearum* is ubiquitous in distribution and is considered as a serious constraint to the cultivation of solanaceous crops in tropical, sub tropical and temperate regions of the world (Zubeda & Hamid, 2011; Begum *et al*., 2012; Shahbaz *et al*., 2015). Seleim *et al.* (2014) made an attempt to isolate and identify the causal pathogen of tomato bacterial wilt in Egypt. The isolates were confirmed through morphological and cultural characteristics as *Ralstonia solanacearum* biovar 2 race 1.

2.6.2 Survey

Aslam *et al*. (2015) conducted to study the incidence and prevalence of bacterial wilt caused by *Ralstonia solanacearum* (Smith) on chilli in five major chilli growing districts of Punjab, Pakistan namely Multan, Kasur, Pakpattan, Bahawalpur and Attock. The study revealed an overall 11% incidence and 85% prevalence of bacterial wilt in Punjab with the highest disease incidence found in Pakpattan district (16%) followed by Kasur (12%) and Attock (12%).

Disease incidence was found to be minimum in Multan and Bahawalpur districts (9% each).

Pepper wilt disease intensity was assessed on-farm in two districts *viz*. Bako Tibbe and Nonno of West Shewa Zone, Ethiopia during the main cropping season of October, 2012. It was recorded that the incidence of wilt disease was 86.4%. Identification and pathogenicity tests revealed that *Ralstonia solanacearum* and four fungal wilt pathogens (*Rhizoctonia solani, Fusarium* spp., *Phytophthora* spp. and *Verticillium* spp.) were associated. The percentage of occurrence of *Rhizoctonia solani, Fusarium* spp., *Phytophthora* spp. and *Verticillium* spp. were 45.0, 17.48, 12.59 and 11.89%, respectively; whereas, the frequency of *R. solanacearum* was 100% (Assefa *et al.*, 2015).

Singh *et al.* (2010) conducted a survey to study the status of bacterial wilt of solanaceous crops (potato, tomato, brinjal, chilli and capsicum) caused by *Ralstonia solanacearum* in Northern and Eastern states of India such as Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Jharkhand and West Bengal. Bacterial wilt disease incidence in tomato and chilli was quite low 1 to 3 percent during summer season, whereas in rainy season, it was 4 to 60 percent in tomato and 3 to 40 per cent in brinjal. Disease incidence in tomato crop was more as compared to other solanaceous crops like brinjal, chilli, capsicum, and potato. Eighty four isolations of *R. solanacearum* were done from all the five states and 65 isolates were used for biovar /race characterization and molecular detection. Out of these 65 isolates, 58 were categorized as biovar 3 and remaining 7 biovar 4. The biovar 3 and 4, causing wilt in solanaceous crops belong to race 1.

2.6.3 Symptomatology

Diseased chilli plants can be found scattered in the field. Bacterial wilt usually occurs in foci associated with water accumulation in lower areas. The initial symptom in mature plants under natural conditions is similar to that observed in tomato or potato. Wilting of leaves, sometimes only few branches of the plant, occurs during hot days followed by recovery throughout the evening and early hours of the morning. The wilted leaves maintain their green color and do not fall as disease progresses. Under favorable conditions complete wilt will occur. The vascular tissues in the lower stem of wilted plants show a dark brown discoloration. These symptoms are very similar to those of Phytophthora blight, induced by *Phytophthora capsici*. However, an extensive external darkening of the lower stem is observed mostly for Phytophthora blight (Momol *et al.*, 2001).

The initial symptom is wilting of terminal leaves, followed by a sudden and permanent wilt. Additional symptoms are vascular browning, water soaking of pith followed by browning and browning of cortex near the soil line during the later stages of infection. Bacterial streaming occurs when a freshly cut stem is suspended in water. The pathogen can survive for long periods of time in a nutrient-depleted environment (Sarkar & Chaudhuri, 2016).

2.6.4 Biochemical test

Dhital *et al.* (2001) characterized seven bacterial strains of potato wilt caused by *Ralstonia Solanacearum* isolated from Nepal and six from Thailand by using the following tests: oxidation/fermentation, starch hydrolysis, indole production and nitrate (NO_3) reduction. Additionally, the tests such as oxygen relation, levan production, urease test, gelatin liquefaction, tween 80 hydrolysis, catalase production, sodium chloride (5 and 7%) tolerance, oxidase test and growth on potato slice were also performed. Furthermore, some tests

were made on arginine dihydrolase, motility, citrate utilization and ammonia production.

Rahman *et al*. (2010) performed Gram's staining and potassium hydroxide solubility test and confirm that all groups of *Ralstonia solanacearum* isolates were Gram negative. The isolates of *R. solanacearum* fermented four basic sugars (dextrose, sucrose, manitol and lactose). These results of all biochemical tests in combination with the pathogenicity test confirmed the isolates were *R. solanacearum* causing bacterial wilt of brinjal. All groups of *R. solanacearum* isolates were found virulent producing pink or light red color or characteristic red center and whitish margin on TZC medium after 24 hours of incubation.

2.7 Chilli veinal mottle

2.7.1 Causal organism

Pepper veinal mottle virus (PVMV), a potyvirus, has been reported to be a major constraint to pepper production, contributing to low yield, reduced fruit quality, and economic loss (Fajinmi & Odebode, 2010). *Chilli veinal mottle virus* (ChiVMV), a potyvirus, is widespread over the world. In China, it was first reported in chili pepper (*Capsicum annuum*) in Hainan Province (south China) in 2006. Subsequently, it was reported in tobacco (*Nicotiana tabacum*) in Yunnan Province (southwest China) in 2011 (Zhao *et al*., 2014).

Zhang *et al.* (2016) also made First Report of *Pepper veinal mottle virus* infecting pepper in mainland China. Banerjee *et al.* (2014) confirmed the occurrence of *Chilli veinal mottle virus* (ChiVMV) under the genus *Potyvirus* in Naga chilli (*Capsicum chinense*) in Meghalaya based on mechanical transmission assay, transmission electron microscopy, RT-PCR

and sequence analysis. This is the first record of Chivmv in Naga chilli in North-East India.

Fajinmi *et al.* (2011) reported that the ability to transmit PVMV to a healthy pepper plant varied between aphid species. *Myzus persicae* had 73.33% transmission ability, *Aphis gossypii*, 80%; *A. craccivora*, 42.22%; *A. spaericola*, 57.77%; and *A. fabae*, 46.66%. Similarly, Alegbejo (1987) reported that these aphids survive on different hosts during dry and wet seasons and that these plants are important refuges and sources of aphids that damage pepper and transmit PVMV. ChiVMV is easily transmitted in the field by many aphid species in a non persistent manner (Ong *et al*., 1979). More than half of the pepper viruses are transmitted by aphids, some are transmitted by nematodes, thrips, leafhopper, beetle and fungi or by contact and through the soil (Green & Kim, 1991). These viral symptoms are caused as a result of aphids feeding on infected pepper plant and transmitting the virus to an uninfected plant as it bites the leaves (Achiangia *et al.*, 2013).

2.7.2 Survey

Achiangia *et al.* (2013) conducted a survey to ascertain the prevalence and field infection rate of *Pepper veinal mottle virus* (PVMV) and *Pepper mottle virus* (PepMoV) on cultivated pepper in three divisions of the Western Highlands of Cameroon in July 2010. The prevalence of PVMV and PepMoV was 100% and 50% respectively.

The incidence, severity and occurrence of four viruses infecting pepper were determined in four locations of Kwara State, Nigeria. The survey indicated the highest virus incidence (97%) in four locations and the lowest incidence (16%) in three locations, with variations in severity scores. The ELISA result indicated the occurrence of all four viruses with the highest

percentage occurrence of virus in the samples as follows: *Pepper Veinal Mottle Virus* (36.3%), *Blackeye Cowpea Mosaic Virus* (16.2%), *Cowpea Aphid borne Mosaic Virus* (7.4%), and *Cucumber mosaic virus* in the locations (4.8%). (Aliyu, 2014)

Olawale *et al.* (2015) made a survey to determine the incidence, diversity and distribution of viruses infecting pepper (*Capsicum* spp.) in six states of South-west Nigeria in 2010 and 2011. The average disease incidence was 79% in 2010 and 76% in 2011; the average disease severity score was 2.9 in both years. Eight viruses in the leaf samples were reported *viz. Potato virus Y* (PVY), *Potato virus* X (PVX), *Pepper veinal mottle virus* (PVMV), *Pepper mild mottle virus* (PMMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Tobacco etch virus* (TEV) and *Tomato mosaic virus* (ToMV). Incidence of PVY was the highest (79%), followed by TEV (67%), CMV (61%), and PVMV (58%); lowest in ToMV (23%). Mixed infections were common in the farmers' fields and high incidence suggests the cultivars are highly susceptible to viral infections.

2.7.3 Symptomatology

Symptoms of PVMV on pepper include mild mottle, mosaic, vein banding, ring spots, various types of necrosis, leaf discoloration, deformation, blistering, and severe stunting of the whole plant (Brunt & Kenten, 1971; Fajinmi *et al*., 1998). Symptoms of virus infection widely vary in expression and severity including mild mottle, mosaic, vein banding, ring spots, necrosis, leaf discoloration, deformation and blistering and severe stunting of the whole plant reported by Soleimani *et al.* (2014) on pepper (*Capsicum annum*). In Nigeria, most of the pepper fields exhibit complex symptoms of mosaic, mottle, leaf distortion, vein chlorosis and stunting that cause considerable losses in yield and plant vigor (Olawale *et al.*, 2015). Symptoms observed on

infected *Capsicum* species includes mild to severe mosaic, mottling, puckering, reduction in leaf size, vein yellowing, leaf and fruit deformation and stunting (Khan *et al.*, 2015).

2.7.4 Molecular characterisation

ChiVMV virions are flexuous filaments 765x13 nm in size (Siriwong *et al.*, 1995), containing a single-stranded RNA genome of *ca.* 9.7 kb with a 3'-terminal poly(A) tail, which encodes a polyprotein of *ca.* 350 kDa cleaved by virus-encoded proteinases into 10 functional proteins (Siriwong *et al.*, 1995; Anindya *et al.*, 2004).

Reddy *et al*. (2004) isolated six viral isolates collected from chilli growing areas of Karnataka and Tamil Nadu states of India. Based on host range, serological relationship, electron microscopy and phylogenetic analysis of coat protein sequences, it was confirmed as *Chilli Veinal Mottle Virus* (CVMV). Using primers specific to CP and 3′ NTR of CVMV, resulted in a PCR fragments of 0.8 to 1.2 kb depending on primer combinations from all six isolates. Comparison of nucleotide sequences of six isolates with already reported sequences of CVMV, showed 91-93% nucleotide identity.

Banerjee *et al.* (2014) confirmed the occurrence of *Chilli veinal mottle virus* (ChiVMV) under the genus *Potyvirus* in Naga chilli (*Capsicum chinense*) in Meghalaya based on mechanical transmission assay, transmission electron microscopy, RT-PCR and sequence analysis. This is the first record of Chivmv in Naga chilli in North-East India.

The occurrence of *Chilli leaf curl virus* (ChLCV) and *Chilli vein mottle virus* (ChiVMV) were detected by using the duplex PCR in the mixed infected Chilli plants (*Capsicum annuum* L.). The duplex PCR was done by using the

specific primer Pot 1 and Pot 2 for CVMV and AVF28 and AV29R for ChLCV. The amplicon and the sequence analysis confirmed the presence of potyvirus and begomovirus in the mixed infection (Sahu *et al.*, 2016).

2.8 Efficacy of bio-control agents against plant pathogens

In vitro test of *Bacillus subtilis* and *Pseudomonas chlororaphis* was reported to be effective in controlling damping-off of chilli (*Capsicum annuum*) caused by *Pythium aphanidermatum*. The talc based formulations of these PGPR isolates proved their efficacies equivalent to the standard fungicide Ridomil to control damping-off disease under glass house conditions. In spite of their direct action, these PGPR isolates also proved to trigger defence related enzymes (Kavitha *et al.*, 2005).

Trichoderma harzianum and *T. longibrachiatum* were found to inhibit the *in vitro* growth of *S. rolfsii* and produced coiling around mycelium of *S. rolfsii* resulting in lysis of hyphae. *T. pseudokoningii, T. polysporum* and *Gliocladium virens* also inhibited the growth of *S. rolfsii*. Where *Aspergillus niger, A. fumigatus, A. flavus, A. terreus, A. nidulans, A. sulphureus, A. parasiticus, A. tamarii, A. versicolar, A. versiclar* and *A. wentii* were used, colonies of *Aspergillus* spp., and *S. rolfsii* met each other but *S. rolfsii* later overgrew the colonies of *Aspergillus* spp. (Yaqub & Shahzad, 2005).

Isolates of *Trichoderma* (*T. harzianum* TR20 and *T. pseudokoningii* TR17) and fluorescent pseudomonads (*Pseudomonas fluorescens* P28 and P51) tested under greenhouse and field conditions for efficacy in suppressing *Rhizoctonia* root rot incidence and promoting plant growth in chilli. The combination, *T. harzianum* (TR20) + *P. fluorescens* (P28), proved most effective in reducing disease incidence (66.7% more efficient than the control), but was at par with copper oxychloride (0.3%). Highest per plant yield was

also recorded in the treatment combination TR20 + P28, followed by combination of *T. pseudokoningii* (TR17) and *P. fluorescens* (P51). Individual application of *T. pseudokoningii* (TR17) and *T. harzianum* (TR20) also significantly increased the yield per plant and was superior to both the *pseudomonads* applied individually (Rini & Sulochana, 2006).

Anand and Bhaskaran (2009) tested eight antagonistic organisms against *Colletotrichum capsici* and *Alternaria alternata*, the causal agents of fruit rot disease of chilli. *In vitro* studies indicated *Trichoderma viride* isolate 3 and *Pseudomonas fluorescens* were very effective in inhibiting mycelial growth of the pathogens *in vitro.*

Belge and Padghan (2009) made *in vitro* test against *Colletotrichum dematium* causing fruit rot of chilli (*Capsicum annum* L) and reported that *Trichoderma viride* inhibited maximum of 40.89% growth of *C. dematium*, whereas *Pseudomonas fluorescence* restricted 38.00% of mycelial growth.

Muthukumar *et al.* (2010) conducted an experiment to study efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. The in vitro studies revealed that combination of *T. viride*, *P. fluorescens* and Zimmu leaf extract showed the highest mycelial growth inhibition over the control. The pot culture studies revealed that seed treatment with combined application of *T. viride*, *P. fluorescens* and Zimmu leaf extract was superior in reducing the pre and post-emergence damping-off incidence (8.3 and 17.0%, respectively), and increased the plant growth and yield (shoot length and root length of 13.7 and 6.3 cm, 146 g/plant, respectively) of chilli when compared to control.

Madhavi and Bhattiprolu (2011) they investigated the in vitro and in vivo efficacy of carbendazim, mancozeb and *T. viride* against chilli wilt disease caused by *Fusarium solani. In vitro* test revealed that combination of carbendazim and mancozeb was effective in inhibiting mycelial growth (93.6%). Integration of different treatments, including seedling dip, with carbendazim, addition of vermicompost, drenching with fungicide, and application of *T. viride* was found to be effective in managing the disease, in comparison to individual treatments.

In an experiment 5 bioagents were screened *in-vitro* against *Sclerotium rolfsii* causal organism of foot rot of ragi. *Trichoderma harzianum* (GKVK) isolate was found to be effective than other biogents (Manu *et al.*, 2012).

Jagtap *et al.* (2013) tested the efficacy of biocontrol agents namely, *T. viride*, *Gliocladium* spp., *T. harzianum*, *T. koningii* and *Pseudomonas fluorescens* against *Colletotrichum capsici* by employing poisoned food technique. The *in vitro* test revealed that per cent inhibition of the test pathogen ranged from 41.18 to 53.33%. and *T. harzianum*, was found to be the most effective antagonist which caused growth inhibition of 53.33%.

Rajput *et al*. (2013) tested eight bioagents using dual culture technique against leaf spot disease (*Alternaria alternata* Keissler) of brinjal (*Solanum melongena* L.). The results revealed that out of all the eight bioagents used, three bioagents viz., *Trichoderma viride* (IARI isolate), *Trichoderma viride* (Navsari isolate) and *T. harzianum* (Junagadh isolate), showed strong antagonistic effect to inhibit the mycelial growth of the pathogen.

Nine antagonistic microbial bioagents *viz., T. viride*, *T. harzianum*, *T. virens, T. pseudokoningii, T. asperellum, Pseudomonas fluorescens,*

Paeicilomyces lilacinus, Beuveria bassiana and Metarhizium anisopliae were evaluated *in vitro* against *Colletortricum capsici*, the causal agent of leaf spot of turmeric. Among the antagonist *T. harzianum* was recorded with the highest per cent inhibition of 83.44%, which was followed by *T. viride* with per cent inhibition of 77.62% (Das *et al.*, 2015).

Patel and Rakholiya (2016) carried out an experiment to test five native bioagents against *Sclerotium rolfsii. viz. Trichoderma harzianum, T. viride, T. fasciculatum, Pseudomonas fluorescens* and *Bacillus subtilis.* Among them *T. harzianum* was reported to have maximum growth inhibition 54.72 per cent of pathogen after 7 days of incubation and also superior to all other bio-agents tested followed by *T. viride* (50.42%), *B. subtilis* (44.09%), *T. fasciculatum* (34.55%) and *P. fluorescens* (30.13%).

Trichoderma spp. are fungal species in certain natural suppressive soil which protected the plant from infectious diseases caused by soil-borne pathogens. Among the soil borne pathogen, the fungus *Rhizoctonia solani* causes serious damages to economically significant crops. *R. solani* persists in soil by producing sclerotia which is a hard-resistant structure. The *Trichoderma* spp. are the potential bio-control agents which inhibit *R. solani* by direct confrontation through mycoparasitic or antibiosis or competition as well as inducing plant defense responses (Abbas *et al., 2017).*

Six fungal and two bacterial bioagents were tested *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease in chickpea. *T. viride* was recorded with highest mycelial growth inhibition (75.55%), followed by *T. harzianum* (73.77%), *T. koningii* (71.88%) and *Pseudomonas fluorescens* (43.77%) (Thaware *et al*., 2017).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The materials used and methodologies employed while carrying out the investigation entitled "Studies on diseases of Naga King Chilli (*Capsicum chinense* Jacq.)" are detailed as below.

3.1 Details of survey sites

Dimapur district: Most part of the district is in the plains with an average elevation of 260m above the MSL. It is situated at 25°48′N latitude and 93°47′E longitude. Villages namely Medziphema, Sirhima and Tsiepama were selected from this district.

Kohima district: It is situated at 25°40′N and latitude 94°07′E longitude with an elevation of 1,444m above the MSL. Three villages namely Thekrujuma, Mengujuma and Zhadima were selected for the investigation from this district.

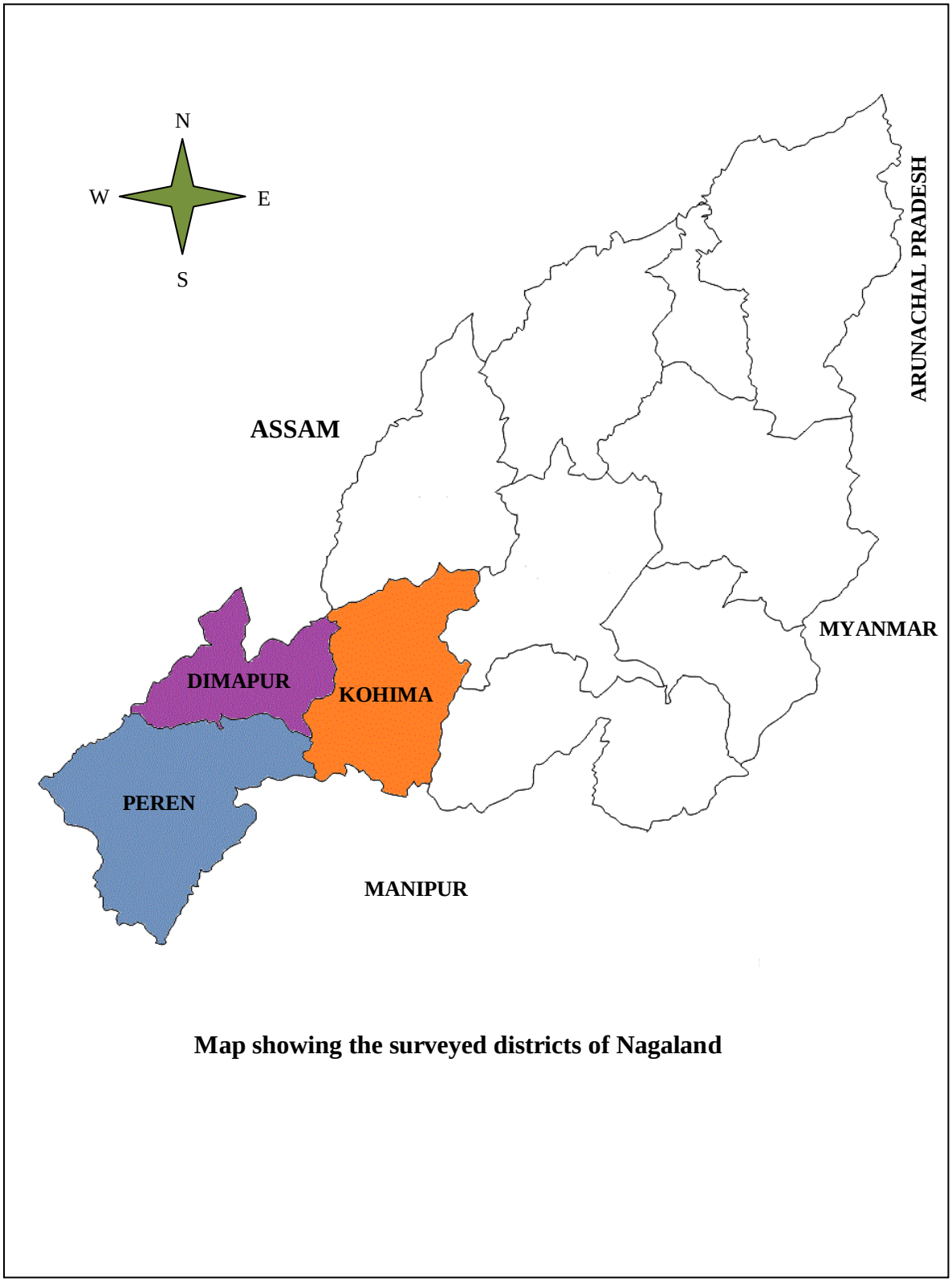
Peren district: The altitude of this district varies from 800-2500 metres above the MSL. It is headquartered at Peren at 1445.40 meters above MSL. It is situated at 25°31′N latitude and 93°44′E longitude. Athibung, Chalkot and Songlhuh villages were selected from this district.

The survey of Naga King Chilli diseases was carried out in the kharif season of 2014 and 2015. Roving survey method (Ekbote, 2002) was employed to carry out the investigation at two stages of the crop *viz.* vegetative stage (60 days after transplanting) *i.e.* during the second week of June to first week of August and at fruiting stage (100 days after transplanting) *i.e.* during second week of September to the first week of November in Naga King Chilli cultivating areas of Dimapur, Kohima and Peren district. For the survey three

Table 3.1 Meteorological observation at monthly interval during the period of investigation (2014 and 2015)

District	Month	2014							2015						
		Temperature ˚C			Relative humidity (%)			Total rainfall (mm)	Temperature ˚C			Relative humidity (%)			Total rainfall (mm)
		Max	Min	Mean	Max	Min	Mean		Max	Min	Mean	Max	Min	Mean	
Dimapur	March	29.40	14.00	21.70	77.00	17.00	47.00	33.90	30.70	14.70	22.70	78.00	12.00	45.00	32.00
	April	32.50	18.20	25.35	72.00	23.00	47.50	41.20	28.90	19.20	24.04	81.00	40.00	60.5	178.2
	May	32.48	22.11	27.29	75.26	40.29	57.77	279.60	30.95	22.62	26.79	81.57	47.11	64.34	221.40
	June	32.62	25.06	28.84	81.60	58.40	70.00	303.80	31.71	24.81	28.26	82.51	58.91	70.71	472.40
	July	31.96	25.40	28.68	83.00	61.80	72.40	770.80	31.64	24.75	28.19	84.20	59.66	71.93	686.40
	August	31.12	25.12	28.12	82.80	61.60	72.20	633.80	31.52	24.96	28.24	82.71	61.83	72.27	474.20
	September	30.91	23.80	27.35	85.43	57.71	71.57	299.00	32.36	24.29	28.32	85.40	59.57	72.49	410.60
	October	30.01	20.75	25.38	83.20	46.37	64.79	178.80	31.25	20.63	25.94	91.91	63.34	77.63	162.20
	November	27.21	14.98	21.09	79.57	24.64	52.11	0.00	28.29	14.26	21.27	92.79	57.00	74.89	02.30
Kohima	March	21.84	8.65	15.24	84.23	24.42	54.32	0.97	27.10	9.87	18.48	66.26	19.94	43.10	1.16
	April	28.87	12.43	20.65	78.07	26.30	52.18	2.80	26.77	12.27	19.52	83.77	39.07	61.42	10.63
	May	27.13	14.26	20.69	92.87	46.65	69.76	133.50	28.58	14.45	21.52	90.23	44.23	67.23	117.70
	June	27.40	16.43	21.92	94.60	58.03	76.30	138.00	24.83	14.20	19.52	89.90	56.27	73.08	232.30
	July	27.19	16.71	21.95	97.61	70.06	83.84	173.50	27.00	16.90	21.95	96.23	65.13	80.68	140.30
	August	26.23	17.39	21.81	98.19	71.61	84.90	170.30	25.94	15.97	20.95	93.29	63.35	78.32	150.60
	September	25.90	15.97	20.93	98.57	57.40	77.90	126.00	27.20	16.93	22.07	94.00	59.07	76.53	87.70
	October	25.84	13.23	19.53	97.74	49.00	73.30	13.20	25.84	12.71	19.27	95.65	47.32	71.48	16.50
	November	22.97	8.80	15.88	98.23	44.37	71.30	00.00	23.17	8.67	15.92	95.23	42.07	68.65	00.00
Peren	March	25.26	9.29	17.27	80.71	24.81	52.56	0.65	30.48	11.87	21.17	66.35	20.32	43.33	0.71
	April	32.83	14.17	23.50	74.63	25.53	50.08	1.53	29.23	15.37	22.30	83.30	38.77	61.03	11.00
	May	31.45	16.87	24.16	91.19	45.71	68.40	113.90	31.68	18.84	25.26	88.87	43.81	66.34	87.70
	June	31.70	20.60	26.15	93.27	55.67	74.40	122.70	30.67	20.10	25.38	94.70	60.20	77.45	207.70
	July	30.13	20.97	25.55	97.00	67.48	82.20	173.20	29.52	20.32	24.92	94.94	65.58	80.26	111.00
	August	28.81	20.13	24.47	97.61	70.13	83.8	160.60	29.29	19.61	24.45	92.32	62.58	77.45	124.80
	September	30.03	18.40	24.22	98.10	62.87	80.48	115.70	30.63	18.93	24.78	94.60	59.70	77.15	80.70
	October	28.65	14.13	21.39	97.48	48.39	72.9	10.30	29.26	15.42	22.34	95.26	47.52	71.39	14.20
	November	25.40	8.60	17.00	97.80	44.73	71.27	00.00	26.23	10.90	18.57	94.13	43.07	68.60	00.00

Source: ICAR Research Complex for NEH Region, Jharnapani, Medziphema, Nagaland.



Map showing the surveyed districts of Nagaland

villages were selected from each district and under each village three different respondent farmers were chosen representing the field sites based on availability of the crop.

3.2 Assessment of the extent of damage caused by the pathogen to Naga King Chilli crop

3.2.1 Assessment of disease incidence

In this survey percent incidence of disease was calculated in the farmers' field of Dimapur, Peren and Kohima district of Nagaland during the kharif season of 2014 and 2015, at two different stages of the crop growth. The disease incidence was assessed by recording the number of plants showing the symptoms and the number of plants examined. In each village, three fields were selected and in each field ten plants were examined randomly and scored for disease incidence by using the formula given by Nene (1972).

$$\textit{Per cent disease incidence} = \frac{Number\ of\ plants\ infected}{Total\ plants\ assesed} \times 100$$

The disease scoring scales used in the course of survey given by different authors are as follows:

Disease rating for Anthracnose disease (Bediako *et al*., 2015)

Rating Scale	Description
0	Healthy/ Healthy fruit on entire plant
1	1-5% of mature leaves with necrotic and chlorotic symptoms/ fruit sunken, light-coloured lesions on exposed fruits lesion can enlarge that may extend to sides
2	6-15% of mature leaves with necrotic and chlorotic symptoms/ Dark

	leathery spot on blossom-end Raised, wartlike brown lesion and small pale halos- "ghost spots"
3	15-50% of young shoots and stems water soaked lesions and minor die back/ Water-soaked, dull green spots covered with cream mould growth
4	51-95% of water-soaked lesions with abundance mycelia growth and fructification, and extensive shoot dieback/ Water-soaked sunken lesions that expand Cloudy, yellow blotches directly below skin
5	Dead plant/ pods soften and quickly collapse

Disease rating for Cercospora leaf spot disease (Bediako *et al*., 2015)

Rating scale	**Description**
0	No disease symptom
1	10% of canopy showing diseased symptoms
3	10-20% of canopy showing disease symptoms
5	25-50% of the canopy showing disease symptoms
7	50-75% of canopy showing disease symptoms
9	>75% of canopy showing disease symptoms

Disease rating for damping-off disease (Abbasi, 2004)

Rating scale	**Description**
0	Healthy
1	Appearance of small water-soaked lesion/yellowing of stem
2	Lesion enlarges and stem becomes soft
3	Post-emergence damping-off
4	Pre-emergence damping-off

Disease rating for Fusarium wilt disease (Abada & Ahmed, 2014)

Rating scale	Description
0	No foliar symptoms
1	Chlorosis and/or wilt restricted to cotyledons or first leaf
2	Chlorosis and/or wilt extending beyond the first leaf
3	Moderate to severe foliar symptoms usually with some abscised leaves
4	Severe foliar symptoms on the entire plant
5	Dead plant

Disease rating for stem rot disease (Doley & Jite, 2013)

Rating scale	Description
0	Healthy
1	Lesions on stem only
2	Up to 25% of the plant symptomic (wilt, dead or dying)
3	26-50% of the plant symptomic
4	>50% of the plant symptomic

Disease rating for bacterial wilt disease (Basu, 2014)

Rating Scale	Description
0	Healthy plants with no effect
1	Yellowing of lower leaves
2	Yellowing, marginal necrosis and defoliation
3	Drying and wilting
4	Complete death of the plants

Disease rating for leaf veinal mottle disease (Bediako *et al*., 2015)

Rating scale	Description
1	No disease symptom
2	Mild mottling
3	Mild mottling with leaf malformation
4	Stunting / severe mottling / leaf bunching
5	Defoliation

3.2.2. Assessment of disease severity

During the survey, disease severity ratings were recorded at two different stages of the crop *i.e.* at vegetative stage and at fruiting stage. For estimation of infected area on the plant, the whole area of the interested part of the plant; whether it is leaf, stem, fruit or the plant as whole was considered as 100 and thereby the infected area was determined by visual assessment methods and pathometric methods. For determining the percentage of diseased area different disease scale ratings were used for different disease. For measuring anthracnose, Cercospora leaf spot and leaf veinal mottle disease scales given by Bediako *et al*. (2015) were adopted. A scale rating given by Abbasi (2004) was adopted for determining damping-off disease. As for Fusarium wilt disease scale ratings given by Abada and Ahmed (2014) was adopted. Stem rot disease was assessed using scale ratings given by Doley and Jite (2013). Lastly, for bacterial wilt disease scale rating given by Basu (2014) was used for severity assessment. In the survey, disease ratings were recorded for the individual plant part/whole plant and the amount of diseases were expressed as the percentage of the plant parts/whole plant covered by the symptoms of the disease.

Severity is often stated as the area or area and volume of plant tissue affected (Horsfall and Cowling 1978). Visual assessment method was used for severity studies to measure areas or volume of infection.

$$\textit{Disease severity (area) \%} = \frac{Area\ of\ plant\ tissuse\ affected\ by\ disease}{Total\ area} \times 100$$

3.2.3 Per cent Disease index

The Disease severity values was converted to (PDI) per cent Disease Index (Wheeler, 1969). PDI was assessed by recording the severity of disease in a locality by adopting the rating scales.

$$PDI = \frac{Sum\ of\ individual\ ratings}{Total\ number\ of\ plants\ observed} \times \frac{100}{maximum\ value\ used}$$

3.3 Identification of disease and collection of disease samples

The diseases in the field were diagnosed based on visual symptoms *i.e.* identification was done on the basis of infected and suspected plant parts showing typical symptoms on the plant parts like leaves, fruits and stem. Plants showing characteristic disease symptoms were collected from the field sites and brought back to the laboratory for further study.

3.4 Isolation and identification of disease causing agents

3.4.1 Isolation and identification of fungal pathogens

3.4.1.1 Cultivation media

Potato Dextrose Agar was prepared by firstly taking 500ml of distilled water and 200g of peeled, sliced potatoes in a one litre beaker and boiled till it became soft. The potato extract was then filtered by using a muslin cloth and 20g of dextrose was added to it. Then, 500ml of distilled water was heated in another beaker and 20g of agar agar was added to it bit by bit and further boiled till agar agar had dissolved completely. The agar agar solution was

mixed with the potato extract and the volume was made up to 1000ml by addition of distilled water. Then, it was transferred into five numbers of 250ml conical flasks and corked with non-absorbent cotton and autoclaved at 121˚C at 15psi for 20 minutes.

PDA slants were prepared by pouring freshly prepared molten PDA into the culture tubes. The volumes of the PDA in the tube were made up to one fourth length of the tube. The tubes were then plugged with non-absorbent cotton wool and autoclaved at121°C at 15psi for 20 minutes. After autoclaving when the medium was still in its liquid form the tubes were placed on the table in a slanting position until solidified.

3.4.1.2 Isolation and purification of fungal pathogens

The isolation of fungal pathogens from diseased sample of Naga King Chilli was done in an aseptic environment inside the laminar air flow chamber. Bits of 5.0x5.0mm^2 size were cut from the infected plant part with the help of a sterile razor blade. The bits were cut from the margin of the lesions so that each bit contained 50% of its area from the lesions and 50% from the healthy tissue. The bits were then surface sterilized by dipping in 5-6% sodium hypochlorite solution for 30 seconds followed by rinsing with three changes of sterile distilled water. With the help of a sterile forcep, the bits were inoculated on the Petri plate containing PDA medium. The inoculated plates were incubated at room temperature (25±2°C).

With the help of an inoculating needle the fungal colonies were transferred into the PDA slants and allowed to grow at room temperature (25±2°C) for 3-5 days, some isolates took longer time to grow. After the fungal isolates gained sufficient growth the slants were stored in the refrigerator at 4°C. Sub-culturing of the fungal isolates were done at an interval of every 60

days. The fungal colony thus obtained were further purified and maintained in PDA slants for further study.

3.4.1.3 Identification of the isolated fungal pathogens

The purified fungal pathogens were observed under the microscope and identification was done based on their morphological characteristics *viz*. hyphal diameter; conidial length, breadth, shape, septation and colour. Morphological characters were studied using a bright field compound microscope under objective lens 10x and 45x.

The typical identifying characters of each of the fungal pathogens were photographed using a digital microscope; model no. NLCD-307, LYNX, Lawrence and Mayo. The cultural, morphological and photographic descriptions, thus obtained were compared with the description given in "Handbook of soil fungi" by Nagamani, A., Kunwar, I. K. and Manoharachary C. and "Pictorial Atlas of Soil and Seed Fungi" by Watanabe T. for identification.

3.4.2 Isolation and identification of bacterial pathogens

3.4.2.1 Cultivation media

Nutrient Agar (NA) medium was used for isolation of bacterial pathogen. Peptone 5g, beef extract 3g and 20g of Agar was boiled in 1000ml of distilled water till all the ingredients dissolved completely. Then it was transferred into 250ml conical flask and corked with non- absorbent cotton wool and autoclaved at 15psi pressure, 121°C for 20 minutes.

For preparation of NA slants freshly prepared NA medium were poured into the culture tubes. The volumes of the NA in the tube were made up to one fourth length of the tube. The tubes were then plugged with non-absorbent

cotton wool and autoclaved at15psi, 121°C for 20 minutes. After autoclaving when the medium was still in its liquid form the tubes were placed on the table in a slanting position until solidified.

3.4.2.2 Isolation and purification of bacterial pathogen

The isolation of bacterial pathogen from diseased sample of Naga King Chilli was done in an aseptic environment inside the laminar air flow chamber. Bits of 5.0x5.0mm^2 size were cut from the infected plant part with the help of a sterile razor blade. The bits were cut from the margin of the lesions so that each bit contained 50% of its area from the lesions and 50% from the healthy tissue. The bits were then surface sterilized by dipping in 5-6% sodium hypochlorite solution for 30 seconds followed by rinsing with three changes of sterile distilled water. After this, the bits were immersed in sterile tube containing 1ml of sterile distilled water and the tissue was macerated with the help of a sterile glass rod. Then, with the help of an inoculating loop a loop full of suspension from the macerated tissue was streaked on the Petri plates containing nutrient agar. The plates were incubated at room temperature (25±2°C). Individual bacterium colonies were transferred into the NA slants and allowed to grow at room temperature (25±2°C) for 3-5 days and then the slants were stored in the refrigerator.

3.4.2.3 Identification of the isolated bacterial pathogens

Ooze test

The infected stem of Naga King Chilli was cut across and the cut end was suspended in clear container containing clean water to see a stream of milky white masses of bacterial cells (ooze) forming a thread in the water. This test was done as quick field diagnosis so as to distinguish bacterial wilt from vascular wilts caused by fungal pathogen and nematode (Ahmed *et al.*, 2013).

Gram staining test

A thin smear of the bacterial wilt pathogen colony was made on the glass slide, air dried and the smear was heat fixed by passing the slide over a flame of spirit lamp. Gently the smear was flooded with crystal violet and let stand for 30 seconds. Then the slide was tilted slightly and gently rinsed with distilled water using a wash bottle. The smear was flooded with Gram's iodine and let stand for 1 minute and was washed with distilled water. The smear was then washed with 95% ethanol by adding drop by drop until no more colour flowed from the smear. The slide was again rinsed with distilled water, and then flooded with safranin for 30 seconds. Lastly, the smear was washed with distilled water and blot dried. The appearance of purple colour indicates the bacteria as Gram positive and if it appears pink colour it indicates that the bacteria are Gram negative (Rahman *et al*., 2010).

Sodium chloride (NaCl) tolerance test

For this test NaCl broth was used that is composed of 5g of peptone, 3g of yeast extract, 5g of glucose, 1000ml of distilled water and 5, 10, 15, 20g of NaCl. The broth was autoclaved at 121°C for 15 minutes and dispensed into 100 ml of flasks. Test strains were inoculated into the flasks and incubated in rotary shaker at 30°C with 100 rpm upto 14 days. Growth was recorded every 2 days for each tube (Hayward 1964).

Starch hydrolysis

Nutrient agar plates containing 0.2% soluble starch were streaked by the test strains of the bacterial wilt pathogen and incubated at 30°C until heavy growth occurs. Then the plates were flooded with IKI solution (iodine 1g, potassium iodide 2g, distilled water 100ml); a clear zone around the colony will be observed as positive reaction (Sands, 1990).

Gelatin hydrolysis

Nutrient agar with 0.4% gelatin was poured into Petri dishes, cooled and dried over night. The following day, strains were inoculated on to each plate and incubated at 30°C. When good growth was observed, the plates were flooded with 5ml of mercuric chloride solution ($HgCl_2$ 12g, distilled water 80ml, conc. HCl 16ml) (Sands, 1990). A clear zone surrounding bacterial growth indicates positive reaction for the test (Dickey & Kelman, 1988).

Catalase Test

This test was performed according to the methods described by He *et al.* (1983). One ml of 3% hydrogen peroxide solution was poured into a test tube. With the help of a glass rod, colonies of the test bacterium were immersed into the hydrogen peroxide solution. Release of bubbles from the culture was recorded as catalase positive (Sands, 1990).

Oxidase test

Freshly grown cultures from nutrient agar with 1% glucose were patched onto a filter paper moistened with a fresh oxidase reagent (1% tetra methyl-para-phenylenediamine dihydrochloride) using a wooden stick. A purple reaction in 30 seconds is recorded as oxidase positive (Sands, 1990).

3.4.3 Isolation and identification of viral pathogens

The isolation, molecular characterization and identification of the viral pathogen were done using the following steps as described below.

3.4.3.1 RNA extraction from leaf tissue of Naga King Chilli

RNA extraction kit (QIAGEN RNeasy Plant Mini Kit, Cat. no. 74904) containing the following components Buffer RLT, RW1, RPE, β-mercaptoethanol, ethanol (96-100%), RNase free water, QIAshredder spin

column (lilac) and RNeasy Mini spin column (pink) was used for extraction of RNA from Naga King Chilli leaf tissue.

Total RNA from the leaf samples was extracted following manufacturer's protocol. 100 mg of the leaf sample was measured and ground well in mortar and pestle using liquid nitrogen. Immediately after crushing, the powder was collected in a micro centrifuge tube (MCT). Then 450µl of buffer RLT was added in the MCT and the mixture was vortexed vigorously for proper homogenization. Before adding buffer RLT to the mixture, β-mercaptoethanol (10µl/1ml) added to buffer RLT. After vortexing the lysate was transferred into a QIAshredder Mini spin column (lilac) placed in 2 ml collection tube and centrifuged for 2 min at 14,000 rpm. After centrifugation the supernatant of the flow-through was transferred to a new MCT without disturbing the cell-debris pellet. Then 0.5 volume of ethanol (96-100%) was added to the cleared lysate, and mixed immediately by pipetting. Then 650µl of mixture was transferred to RNeasy Mini spin column (pink) in a 2ml collection tube. The mixture was centrifuged again for 30 s at ≥ 10,000 rpm. Then, the flow-through was discarded and 700µl buffer RW1 was added to RNeasy Mini spin column and centrifuged for 30 s at ≥8000 x g (≥ 10,000 rpm). After that, the flow-through was discarded and 500µl buffer RPE was added to RNeasy Mini spin column and again centrifuged for 30 s at ≥ 10,000 rpm. The step was repeated twice with a final centrifugation for 2 min at ≥ 10,000 rpm. Then, the RNeasy Mini spin column was placed in a new 2 ml collection tube, centrifuged at full speed *i.e.* 13,000 rpm for one minute to dry the membrane. Then the spin column was transferred into a 1.5 ml collection tube and 30 µl of RNase-free water was added directly on to the spin column and then centrifuged for 1 min at 10,000 rpm) to elute the RNA. The extracted RNA samples were stored in deep freezer (-20ºC).

3.4.3.2 Reverse transcription polymerase chain reaction (RT-PCR)

Materials used

Sample (extracted total RNA from infected leaf tissues of Naga King Chilli). RT-PCR was carried out using *Potyvirus* genus-specific degenerate primers *viz*., CIFor and CIRev (Ha *et al*., 2008). The primer set was reported to amplify a ~700 bp region of cylindrical inclusion protein (CI) domain (Ha *et al*., 2008) of *Potyvirus* open reading frame (ORF). All the primers used in this study were synthesized from GCC Biotech Pvt. Ltd.,Kirtankhola, Joychandipur, West Bengal. One-step RT-PCR kit (Qiagen, Germany; Cat# 210212) containing the following components: 5X one step RT-PCR buffer containing 12.5 mM $MgCl_2$, 10 mM dNTP-mix, One step RT-PCR enzyme mix 1µl/reaction and RNase free water was used for RT-PCR experiment

Primers	**Sequence (5'-3')**	**Working Concentration**	**Annealing Temperature**
CI For	GGIVVIGTIGGIWSIGGIAARTCIAC	10µM	40°C
CI Rev	ACICCRTTYTCDATDATRTTIGTIGC	10µM	

RT-PCR reaction mixture

The PCR amplifications were carried out in thermal cycler (AB applied Biosystems, Life technologies, Singapore). The reaction mixture contained 1 µl of total RNA, 1 µl each of 10µM forward and reverse primers, 5 µl of 5X one step RT-PCR buffer containing 2.5 mM $MgCl_2$,1 µl of 10 mM dNTPs,1 µl of one-step RT-PCR enzyme mix and RNase free water to make up the volume up to 25µl to target the CP and HC-Pro gene.

PCR Cycles

PCR cycles were composed of reverse transcription at 50°C for 30 min, followed by an initial PCR activation at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 40°C for 30 sec and extension for 1 min at 68°C. A final extension was allowed for 5 min at 68°C. Then, after the completion of the PCR the amplified products were stored in -20°C.

3.4.3.3 Gel electrophoresis and documentation

Materials used

Tank buffer: Comprising of 1X TAE (Tris-Acetic acid-EDTA) buffer prepared from 10X TAE stock (GCC Biotech Pvt. Ltd.) by 10 times dilution, Ethidium bromide: 1.0% concentration (10mg/ml) (SRL; Cat#313761), Agarose (HiMedia; Cat# 9012-36-6), 1kb DNA ladder (Thermo scientific 0' Gene Ruler™, Concentration 0.1 μg/μl, Cat# SM1163), 6X Gel loading dye (GCC Biotech Pvt. Ltd; Cat# GCR-22A).

The amplified products obtained through RT-PCR were subjected to electrophoresis on 1% agarose gel. The gel was prepared by melting 1g of agarose in 100 ml 1X TAE buffer in a conical flask with frequent shaking. Then it was kept in room temperature for cooling down to 65-70°C. After that 4 μl of ethidium bromide was added, mixed thoroughly by gentle swirling and poured into the gel plate to solidify. Then the amplified products were loaded into the wells by mixing with loading dye (final concentration 1X) along with the 1kb DNA ladder as a marker and the gel was run for 1 hr at a fixed voltage (7V/cm). The gel was examined under a UV trans illuminator (GelDoc XR, Biorad, Germany) and photographed.

3.4.3.4 Purification of the PCR amplicons

The amplified fragments (~>1kb and ~850 bp) of the representative isolate were gel purified using following protocol.

Materials used

PCR products, Agarose, Scalpel, 1X TAE (Tris-Acetic acid-EDTA) buffer prepared from 10X TAE stock (GCC Biotech Pvt. Ltd.) by 10 times dilution, Gel electrophoresis system, Gel Extraction kit (QIAGEN QIAquick; Cat# 28706), Components: Buffer QG, PE, EB, isopropanol, 6X Gel loading dye (GCC Biotech Pvt. Ltd; Cat# GCR-22A), 1kb DNA ladder (Thermo scientific 0' Gene Ruler™, Concentration 0.1 µg/µl, Cat# SM1163).

The fragment was excised from the agarose gel with a sterile sharp scalpel and placed in MCT. The weight of the gel slice was measured in a digital balance. Then 3 volumes of Buffer QG was added to 1 volume (V=W) of the gel and incubated at 50°C in water bath until the gel slice completely dissolved. After that 1 volume of isopropanol was added into the tube and mixed properly by pipetting. To bind RNA, the sample was transferred to QIAquick column and centrifuged for 1 min at 13,000 rpm. The lysate was discarded. Then 500 µl of Buffer QG was added and centrifuged again for 1 min at same speed. For washing, 750 µl of Buffer PE was added to the column and centrifuged for 1 min at 13,000 rpm. The clean lysate discarded again and the column was kept in the new MCT. To elute the RNA 30 µl of Buffer EB was added in the center of the column and incubated in room temperature for 1 min and then centrifuged for 1 min at 13,000 rpm. The eluted products were checked again for confirmation by running a gel with 1kb ladder as described earlier.

Sequencing: The gel-purified amplicons for ~700 bp was sequenced bi-directionally from Xcelris Genomics, Ahmedabad, Gujarat, India.

3.5 Testing effectiveness of bio-control agents *in vitro* against *Colletotrichum capsici, Fusarium oxysporum, Sclerotium rolfsii* and *Rhizoctonia solani*

Seven biocontrol agents *Trichoderma asperellum, T. harzianum, T. koningii, T. virens, T. viride, Bacillus subtilis* and *Pseudomonas fluorescens* were studied *in vitro* for their antagonistic effect on the test pathogens by dual culture technique. Mycelium of the antagonist and the pathogen measuring 10 mm in diameter was corked out with a sterile cork borer from the periphery of the actively growing culture and they were inoculated on Petri plates (90mm diameter) containing PDA medium with the help of an inoculating loop. The discs were placed upside down on the PDA plates, so that the mycelia are in direct contact with the medium. Control plates having only the test pathogen was also kept for comparison. In case of bacterial antagonist, the bacterial culture was streaked against the test pathogen. The loaded plates were then incubated at 25±1°C and the observation was taken 3 days after inoculation. The experiment was laid in a Complete Randomized Design (CRD) and each treatment was replicated three (3) times. The treatment combinations for the experiments were as follows.

Treatments (*Colletotrichum capsici*)

T 1	-	*Colletotrichum capsici + Trichoderma koningi*
T 2	-	*C. capsici + T. viride*
T 3	-	*C. capsici + T. harzianum*
T 4	-	*C. capsici + T. asperellum*
T 5	-	*C. capsici + T. virens*
T 6	-	*C. capsici + Bacillus subtilis*
T 7	-	*C. capsici + Pseudomonas fluorescens*
T 8	-	*Colletotrichum capsici* (control)

Treatments (*Fusarium oxysporum*)

T 1	-	*Fusarium oxysporum + Trichoderma koningi*
T 2	-	*F. oxysporum + T. viride*
T 3	-	*F. oxysporum + T. harzianum*
T 4	-	*F. oxysporum + T. asperellum*
T 5	-	*F. oxysporum + T. virens*
T 6	-	*F. oxysporum + Bacillus subtilis*
T 7	-	*F. oxysporum + Pseudomonas fluorescens*
T 8	-	*Fusarium oxysporum* (control)

Treatments (*Sclerotium rolfsii*)

T 1	-	*Sclerotium rolfsii + Trichoderma koningi*
T 2	-	*S. rolfsii + T. viride*
T 3	-	*S. rolfsii + T. harzianum*
T 4	-	*S. rolfsii + T. asperellum*
T 5	-	*S. rolfsii + T. virens*
T 6	-	*S. rolfsii + Bacillus subtilis*
T 7	-	*S. rolfsii + Pseudomonas fluorescens*
T 8	-	*Sclerotium rolfsii* (control)

Treatments (*Rhizoctonia solani*)

T 1	-	*Rhizoctonia solani + Trichoderma koningi*
T 2	-	*R. solani + T. viride*
T 3	-	*R. solani + T. harzianum*
T 4	-	*R. solani + T. asperellum*
T 5	-	*R. solani + T. virens*
T 6	-	*R. solani + Bacillus subtilis*
T 7	-	*R. solani + Pseudomonas fluorescens*
T 8	-	*Rhizoctonia solani* (control)

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The results recorded during the course of the investigation entitled "Studies on diseases of Naga King Chilli (*Capsicum chinense* Jacq.)" are presented and discussed in this chapter under the following heads.

4.1 Survey of Naga King Chilli diseases

A survey was carried out in Naga King Chilli growing districts of Nagaland *viz.* Dimapur, Kohima and Athibung in the kharif season of 2014 and 2015. Roving survey method (Ekbote, 2002) was employed to carry out the investigation at two stages of the crop *viz.* vegetative and at fruiting. Seven Naga King Chilli diseases *viz*. anthracnose, Cercospora leaf spot, damping-off, Fusarium wilt, stem rot, bacterial wilt and leaf veinal mottle disease were identified during this investigation.

4.1.1 Anthracnose of Naga King Chilli

The symptoms of anthracnose disease of Naga King Chilli appeared on the leaves, fruits and twigs and are depicted in Plate 1. On the leaf the disease appeared in the form of small dark brown to black irregular spots. At the later stage of infection the spots coalesced forming large necrotic patches (Plate 1a). The symptoms on the fruit appeared as small, circular, water soaked and sunken lesions with dark margin (Plate 1b). The sunken lesions were covered with mass of conidia. As the disease progressed the lesions enlarged forming concentric markings with dark fructification (acervuli). Severely infected fruits were observed to drop off prematurely. In the die-back phase, necrosis of the tender twigs from the tip downwards was observed (Plate 1c). In the advanced stage of the disease, the twigs became straw coloured, with large number of visible black dots (acervuli). The top branches and the side branches were

observed to be killed and in cases of heavy infection, the whole plant withered away completely.

Various authors have described the symptoms of anthracnose disease. Symptoms of the disease observed in this study were found to be in agreement with typical symptoms of the disease described earlier by various workers (Singh, 2005; Than *et al*., 2008; Ismail *et al*., 2015; Oo & Oh, 2016).

4.1.1.1 Disease incidence

Survey on anthracnose of Naga King Chilli during the year 2014 (Table 4.1) reveals that Songlhuh of Peren district recorded highest incidence of anthracnose (51.00%), while the lowest incidence (8.33%) was recorded from Mengujuma (Kohima district) among the villages. At district level, the highest incidence was recorded from Dimapur (47.59%) followed by Peren (47.56%) and Kohima (23.67%). In the year 2015, among the villages Athibung (48.67%) recorded the highest incidence of anthracnose followed by Sirhima (45.33%) of Dimapur district and the least incidence was recorded from Mengujuma (11.00%) of Kohima district. At district level, Peren (42.22%) recorded highest incidence followed by Dimapur (41.22%) and Kohima (22.56%). According to the pooled data among all the villages, Athibung (Peren district) recorded highest incidence of 49.00% followed by Sirhima (47.50%) of Dimapur district and the lowest incidence was recorded from Mengujuma (Kohima district) (9.67%). Among the districts, Dimapur (44.39%) recorded highest incidence of anthracnose followed by Peren (43.83%) and Kohima (23.11%).

It was observed that the incidence of anthracnose of Naga King Chilli declined in second year of survey in comparison to that of first year survey, Ekbote (2002) reported the mean disease incidence of chilli fruit rot (36.4%)

caused by *Colletotrichum capsici* in the Haveri district of Karnataka. In the present study Dimapur district (44.39%) recorded highest incidence of anthracnose disease in Naga king Chilli. This may be due to higher temperature and rainfall (Table 3.1) received during the course of investigation. Anthracnose disease caused by *Colletotrichum* spp. are favoured by windblown rain, high temperature and requires a film of water on the plant or high relative humidity in almost every stage of their life cycle (Agrios, 2005; Prasad, 2016).

4.1.1.2 Per cent disease index

PDI of anthracnose disease of Naga King Chilli during the year 2014 and 2015 are given in Table 4.2. During the year 2014, the highest mean PDI was recorded from Tsiepama village (51.00) of Dimapur district among the villages. Of the three districts, Dimapur (48.33) recorded the highest PDI followed by Peren (41.33) and Kohima (27.77). Similarly, during the year 2015, it was observed that PDI of anthracnose at vegetative stage significantly increased when the plant attained fruiting stage. It was also observed that among the villages Medziphema (54.06) of Dimapur district recorded highest PDI of anthracnose disease in Naga King Chilli followed by Athibung (53.00) village of Peren district. Among the districts Dimapur (49.40) recorded the highest PDI followed by Peren and Kohima (42.44 and 31.41 respectively). From the pooled data it indicates that Athibung (51.00) village of Peren district recorded the highest PDI and lowest was recorded from Mengujuma (20.48) village of Kohima district among all the villages. The highest PDI among the districts was recorded from Dimapur (48.87) followed by Peren (41.89) and lowest PDI was recorded from Kohima (29.59).

From the survey data in Table 4.2, it was observed that there was significant difference of PDI of anthracnose in the vegetative and fruiting stage

Table 4.1 Incidence of anthracnose disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of anthracnose disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	50.00	41.00	47.00	47.56	42.00	41.22	45.50	44.50	44.39
		F2	42.00	38.00					40.00		
		F3	49.00	47.00					48.00		
	Sirhima	F1	55.00	34.00	49.67		45.33		44.50	47.50	
		F2	33.00	58.00					45.50		
		F3	61.00	44.00					52.50		
	Tsiepama	F1	25.00	32.00	46.00		36.33		28.50	41.17	
		F2	45.00	22.00					33.50		
		F3	68.00	55.00					61.50		
Kohima	Thekrujuma	F1	25.00	44.00	28.33	23.67	31.33	22.56	34.50	29.83	23.11
		F2	25.00	27.00					26.00		
		F3	35.00	23.00					29.00		
	Zhadima	F1	30.00	42.00	34.33		25.33		36.00	29.83	
		F2	38.00	4.00					21.00		
		F3	35.00	30.00					32.50		
	Mengujuma	F1	15.00	10.00	8.33		11.00		12.50	9.67	
		F2	0.00	5.00					2.50		
		F3	10.00	18.00					14.00		
Peren	Athibung	F1	30.00	55.00	49.33	45.44	48.67	42.22	42.50	49.00	43.83
		F2	65.00	37.00					51.00		
		F3	53.00	54.00					53.50		
	Chalkot	F1	28.00	30.00	36.00		34.33		29.00	35.17	
		F2	45.00	29.00					37.00		
		F3	35.00	44.00					39.50		
	Songlhuh	F1	58.00	28.00	51.00		43.67		43.00	47.33	
		F2	45.00	55.00					50.00		
		F3	50.00	48.00					49.00		

Table 4.2 Percent disease index (PDI) of anthracnose of Naga King Chilli at different stages of the crop growth during 2014 and 2015

District	Village	2014				2015				Pooled	
		Vegetative Stage	Fruiting Stage	Mean PDI		Vegetative Stage	Fruiting Stage	Mean PDI		Village	District
				Village	District			Village	District		
Dimapur	Medziphema	22.66	66.66	44.66	48.33	41.00	67.11	54.06	49.40	49.36	48.87
	Sirhima	40.00	58.66	49.33		36.11	62.21	49.16		49.25	
	Tsiepama	41.33	60.66	51.00		34.33	55.66	45.00		48.00	
Kohima	Thekrujuma	30.66	42.66	36.66	27.77	32.33	39.43	35.88	31.41	36.27	29.59
	Zhadima	33.33	28.66	31.00		30.00	36.10	33.05		32.02	
	Mengujuma	5.33	26.00	15.67		15.66	34.92	25.29		20.48	
Peren	Athibung	26.00	72.00	49.00	41.33	37.00	69.00	53.00	42.44	51.00	41.89
	Chalkot	29.33	50.00	39.67		23.00	45.66	34.33		37.00	
	Songlhuh	30.66	40.00	35.33		29.00	51.00	40.00		37.67	

of all the villages during the year 2014 and 2015 which is in conformity with the findings of Darge *et al.* (2016) who reported significant difference of severity of anthracnose. The difference may be attributed due to the higher relative humidity associated with high temperature (Table 3.1) during fruiting stage, which provides favourable environmental conditions for anthracnose development after infection (Estrada *et al.* 2000).

4.1.2. Cercospora leaf spot of Naga King Chilli

Initially small brownish spots appeared on the leaves and which gradually developed into big circular greyish spots with whitish centre (Plate 2) marginated by definite darker zone, giving its signature frog eye appearance. Later they formed into large lesions due to coalescing of the spots and the centre portion droped from the older leaves. In heavily affected plant, leaves turned yellow and finally dropped off.

Typical symptoms of Cercospora leaf spot disease *viz.* frog eye symptoms, necrotic spots with whitish centre marginated by definite darker zone have been described by other workers (Patel *et al.* 2001; Bhat *et al.* 2008) which were found in agreement with the symptoms that were observed in Cercospora leaf spot disease of Naga King Chilli.

4.1.2.1. Disease incidence

The survey data (Table 4.3) suggests that during the year 2014, highest incidence of Cercospora leaf spot disease (14.50%) was recorded from Medziphema village (Dimapur district) that was followed by Zhadima and Mengujuma (Kohima district) villages each recording incidence of 10.00%, while the lowest incidence (1.67%) was recorded from Songlhuh (Peren district) at village level. Among the districts, Dimapur (10.11%) recorded the highest mean incidence followed by Kohima (8.67%) and Peren (6.39%) in the

year 2014. During the year 2015, Medziphema village of Dimapur district again recorded the highest incidence (12.83%) of Cercospora leaf spot disease and lowest incidence (1.83%) was recorded from Songlhuh village (Peren district). Among the districts, Dimapur (9.83%) recorded the highest mean incidence followed by Kohima and Peren (7.94 and 5.61% respectively). Pooled data depict that Dimapur recorded the highest mean incidence of 9.97% followed by Kohima (8.31%) and lowest recorded from Peren (6.00%).

The data on survey revealed that Cercospora leaf spot incidence varied from locality to locality. This may be due to type of cultivar grown, topography geographic location and meteorological factors (Table 3.1). Highest incidence of Cercospora leaf spot in Naga King Chilli was recorded from Dimapur district. The pathogen *Cercospora* is favored by high temperatures and therefore is most destructive in the summer months and in warmer climatic areas (Agrios, 2005). Farooq *et al*. (2008) also reported frog eye leaf spot disease incidence on bell pepper ranged from 20.06 to 37.20% in Kashmir.

4.1.2.2 Per cent disease index

Table 4.4 represents the PDI for Cercospora leaf spot of Naga King Chilli recorded during the year 2014 and 2015. During the course of survey in 2014, the mean PDI of Chalkot village (9.99) of Peren district was highest among the villages, followed by Sirhima (9.25) of Dimapur district. At district level, Dimapur (7.53) recorded the highest PDI followed by Peren (7.15) and lowest recorded from Kohima (5.61). Similarly for the year 2015, Chalkot village of Peren district recorded the highest PDI (10.71), followed by Songlhuh village (10.11) of Peren district, the lowest PDI was recorded from

Table 4.3 Incidence of Cercospora leaf spot disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of Cercospora leaf spot disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	17.50	13.00	14.50	10.11	12.83	9.83	15.25	13.67	9.97
		F2	13.50	7.50					10.50		
		F3	12.50	18.00					15.25		
	Sirhima	F1	17.50	15.00	9.17		8.83		16.25	9.00	
		F2	7.50	6.50					7.00		
		F3	2.50	5.00					3.75		
	Tsiepama	F1	10.00	8.50	6.67		7.83		9.25	7.25	
		F2	10.00	0.00					5.00		
		F3	0.00	15.00					7.50		
Kohima	Thekrujuma	F1	7.50	2.50	6.00	8.67	7.33	7.94	5.00	6.67	8.31
		F2	0.00	6.50					3.25		
		F3	10.50	13.00					11.75		
	Zhadima	F1	22.50	15.50	10.00		6.83		19.00	8.42	
		F2	5.00	2.50					3.75		
		F3	2.50	2.50					2.50		
	Mengujuma	F1	15.00	10.50	10.00		9.67		12.75	9.83	
		F2	10.00	2.50					6.25		
		F3	5.00	16.00					10.50		
Peren	Athibung	F1	10.00	15.00	9.17	6.39	9.50	5.61	12.50	9.33	6.00
		F2	2.50	8.00					5.25		
		F3	15.00	5.50					10.25		
	Chalkot	F1	12.50	0.00	8.33		5.50		6.25	6.92	
		F2	2.50	16.50					9.50		
		F3	10.00	0.00					5.00		
	Songlhuh	F1	2.50	5.50	1.67		1.83		4.00	1.75	
		F2	2.50	0.00					1.25		
		F3	0.00	0.00					0.00		

Table 4.4 Percent disease index (PDI) of Cercospora leaf spot of Naga King Chilli at different stages of the crop growth during 2014 and 2015

District	Village	2014				2015				Pooled	
		Vegetative Stage	Fruiting Stage	Mean PDI		Vegetative Stage	Fruiting Stage	Mean PDI		Village	District
				Village	District			Village	District		
Dimapur	Medziphema	13.33	2.22	7.78	7.53	12.21	5.21	8.71	7.09	8.24	7.31
	Sirhima	15.91	2.59	9.25		14.23	3.32	8.78		9.01	
	Tsiepama	8.51	2.59	5.55		5.21	2.33	3.77		4.66	
Kohima	Thekrujuma	3.33	5.92	4.63	5.61	5.24	4.56	4.90	4.95	4.76	5.28
	Zhadima	4.44	6.29	5.37		8.22	2.22	5.22		5.29	
	Mengujuma	4.44	9.25	6.85		3.33	6.12	4.73		5.79	
Peren	Athibung	2.22	8.51	5.37	7.15	5.24	6.00	5.62	8.81	5.49	7.98
	Chalkot	9.25	10.73	9.99		11.21	10.21	10.71		10.35	
	Songlhuh	2.59	9.62	6.11		12.11	8.11	10.11		8.11	

Tsiepama (3.77) (Dimapur district) among the villages. At district level, Peren (8.81) recorded the highest mean PDI for Cercospora leaf spot of Naga King Chilli followed by Dimapur (7.09) and lowest was recorded from Kohima (4.95). According to pooled data, among the districts Peren (7.98) was observed to have the highest PDI of Cercospora leaf spot of Naga King Chilli followed by Dimapur (7.31) and Kohima (5.28) and at village level, Chalkot village of Peren District recorded the highest mean PDI (10.35) and the lowest recorded from Tsiepama (4.66) (Dimapur district).

The mean PDI of Cercospora leaf spot of Naga King Chilli recorded during the year 2014 and 2015 ranged from 3.77 to 10.71; this was found to be in agreement with the findings of Suresh (2013) who also recorded disease severity ranging from 5 to 20 per cent irrespective of location surveyed in Southern parts of Karnataka. Further, the disease severity varied in different locations indicating the probable role of environment and/or existence of physiological races. These observations were also found to be in agreement with the earlier report of Hossain *et al.* (2011) on Cercospora leaf spot of chilli.

4.1.3 Damping-off in Naga King Chilli

During the course of study, toppling down (Plate 3) of the infected Naga King Chilli seedlings was observed as the most prominent symptom. In infected seedlings reddish brown to black lesions were observed on the stems. Later, the infected stem became water-soaked and soft, causing the plant to fall over and in some case the infected stem girdled. In the nursery seed bed the seedlings began to topple down in patches and within few days the entire seedlings were killed. The symptoms described in this investigation were found to be in agreement with typical symptoms of damping-off disease as described by Olsen (1988).

4.1.3.1. Disease incidence

The data on the incidence of damping-off disease of Naga King Chilli in three growing districts of Nagaland are presented in Table 4.5. In the year 2014, incidence of damping-off disease was found highest in Medziphema village (10.83%) of Dimapur district. At district level, Dimapur recorded the highest incidence (7.33%) followed by Kohima (4.50%) and Peren (4.11%). Similarly, in the year 2015, the incidence of damping-off disease of Naga King Chilli was recorded highest from Medziphema (9.17%) and at district level the highest incidence of 6.83% was recorded from Dimapur followed by Peren at 5.00% and Kohima (4.83%). It is evident from the pooled data that among all the villages, Medziphema (10.00%) recorded highest incidence followed by Sirhima (7.08%) of Dimapur district and the lowest incidence of damping-off was recorded from Athibung (2.25%) of Peren district. Among the three districts, Dimapur (7.08%) recorded the highest incidence, followed by Kohima and Peren (4.67% and 4.56%, respectively).

From Table 4.5 it is observed that, there is not much difference in the incidence of damping-off among the different locations. One reason maybe damping-off occurs in both cool and warm soils. The finding was found to be in agreement with the findings of Olsen (1988) who reported that *Rhizoctonia solani*, the causal agent of damping-off disease is active at different soil temperature. Jiskani *et al.* (2007) reported that *Rhizoctonia solani* Kuhn was isolated as the predominant damping-off fungus with highest frequency (60.0%) from the overall tomato fields of Hyderabad districts.

4.1.3.2. Per cent disease index

During the year 2014 and 2015, survey on damping-off in Naga King Chilli was carried out in the villages of Dimapur, Kohima and Peren district. PDI calculated during the course of investigation are presented in Table 4.6. In

the year 2014, the highest mean PDI was recorded from Sirhima village (31.66) of Dimapur district while the lowest mean PDI was recorded from Zhadima (8.33) of Kohima district at the village level. While at district level, highest mean PDI was recorded from Dimapur (24.72) followed by Peren (18.89) and Kohima (15.28). During the year 2015, the highest mean PDI of damping-off in Naga King Chilli was recorded from Chalkot (34.22) village of Peren district followed by Sirhima village (Dimapur district) (30.00) among the villages. At district level Dimapur (25.14) recorded the highest mean PDI followed by Peren (20.85) and Kohima (16.74). It is evident from pooled data that Chalkot village (31.69%) of Peren district was recorded with highest PDI and lowest was recorded from Zhadima village (9.45) of Kohima district. Among the district highest PDI was recorded from Dimapur (24.93) followed by Peren (19.87) and Kohima (16.01).

The PDI recorded during the year 2014 and 2015 for damping-off on Naga King Chilli indicates that there was significant variation of mean PDI among the districts of Nagaland. The reasons maybe cause of the variations in the topography, climatic conditions (Table 3.1) and other factors as it has been reported by earlier worker Cram (2003) that the severity of damping-off is highly dependent on whether the soil moisture, temperature, and pH are more beneficial to the growth of the host or the pathogen and other factors that can affect development of the disease, aside from host susceptibility and pathogen populations, including the level of available nitrogen, presence of antagonistic microorganisms, and variation in pathogenicity within a fungal species.

Table 4.5 Incidence of damping-off disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of damping-off disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	15.00	12.00	10.83	7.33	9.17	6.83	13.50	10.00	7.08
		F2	0.00	5.50					2.75		
		F3	17.5	10.00					13.75		
	Sirhima	F1	10.00	8.50	7.17		7.00		9.25	7.08	
		F2	6.50	2.50					4.50		
		F3	5.00	10.00					7.50		
	Tsiepama	F1	5.00	0.00	4.00		4.33		2.50	4.17	
		F2	0.00	8.00					4.00		
		F3	7.00	5.00					6.00		
Kohima	Thekrujuma	F1	5.50	7.50	5.17	4.50	6.00	4.83	6.50	5.58	4.67
		F2	0.00	8.00					4.00		
		F3	10.00	2.50					6.25		
	Zhadima	F1	0.00	8.00	1.67		3.50		4.00	2.58	
		F2	0.00	0.00					0.00		
		F3	5.00	2.50					3.75		
	Mengujuma	F1	5.00	5.00	6.67		5.00		5.00	5.83	
		F2	0.00	5.00					2.50		
		F3	15.00	5.00					10.00		
Peren	Athibung	F1	0.00	2.50	1.67	4.11	2.83	5.00	1.25	2.25	4.56
		F2	5.00	6.00					5.50		
		F3	0.00	0.00					0.00		
	Chalkot	F1	5.00	0.00	5.67		4.50		2.50	5.08	
		F2	7.00	5.50					6.25		
		F3	5.00	8.00					6.50		
	Songlhuh	F1	10.00	8.00	5.00		7.67		9.00	6.33	
		F2	5.00	10.00					7.50		
		F3	0.00	5.00					2.50		

Table 4.6 Percent disease index (PDI) of damping-off of Naga King Chilli during 2014 and 2015

District	Village	2014		2015		Pooled	
		PDI of the village	Mean PDI of the district	PDI of the village	Mean PDI of the district	Village	District
Dimapur	Medziphema	20.83	24.72	22.10	25.14	21.47	24.93
	Sirhima	31.66		30.00		30.83	
	Tsiepama	21.66		23.33		22.50	
Kohima	Thekrujuma	12.50	15.28	11.00	16.74	11.75	16.01
	Zhadima	8.33		10.56		9.45	
	Mengujuma	25.00		28.66		26.83	
Peren	Athibung	10.00	18.89	13.33	20.85	11.67	19.87
	Chalkot	29.16		34.22		31.69	
	Songlhuh	17.50		15.00		16.25	

4.1.4 Fusarium wilt of Naga King Chilli

One of the most prominent symptoms observed in this course of investigation was the upward and inward rolling of leaves followed by wilting of the infected Naga King Chilli plants. In infected plants (Plate 4), leaves turned yellow, discolouration of the stem vascular tissues, the plant growth was stunted and lastly the plant wilted and died. Joshi *et al.* (2012) and Shahzad (2014) also reported similar symptoms of Fusarium wilt disease in chilli which included leaf chlorosis, vascular discoloration, and wilting of plants.

4.1.4.1 Disease incidence

Survey data on incidence of Fusarium wilt on Naga King Chilli are presented in Table 4.7. The table depicts that the incidence of Fusarium wilt was highest (4.17%) in Medziphema (Dimapur district) and Athibung (Peren district) at village level, while Dimapur (2.78%) recorded the highest incidences among the districts, followed by Kohima (2.17%) and Peren (2.11%) during the surveyed year of 2014. Similarly in 2015, Medziphema (6.00) recorded the highest incidence of Fusarium wilt disease among the villages. And among the districts, Dimapur (4.28%) was observed with the highest mean incidence followed by Kohima (2.28%) and Peren (2.22%). Pooled data of 2014 and 2015 showed that the highest incidence of Fusarium wilt of Naga King Chilli was recorded from Medziphema village (5.08%) followed by Athibung (4.33%) and the lowest in Chalkot (0.42%) village of Peren district. At district level Dimapur (3.53%) recorded the highest incidence that was followed by Kohima (2.22%) and Peren (2.17%).

Joshi *et al*. (2012) reported that wilt disease is influenced by environmental conditions like soil temperature, soil moisture, soil type which influences soil microbial populations. So this maybe the reason for the variation in incidence among the different locations of the three districts of

Nagaland under study. Manikandan and Raguchander (2014) reported that Fusarium wilt incidence ranged from 19 % to 45% during the survey at different growth crop stages of tomato in ten districts of Tamil Nadu.

4.1.4.2 Per cent disease index

Table 4.8 represents the PDI of Fusarium wilt disease recorded from Naga King Chilli growing villages of Dimapur, Kohima and Peren district during the year 2014 and 2015. During 2014, Thekrujuma village (Kohima district) (15.22) recorded the highest mean PDI of Fusarium wilt among the villages whereas no PDI was recorded from Chalkot (Peren district) at village level. Among the districts, Dimapur (10.56) recorded the highest mean PDI followed by Kohima (9.81) and Peren (8.11). In 2015, Medziphema village (19.83) of Dimapur district recorded the highest mean PDI among the villages, whereas the lowest mean PDI (0.83) was recorded from Chalkot. Among the districts, Dimapur (11.84) was recorded with the highest mean PDI seconded by Kohima (9.07) and the lowest mean PDI was recorded from Peren (8.70). Pooled data reveals that Medziphema (Dimapur district) (16.75) was recorded with highest PDI for Fusarium wilt disease in Naga king Chilli among the villages whereas lowest was recorded from Chalkot (Peren district) (2.08). Among the districts, Dimapur (11.20) recorded the highest mean PDI seconded by Kohima (9.12) and lowest mean PDI was recorded from Peren (8.40).

From the survey data (Table 4.8) it was observed the PDI of Fusarium wilt in Naga King Chilli recorded from different villages of Kohima, Dimapur and Peren districts of Nagaland during 2014 and 2015 ranged from 0.00 to 27.33, which indicates significant variation in the PDI. Goldberg (2010) reported that the disease incidence and severity vary from year to year and

Table 4.7 Incidence of Fusarium wilt disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of Fusarium wilt disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	0.00	3.50	4.17	2.78	6.00	4.28	1.75	5.08	3.53
		F2	7.50	7.00					7.25		
		F3	5.00	7.50					6.25		
	Sirhima	F1	5.00	4.00	3.33		3.50		4.50	3.42	
		F2	2.50	6.50					4.50		
		F3	2.50	0.00					1.25		
	Tsiepama	F1	0.00	4.50	0.83		3.33		2.25	2.08	
		F2	2.50	0.00					1.25		
		F3	0.00	5.50					2.75		
Kohima	Thekrujuma	F1	7.50	8.00	3.33	2.17	4.17	2.28	7.75	3.75	2.22
		F2	2.50	0.00					1.25		
		F3	0.00	4.50					2.25		
	Zhadima	F1	2.00	0.00	0.67		1.83		1.00	1.25	
		F2	0.00	5.50					2.75		
		F3	0.00	0.00					0.00		
	Mengujuma	F1	0.00	0.00	2.50		0.83		0.00	1.67	
		F2	0.00	2.50					1.25		
		F3	7.50	0.00					3.75		
Peren	Athibung	F1	7.50	6.00	4.17	2.11	4.50	2.22	6.75	4.33	2.17
		F2	2.50	6.00					4.25		
		F3	2.50	1.50					2.00		
	Chalkot	F1	0.00	0.00	0.00		0.83		0.00	0.42	
		F2	0.00	0.00					0.00		
		F3	0.00	2.50					1.25		
	Songlhuh	F1	0.00	1.50	2.17		1.33		0.75	1.75	
		F2	0.00	0.00					0.00		
		F3	6.50	2.50					4.50		

Table 4.8 Percent disease index (PDI) of Fusarium wilt of Naga King Chilli at different stages of the crop growth during 2014 and 2015

District	Village	2014				2015				Pooled	
		Vegetative Stage	Fruiting Stage	Mean PDI		Vegetative Stage	Fruiting Stage	Mean PDI		Village	District
				Village	District			Village	District		
Dimapur	Medziphema	0.00	27.33	13.67	10.56	6.33	33.33	19.83	11.84	16.75	11.20
	Sirhima	0.00	26.00	13.00		0.00	18.15	9.08		11.04	
	Tsiepama	0.00	10.00	5.00		0.00	13.21	6.61		5.80	
Kohima	Thekrujuma	21.11	9.33	15.22	9.18	12.33	12.55	12.44	9.07	13.83	9.12
	Zhadima	0.00	10.66	5.33		0.00	11.20	5.60		5.47	
	Mengujuma	6.66	7.33	7.00		8.32	10.00	9.16		8.08	
Peren	Athibung	7.33	20.66	14.00	8.11	12.00	16.11	14.06	8.70	14.03	8.40
	Chalkot	0.00	0.00	0.00		0.00	8.31	4.16		2.08	
	Songlhuh	8.66	12.00	10.33		7.33	8.45	7.89		9.11	

from one location to another. The disease significance also varies with host susceptibility, pathogen virulence, soil type, and environmental conditions. The highest PDI was recorded from Dimapur district (11.20). This may be due to Dimapur district having higher temperature (Table 3.1) during the course of investigation as compared to other districts. This was found to be in agreement with the findings of Agrios (1969) who reported higher severity of Fusarium wilt diseases of tomato in warm climatic regions.

4.1.5 Stem rot of Naga King Chilli

In Naga King Chilli plant, the symptoms were predominant during the seedling stage which is depicted in Plate 5. The initial symptoms included mycelium covering the plant stem just near the soil surface sometimes even extending into the soil and on organic debris, formation of small brown spherical sclerotia followed by rotting of the stem tissues causing the seedling to break at the rotting part and fall down. The production of abundant white mycelium, and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease.

Mehrotra and Aneja (1990) also reported the cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debris. Wilson (1953) also described similar symptoms of stem rot in groundnut caused by *Sclerotium rolfsii*.

4.1.5.1 Disease incidence

The incidence of stem rot of Naga King Chilli under different districts of Nagaland recorded during the year 2014 and 2015 are presented in Table 4.9. During the kharif season of 2014, among the villages Sirhima (3.00%) recorded highest mean incidence of stem rot disease followed by Medziphema and Tsiepama both at 2.50% (Dimapur district), while in Zhadima (Kohima

district), Athibung and Songlhuh (Peren district) no incidence was recorded. Dimapur (2.67%) recorded the highest incidence of stem rot followed by Kohima (1.22%) and lowest incidence was recorded from Peren (0.28%) among the districts. During the following year 2015, Thekrujuma village (2.33%) of Kohima district recorded the highest incidence, whereas no incidences were recorded from Chalkot and Songlhuh villages (Peren district). And at district level Dimapur (1.56%) recorded the highest incidence followed by Kohima (1.17%) and Peren (0.28%). Pooled data of 2014 and 2015 indicates that, of all the villages, Tsiepama (2.33%) (Dimapur district) reported the highest incidence followed by Thekrujuma (2.17%) (Kohima district), whereas no incidence was recorded from Songlhuh of Peren District. Among the districts highest incidence was observed from Dimapur (2.11%) followed by Kohima (1.19%) and lowest incidence from Peren (0.28%).

The mean incidence of stem rot of Naga King Chilli recorded during 2014 and 2015 ranged from 0.28% to 2.11% which was found to be in agreement with the findings of Dange (2006) and Rani (2016) who also reported stem rot incidence ranging from 4% to 12.8% from the districts of Andhra Pradesh and Telangana. The data on survey revealed that the incidence of stem rot of Naga King Chilli varied from locality to locality; it may be varied due to agro climatological situations, cropping patterns and also cultural practices followed. Even, it could also be attributed to the existence of variability or pathogenic diversity present in the fungus (Muthukumar and Venkatesh 2013).

4.1.5.2 Per cent Disease Index

PDI of stem rot disease recorded in Naga King Chilli from three districts of Nagaland are presented in Table 4.10. From the Table it is noticed that during the 2014 Medziphema village (Dimapur district) recorded the highest

(17.50) mean PDI among the surveyed villages, no PDI was recorded from Zhadima (Kohima district), Athibung and Songlhuh village of Peren district. Among the districts, Dimapur (11.39) recorded the highest mean PDI followed by Kohima with PDI of 8.33 and Peren (3.61). During the year 2015 survey it was noticed that no PDI was recorded from Songlhuh village of Peren district, while Tsiepama (17.00) recorded the highest mean PDI among the villages and from the districts, Dimapur (11.85) recorded the highest PDI followed by Kohima (8.81) and Peren with the lowest PDI of 4.50. In pooled data, Thekrujuma (Kohima district) (14.08) recorded the highest mean PDI of stem rot disease in Naga king Chilli whereas there was no record of PDI from Chalkot of Peren district among the villages. Among the districts Dimapur (11.62) recorded the highest PDI which was seconded by Kohima (8.57) and least PDI recorded from Peren (4.06).

Dimapur district (11.62) recorded the highest PDI of stem rot in Naga King Chilli caused by *Sclerotium rolfsii* during the survey of 2014 and 2015. Villages under Dimapur district have higher temperature as given in Table 3.1, so this might be one of the reasons for the significantly higher PDI as compared to villages of other districts which have cooler climate. Harlapur, (1988) reported that *Sclerotium rolfsii* is well known polyphagous, non-target fungus, generally distributed in tropical and sub-tropical regions where average soil temperature of 30˚C prevails. Variations were also observed in the PDI recorded from different locations that were surveyed under different districts.

Table 4.9 Incidence of stem rot disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of stem rot disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	2.50	0.00	2.50	2.67	1.67	1.56	1.25	2.08	2.11
		F2	5.00	0.00					2.50		
		F3	0.00	5.00					2.50		
	Sirhima	F1	5.00	0.00	3.00		0.83		2.50	1.92	
		F2	0.00	2.50					1.25		
		F3	4.00	0.00					2.00		
	Tsiepama	F1	2.50	0.00	2.50		2.17		1.25	2.33	
		F2	2.50	3.50					3.00		
		F3	2.50	3.00					2.75		
Kohima	Thekrujuma	F1	0.00	3.50	2.00	1.22	2.33	1.17	1.75	2.17	1.19
		F2	2.50	2.00					2.25		
		F3	3.50	1.50					2.50		
	Zhadima	F1	0.00	0.00	0.00		0.50		0.00	0.25	
		F2	0.00	0.00					0.00		
		F3	0.00	1.50					0.75		
	Mengujuma	F1	0.00	2.00	1.67		0.67		1.00	1.17	
		F2	0.00	0.00					0.00		
		F3	5.00	0.00					2.50		
Peren	Athibung	F1	0.00	2.50	0.00	0.28	0.83	0.28	1.25	0.42	0.28
		F2	0.00	0.00					0.00		
		F3	0.00	0.00					0.00		
	Chalkot	F1	0.00	0.00	0.83		0.00		0.00	0.42	
		F2	2.50	0.00					1.25		
		F3	0.00	0.00					0.00		
	Songlhuh	F1	0.00	0.00	0.00		0.00		0.00	0.00	
		F2	0.00	0.00					0.00		
		F3	0.00	0.00					0.00		

Table 4.10 Percent disease index (PDI) of stem rot of Naga King Chilli during 2014 and 2015

District	Village	2014		2015		Pooled	
		PDI of the village	Mean PDI of the district	PDI of the village	Mean PDI of the district	Village	District
Dimapur	Medziphema	17.50	11.39	10.33	11.85	13.92	11.62
	Sirhima	5.83		8.22		7.03	
	Tsiepama	10.83		17.00		13.92	
Kohima	Thekrujuma	15.83	8.33	12.32	8.81	14.08	8.57
	Zhadima	0.00		3.12		1.56	
	Mengujuma	9.16		11.00		10.08	
Peren	Athibung	0.00	3.61	8.40	4.50	4.20	4.06
	Chalkot	10.83		5.11		7.97	
	Songlhuh	0.00		0.00		0.00	

4.1.6 Bacterial wilt of Naga King Chilli

Symptoms (Plate 6a) of bacterial wilt disease in Naga King Chilli (*Capsicum chinense*) plant was similar to that of *Capsicum annum*. The disease occurred in scattered plants or groups of plants in the field. Wilting started with the youngest leaves, there was inward rolling of leaves and yellowing, followed by sudden and permanent wilt. The vascular tissues in the lower stem of wilted plants showed a dark brown discoloration. Diseased stems when cut and placed in a clear glass of water showed steady, grey bacterial ooze (Plate 6b) coming from the cut end. This bacterial ooze was one of the key features in diagnosing this disease and distinguishing it from Fusarium wilt disease.

Similar symptoms of bacterial wilt disease observed in Naga King Chilli were also reported by other workers *viz.* Momol *et al.* (2001) described similar type of symptoms of bacterial wilting in tomato plants. Sarkar and Chaudhuri (2016) also reported solanaceous crops exhibiting similar type of symptoms.

4.1.6.1 Disease incidence

Incidence of bacterial wilt of Naga King Chilli in different districts under study during the year 2014 and 2015 are presented in Table 4.11. During the year 2014, the highest incidence of bacterial wilt was recorded from Medziphema (23.33%) of Dimapur district among the villages. And at district level Dimapur (9.44%) recorded the highest incidence followed by Peren (1.11%) while no incidence was recorded from Kohima. It was observed that some villages *viz.* Tsiepama (Dimapur district), Songlhuh (Peren district) and Tsiepama, Zhadima and Mengujuma of Kohima district did not record any incidence of stem rot in Naga King Chilli. Similarly, in the year 2015, no incidence was recorded from Tsiepama of Dimapur district and Zhadima, Mengujuma of Kohima district, while Medziphema (23.33) of Dimapur district showed the highest incidence among the villages. Of all the districts Dimapur

(8.89%) recorded the highest incidence seconded by Peren (2.78%) and the lowest incidence was recorded from Kohima (0.83%). Pooled data of 2014 and 2015 indicate that Medziphema (23.33) (Dimapur district) had the highest incidence of bacterial wilt disease of Naga King Chilli among the villages and no incidence was recorded from Tsiepama (Dimapur district), Zhadima and Mengujuma of Kohima district. At district level Dimapur (9.17%) recorded highest incidence followed by Peren (1.94) and Kohima (0.42%).

The survey data on bacterial wilt disease caused by *Ralstonia solanacearum* in Naga King Chilli during the year 2014 and 2015 reveals that the disease incidence varied significantly from locality to locality under different district of Nagaland. Variations in the incidence and prevalence of bacterial wilt are attributable to the diversity of *R. solanacearum* strains, variations in soil types (Aslam *et al.* 2015). Dimapur district (9.17%) recorded the highest incidence of the disease and this may be due to higher prevailing temperature (Table 3.1) as compared to other districts. The bacterial wilt pathogen *R. solanacearum* has been reported to survive in the soil for longer periods of time and disease development is favoured by warm and humid soil conditions (Ahmed *et al*., 2013).

4.1.6.2 Per cent Disease Index

Table 4.12 represents the PDI of bacterial wilt of Naga King Chilli recorded from different villages under Dimapur, Kohima and Peren districts of Nagaland during 2014 and 2015. In 2014, Medziphema village (25.83) recorded highest mean PDI among the villages and no PDI was recorded from any of the villages at vegetative stage. At fruiting stage no PDI were recorded from village's *viz.* Tsiepama (Dimapur district), Thekrujuma, Zhadima, Mengujuma (Kohima district) and Songlhuh (Peren district). Among the districts Dimapur (11.80) was recorded the highest mean PDI followed by

Peren (10.83) and no PDI recorded from Kohima. During the following year in 2015, except for Medziphema (Dimapur district) (22.00) no PDI was recorded from other villages at vegetative stage. At fruiting stage there were no record of PDI in Tsiepama (Dimapur district), Zhadima and Mengujuma village (Kohima district), whereas mean PDI of Medziphema (44.61) was recorded highest among the villages. At district level Dimapur (19.11) recorded the highest PDI followed by Peren (15.70) and Kohima (5.61). Under pooled data, there was no record of PDI from Tsiepama (Dimapur district), Zhadima and Mengujuma village (Kohima district), while highest mean PDI was recorded from Medziphema (Dimapur district) (35.22). Among the districts, Dimapur (15.46) was with the highest PDI seconded by Peren (13.27) and lowest recorded from Kohima (2.81).

From Table 4.12 it is observed that there is significant difference of PDI of bacterial wilt in Naga King Chilli caused by *Ralstonia solanacearum* in all the survey locations of Nagaland during the year 2014 and 2015. These variations of wilt severity may be attributed to the diversity of *R. solanacearum* isolates and also due to the variations in soil factors prevailing in different locations surveyed. Differences of wilt incidence and severity were also reported in eggplant due to the great diversity of host plants affected by this pathogen, phenotype and genotype of *R. solanacearum*, its wide geographical distribution, and the range of environmental conditions conducive to bacterial wilt (Rahman *et al*., 2010). Dimapur district (15.46) recorded the highest PDI this may be severity of disease is related with the prevailing temperature and soil moisture at the time of transplanting and sowing (Haywad, 1991).

Table 4.11 Incidence of bacterial wilt disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of bacterial wilt disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	10.00	5.00	23.33	9.44	23.33	8.89	7.50	23.33	9.17
		F2	15.00	55.00					35.00		
		F3	45.00	10.00					27.50		
	Sirhima	F1	0.00	0.00	5.00		3.33		2.50	4.17	
		F2	5.00	10.00					5.00		
		F3	10.00	0.00					5.00		
	Tsiepama	F1	0.00	0.00	0.00		0.00		0.00	0.00	
		F2	0.00	0.00					0.00		
		F3	0.00	0.00					0.00		
Kohima	Thekrujuma	F1	0.00	0.00	0.00	0.00	1.67	0.83	0.00	1.25	0.42
		F2	0.00	5.00					2.50		
		F3	0.00	0.00					0.00		
	Zhadima	F1	0.00	0.00	0.00		0.00		0.00	0.00	
		F2	0.00	0.00					0.00		
		F3	0.00	0.00					0.00		
	Mengujuma	F1	0.00	0.00	0.00		0.00		0.00	0.00	
		F2	0.00	0.00					0.00		
		F3	0.00	0.00					0.00		
Peren	Athibung	F1	0.00	0.00	1.67	1.11	3.33	2.78	0.00	2.50	1.94
		F2	5.00	0.00					2.50		
		F3	0.00	10.00					5.00		
	Chalkot	F1	0.00	5.00	1.67		3.33		2.50	2.50	
		F2	0.00	5.00					2.50		
		F3	5.00	0.00					2.50		
	Songlhuh	F1	0.00	0.00	0.00		1.67		0.00	0.83	
		F2	0.00	5.00					2.50		
		F3	0.00	0.00					0.00		

Table 4.12 Percent disease index (PDI) of bacterial wilt of Naga King Chilli at different stages of the crop growth during 2014 and 2015

District	Village	2014				2015				Pooled	
		Vegetative Stage	Fruiting Stage	Mean PDI		Vegetative Stage	Fruiting Stage	Mean PDI		Village	District
				Village	District			Village	District		
Dimapur	Medziphema	0.00	51.66	25.83	11.80	22.00	67.22	44.61	19.11	35.22	15.46
	Sirhima	0.00	19.16	9.58		0.00	25.42	12.71		11.15	
	Tsiepama	0.00	0.00	0.00		0.00	0.00	0.00		0.00	
Kohima	Thekrujuma	0.00	0.00	0.00	0.00	0.00	33.68	16.84	5.61	8.42	2.81
	Zhadima	0.00	0.00	0.00		0.00	0.00	0.00		0.00	
	Mengujuma	0.00	0.00	0.00		0.00	0.00	0.00		0.00	
Peren	Athibung	0.00	20.00	10.00	10.83	0.00	21.22	10.61	15.70	10.31	13.27
	Chalkot	0.00	45.00	22.50		0.00	55.00	27.50		25.00	
	Songlhuh	0.00	0.00	0.00		0.00	18.00	9.00		4.50	

4.1.7 Leaf veinal mottle of Naga King Chilli

Most prominent symptoms (Plate 7) in Naga King Chilli plants suffering from leaf veinal mottle disease included mottling, vein banding, narrowing, crinkled and distortion of leaves followed by stunted growth. Infected young plants become stunted and had dark-green streaks on their stems and branches. The fruits produced by infected plants were mottled, distorted and size of some fruits was greatly reduced. Green peach aphid (*Myzus persicae*) (Plate 8) was prominently noticed to be in close association with the plants infected by *Chilli veinal mottle virus* (ChiVMV) throughout the course of investigation. This created an assumption that green peach aphid (*Myzus persicae*) is facilitating as vector for transmission of ChiVMV. In support to the made assumption, Ong *et al*. (1979) and Fajinmi *et al.* (2011) reported of aphids species transmitting ChiVMV in chilli in non-persistent manner. Symptoms recorded during the study of Naga King Chilli plants suffering from leaf veinal mottle disease were found to be in agreement with the findings of other workers (Brunt & Kenten, 1971; Fajinmi *et al*., 1998; Olawale *et al.*, 2015)

4.1.7.1 Disease incidence

Survey on incidence (Table 4.13) of leaf veinal mottle disease of Naga King Chilli was carried out during 2014 and 2015. In the year 2014, Athibung from Peren district (44.17%) recorded the highest incidence at village level and lowest was recorded from Mengujuma (18.33%) (Kohima district). Peren (34.72%) recorded highest incidence of leaf veinal mottle followed by Dimapur (28.39%) and Kohima (26.39%) at district level. For the year 2015, among all the villages, Athibung (36.33%) recorded highest incidence and lowest incidence (15.27%) was recorded from Mengujuma village. While, among the districts Peren (32.89%) recorded highest incidence followed by Dimapur (27.89%) and the lowest was recorded from Kohima (26.28%). From

the pooled data it is clear that Athibung (40.25%) recorded the highest incidence of leaf veinal mottle disease of Naga King Chilli followed by Zhadima (33.42%) (Kohima district) and lowest was recorded from Mengujuma (16.75%) at the village level. Among the districts Peren (33.81%) recorded highest incidence seconded by Dimapur (28.14%) and lowest recorded from Kohima (26.33%).

Banerjee *et al.* (2014) made the first report of occurrence of *Chilli veinal mottle virus* (ChiVMV) under the genus *Potyvirus* in Naga chilli (*Capsicum chinense*) in Meghalaya (North-East India). From Table 4.13 it is observed that there is a variation in the incidence of leaf veinal mottle in Naga King Chilli under different district of Nagaland during the year 2014 and 2015. Fajinmi and Odebode (2010) reported that alternative weed host coupled with a good breeding environment for the vectors of the virus aid effective transmission which leads to the high prevalence and incidence of leaf veinal mottle disease. Hidayat *et al*. (2012) reported incidence of *chili veinal mottle virus* ranging from 22 to 77% in areas surveyed in Indonesia during 2008 and 2009.

4.1.7.2 Per cent Disease Index

The PDI values for leaf veinal mottle of Naga King Chilli recorded during the year 2014 and 2015 are presented in Table 4.14. In the year 2014, highest mean PDI was recorded from Zhadima village (Kohima district) (72.00) among the villages, which was closely followed by Sirhima (71.33) of Dimapur district and the least mean PDI was recorded from Mengujuma (Kohima district) (45.33). At district level, Peren (66.78) recorded highest mean PDI followed by Dimapur (63.11) and Kohima (61.00). During 2015, among the villages the highest mean PDI was recorded from Zhadima (71.83) of Kohima district closely followed by Medziphema (Dimapur district) (70.12). Dimapur district (66.04) recorded the highest mean PDI followed by

Peren and Kohima district (64.86 and 56.53 respectively). According to the pooled data the highest PDI (71.91) of leaf veinal mottle disease of Naga King Chilli was recorded from Zhadima village (Kohima district) among all the surveyed villages and the lowest mean PDI was recorded from Mengujuma village (46.58) of Kohima district. And at district level, the highest mean PDI was recorded from Peren (65.82) which was followed by Dimapur (64.57) and Kohima (58.76) recorded the lowest PDI.

All the districts recorded significantly different mean PDI during the survey of leaf veinal mottle in Naga King Chilli for the year 2014 and 2015. The mean PDI ranged from 56.53 to 66.78. Fajinmi (2010) also reported severity of pepper (*Capsicum annuum* L.) in Nigeria caused by *Pepper veinal mottle virus* ranging from 34.48 to 43.85%. Climate and vegetation in the different regions play a major role in determining the incidence and severity of PVMV infection on pepper in the fields. Aldyhim, and Khalil (1993) reported the ability of the isolated PVMV strain to cause infection on alternative host plants and adaptive ability of the virus on weed host plants in the two agro-ecological zone characterized by thick vegetation and warm humid climate, with the presence of many secondary host plants for the virus and vectors aids its infective capability and spread of the virus on pepper plants.

Table 4.13 Incidence of leaf veinal mottle disease of Naga King Chilli in Nagaland under different districts during 2014 and 2015

District	Village	Field	2014	2015	Mean incidence of leaf veinal mottle disease				Pooled of field	Pooled of village	Pooled of district
					2014		2015				
			DI%	DI%	Village	District	Village	District			
Dimapur	Medziphema	F1	27.50	33.50	30.83	28.39	30.00	27.89	30.50	30.42	28.14
		F2	22.50	30.50					26.50		
		F3	42.50	26.00					34.25		
	Sirhima	F1	15.50	21.50	27.67		27.00		18.50	27.33	
		F2	37.50	22.00					29.75		
		F3	30.00	37.50					33.75		
	Tsiepama	F1	35.00	10.00	26.67		26.67		22.50	26.67	
		F2	30.00	35.00					32.50		
		F3	15.00	35.00					25.00		
Kohima	Thekrujuma	F1	35.00	42.00	26.67	26.39	31.00	26.28	38.50	28.83	26.33
		F2	20.00	28.50					24.25		
		F3	25.00	22.50					23.75		
	Zhadima	F1	42.50	34.50	34.17		32.67		38.50	33.42	
		F2	27.50	35.50					31.50		
		F3	32.50	28.00					30.25		
	Mengujuma	F1	17.50	15.50	18.33		15.17		16.50	16.75	
		F2	20.00	19.50					19.75		
		F3	17.50	10.50					14.00		
Peren	Athibung	F1	35.00	45.50	44.17	34.72	36.33	32.89	40.25	40.25	33.81
		F2	55.00	22.00					38.50		
		F3	42.50	41.50					42.00		
	Chalkot	F1	30.00	40.00	27.50		29.17		35.00	28.33	
		F2	25.00	22.00					23.50		
		F3	27.50	25.50					26.50		
	Songlhuh	F1	40.00	35.50	32.50		33.17		37.75	32.83	
		F2	27.50	35.50					31.50		
		F3	30.00	28.50					29.25		

Table 4.14 Percent disease index (PDI) of leaf veinal mottle of Naga King Chilli at different stages of the crop growth during 2014 and 2015

District	Village	2014				2015				Pooled	
		Vegetative Stage	Fruiting Stage	Mean PDI		Vegetative Stage	Fruiting Stage	Mean PDI		Village	District
				Village	District			Village	District		
Dimapur	Medziphema	52.66	73.33	63.00	63.11	62.12	78.11	70.12	66.04	66.56	64.57
	Sirhima	70.00	72.66	71.33		58.44	59.56	59.00		65.17	
	Tsiepama	61.33	48.66	55.00		70.34	67.67	69.01		62.00	
Kohima	Thekrujuma	59.33	72.00	65.67	61.00	45.66	54.21	49.94	56.53	57.80	58.76
	Zhadima	70.66	73.33	72.00		66.43	77.22	71.83		71.91	
	Mengujuma	32.66	58.00	45.33		29.34	66.32	47.83		46.58	
Peren	Athibung	56.00	81.33	68.67	66.78	45.78	78.81	62.30	64.86	65.48	65.82
	Chalkot	48.00	78.66	63.33		52.11	80.00	66.06		64.69	
	Songlhuh	58.00	78.66	68.33		54.78	77.67	66.23		67.28	

4.2 Identification of disease causing agents

4.2.1 *Colletotrichum capsici* (anthracnose disease)

The colony colour of *Colletotrichum capsici* on PDA plates (Plate 9a) appeared as dark grey. Under the microscopic view, setae were found abundantly which was long, rigid, bristle like, septate and dark brown in colour (Plate 9b). The conidia **(**Plate 9c) appeared falcate, fusiform, hyaline, measuring 16-25 in length and 3.25-3.8µm in breadth.

Ghosh *et al.* (2016) also reported that the conidia of *Colletotrichum* isolate from soybean and chilli were falcate, fusiform with acute apices and their size ranged from 19.65–21.00µm in length and 3.0–3.5µm in breadth. Similar findings were also reported by Sangdee *et al.* (2011) and Nagamani *et al.* (2006).

4.2.2 *Cercospora capsici* (Cercospora leaf spot disease)

Colony colour of *Cercospora capsici* appeared whitish grey with more greyish at the centre potion on PDA plates as illustrated in Plate 10a. Conidia (Plate 10b) appeared long, cylindrical to filiform, straight to slightly curved, indistinctly multiseptate, pale olivaceous, measuring 81.25-162.5µm in length and 3.25-4µm in breadth. These characteristics were found in agreement with the findings of Meon (1990) and Bhat *et al.* (2008).

4.2.3 *Rhizoctonia solani* (damping-off disease)

Cultures of *Rhizoctonia solani* appeared brown colour on PDA plates (Plate 11a**)**. Under microspore, hyphae appeared pale brown, branched with nearly right-angled side branches constricted basally, septated closely between main hyphae and side branches as depicted in Plate 11b. The hyphal breadth measuring 3.25-4.5µm. Sclerotia brown to dark brown, various in shape measuring 1-3mm in diameter. The morphological characteristics described by

Moni *et al.* (2016) and Watanabe (2002) were in confirmation with the morphological characteristics observed in this investigation.

4.2.4 *Fusarium oxysporum* (Fusarium wilt disease)

Fusarium oxysporum appeared white in colour with light pinkish tinge on PDA plates (Plate 12a). Macroconidia (Plate 12b) 2-6 septate, spindle to fusiform, curved or almost straight, pointed at both ends. The conidia measured 25-58µm in length and 3.45-5.40µm in breadth. Conidiophores (Plate 12c) unbranched or sparsely branched, hyaline, simple, short, not well differentiated from hyphae. Hussain *et al.* (2012) also reported slightly similar type of macroconidial size of *Fusarium oxysporum* ranged from 20.27 - 40.50 µm and 5.00 - 6.75µm. Booth (1971) also reported morphological characteristics which were in agreement with that of *Fusarium oxysporum* observed during the course of this investigation.

4.2.5 *Sclerotium rolfsii* (stem rot disease)

Sclerotium rolfsii when cultured in PDA plates (Plate 13a), appeared as whitish colony with filamentous type of growth. Sclerotia initially white in colour, later becoming light to dark brown at maturity with diameter of 1-2mm. *Sclerotium rolfsii* lacks asexual fruiting bodies and conidia. The hyphae are branched, with side branches septated very closely near the main hyphae and constricted basally as depicted in Plate 13b. Size of hyphae measured 3.25-5µm in diameter. The morphological characteristics of *Sclerotium rolfsii* causing stem rot disease in Naga King Chilli was found in agreement with the findings of Kumar *et al*. (2014) and Mahadevakumar *et al.* (2016).

4.2.6 *Ralstonia solanacearum* (bacterial wilt disease)

In Gram staining test, the isolate of *Ralstonia solanacearum* retained pink colour which indicates that the bacteria are Gram negative (Plate 14b)

which was also reported by Rahman *et al*. (2010). Heavy growth of *R. solanacearum* was observed in 2% NaCl medium with weak growth in 5% and 10% NaCl medium and no visible growth of *R. solanacearum* was observed in 20% NaCl medium (Plate 14c). The absence of growth in 20% NaCl medium confirmed the characteristics of *R. solanacearum* as reported by Ocho (2006). *R. solanacearum* did not hydrolyse starch and gelatine as no clear zone zone surrounding the bacterial growth was observed when the plates were flooded with IKI solution (for starch hydrolysis) or with mercuric chloride solution (for gelatin hydrolysis), this was found in agreement with the findings of Seleim *et al.* (2014) and Ocho (2006). Catalase test (Plate 14d) for *R. solanacearum* was recorded as positive since it produced bubbles upon immersion into the hydrogen peroxide solution which is in agreement with the results of Sands (1990) and Ocho (2006). Appearance of purple colour soon after the colonies of *R. solanacearum* were patched onto the filter paper moistened with fresh oxidase reagent (1% tetra methyl-para-phenylenediamine dihydrochloride) (Plate 14e)confirmed the test as positive. This purple colour appearing within 30 seconds after addition of culture to the oxidase reagent was confirmed as one of the biochemical characteristics of *R. solanacearum* by Seleim *et al.* (2014).

4.2.7 *Chilli veinal mottle virus* (leaf veinal mottle disease)

The total RNA extracted from the leaf sample with symptoms of leaf veinal mottling was amplified by reverse transcription – polymerase chain reaction (RT-PCR). RT-PCR was carried out using *Potyvirus* genus specific degenerate primers *viz*., CIF/CIRev to amplify a ~700 bp region (Plate 15) of cylindrical inclusion protein (CI) domain (Ha et al. 2008) of *Potyvirus* open reading frame (ORF) (Ha *et al*. 2008). Agarose gel documentation confirmed the presence of PRSV (*Papaya ring spot virus*) was confirmed with the presence of >1 kb band for CP and 850bp for HC-Pro. The gel-purified

amplicons for ~700 bp was sequenced bi-directionally from Xcelris Genomics, Ahmedabad, Gujarat, India and it is given as below

CHILLI_CIF_S015716_E07_055

GMGGAMATCGGRTGTATATGCAGAGAGGGAGGGTATTATTGTTGAACCCACACGACCAC
TTGCAGAGAATGTGCATGCACAGTTATCTGCCAGTCCATTCCATCTAAACCCAACCCTCAT
GATGAGGAACAAGTCCGTGTTTGGTTCGACTCCAATCACGATCATGACAAGTGGATACGC
ACTGCATTATCTTGCAAACAATGCACAGCGCCTTAAAGAGTTCAGCTTTATACTGTTCGAT
GAGTGCCATGTTTTGGATGCTAGCGCAATGGCATTTAGATGTTTGTTGGCTGACAGGGAGT
TTGAAGGGAAGATTCTTAAGGTGTCAGCTACACCACCAGGGCGTGAAACTGAATTTTCCA
CACAGTATCCCGTCGAGCTTAAAACTGAAGAGCACCTTAGCTTCCAACAGTTTGTGGATG
CACAGGGCACGGGCGCTAATGCAGATGTTATAGATATTGCAGACAACATCCTCGTGTACG
TATCAAGCTATAATGAAGTGGATCAACTGTCAAAGCTGCTAGTTGACAGGAAGTTCAAAG
TGACGAAAGTTGATGGGAGGACAATGAAATCGGGAGCAACAGAGATTAAGACAGTTGGG
TCAAAACATAGGAAACACTTTATAGTTGCCACCAACATAATMGAAAAACGSCSGTMCCA
AAAA.

CHILLI_CIR_S015716_F07_053

CATTTWWRGTKGTTTCTATGTTTTGACCAACTGTCTTAATCTCTGTTGCTCCCGATTTCATT
GTCCTCCCATCAACTTTCGTCACTTTGAACTTCCTGTCAACTAGCAGCTTTGACAGTTGAT
CCACTTCATTATAGCTTGATACGTACACGAGGATGTTGTCTGCAATATCTATAACATCTGC
ATTAGCGCCCGTGCCCTGTGCATCCACAAACTGTTGGAAGCTAAGGTGCTCTTCAGTTTTA
AGCTCGACGGGATACTGTGTGGAAAATTCAGTTTCACGCCCTGGTGGTGTAGCTGACACC
TTAAGAATCTTCCCTTCAAACTCCCTGTCAGCCAACAAACATCTAAATGCCATTGCGCTAG
CATCCAAAACATGGCACTCATCGAACAGTATAAAGCTGAACTCTTTAAGGCGCTGTGCAT
TGTTTGCAAGATAATGCAGTGCGTATCCACTTGTCATGATCGTGATTGGAGTCGAACCAA
ACACGGACTTGTTCCTCATCATGAGGGTTGGGTTTAGATGGAATGGACTGGCAGATAACT
GTGCATGCACATTCtCTGCAAGTGGTCGTGTGGGTTCCAACAATAATACCCTCCCTCTCTTG
CATAATACACTCGGAAGTCCAGTCGAYTTCCCCSWCCCMMMCSSCCCCCACAATG.

Blast search confirmed 92% similarity with *Chilli veinal mottle virus* isolate Meghalaya-4, the isolate shared 79-97% identity with those of previously reported ChiVMV isolates including PRSV isolate. Reddy *et al*. (2004) reported six *Chilli veinal mottle virus* (CVMV) isolates collected from chilli growing areas of Karnataka and Tamil Nadu states of India and

confirmed its identity basing on host range, serological relationship, electron microscopy and phylogenetic analysis of coat protein sequences. Also the occurrence of *Chilli veinal mottle virus* (ChiVMV) in Naga King Chilli (*Capsicum chinense*) was also reported by Banerjee *et al.* (2014) in Meghalaya based on mechanical transmission assay, transmission electron microscopy, RT-PCR and sequence analysis.

4.3 Efficacy of bio-control agents *in vitro* against important pathogens of Naga King Chilli

Seven bio control agents *viz. Trichoderma asperellum, T. harzianum, T. koningii, T. virens, T. viride, Bacillus subtilis* and *Pseudomonas fluorescens* were tested *in vitro* using dual culture technique for their efficacy against some disease causing pathogens of Naga King Chilli *viz. Colletotrichum capsici* (anthracnose), *Fusarium oxysporum* (Fusarium wilt), *Sclerotium rolfsii* (stem rot) and *Rhizoctonia solani* (damping-off).

4.3.1 *In vitro* test for efficacy of bio control agents against *Colletotrichum capsici*

All the test bio control agents were found to have significant effect on radial growth of *Colletotrichum capsici*, as given in Table 4.15 and Plate16. The radial growth of *Colletotrichum capsici* in all the treatments ranged from 7.00-16.67mm with control having radial growth of 34.33mm. Of all the bio control agents, *Trichoderma harzianum* (79.61%) recorded highest per cent inhibition of the pathogen *C. capsici* followed by *T. viride* (77.67%), *T. virens* (74.76%), *T. koningii* (73.69%), *T. asperellum* (66.99%), *Bacillus subtilis* (65.05%) and the lowest per cent inhibition was recorded from *Pseudomonas fluorescens* (51.46%). Jagtap *et al.* (2013) reported that *T. harzianum* was found to be the most effective antagonist against *C. capsici* causing leaf spot disease in turmeric. Das *et al*. (2015) also reported that *T. harzianum* recorded

the highest per cent inhibition of 83.44%, which was followed by *T. viride* with per cent inhibition of 77.62%, *in vitro* against *C. capsici*, the causal agent of leaf spot of turmeric.

4.3.2 *In vitro* test for efficacy of bio control agents against *Fusarium oxysporum*.

Results on the *in vitro* test screened for the efficacy of bio control agents against *Fusarium oxysporum* are given in Table 4.16 and depicted in Plate 17.

Trichoderma koningii and *T. viride* recorded 100 per cent inhibition towards *Fusarium oxysporum* followed by *T. harzianum* (74.73%), *T. asperellum* (60.44%), *T. virens* (53.85%), *Pseudomonas fluorescens* (45.05%) and the lowest per cent inhibition was recorded from *Bacillus subtilis* (42.86%). Thaware *et al*. (2017) reported that *T. koningii* and *T. viride* caused 71.88% and 75.55% inhibition respectively, *in vitro* test against *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease in chickpea. Gupta *et al*. (2012) also reported that *T. viride*, inhibited the growth of *Fusarium oxysporum* f. sp. *melongenae* (Fusarium wilt of brinjal) by 90.30 per cent over control.

4.3.3 *In vitro* test for efficacy of bio control agents against *Sclerotium rolfsii*

In Table 4.17 the data presented are the results of *in vitro* test for efficacy of bio control agents against *Sclerotium rolfsii*. Here, the highest per cent inhibition was recorded from *Trichoderma harzianum* (85.07%) which was followed by *T. viride* with inhibition of 79.90%, *T. virens* and *Trichoderma koningii* with 77.61%, *T. asperellum* (75.62%) while lowest were recorded from *Pseudomonas fluorescens* and *Bacillus subtilis* with growth inhibition of 16.92% and 15.42% respectively (Plate 18). The present findings were found to be in agreement with the findings of Manu *et al.* (2012)

Table 4.15. *In vitro* test for efficacy of bio control agents against *Colletotrichum capsici* causing anthracnose disease of Naga King Chilli

Treatments	Radial growth (mm) and per cent inhibition of *Colletotrichum capsici* at 3 DAI	
	Growth (mm)	**PI (%)**
Treatment 1 = *Colletotrichum capsici* + *Trichoderma koningii*	9.00 (17.46)*	73.79
Treatment 2 = *C. capsici* + *T. viride*	7.67 (16.07)	77.67
Treatment 3 = *C. capsici* + *T. harzianum*	7.00 (15.34)	79.61
Treatment 4 = *C.capsici* + *T. asperellum*	11.33 (19.67)	66.99
Treatment 5 = *C. capsici* + *T. virens*	8.67 (17.12)	74.76
Treatment 6 = *C. capsici* + *Bacillus subtilis*	12.00 (20.27)	65.05
Treatment 7 = *C. capsici* + *Pseudomonas fluorescens*	16.67 (24.09)	51.46
Treatment 8 = *Colletotrichum capsici* (control)	34.33 (35.87)	
SEm±	0.84	
CD (P=0.05)	2.50	

*Figures in the parentheses are in arcsine transformed values

Table 4.16 *In vitro* test for efficacy of bio control agents against *Fusarium oxysporum* causing Fusarium wilt disease of Naga King Chilli

Treatments	Radial growth (mm) and per cent inhibition of *Fusarium oxysporum* at 3 DAI	
	Growth (mm)	**PI (%)**
Treatment 1 = *Fusarium oxysporum* + *Trichoderma koningii*	0.00 (5.85)*	100.00
Treatment 2 = *F. oxysporum* + *T. viride*	0.00 (5.85)	100.00
Treatment 3 = *F. oxysporum* + *T. harzianum*	7.67 (16.07)	74.73
Treatment 4 = *F. oxysporum* + *T. asperellum*	12.00 (20.27)	60.44
Treatment 5 = *F. oxysporum* + *T. virens*	14.00 (21.97)	53.85
Treatment 6 = *F. oxysporum* + *Bacillus subtilis*	17.33 (24.60)	42.86
Treatment 7 = *F. oxysporum* + *Pseudomonas fluorescens*	16.67 (24.09)	45.05
Treatment 8 = *Fusarium oxysporum* (control)	30.33 (33.42)	
SEm±	**1.36**	
CD (P=0.05)	**4.09**	

*Figures in the parentheses are in arcsine transformed values

Table 4.17 *In vitro* test for efficacy of bio control agents against *Sclerotium rolfsii* causing stem rot disease of Naga King Chilli

Treatments	Radial growth (mm) and per cent inhibition of *Sclerotium rolfsii* at 3 DAI	
	Growth (mm)	**PI (%)**
Treatment 1 = *Sclerotium rolfsii* + *Trichoderma koningii*	15.00 (22.79)*	77.61
Treatment 2 = *S. rolfsii* + *T. viride*	13.47 (21.53)	79.90
Treatment 3 = *S. rolfsii* + *T. harzianum*	10.00 (18.43)	85.07
Treatment 4 = *S. rolfsii* + *T. asperellum*	16.33 (23.84)	75.62
Treatment 5 = *S. rolfsii* + *T. virens*	15.00 (22.79)	77.61
Treatment 6 = *S. rolfsii* + *Bacillus subtilis*	56.67 (48.83)	15.42
Treatment 7 = *S. rolfsii* + *Pseudomonas fluorescens*	55.67 (48.25)	16.92
Treatment 8 = *Sclerotium rolfsii* (control)	67.00 (54.94)	
SEm±	**0.79**	
CD (P=0.05)	**2.37**	

*Figures in the parentheses are in arcsine transformed values

who also reported that among 5 bio control agents screened *in-vitro* against *S. rolfsii* causal organism of foot rot of ragi, *T. harzianum* (GKVK) isolate was found to be the most effective. Similarly Patel and Rakholiya (2016) also reported that *T. harzianum* recorded highest growth inhibition of 54.72% against *S. rolfsii*.

4.3.4 *In vitro* test for efficacy of bio control agents against *Rhizoctonia solani*

The inhibitory actions of bio control agents on the growth of *Rhizoctonia solani* are presented in Table 4.18 and illustrated in Plate 19. *Trichoderma koningii*, *T. viride*, *T. harzianum* and *T. virens* recorded the highest per cent inhibition of 92.20% followed by *T. asperellum* (87.50%), *Bacillus subtilis* (55.50%) whereas lowest per cent inhibition was recorded from *Pseudomonas fluorescens* (36.25%). Except for *T. asperellum* other four *Trichoderma* spp. were statistically at par and recorded the highest inhibition over the mycelia growth of *Rhizoctonia solani* (control). Kumar *et al.* (2016) reported *Trichoderma* spp. isolated from Jharkhand showed strong antagonistic potential which inhibited more than 50% mycelia growth of *Rhizoctonia solani*. Similarly, it has also been reported that *Trichoderma* spp. are the potential biocontrol agents which inhibit *R. solani* by direct confrontation through mycoparasitic or antibiosis or competition as well as inducing plant defense responses (Abbas *et al., 2017).*

Table 4.18 *In vitro* test for efficacy of bio control agents against *Rhizoctonia solani* causing damping-off disease of Naga King Chilli

Treatments	Radial growth (mm) and per cent inhibition of *Rhizoctonia solani* at 3 DAI	
	Growth (mm)	**PI (%)**
Treatment 1 = *Rhizoctonia solani* + *Trichoderma koningii*	1.04 (5.85)*	92.20
Treatment 2 = *R. solani* + *T. viride*	1.04 (5.85)	92.20
Treatment 3 = *R. solani* + *T. harzianum*	1.04 (5.85)	92.20
Treatment 4 = *R. solani* + *T. asperellum*	1.67 (7.42)	87.50
Treatment 5 = *R. solani* + *T. virens*	1.04 (5.85)	92.20
Treatment 6 = *R. solani* + *Bacillus subtilis*	5.93 (14.10)	55.50
Treatment 7 = *R. solani* + *Pseudomonas fluorescens*	8.50 (16.95)	36.25
Treatment 8 = *Rhizoctonia solani* (control)	13.33 (21.42)	
SEm±	**0.53**	
CD (P=0.05)	**1.57**	

*Figures in the parentheses are in arcsine transformed values

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSSIONS

Naga king chilli is one of the important native crops of Nagaland. Naga King Chilli is a very sensitive and vulnerable crop which does not grow well in all areas and like any other cultivated crops it does suffer from several diseases. In order to understand the diseases better and in view of lack of extensive study on occurrence of various diseases in Naga King Chilli in Nagaland, a disease survey was carried out during the kharif season of 2014 and 2015 in three Naga King Chilli growing districts of Nagaland *viz*. Dimapur, Kohima and Peren under the title "Studies on diseases of Naga King Chilli (*Capsicum chinense* Jacq.)".

The survey data collected on the incidence of diseases in Naga King Chilli reveals that Dimapur district recorded the highest disease incidence for anthracnose disease (44.39%), Cercospora leaf spot disease (9.97%), damping-off disease (7.08%), Fusarium wilt disease (3.53%), stem rot disease (2.11%) and bacterial wilt disease (9.17%). Whereas incidence of leaf veinal mottle disease was highest in Peren district (33.81%).

PDI (Per cent disease incidence) of all the diseases were collected from three districts of Nagaland. The collected data revealed that Dimapur district recorded the highest PDI for anthracnose (48.87), damping-off (24.93), Fusarium wilt (11.20), stem rot (11.62) and bacterial wilt (15.46). Peren district recorded the highest PDI for Cercospora leaf spot (7.98) and leaf veinal mottle disease (65.82). Kohima district was observed with the lowest PDI for all the seven diseases throughout the investigation.

In case of identification of the disease causing agents, the fungal pathogens *viz*. *Colletotrichum capsici* (anthracnose), *Cercospora capsici* (Cercospora leaf spot), *Rhizoctonia solani* (damping-off), *Fusarium oxysporum* (Fusarium wilt) and *Sclerotium rolfsii* (stem rot) were identified by studying their morphological and microscopic characteristics. Biochemical test *viz*., Gram staining test, NaCl tolerance test, catalase test and oxidase reagent test confirmed positive test result in confirmation of *Ralstonia solanacearum* (bacterial wilt). For identification of the viral pathogen; leaf veinal mottle (*Chilli veinal mottle virus*), reverse transcription – polymerase chain reaction (RT-PCR) PCR was carried out using *Potyvirus* genus specific degenerate primers *viz*., CIF/CIRev to amplify a ~700 bp region of cylindrical inclusion protein (CI) domain which confirmed 92% similarity with that of *Chilli Veinal Mottle virus* isolate Meghalaya-4.

Seven bio-control agents were screened *in vitro* using dual culture technique for their efficacy against disease causing pathogens of Naga King Chilli. Among the bio control agents *Trichoderma harzianum* was found to be the most effective antagonist against *Colletotrichum capsici* (anthracnose) recording 79.61% inhibition of mycelia growth, whereas *T. koningii* and *T. viride* recorded 100 per cent inhibition over the growth of *Fusarium oxysporum* (Fusarium wilt). Highest inhibition of mycelia growth of *Sclerotium rolfsii* (stem rot) *in vitro* was recorded from *T. harzianum* (85.07%). In case of *Rhizoctonia solani* (damping-off) highest inhibition of 92.20% were recorded from *T. koningii*, *T. viride*, *T. harzianum* and *T. virens.*

CONCLUSIONS:

It can be concluded from the present investigation that Naga King Chilli suffers from different plant pathogenic diseases. From the findings it was noticed that there is variation in prevalence and severity of different diseases under different Naga King Chilli growing district.

The future prospect of the present findings is to encourage other investigators to carry out more extensive studies in other remaining districts of Nagaland. This will give broader idea about the prevalence and severity of other different pathogenic as well as non-pathogenic diseases of Naga King Chilli under different topographic, climatic and environmental conditions. Disease survey data will help grower and concerned stakeholders to prioritize the strategies for managing disease of Naga King Chilli crop on which the state of Nagaland has acquired GI rights. The crop has large export potential owing to its high pungency level and its organic method of cultivation. In this direction, the present study will help selecting bio-control agents against important diseases of Naga King Chilli.

CHAPTER VI

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1a. Symptoms on the leaves

1b. Symptoms on the fruit

1c. Die-back symptoms

Plate 1: Symptoms of anthracnose disease in Naga King Chilli

Plate 2. Symptoms of Cercospora leaf spot disease on the leaves of Naga King Chilli

Plate 3. Naga King Chilli seedlings showing damping-off symptom

Plate 4. Naga King Chilli plants showing Fusarium wilt symptom

Plate 5. Symptom of stem rot on Naga King Chilli seedlings

Plate 6a. Plants showing bacterial wilt symptom

Plate 6b. Ooze test

Plate 6: Bacterial wilt disease in Naga King Chilli caused by *Ralstonia solanacearum*

Plate 7. Symptoms of leaf veinal mottling on Naga King Chilli

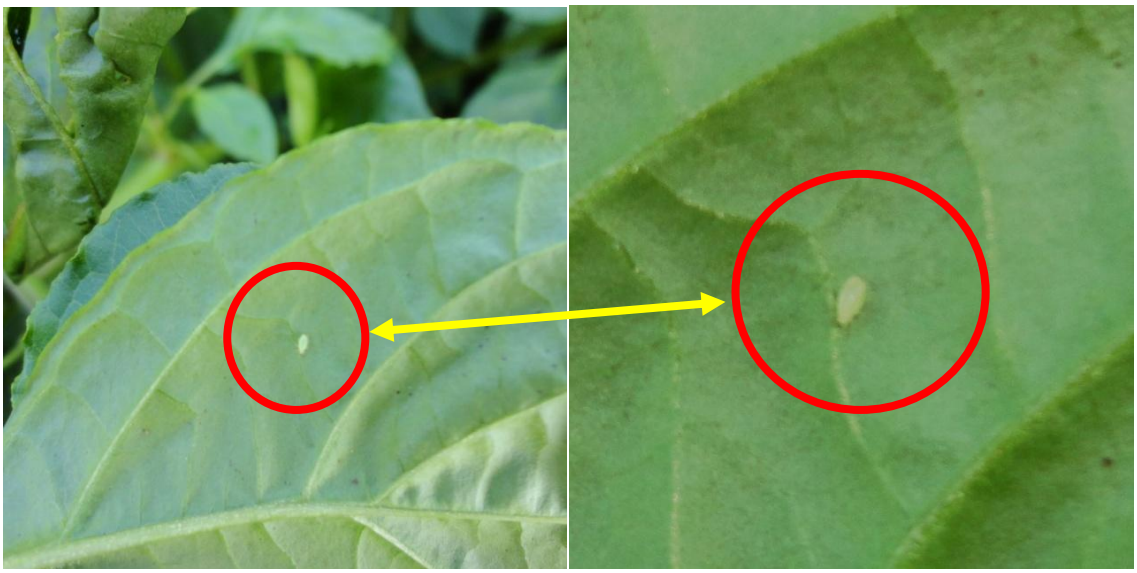
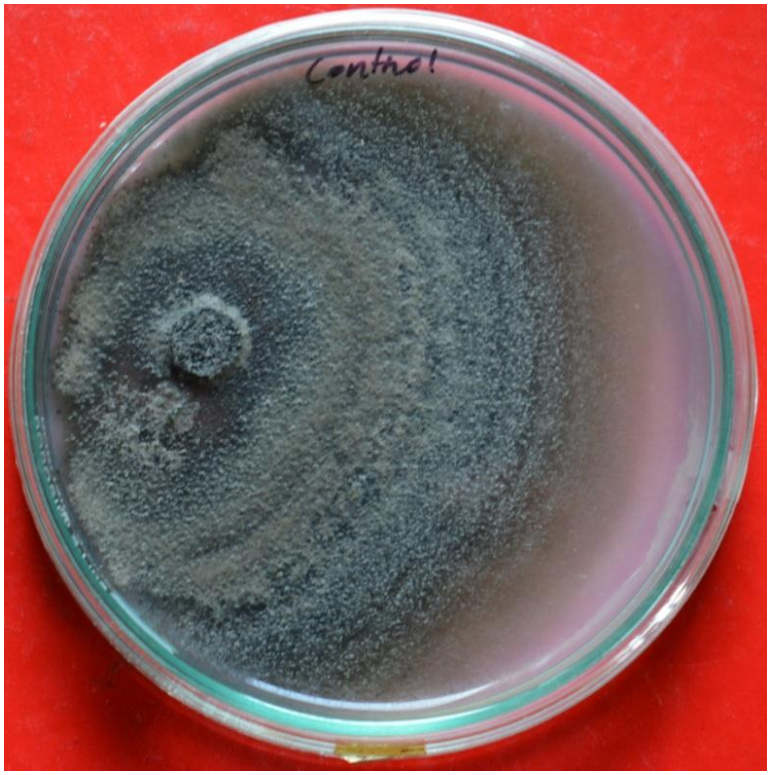
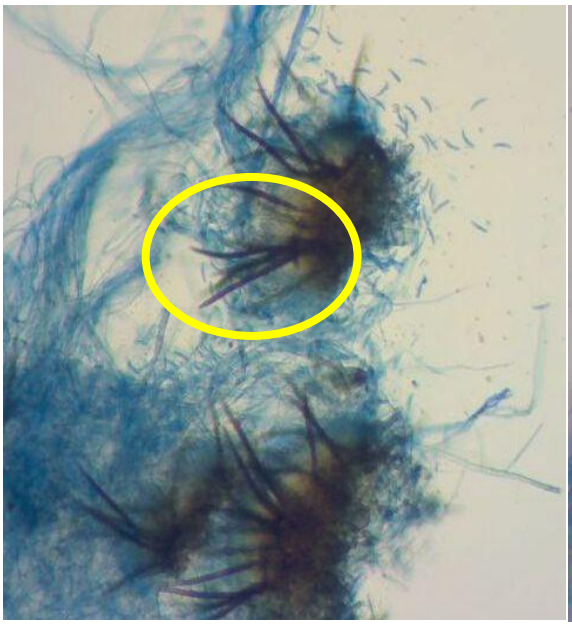


Plate 8. Green peach aphid vector of *Chilli veinal mottle virus*



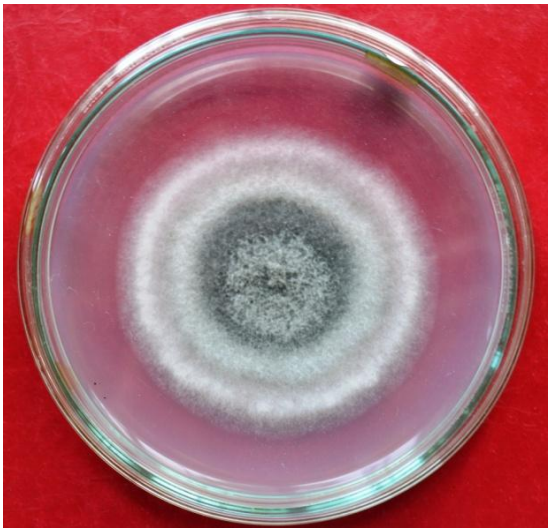
9a. *Colletotrichum capsici* in PDA plate



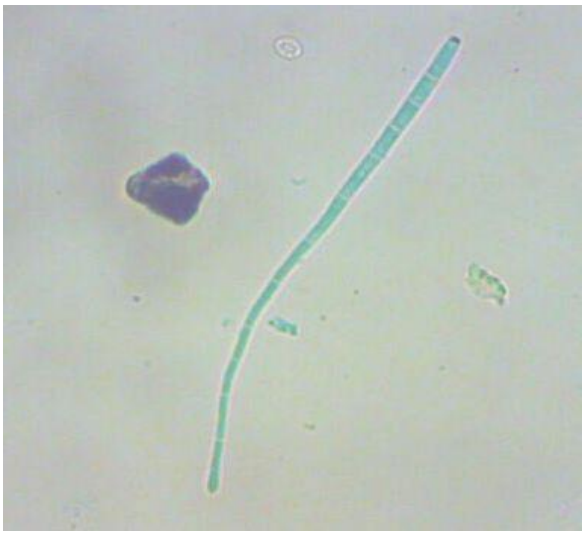
9b. Setae of *Colletotrichum* under 45X

9c. Conidia of *Colletotrichum capsici capsici* under 45X

Plate 9: *Colletotrichum capsici* (anthracnose disease in Naga King Chilli)

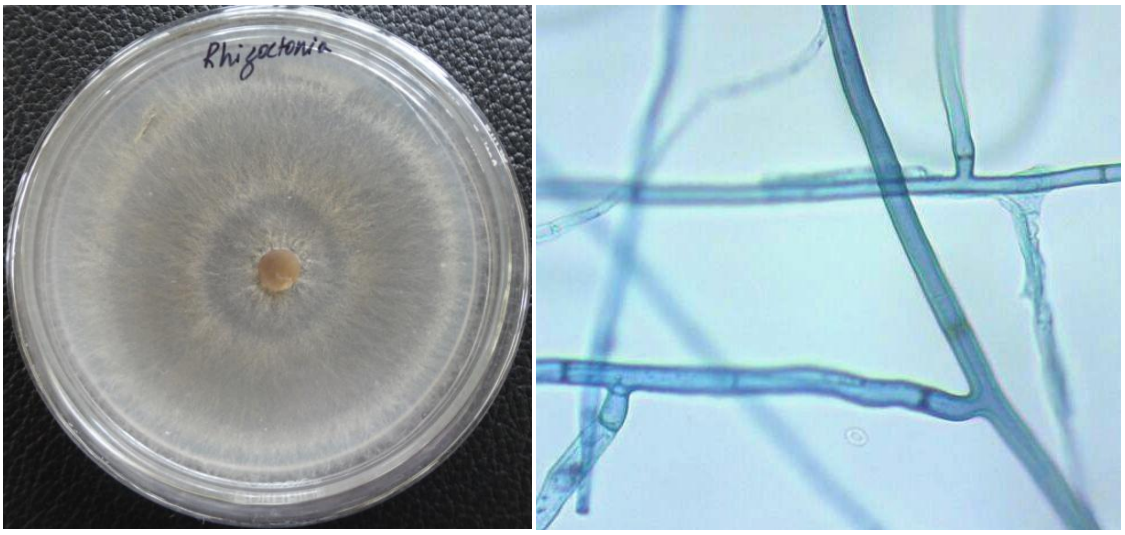


10a. *Cercospora capsici* in PDA plate



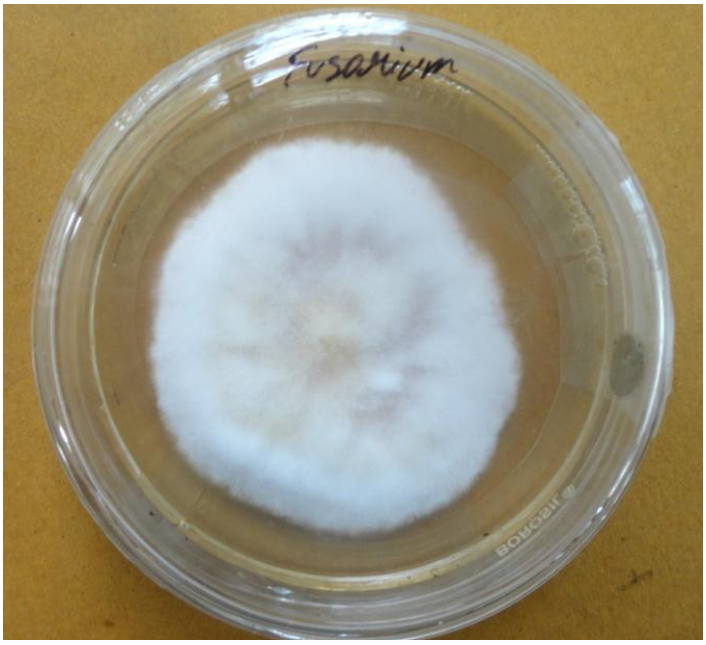
10b. Conidium of *Cercospora capsici* (45X)

Plate 10: *Cercospora capsici* (Cercospora leaf spot disease in Naga King Chilli)

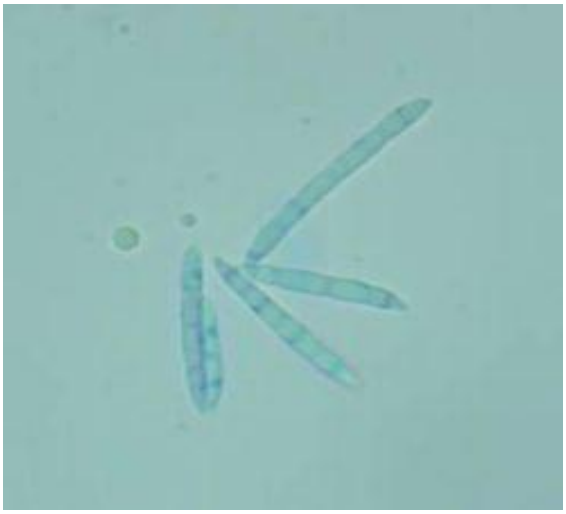


11a. *Rhizoctonia solani* in PDA plate **11b. *Rhizoctonia solani* (45X)**

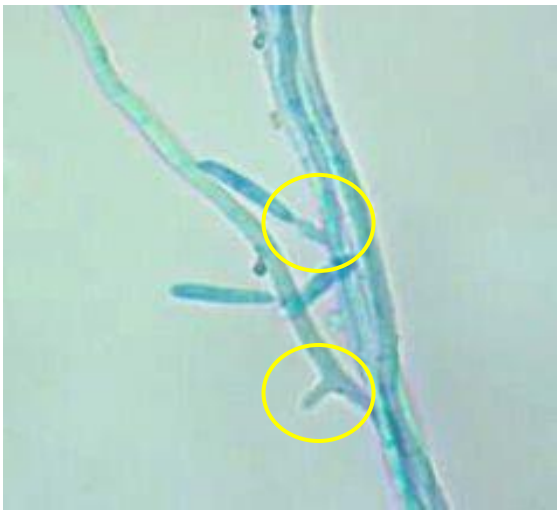
Plate 11: *Rhizoctonia solani* (Damping off disease in Naga King Chilli)



12a. *Fusarium oxysporum* in PDA plate

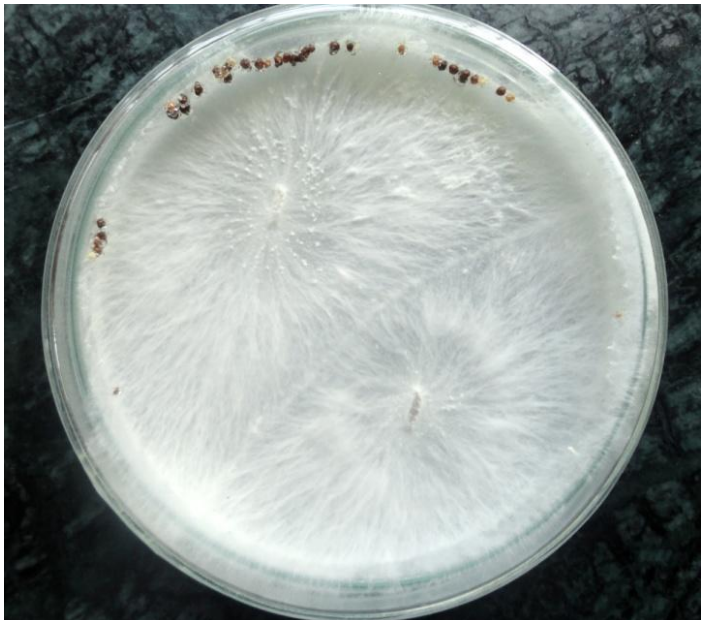


12b. Macroconidia (45X)

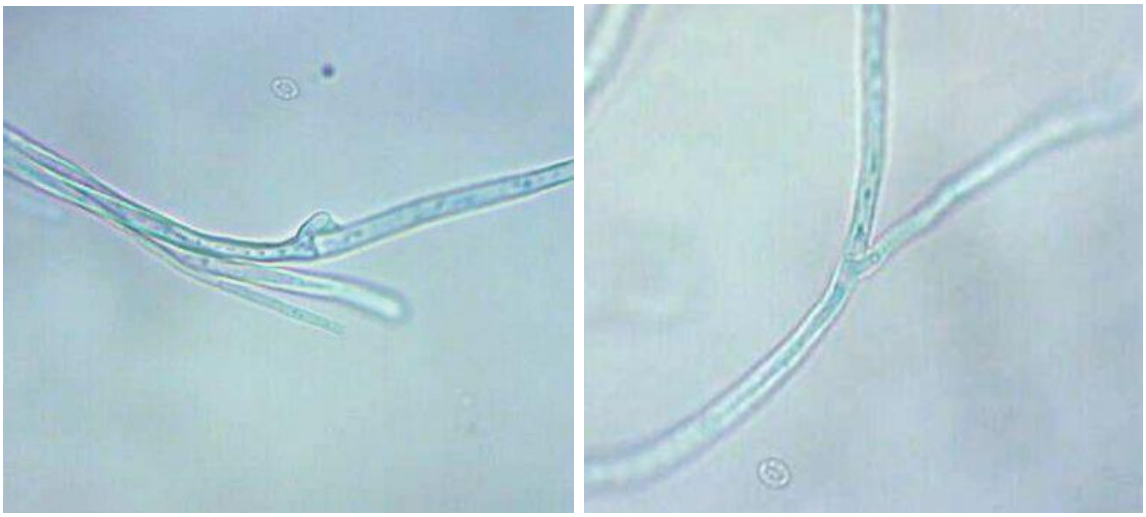


12c. Conidiophore (45X)

Plate 12: *Fusarium oxysporum* (Fusarium wilt disease in Naga King Chilli)

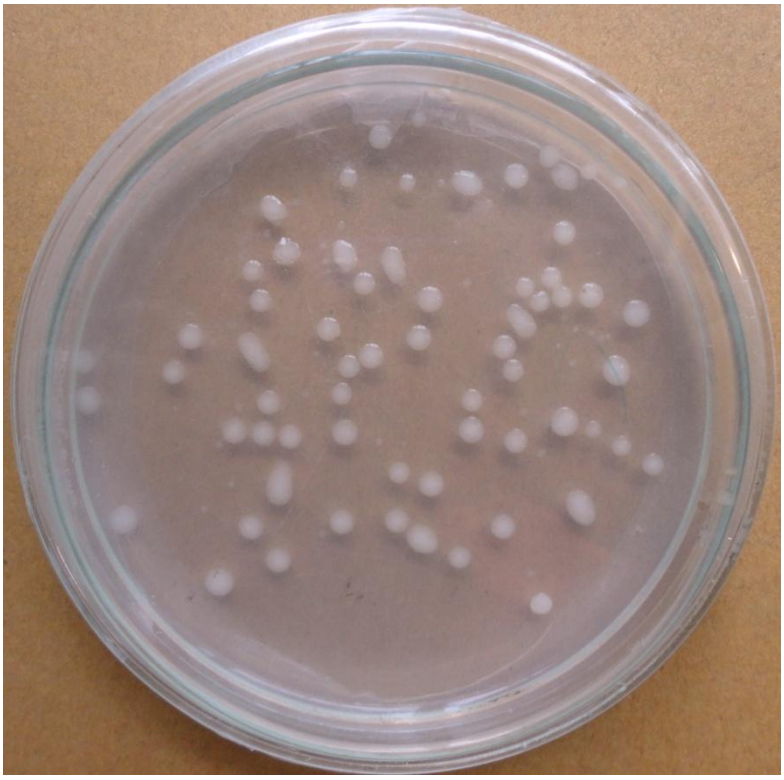


13a. *Sclerotium rolfsii* in PDA plate

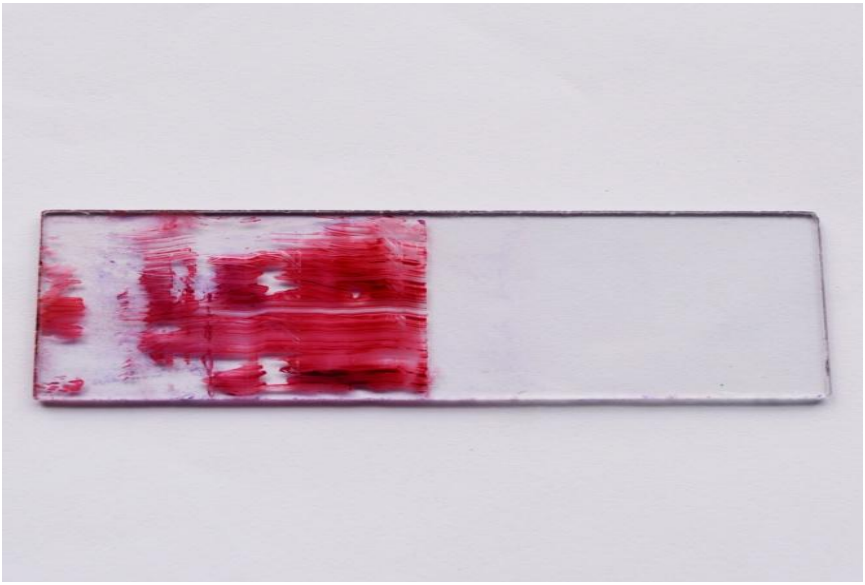


13b. Photo micrograph of *Sclerotium rolfsii* mycelium (45X)

Plate 13: *Sclerotium rolfsii* (Stem rot disease in Naga King Chilli)

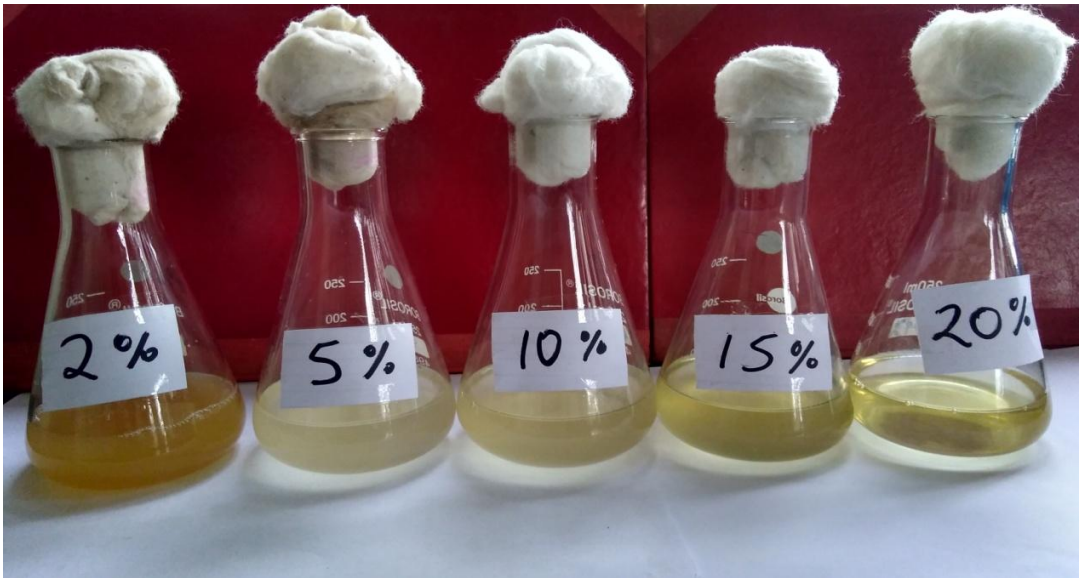


14a. *Ralstonia solanacearum* in nutrient agar plate

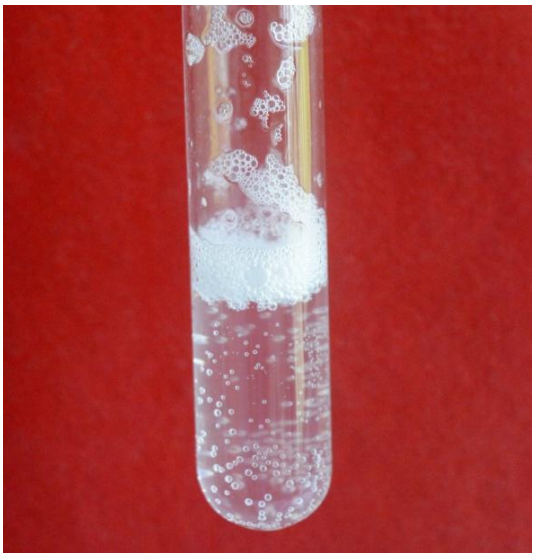


14b. Gram staining test (Gram negative)

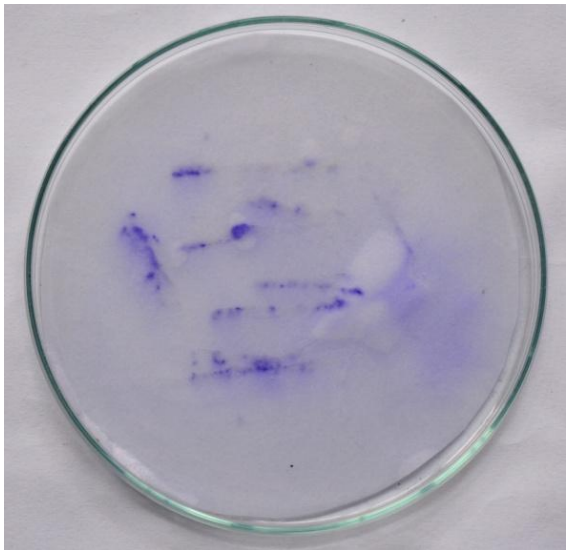
Plate 14: Biochemical test on *Ralstonia solanacearum* (continued..)



14c. Sodium chloride (NaCl) tolerance test (test positive)



14d. Catalase test (test positive)



14e. Oxidase test (test positive)

Plate 14: Biochemical test on *Ralstonia solanacearum*

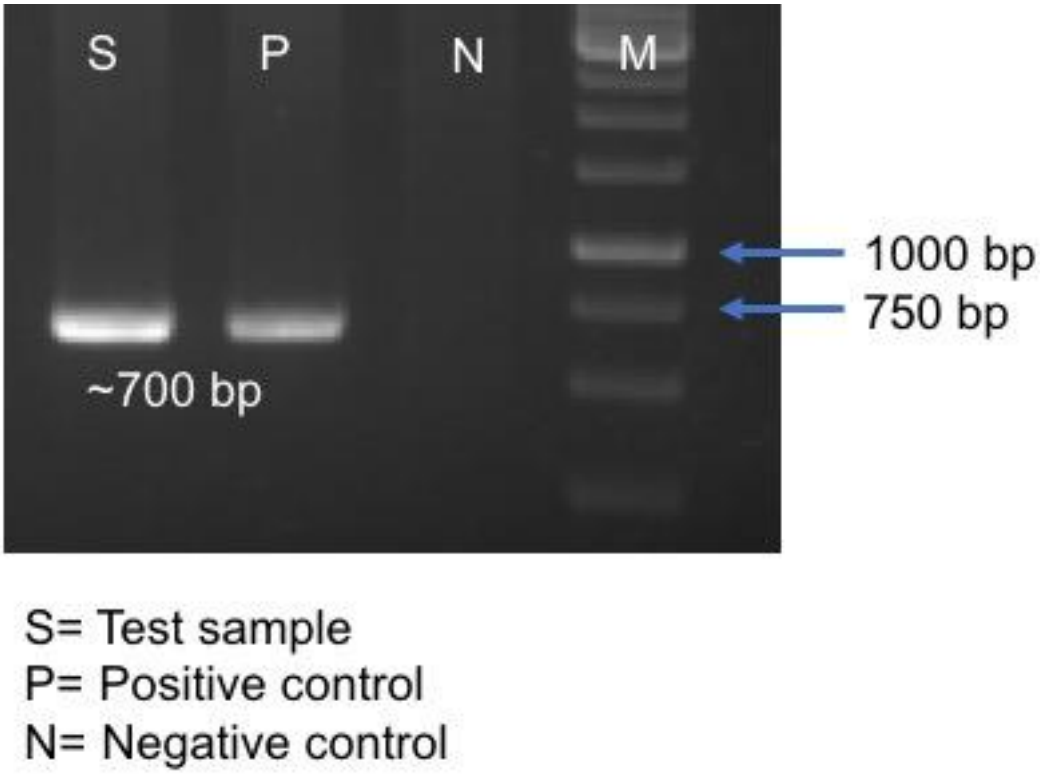
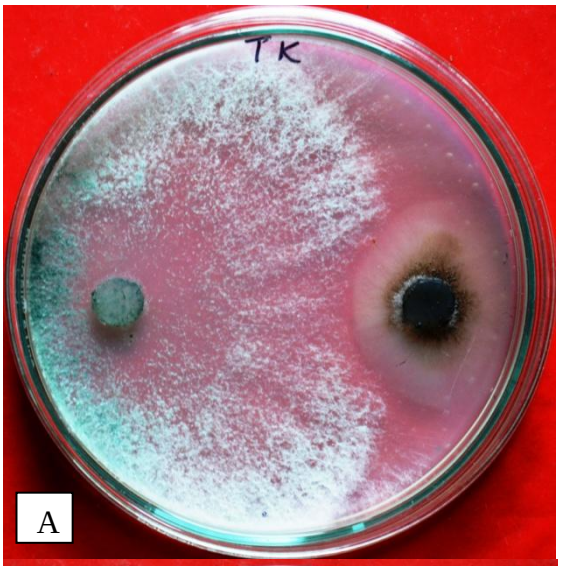


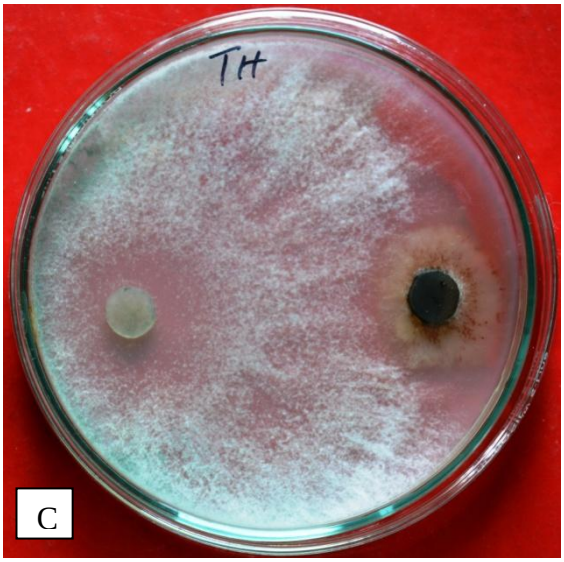
Plate 15: Amplified 700 bp region of cylindrical inclusion protein (CI) domain of *Chilli veinal mottle virus* (ChiVMV)



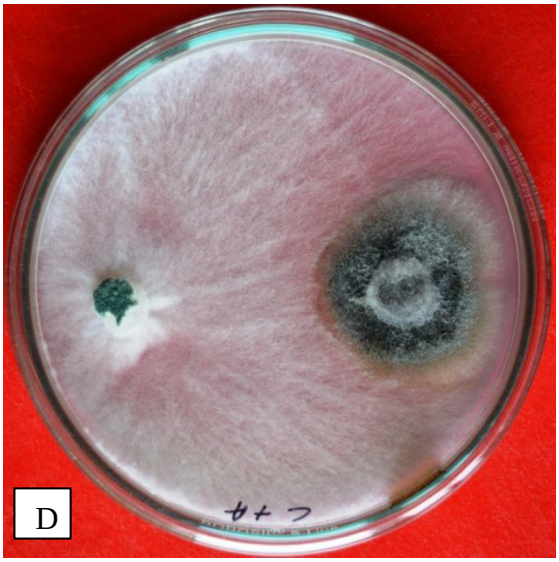
B

Colletotrichum capsici + Trichoderma koningi

C. capsici + T. viride

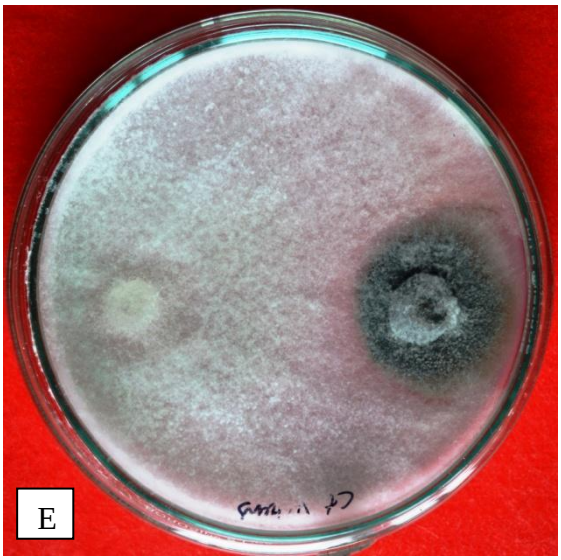


C. capsici + T. harzianum

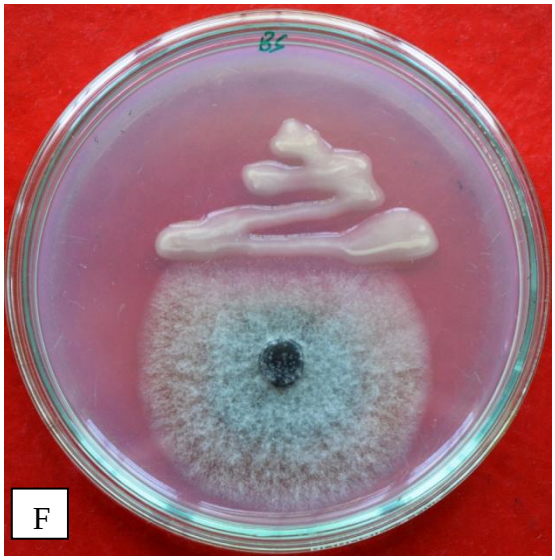


C. capsici + T. asperellum

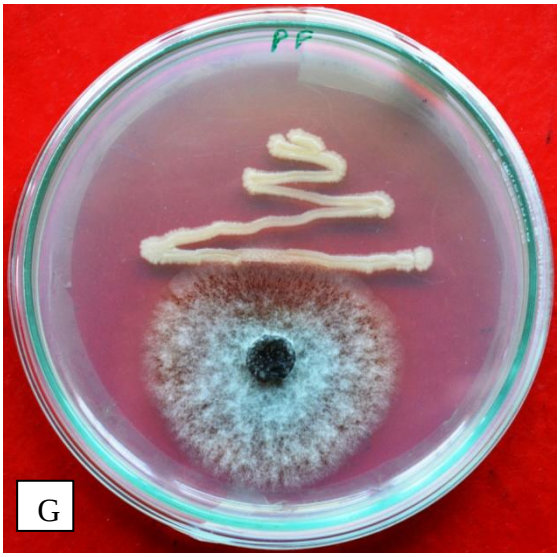
Plate 16: Antagonistic effect of bio control agents against *Colletotrichum capsici* (continued.....)



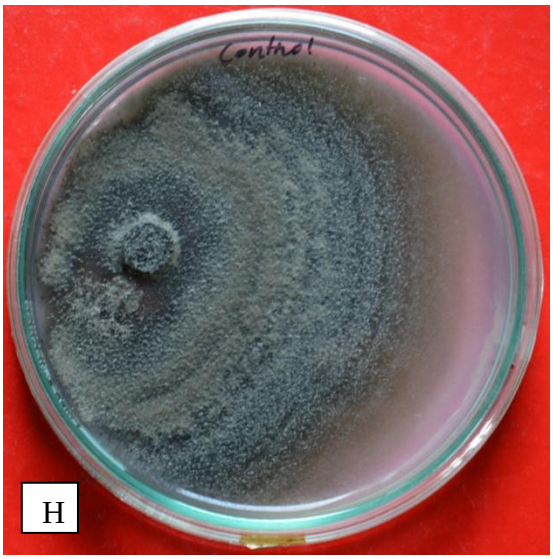
C. capsici* + *T. virens



C. capsici* + *Bacillus subtilis

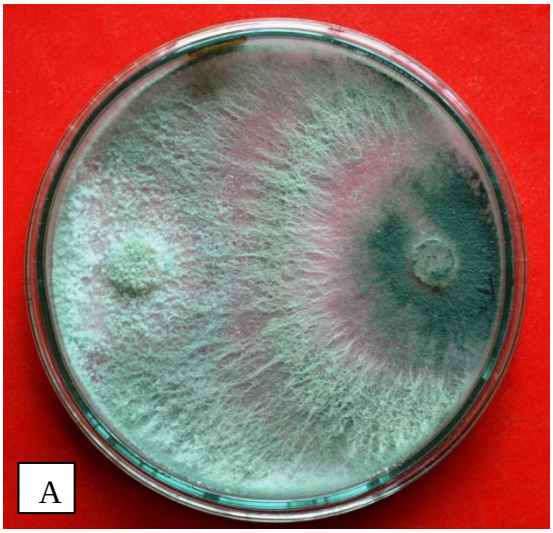


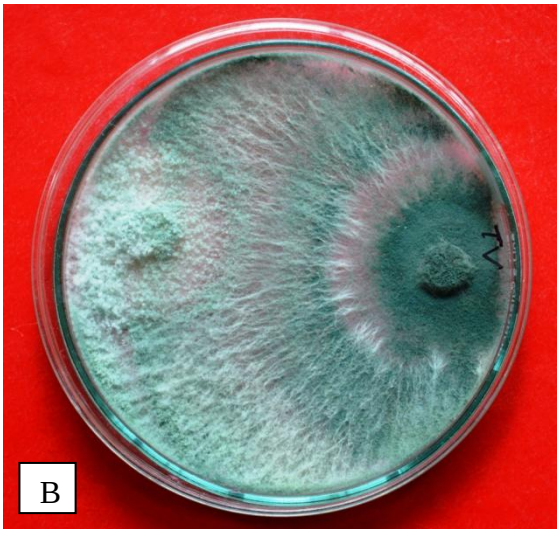
C. capsici* + *Pseudomonas fluorescens



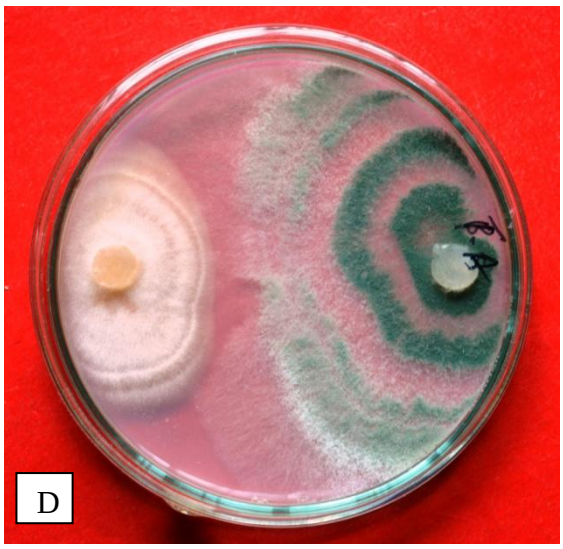
***Colletotrichum capsici* (control)**

Plate 16: Antagonistic effect of bio control agents against *Colletotrichum capsici*.



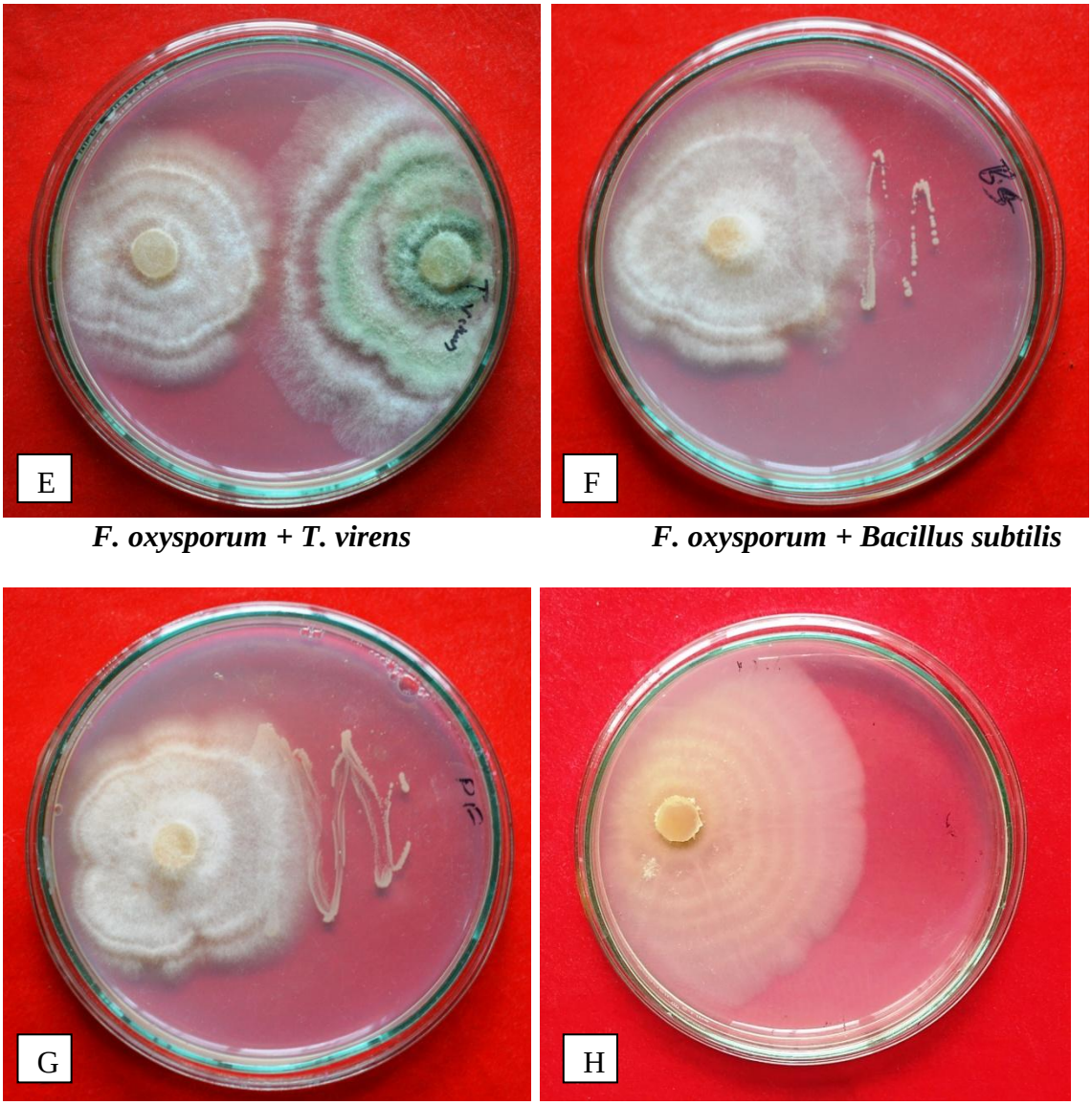


Fusarium oxysporum + Trichoderma koningii viride ***F. oxysporum + T.***



F. oxysporum + T. harzianum ***F. oxysporum + T. asperellum***

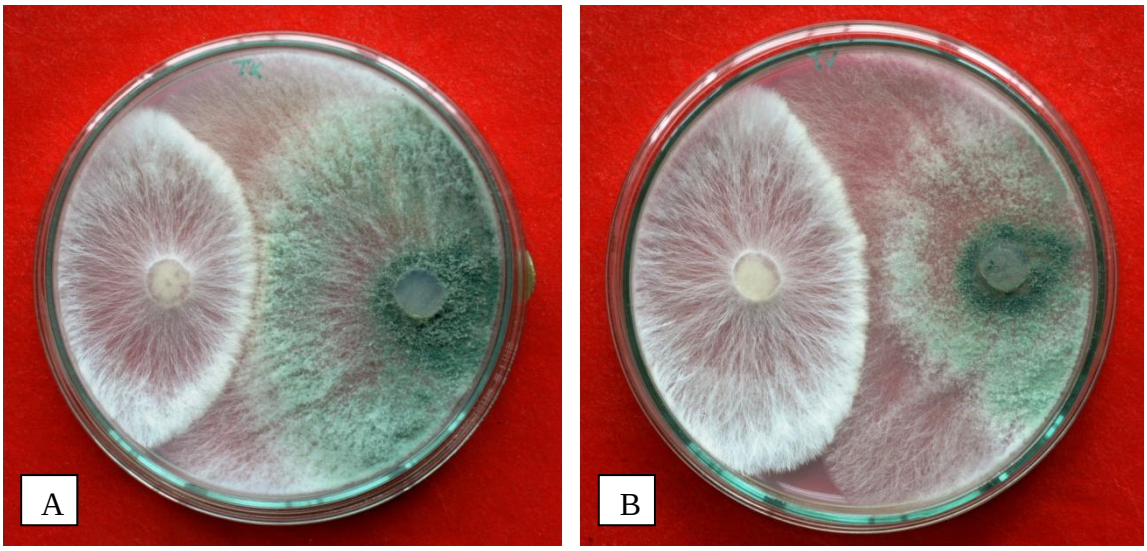
Plate 17: Antagonistic effect of bio control agents against *Fusarium oxysporum* (continued….)



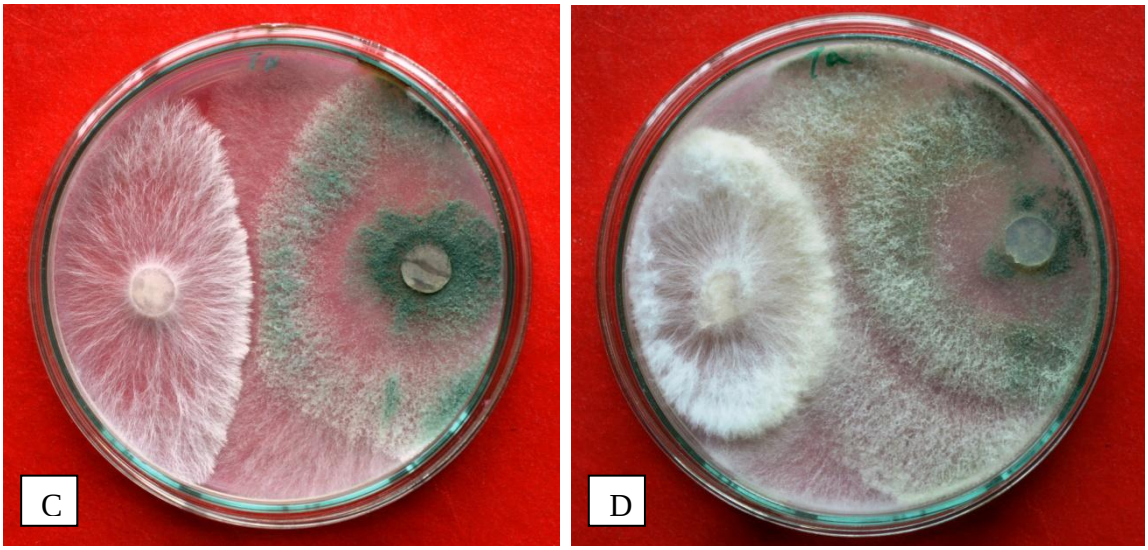
F. oxysporum + T. virens ***F. oxysporum + Bacillus subtilis***

F. oxysporum + Pseudomonas fluorescens ***Fusarium oxysporum* (control)**

Plate 17: Antagonistic effect of bio control agents against *Fusarium oxysporum*

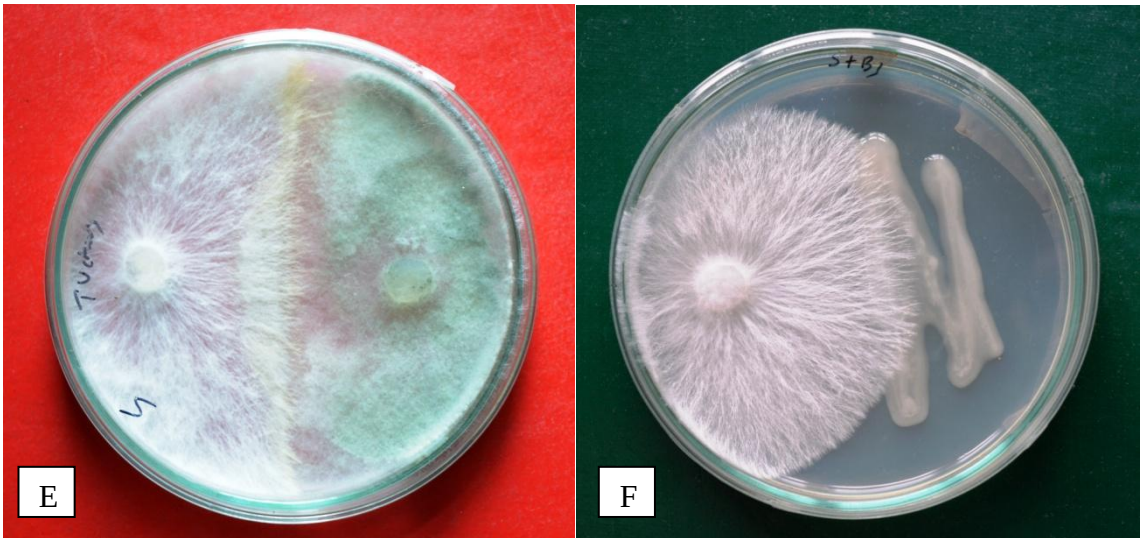


Sclerotium rolfsii* + *Trichoderma koningii ***S. rolfsii* + *T. viride***

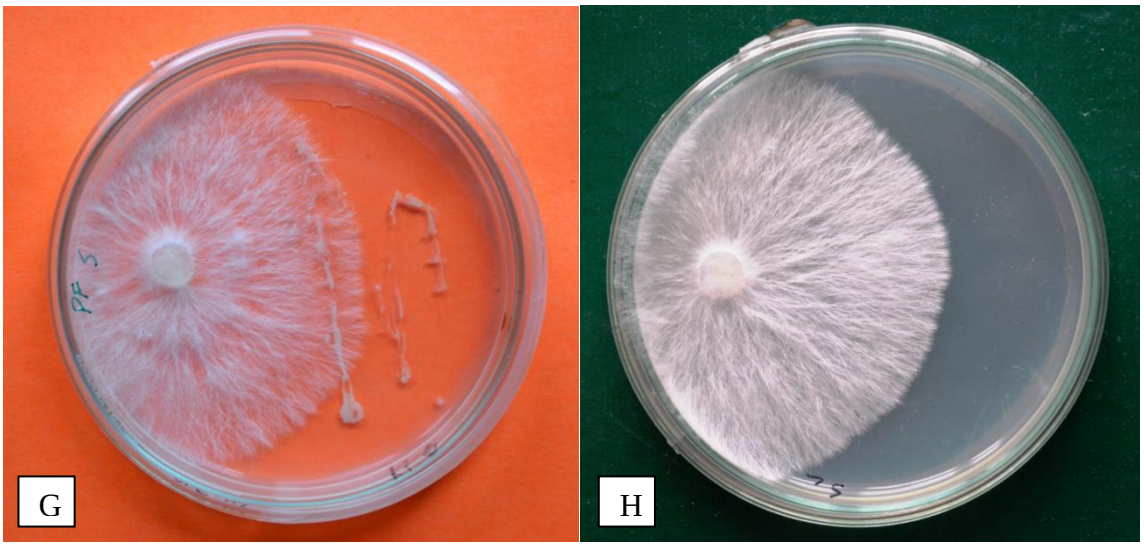


S. rolfsii* + *T. harzianum ***S. rolfsii* + *T. asperellum***

Plate 18: Antagonistic effect of bio control agents against *Sclerotium rolfsii* (continued….)

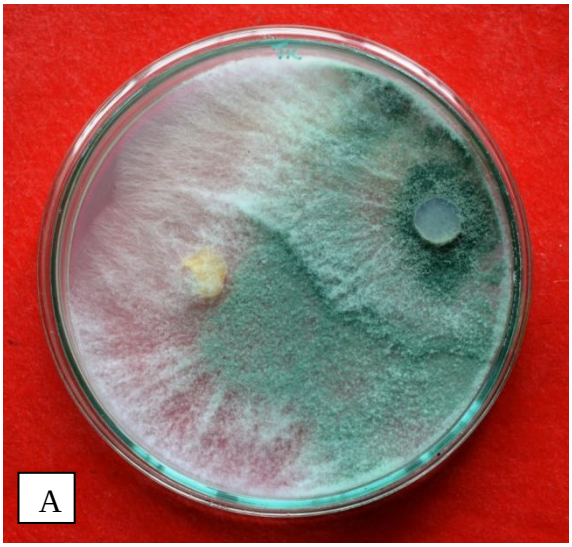


S. rolfsii* + *T. virens ***S. rolfsii* + *Bacillus subtilis***

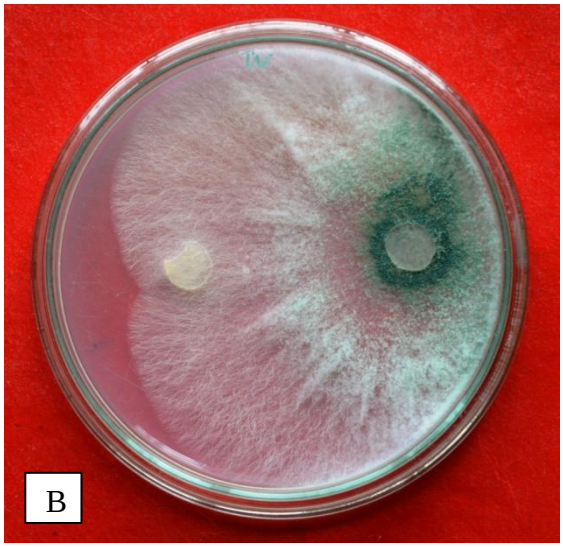


S. rolfsii* + *Pseudomonas fluorescens ***Sclerotium rolfsii* (control)**

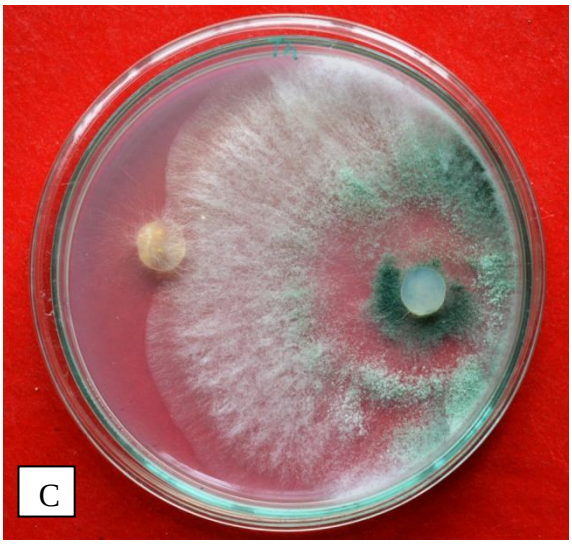
Plate 18: Antagonistic effect of bio control agents against *Sclerotium rolfsii*



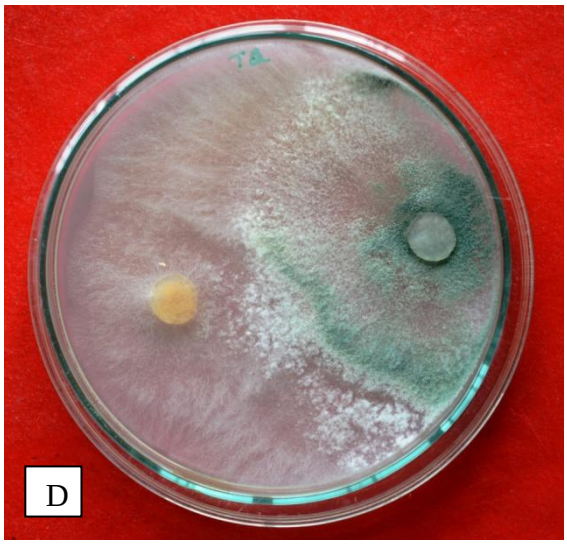
Rhizoctonia solani + Trichoderma koningii



R. solani + T. viride

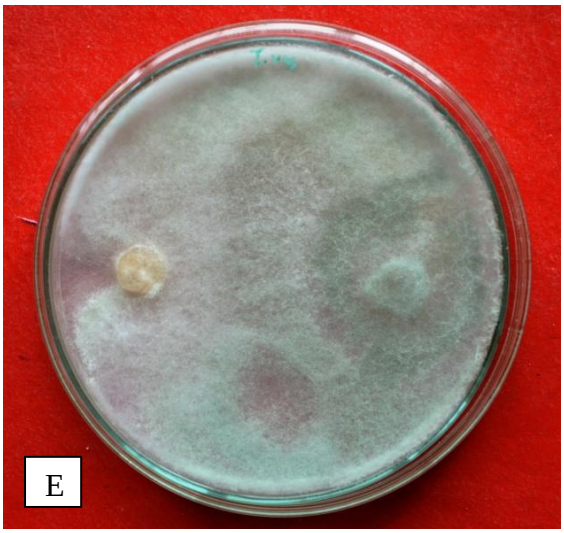


R. solani + T. harzianum

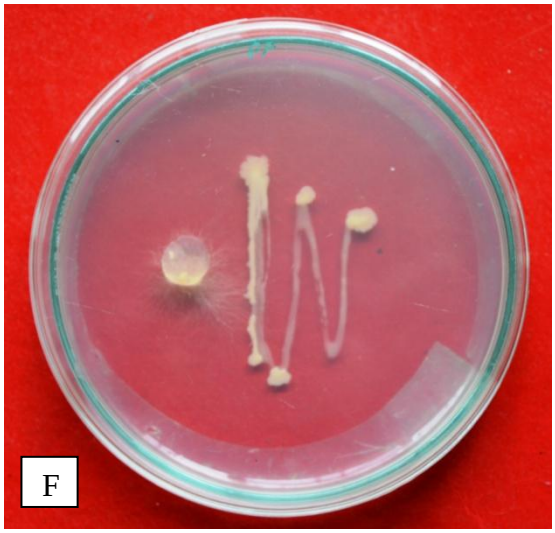


R. solani + T. asperellum

Plate 19: Antagonistic effect of bio control agents against *Rhizoctonia solani* (continued....)

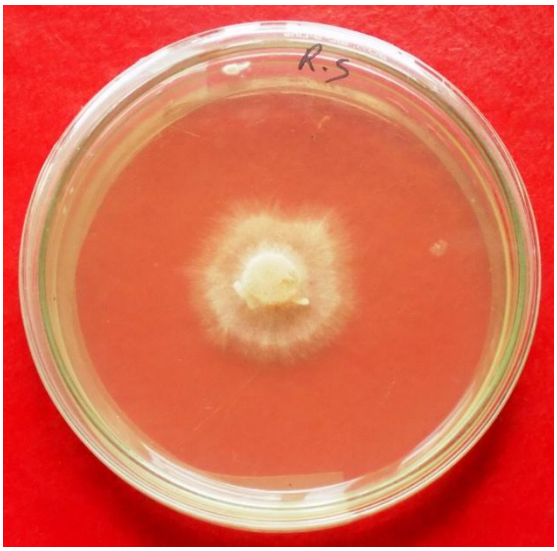


R. solani + T. virens



R. solani + B. subtilis

R. solani + Pseudomonas fluorescens



***Rhizoctonia solani* (control)**

Plate 19: Antagonistic effect of bio control agents against *Rhizoctonia solani*