PREVALENCE AND INCIDENCE OF BASAL STEM ROT DISEASE OF COCONUT IN SELECTED COASTAL REGIONS OF BANGLADESH AND ITS *IN-VITRO* MANAGEMENT

RABEYA BUSRI RODELA

DEPARTMENT OF PLANT PATHOLOGY

FACULTY OF AGRICULTURE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY DHAKA-1207

December, 2018

PREVALENCE AND INCIDENCE OF BASAL STEM ROT DISEASE OF COCONUT IN SELECTED COASTAL REGIONS OF BANGLADESH AND ITS *IN-VITRO* MANAGEMENT

BY

RABEYA BUSRI RODELA

REGISTRATION NO.: 17-08253

A Thesis

Submitted to the Faculty of Agriculture,

Sher-e-Bangla Agricultural University, Dhaka,

In partial fulfillment of the requirements for

the degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

SESSION: JULY-DECEMBER, 2016

Approved by:

Dr. Md. Belal Hossain
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Supervisor

Dr. F. M. Aminuzzaman
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Co-Supervisor

Professor Dr. Khadija Akhter
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka


DEDICATED
TO
MY BELOVED PARENTS
AND
TO THE FARMERS WHO
FEED THE NATION

ACKNOWLEDGEMENTS

All praises to the ''Almighty Allah'' for best owing mercy upon the author and for imbibing confidence on her to materialize the research work. It is a great gratification to articulate her gratitude to her respected parents, who digest much hardship inspiring for prosecuting her studies, thereby receiving proper education.

The author feels her dictionary is too poor to translate the sense of gratitude into words and heartfelt reverence to her research supervisor ***Dr. Md. Belal Hossain****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his sustained encouragement, valuable suggestions, unvarying inspiration and timely advice besides providing an atmosphere of freedom and all through the research work and for taking massive care in preparing this manuscript. His insight and professional skill have made a distinctive contribution to complete this piece of research.*

The author expresses her sincere appreciation, sense of gratitude, respect and immense indebtedness to the respected Co-Supervisor ***Dr. F. M. Aminuzzaman****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for his valuable suggestions and constructive criticism during research work.*

The author is impressively gratified to her respected teacher ***Dr. Khadija Akhter*** *Professor and Chairman and also to all respected teacher, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, encouragement and co-operation during the entire study period.*

The author would like to express her special gratitude to her beloved ***Parents*** *, whose always inspired and assist during the research work. The author would like to expresses cordial thanks to her friends and well-wishers Md. Jahirul Islam, Md. Khairul Islam, Asikur Rahaman , Nurunnahar laboni, Sumona Rahman, Neela Sultana, Afsana Akhter, Suborna Akhter, Laila Asrafi Akhi, Moury kabir, Ferdous jahan urmi, Sabjana akhter, who helped her with their valuable suggestions and directions during the research work.*

The author has taken an opportunity to express her cordial thanks and sincere gratitude to the laboratory staff of the Department of Plant Pathology of SAU for their co-operation and help to complete this piece of research.

The author expresses her massive thankfulness to all of them who supported and encouraged her to pursue higher education and regret for her inability for not to mention every one by name who also contributed in pursuing the research works.

The Author

PREVALENCE AND INCIDENCE OF BASAL STEM ROT DISEASE OF COCONUT IN SELECTED COASTAL REGIONS OF BANGLADESH AND ITS *IN-VITRO* MANAGEMENT

ABSTRACT

Basal stem rot (BSR) caused by basidiomycotina fungi, *Ganoderma* is one of the most devastating diseases of numerous perennial, coniferous and palmaceous plants. In forest systems, *Ganoderma* has an ecological role in the breakdown or delignification of woody plants. Symptoms of BSR disease can take several years to develop, and the presence of the pathogen (such as indicated by fruiting bodies) is often only visible when the fungus is well established and more than half of the bole tissue has been decayed, leaving no chance for the grower to cure the infected palms. A limiting factor in controlling the BSR disease is the lack of reliable diagnostic method(s) for early diagnosis. The present study was aimed to evaluate the occurrence and incidence of basal stem rot disease and identify its causal organism and to perform a bio-control based *in-vitro* management practices. The survey study was conducted in three selected coastal districts *viz.* Patuakhali, Barishal and Pirojpur during January 2018 to October 2019. The *in-vitro* management study was conducted in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka . The disease incidence of basal stem rot was measured 69.33%, 68.33%, 57% and the disease severity was 36.89%, 30.79%, 29.5% in Pirojpur, Patuakhali and Barishal respectively. The highest disease incidence (29.5%, 35.75% and 36.75%) was found in loamy soil, at 6 to 7 pH and in plant age group 36-40 years respectively. In total 45 root samples, 14 sporophore and 12 disease stem bits/barks were collected for aseptic isolation. Diseased root bits and sporocarp were good source(s) for isolation of *Ganoderma* with Potato Dextrose Agar and Potato Sucrose Agar that supported the highest radial growth and biomass production. The isolated pathogens from root samples were identified as *Ganoderma applanatum* and *Ganoderma lucidum*. The bio-control based management practices like *Trichoderma* suspension as bio-agent, Cattle urine as bio-product and Neem oil and Garlic extract as botanicals were applied for controlling the basal stem rot disease studied under *in-vitro* condition. Among the four bio-control agents cattle urine gave the best inhibitory actions against BSR disease under *in-vitro* condition which accounted lower radial mycelial growth at 9 DAI of *Ganoderma* (3 mm) with 3 ml doses of application.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

%	**=**	**Percentage**
FAO	**=**	**Food and Agricultural Organization**
et al.,	**=**	**And others**
etc.	**=**	**Etcetera**
i.e.	**=**	**id est (L), that is**
viz.	**=**	**Videlicet, it is permitted to see**
DI	**=**	**Disease Incidence**
DS	**=**	**Disease Severity**
DSI	**=**	**Disease severity index**
SL	**=**	**Serial**
No.	**=**	**Number**
CFU	**=**	**Colony forming unit**
µL	**=**	**Micro-Liter**
ml	**=**	**Milliliter**
SAU	**=**	**Sher-e-Bangla Agricultural University**
Kg	**=**	**Kilogram**
L	**=**	**Liter**
CV	**=**	**Percent Coefficient of Variation**
LSD	**=**	**Least Significant Difference**
DAI	**=**	**Days After Inoculation**
PDA	**=**	**Potato Dextrose Agar**
PSA	**=**	**Potato Sucrose Agar**
^{o}C	**=**	**Degree Celsius**
^{o}F	**=**	**Degree Fahrenheit**
M.S	**=**	**Master of Science**
BBS	**=**	**Bangladesh Bureau of Statistic**

INTRODUCTION

Coconut (*Cocos nucifera L.*) belonging to family Arecaceae is an important commercial crops of Bangladesh providing livelihood to a substantial number of farm families. Coconut, the versatile palm popularly known as , 'King of Palms' , 'Tree of Heaven', 'Tree of life', 'Tree of Abundance', as well as Allah gift to mankind , is grown in more than 90 countries around the world within an area of 14.231 million hectares producing about 57.514 billion nuts or 10.52 million tonnes of copra (Athira, 2017). The total area and the production in Asian Pacific Coconut Committee (APCC) countries are estimated at 11.4 million ha and 9.2 million MT, which is 90 and 84 percent of world area and production (Rethinam, P. and Taufikkur Rahman, L. 2002).

Coconut palm is often described as "Kalpavriksha" because of the multifarious uses of every part of it in the commercial sector. It is equally important in the therapeutical sector, especially for the medicinal purposes. Inflorescence, tender coconut and unfermented sweet toddy are rich sources of sugars, minerals, essential amino acids, vitamins and digestive enzymes. Coconut milk is a good source of protein and fat. Coconut oil contains capric, myristic and stearic acids in addition to glycerides and is considered as the safest vegetable oil for human consumption. In a 100 gram reference amount, coconut oil supplies 354 Calories. Half of the saturated fat content of coconut oil is lauric acid (41.8 grams per 100 grams of total composition), while other significant saturated fats are myristic acid (16.7 grams), palmitic acid (8.6 grams) and caprylic acid (6.8 grams) (Kandana, R. Bhaskaran and R. Samiyappan. 2008 and wikipedia).

The natural habitat of coconut is the coastal belt of tropics where it flourishes in sea-washed littoral sand with constant motion of underground current of water in rhizosphere zone. It is the most valuable antique plantation crops in coastal regions of Bangladesh. Sandy soil is suitable for coconut plantation and it is highly tolerant to Salinity. It prefers mainly abundant of sunlight and regular rainfall areas.

Coconuts also need high humidity (70–80%) for optimum growth that is why rarely seen in low humidity areas with low annual precipitation. As red sandy loam, alluvial, literate and coastal sandy well-drained soils rich in organic matter and with a pH ranging from 5.0 to 8.0 are best for its growth and higher yield, the coconut can be grown on a wide variety of soils across the world. In Bangladesh Coconut palms are grown 466975 MT's in 10365 acres of land per year in different districts or areas throughout the country (*Yearbook of Agricultural Statistics-2018*). Among the Coastal regions of Bangladesh, Barisal district ranks first in total production of inside and outside garden followed by Chittagang, Khulna, Dhaka, Mymensing, Rajshahi, Rangpur, Sylhet. In world coconut's production Bangladesh has share about 89,400 tons . Coconut plant plays a very indicative role in the economy of the southern and coastal regions of Bangladesh. Coconut farming could a great milestone for the economy of Bangladesh. It provides nutritious drink, many edible nutritious products, oil for edible and non-edible uses, fiber of commercial value, shell for fuel and industrial uses, beverage, timber and a variety of miscellaneous products for use such as handicrafts etc.

Coconut is considered as a crop of high economic value because of its variegated uses. It is an essential and dominant component of the homesteads and garden lands along the coastal parts of Bangladesh and plays a vital role in the socio-cultural and economic life of large number of small and marginal farmers (Dagar *et al.* 2014). Many smallholder's households generally depend on the coconut for their livelihood as it provides balanced incomes. The coconut is a benevolent crop and a perfect gift to mankind. Coconut palm exerts a confidential influence on the rural economy of the many areas where it is grown extensively and provides sustenance to more than million people in the country. In the present situation of growing coconut plantation has a great prospect in our economic growth.

Coconut palm is usually affected by various biotic and abiotic stresses resulting in drastic reduction in yields. Even though coconut palm is hardy in nature and

adaptable to varied climatic conditions, it is affected by many diseases (Nambiar 1994, Henry Louis 2002). Root (wilt) , basal stem rot (BSR), bud rot , stem bleeding, leaf blight and grey leaf spot are the major diseases of coconut is reported throughout the world. Coconut sector in the country are faced with frequent challenges like palm senility, natural calamities such as floods, drought, pest and diseases that not only deteriorate the quality of fruits but also reduced the vigor and yield of palms. Among the noxious diseases, Basal stem rot (BSR) disease caused by *Ganoderma applanatum* and *Ganoderma lucidum* is the most deleterious disease accounting to severe yield loss in coastal parts of Bangladesh.

The disease is reported from various places all over the tropical world *viz*., India, Srilanka, West Indies, Seycheles , Guam etc. Though the disease was first recorded by Dr. Butler in the begining of 20th century and later by Venkatanarayan (1936) from Karnataka, a severe outbreak occurred in 1652 in Thanjavur district of Tamil Nadu, and hence named as Thanjavur wilt. The genus *Ganoderma* was first introduced by Finnish mycologist Peter Adolf Karsten in 1881 (Karsten, 1881), with *G. lucidum* (Curtis:Fr.) P. The genus belongs to the family Ganodermataceae that remains in the order Polyporales of the Basidiomycetes. The family (Ganodermataceae) includes eight genera that are distinguished by their unique double-walled basidiospores. The genus *Ganoderma* was further subdivided into two subgenera: subgenus *Ganoderma* based on *G. lucidum* for the laccate species and subgenus *Elfvingia* based on *G. applanatum* for the species with a non-laccate fruiting body (Moncalvo and Ryvarden, 1997). In this way, *G. lucidum* and *G. applanatum* are the two important species complexes in the history and nomenclature of the genus. These are two of the most poorly understood species of *Ganoderma* and most frequently with misapplied names (Seo and Kirk, 2000).

Ganoderma species are important wood decaying fungi occurring throughout the world. They are diverse in the tropics affecting plantation crops such as coconut, arecanut and oil palm by causing Basal Stem rot (Singh, 1991; Ariffin *et al*., 2000;

Flood *et al*., 2000; Pilotti *et al*., 2003; Pilotti, 2005) and they also affect ornamental and forest trees in tropical and temperate areas causing disease and wood rots of timber (Lee, 2000). These wood decaying fungi cause white-rot of hardwoods through delignification (Adaskaveg *et al*., 1991, 1993; Schwarze *et al*., 1995). In the early stages of decay caused by *Ganoderma* species, bleached zones usually appear in the wood, as a result of selective delignification. As the decay progresses, the wood becomes softer and loses its tensile strength until a late stage where the wood disintegrates and becomes soft or spongy (Schwarze and Ferner, 2003).

The characteristic symptom of the disease is extensive discolouration and rotting of root systems, which leads to tissue disintegration and the stele turns brown. The roots become watery with a typical smell of alcohol. More often, the formation of new root produced is progressively reduced. In the advanced stages of infection, the fungus produces fruiting bodies (sporocarps) which may or may not develop before foliar symptoms. Sporocarps may develop at the trunk base and the appearance of sporocarps is the most diagnostic symptom of the disease. The sporophores initially appear as small, white buttons of fungal tissues that develop rapidly into the familiar bracket-shaped mature sporophore. The young sporophore is white or yellow, whereas the mature sporophore upper surface can be light to dark brown, with a light margin and a shiny lacquered finish. The undersurface is whitish in colour and has numerous minute pores (Bhaskaran *et al*., 1982; Rethinam, 1984; Bhaskaran, 1986; Samiyappan *et al*., 1996). The disease is generally observed in sandy or sandy loam soils in coastal regions on the east coast where coconut is grown under rainfed conditions and also in neglected plantations. Khairudin (1990) reported that most of the soil series found on coastal areas are susceptible to BSR. Soils with poor drainage and water stagnation during rainy season were found to favour the disease. The Basal stem rot disease incidence was very high between March and August. It was directly related to mean soil temperature, rainfall and relative humidity.

Naturally *Ganoderma spp.* has a wide host range attacking variety of palms and several forest, avenue and fruit trees (Naidu *et al* ,1966; Govindu *et al*., 1983; Bhaskaran, 1994). It usually attacks old or weak palms growing under unfavorable conditions. It is a soil dweller inhabiting dead as well as living plant material in the soil, enters through the wounds and spread of the disease takes place mainly through soil. That disease incidence and severity was observed maximum (up to 29.5%) in coconut gardens raised in loamy and sandy soils in coastal areas. The presence of old infected stumps in the garden and non-adoption of recommended cultural practices will pave the way for disease spread. Basically, *Ganoderma* is a soil-borne pathogen and it survives well in the soil for a long time. The formation of chlamydospores during adverse conditions helps survival of pathogen and chlamydospores become more resistant to environmental factors than basidiospores and could be responsible for dissemination of the disease. Irrigation water and rain water help in the spread of the fungus from one field to others. The trend of research on Coconut in coastal regions of Bangladesh seems to be indiscriminate, unsystematic and inconclusive with very few exceptions. Though basal stem rot is the one of the most remarkable disease of coconut but no works found on it before in our country. The present study paper updates the status of BSR in India along with recent developments in early detection, molecular identification and integrated disease management methods and to identify research priorities and knowledge gaps. Due to seed borne, soil borne as well as air borne nature of these fungal diseases, the plants are infected from seedling to older stage and are suffering from immense losses in fruit yield of coconut. Hence, it is customary for the farmers to efficiently manage these setback diseases, for the farmers " income to attain".

Investigations on Basal Stem Rot of coconut with respect to pathogen variability and bio-control based disease management practices were under taken with the following objectives:

- To estimate the incidence and severity of basal stem rot disease of coconut in selected coastal regions of surveyed area.
- To identify and characterize as an isolate on the basis of cultural and morphological study.
- To evaluate the efficacy of selected bio-agent, botanicals and bio-product against *Ganoderma sp.* in *In-vitro*.

REVIEW OF LITERATURE

Basal Stem Rot (BSR) disease of coconut caused by the different species of *Ganoderma* is one of the most devastating diseases of numerous perennial, coniferous and palmaceous hosts which causes white rots of hardwoods in many woody plants by decomposing lignin as well as cellulose and related polysaccharides (Hepting, 1971). In forest systems, *Ganoderma* has a harmful ecological role in the breakdown or delignification of woody plants. BSR disease takes several years to develop inside the plant and express the symptoms, presence of the pathogen is often only visible by fruiting bodies when the fungus is well established and more than half of the bole tissue decayed, leaving no chance for the grower to cure the infected palms resulting in excessive reduction in production and productivity of the palms (Kandan *et al*., 2010). A literature review concerning occurrence and distribution, disease development and symptoms, isolation and establishment of pure culture, disease diagnosis, taxonomy, morphological, cultural and molecular characterization besides bio-control based management practices are reviewed here under:

Barman and Ahmed (1998) in their study examined the performance of production and productivity of coconut in Bangladesh and also state that there is considerable expansion in the coastal regions in Chittagong and Khulna divisions. These two divisions account for about 81 per cent of coconut area and 83 per cent of production.

2.1. Occurrence and distribution of BSR

The distributions of *Ganoderma* species are worldwide in green ecosystem both to tropical and temperate regions (Pilotti, 2005). Among the various fungal diseases affecting coconut palm, basal stem rot (BSR) or root rot caused by *Ganoderma spp*. viz., *G. lucidum, G. boninense, G. applanatum* etc., is the most destructive fungus. *Ganoderma spp*. has a wide host range attacking a variety of palms and several

forest and fruit trees. According to Naidu *et al*. (1986), hosts belonging to 19 families, 36 genera and 48 species have been reported to be affected by *Ganoderma spp.* Coconut palms in the age group of 10–30 years are easily attacked by the pathogen. Hasan and Turner (1998) described that, above informations included as the coconut plant is the main hosts for Basal Stem Rot disease. The disease has been reported from various places all over the tropical world viz., India, Sri Lanka, West Indies, Seychelles, Guam, etc.

Turner (1981) reported that, there are fifteen species of *Ganoderma* isolated from various parts of the world such as North America, Africa, India, Malaysia, Indonesia, Papua New Guinea and Thailand as being associated with Basal Stem Rot, which includes, *G. applanatum, G. boninense, G. chalceum, G. cochlear, G. lucidum, G. miniatocinctum, G. pseudoferreum, G. tornatum, G. tropicum and G. zonatum.* Within these species *G. boninense, G. chalceum, G. lucidum, G. miniatocinctum, G. pseudoferreum, G. tornatum* in diseased oil palms from different areas of Peninsular Malaysia, *G. boninense* is the most aggressive pathogen to causing the basal stem rot in oil palm (Wong *et al*., 2012; Turner 1981).

Under controlled conditions, *Ganoderma boninense* are found to grow at an optimum pH of between 3.7 to 5.0 and optimum temperature between 27-30°C (Nawawi and Ho, 1990). At the field conditions, this may represent huge range of oil palm growing condition and soil types. The influence of soil types were not yet fully understood or reported on BSR disease occurrence and distribution. High soil moisture condition of coastal soils might have benefited *Ganoderma* to grow without much competition over other, antagonistic soil fungi (Gurmit, 1991). Basal Stem Rot disease is widely prevalent in coastal sandy soils or sandy loam soils where coconut is raised under rainfed conditions and much attention was not paid for cultural practices. However, the disease is not confined to any particular soil type (Bhaskaran and Ramanathan ,1984). Clay soils with poor drainage and a high water retention capacity might favour to cause Basal Stem Rot disease.

Snehalatharani *et al*. (2016) informed that, the disease in Indian subcontinent is reported to be caused by *G. lucidum* (Leys.) Karst., *G*. *applanatum* (Pers.) Pat. and *G. boninense*. If any adequate measures should not be taken, the disease is becoming a major threat to coconut production in Andhra Pradesh, Karnataka and Tamil Nadu states. The fungus usually attacks old or weak palms, which growing under unfavorable conditions. The pathogen is a soil dweller inhabiting in dead as well as living plant material in the soil, which enters into the plants through the wounds and disease spread mainly through soil.

2.2. Disease development and Symptomology

Singh *et al*. (1991) described that *Ganoderma* infects coconut palm from seedlings to old plants, the disease progresses slowly and every infected plant ultimately dies soon or later. The infection begins from the roots but the external symptoms appear on young palm as one-sided yellowing or molting of lower fronds, followed by necrosis. The infected coconut palms roots were very friable and their internal tissues become very dry and powdery. The cortical tissue was brown and disintegrated easily, while the stele becomes black in color.

Due to the infection, the newly unfolded leaves were shorter and chlorotic and sometimes the tips necrotize. Infected older plants produced several unopened spear leaves that were usually white in color. By the time the foliar symptoms apparent, the fungus killed half of the plant's tissues. Infected young palms died within 6 – 24 months, whereas mature palms took 2–3 years (Arrifin *et al.*, 2000).

Naher *et al* , (2012a) founded that from a glasshouse trial study, the disease severity of BSR is 8.3% in roots on six month-old oil palm seedlings however, leaf with no external symptoms. BSR is called a silent killer of coconut palms as the basal tissues in cross section showed as brown areas of rotting, wherein leaves appeared as alive. Turner (1981) and Ariffin *et al* , (1989) reported that areas of rotting tissues or darker yellow zones are called as "reaction zones" that is resulted from defense mechanisms of the palm to form infection.

Karthikeyan *et al* , (2006) reported that, the disease caused 15 to 25% damage to roots and bole below the ground level by the time external symptoms are visible. The internal tissues of the affected stem turn brown in color and rotting in the stem can be seen up to the height of the bleeding. Bleeding on the stem begins at the base and may extend up to 15 feet in severe cases (Vijayan and Natarajan, 1972). Occasionally, some infected palms do not show bleeding symptoms (Thirumalaiswamy *et al* ,1992). The bark from the base of the stem peels off. Infestation of scolytid beetle, *Xyloborus perforans* and the weevil, *Diocalandra stigmaticollis* are found infesting the stem in severely infected palms.

Ganoderma sp. causes root and stem rot diseases of different trees and crops resulting in losses of crops yield such as oil palm, rubber and other trees worldwide (Chee, 1990; Ariffin *et al* , 2000; Lee, 2000; Sankaran *et al* , 2005). The BSR disease could easily be detected when fruiting bodies (basidiocarps) appear on the stem of a tree, which release basidiospores (Pilotti *et al* , 2003; Pilotti, 2005; Sanderson, 2005) that causes root infection besides spread through root to root contact among trees (Turner, 1965 ; Ariffin *et al* , 2000).

Hennessy and Daly (2007) reported that at the advanced stage, the disease could be generally identified by the distinctive fruiting body or bracket or basidiocarp, which grows on the trunk of infected plants or which may or may not develop before foliar symptoms appear. The sporophores initially appear as small, white buttons of fungal tissues that develop rapidly into the familiar bracket-shaped mature sporophore. The young sporophore is white or yellow, whereas the mature sporophore upper surface can be light to dark brown, with alight margin and a shiny lacquered finish . The undersurface is whitish in color and has numerous minute pores (Bhaskaran *et al* , 1982; Rethinam , 1984). Brackets emerge once in the infected coconut palm, which has spread significantly through the plant, resulting in its death.

Schwarze and Ferner (2003) said that, spores of *Ganoderma sp.* incorporated into the soil, germinate and then the hyphae grew over the roots. The fungus moved from

the roots to the woody trunk tissue where it destroyed the xylem (**water conducting tissue**). Primary symptoms of that disease observed,which included a mild to severe wilting of leaves and die-back of some branches. Infected trees died gradually depending on the environmental conditions.

Recently, Vinayaka and Prathibha (2013) described the BSR disease symptoms that are characterized by yellowing of the lower leaves and decay/death of fine roots. Bleeding patches appeared at the base of the stem near the ground level with lesions gradually extending upwards, white crust appeared on the infected stem, leaves drooping followed by button shedding and barren nuts. They have also reported stem decay that traverse upwards, outer leaf whorl dying and drooping off followed by spindle leaf drooping except erect and healthy two/ three leaves that also fall off leaving the decapitated stem finally.

2.3. Weather factors

It was found that the disease incidence was reported to be more between **March and August** in the coastal regions of Bangladesh. The disease incidence was directly related with mean maximum soil temperature and it was not related with minimum temperature, rainfall and relative humidity (Bhaskaran *et al*., 1985). But Karthikeyan (2006) reported that there was a positive correlation with relative humidity.

2.4. Isolation and establishment of pure culture

Venkatarayan (1936) reported that, *G. lucidum* grew well on **malt agar** medium. Adaskaveg and Gilbertson (1987); Biley *et al* , (2000); Lomberh *et al* , (2002) also suggested that, **malt extract** medium also supported the good growth of *G. lucidum*. Booth (1971) informed that, **Potato Dextrose Agar** has been found good medium except for a slightly more time required for growth. Sharma and Thakur (2010) reported that, radial growth of *G. lucidum* was higher in **malt extract agar** added with **linseed extract** medium. Palanna *et al* , (2009) reported that, isolation of

Ganoderma sp. from sporophore and diseased root bits that were found good inoculum sources.

2.5. Identification of pathogen species

The taxonomy of **Basidiomycota** (one of phylum of fungi) is traditionally has been based on the morphological features of the basidiocarps or brackets. Identification based on these basidiocarp features, however, is prone to problems such as absence of basidiocarps during certain times of the year their morphological plasticity and presence of cryptic species (Moncalvo and Ryvarden, 1997; Gottlieb and Wright, 1999a). For these reasons, contemporary taxonomy and identification of *Ganoderma* species employ morphological studies, mating tests, analyses of biochemical and DNA sequence, or combinations. The fungus *Ganoderma lucidum* was first described under the name *Fomes lucidus* (Leys.) Fr. and Butler described the fungus in detail in 1909.

Taxonomically, *Ganoderma* belongs to the Phylum: Basidiomycota; Order: Aphyllophorales and Family: Ganodermataceae (Alexopoulous *et al*. 1996) the family: Polyporaceae (Ganodermataceae) and the genus: *Ganoderma* (Wasser and Weis, 1999).In all, **219 species** within the family have been assigned to the genus *Ganoderma*, of which *G. lucidum* (W. Curt.: Fr.) P. Karsten is the type species (Moncalvo, 2000).

The family, Polyporaceae is classified on the basis of tiny holes on the underside of the fruiting body which contain reproductive spores. They have a woody or leathery feel and the presence of these pores are obvious characteristics that distinguish polypores from other common types of mushrooms. Polypores, like other fungi, grew on wood as an expansive network of microscopic tubes known as mycelium. They degrade the wood over time and produce a fruiting body (conk) on the surface of the wood. *Ganoderma* species are among those fungi that can thrive under hot and humid conditions and are usually found in subtropical and tropical regions (Moncalvo and Ryvarden, 1997).

The *Ganoderma* was further subdivided into two subgenera on the basis of presence of laccate (*G. lucidum* complex) and non-laccate (*G. applanatum*) (Moncalvo and Ryvarden, 1997). More than 250 *Ganoderma* species have been described worldwide, and most of these descriptions have been based on only pleomorphic characters and therefore uncertainty exists about the taxonomic status . Such taxonomic problems lead to the misuse of names, absence of type specimens, the large number of synonymies and differences in morphological characters.

Due to such problems, Moncalvo *et al*, 1995a, b; Gottlieb *et al*, 2000; Hong and Jung, (2004) proposed methods to determine the identity of *Ganoderma* species including cultural characters, sexual compatibility studies, isozyme studies and DNA based techniques.

2.5.1. Cultural characteristics

Morphology and cultural characteristics of basiodiocarp such as chlamydospore production, growth rate and thermophily have been used to differentiate *Ganoderma* species (Adaskaveg and Gilbertson, 1986 & 1989).

Seo and Kirk (2000) reported that hyphal structures in culture such as generative hyphae with clamp connections, hyaline, thin walled, branched, 1.4 to 2m in diameter, abundantly formed chlamydospores which are slightly thick-walled, terminal or intercalary, ellipsoid and sometimes in chains. 8.8-11.8 m × 3.7-5.9 m in size; cuticular cells from crustose layer are hyaline to light brown, round to irregular in shape and closely packed, presence of staghorn hyphae with projections in some isolates. Submerged mycelium is thin walled, hyphae and chlamydospores as in aerial mycelium (Govindu *et al*. 1983) and also produce fibre or skeletal hyphae, cuticular cells and vesicles, and hyphal rosettes as well as chlamydospores production besides growth rate and thermophily of the cultures distinguished *Ganoderma* species.

Rajendran *et al* , (2007) suggested that most of the *Ganoderma* isolates produced a dense mycelial growth on PDA medium and a few isolates showed sparse mycelial growth. Most of the isolates appeared white in the initial stage of growth and later the colony colour changed from white to pale yellow or light brown.

2.5.2. Morphological characteristics

Ganoderma is morphologically most complex genus of family Ganodermataceae of Aphyllophorales. Different characteristics, such as shape and colour (red, black, blue/green, white, yellow and purple) of fruit body, host specificity and geographical origin, were used to identify individual members of the species. Fruiting bodies of *G. lucidum* were stipitate, dimidiate or reniform and rarely suborbicular, thick, corky, yellowish in margin which turn brownish with shining laccate on the surface (Wang *et al* , 2012).

The shape and size of the basidiospores and the cuticle cells have been considered as the two most important characters in the genus *Ganoderma*. All *Ganoderma* species lack cystidia, have echinucleate basidiospores and often cause white rot in the substrate/host (Steyaert, 1972). Basidiospores of *G. lucidum* were brown, ovate with a rounded base and truncate to narrowly rounded apex.

Usually, different species of *Ganoderma* produce different feature and pathogenecity . The species identification or differentiation of *Ganoderma* is still limited; thus, this lack of information causes crucial problem for disease management. Preliminary studies on scanning electronic microscopy of *Ganoderma applanatum* and *G. lucidum* isolated from basal stem rot infected coconut could able to differentiate both the species. Spores of *G. applanatum* are single, dumbbell shaped where as that of *G. lucidum* are bundled together in round balls (Anonymous 2005).

Association of *Ganoderma lucidum* with BSR disease of coconut was reported by Vijayan and Natarajan (1986) and Bhaskaran *et al.* (1990a). Pathogenicity of

Ganoderma spp. isolated from Ganoderma wilt infected coconut palm was proved by many researchers. Both *G. applanatum* and *G. lucidum* were isolated from the diseased root bits of coconut. However, colonization of *G. lucidum* was very fast when compared to *G. applanatum.* Root rotting up to 21% was observed in palms inoculated with *G. lucidum* and only colonization up to 8 to 10 cm on either side of inoculation point was observed with *G. applanatum* (Bhaskaran *et al*, 1991; Srinivasulu *et al*, 2003). Naik *et al*, (2000) reported that disease symptoms developed in coconut seedlings after 9 to 11 months under artificial inoculation conditions. Occurrence of both the species as the causal organism of basal stem rot of coconut was reported and there was wider variation morphologically and genetically among the isolates of *Ganoderma* collected from various districts of the states (Anonymous, 2014).

2.6. Virulence of *Ganoderma* isolates

Various methods of artificial inoculation of oil palm seedlings had been carried out. Lim *et al*. (1992) successfully inoculated injured roots of healthy 15-month-old palms by placing wheat-oat medium inoculated with *Ganoderma*. Idris *et al.* (2004) employed root inoculation method where primary roots of oil palm seedlings in polybag were exposed and inserted into test tubes containing various *Ganoderma spp*. isolates grown in POPW medium (mixture of paddy, oil palm, wood sawdust, supplemented with sucrose, ammonium sulphate, calcium sulphate, and bacto peptone). Of a total of 344 isolates tested, 304 isolates were found pathogenic and 40 isolates were non-pathogenic.

Breton *et al*, (2006) observed that artificial inoculation methods of *Ganoderma boninense* isolates to oil palm seedlings were one of the main parameters affecting the level of disease severity and aggressiveness of isolates collected from three different estates in Indonesia. They have suggested investigating growth rates of different *G. boninense* isolates under *in-vitro* conditions and variations in the degree of virulence in oil palm seedlings.

2.7. Disease Management

Originally, the *Ganoderma* is a soil-borne pathogen and it survives well in the soil for a long time. The incubation period of this disease has been determined to be several years (Turner, 1981). The visible disease symptoms of this pathogen appear at a very late stage of infection when more than half of the root tissues have been decayed. Basal stem rot disease could be arrested by different management practices with the integration of cultural, chemical and biological methods if the disease is detected in the early stages.

2.7.1. Early detection

Basal Stem Rot disease is like a "cancer" in palms especially in coconut, as well as in oil palms, and it is very difficult to detect at the early stages of the disease. The limiting factor in the control of BSR is the lack of early disease detection. Some conventional diagnostic tools have been developed for early diagnosis of BSR such as (i) the colorimetric method, using Ethylene Diamine-Tetraacetic Acid (EDTA), which is used to detect *G. lucidum* in coconut (Natarajan *et al*., 1986), (ii) semi-selective media for *Ganoderma* cultures from oil palms (Darus *et al*., 1993), and (iii) Ganoderma-selective media (GSM) which detect the pathogen from any infected tissues. According to Ariffin *et al.* (1996), GSM detected *Ganoderma* in oil palms that were infected but not shown any external symptoms. Due to the low accuracy these conventional methods have not been recommended.

Kandan *et al*, (2009) ; Priestley *et al.*, (1994) ; Fox and Hahne, (1989) reported that, recently, advance molecular techniques have been innovated with more accuracy of detection and fungal identification. Two diagnostic methods used to detect *Ganoderma,* such as (i) the use of polyclonal antibodies (PAbs) in the pathogen using enzyme-linked immunosorbent assay (ELISA), and (ii) the use of polymerase chain reaction (PCR) methods using specific deoxyribose nucleic acid sequences of the pathogen. This laboratory based techniques were suitable for small scale samples which were not applied for field condition.

Santoso *et al* , (2011; Azahar *et al* , (2011); Markom *et al* , (2009) informed that the development of device system in agriculture technology such as remote sense system is being used to detect real-time disease monitoring. However, the results showed that the remote sense system could discriminate the *Ganoderma* infected plant in field condition but not able to detect the stages of infection levels or early infection of the disease.

For detecting the presence of *G. lucidum* inoculum in coconut gardens, various plant species were tested of which Subabul (*Leucaena leucocephala*) and *Glyricidia maculata* were very useful as indicator plants since these plants showed natural infection under field conditions at least six months earlier to infection on coconut palms (Anon , 1989).

Karthikeyan *et al* , (2006) reported that molecular and immunological methods for detecting the Ganoderma disease of coconut. Rajendran *et al* , (2014) reported PCR as a new technique that has gained broad acceptance very quickly in many areas of science and application of the two Gan1 and Gan2 primers generated from the ITS1 sequence proved useful for the specific detection of plant pathogenic *Ganoderma* isolates.

2.7.2. *In-vitro* evaluation of antagonists against Ganoderma

Dharmaputra *et al* , (1989) reported that *Penicillium citrinum* inhibited the growth of the pathogen and formed a zone of inhibition on the agar media. *Trichoderma harzianum* and *T. viride* not only repressed the growth of the pathogen but also caused lysis of the hyphae, and the colony was totally overgrown by the antagonists.

Bansali (2003) reported that in dual-culture technique *Trichoderma* inhibited the mycelial growth of *Ganoderma lucidum* on potato dextrose agar under ***in vitro*** conditions. Among the three strains of *T. harzianum* and *T. viride* inhibited the maximum mycelial growth by acting as antagonists to *G. lucidum*.

Iyer *et al.* (2004) reported that four fungal cultures viz., an unidentified sterile white fungus (77.80), *Trichoderma harzianum* (72.20), *T. viride* (62.0) and *Pencillium sp. (*42.0) were found to be inhibitory on the mycelial growth of pathogen at 96 hrs. Srinivasulu *et al*. (2004) reported that *Trichoderma viride* completely inhibited *Ganoderma applanatum* and *Ganoderma lucidum*.

Reports of CPCRI revealed that the culture filtrates of *Trichoderma harzianum* and *Bacillus amyloliquefaciens* showed antagonistic property against *Ganoderma*. Culture filtrate of *T. harzianum* inhibited the mycelial growth of *Ganoderma* (54 per cent), while culture filtrate of *B. amyloliquefaciens* showed 80 per cent inhibition.

Anon (2007) revealed that among the three *Trichoderma spp.* tested, the maximum inhibitory effect on the pathogen was exerted by *T. virens* (74%), followed by *T. viride* (69%) and *T. harzianum* (58.5%). Among the bacterial antagonists tested, the maximum inhibition was produced by *Pseudomonas fluorescens* (83.1%), followed by *Bacillus subtilis* (83.0%), *P. fluorescens* (50.5%) and *Bacillus sp*. (43.0%).

Among the 17 bio-control agents screened, native *Trichoderma sp.* recorded minimum radial growth of 1.72 cm by exerting 81 percent reduction over control, it was followed by *Trichoderma sp*. that recorded 2.30 cm radial growth with 74 percent reduction over control (Palanna *et al* , 2013).

Rajendran *et al*. (2007) reported that 55 endophytic bacterial strains isolated from coconut roots of different regions, isolate EPC5 (Endophytes coconut), EPC8, EPC15, EPC29, EPC52 and Pf1 (Plant growth promoting rhizobacteria) promoted the rice seedling growth in roll towel and pot culture method. EPC5 (Plant growth promoting endophytic bacteria), Pf1 and *Trichoderma viride* (Plant growth promoting fungus) effectively inhibited the *G. lucidum* growth ***in-vitro.***

Chakrabarty and Ray (2007) reported that dual culture studies revealed that the three fungal cultures viz., *Trichoderma harzianum* (63.99%), *Trichoderma viride*

(66.55%) and *Gliocladium virens* (62.12%) have inhibitory effect on the mycelial growth of the pathogen after 96 hours of incubation.

Susanto *et al* , (2005) suggested that *in vitro* studies have shown the fungi *Trichoderma spp*., *Aspergillus spp*., and *Penicillum spp*.antagonistic towards *Ganoderma* and were found good bio-control agents against *Ganoderma.*

2.7.3. *In-vitro* evaluation of botanicals against Ganoderma

Srivastava *et al.,* (1994) ; Singh *et al*., (1999) described that the plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics, etc. Plant extracts are effective against plant pathogens as they have unique antimicrobial properties that act in a holistic manner due to presence of certain secondary metabolites, *viz*., alkaloids, terpenoids, glycocides and phenolic acids.

Garlic extract of 10 per cent concentration completely arrested the growth of *T.viride, T. harzianum* and *T. hamatum* and both the species of *G. lucidum* and *G. applanatum* (Srinivasalu *et al*., 2002). Neem cake extract, banana rhizome extract and *Tephrosia purpurea* extract gave 100, 86 and 54 per cent inhibition respectively.

Kharkwal *et al*, (2012) determined the antifungal activity of the dealcoholized extract of the leaves of *Clerodendron infortunatum* Retz.against four fungal organisms *i.e. A.niger, P. frequentance, P. notataum* and *B. cinera*. Bhardwaj (2012) carried out test of aqueous extract of twenty plants for their antifungal activity against *Fusarium solani*, the causal organism of dry rot disease of potato.

Among 10 botanicals tested under laboratory conditions, only *Glyricidia* was found inhibitory against *G. applanatum*, by recording radial growth of 5.4 cm as against 9.0 cm in control (Palanna *et al*., 2013). Garlic was found to be fungitoxic to a number of plant pathogen (Iyer *et al*, (2004) ; Gowda and Nambiar, (2006); Chakrabarty *et al* , 2013).Crude extract of different plant parts of *Solanum nigrum* obtained using solvents *viz*., petroleum ether, chloroform, acetone, ethanol and

methanol showed that leaf aqueous extract was more effective against all the microbes tested (Ramya *et al* , 2012).

Antifungal activity of a perennial aquatic herb (*Eichhornia crassipes*) was tested against *Ganoderma lucidum*, basal stem rot pathogen of coconut at two concentrations (150 mg/ml and 300 mg/ml). Among these concentrations, 300mg/ml was found to be most effective in inhibition of pathogen (Deepatharshini and Ananthi, 2015).

2.7.4. *In-vitro* evaluation of chemicals against Ganoderma

Results of experiments carried out in CPCRI, Kasargod revealed that six fungicides viz., Calixin (Tridemorph 80% WP), Bavistin (Carbendazim 50% WP), Captra (Captan 50% WP), Companion (Carbendazim 12% + Mancozeb (63% WP), Diodine (65% WP), Matco-8-64 (Metalaxyl 8% + Mancozeb 64%) and Indofil-M-45 (Mancozeb 75% WP) tested for their efficacy on *Ganoderma* isolates, Tridemorph 80% EC and Companion (Carbendazim 12% + Mancozeb (63% WP) proved to be the most effective (Anon, 2000). Among the fungicides tested, copper oxychloride (0.3%), Bordeaux mixture (1%), Calixin (0.1%) and Contaf (0.1%) exerted complete inhibition (Anon., 2013).

2.7.5. Integrated Disease Management of BSR disease of Coconut

Palanna *et al.,* (2005a) reported that manganese sulphate, magnesium sulphate, ammonium molybdate, calcium sulphate and ferrous sulphate are found to be supportive for the growth of *T. viride* under *in vitro* conditions, whereas copper sulphate and sodium borate exerted 100 % inhibitory effect. The growth of *T. viride* was significantly increased by Na, K and Mg salts compared to medium without salts. Srinivasalu *et al.,* (2002) reported that Bordeaux mixture (1%), Copper oxychloride (0.3%) Bitertanol (0.1%), Tridomorph (0.1%), Hexaconazoal (0.1%)

and Traidemifon (0.1%) were found completely inhibiting the growth of *G. lucidum, G. applanatum* and three species of *Trichoderma.*

Basal stem rot disease caused by *Ganoderma* considered most destructive disease of palms. Resistant mycelium, basidiospores, chlamydospores, and pseudosclerotia present in *G. boninense* influence the control of *Ganoderma* (Susanto *et al*., 2005; Naher *et al*., 2012b).

Sanderson (2005) and Paterson (2007) also revealed that , a dead or felled oil palm could spread the disease by spore or root contact. Hence, no satisfactory method exists to control BSR in the field condition. However, studies using various methods such as the trenching, replanting techniques and chemical control had given different paths with great potential. Digging trenches around infected palms to prevent mycelium spread by root contact with neighboring healthy palms has been recommended as a management practice. Sanitation during replanting regarded as an important practice for controlling BSR. The result showed that this method could only lower the disease incidence. Biological control of BSR disease or *Ganoderma spp*. achieved with prophylactic application of *Trichoderma* at early stages of the disease (Abdullah *et al*., 2003).

Gunasekaran *et al*., 1986 described that biocontrol agents like *Trichoderma harzianum* and *T. viride* were reported to be antagonistic to *Ganoderma lucidum*. Nur Ain Izzati and Abdullah (2008) reported that disease suppression in *Ganoderma* infected oil palm seedlings treated with a conidial suspension of *Trichoderma harzianum* was tested in plant house conditions to determine the effectiveness of the fungus as a biocontrol agent. The highest efficacy of control was achieved by treatment right after artificial infection; the total number of infected plants was reduced to give the lowest disease severity index (DSI) value of 5.0 per cent, compared to the infected and nontreated control that had the highest DSI of 70.0 per cent. Jayarajan *et al.* (1987) stated that Neem cake is effective in reducing Ganoderma wilt of coconut.

Sampath Kumar and Nambiar (1990) reported that, drenching the base of the palm with Captan or Carbendazim at 0.3 per cent concentration was effective in preventing the spread of the disease to the neighbouring palms. Bhaskaran (1993) reported that root treatment with Tridemorph (2ml/100ml) at quarterly interval for one year combined with application of 5kg neem cake per palm per year controlled the basal stem rot of coconut effectively. The chemical treatment (carboxin and quintozene fungicides) showed significant reduction in BSR incidence (George *et al*., 1996).

Naik (2001) reported the lowest BSR index with the application of Tridemorph root feeding (2%) + soil drenching (0.3%) followed by Hexaconazole root feeding (l%) + soil drenching (0.2%); soil drenching with Tridemorph (0.3%) and Hexaconazole (0.2%) compared to root feeding Tridemorph (2%) or Hexaconazole (1%) alone in the gardens. Naik and Venkatesh (2001) reported that use of 2 per cent Tridemorph for root feeding and 0.1 per cent as soil drench in combination with neem-cake application for managing basal stem rot of coconut.

Srinivasalu *et al*, (2004) reported that native bio control agents *viz*., *T. viride, T. harzianum, T. hamatum* were found to be inhibitory to *G. applanatum* and *G. lucidum*. Tridemorph (0.1%) and Hexaconazole (0.1%) were found to completely inhibit both *G. applanatum* and *G. lucidum* under *in vitro* condition.

Karthikeyan *et al*, (2009) reported that in integrated disease management (IDM), fungicide tridemorph treated palms showed low infection level (O.D value) within seven months and *T. harzianum* and *P. fluorescens* + *T. viride* treated palms showed below infection level (OD value) of the disease in eighth month. Combination of *T. viride (*50g*)* and neem cake @ 5kg/palm/year was found to be highly effective in the management of BSR disease of coconut (Srinivasalu *et al*., 2004).

Bhaskaran *et al*, (1990a) stated that incorporation of organic manures, especially neem cake into the soil and irrigation during summer reduced disease severity. Root treatment of coconut palm infected by *Ganoderma lucidum* with Tridemorph

(2ml/100ml water) at quarterly intervals for one year combined with annual application of 5 kg neem cake/palm reduced disease incidence and increased yields by 132% (Bhaskaran, 1993). Application of Neem cake @ 10kg/palm/year increased the total population of fungi in rhizosphere and inhibited the growth of *G. lucidum* (Gunasakaran *et al*., 1986). Srinivasalu *et al.* (2001) stated that 50gm *T. viride* + Neem cake (1kg) per palm per year controlled the linear spread of *Ganoderma* to the extent (22cm) against 77.6 cm in untreated palms.

The lowest disease index was recorded in treatment with Tridemorph root feeding (2%) + Soil drenching (0.3%), followed by Hexaconazole root feeding (1%)+ soil drenching (Naik, 2001). Karthikeyan *et al*, (2006) stated that, the mixture of two antagonists (*P. fluorescens* + *T. viride*) suppressed Ganoderma disease development in coconut.

MATERIALS AND METHODS

To conduct the study, a survey was done in selected locations of coastal regions *viz*., Patuakhali, Pirojpur and Barishal district. For the management study, an *in-vitro* experiment was conducted in Molecular Biology and Plant Virology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The study was done during the period of January 2018 to October 2019. The details of materials used and methodology followed during the study are described in this chapter.

3.1. Survey study

3.1.1. Survey study for the prevalence and incidence of basal stem rot (BSR) disease of coconut in selected location of coastal regions

For the survey study, three upazillas of three districts were selected from coastal areas of Bangladesh *viz*. Patuakhali, Pirojpur, Barisal (Table 1). Based on simple random sampling technique in each garden a number of coconut plants were uniformly observed and number of plants infested with basal stem rot disease were recorded (Plate 1-4). The disease incidence was estimated by using the following formula.

$$\text{Disease Incidence (\%)} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

During the course of survey study, different cropping pattern followed by the farmers with coconut, soil type, method of irrigation etc. were recorded and correlated with disease incidence. In addition, farmer's practices for management of BSR performed in orchard of coconut and other related informations were also documented as per the structural questionnaire (Appendix I & II).

Map showing the experimental site under the study

★**The experimental site (Barishal, Patuakhali and Pirojpur district, SAU campus) under the study.**

Table 1. Survey details of Selected areas for the survey of BSR disease of coconut

Sl No.	Locations	Areas	No. of garden observed	Total no. of plant in observed garden	No. of plant observed
01.	Patuakhali	Kalapara	03	530	60
		Dashmina	02	290	60
		Patuakhali sadar upazila	03	420	60
		Sub-total	08	1240	180
02.	Barishal	Babuganj	03	750	60
		Gaurnadi	03	335	60
		Mehendiganj	03	750	60
		Sub-total	09	1830	180
03.	Pirojpur	Bhandaria	03	625	60
		Nesarabad	03	525	60
		Kawkhali	03	450	60
		Sub-total	09	1600	180

3.1.2. Disease severity index (DSI)

The plants were scored for disease class on a scale of 0 to 4 (Table 6). After recording the disease class for each control and treatment, the Disease Severity Index (DSI) was calculated using a modified method of Abdullah *et al*. (2003).

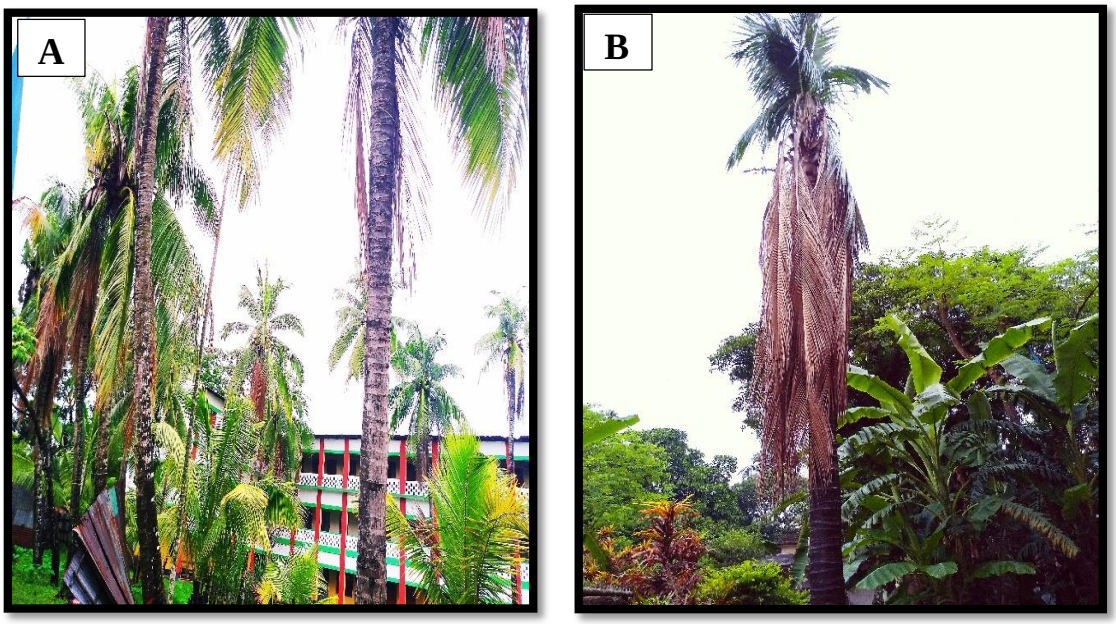
The DSI was calculated based on the following formula:

$$\text{Disease Severity Index/ (DSI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plants assessed x maximum disease grade}} \times 100$$

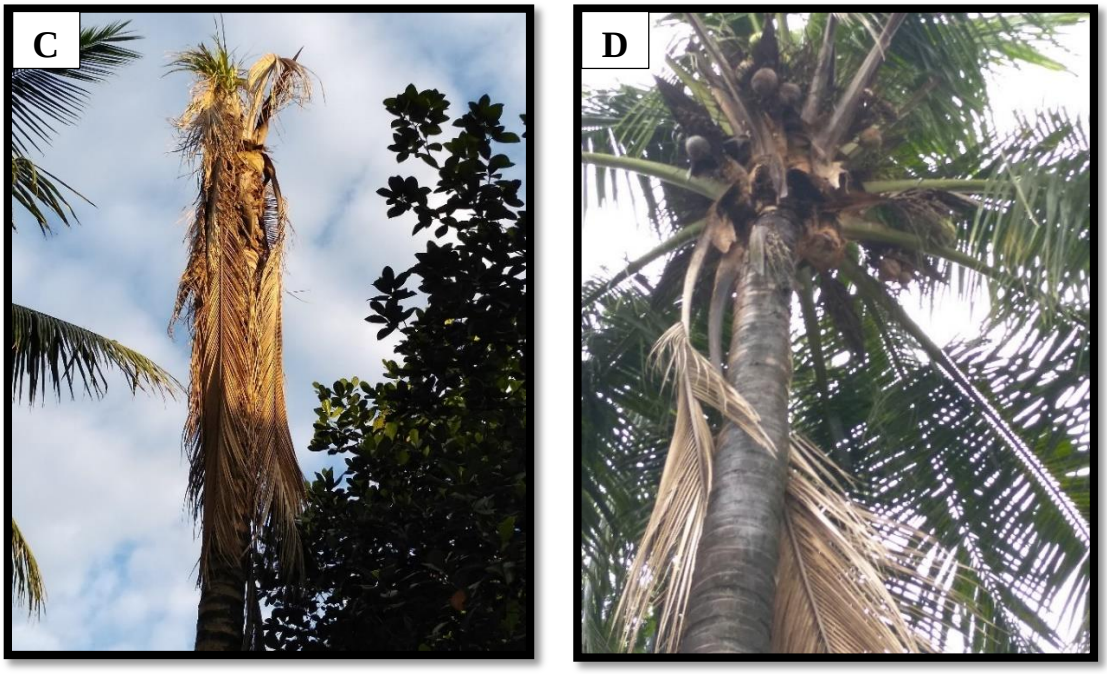
Where:

Disease grade (0, 1, 2, 3 or 4) are taken from Disease scale for Basal Stem Rot 0-4, Abdullah *et al* , (2003).

Disease class	Signs and symptoms of infection
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on any part of plants , with or without chlorotic leaves
2	Appearance of fungal mass/mycelium on any part of plants with chlorotic leaves (1-3)
3	Appearance of fungal mass / mycelium on any part of plants with chlorotic leaves(> 3)
4	Formation of well-developed basidioma and plants dried /wilted



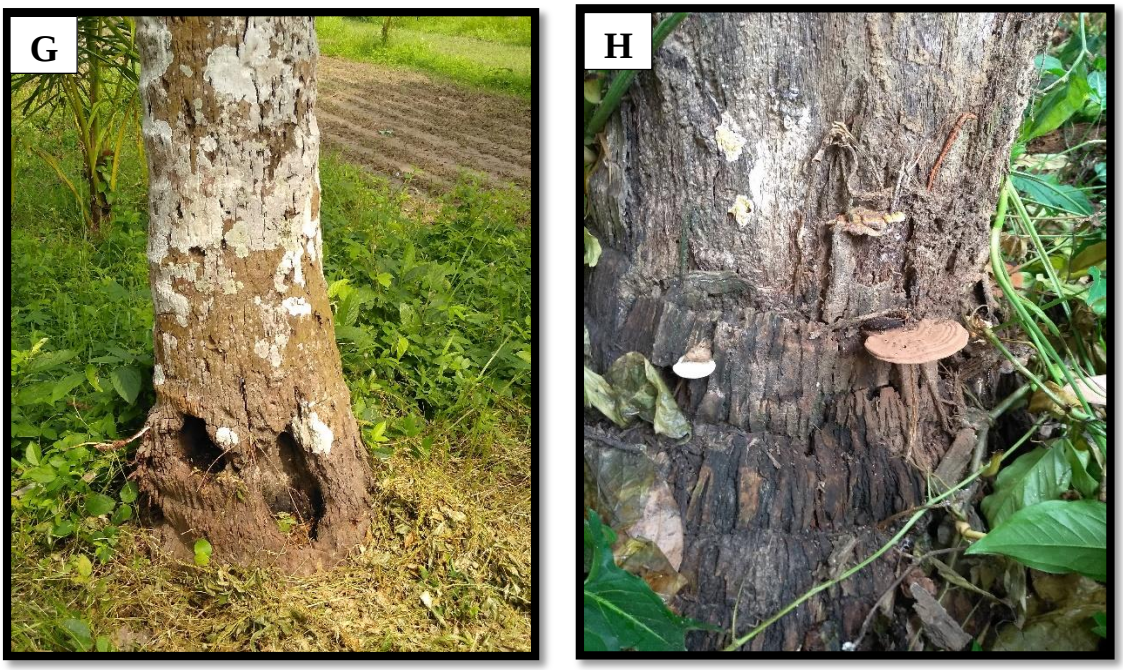
(A &B) Yellowing of leaves and reduction in crown size



(C & D) Withering and drooping of leaves around the crown and skirt formation

Plate 1. Yellowing and withering leaves symptoms of Basal stem rot disease of coconut found in survey areas

(E & F) Plants with necrotic leaves



(G & H) Wood decaying started by *Ganoderma sp.*

Plate 2. Symptoms of basal stem rot found in survey areas

(I & J) Formation of white crust on the BSR disease infected coconut stem

(K & L) *Ganoderma* infected coconut root tissue (white crust)

Plate 3. Symptoms of basal stem rot found in survey area

Stem bleeding of BSR disease infected plant **_Ganoderma_ infected dead plant**

Formation of basidioma on the base of coconut plant

Plate 4. Symptoms of basal stem rot found in survey area

The most common symptoms observed during course of investigation are yellowing of outer whorl leaves, reduction in crown size, drooping of leaves, decay of wood and severely affected palms, formation of basidiocarp (Plate 1, 2, 3, 4). The farmers are not conscious about the basal stem rot disease of coconut. So, the disease rate is increasing day by day. In Pirojpur district some commercial garden were so much affected that the yield of coconut about to be stopped. A farmer of Barisal BADC informed that about 20 plants were dead at a time. As they didn't know any control measure, they were unable to treat the plants.

3.1.3. Collection and preservation of diseased roots of BSR disease infected coconut plant from different Survey areas

Different parts of the coconut palms such as diseased root bits, diseased barks and sporocarps were collected from infected plants from survey areas. The samples were labeled properly and packed in polythene bags to bring the lab for further study. In the lab the samples were preserved in refrigerator at 4°C.

Collection of diseased roots, barks and sporophore of coconut from different Survey area

Plate 5. Samples of basal stem rot collected from different survey area.

3.1.4. Cleaning and sterilization of laboratory materials

The glassware was washed and dried in hot air oven. The cleaned and dried petri dishes were rapped with foil paper and sterilized in the oven drier. Test tubes and flasks containing liquid media plugged with non-absorbent cotton covered with foil paper were autoclaved.

3.1.5. Sterilization of the laminar air flow

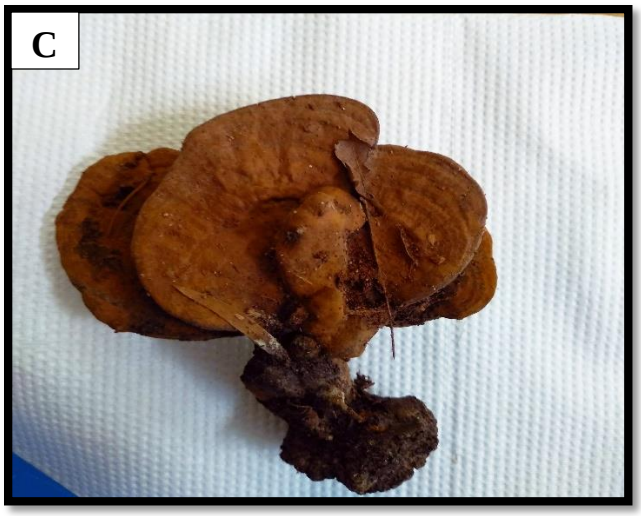
All the experiments namely isolation, cultural studies and *in vitro* evaluation of bio control agents, botanicals were conducted under aseptic conditions in the laminar air flow cabinet. Before and after working UV- ray was given for sterilization of the laminar air flow. Before working under the hood, the working surface was uniformly sterilized by swabbing with 70 per cent ethanol. Any other material brought from outside was also sterilized with ethanol. In case of glassware, mouth of the bottles, etc. was flamed before and after use. The blades, forceps, inoculation loop etc. were sterilized by autoclave or heating in the flame. Also, before starting the experiments the hands were cleaned well with 70 per cent ethanol.

3.1.6. Isolation and identification as isolate of the identified causal organisms

Infected roots/stems collected from infected coconut plants were washed thoroughly with sterile water and cut into small pieces and were surface sterilized in 70% ethanol for 30 seconds and washed three times serially in sterile distilled water to remove the traces of ethanol. After surface sterilization diseased specimens were placed in sterilized Petri dishes along with wet blotter papers under room temperature for about 5 to 7 days. After 5 to7 days of incubation period, slight mycelial growth was observed and that was transferred into Potato Dextrose Agar (PDA) and Potato Sucrose Agar (PSA) medium (Plate-6,7,8). The inoculated petri plates were incubated at room temperature (28 °C ± 2 °C) for 30 days to facilitate growth of the fungus. The radial growth was measured 2 days interval for 3 times. The pure culture of the fungus was obtained by following

hyphal tip culture technique under aseptic conditions. The isolated *Ganoderma* isolates of coconut were designated as Bbg, Bgd, Bmg, Psp, Pds, Pkp, Pikl, Pins, Pibh and GSAU (Table.2).

Diseased root samples **Diseased bark samples**



Basidioma of *Ganoderma sp.*

Plate 6. Collected Samples for *Ganoderma* isolation

Table 2. Identification and designation of *Ganoderma* isolates

SI NO.	Source of isolation	Place of collection	Identity and designation of *Ganoderma* isolates
01	Root sample and bark bits	Babuganj, Barisal Dist.	Bbg
02	Root sample	Gaurnadi, Barisal Dist.	Bgd
03	Root sample	Mehendiganj, Barisal Dist.	Bmg
04	Root sample and bark bits	Sadar upazila, Patuakhali Dist.	Psp
05	Root sample	Kalapara, Patuakhali Dist	Pkl
06	Root sample	Dashmina, Patuakhali Dist	Pds
07	Root sample	Nesarabad, Pirojpur Dist.	Pins
08	Root sample	Bhandaria, Pirojpur Dist.	Pibh
09	Root sample	Kawkhali , Pirojpur Dist.	Pikl
10	Root sample, bark bits and Sporocarp	SAU campus , Dhaka	GSAU

3.1.7. Maintenance of pure cultures of different identified isolates from different coastal regions

The isolated fungus was sub-cultured on PDA and PSA medium and allowed to grow at 28 °C ± 2°C temperature for 5-7 days. The cultures obtained were stored in refrigerator at 4°C for further studies and they were cultured periodically once in month.

3.1.8. Study on variability of *Ganoderma* isolates of coconut

Ten *Ganoderma* isolates of coconut each isolated during course of investigation were used in variability study.

Plate 7. Collected root and bark samples are incubated in blotter paper for the identification of causal organism

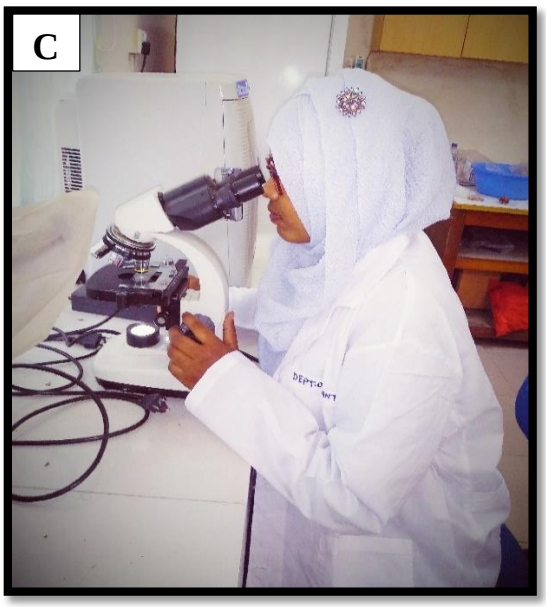
3.1.9. Cultural and morphological variability of *Ganoderma* isolates

3.1.9.1. Growth on Potato Dextrose Agar

PDA media was prepared according to standard protocol. To carry out the study, 15-18 ml of the medium was poured in 90 mm petriplates. Such petriplates were inoculated with 5 mm disc cut from periphery of actively growing seven-day old culture of the individual isolate grown on PDA and PSA media in petriplate and incubated at 28± 2 ^{o}C. Each treatment was replicated thrice. Ten *Ganoderma* isolates (Bbg, Bgd, Bmg, Psp, Pds, Pkp, Pikl, Pins, Pibh and GSAU) were collected from different geographic locations and then cultured on PDA and PSA media. The morphological characters like colony diameter/growth biomass production, colony colour, colony margin, mycelial density, appearance of zones, reverse pigmentation and conidial structure etc were studied (Plate 7-10). The experiment was conducted in three replications. Mycelia from seven days old active culture was transferred onto the centre of a standard 9 cm PDA plate and incubated for 7 days at an ambient

temperature. The test for all isolates was run simultaneously to avoid prejudice due to outward factors. The diameter was measured daily and the number of days required for maximum growth of mycelium was also recorded. The colony texture, appearance of zone, type of colony margin, reverse pigmentation colour and mycelial density were recorded after seventh day of incubation (Table 3). Observations were taken when the pathogen covered completely in petriplate. The colony diameter was recorded by averaging the radial growth of the colony. The data on radial growth were analyzed statistically.

Incubated samples are inoculate into selective media



Diagnosis the isolates and causal organisms of BSR disease

Plate 8. Incubated samples are inoculate on the media under laminar airflow and diagnosed on microscope .

Table 3. Cultural and morphological characters and their corresponding codes used to describe *Ganoderma* isolates

SI NO.	Characters	Description	Code
01.	Days for full plate	< 6	1
		6-9	2
		9-15	3
		>15	4
02.	Colony colour	White	5
		Pale white	6
03.	Mycelia texture	Smooth	7
		Leathery	8
		Fluffy	9
04.	Reverse pigmentation	No pigmentation (White)	10
		Pale yellow	11
		Yellowish	12
		Pinkish	13
05	Mycelia density	Thin	14
		Dense	15
		Thin at center & dense at corner	16
		Irregular density	17
		Dense at center	18
06.	Margin	Filamentous	19
		Even	20
		Undulate	21

Source: Palanna, K. B. (2016).

3.2. Management study (*In-vitro*)

3.2.1. Biocontrol-based disease management of basal stem rot disease of coconut

3.2.1.1. Treatment Details

Bio-control based *in-vitro* management treatments was conducted in the Molecular Biology and Plant Virology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University. *Ganoderma* Isolate from SAU campus was selected for *in-vitro* management practices during December, 2018 and data was collected in the certain time interval. Treatment details are given in Table 10.

Table. 4. List of treatments

Treatments	Name of the treatments
T_1	*Trichoderma viride* (suspension)
T_2	Neem oil
T_3	Cattle urine
T_4	Garlic solution
T_5	Control

3.2.1.2. Preparation of treatment particulars

For the treatment purpose *Trichoderma viride* solution was collected from the available sources and diluted with distilled water. The diluted solution was applied in petriplates both in Cup method and Pour technique method. Neem oil was collected and applied also by both methods . Garlic extract was made by fresh garlic then it is blended by blender. The Garlic extract was applied by both methods for *in-vitro* evaluation. Neem oil is collected from nearest market. Fresh cattle urine was collected from sher-e-bangla agricultural university farm and applied by both methods.

***Trichoderma* suspension**

Neem oil

Garlic extract

Cattle urine

Plate 9. Different bio-control treatment particles

3.2.2. Analysis of data

The survey data were analyzed by using computer package program SPSS and data from lab experiment were analyzed by using Statistix-10 software. Treatment means were compared by LSD at 0.05 level of significance. The data obtained in the present investigation for various parameters were subjected to ANOVA for a completely randomized design for *in -vitro* studies in lab.

RESUTLS AND DISCUSSIONS

Experimental results pertaining to isolation of the causal organism of basal stem rot disease of coconut from roots, stem barks and sporocarps that were collected from different surveyed areas; the cultural and morphological variability of those isolates; bio-agents and botanicals against *Ganoderma sp.*; the *in-vitro* management program to control the Basal Stem Rot disease of coconut have been presented in this chapter.

4.1. Survey study

4.1.1. Survey study for the incidence and severity analysis of basal stem rot disease of coconut in selected coastal regions

4.1.1.1. (%) Disease incidence and severity of basal stem rot disease in coconut

A Survey was carried out through random sampling technique in selected coastal regions; the (%) disease incidence and severity of basal stem rot disease in coconut were measured. The location wise disease incidence of BSR was ranged from 42% - 78%. Among surveyed locations, maximum disease incidence (78%) of basal stem rot (BSR) was recorded in Bhandaria of Pirojpur district and minimum disease incidence (42%) was recorded in Mehendiganj of Barisal district.

The location wise disease severity of BSR was ranged from 23.18 - 45%. Maximum disease severity (45%) of BSR was recorded in Bhandaria of Pirojpur district and minimum disease severity (23.18%) was recorded in Nesarabad of Pirojpur district. Results of (%) disease incidence and severity are present in table 5 and figure 1.

Table 5. (%) Disease incidence and severity of basal stem rot disease of coconut in selected coastal regions

SI NO.	District	Upazila	No of Plants observed	BSR*	
				DI (%)*	DSI (%)*
01	Pirojpur	Bhandaria	60	78	45
		Nesarabad	60	55	23.18
		Kawkhali	50	75	42.5
02	Barishal	Babuganj	60	67	28.5
		Gaurnadi	40	62	31.25
		Mehendiganj	60	42	28.75
03	Patuakhali	Kalapara	60	68	25.38
		Dashmina	40	72	31.25
		Patuakhali police line	60	65	35.75
			Total= 490		

***(N.B: BSR- Basal Stem Rot, DI- Disease Incidence, DSI- Disease Severity Index)**

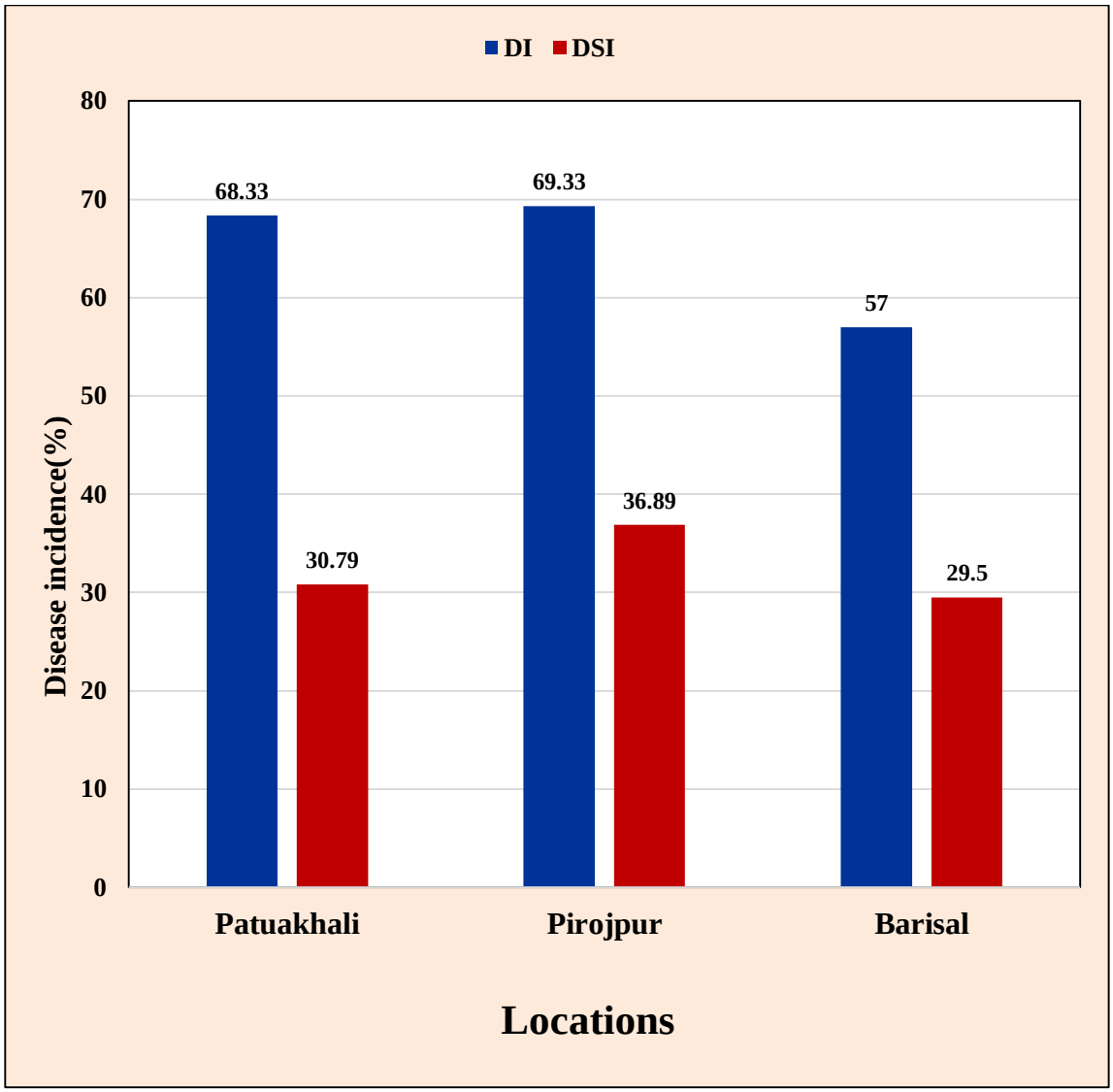


Figure 1. Disease incidence and severity of basal stem rot of coconut in surveyed areas (Patuakahli, Pirojpur and Barishal district)

4.1.1.2. Disease incidence of basal stem rot disease according to soil type, soil pH, plant age and garden type

The basal stem rot (BSR) disease incidence with respect to major soil type, soil pH and plant age were also recorded. The disease incidence with respect to soil types, maximum disease incidence (29.5%) was recorded in loamy soils while minimum incidence (21.25%) was found in clay soils.

The disease incidence with respect to soil pH, maximum disease incidence (35.75%) was noticed in soil pH ranged 6-7 and minimum (22.5%) was in 7-8 ranged.

The disease incidence with respect to plant age, maximum disease incidence (40%) was observed in age group of 36-40 years old plants and minimum disease incidence (21%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (61.75%) was found in yard garden while minimum disease incidence (32.75%) was recorded in road side plants. Results are present in table 6. The survey study revealed that basal stem rot disease of coconut have a relationship with soil type, soil pH, age and also garden type. For this reason, the disease occurrence and distributions differ from region to region. Also which is show below in graphical view. These results are presented as graphical view in figure 2-5.

Table 6. (%) Disease incidence of basal stem rot disease of Coconut with respect to soil types, soil pH, age, and garden types

SI NO.	Particulars	Disease Incidence (%)
01	**Soil type**	**BSR**
	Loam	29.5
	Sandy	25.23
	Clay	21.25
	Sandy loam	18.25
02	**Soil pH**	**BSR**
	5-6	28.75
	6-7	35.75
	7-8	22.5
03	**Age**	**BSR**
	20-25	21
	26-30	26.75
	31-35	31.25
	36-40	36.75
04	**Garden Type**	**BSR**
	Commercial	23.25
	Yard Garden	61.75
	Road Side	32.75

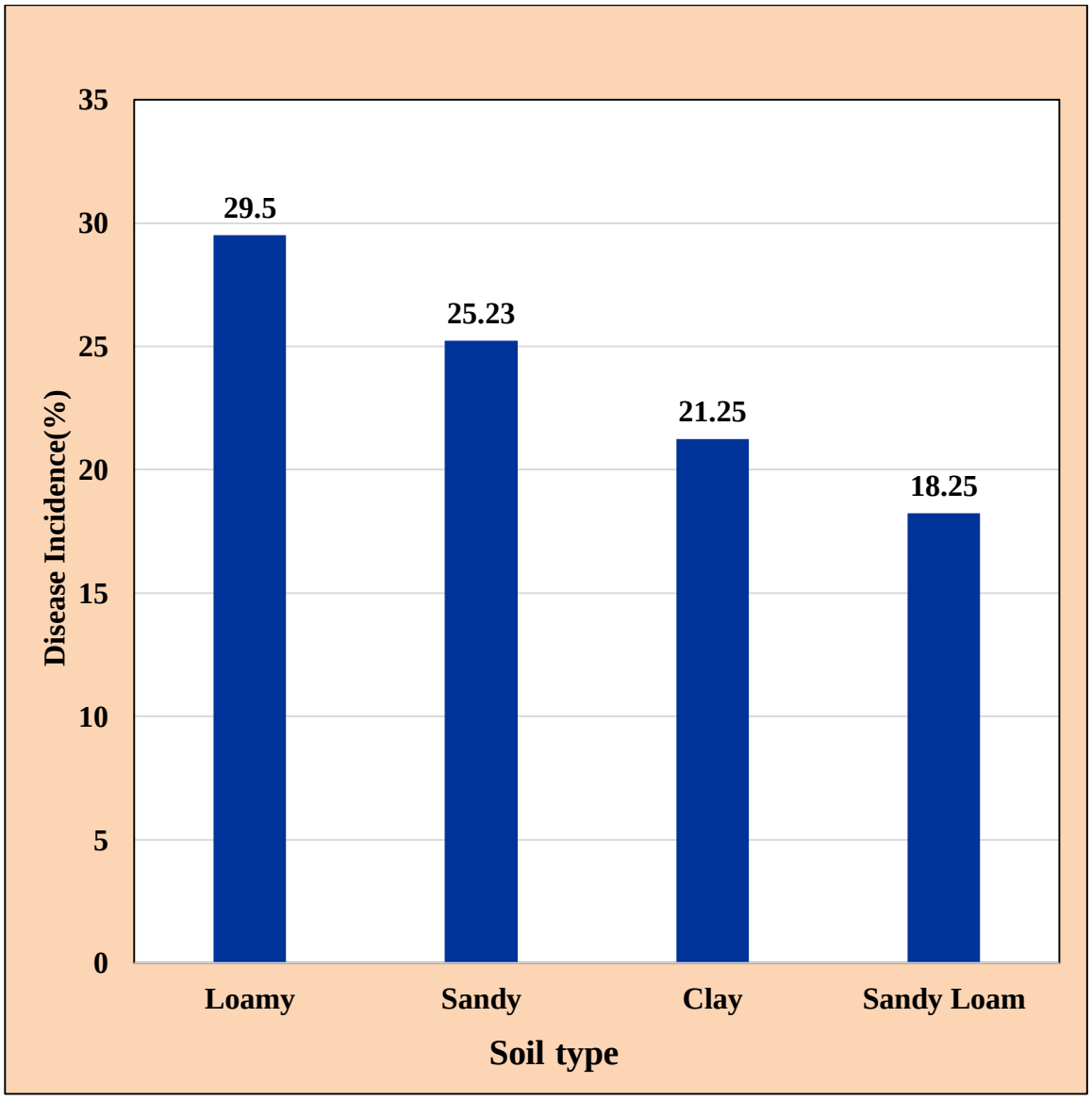


Figure 2. (%) Disease incidence of basal stem rot disease of coconut according to soil type

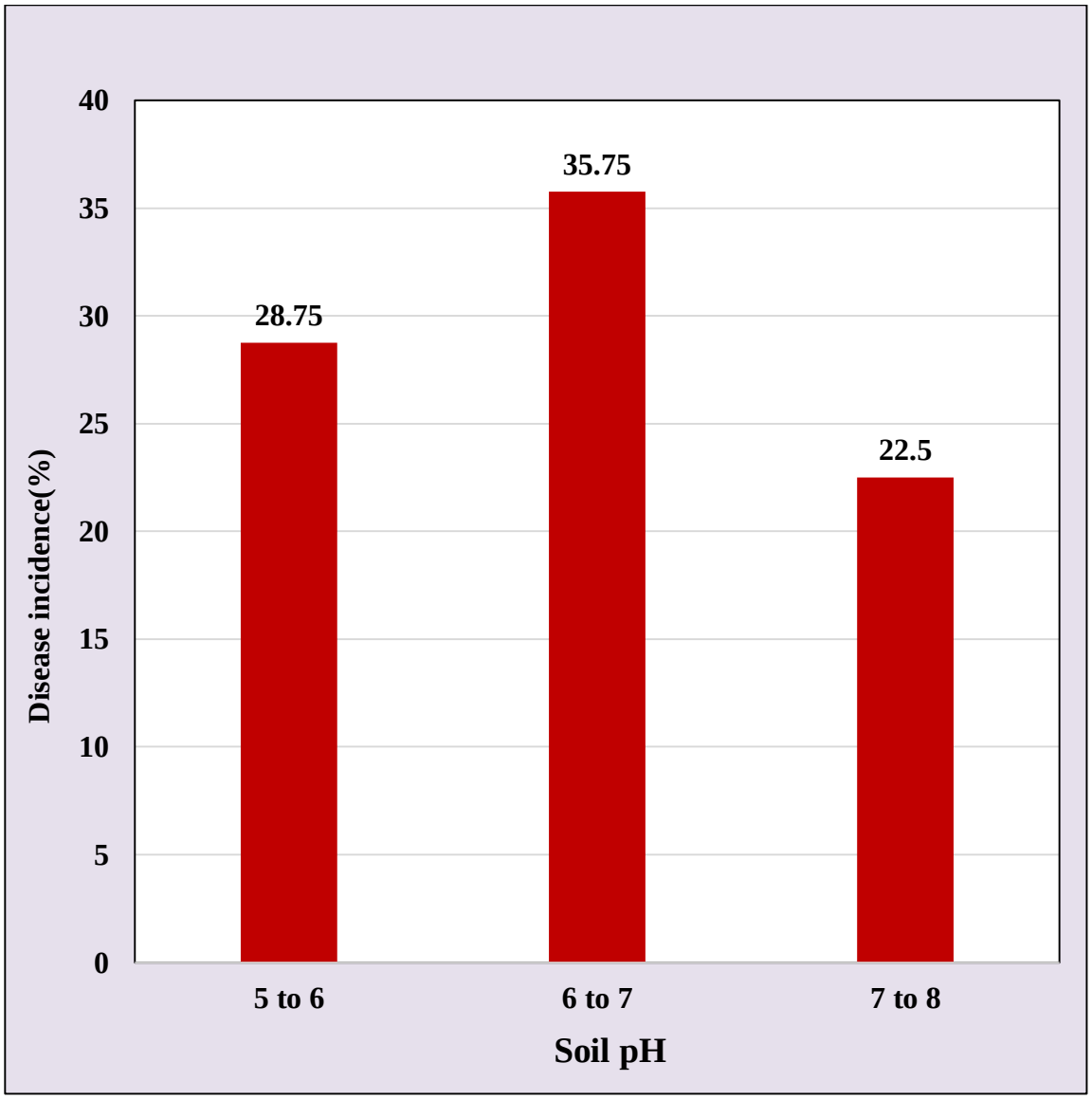


Figure 3. (%) Disease incidence of basal stem rot diseases of coconut according to soil pH

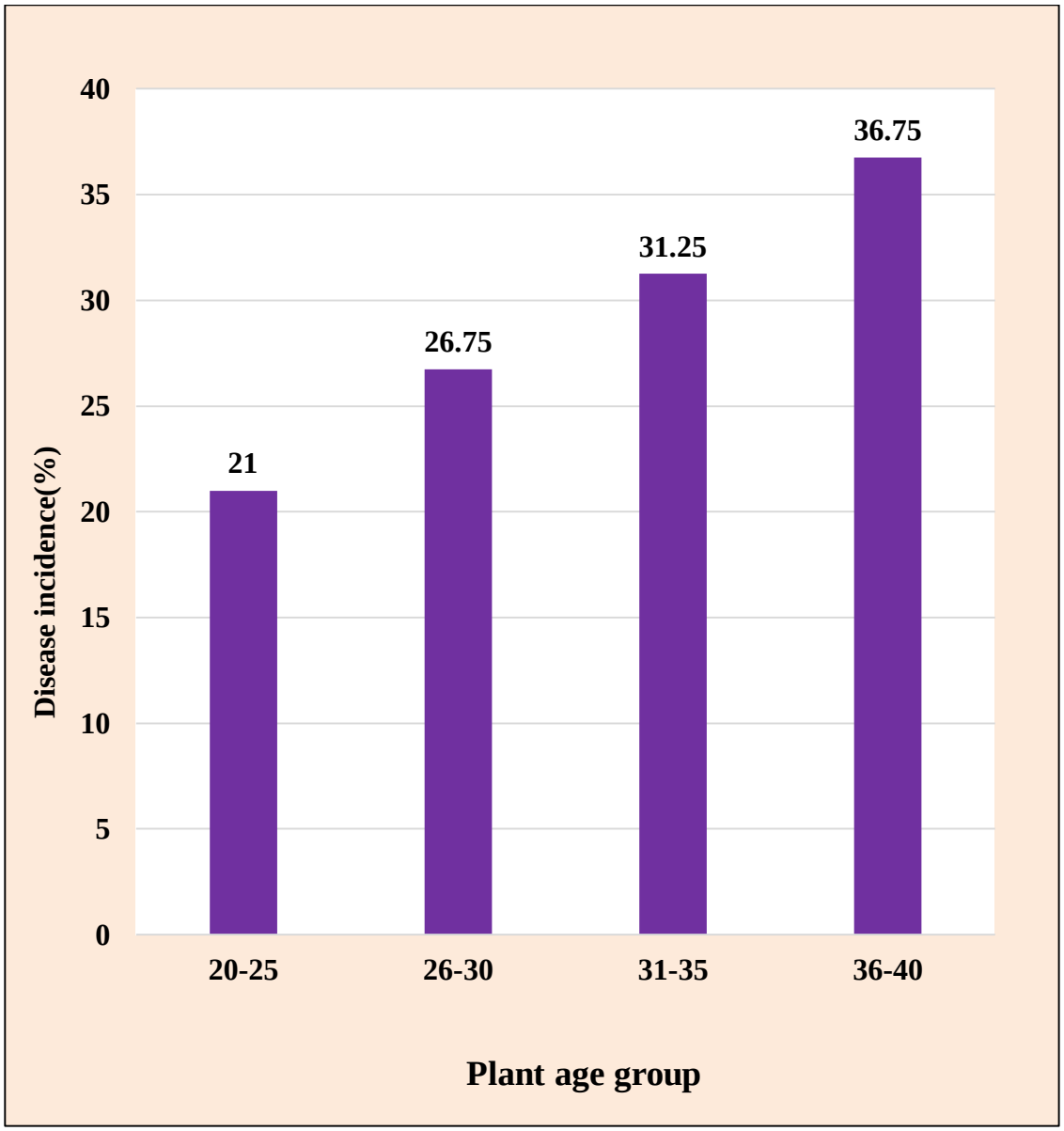


Figure 4. (%) Disease incidence of basal stem rot diseases of coconut according to plant age group

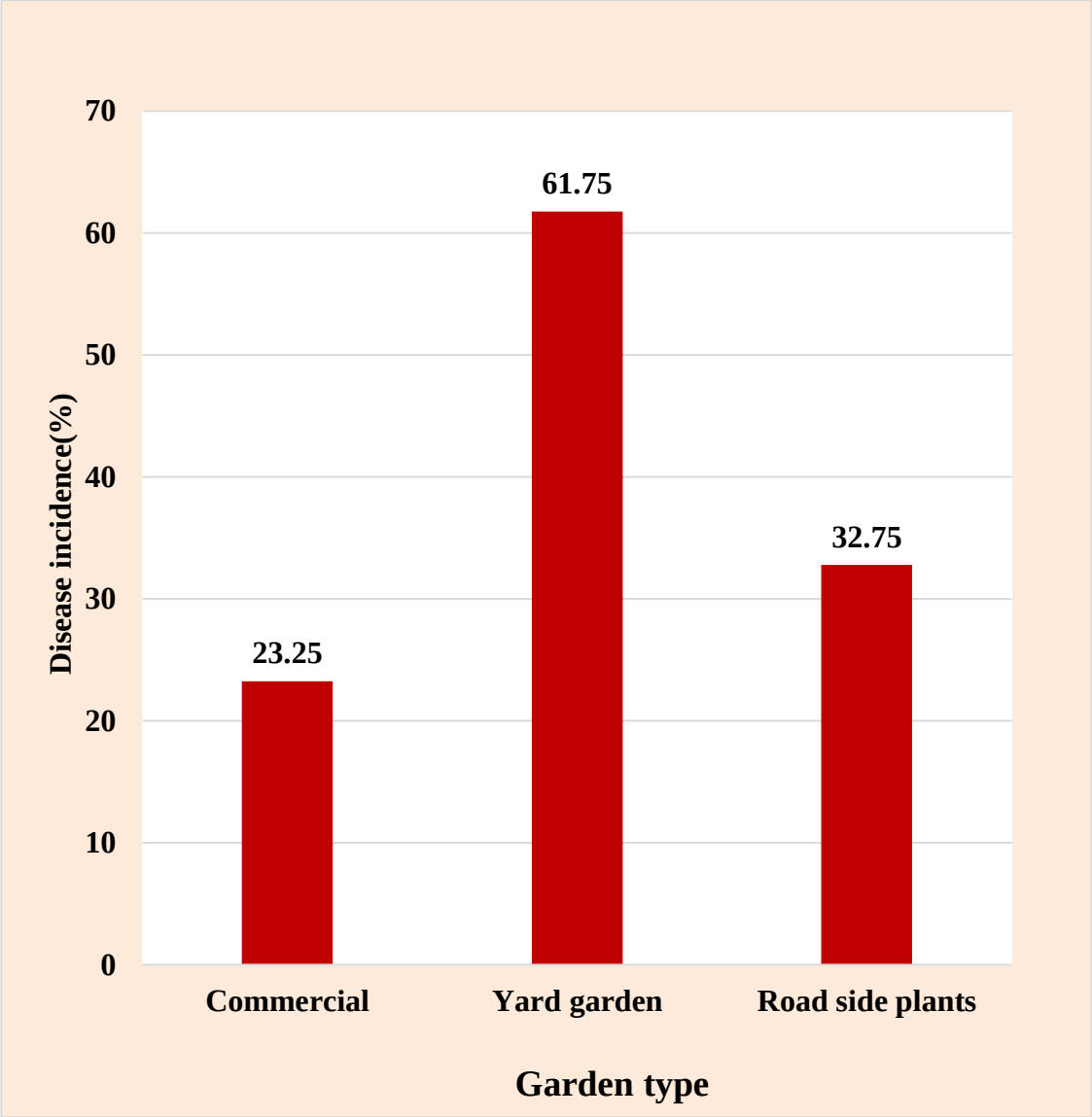


Figure 5. (%) Disease incidence of basal stem rot disease of coconut according to garden type

4.2. Management study (*in-vitro*)

4.2.1. Isolation and identification of the causal organism of basal stem rot disease of coconut

Stem barks, root bits and sporophore were found a good sources for aseptic isolation of BSR pathogen. In total 45 root samples were collected for isolation and pathogen association was found in 23 samples out of 45 samples. Also 17 sporophore and 12 disease stem bits were collected for isolation and Pathogen association was found in 8 and 3 samples out of the collected samples respectively. About 51.11% *Ganoderma* isolates was obtained from root bits, 47.06% obtained from sporophore and 25% obtained from diseased stem barks. Results are presented in table 7. Different isolates are identified from selected locations that are arranged in district wise and revealed in plate 10 -13. After studying of microscopic view two types of *Ganoderma* species were identified. viz., *Ganoderma applanatum* and *Ganoderma lucidum.* Conidial morphology of *Ganoderma applanatum;* spore size 5.58 × 5.3 µm , ellipsoid, smooth and thick walled in shape and spores are brownish in color. Conidial morphology of *Ganoderma lucidum*; spore size 3.1 × 3.5 µm , oval to oblong in shape, smooth and thin walled and spores are yellowish in color. The microscopic view conidial structures of identified *Ganoderma* species are revealed in plate 14.

Table 7: Isolates of *Ganoderma* from different type samples of coconut

Sl. No.	Type of sample	Number of samples	Ganoderma obtained	% isolates obtained
1	Sporophore	17	8	47.06
2	Root samples	45	23	51.11
3	Disease Stem Bits/Bark	12	3	25
Sub Total		**74**	**34**	**--**

Bbg

Bgd

Bmg

Plate 10. Isolates of *Ganoderma sp.* of collected from Barishal district (Appendix-III)

PDs

PKp

PSp

Plate 11. Isolates of *Ganoderma sp.* of collected from Patuakhali district(Appendix-III)

PiBh

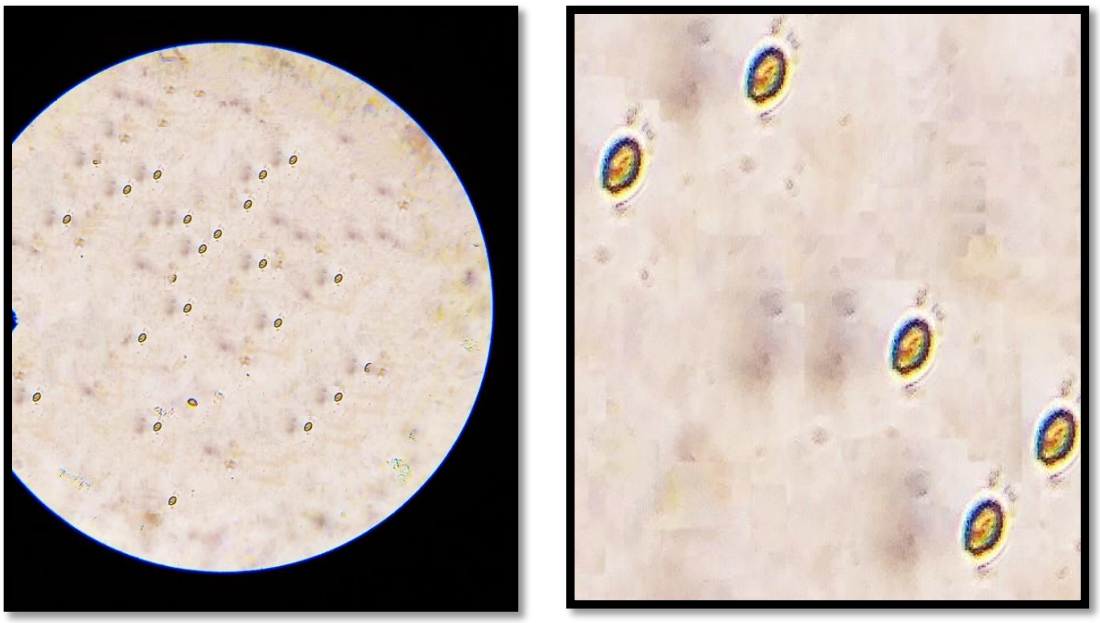
PiKl

PiNs

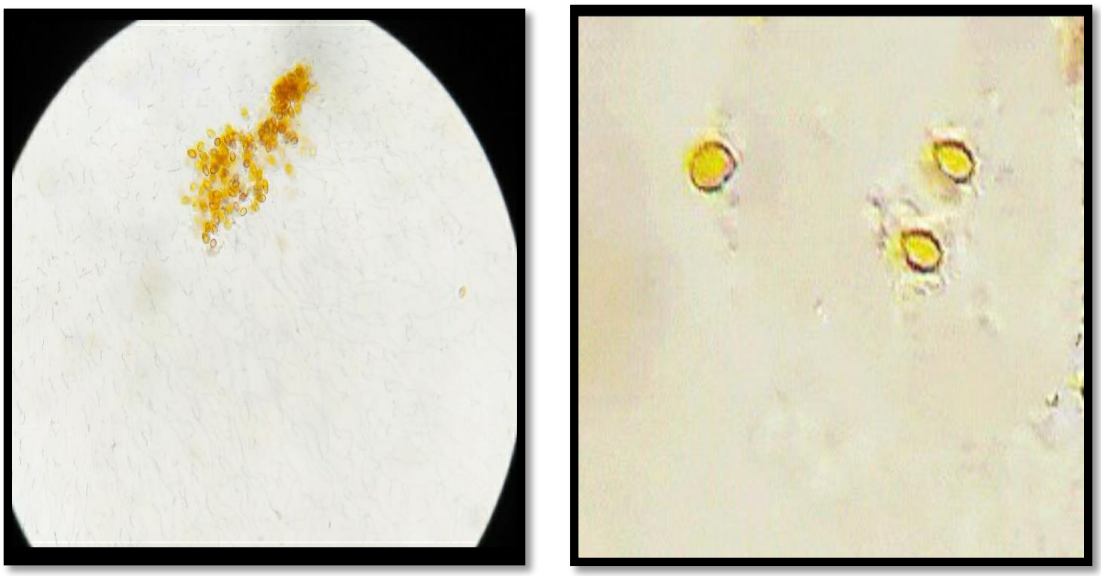
Plate 12. Isolates of *Ganoderma sp.* of collected from Pirojpur district(Appendix-III)

GSAU

Plate 13. Isolates of *Ganoderma sp.* of collected from SAU Campus(Appendix-III)



A. *Ganoderma applanatum*



B. *Ganoderma lucidum*

Plate 14. Microscopic view of conidial structure of *Ganoderma sp.*

4.2.2. Study of cultural and morphological variability of *Ganoderma* isolates

They were subjected for the cultural and morphological characters *viz*., mycelial radial growth and texture, appearance zone, reverse pigmentation color, type of colony margin, mycelial density on PDA media were recorded at seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates (Table 8). The results revealed that among the isolates of *Ganoderma,* there were significantly variation in cultural and morphological characters. The growth diameter of mycelium after 3, 6 and 9 days of inoculation was also significantly varied, the radial mycelial growth ranged was 31.83 to 39.67 mm at 3 DAI, 51-63.10 mm at 6 DAI and 77.50-87.42 mm at 9 DAI respectively. The number of days taken to cover full plate was ranged from 7 to 15 days. However, some of isolates taken <10 days to cover entire plate (PSp, BGd, BMg, PiNs, SAU). There were lot of variations were observed with respect to mycelial characteristics *viz*., reverse pigmentation, density of mycelium and colony margin. However, there were no too much variations observed with respect to color and texture of the colony (Figure 6 , Plate 10-13, Appendix-III).

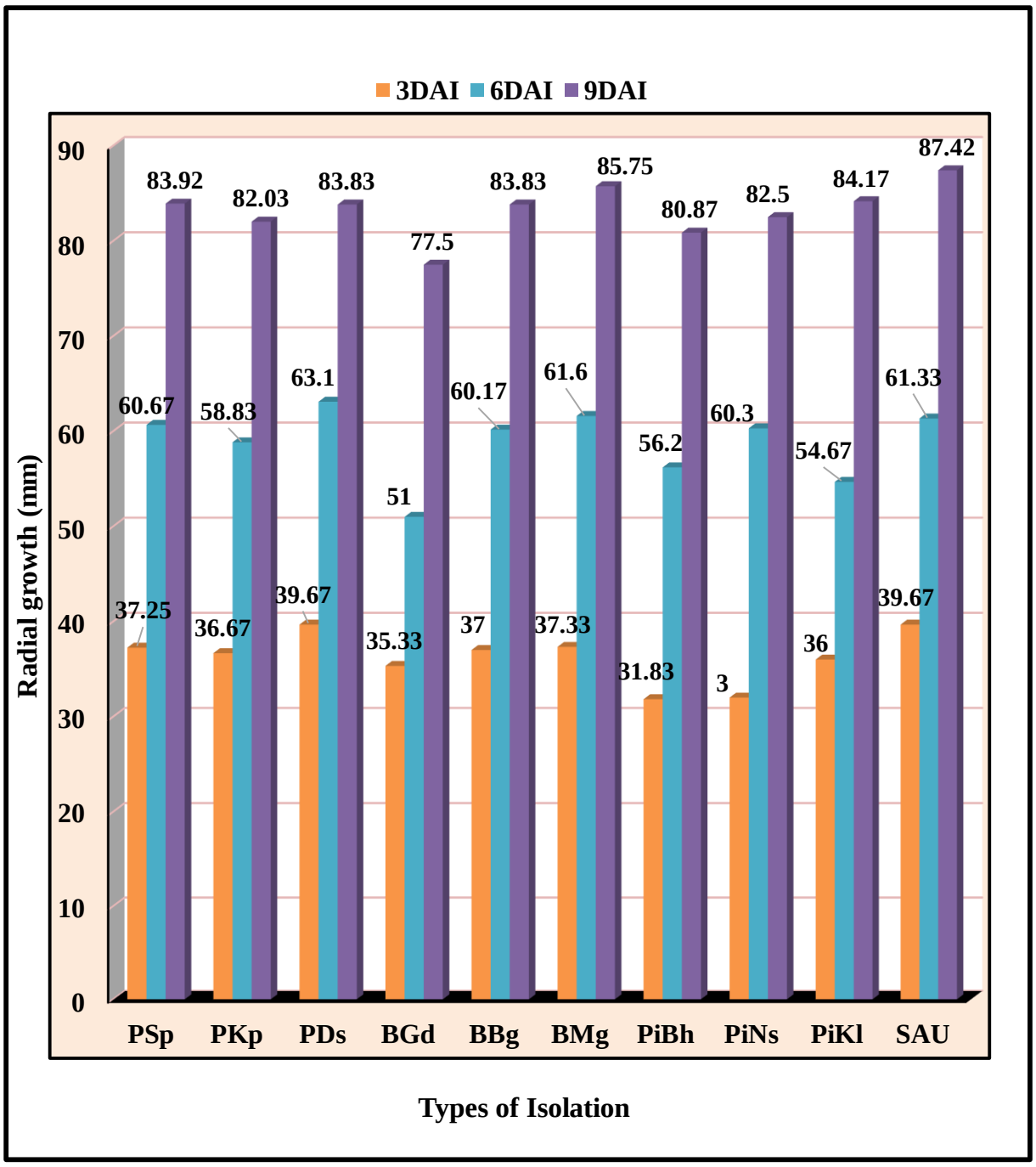


Figure 6. Radial mycelial growth of *Ganoderma* isolates on PDA media

4.2.3. *In-vitro* evaluation of bio-agent, botanicals and bio-product against *Ganoderma* isolate (GSAU)

Efficacy of bio control agent (*Trichoderma* sp. suspension), botanicals (Garlic extact & Neem oil) and bio-products (Cattle urine) was studied under *in-vitro* condition. For the evaluation study, cup method and pour technique method was followed with different doses of applications (1ml, 2ml & 3 ml) against *Ganoderma* isolate of coconut.

4.2.4. *In-vitro* efficacy of different selected treatments with different doses through Pour technique method

The effective inhibition ability of selected bio-control based treatments under *in-vitro* condition like bio-agent (*Trichoderma viride* suspension), bio-product (Cattle urine) and botanicals (Garlic extract and Neem oil) against basal stem rot disease caused by *Ganoderma sp*. stated through Pour technique method. Among the selected bio-control agent, bio-product (cattle urine) gave superior result. The results were recorded 3 DAI three times at 3 ,6 & 9 Days after inoculation. After 3 DAI, it was observed that radial mycelial growth inhibition was 12 mm, 9 mm, 8.17 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 6 DAI, radial mycelial growth inhibition was 11.5 mm, 8.5 mm and 4.33 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 9DAI, radial mycelial growth inhibition was 12 mm, 4.67 mm and 3mm in respect to 1ml, 2 ml and 3 ml doses of application, respectively.

The bio-agent (*Trichodema viride* suspension) also gave very effective inhibitory result that recorded after three days after inoculation, it was observed that radial mycelial growth inhibition was 14.83mm, 12.5mm and 8.33mm doses of application, respectively. After 6 DAI, it was observed that radial mycelial growth inhibition was 22.67mm, 18.67mm and 13.67mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 9 DAI, it was observed that radial mycelial

growth inhibition was 28.67 mm, 20.67 mm and 18.33 mm in respect to 1 ml, 2 ml and 3 ml doses of application, respectively.

The botanicals (Garlic extract and Neem oil) also gave very effective inhibitory result that recorded after three days of inoculation (Garlic extract), observed results showed radial mycelial growth inhibition was 7.17 mm , 5.33 mm , 4.67 mm doses of application, respectively. After 6DAI, radial mycelial growth inhibition was 17 mm, 13 mm and 6.67 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 9DAI, there also radial mycelial growth inhibition was 16.5 mm, 9.67 mm and 6.67 mm in respect to 1ml, 2ml and 3ml doses of application, respectively.

The botanicals Neem oil also gave very effective inhibitory result that recorded after three days of inoculation , it was observed that radial mycelial growth inhibition was 10.67mm , 9mm and 9mm doses of application, respectively. After 6DAI, radial mycelial growth inhibition was 15.67 mm, 12.67 mm and 12 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 9DAI, radial mycelial growth inhibition was 18.67 mm, 15.33 mm and 13.33 mm in respect to 1 ml, 2 ml and 3 ml doses of application, respectively. All the above results are presented in table-9,10,11; figure 7,8,9 and plate 15,16,17 (Appendix IV) .

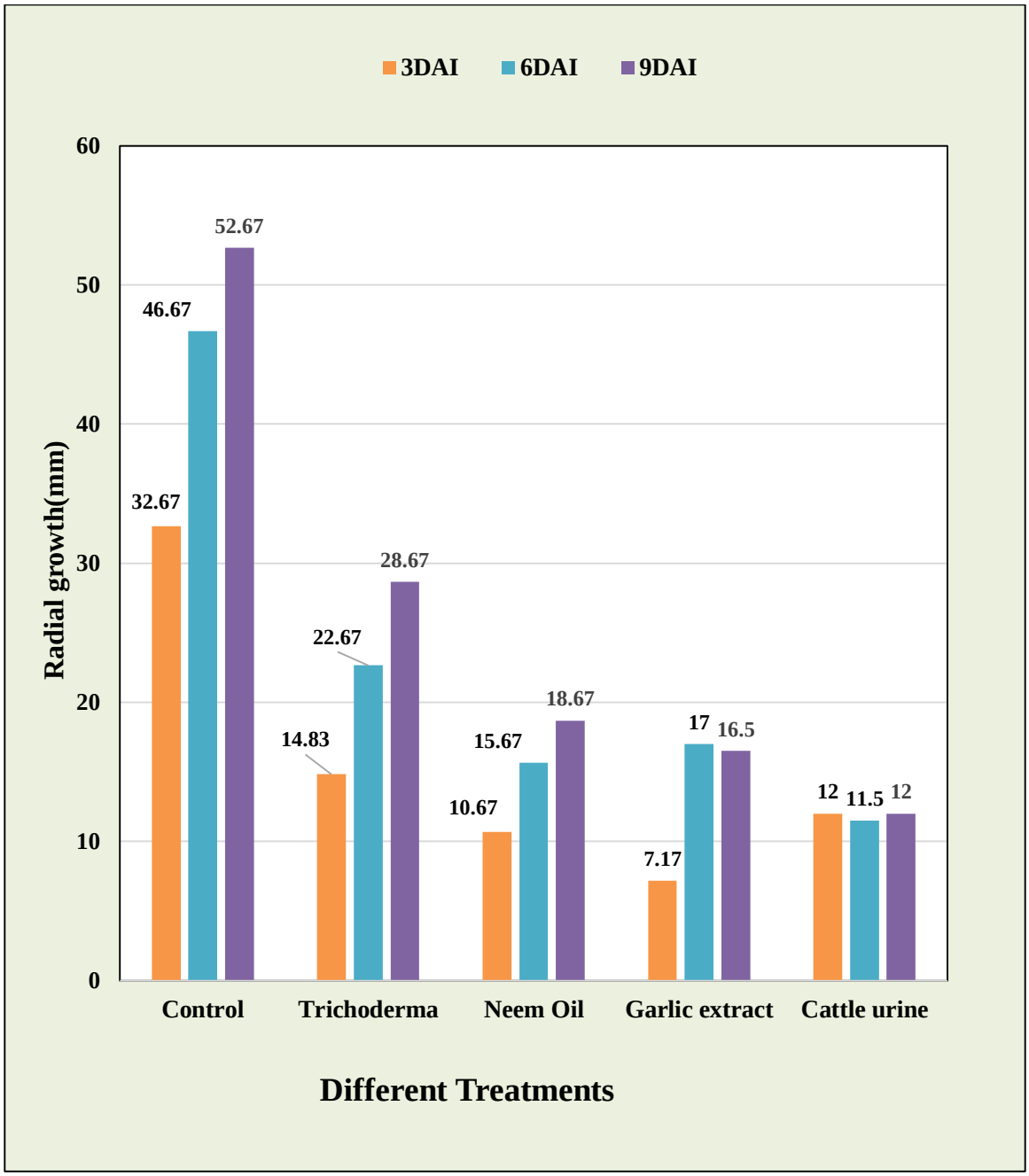
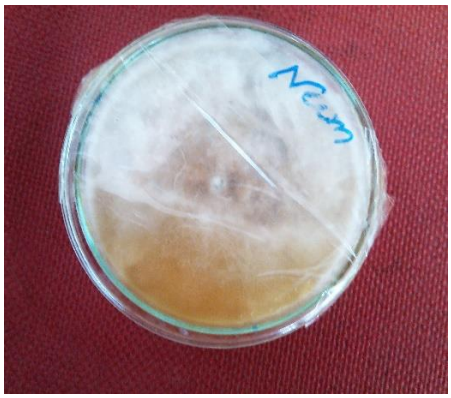


Figure 7. Efficacy of different treatments against *Ganoderma* isolate with 1ml doses of application

Trichoderma viride



Neem oil

Garlic extract

Cattle urine

Control

Plate 15: Inhibition effect of radial growth of *Ganoderma* isolate of coconut with 1ml doses of application under *in-vitro* condition by bio-control agents

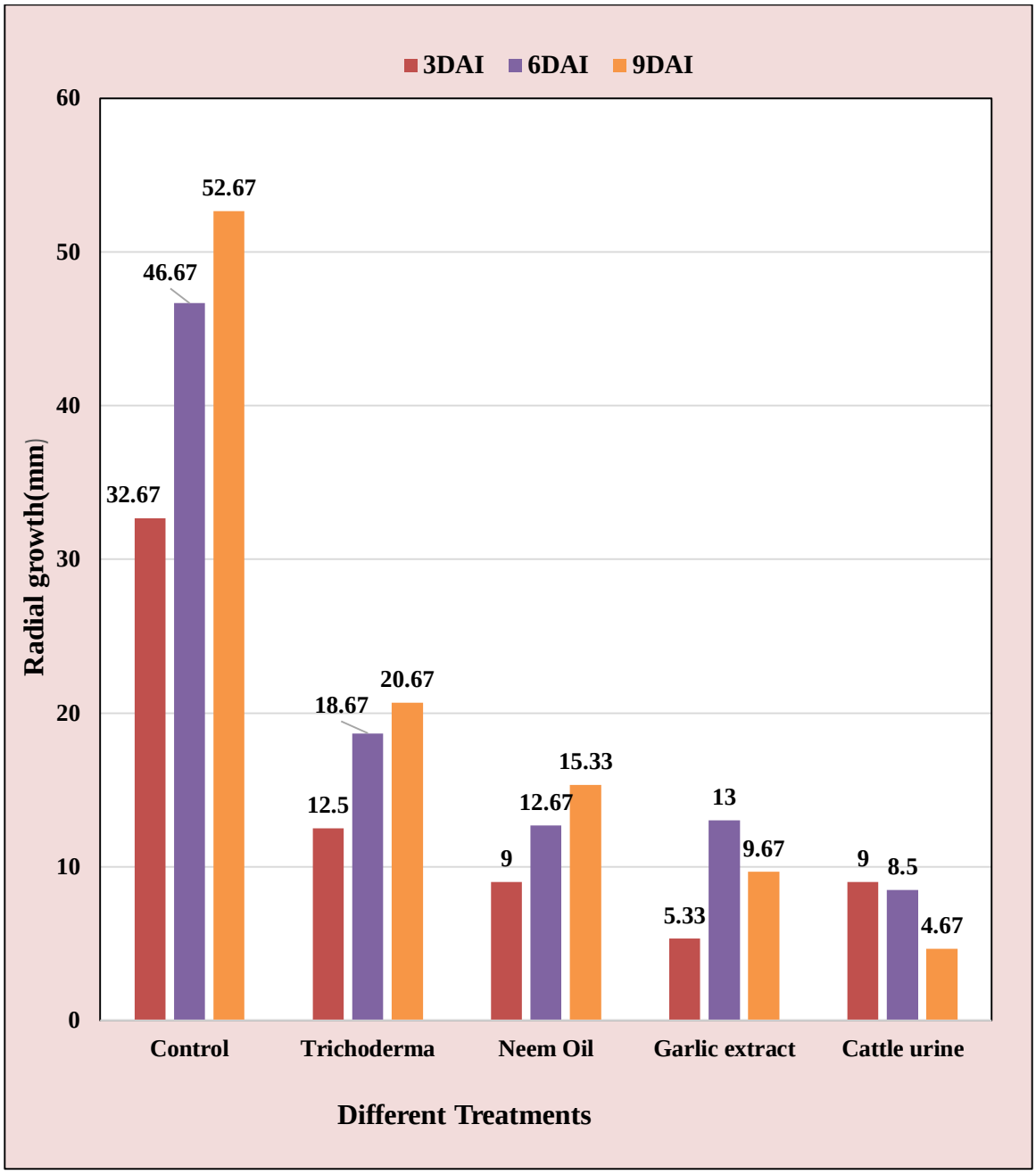
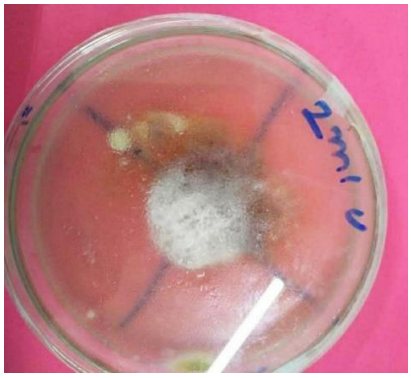


Figure 8. Efficacy of different treatments against *Ganoderma* isolate with 2ml doses of application

Trichoderma viride

Neem oil

Garlic extract



Cattle urine

Control

Plate 16: Inhibition effect of radial growth of *Ganoderma* isolate with 2ml doses of application of coconut under *in-vitro* condition by biocontrol agents

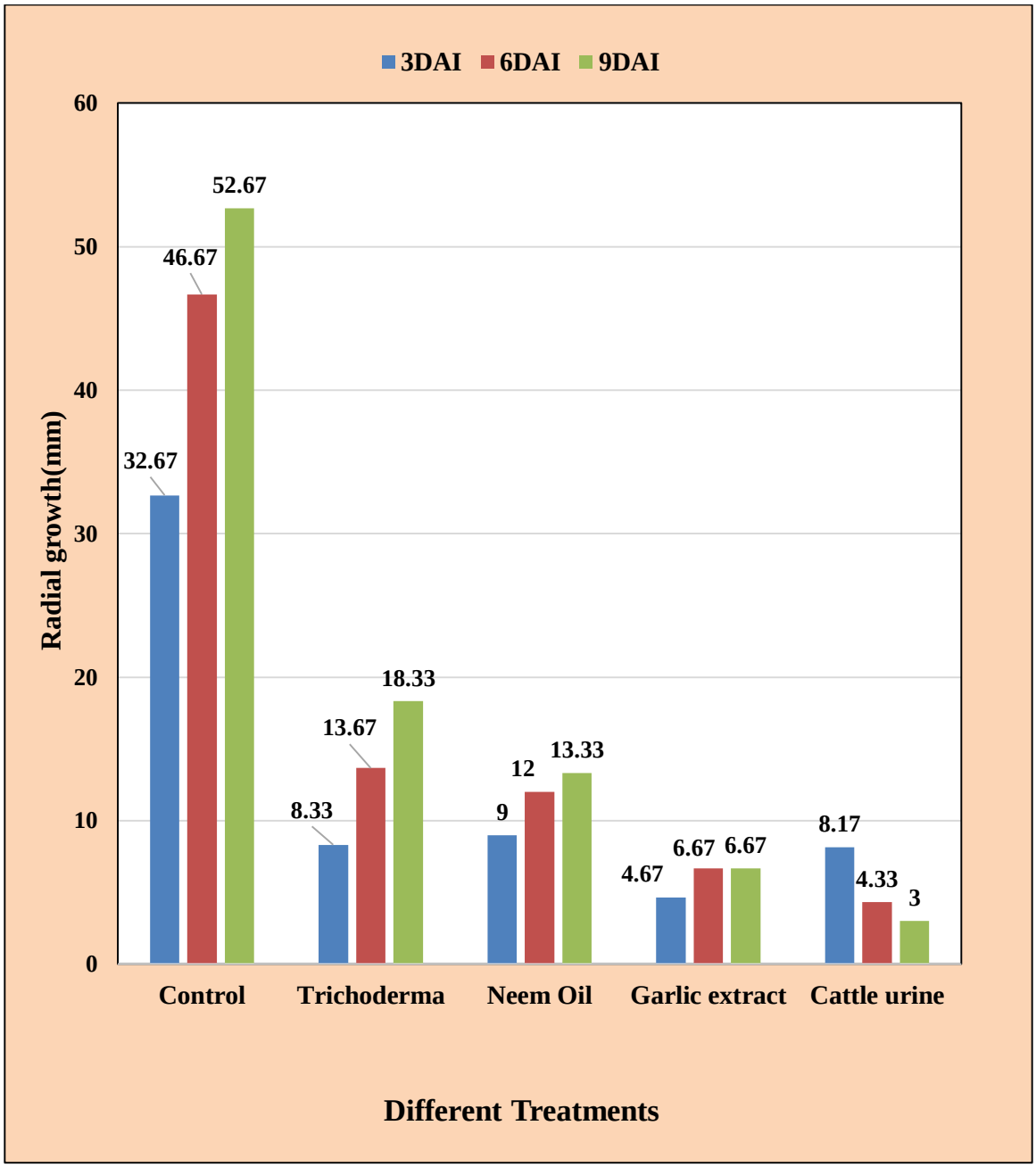
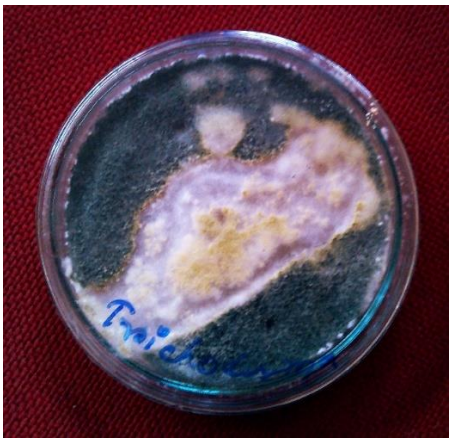
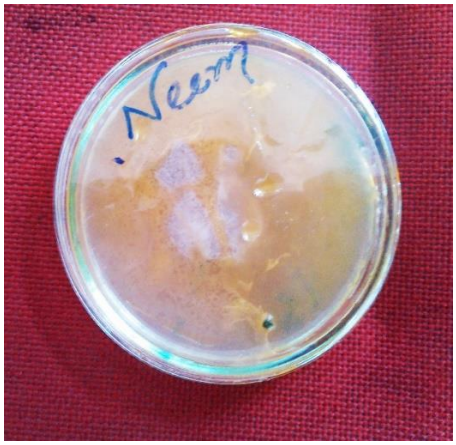


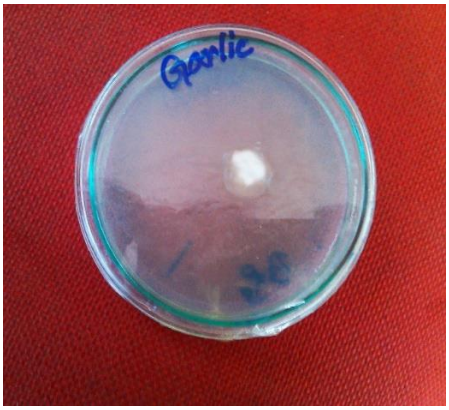
Figure 9. Efficacy of different treatments against *Ganoderma* isolate with 3ml doses of application



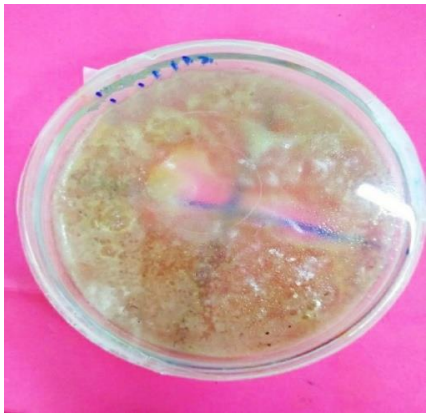
Trichoderma viride



Neem oil



Garlic extract



Cattle urine

Control

Plate 17: Inhibition effect of of radial growth of *Ganoderma* isolate with 3ml doses of application of coconut by Pour technique method under *in-vitro* condition biocontrol agents.

4.2.5. Efficacy of different treatments result in different doses are stated below through Cup method in *in-vitro* :

The effective inhibition ability of selected bio-control based treatments under *in-vitro* condition like bio-agent (*Trichoderma viride* suspension), bio-product (Cattle urine) and botanicals (Garlic extract and Neem oil) against basal stem rot disease caused by *Ganoderma sp*. stated through cup method. Among the selected bio-control agent, bio-product (cattle urine) gave superior result compaired to other treatment. The results were recorded 3 DAI three times at 3, 6 & 9 Days. After 3 DAI, it was observed that radial mycelial growth inhibition was 14.17 mm, 13.5 mm, 5.58 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After 6 DAI , it was observed that radial mycelial growth inhibition was 15.42 mm, 14.17 mm and 5.67 mm in respect to 1ml, 2ml and 3ml doses of application respectively. After nine days of inoculation, it was observed that radial mycelial growth inhibition was 17.83 mm, 16.17 mm and 6.17 mm in respect to 1ml, 2ml and 3ml doses of application, respectively.

The bio-agent (*Trichodema viride* suspension) also gave very effective inhibitory result that recorded after three days of inoculation. It was observed that radial mycelial growth inhibition was 21.5 mm , 16.08 mm , 12.5 mm doses of application respectively. After six days of inoculation, radial mycelial growth inhibition was 25.33 mm, 16.42 mm and 12.83 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After nine days of inoculation, radial mycelial growth inhibition was 34.17 mm, 20.67 mm and 16.17 mm in respect to 1ml, 2ml and 3ml doses of application, respectively.

The botanicals also gave very there were effective inhibitory result found by using Garlic extract and Neem oil. After three days of inoculation (Garlic extract) , radial mycelial growth inhibition was 18.33 mm , 13.5 mm , 9.17 mm doses of application, respectively. After six days of inoculation, radial mycelial growth inhibition was

25.67 mm, 14.17 mm and 11.33 mm in respect to 1ml, 2ml and 3ml doses of application, respectively.

After nine days of inoculation, radial mycelial growth inhibition was 31 mm, 16.17 mm and 13 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. The botanicals Neem oil also gave very effective inhibitory result that recorded after three days of inoculation, radial mycelial growth inhibition was 23.17 mm , 15.5 mm , 11.83 mm doses of application, respectively. After six days of inoculation, radial mycelial growth inhibition was 31.67 mm, 22.33 mm and 12.83 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. After nine days of inoculation, radial mycelial growth inhibition was 38.67 mm, 15.67 mm and 16.5 mm in respect to 1ml, 2ml and 3ml doses of application, respectively. All the above results are presented in figure 10 & 11 and plate 18 and in table 12,13 & 14 (Appendix V).

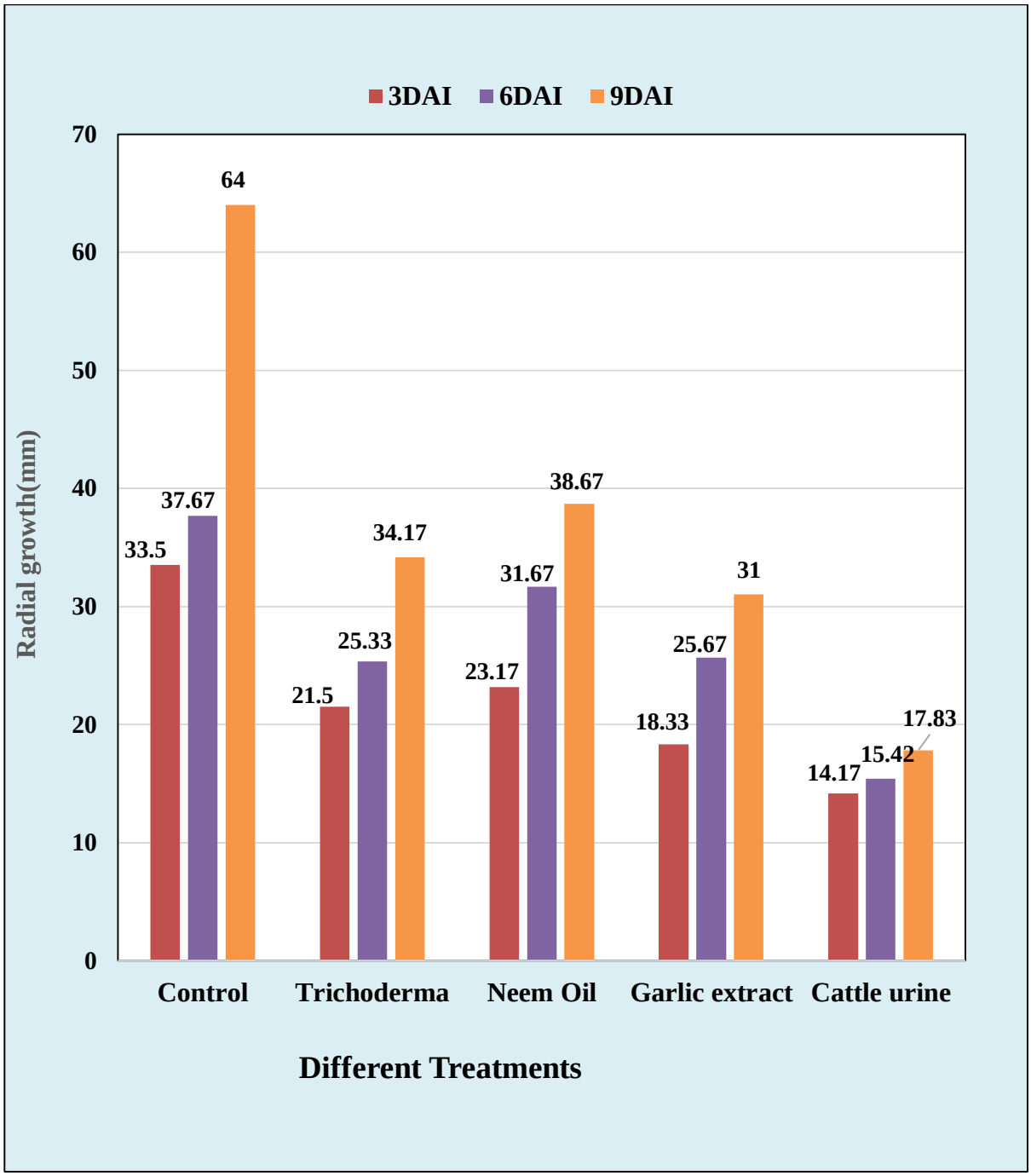


Figure 10. Efficacy of different treatments against *Ganoderma* isolate with 1ml doses of application (cup method & pour technique method)

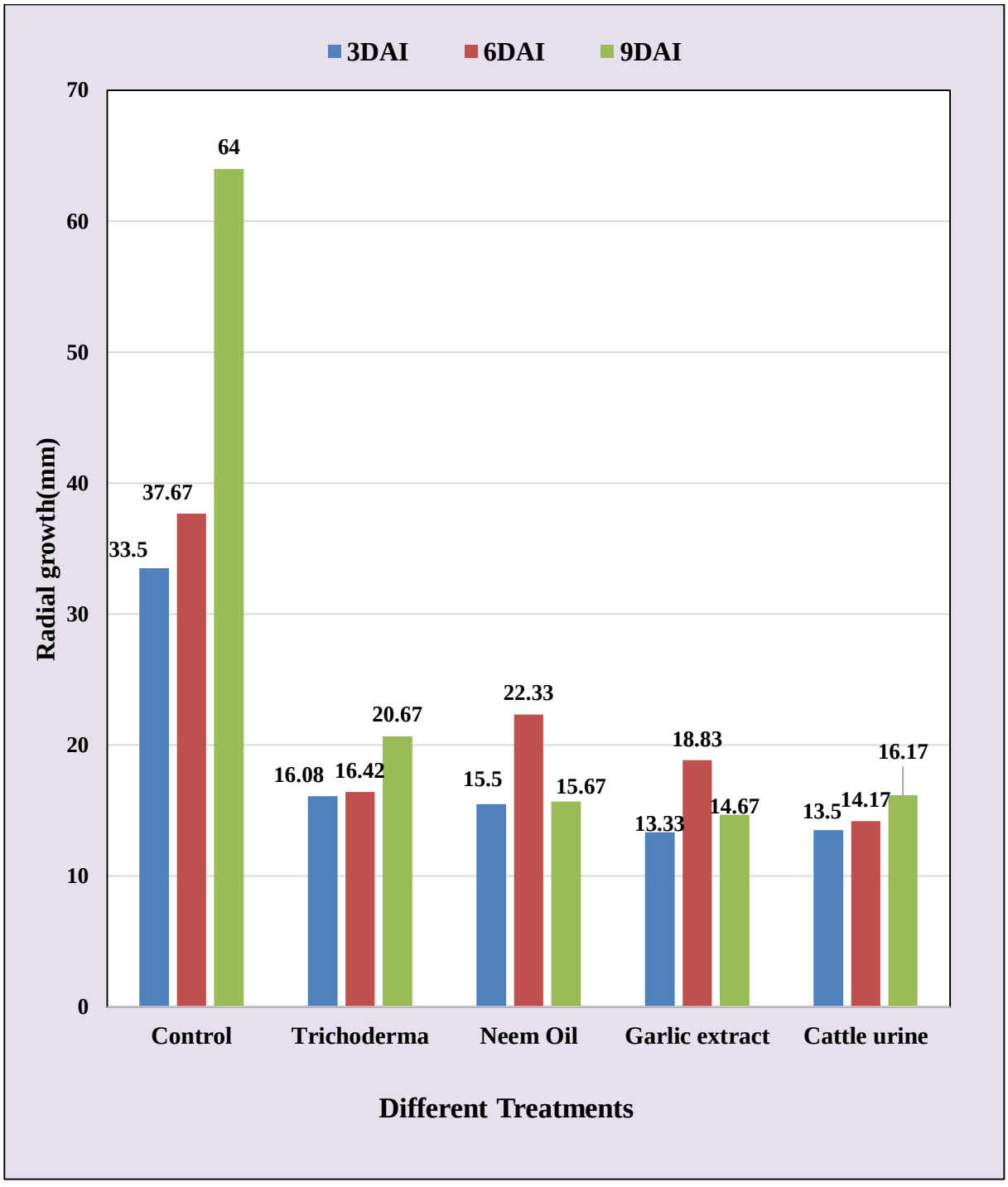


Figure 11. Efficacy of different treatments against *Ganoderma* isolate with 2ml doses of application

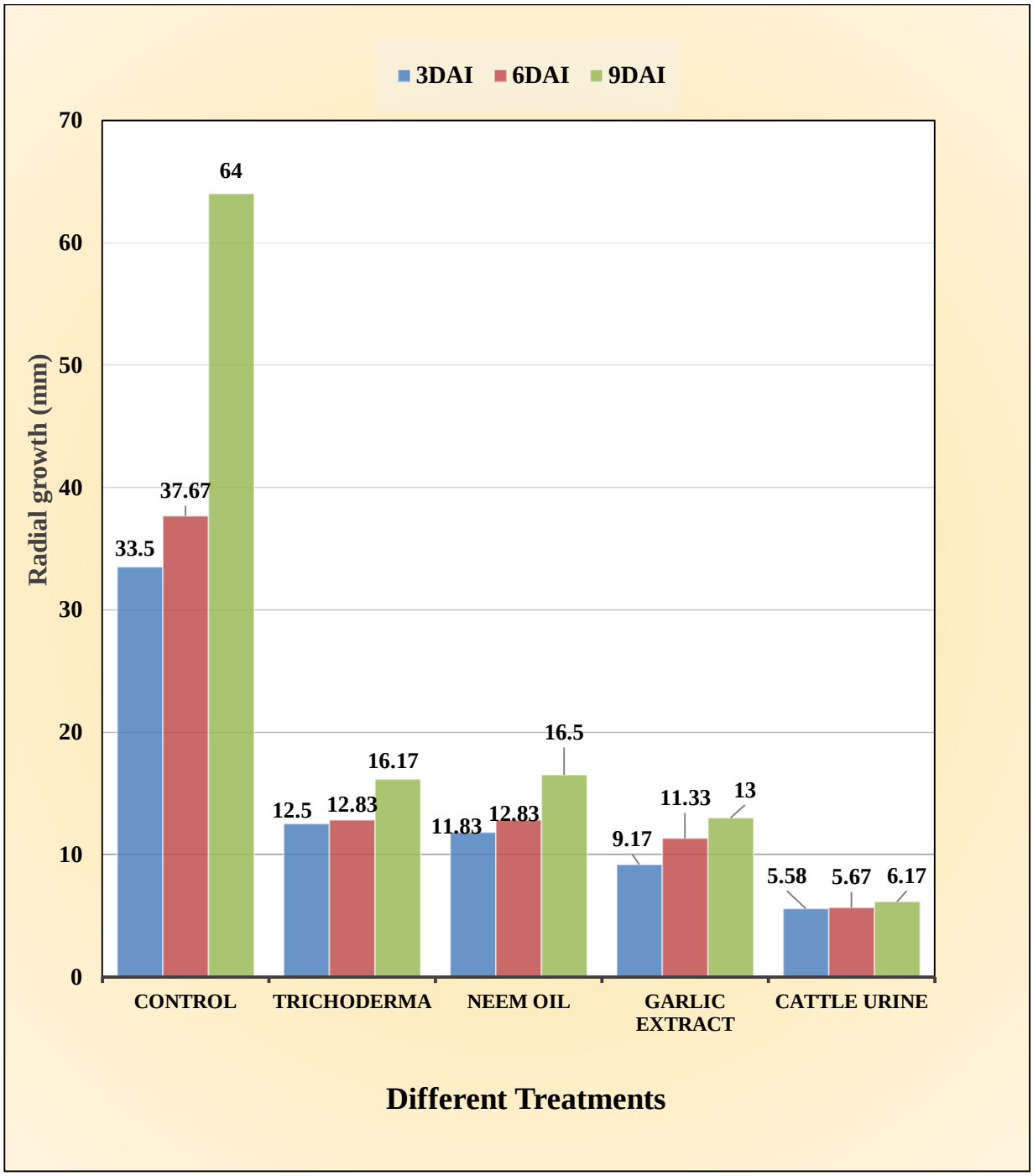
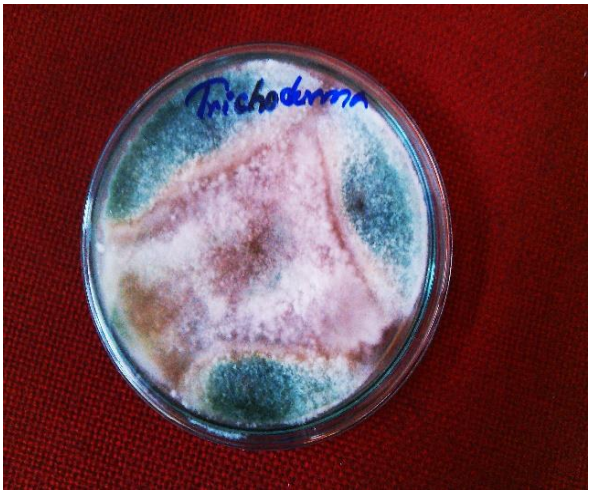
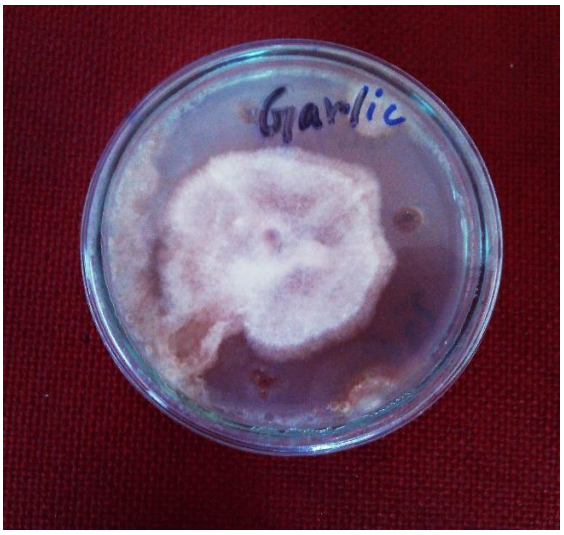


Figure 11. Efficacy of different treatments against *Ganoderma* isolate with 3ml doses of application



Trichoderma viride

Neem oil



Garlic extract

Cattle urine

Plate 18: Inhibition effect of radial growth of *Ganoderma* isolate with 3ml doses of application through Cup method under *in-vitro* condition by bio-control agents

Discussion

Basal Stem Rot disease of Coconut is newly reported soil borne disease in Bangladesh particularly in Coastal regions. There were conducted a few research on BSR disease of coconut. The present study was carried out to estimate the occurrence and disease incidence of basal stem rot of coconut in selected coastal regions of Bangladesh. For that purpose a structural questioner based survey was done in the selected coastal regions and disease incidence and severity was measured. The prime aim of the study was to find out the effective bio-control based management practices to control BSR disease of Coconut.

The competitive ability of bio-control agent which are experimented under *in-vitro* condition. This studies have shown that different biocontrol-based management practices was introduced by using bio-product (Cattle urine), botanicals (garlic extract and neem oil) and bio-agent (*Trichoderma* suspension) against BSR of Coconut which resulted maximum inhibition efficacy in lower radial growth of *Ganoderma.*

4.3.1. Disease incidence and severity of Basal Stem Rot (BSR)

A Survey was carried out through random sampling technique in selected coastal regions; the (%) disease incidence and severity of basal stem rot disease in coconut were measured. The location wise disease incidence of BSR was ranged from 42% - 78%. Among surveyed locations, maximum disease incidence (78%) of basal stem rot (BSR) was recorded in Bhandaria of Pirojpur district and minimum disease incidence (42%) was recorded in Mehendiganj of Barisal district. On the other hand, location wise disease severity of BSR was ranged from 23.18 - 45%. Maximum disease severity (45%) of BSR was recorded in Bhandaria of Pirojpur district and minimum disease severity (23.18%) was recorded in Nesarabad of Pirojpur district.

The basal stem rot (BSR) incidence with respect of major soil type, soil pH and plant age were also recorded. The disease incidence with respect to soil types,

maximum disease incidence (29.5%) was recorded in loamy soils while minimum incidence (21.25%) was found in clay soils. The disease incidence with respect to soil pH, maximum disease incidence (35.75%) was noticed in soil pH ranged 6-7 and minimum (22.5%) was in 7-8 ranged. The disease incidence with respect to plant age, maximum disease incidence (40%) was observed in age group of 36-40 years old plants and minimum disease incidence (21%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (61.75%) was found in yard garden while minimum disease incidence (32.75%) was recorded in road side plants. The investigation results regarding the percent disease incidence and severity of BSR observed in the study is almost agree with as observed by Srinivasulu *et al.* (2003), Palanna *et al.* (2013), Naik *et al.* (2000).

4.3.2. Isolation and identification of the causal organism of BSR disease of coconut

Stem barks, root bits and sporophore were found a good sources for aseptic isolation of BSR pathogen. In total 45 root samples were collected for isolation. Pathogen association was found in 23 samples out of 45 samples. Also 17 sporophore and 12 disease stem bits were collected for isolation and pathogen association was found in 8 and 3 samples out of the collected samples, respectively. About 51.11% *Ganoderma* isolates was obtained from root bits, 47.06% obtained from sporophore and 25% obtained from diseased stem barks. After studying of microscopic view two types of *Ganoderma* species were identified. viz., *Ganoderma applanatum* and *Ganoderma lucidum.* Conidial morphology of *Ganoderma applanatum;* spore size 5.58 × 5.3 µm , ellipsoid, smooth and thick walled in shape and spores are brownish in color. Conidial morphology of *Ganoderma lucidum*; spore size 3.1 × 3.5 µm , oval to oblong in shape, smooth and thin walled and spores are yellowish in color. The results recorded in this study regarding the isolation and identification is almost

similar as observed by Kandan *et al*. (2010), Vinayaka and Prathibha (2013), Palanna *et al.* (2013).

4.3.3. Biocontrol-Based Management studies for Basal Stem Rot of coconut

Chemical fungicides are not thought of as a long term solution to crop health management. Requirement for repeated application, residue problems, health and environmental hazards and development of fungicide resistance in the pathogen are the major problems associated with the use and overuse of chemical fungicides (Mukhopadhyay and Mukherjee, 1996). As a result, in recent years, the focus has been shifted in finding out safer alternatives like boi-control based management practices. In this study, in *in-vitro* efficacy of bio-control agents was evaluated against *Ganoderma* at three doses of applications *viz.*,1ml, 2ml and 3ml by Pour technique and Cup method. The radial growth of the mycelium was recorded on three days after inoculation three times at 3, 6, 9 DAI. The inhibitory efficacy of Cattle urine gave superior result than other treatments against *Ganoderma* at 3 ml of concentrations. This bio-product is available for the farmers to apply against BSR disease than other management practices or treatments.

Bio-agent (*Trichoderma viride*) gave effective inhibition in radial mycelial growth of *Ganoderma*.The present findings are in agreement with Srinivasalu *et al*. (2004) who reported that, native BCAs (*T. viride, T. harzianum and T. hamatum*) were effective in controlling basal stem rot pathogens (*G. lucidum and G. applanatum*) *in-vitro*. *T. harzianum* and *T. viride* were reported to be antagonistic to *G. lucidum* (Gunasekaran *et al*., 1986; Bhaskaran, 1990). Bansali (2003) reported that in dual culture technique *Trichoderma* was found to inhibit the mycelial growth of *Ganoderma lucidum* on potato dextrose agar under *in-vitro* conditions. The mycelial growth and conidia production rate of *Ganoderma sp*. were higher in potato sucrose agar than potato dextrose agar. Among the three strains of *T. harzianum* and *T. viride* inhibited the maximum mycelial growth by acting as antagonists to *G. lucidum* within 96 hrs of inoculation.

Several studies were carried out on the biological control of *Ganoderma* associated with oil palm basal stem rot (BSR) and its mode of action. The growth of *Ganoderma applanatum* and *Ganoderma lucidum* was inhibited when exposed to the trapped head space of volatile compounds produced in the presence of *Trichoderma sp*. isolates. The inhibition started after 24 hours and increased until end of the experiment. Inhibitory effect on the presence of head space gases on agar petri dishes indicated that the unidentified volatile substances suppressed the pathogenic fungus of *Ganoderma sp*. These results are consistent with the study conducted by Mumpuni *et al*., 1998, that the volatile compounds from *Trichoderma spp*. impeded the hyphal growth of different fungal pathogens on agar plates. They reported that *T. viride* produced large amount volatile compounds to affect the hyphal tips of *Ganoderma applanatum* and *Ganoderma lucidum*.

In-vitro efficacy of Botanicals evaluated against *Ganoderma* isolate (GSAU) are found to be inhibitory result at 3ml doses of application. Neem oil and Garlic extract inhibit the mycelial growth of *Ganoderma* in a desirable level over control. The plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics, etc. Plant extracts are effective against plant pathogens as they have unique antimicrobial properties that act in a holistic manner due to presence of certain secondary metabolites, viz., alkaloids, terpenoids, glycocides and phenolic acids (Srivastava *et al*., 1994; Singh *et al*., 1999). The result are conformity with the work of Rashmi and Yadav (1999), they reported that all the plant extracts were less effective at lower concentrations, there was a positive correlation between concentration and mycelial growth inhibition percentage.

In comparison of two methods of inhibition efficacy, it was stated that Pour technique method is best than Cup method of treatment application against *Ganoderma sp*. causes Basal Stem Rot disease of Coconut.

Finally, it may be stated that lot of variability was observed with respect to cultural and molecular aspects of *Ganoderma*. Basal Stem Rot disease of coconut cannot

be kept under control with just a single management strategy. In the present study, different treatments like bio-products, bio-agents and botanicals showed in a desirable level of inhibitory result against BSR disease of coconut.

SUMMARY AND CONCLUSION

The natural habitat of coconut is the coastal belt of tropics where it flourishes in sea-washed littoral sand with constant motion of underground current of water in rhizosphere zone. It is the most valuable antique plantation crops in coastal regions of Bangladesh. Coconut palm is usually affected by various biotic and abiotic stresses resulting in drastic reduction in yields. Even though coconut palm is hardy in nature and adaptable to varied climatic conditions, it is affected by many diseases (Nambiar 1994, Henry Louis 2002). Root (wilt) , basal stem rot (BSR), bud rot , stem bleeding, leaf blight and grey leaf spot are the major diseases of coconut is reported throughout the world. Among those diaseases, Basal stem rot (BSR) disease caused by *Ganoderma applanatum* and *Ganoderma lucidum* is the most deleterious disease accounting to severe yield loss in coastal parts of Bangladesh. To conduct the study, a survey was done in selected locations of coastal regions *viz*., Patuakhali, Pirojpur and Barishal district.

The results of the studies conducted with Basal Stem Rot disease in Coastal regions of Bangladesh with respect to survey, isolation and identification and bio-control based disease management are summarized in this chapter.

The study was conducted in Molecular Biology and Plant virology laboratory of causal organism under the Department of Plant Pathology, Sher-e-Bangla Agricultural University and survey was done in coastal region. The objectives of the study was to measure the disease incidence and severity of basal stem rot disease of coconut and also isolation and identification of causal organism and bio-control based management practices for controlling the Basal Stem Rot disease of coconut under *in-vitro* condition.

Basal Stem Rot disease incidence in major coconut growing three districts in coastal regions ranged from 0 to 69.33 per cent. The maximum disease incidence (69.33) was measured in Pirojpur district followed by Patuakhali and Barisal with disease

incidence 68.33% and 57% ,respectively. The disease incidence was maximum in both loamy and sandy soils. The severity of BSR in surveyed area of Coastal regions ranged from 0 to 36.89 per cent. In Pirojpur district the maximum disease severity (36.89%) was recorded followed by Patuakhali and Barishal with 30.79% and 29.5% disease severity, respectively. The percent disease incidence and severity of BSR also varied greatly from soil temperatue, relative humidity, garden to garden type and soil pH. Its occurrence and distribution in major coconut growing regions revealed that the disease is not confined to any soil types, however is more prevalent in lighter soils. Sporocarp and diseased root bits were found good source as maximum isolates procured under aseptic conditions. The percentage of isolates obtained from sporocarp, diseased stem barks and diseased root bits was 47.06, 25 and 51.11, respectively.

The growth study of *Ganoderma* in PDA/PSA medium revealed that the radial growth on 3rd, 6th and 9th day after inoculation varied significantly and the radial growth ranged from 31.83 to 39.67 mm on 3DAI . Similarly, on 6th and 9th day it ranged from 51 to 63.08 mm and 77.50 to 87.42 mm, respectively. Potato sucrose Agar (90 mm) supported the highest radial growth of *Ganoderma* than Potato dextrose agar (75mm) on 9 Days after inoculation.

The isolated samples were identified based on mycelial morphology and characteristics *viz*., radial mycelial growth, mycelial texture, appearance of zone, reverse pigmentation colour, type of mycelial margin, mycelial density and conidial structure. All these isolates were observed for 7-15 days. The radial growth was taken at 3 days interval after inoculation. Two species of *Ganoderma* were identified *viz., Ganoderma lucidum* and *Ganoderma applanatum.* High degree of variation was observed between the pathogen isolates through morphological and cultural studies.

The bio-control based management practices like fungal bioagent as *Trichoderma viride* suspension, the bio-product (Cattle urine), the botanicals (Neem oil and

Garlic extract) gave maximum inhibition against *Ganoderma applanatum* and *Ganoderma lucidum* . Among the four bio-control agents the bio-product Cattle urine showed satisfactory result of controlling *Ganoderma* under *in-vitro* condition which gives a hopeful outcome in case of overcome the basal stem rot disease of coconut. However, thorough research is needed in molecular identification and differentiation of the pathogen species as confusion in identification process leads to inefficient disease management. Further, identification of diversity existing between and within *Ganoderma* species in coconut assists in developing early detection molecular markers. Continuous research for biocontrol agents and testing their on farm efficacy helps in management of this virulent soil borne pathogen in coconut.

VI REFERENCES

Abdullah F., Ilias G.N.M., Nelson M., Nur Ain Izzati M.Z., Umi Kalsom Y. (2003). Disease assessment and the efficacy of Trichoderma as a biocontrol agent of basal stem rot of oil palms. *Research Bulletin Science Putra*, **11**: 31–33

Adaskaveg, J.E. and Gilbertson, R.L. (1989). Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G.tsugae. Mycol. Res*., **92**: 182-191.

Adaskaveg, J.E. and Gilbertson, R.L.(1986). Cultural studies and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of *G. lucidum* complex. *Mycologia*, **78**: 694-705.

Adaskaveg, J.E. and Gilbertson, R. L. (1987). Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *G. tsugae. Mycologia*., **79**: 603-613.

Adaskaveg, J.E., Blanchette, R.A. and Gilbertson, R.L. (1991). Decay of date palm wood by hite-rot and brown-rot fungi. *Canadian J. bot*., **69**:615-629.

Adaskaveg, J.E., Miller, R.W. and Gilbertson, R.L. (1993). Wood decay, ligncolous fungi, and decline of peach trees in South Carolina. *Plant disease* . **77**: 707-711.

Alexopoulous, C.J., Mims, C.W. and Blackwell, M. (1996). Introductory Mycology. John Wiley and Sons, Inc. p 869.

Anonymous, (1989). coconut leaf diseases in Brazl, *Olegineux*. **44**(4): 117-118.

Anonymous, (2005). All India Co-ordinated Research Project on Palms, Project Report 2005. *Central Plantation Crops Research Institute, Kasaragod*, p 49.

Anonymous, (2013). Annual Progress Report for 2012-2013. Central Plantation Crops Research, *Kasaragod, Kerela India*. PP: 142.

Anonymous, (2014). All India Co-ordinated Research Project on Palms, Project Report 2014. *Central Plantation Crops Research Institute, Kasaragod*, p 40.

Ariffin, D., Idris, A.S. and Abdul, H. H. (1989). Significance of the black line within oil palm tissue decay by Ganoderma boninense. *Elaeis*., **1**: 11-16.

Ariffin, D., Idris, A.S. and Singh, G. (2000). Status of *Ganoderma* in oil palm. [In "*Ganoderma* Diseases of Perennial Crops" by Flood, J., Bridge, P. D. and Holdesness, M.(Ed‟s)] CAB International, Oxon, UK. pp 49-68.

Ariffin , D., Idris , A. S. and Marzuki, A. (1996). Spread of Ganoderma boninense and vegetative compatibility studies of a single field palm isolates. In: Ariffin, D (Eds.) PORIM International Palm oil Congress (Agriculture). Palm oil Research Institute of Malaysia Bangi, Malaysia, p 317-329.

Athira, K. (2017). International Journal of Current Microbiology and Applied Sciences, Volume **6** Number 12 (2017) pp. 416-423.

Azahar , T. M., Mustapha , C. J., Mazliham , S. and Patrice , B. (2011). Temporal analysis of basal stem rot disease in oil palm plantations: An analysis on peat soil. *Int. J. Eng. Tech.* **3**:96-101.

Bansali, R. (2003). Rejuvenation of *Ganoderma* affected khejri trees through biocontrol agents BCAs "success story'' Div III CAZRI, Jodhpur.

Barman, S.C., Ahmed, M. (1998). Coconut and its trend in area, production and productivity in Bangladesh. *Indian Coconut Journal*., **29**(2):6-10.

BBS **2019** (*yearbook of Agricultural statistics-2018*).

Bhardwaj Surender, k. (2012). Evaluation of plant extracts as antifungal agents against Fusarium solani (Mart.) Sacc. *World J. Agricultural Sci*., **8**(4): 385-388.

Bhaskaran, R., Ramanathan, T. and Ramiah, M. (1982). The Thanjavur wilt. *Intensive Agri.*, **20**:19–21.

Bhaskaran, R. and Ramanathan, T. (1984). Occurrence and spread of Thanjavur wilt disease of coconut. *Indian Coconut Journal*., **15**(6): 12–14.

Bhaskaran, R., Chandrasekar, G. and Shnmugam, N. (1985). Problems and priorities in the management of Thanjavur wilt of coconut. pp 183-187 In: Jayaraj, S (ed) Integrated Pest and Disease Management. Proc. Nat. Sem. Tamil Nadu Agric Univ. Coimbatore

Bhaskaran, R. (1986). Coconut diseases and their management. [In Pest and disease management: oilseed, pulses, millets and cotton. Jayaraj S, (Eds). Coimbatore (India)]: Tamil Nadu Agricultural University; p. 81–89.

Bhaskaran, R. (1990a). Biological control of Thanjavur wilt disease of coconut. In: National Symposium on Biocontrol of Root Disease. Annamalai University, Annamalainagar: 7–8.

Bhaskaran, R., Suriachandraselvan, M. and Ramachandran, T. K. (1990b). Ganoderma wilt disease of coconut-a threat to coconut cultivation in India. *The Planter*. **66** (774): 467- 471.

Bhaskaran, R., Ramadoss, N. and Suriachandraselvan, M. (1991). Pathogenicity of Ganoderma Spp. Isolated from Tanjavur wilt infected coconut (Cocos nucifera L.). *Madras Agric. J*., **78** (1-4): 137-138.

Bhaskaran , R. (1993). Integrated management of basal stem rot disease of coconut Indian Coconut. J., **24** (4): 5-8.

Bhaskaran, R. (1994). Biofertilizers for the management of basal stem rot disease of coconut. Indian Coconut J., **25**: 7–11.

Bridge and M. Holderness, (Eds.), CAB International, Oxon, UK. pp 322.

Biley, V.T.,Solomko, E.F. and Buchalo, A.S. (2000). Growth of edible and medicinal mushrooms on commercial agar. In: Van Griensven LJLD, (Eds.) Cultivation of edible fungi, 15th International Science Congress, Masstricht, Balkena, Rotterdam pp 779-782.

Booth, C. (1971). Fungal culture media. In: Methods in Microbiology C Booth (Eds.). Academic Press, London, New York. P 49-94.

Breton, F., Hasan, Y., Hariadi, Z., Lubis, and DE, H., Franqueville. (2006). Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *J. Oil Palm Res*., Special Issue: 24-36.

Butler, E.J. and Bisby, G.R. (1931). The Fungi of India. *Central Publication Branch, Calcutta* : 237pp.

Chakrabarty, R. and Ray, A.K. (2007). In vitro studies on management of basal stem rot of arecanut caused by *Ganoderma lucidum* (Curtis ex.Fr.) Karst. *J. Plant. Crops.* **35**(1): 39-41.

Chakrabarty , R., Acharya , G. C. and Sarma , T. C. (2013). Effect of fungicides, Trichoderma and plant extracts on mycelial growth of Thielaviopsis paradoxa under in vitro condition. *The Bioscan*. **8**(1):55-58.

Chee, K. H. (1990). Present status of rubber diseases and their control. Review Pl. *Pathol.*, **69**: 423-430.

Classification. In "*Ganoderma* Diseases of Perennial Crops" - J. Flood, P. D.coconut palms. *Phytopathology.*, **26**: 153–175.

Dagar, J. C., Pandey, C .B. and Chaturvedi, C. S. (2014). Agroforestry: A Way Forward for Sustaining Fragile Coastal and Island Agro-,Ecosystems. (In): Agroforestry Systems in India: Livelihood Security and Ecosystem Services, Advances in Agroforestry 10, pp 185–232.

Dagar, J.C., Singh, A.K. and Arunachalam, A. (Eds). *Springer Publisher, India.*

Darus, A., Seman, A. and Khairudin, H. (1993). Confirmation of *Ganoderma* infected palm by drilling technique. PORIM international palm oil congress: Update and Vision (Agriculture). *PORIM, Malaysia*, p 735-738.

Dharmaputra , O.S., Tjitrosomo , H. S. and Abadi , A. L. (1989). Antagonistic effect of four fungal isolates to Ganoderma boninense, the causal agent of basal stem rot of oil palm. *J. Biotrop.*, **3**: 41–49.

Deepatharshini, D. and Ananthi, E. (2015). Fungal activity of leaf extract of *Eichhorinia crassipes* against *Ganoderma lucidum* causing basal stem rot disease in coconut tree world. *J. Pharmacy and pharm. sci*., **4**(6): 859-864.

Flood, J., Hasan, Y., Turner, P.D. and Ogrady, E.B. (2000). The spread of *Ganoderma* from infective sources in the field and its implications for management of the disease in oil palm. [In "*Ganoderma* Diseases of Perennial Crops" - J. Flood, P.D. Bridge AND M. Holderness, (Eds.)], CAB International, Oxon, UK. pp 101-113.

Fox RTV. and Hahne, K. (1989). Prospects for the rapid diagnosis of Armillaria by monoclonal antibody ELISA. In: Morrison DJ (Eds.) proceeding of the seventh international conference on root and butt rots. Pacific Forestry centre, Columbia, p 458-469.

Gottlieb, A. M. and Wright, J.E.(1999a). Taxonomy of Ganoderma from southern South America: subgenus Ganoderma. *Mycological Research*.**103**: 661673.

Gottlieb, A. M., Ferrer, E. and Wright, J. E. (2000). rDNA analyses as an aid to the taxonomy of species of Ganoderma. *Mycological Research*. **104**: 10331045.

George , S. T., Chung , G. F. and Zakaria , K. (1996). Updated results (1990– 1995) on trunk injection of fungicides for the control of Ganoderma basal stem rot. In: Ariffin D et al., (Eds) PORIM International palm oil congress (Agriculture). Palm Oil Research Institute of Malaysia, Bangi., p 508-515.

Govindu, H.C., Rao, A.N.S. and Kesavamurthy, K.V. (1983). Biology of *Ganoderma lucidum* (Leys.) Karst. and control of Anabe Roga of coconut. [In Coconut Research and Development - N. M. Nayar, (Eds.)]. Wiley Eastern Ltd., pp. 325-32.

Gowda, P. V. and Nambiar, K. K. N. (2006). Antifungal activity of garlic (Allium sativum Linn.) extracts on Thielaviopsis paradoxa (de Seynes) von Hohnel, the pathogen of stem bleeding disease of coconut. J. *Plant. Crops*. **34**(3): 472-475.

Gunasekaran, M., Ramadoss, N., Ramiah, M., Bhaskaran, R. and Ramanathan, T. (1986). Role of neem cake in the control of Thanjavur wilt of coconut. *Indian Coconut J*., **17**(1): 7-12.

Gurmit, S. (1991). *Ganoderma* - the scourge of oil palm in the coastal area. *Planters,* **67**: 421-444.

Hasan, Y. and Turner, P.D. (1998). The comparative importance of different oil palms tissues as infection sources for basal stem rot in replanting. *The Planter*. **74**:119-135.

Hennessy, C. and Daly, A. (2007). *Ganoderma* diseases. *Agnote*, No:167, 2pp.

Hepting, G.H.(1971). Diseases of forest and shade trees of the United States. In: Flood, J., Bridge P. D., Holderness, M., (Eds.) *Ganoderma* diseases of perennial crops. CABI Publisher, UK.

Hong, S.G. and Jung, H.S. (2004). Phylogenetic analysis of *Ganoderma* based on nearly complete mitochondrial small subunit ribosomal DNA sequences. *Mycologia*., **96**:742-755.

Henry, L.I (2002). Coconut – The Wonder Palm, pp. 206–18. Hi-Tech Corporation Ramanputhoor, Nagercoil.

Idris, A. S., Kushairi, A., Ismail, S. and Ariffin, D. (2004). Selection for partial resistance in oil palm progenies to Ganoderma basal stem rot. *J. Oil Palm Res*., **16**: 12-18.

Iyer , Rohini , Parvathy , Meera , Lekha , G., Hegde , Vinayak and Gunasekharan , M. (2004). Management of basal stem rot disease of Areca catechu L. in India. *J. Plantn Crops.,* **32**(1): 25-27

ICAR-CPCRI. (2015). Vision 2050. ICAR - Central Plantation Crops Research Institute, Kasaragod, p 71.

Jayarajan , R., Sabitha . D., Baskaran , R. and Jayarajan , S. (1987). Effect of neem (Azadirachta indica) and other plant products in the management of plant disease in India. Natural pesticides from the neem tree Azadirachta indica, A juss and Other tropical plants.

Kandan, A., Bhaskaran, R. B. and Samiyappan, R. C. (2010). *Ganoderma* – A basal stem rot disease of coconut palm in South Asia and Asia pacific regions. *Archives of Phytopathology and Plant Protection*. **43** (15):1445–1449.

Kandana, A., Bhaskaranb, R. and Samiyappanc, R. (2008).*Ganoderma* – a basal stem rot disease of coconut palm in south Asia and Asia pacific regions.Vol. **00**, No.1–5.

Kandan, A., Radjacommare, R., Ramanathan, A., Raguchander, T., Balasubramanian, P. and Samiyappan , R. (2009). Molecular biology of Ganoderma pathogenicity and diagnosis in coconut seedling. *Folia Microbiol*., **54**: 147-152.

Karthikeyan, M., Bhaskaran, R., Radhika, K., Mathiyazhagan, S., Jayakumar, V., Sandosskumar, R. and Valazhahan, R. (2006) . Development and comparison of ELISA and PCR methods for the early detection of Ganoderma disease of coconut.

Karthikeyan, M., Adhika, K., Bhaskaran, R., Mathiyazhagan, S. and Velazhahan, R. (2009). Rapid detection of Ganoderma lucidum and assessment of inhibition effect of various control measures by immunoassay and PCR. African J. *Biotechnol.*, **8** (10): 2202-2208.

Karsten, P. (1881). Enumeratio Boletinearumet Polyporearum Fennicarum, Systemate novo dispositarum. *Revue de Mycologie*. **3**: 16-19.

Khairudin, H. (1990). Basal stem rot of oil palm: incidence, etiology and control. MSc Thesis. Universiti Pertanian Malaysia, Selamgor, Malaysia.

Kharkwal, H., Joshi, D.D., Kharkwal, A.C. and Prasad, R. (2012). Antifungal activity of the leaf extract of *Clerodendron infortunatum* Retz. *World Applied Science J*., **20**(11): 1538-1540.

Lee, S.S. (2000). The current status of root diseases of *Acacia mangium* Willd. [In "*Ganoderma* Diseases of Perennial Crops" (J. FLOOD, P. D. BRIDGE AND M. HOLDERNESS, (Eds.)], CAB International, Oxon, UK. pp 71-79.

Lomberh, M.L. (2002). Study on Hypsizygus marmoreus (Peck) Bigelow in culture. Ukrayins'kyi Botanichnyi Zhurnal. **59**: 292-298.

Lim, T. K., Chung, G. F. and Ko, W. H. (1992). Basal stem rot of oil palm caused by Ganoderma boninense. *Plant Pathology Bulletin*. **1**:147-152.

Markom , M. A., Shakaff , A. Y., Adom , A. H., Ahmad , M. N., Hidayat , W., Abdullah , A. H. and Fikri , A. N. (2009). Intelligent electronic nose system for basal stem rot disease detect. *Com Electron Agri*., **66**: 140-146.

Moncalvo, J. M. (2000). Systematics of *Ganoderma*. [In: Flood, J., Bridge P. D., Holderness, M., (Eds.)]. *Ganoderma* Diseases of Perennial Crops. CABI Publishing, UK. pp: 23–45.

Moncalvo, J.M., Wang. H.H. and Hseu, R.S. (1995a). Phylogenetic relationships in Ganoderma inferred from the internal transcribed spacers and 25s ribosomal DNA sequences. *Mycologia*., **87**: 223-238.

Moncalvo, J. M. and Ryvarden, L. (1997). A nomenclature study of the *Ganoderma* taceae Donk. Sinopsis Fungorum. **11**: 1-14.

Mumpuni, A., Sharma, H.S.S. and Brown, A.E. (1998). Effect of metabolites produced by *Trichoderma harzianum* biotypes and *Agaricus bisporus* on their respective growth radii on culture. Appl. Environ. *Microbiol*., **64**:5053-5056.

Naher, L., Tan, S.G., Yusuf, K.U., Ho, C.L. and Abdullah, F. (2012a). Biocontrol agent *Trichoderma* strain FA 1132 as an enhancer for oil palm growth. *Pertanika J Tropical Agri Sci.* **35**: 173-182.

Naidu .GVB., Kumar, SNS., Sannamarappa , M. (1986). Anaberoga, *Ganoderma lucidum* (Leys.). Karst on arecanut palm: a review and further observations. *J Mysore Hort Soc.* **1**(3):14–20.

Nambiar, K.K.N. (1994). Diseases and disorders of coconut. (In) Advances in Horticulture, Vol.X – Plantation and Spice Crops Part – 1, pp 857–82. Chadha, K.L.and Rethinam, P. (Eds). Malhotra Publishing House, New Delhi.

Natarajan, S., Bhaskaran, R. and Shanmugam, N. (1986). Preliminary studies to develop techniques for early detection of Thanjavur wilt in coconut. *Indian Coconut J*., **17**: 3-6.

Naik, R. G., Palanimuthu, V., Hanumanthappa, M. and Indiresh, K. M. (2000). Prevalence and intensity of basal stem rot disease of coconut in Arsikere taluk of Karnataka. *Indian Coconut J.*, **31** (1): 8-10.

Naik, R. G. (2001). Chemical control of basal stem rot of coconut (Cocos nucifera (L.)) *Agricultural Sciences Digest*, **21** (4): 247-249.

Nawawi, A. and Ho,Y.W. (1990). Effect of temperature and pH on growth pattern of *Ganoderma boninense* from oil palm in Peninsular Malaysia. *Pertanika* ., **13**: 303-307.

Palanna, K.B., Boraiah, B., Nagarj, M.S., Thyagaraj, N.E. and Ramaswamy, G.R. (2013). Effect of bio-control agents and botanicals on in vitro growth and development of *Ganoderma applanatum*. *J. Plant. Crops*. **41**(2): 0-0.

Palanna, K.B., Childananda swamy, B.S. and Mathinilanm. (2005a).Effect of Chemical fertilizers on growth, sporulation and antifungal activity of *Trichoderma viride* in vitro. *Mysore J. Agric. Sci*., **39**(4): 570-573.

Palanna, K.B., Ganesh Naik, R., Basavaraj, T. B., Boraiah, B. and Tyagaraj, N.E. (2009). Etiology and management of coconut Basal Stem Rot (Ganoderma Wilt) in Sandy soils of Karnataka. *Journal of Plant*. Crops. **37** (1): 26-29.

Pilotti, C.A., Sanderson, F.R. and Aitken, E.A.B. (2003).Genetic structure of a population of *Ganoderma boninense* on oil palm. *Plant Pathol*., **52**: 455-463.

Pilotti, C.A. (2005). Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen biology and epidemiology. *Mycopathologia*., **159**: 129-137.

Palanna , K. B., Chidananda Swamy , B. S. and Mathinil , (2005a). Effect of Chemical fertilizers on growth, sporulation and antifungal activity of Trichoderma viride in vitro. *Mysore J. Agric. Sci*., **39**(4): 570-573.

Palanna, K. B., Boraiah, B., Nagarj, M. S., Thyagaraj, N. E. and Ramaswamy, G. R. (2013). Effect of bio-control agents and botanicals on in vitro growth and development of Ganoderma applanatum. J. *Plant. Crops*. **41**(2): 0-0.

Paterson , R. R. M. (2007). Ganoderma disease of oil palm- a white rot perspective necessary for integrated control. *Crop Protect*., **26**: 1369–13.

Priestley, R., Mohammed, C. and Dewey, F. M. (1994). The development of monoclonal antibody based ELISA and dipstick assays for the detection and identification of Armillaria species in infected wood. In: Schots A, Dewey F. M, Oliver UR (Eds) Modern assays for plant pathogenic fungi: Identification, detection and Quantification, CAB international: Wallingford, UK, p. 149-156.

Ramya, S., Krishnasamy, G., Jayakumararaj, R., Periathambi, N. and Devaraj, A. (2012). Bioprospecting *Solanum nigrum* Linn. (Solanaceae) as a potential source of Anti-Microbial agents against selected bacterial strains. *Asian J. Biomedi. and Pharm. Sci.,* **2**(12): 65-68.

Rethinam, P. and Taufikkur Rahman, L. (2002). Global scenario of coconut oil. *Indian Coconut J.,* **33**(7):1–8.

Rethinam, P. (1984). Thanjavur wilt disease of coconut in Tamil Nadu. *Indian Coconut J*. **15**: 3-11.

Rajendran, L., Gandhi, K., Thiruvengadam, R. and Ramasamy, S. (2007). In vitro evaluation of bacterial endophytes influence on Ganoderma lucidum (leys) karst. mycelial growth. *Journal of Plant Protection Research*., **47**(4): 425-436.

Rajendran, L., Akila, R., Karthikeyan , G., Raguchander , T., Saravanakumar , D. and Samiyappan, R. (2014). Nucleic acid based detection technique for

Ganoderma lucidum in coconut., Arch. *Phytopath. Plant Protect*., **47** (6): 690-702

Samiyappan, R., Bhaskaran, R. and Rethinam, P. (1996). Diagnosis for early detection of *Ganoderma* diseases in perennial crops: *approaches and prospects. J. Plant Dis. Prot.,* **103**(1): 85-93.

Sanderson, F.R. (2005). An insight into spore dispersal of *Ganoderma boninense* on oil palm. *Mycopathologia*., **159**: 139-141.

Sangal, P.M., Mukherji, S.K. and Singh, B. (1961). A short note on the fungus flora of Nicobar Islands. *Indian Forester*., **87**: 766-767.

Sankaran, K.V., Bridge, P. D. and Gokulapalan, C. (2005). Ganoderma diseases of perennial crops in India- an overview. *Mycopathologia*., **159**: 143-152.

Sampath K. S. N. AND Nambiar, K K. N. (1990). Ganoderma disease of arecanut-isolation, pathogenicity and control. J. *Plant. Crops*. **18**: 14-18.

Schwarze, F. W. M .R., Londsdale, D. and Mattheck, C. (1995).Detectability of wood decay caused by *Ustulina deusta* in comparison with other tree-decay fungi. *European J. Forest Pathology*., **25**: 327-341.

Schwarze, F. W. M. R. and Ferner, D. (2003). *Ganoderma* on trees Differentiation of species and studies of invasiveness. www.enspec.com/articles/ research.htm. pp 1- 21.

Sharma, D. and Thakur, M. P. (2010). Effect of substrates on vegetative growth and fruiting induction of *Ganoderma* species. J. *Mycology and Plant Pathol*., **40**: 425- 431.

Steyaert, R. L. (1972). Species of Ganoderma and related genera mainly of the Bogor and Leiden herbaria. *Persoonia*., 7: 55-118.

Seo, G.S. and Kirk, P.M. (2000). Ganodermataceae: Nomenclature and Classification. In “Ganoderma Diseases of Perennial Crops” - J. Flood, P. D. Bridge and M. Holderness, (Eds.), CAB International, Oxon, UK. pp 322.

Singh, G. (1991). *Ganoderma*- the scourge of oil palms in the coastal areas. *The Planter*. **67**: 421-444.

Singh, P. P., Shin, Y. C., Park, C. S. and Chung, Y. R. (1999). Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. *Phytopathology.,* **89**: 92– 99.

Snehalatharani, A., Maheswarappa, H.P., Devappa, V. and Malhotra, S.K., Ysrhu, Dr., Ambajipeta. (2016). A Review Article Status of coconut basal stem rot disease in India .AICRP on Palms, Horticultural Research Station, *Indian Journal of Agricultural Sciences* **., 86** (12): 1519–29.

Srinivasalu, B., Aruna, K., Sabitha, D. and RAO, D. V. R. (2001). Occurrence and Bio-control of Ganoderma wilt disease of coconut in Coastal Agro-Ecosystem of Andhra Pradesh. J. *Indian Society of Coastal Agric*. Res., **19**(1&2): 191-195.

Srinivasalu, B., Aruna, K., Lakshimi, B.K.M., Sabitha Doraiswamy., Rao, D. V. R. and Hameed khan, H. (2002).*Trichoderma hamatum*- a potential bio control agent for basal stem rot (Ganoderma wilt) disease of coconut. *Proceedings of Placrosym* **(XV):** 541-544.

Srinivasalu, B., Sabitha Doraisamy., Aruna, K., Rao, D.V.R. and Rabindran, R. (2002). Efficacy of biocontrol agent, chemicals and botanicals on *Ganoderma* sp, the coconut basal stem rot pathogen. *Journal of Plantation Crops* ., **30** (3): 57-59.

Srinivasalu, B., Aruna, K., Rao, D.V. R. and Hameed Khan, H. (2003). Epidemioligy of Basal Stem Rot (Ganoderma wilt) disease of coconut in Andra Pradesh. Ind. J. *Pl. Prot.*, **31**(1): 48-50.

Srinivasalu , B., Vijay krishna kumar , K., Aruna , K. and Rao , D. V. R. (2004). Bio control of major pathogens of coconut. *J. Plant. Crops*. **32**: 309313.

Srivastava, B. P.,Singh, K. P., Singh, U.P. and Pandey, V. B.(1994). Effect of some naturally occuring alkaloids on conidial germination of *Botrytis cinerea*. *Bioved*., **5**:69-72.

Susanto, A., Sudharto, P.S. and Purba, R.Y. (2005). Enhancing biological control of basal stem rot diseases (*Ganoderma boninense*) in oil palm plantations. *Mycopathol*., **159**: 153–157.

Santoso, H., Gunawan , T., Jatmiko , H. R., Darmosarkoro , W. and Minasny , B. (2011).Mapping and identifying basal stem rot disease in oil palms in North Sumatra with Quick Bird image. *Precision Agri.,* **12**:233-248.

Thirumalaiswamy, K., Vijayaraghavan, H. and Savery, J. (1992). Early diagnosis of Thanjavur wilt of coconut, *Indian Coconut Journal* **23**: 5, 9–10, and *Planter* **68**: 599–601.

Turner, P.D. (1981). Oil Palm diseases and disorders. Oxford University Press, Kuala Lumpur.

Turner, P. D. (1965). The incidence of Ganoderma disease of oil palms in Malaya and its relation to previous crops. Ann. App. Biol., **55**: 417-423.

Venkatarayan, S. V. (1936). The biology of *Ganoderma lucidum* on areca and coconut palms. *Phytopathology*., **26**: 153–175.

Vijayan, K. M. and Natarajan, S. (1972). Some observations on the coconut wilt disease of Tamil Nadu. *Coconut Bulletin* **., 2**(12): 2–4.

Vinayaka, H. and Prathibha, V. H. (2013).Integrated disease management in coconut;Volume **6** Number 12 (2017) pp. 416-423.

Wang, X. C., XI, R. J., LI, Y., Wang, D. M. and Yao, Y. J.(2012b). The species identity of the widely cultivated *Ganoderma*, '*G. lucidum'* (Ling-zhi), in China http://plosone.org (2nd August, 2012).

Wasser, S.P. and Weis, L.A. (1999). Medicinal properties of substrates occurring in higher Basidiomycetes mushrooms: Current perspectives. *Int. J. Med. Mush*., **1**: 31-62.

Wong, L. C., Bong, C. F. J. and Idris, A. S. (2012). *Ganoderma* species associated with basal stem rot disease of oil palm. *Amer. J. Ppl. Sci*., **9**: 879-885.

APPENDICES

Appendix-I. Survey questioner

Structural questioner for survey study of Basal Stem Rot disease

Survey on basal stem rot disease of coconut in Bangladesh

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Name of the District:
Name of the upazilla:
Type of the orchard:
Name of the Owner:
Area of the orchard:
Total No. of plant:
No. of plant Observed:

Table : Disease scale for Ganoderma wilt 0-4 (Abdullah *et al*. 2003;Ilias 2000)

Disease class	Signs and symptoms of infection
0	Healthy plants with green leaves without appearance of fungal mycelium on any part of plants
1	Appearance of white fungal mass on any part of plants , with or without chlorotic leaves
2	Appearance of fungal mass/mycelium on any part of plants with chlorotic leaves (1-3)
3	Appearance of fungal mass / mycelium on any part of plants with chlorotic leaves($>$ 3)
4	Formation of well-developed basidioma and plants dried /wilted

SL	G.W	Age
1	1	20
2	2	
3	1	
4	0	
5	0	
6	1	
7	0	
8	1	
9	1	
10	0	
11	1	
12	2	
13	0	
14	0	
15	1	
16	0	
17	2	
18	1	
19	1	
20	0	
Total		

%D.I= **% D.S =**

Cultural practices:

1. **Cleaning**
2. **…..**
3. **……**
4. **…….**
5. **……**

Use of fertilizer

1. **Chemical:**
   No
   ……..
   ……
   ……

2. **Biological:**
   Household waste
   ……..
   ……..
   ……..

Other information:

Type of soil of the area:

GPS of the area:

Soil pH:

Relay crops with coconut: Banana, rain tree.

Problems faced by the cultivar:
Very unconscious about the agricultural practices.
Comments after survey
……
……
……

Survey held by

Rabeya Busri Rodela
MS student
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Cell: 01766808043
Email: ayarodela194142@mil.com

Supervised by

Dr. Md. Belal Hossain
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Cell: +8801711988444
E-mail:dr.mbhossain@sau.edu.bd

Appendix-II. Plants grew with coconut and cultural practices in the coconut orchard

During survey various types of plants were seen growing with coconut. The plants are listed below –

A. List of the plants grown with coconut

SI. No.	Area	Plants growing with coconut
01	Pirojpur	Arecanut,palmyras,date,mango,Banana,guava
02	Barisal	Banana, Rain tree, jujube,guava,jackfruit
03	Patuakhali	Berry,Banana,Arecanut,Jackfruit,guava,mango

Different types of cultural practices were seen in the surveyed area. But some farmers are so much unconscious about the practices. Some farmers had little training on coconut growing but it was not enough for getting enough outcome.

B. List of cultural practices performed in coconut garden

SI. No.	Area	Culture practices
01	Pirojpur	Cleaning,irregation,fertilization
02	Barisal	Cleaning,irregation,fertilization
03	Patuakhali	Cleaning

Appendix-III. Cultural and morphological characteristics /variability of isolated Pathogens

Table 8: Cultural and morphological characteristics/variability of *Ganoderma* isolates of coconut

SI No.	Isolates	Rdial growth(mm)			Days Taken for full plate	Colony/ Mycelial characters		
		3DAI	6DAI	9DAI		Colony Color /Reverse pigmentation	Mycelia Texture/ Mycelia density	Margin
01	PSP	37.25ab	60.67ab	83.92a	9	White/white	Fluffy/ Leathery/ Irregular	Filamentous
02	PKp	36.67ab	58.83ab	82.03a	11	White/yellowish	Fluffy/ dense	Even
03	PDs	39.67a	63.08a	83.83a	10	Creamy White/white	Fluffy/ Thin	Even
04	BGd	35.33ab	51a	77.50a	7	Pale white/NP	Leathery /Thin	Filamentous
05	BBg	37ab	60.17ab	83.83a	15	White/NP	Leathery/ dense	Filamentous
06	BMg	37.33ab	61.58ab	85.75a	14	Pale white/NP	Leathery/ Thin at centre and dense at centre	Even
07	PiBh	31.83b	56.17ab	80.87a	15	White/pinkish	Leathery/ dense at centre	Even
08	PiNs	32b	60.30ab	82.50a	7	White/yellowish	Fluffy/ Dense/ Irregular	Undulate
09	PiKl	36ab	54.67ab	84.17a	7	Pale White/NP	Fluffy/ irregular	Undulate
10	SAU	39.67a	61.33ab	87.42a	15	Creamy white/yellowish	Leathery/ Thin at centre and dense at centre	Even
11	LSD (0.05)	7.172	11.17	13.42				
12	CV(%)	11.61	11.16	9.50				

Note: DAI-Days After Inoculation

Appendix-IV. *In-vitro* efficacy of different selected treatments with different doses through Pour technique method

Table 9: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 1ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	**5DAI**	**7DAI**
1	Control	32.67a	46.67a	52.67a
2	Trichoderma	14.83b	22.67b	28.67b
3	Neem Oil	10.67bc	15.67bc	18.67c
4	Garlic extract	7.17c	17bc	16.5c
5	Cattle urine	12b	11.5c	12c
6	**LSD (0.05)**	4.368	8.023	8.687
7	**CV(%)**	15.53	19.43	18.58

Note: DAI-Days After Inoculation

Table 10: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 2ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	**5DAI**	**7DAI**
1	Control	32.67a	46.67a	52.67a
2	Trichoderma	12.5b	18.67b	20.67b
3	Neem Oil	9bc	12.67bc	15.33bc
4	Garlic extract	5.33c	13bc	9.67cd
5	Cattle urine	9bc	8.5c	4.67d
6	**LSD (0.05)**	3.8948	7.5997	8.0816
7	**CV(%)**	15.63	20.99	21.56

Note: DAI-Days After Inoculation

Table 11: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 3ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	5DAI	7DAI
1	Control	32.67a	46.67a	52.67a
2	Trichoderma	8.33b	13.67b	18.33b
3	Neem Oil	9b	12bc	13.33bc
4	Garlic extract	4.67c	6.67cd	6.67cd
5	Cattle urine	8.17b	4.33d	3d
6	**LSD (0.05)**	3.0533	6.6762	7.5596
7	**CV(%)**	13.36	22.02	22.10

Note: DAI-Days After Inoculation

Appendix-V. *In-vitro* efficacy of different selected treatments with different doses through Cup method

Table 12: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 1ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	6DAI	9DAI
1	Control	33.5a	37.67a	64a
2	Trichoderma	21.5bc	25.33c	34.17b
3	Neem Oil	23.17b	31.67b	38.67b
4	Garlic extract	18.33cd	25.67c	31b
5	Cattle urine	14.17d	15.42d	17.83c
6	**LSD (0.05)**	4.529	2.649	8.4356
7	**CV(%)**	11.25	5.36	12.49

Note: DAI-Days After Inoculation

Table 13: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 2ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	**5DAI**	**7DAI**
1	Control	33.5a	37.67a	64a
2	Trichoderma	16.08b	16.42cd	20.67b
3	Neem Oil	15.5b	22.33b	15.67b
4	Garlic extract	13.33b	18.83c	14.67b
5	Cattle urine	13.5b	14.17d	16.17b
6	**LSD (0.05)**	4.427	3.290	7.281
7	**CV(%)**	13.24	8.26	15.26

Note: DAI-Days After Inoculation

Table 14: Efficacy of different treatments against *Ganoderma* isolate of Coconut with 3ml doses of application.

SI No.	Treatments	Application interval & doses (mm)		
		3DAI	5DAI	7DAI
1	Control	33.5a	37.67a	64a
2	Trichoderma	12.5b	12.83b	16.17b
3	Neem Oil	11.83b	12.83b	16.5b
4	Garlic extract	9.17bc	11.33b	13bc
5	Cattle urine	5.58c	5.67c	6.17c
6	**LSD (0.05)**	4.936	5.156	7.563
7	**CV(%)**	18.69	17.64	17.95

Note: DAI-Days After Inoculation