INCIDENCE OF COCONUT DISEASES IN SELECTED AREA OF BANGLADESH AND STUDY OF BIOCONTROL BASED MANAGEMENT PACKAGE AGAINST BASAL STEM ROT OF COCONUT

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CERTIFICATE

This is to certify that the thesis entitled '**INCIDENCE OF COCONUT DISEASES IN SELECTED AREA OF BANGLADESH AND STUDY OF BIOCONTROL BASED MANAGEMENT PACKAGE AGAINST BASAL STEM ROT OF COCONUT'**. Submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology, embodies the results of a piece of bona fide research work carried out by Registration No. 17-08254 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2018
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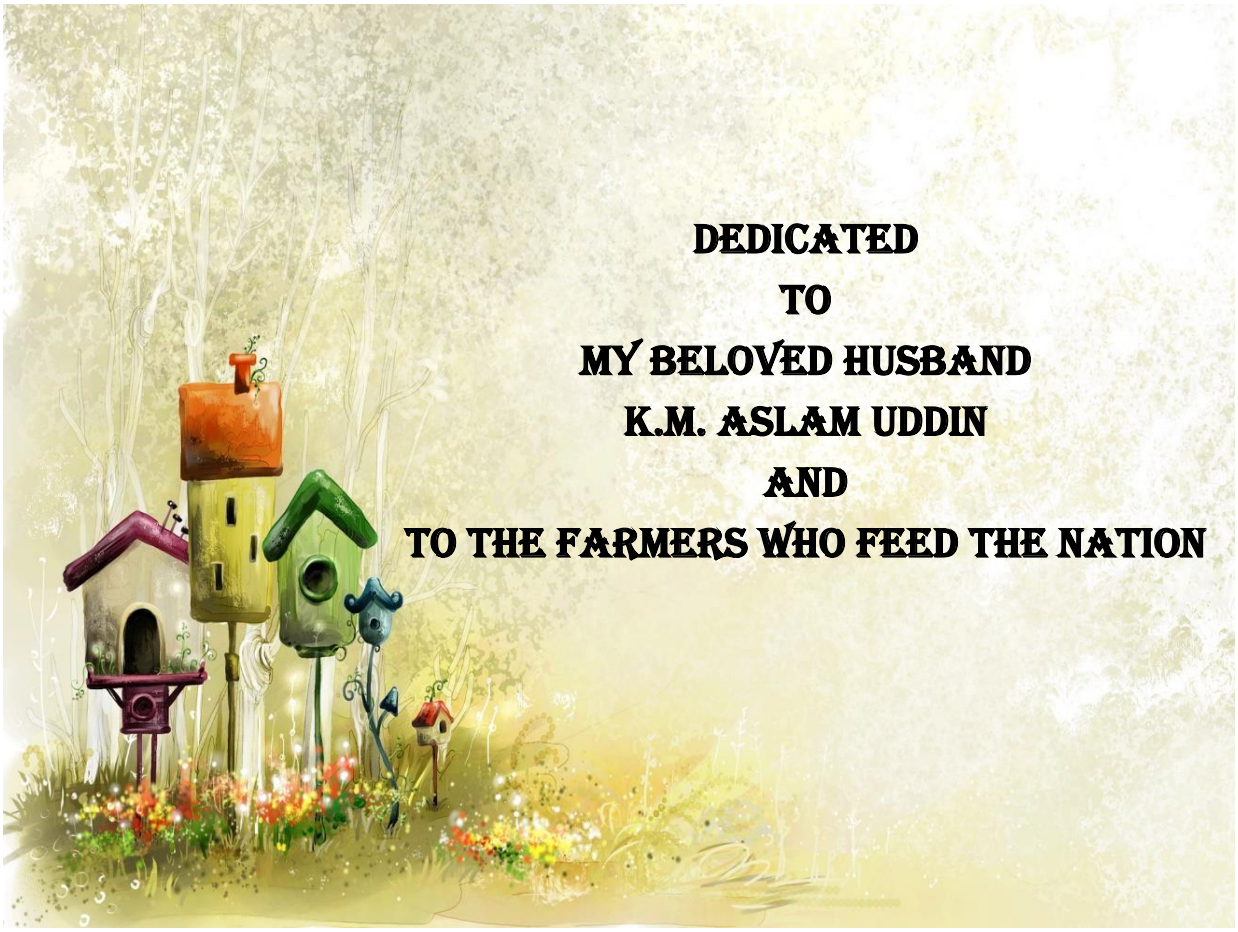

DEDICATED

TO

MY BELOVED HUSBAND

K.M. ASLAM UDDIN

AND

TO THE FARMERS WHO FEED THE NATION

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The Author

Incidence of Coconut Diseases in Selected Area of Bangladesh and Study of Biocontrol Based Management Package against Basal Stem Rot of Coconut

ABSTRACT

A survey study and lab experiment was conducted in this study. The survey study was held on four district. Three are coastal area viz. Noakhali, Barisal and Patuakhali and another is in SAU campus in Dhaka. The lab experiment was conducted in Molecular Biology and Plant Virology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, during January 2018 to March 2019. The study was aimed to demonstrate occurrence and incidence of basal stem rot and foliar diseases of coconut in surveyed area and to develop a biocontrol-based management package against basal stem rot. The disease incidence of basal stem rot was measured 65%, 64.44%, 60.83%, 49.17%, in SAU, Patuakhali Noakhali and Barisal respectively. The disease severity was 33.88%, 30%, 26.67%, and 24.58% respectively in Noakhali, SAU, Patuakhali and Barisal. The incidence of foliar diseases was estimated 85%, 85%, 78.61%, and 77.78% respectively in Noakhali, SAU, Patuakhali and Barisal. The disease severity was 39.13%, 33.56%, 28.33% and 24.13% respectively in Noakhali, Patuakhali, SAU and Barisal. The highest incidence of basal stem rot (27.5%) and leaf disease (19.2%) was found in loamy soil. The highest disease incidence was found at the soil pH 6-7 and the incidence were 26.75% and 22.45 respectively. The highest incidence of basal stem rot (24%) and foliar diseases (18%) was found in plant age group 36-40 years. In total 120 root samples and 90 leaf samples were collected for aseptic isolation. The isolated pathogens from root samples were identified as *Ganoderma sp*. while three pathogens were isolated from leaf samples which are *Pestalotia sp*., *Curvularia sp*., and *Alternaria sp*. In total 32 *Ganoderma* infected plants from SAU campus were selected and treated with 3 management package of different bioagent and botanicals viz., *Trichoderma viridae*, cattle urine, masturd oil cake, neemseed extract and garlic extract. After 5 months of treatment root samples were collected and investigated for existence of pathogen .Among the three combinations mustard oil cake + neemseed extract + *Trichoderma viridae* and cattle urine gave the best performance and disease inhibition were 35% and 52.5% over control. The result of the present study revealed that basal stem rot and foliar diseases are frequently occurred in the survey areas and cattle urine was found effective in the management of this disease in coconut.

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ABBREVIATIONS AND ACRONYMS

%	**=**	**Percentage**
FAO	**=**	**Food and Agricultural Organization**
et al.,	**=**	**And others**
etc.	**=**	**Etcetera**
i.e.	**=**	**id est (L), that is**
viz.	**=**	**Videlicet, itss 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**

INTRODUCTION

The coconut (*Cocos nucifera*) is an important horticulture crop which provides food, oil, beverage, medicine fiber and variety of raw materials for the production of an array of products of commercial importance worldwide. Coconut, the versatile palm popularly known as “King of Palms”, “Tree of Heaven”, “Tree of life”, “Tree of Abundance”, is grown in more than 90 countries across the world in an area of 14.231 million hectares producing about 57.514 billion nuts or 10.52 million tonnes of copra (Athira, 2017). The natural habitat of coconut in the coastal belt of tropics where it flourishes in sea-washed littoral sand with constant motion of underground current of water in rhizosphere. In Bangladesh, coconut is produced abundantly in the coastal area. In Bangladesh the area under coconut production is about 41711 ha, the total production being about 89400 tons (FAO,2017).

The economic importance of this tree crop is evident from the fact that in Bangladesh also has a handsome coconut growing profile especially in the coastal zone. coconut is considered as a crop of high economic value due to its diversified uses. The crop is commonly grown in homesteads with efficient utilization of land. Many smallholder’s households generally depend on the coconut for their livelihood as it provides regular incomes. The coconut palm exerts a profound 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 coconut has a great prospect in our economic growth. Coconut is normally affected by various abiotic and biotic stresses resulting in drastic reduction in yields even few noxious pathogens are hampering the production by killing the coconut plants. Coconut sector in the country are faced with umpteen challenges like palm senility, natural calamities such as floods, drought, pest and diseases. Among the diseases, basal stem rot, bud rot, leaf spot, stem bleeding are prominent diseases of coconut. Among the diseases basal stem rot is one of the major constraints limiting production and productivity of the palms. It is caused by *Ganoderma* sp. Finnish mycologist Peter Adolf Karsten in 1881, introduce the genus *Ganoderma.*) The genus belongs to the family Ganodermataceae that resides in the order Polyporales of the Basidiomycetes (Moncalvo and Ryvarden , 1997). The family 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.

It is an important wood decaying fungus 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 (Pilotti, 2005). The characteristic symptom of the disease is extensive discoloration and rotting of root systems, which leads to tissue disintegration and the stele turns brown. The roots become watery with a distinctive smell of alcohol. More often, the development 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 most diagnostic symptom of the disease. The sporophores initially appear as small, white buttons of fungal tissues that develop rapidly into the accustomed 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.Basal stem rot is generally observed in sandy or sandy loam soils in coastal zone on the east coast where coconut is grown under rainfed conditions and also in neglected plantations. The presence of old infected stubs 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.

These fungi cause white-rot of hardwoods through delignification it causes total death of coconut plant. The disease first starts in the root system. Initially, a few roots get infected and rot. Decay and death of the fine roots is the first underground symptom of the disease. Normal development of flowers and bunches is arrested with the progress of the disease and caused death of the plant.

In the coastal areas leaf diseases are also prominent and found in most of the plant. Several pathogens are responsible for leaf disease of coconut. Among the foliar diseases of coconut, grey leaf spot caused by *Pestalotia palmarum* is very common. The characteristics symptoms of grey leaf spots are larger lesion which up to 15mm long, grey in color with a tinny dark brown margin. Sometimes, the spots are merged together and are surrounded by yellow haloes. The diseases occur on older leaves and are unlikely to decrease yields remarkably). Grey leaf spot or blight caused by *Pestalotia palmarum* had no immune or resistant sources against the disease (Khan and Hossain, 2013). Besides we have little information about the incidence, prevalence, epidemiology and management of seedling diseases of coconut in Bangladesh.

Curvularia leaf spot is characterized by appearance of small, circular yellow spots on leaves in plants. Spots gradually turns brown, with centre of the spot drying up having a sunken impression. The disease turns serious in neglected ill drained gardens and in nurseries raised under heavy shade. Leaf spots of coconut begins as tiny water soaked flecks. These flecks are expanded into larger, tan to brown spots which are circular to oval having a darker brown border. The surrounding area of leaf spots become slightly yellow. As larger areas of the leaf are diseased, the dead leaf tissue becomes tan and a more distinctly yellow area surrounds the lesions. Diseased tissue becomes brittle and parts of the infected area is lost giving the leaf its tattered appearance Dr. Budhadev Mishra (2014). This pathogen produces spores on the upper part of blighted coconut leaves. Moisture favors the production of hundreds of conidiophores that develop from the epidermis of the coconut leaves. Renewed high moisture levels or continuous moisture results in the development of spores by the conidiophores. The temperatures (25°C± 1) favor spore formation while higher temperatures (31°C or 88°F) are not conducive.

Alternaria leaf spot of coconut also found on coconut plants. The affected plants showed that symptoms initially appear as small circular, water-soaked lesions approximately 1-2 mm. As the leaf spots enlarge, the affected tissues within the lesion become olive-green, eventually turning dark-brown and may coalesce to involve considerable portions of the laminae. The size of individual lesion was variable and some were up to 0-5 cm in diameter (Dr. Budhadev Mishra, 2014).

In the present study basal stem rot and foliar diseases were the major concern. Though basal stem rot is a cancerous disease of coconut but no work is found on it in our country.

Therefore, investigations on basal stem rot and foliar diseases of coconut with respect to pathogen variability and disease management were under taken with the following specific objectives.

- To estimate the incidence and severity of basal stem rot and foliar diseases of coconut at surveyed area.
- To identify and characterize as an isolate on the basis of cultural and morphological study.
- To study on biocontrol based management package against basal stem rot in coconut.

REVIEW OF LITERATURE

A literature review concerning occurrence and distribution, disease development and symptoms, isolation and establishment of pure culture, disease diagnosis, morphological, cultural characteristics of basal stem rot and foliar diseases of coconut and integrated disease management practices against basal stem rot wilt 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

Kandan *et al*. (2010) described that basal stem rot (BSR), also called *Ganoderma* wilt, of coconut by the different species of *Ganoderma* is one of the most devastating diseases of coconut. In forest systems, *Ganoderma* has a very harmful ecological role in the breakdown or delignification of woody plants. BSR disease takes several years to develop and for expression of 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 drastic reduction in production and productivity of the palms.

Pilotti (2005) described the distributions of *Ganoderma* species are worldwide in green ecosystem both to tropical and temperate regions. In West Africa Wakefield (1920) identified the causal pathogen was originally as *Ganoderma lucidum.*

Arrifin *et al*. (2000) reported, In Malaysia, the causal pathogen was first recognised as *G. lucidum* by Thompson (1931). They also reported the wide host range of *Ganoderma,* where more than 44 species from 34 genera of plants have been identified as potential hosts. This includes the coconut and oil palms as the leading hosts for basal stem rot.

Turner (1981) reported fifteen species of *Ganoderma* from different parts of the world such as Africa, Indonesia, India, Malaysia, North America, Papua New Guinea and Thailand as being associated with BSR, that includes, *G. applanatum*, *G. cochlear*, *G. lucidum*, *G.*

miniatocinctum, *G. pseudoferreum* ,*G. boninense*, *G. chalceum*, *G. tornatum*, *G. tropicum* and *G. zonatum*. Within these species *G. lucidum*, *G. miniatocinctum*, *G. boninense*, *G. chalceum*, *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) reported that the disease found in different places all over the tropical world viz., West Indies, India, Sri Lanka, Seycheles, Guam, etc.

Asnehalatharani *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 are not taken, the disease is becoming a major threat to coconut production in Andhra Pradesh, Karnataka and Tamil Nadu states.

Doraiswamy *et al*. (2003) informed that about 40 leaf disease causing fungi were reported from the major coconut growing countries of the world. Out of which, grey leaf blight was the most common disease reported from 28 countries.

K. Athira. (2017) reported that the incidence of grey leaf blight is noticed in south farm and the causal organism is *Pestalotia palmarum*. The most favorable condition for this pathogen is well drained soils or soils with potash deficiency, continuous rainy weather for 4-5 days and strong winds.

2.2 Disease development and Symptomology

Kandan *et al*. (2010) informed that *Ganoderma* infects coconut plants from seedlings to old plants, the disease progresses slowly and every infected plant eventually 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.

Arrifin *et al*. (2000) reported that 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 plants tissues. The roots of the infected palms were very friable

and their internal tissues become very dry and powdery. Infected young palms died within 6 – 24 months, whereas mature palms took 2–3 years.

Susanto *et al*. (2005) and Naher *et al.* (2012) said, basal stem rot disease caused by *Ganoderma* considered most destructive disease in oil palm. This is because the disease does not show symptoms at the early stage. In addition, resistant mycelium, basidiospores, chlamydospores, and pseudosclerotia at these resistance stages present in *G. boninense* which are stimulus to inhibit control of *Ganoderma.*

Naher *et al*. (2012) founded in a glasshouse trial that the disease severity of 8.3% in roots on six-month old oil palm seedlings however, leaf with no external symptoms.

Singh (1991) found basal stem rot infection supposed to occur by mycelial development through root contact; He suggested sanitation during replanting regarded as an important practice for controlling BSR. The result showed that this method lowers the disease incidence but not reduced BSR with satisfactory.

Hennessy and Daly (2007) reported that at the advanced stage, the disease could be generally identified by the distinctive fruiting body or bracket, which grows on the trunk of infected plants. Brackets emerge once the infection has spread significantly through the plant, resulting in its death.

Vinayaka and Prathibha (2013) described the 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, 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.

Mishra (2014) reported Pestalotia leaf blight in adult coconut palm, first symptom of grey leaf blight appears on the outer whorl of the lower most leaves as small yellowish brown spots. Spots gradually become oval in shape encircled by greyish brown band. Centre of the spot turns greyish white, brown bands darkens and gets surrounded by a yellowish halo. In advance stage spots coalesce to form large irregular necrotic patches. Minute black spot

of fungal pycnidia appears on blighted surface. Leaflets shrivel, dry completely and show burnt and blighted appearance. *Curvularia* leaf spot is characterized by appearance of small, circular yellow spots on leaves in nursery and very young plants. Spots gradually turns brown, with centre of the spot drying up having a sunken impression. The disease turns serious in neglected ill drained gardens and in nurseries raised under heavy shade. *Alterneria* leaf spot is found in nursery and in very young plants as black and oval spots mostly during summer. Adjacent spots coalesce to form larger patches.

2.3 Isolation and establishment of pure culture

Venkatarayan (1935) reported that *G. lucidum* grew well on malt agar medium. Malt extract medium also supported the good growth of *G. lucidum*.

Biley *et al*. (2000) reported potato Dextrose Agar has been found good medium except for a slightly more time required for growth. Palanna *et al.* (2016) reported isolation of *Ganoderma* from sporophore and diseased root bits that were found good inoculum sources.

2.4 Identification of pathogen species

Moncalvo and Ryvarden (1997) described that the taxonomy of Basidiomycota is traditionally has been based on the morphological features of the basidiocarps. 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. For these reasons, contemporary taxonomy and identification of *Ganoderma* species employ morphological studies, mating tests, analyses of biochemical and DNA sequence, or combinations.

Butler and Bisby (1931) explain that the fungus *Ganoderma lucidum* was first described under the name *Fomes lucidus* (Leys.). The following are considerd as synonyms: *Fomes lucidus* (eys.) Fr., *F amboinensis* (Lam.), *Polyporus lucidus* Fr. *P. amboinensis* Fr., *Polystictus egregious Masse, G. amboinensis* (am.) Pat., *G. resinaceum, G. sessile Murrill, Polyporus fulvellus Bres., P. resinosus Schraeder, Pcurtisil* (Berk.) *Murill*, and *G. mangiferae* (Lev.)

The *Ganoderma* was further subdivided into two subgenera on the basis of presence of laccate (*G. lucidum* complex) and non-laccate (*G. applanatum*).

Hong and Jung (2004) explained that 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.

Smith and Sivasithamparam (2000) proposed methods to determine the identity of *Ganoderma* species including cultural characters, sexual compatibility studies, isozyme studies and DNA based techniques.

2.4.1 Cultural characteristics

Seo and Kirk (2000) reported that in addition to basidiocarp morphology, cultural characteristics such as chlamydospore production, growth rate and thermophily have been used to differentiate *Ganoderma* species structures in culture such as generative hyphae with clamp connections, fibre or skeletal hyphae, staghorn 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*. (2013) reported 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

2.4.2 Morphological characteristics

Wong *et al.* (2012) reported that *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.

Steyaert (1972) found that 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. Basidiospores of *G. lucidum* were brown, ovate with a rounded base and truncate to narrowly rounded apex.

Chang and Miles (2004) reported that the epispores were smooth and the endospores were rough with large central gutta Surface of the basidiospores were dimpled and the wall composed several layers. The two walls of the basidiospores were separated by columns and the spore surface appeared punctate.

2.5 Biocontrol-Based Integrated Disease Management

George *et al*. (1996) found that the chemical treatment result showed significant reduction in BSR incidence when the oil palm trunk was injected with a combination of the fungicides carboxin and quintozene fungicides.

Bruce and Highley (1991) showed *in vitro* studies have shown that the fungi *Trichoderma* spp., *Aspergillus* spp., and *Penicillum* spp. are antagonistic agents towards *Ganoderma.* When mass produced by antagonistic activity, these antagonists, especially *Trichoderma* spp., are good bio-control agents for *Ganoderma.*

Cooper *et al.* (2011) said that the most benefits of bio-fungicide reside in their biocompatibility and biodegradability that it controls the disease without causing any damage to the plant and leaving any toxic elements which are harmful for microoranism and soil environment.

Sariah *et al*. (1999) took attempt in the use of fungicides for BSR control in the field have not been successful, although *in vitro* screening had identified chemicals effective against *Ganoderma*. The inadequacy of *in vitro* screening had prompted research into possible *in vivo* screening assays.

Haram *et al. (1996*) suggested different mechanisms were responsible for the effects of biocontrol agents; they included competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of an inhibitory compound.

Bhansali (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*.

Bhaskaran (1990) reported that the soil application of *Trichoderma harzianum* at 500g inoculums along with various organic manures *viz.*, green leaves (50kg) or Farm yard manure (50kg) or neem cake (5kg) significantly reduces the BSR disease intensity and increases the nut yield. Application of *Trichoderma viride* and *P.fiuorescens* talk formulations at the rate of 200g each /palm in combination with 50kg FYM found effective against the disease.

Shanmugam *et al.* (2018) found that The management practices viz., sowing of green manure crop sunhemp @ 50 Kg/ha, insitu ploughing of sunhemp on 4045th day after sowing, Neem cake 5 Kg/tree along with *Pseudomonas fluorescens* @ 5Kg/ha and *Trichoderma viride* @ 5Kg/ha reduced the incidence of *Ganoderma* wilt and increased the yield.

MATERIALS AND METHODS

The *in-vitro* experiment was conducted in Molecular Biology and Plant Virology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The survey was done in several selected locations of coastal zone viz. Noakhali, Patuakhali and Barisal district and SAU Campus. The study was done during the period of January 2018 to March 2019. The details of materials used and methodology followed during the study are described in this chapter.

3.1 Survey study for the incidence of coconut diseases in selected Locations of Bangladesh

Three districts from coastal area of Bangladesh and SAU campus were selected for the survey study. The survey was done in the major coconut growing areas of coastal zone viz. Noakhali, Patuakhali, Barisal and SAU campus (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 and foliar diseases were recorded (Plate 1-5). The disease incidence was estimated by using the following formula.

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

During the 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 performed in orchard of coconut and related information were also documented as per the survey data sheet (appendix I & II).

Table 1. Survey details of selected areas for survey

SL NO.	Locations	Areas	No. of garden observed	Total no. of plant in observed garden	No. of plant observed
01.	Noakhali	Subornochor	03	630	60
		Begumgong	03	390	60
		Sudharampur	03	620	60
		Sub-total	09	1640	180
02.	Patuakhali	Dumki	03	200	50
		Patuakhali Sadar	03	250	60
		Bauphal	03	280	60
		Sub-total	09	730	180
03.	Barisal	Bakergong	03	200	60
		Kashipur	03	140	60
		Barisal sadar	03	230	60
		Sub-total	09	570	180
04.	SAU campus (Dhaka)			250	60

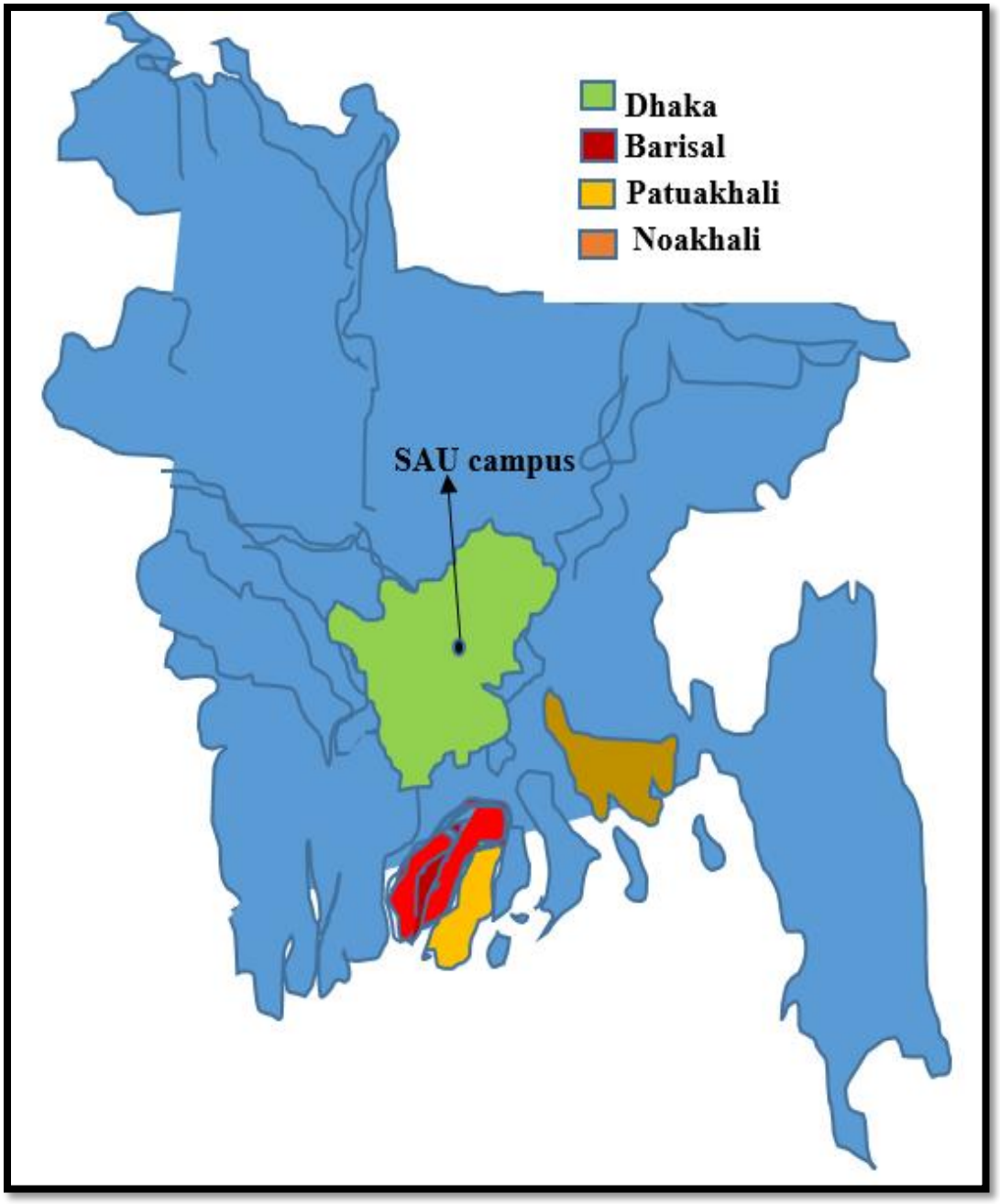


Figure 1. Surveyed area of basal stem rot and foliar diseases of coconut

3.2. 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:

$$\textbf{Disease Severity Index (DSI)} = \frac{\textbf{Sum of all disease rating}}{\textbf{Total number of rating} \times \textbf{maximum disease grade}} \times 100$$

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

Grade Description (% leaf area infected) for foliar diseases of coconut is taken from (K. Athira, 2017).

Disease class	leaf area infected (%)
0	No sign or symptoms
1	0-10 % infection
3	11-15% infection
5	16-25% infection
7	26-50% infection
9	>50%infection

Yellowing and reduction in crown size

Drooping of leaves around the crown and skirt formation

Plants with necrotic leaves

Decaying of wood by *Ganoderma sp*

Plate 1. Symptoms of *Ganoderma* wilt found in survey areas

Decaying of wood by *Ganoderma*

Decaying of wood by *Ganoderma* sp.

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

Formation of basidioma on the base of coconut plant

Plate 3. 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). The farmers are not conscious about the disease. So the disease rate is increasing day by day. In Noakhali district some commercial garden were so much affected that the yield of coconut about to be stopped. A farmer of Shudharampur 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.

Plate 4. Coconut leaves showing leaf spot and leaf blight

Plate 5. Coconut leaves showing leaf spot and leaf blight

3.3 Collection of diseased roots and leaves of coconut from different Survey area and preservation

Different parts of the coconut palms such as diseased root bits, affected leaves showing typical symptoms 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.

3.4 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 autoclaved. Test tubes and flasks containing liquid media plugged with non-absorbent cotton covered with foil paper were autoclaved.

3.5 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 heating in the flame. Also, before starting the experiments the hands were cleaned well with 70 per cent ethanol.

3.6 Isolation and designation as isolate of the identified causal organisms

Infected roots/leaves 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) medium (Plate 6, Appendix-III). The inoculated plates were incubated at room temperature (28 °C ± 2 °C) for 30 days to facilitate growth of the

fungus. The radial growth was measured 3 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 GNSu, GNSd, GNBg, GPDm, GPBl, GPSr, GBBg, GBKr, GBSr, GSAU. (Table.2). Similarly, *Pestalotia* isolates were designated as PNSu, PNSd, PNBg, PPDm, PPBl, PPSr, PBBr, PBKr, PBSr, PSAU(Table.3) *Curvularia* isolates are designated as CNSu, CNSd, CNBg, CPDm, CPBl, CPSr, CBBr, CBKr, CBSr,CSAU (Table 4.). *Alternaria* isolates are designated as ANSu, ANSd, ANBg, APDm, APBl, APSr, ABBr, ABKr, ABSr, ASAU (Table 5.).

Root samples

Sporocarp

Root samples on blotter papers

Plate 6. Samples for *Ganoderma* isolation

Table 2. Identity and designation of *Ganoderma* isolates

SL NO.	Source of isolation	Place of collection	Identity and designation of *Ganoderma* isolates
01	Root sample	Subornochor, Noakhali Dist.	GNSu
02	Root sample	Sudharampur, Noakhali Dist	GNSd
03	Root sample	Begumgong, Noakhali Dist.	GNBg
04	Root sample	Dumki, Patuakhali Dist.	GPDm
05	Root sample	Bauphal, Patuakhali Dist	GPBl
06	Root sample	Patuakhali sador, Patuakhali Dist	GPSr
07	Root sample	Bakergong, Barisal Dist	GBBg
08	Root sample	Kashipur , Barisal Dist.	GBKr
09	Root sample	Barisal sador, Barisal Dist.	GBSr
10	Sporocarp	SAU campus , Dhaka	GSAU

Table 3. Identity and designation of *Pestalotia* isolates

SL No.	Source of isolation	Place of collection	Identity and designation of *Pestalotia* isolates
01	Leaf sample	Subornochor, Noakhali Dist.	PNSu
02	Leaf sample	Sudharampur, Noakhali Dist	PNSd
03	Leaf sample	Begumgong, Noakhali Dist.	PNBg
04	Leaf sample	Dumki, Patuakhali Dist.	PPDm
05	Leaf sample	Bauphal, Patuakhali Dist	PPBl
06	Leaf sample	Patuakhali sador, Patuakhali Dist	PPSr
07	Leaf sample	Bakergong, Barisal Dist	PBBg
08	Leaf sample	Kashipur , Barisal Dist.	PBKr
09	Leaf sample	Barisal sador, Barisal Dist.	PBSr
10	Leaf sample	SAU campus , Dhaka	PSAU

Table 4. Identity and designation of *Curvularia* isolates

SL No.	Source of isolation	Place of collection	Identity and designation of *Curvularia* isolates
01	Leaf sample	Subornochor, Noakhali Dist.	CNSu
02	Leaf sample	Sudharampur, Noakhali Dist	CNSd
03	Leaf sample	Begumgong, Noakhali Dist.	CNBg
04	Leaf sample	Dumki, Patuakhali Dist.	CPDm
05	Leaf sample	Bauphal, Patuakhali Dist	CPBl
06	Leaf sample	Patuakhali sador, Patuakhali Dist	CPSr
07	Leaf sample	Bakergong, Barisal Dist	CBBg
08	Leaf sample	Kashipur , Barisal Dist.	CBKr
09	Leaf sample	Barisal sador, Barisal Dist.	CBSr
10	Leaf sample	SAU campus , Dhaka	CSAU

Table 5. Identity and designation of *Alternaria* isolates

Sl No.	Source of isolation	Place of collection	Identity and designation of *Alternaria* isolates
01	Leaf sample	Subornochor, Noakhali Dist.	ANSu
02	Leaf sample	Sudharampur, Noakhali Dist	ANSd
03	Leaf sample	Begumgong, Noakhali Dist.	ANBg
04	Leaf sample	Dumki, Patuakhali Dist.	APDm
05	Leaf sample	Bauphal, Patuakhali Dist	APBl
06	Leaf sample	Patuakhali sador, Patuakhali Dist	APSr
07	Leaf sample	Bakergong, Barisal Dist	ABBg
08	Leaf sample	Kashipur , Barisal Dist.	ABKr
09	Leaf sample	Barisal sador, Barisal Dist.	ABSr
10	Leaf sample	SAU campus , Dhaka	ASAU

Note: G= *Ganoderma*,P= *Pestalotia* , C= *Curvularia* ,A= *Alternaria* .

3.7 Maintenance of pure cultures of different isolates of identified Organisms

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

3.8 Study on variability of *Ganoderma* isolates of coconut

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

3.8.1 Cultural morphological variability of *Ganoderma* isolates

3.8.1.1 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 in petriplate and incubated at 28± 2 ^{o}C. Each treatment was replicated thrice. Ten *Ganoderma* isolates (GNSu, GNSd, GNBg, GPDm, GPBl, GPSr, GBBr, GBKr, GBSr, GSAU) collected from different geographic locations were cultured on PDA. 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 6). Observations were taken when the pathogen covered complete petriplate. The colony diameter was recorded by averaging the radial growth of the colony. The data on radial growth were analyzed statistically.

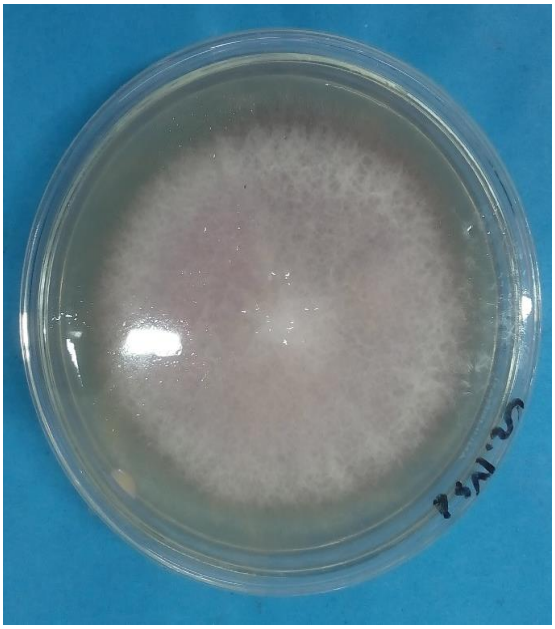
Table 6. Cultural morphological characters and their corresponding codes used to describe *Ganoderma* isolates

SL 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

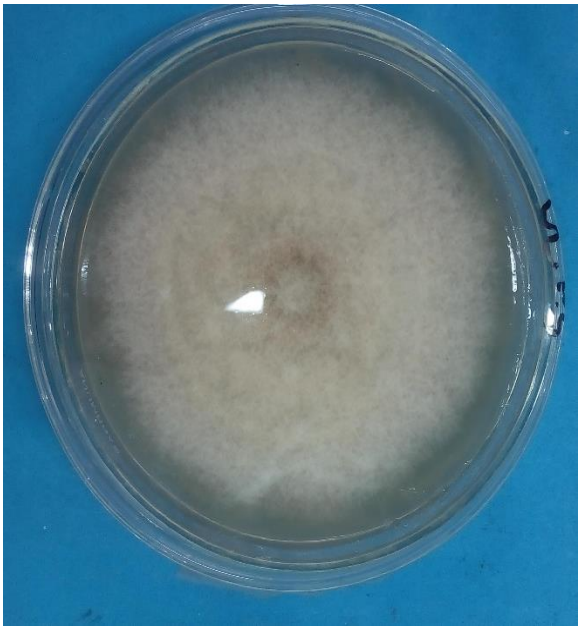
3.8.1.2 Spore count

Spore of *Ganoderma* isolates was counted at 5-fold dilution by using hemacytometer.100µL diluted solution was taken in haemacytometer and observed under microscope. Out of 16 small square conidia of 5 small square was counted randomly and average value was estimated as the no. of conidia per micro liter.

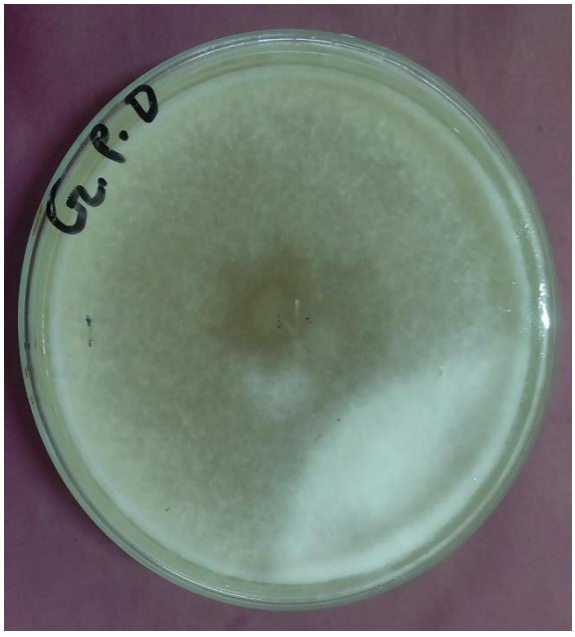
GNBg



GNSu

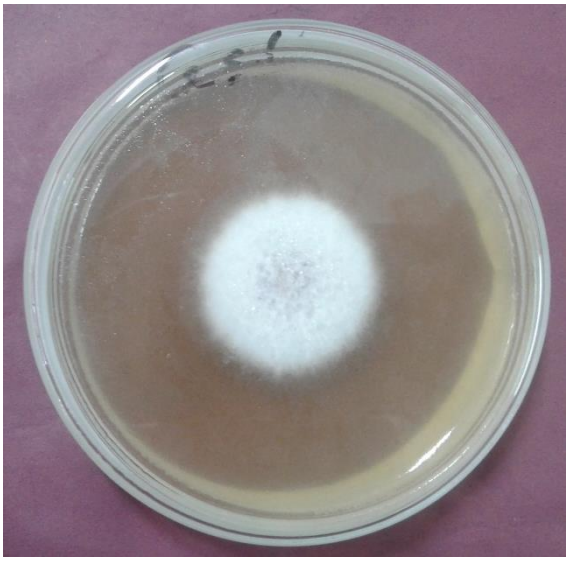


GNSd

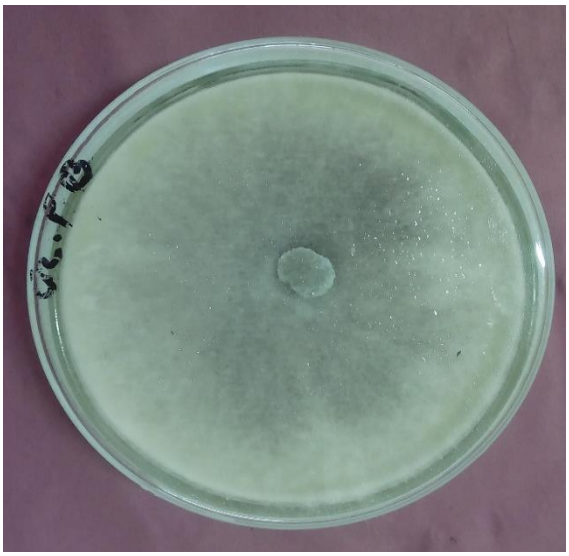


GPDm

Plate 7. Isolates of *Ganoderma sp.*

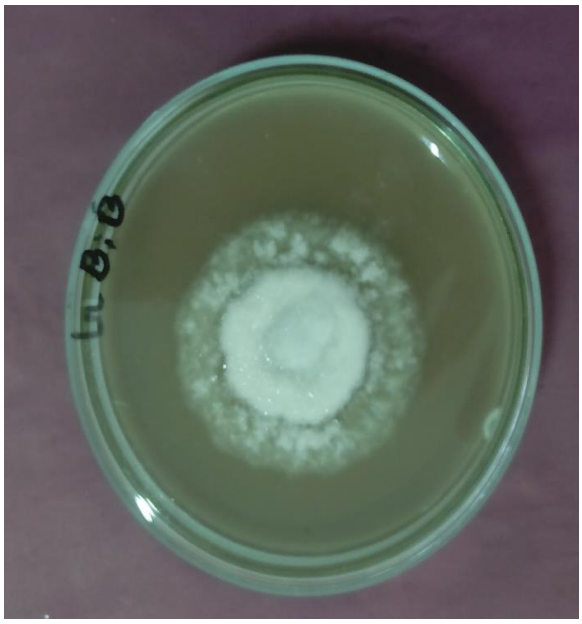


GPSr



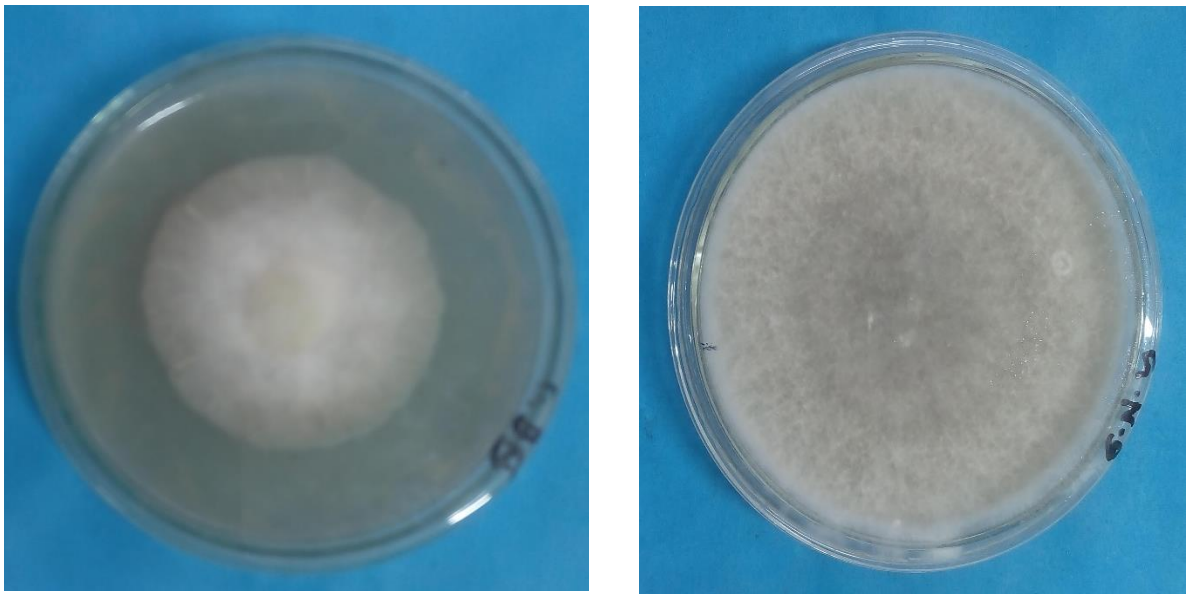
GPBl

GBKr



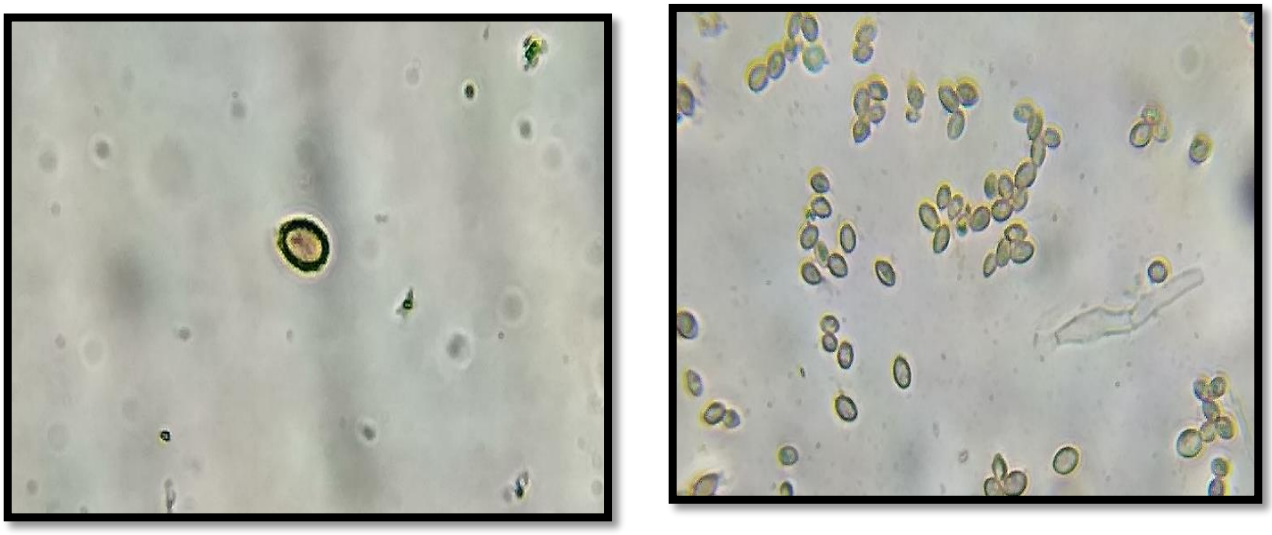
GBBg

Plate 8. Isolates of *Ganoderma sp.*

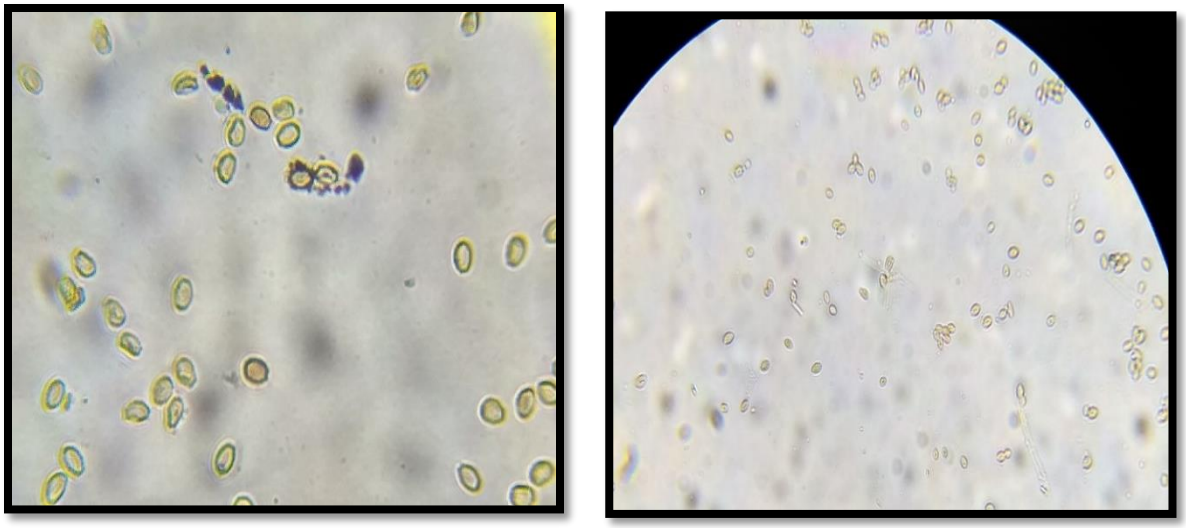


GBPr **GSAU**

Plate 9. Isolates of *Ganoderma sp.*



Ganoderma applanatum



Ganoderma lucidum

Plate 10. Microscopic view of conidia of *Ganoderma spp.*

3.9 Study on variability of *Pestalotia* isolates of coconut

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

3.9.1 Cultural morphological variability of *Pestalotia* isolates

3.9.1.1 Growth on Potato Sucrose Agar

Composition of PSA media

Potato Sucrose Agar (PSA)

Potato (unpeeled) 200 g

Sucrose 20 g

Agar- 20 g

Distilled water 1000 ml (volume to make up)

Two hundred gram of unpeeled potatoes were cut into small pieces and boiled in distilled water and then extract was collected by filtering. Sucrose 20.0 g and agar 20.0 g each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized in autoclave. 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 in petriplate and incubated at 28± 2 ^{o}C. Each treatment was replicated thrice.

Ten *Peatalotia* isolates (PNSu, PNSd, PNBg, PPDm, PPBl, PPSr, PBBr, PBKr, PBSr, PSAU) collected from different geographic locations were cultured on PSA. 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. The experiment was conducted in three replications. Mycelia from seven days old active culture was transferred onto the centre of a standard 9 cm PSA plate and incubated for 9 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 7, Plate 11-13). Observations were taken when the pathogen covered

complete petriplate. The colony diameter was recorded by averaging the radial growth of the colony. The data on radial growth were analyzed statistically.

Table 7. Cultural morphological characters and their corresponding codes used to describe *Pestalotia* isolates

SL NO.	Characters	Description	Code
01.	Days for full plate	< 5	1
		5-7	2
		7-10	3
		>10	4
02.	Colony color	Ash	5
		White	6
		Pale white	7
03.	Mycelia texture	Smooth	8
		Leathery	9
		Fluffy	10
04.	Reverse pigmentation	No pigmentation	11
05	Mycelia density	Thin	12
		Dense	13
		Dense at center & thin at corner	14
		Dense at center	15
06.	Margin	Filamentous	16
		Even	17
		Undulate	18

3.9.1.2 Spore count

Spore of *Pestalotia* isolates was counted at 3-fold dilution by using hemacytometer.100µL diluted solution was taken in haemacytometer and observed under microscope. Out of 16 small square conidia of 5 small square was counted randomly and average value was measured as the no. of conidia per micro liter.

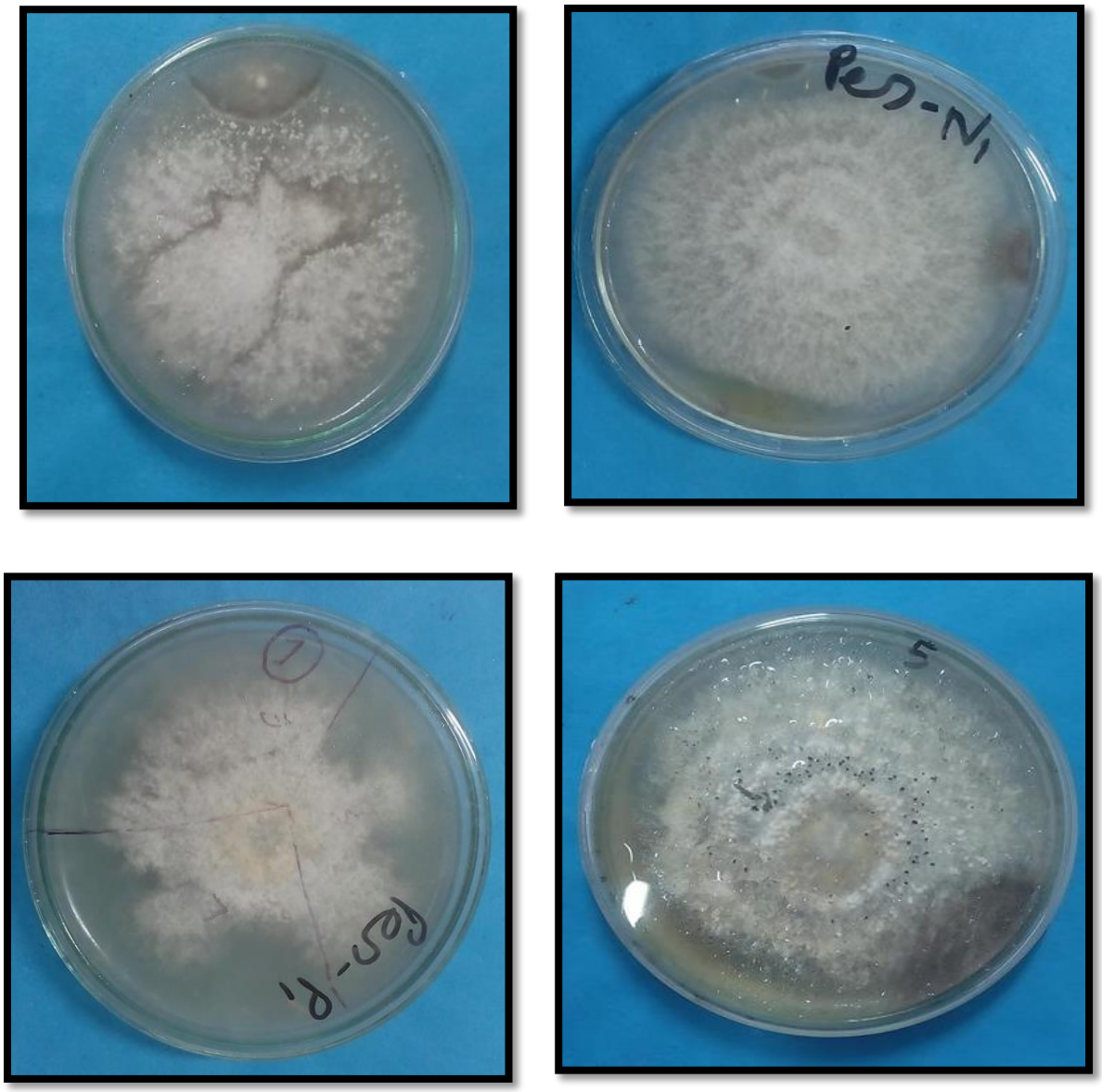


Plate 11. Isolates of *Pestalotia sp.*

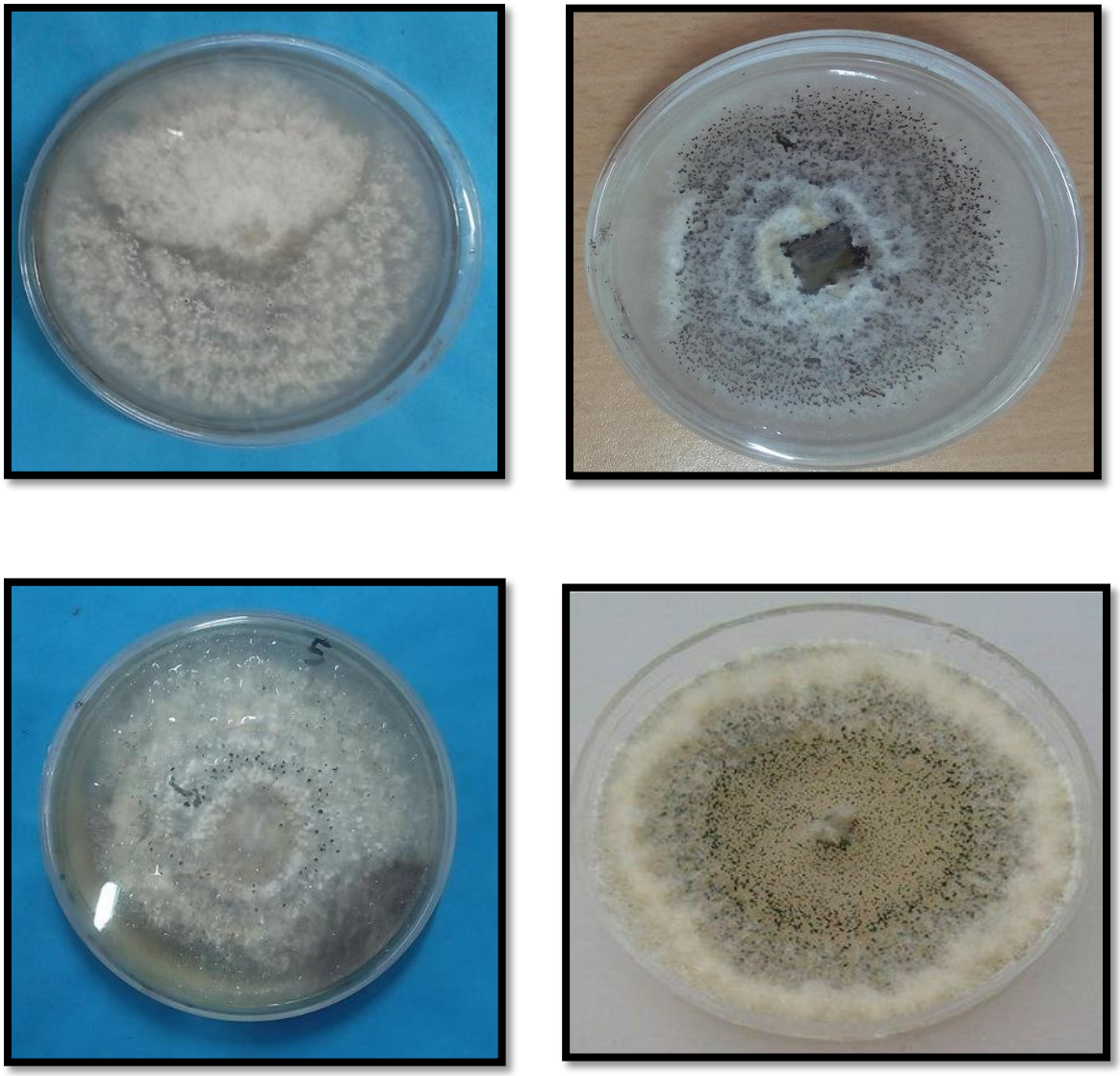


Plate 12. Isolates of *Pestalotia sp*.

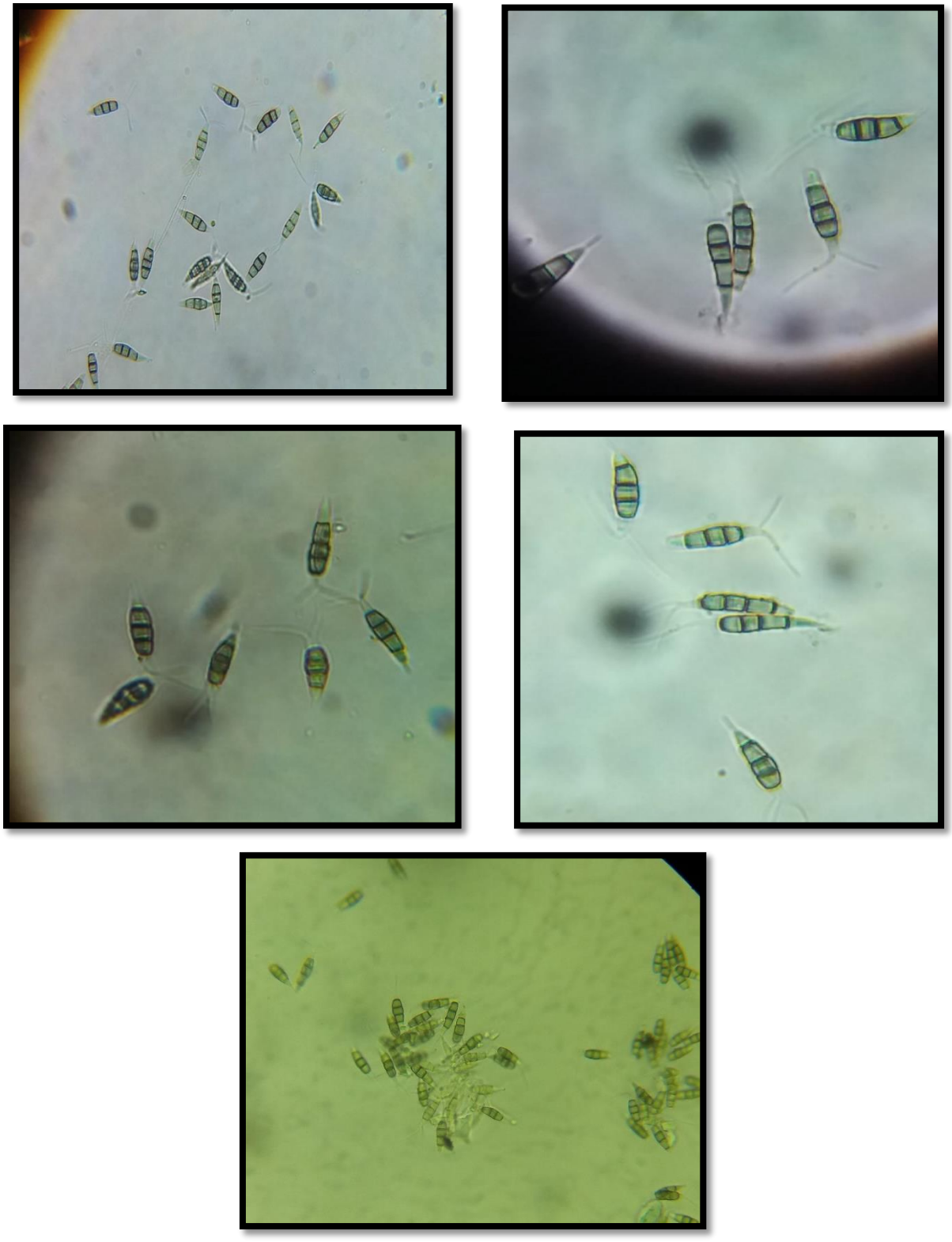


Plate 13. Microscopic view of conidia of *Pestalotia sp.*

3.10 Study on variability of *Curvularia* isolates of coconut

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

3.10.1 Cultural morphological variability of *Curvularia sp* isolates

3.10.1.1 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 in petriplate and incubated at 28± 2 ^{o}C. Each treatment was replicated thrice. Ten *Curvularia* isolates (CNSu, CNSd, CNBg, CPDm, CPBl, CPSr, CBBr, CBKr, CBSr, CSAU) collected from different geographic locations were cultured on PDA. 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.

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 9 days at an ambient temperature. (Table 8, plate 14). 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 color and mycelial density were recorded after 9 days of incubation. Observations were taken when the pathogen covered complete petriplate. The colony diameter was recorded by averaging the radial growth of the colony. The data on radial growth were analyzed statistically.

Table 8. Cultural morphological characters and their corresponding codes used to describe *Curvularia* isolates

SL NO.	Characters	Description	Code
01.	Days for full plate	< 5	1
		5-7	2
		7-10	3
		>10	4
02.	Colony colour	Black	5
		Ash	6
03.	Mycelia texture	Smooth	7
		Leathery	8
		Fluffy	9
04.	Reverse pigmentation	No pigmentation	10
05	Mycelia density	Thin	11
		Dense	12
		Thin at center & dense at corner	13
		Dense at center	14
06.	Margin	Filamentous	15
		Even	16
		Undulate	17

3.10.1.2 Spore count

Spore of *Curvularia* isolates was counted at 5-fold dilution by using haemacytometer.100µL diluted solution was taken in haemacytometer and observed under microscope. Out of 16 small square conidia of 5 small square was counted randomly and average value was estimateds as the no. of conidia per micro liter.

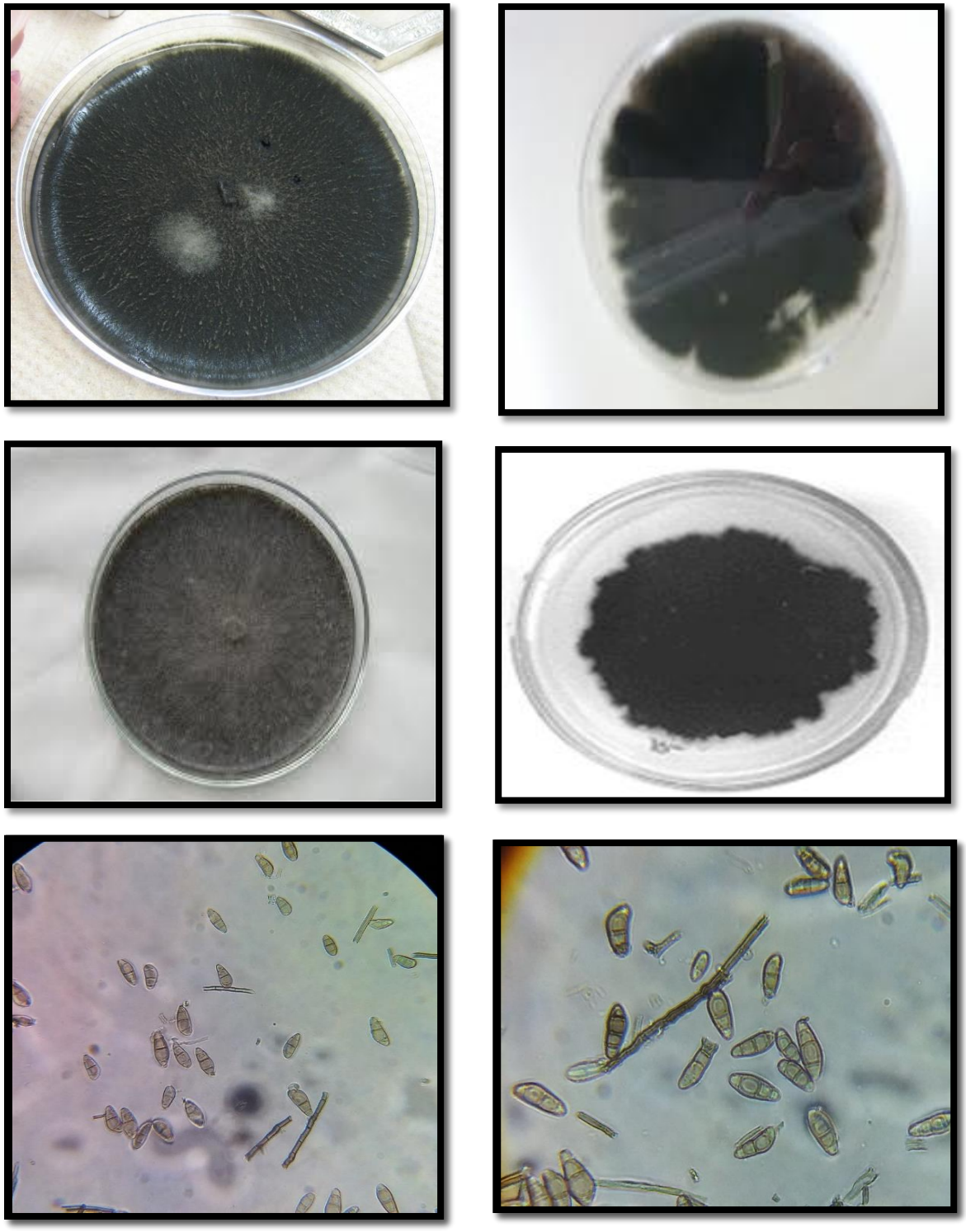


Plate 14. Isolates of *Curvularia sp.* and microscopic view of

3.11 Study on variability of *Alternaria* isolates of coconut

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

3.11.1 Cultural morphological variability of *Alternaria* isolates

3.11.1.1 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 in petriplate and incubated at 28± 2 ^{o}C. Each treatment was replicated thrice. Ten *Alternaria* isolates (ANSu, ANSd, ANBg, APDm, APBl, APSr, ABBr, ABKr, ABSr, ASAU) collected from different geographic locations were cultured on PDA. The morphological characters like colony diameter/growth biomass production, colony color, colony margin, mycelial density, appearance of zones, reverse pigmentation and conidial structure etc. were studied.

The experiment was conducted in three replications. Mycelia from seven days old active culture was transferred onto the center of a standard 9 cm PDA plate and incubated for 9 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 color and mycelial density were recorded after 9 days of incubation (Table 9, Plate 15). Observations were taken when the pathogen covered complete petriplate. The colony diameter was recorded by averaging the radial growth of the colony. The data on radial growth were analyzed statistically.

Table 9. Cultural morphological characters and their corresponding codes Used to describe *Alternaria* isolates

SL NO.	Characters	Description	Code
01.	Days for full plate	< 5	1
		5-7	2
		7-10	3
		>10	4
02.	Colony colour	Light ash	5
		Dark Ash	6
03.	Mycelia texture	Smooth	7
		Leathery	8
		Fluffy	9
04.	Reverse pigmentation	No pigmentation	10
05.	Mycelia density	Thin	11
		Dense	12
		Thin at center & dense at corner	13
		Dense at center	14
06.	Margin	Filamentous	15
		Even	16
		Undulate	17

3.11.1.2 Spore count

Spore of *Alternaria* isolates was counted at 4-fold dilution by using hemacytometer.100µL diluted solution was taken in haemacytometer and observed under microscope. Out of 16 small square conidia of 5 small square was counted randomly and average value was measured as the no. of conidia per micro liter.

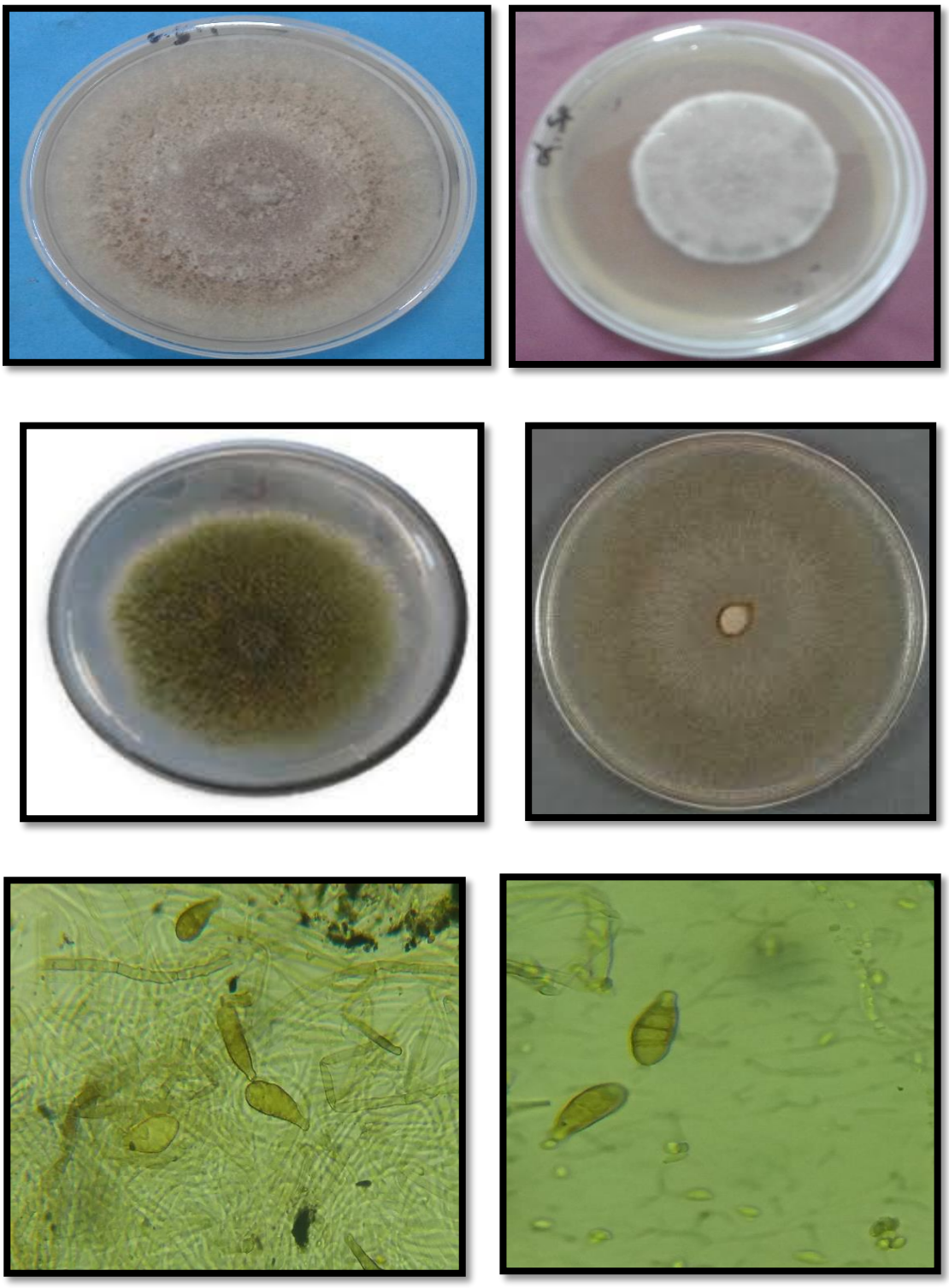


Plate 15. Isolates of *Alternaria sp.* and microscopic view of conidia

3.12 Biocontrol-based disease management of basal stem rot of coconut

3.12.1 Pre-treatment observations

Before giving treatment the disease affected plants were identified by isolating the pathogen from the root sample. Only disease affected plants were taken for the treatment.

3.12.2 Treatment Details

The biocontrol based disease management trial was conducted in Sher-e-Bangla Agricultural University campus. About 32 infected plants were selected and treatment was given during October, 2018 and data was collected in March, 2019. Treatment details is given Table 10.

Table.10 List of treatments and combination

Treatments	Particulars/plant
T_1	5kg mustard oil cake + 1kg neemseeds + 250ml *Trichoderma viride*
T_2	5kg mustard oil cake +1kg neemsseeds + 500ml garlic extract + 1L water
T_3	Cattle urine 1L/1L water
T_4	Absolute control

3.12.2.1 Preparation of plant for treatment

Before application of treatment 32 infected plants of SAU campus was prepared. The Soil around the trunk of each plant was loosen with spade. A furrow about 60 cm far from the trunk and 10 cm deep furrow was made around the trunk (Plate 15.).

3.12.2.2 Preparation of treatment particulars

For treatment *Trichoderma viride* was cultured (Figure 2.) in PDA media in pour plate method by using *Trichoderma viride* solution. *Trichoderma viride* solution was prepared from the pure culture and diluted with distilled water. The diluted solution was applied with mustard oilcake and grinded neemseeds.

Neemseeds were collected, grinded and soaked overnight into water with mustard oilcake. Garlic extract was made by fresh garlic by blinding. *in-vitro* evaluation of garlic extract

combination with *Trichoderma viridae* was done before application (Appendix- IV). The garlic extract was applied with mustard oilcake and grinded neemseeds (Plate 16).

Fresh cattle urine was collected from nearby farm. Cattle urine was applied separately diluted with water. Three types of treatment combinations were made before application.

3.12.2.3 Application of treatment particulars

The treatment was given on the loosen part and on the furrow around the trunk (Plate 17).

Plate 16. Preparation of coconut root zone for application of Treatments.

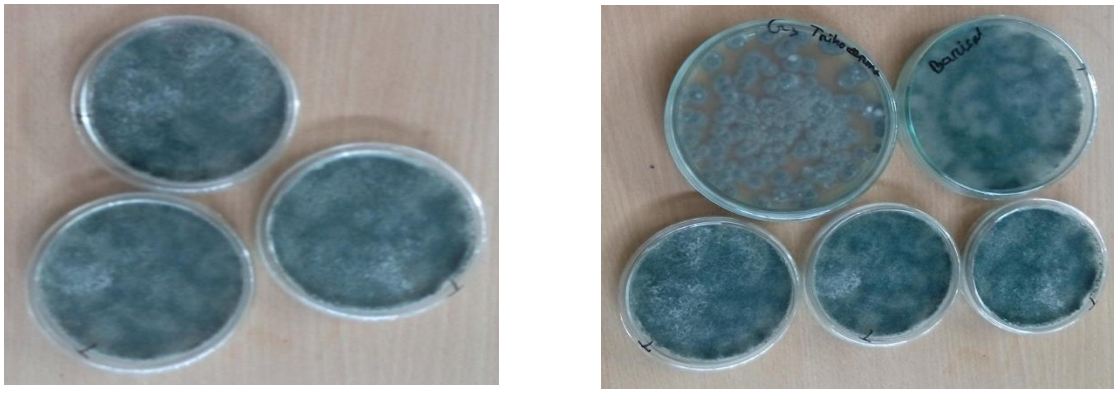


Figure 2. *Trichoderma viridae* cultures on PDA

Plate 17. Preparation of Treatment particulars before application

Plate 18. Application of Treatment particulars on coconut plant

3.10 Analysis of data

The data were statistically analyzed using computer package program. 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.

RESUTLS

Experimental results pertaining to isolation of *Ganoderma, Pestalotia, Curvularia, Alternaria* from leaf and root samples that were collected from different selected areas; the cultural and morphological variability of those isolates; bio-agents and botanicals against *Ganoderma*; the management schedule for basal stem rot of coconut in Shere-e- Bangla Agricultural University have been presented in this chapter.

4.1 Survey for the incidence of Basal Stem Rot and foliar diseases of coconut in coastal Zone and SAU Campus

A Survey was carried out through random sampling technique in coastal zone; Noakhali, Patuakhali, Barisal District and in Sher-e- Bangla Agricultural University campus during Jan-Jun, 2018. Incidence and severity of basal stem rot and foliar diseases of coconut were measured.

4.1.1 Disease incidence and severity of basal stem rot and foliar diseases in coconut

Among surveyed locations, Maximum disease incidence (70%) of basal stem rot (BSR) was recorded in Begumgong of Noakhali district and minimum disease incidence (20%) was recorded in Bakergong of Barisal district. The locations wise disease incidence of BSR was ranged from 49.17 - 65%. Maximum disease severity (33.75%) of BSR was recorded in Begumgong of Noakhali district and minimum disease severity (22%) was recorded in Bauphal of Patuakhali district. The locations wise disease severity of BSR was ranged from 24.58 - 33.88%.

Among the surveyed areas, maximum disease incidence (88.33%) of foliar diseases was recorded in Begumgong of Noakhali district and minimum disease incidence (65%) in Dumki of Patuakhali district. The location wise disease incidence of foliar diseases was ranged from 65 - 88.33% in surveyed areas. The foliar disease severity was also measured in surveyed locations. The maximum disease severity was found in Begumgong (45.92%) of Noakhali district and minimum disease severity (21.48%) was recorded in Dumki of Patuakhali district. The location wise disease incidence of foliar diseases was ranged from 21.48 - 45.92% in surveyed areas. The results are presented in (Table 11. and Figure 3-4).

Table 11. Disease incidence of basal stem rot and foliar disease of coconut

SL NO.	District	Upazila	No of Plants observed	BSR		Foliar Diseases	
				DI(%)	DSI (%)	DI(%)	DSI(%)
01	Noakhali	Subornachor	60	65	28.75	88.13	42.4
		Shudharampur	60	47.5	33.13	78.33	29.07
		Begumgong	40	70	33.75	88.33	45.92
02	Barisal	Bakergong	60	20	26.88	73.33	26.48
		Barisal sadar	30	67.5	26.87	87.5	42.55
		Kashipur	60	60	26.25	75	31.66
03	Patuakhali	Dumki	60	61.67	22.67	65	21.48
		Bauphal	30	63.33	22	83.33	24.07
		Patuakhali sadar	60	68.33	29.08	85	26.85
04	SAU campus		40	65	30	85	28.33
			Total=480				

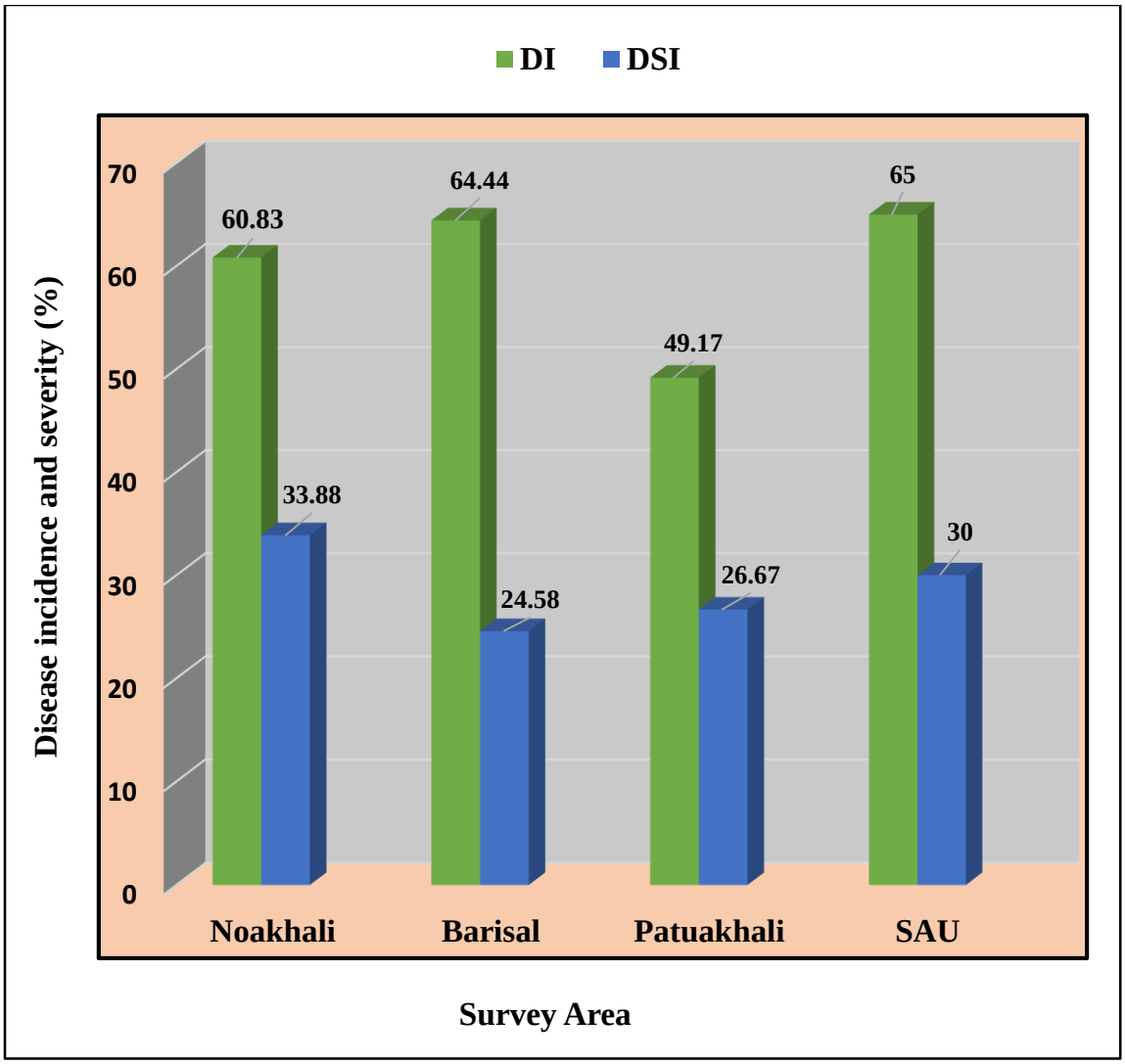


Figure 3. Disease incidence and severity of basal stem rot of coconut in surveyed areas

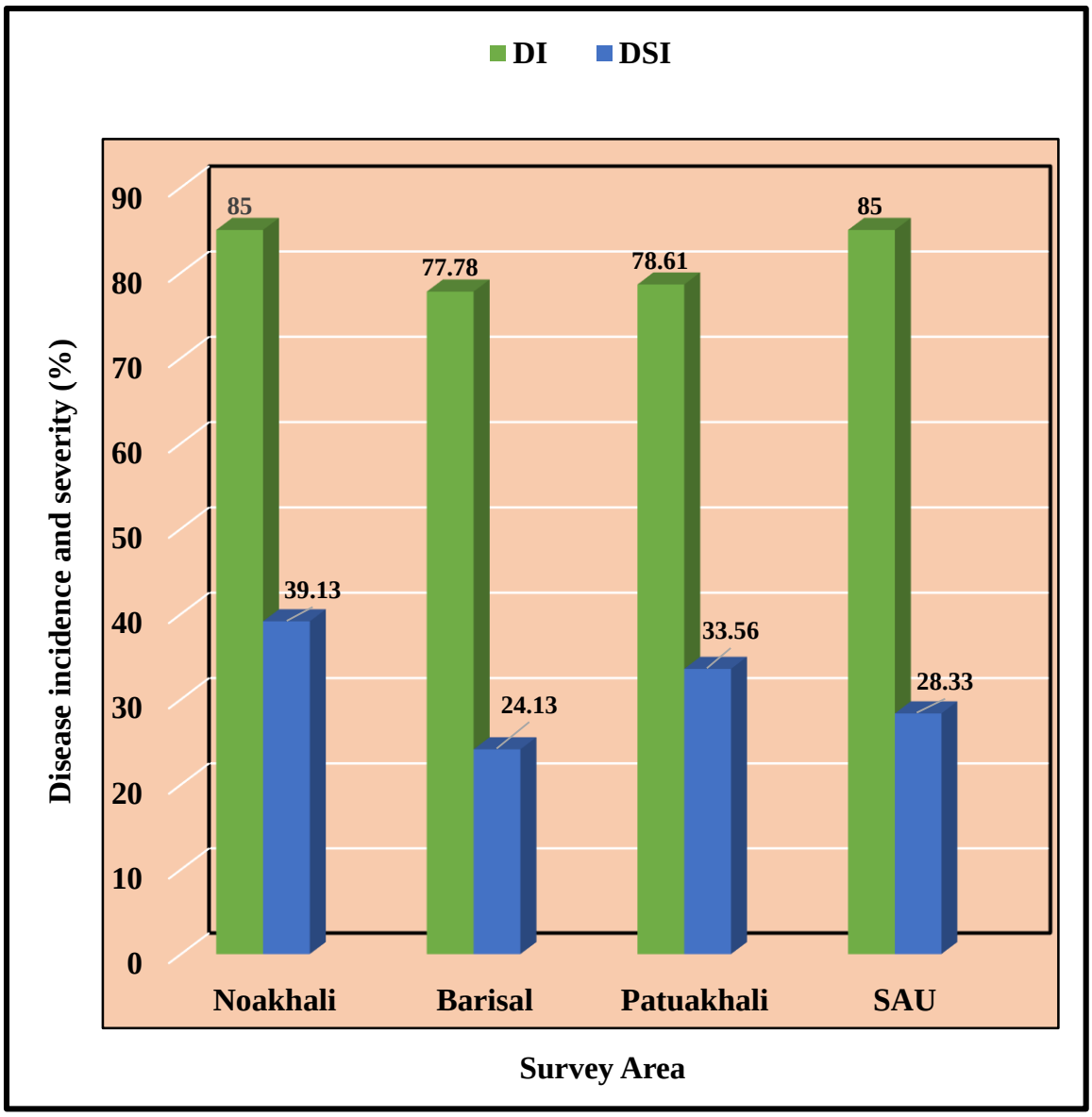


Figure 4. Disease incidence and severity of foliar diseases of coconut in surveyed area

4.1.2 Disease incidence of basal stem rot and foliar diseases according to soil type, soil pH, plant age and garden type

The Basal Stem Rot (BSR) incidence with respect major soil type, soil pH and plant age were also recorded. The disease incidence with respect to soil types, maximum disease incidence (27.5%) was recorded in loamy soils while minimum incidence (11.25%) was found in clay soils. The disease incidence with respect to soil pH, maximum disease incidence (26.75%) was noticed in soil pH ranged 6-7 and minimum (20.5%) was in 7-8 ranged. The disease incidence with respect to plant age, maximum disease incidence (24%) was observed in age group of 36-40 years old plants and minimum disease incidence (13%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (45.75%) was found in yard garden while minimum disease incidence (12.75%) was recorded in road side plants.

The foliar diseases incidence with respect major soil type, soil pH and plant age were also recorded. According to soil type, maximum leaf disease incidence (19.2%) was recorded in loamy soil and minimum leaf disease incidence (9%) was recorded in sandy soil. The disease incidence with respect to soil pH, maximum disease incidence was recorded (22.4%) in soil pH 5-6 and minimum (14.8%) was in soil pH 7-8. The disease incidence with respect to plant age, maximum disease incidence (18%) was observed in age group of 36-40 years old plants and minimum disease incidence (11%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (33.4%) was found in yard garden while minimum disease incidence (9.4%) was recorded in road side plants. The result was presented in (Table 12 and Figure 5-8).

Table 12. Disease incidence of basal stem rot and foliar disease of Coconut respect to soil types, soil pH, age, and garden types

SI	Particulars	Disease Incidence (%)	
01	**Soil type**	**BSR**	**Foliar disease**
	Loam	27.5	19.2
	sandy	23.25	9
	clay	11.25	16.8
	Sandy loam	17.75	13
02	**Soil pH**	**BSR**	**Foliar disease**
	5-6	26.25	20
	6-7	26.75	22.4
	7-8	20.5	14.8
03	**Age**	**BSR**	**Foliar disease**
	20-25	13	11
	26-30	19.75	13
	31-35	23	17.2
	36-40	24	18
04	**Garden Type**	**BSR**	**Foliar disease**
	Commercial	21.25	14.8
	Yard Garden	45.75	33.4
	Road Side	12.75	9.4

The survey revealed that basal stem rot and foliar diseases of coconut have a relationship with soil type, soil pH, age and also garden type. For this reason, the disease occurance and distribution differ from region to region.

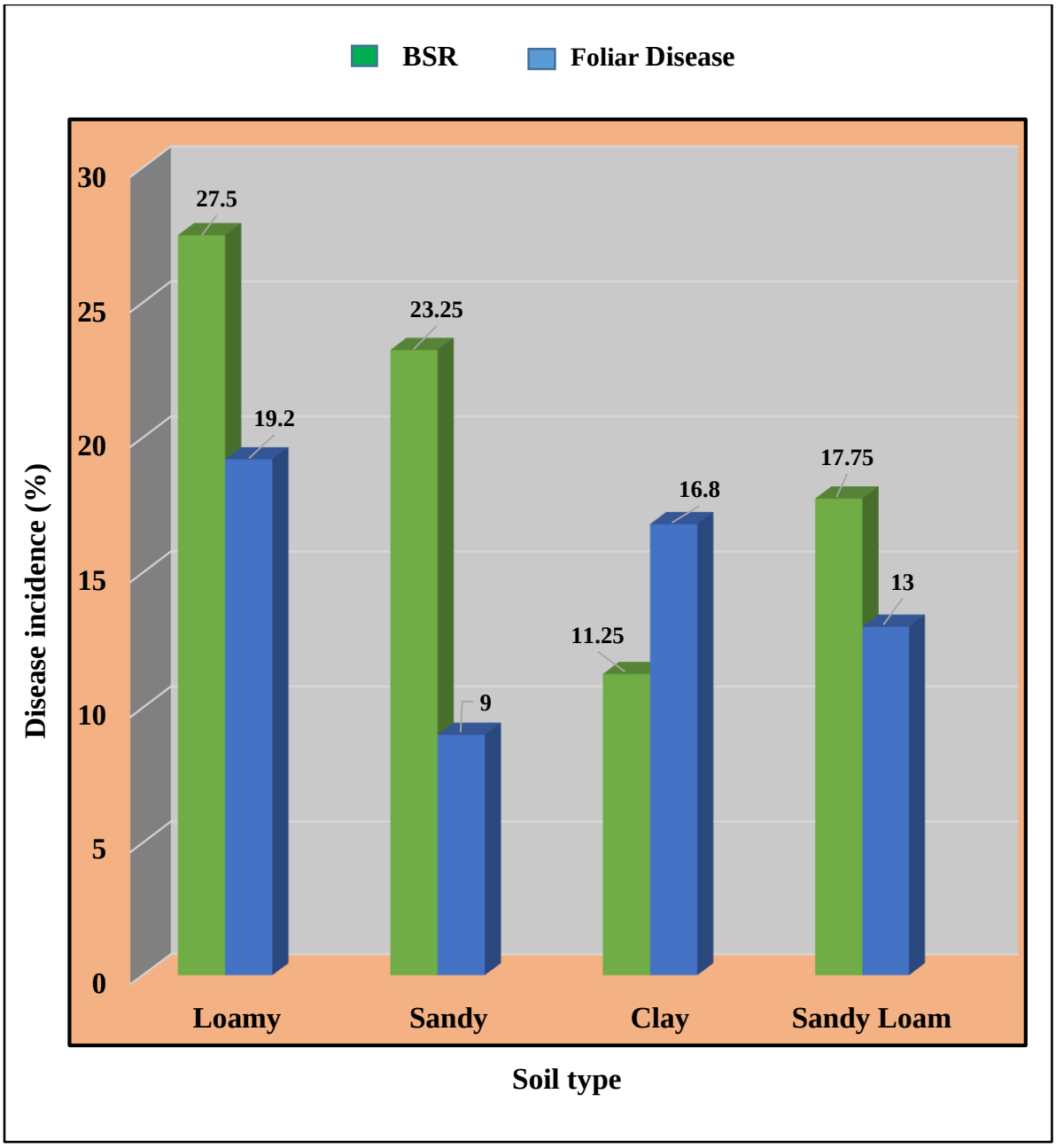


Figure 5. Disease incidence of basal stem rot and foliar diseases of coconut according to soil type

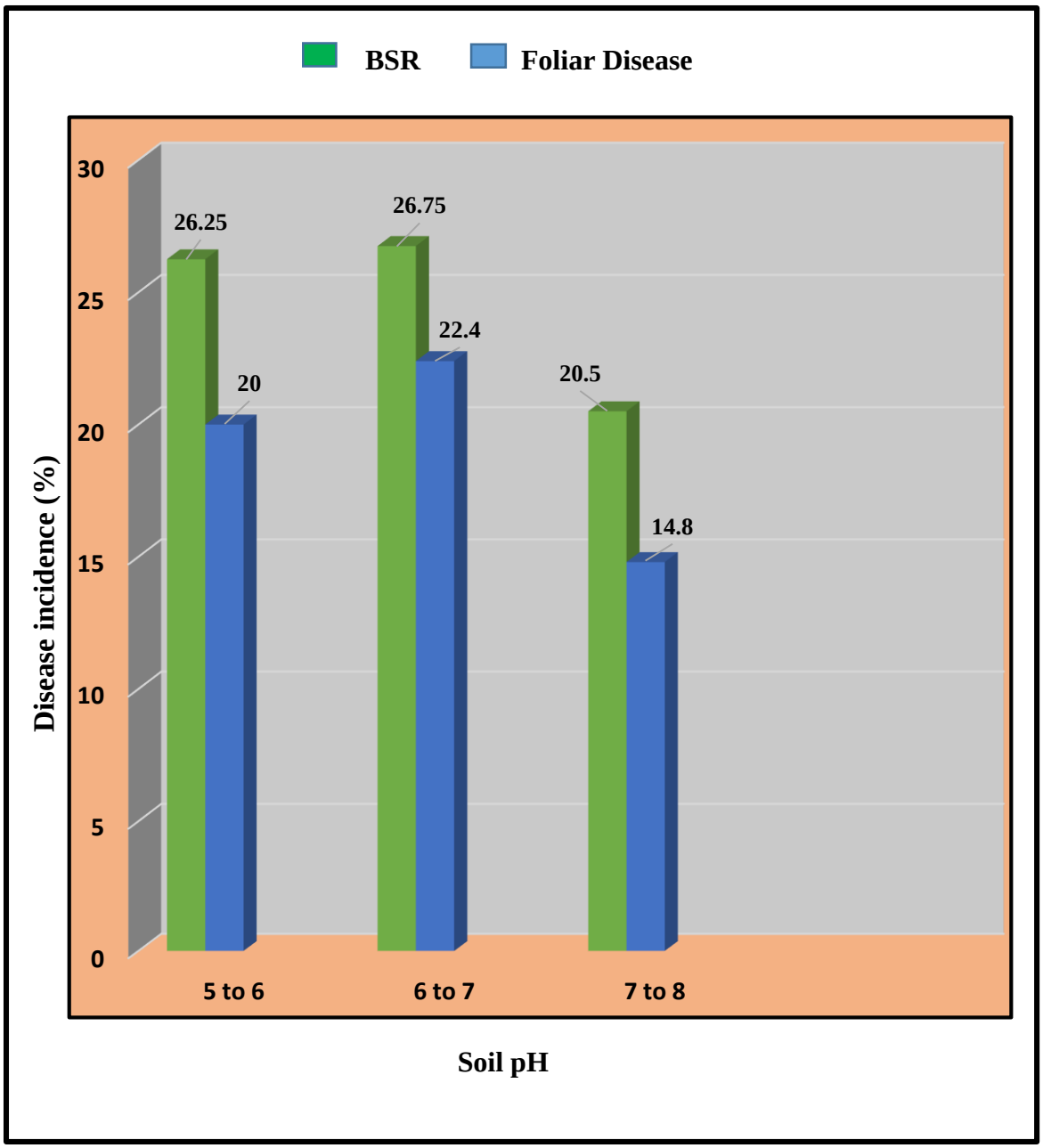


Figure 6. Disease incidence of basal stem rot and foliar diseases of coconut according to soil pH

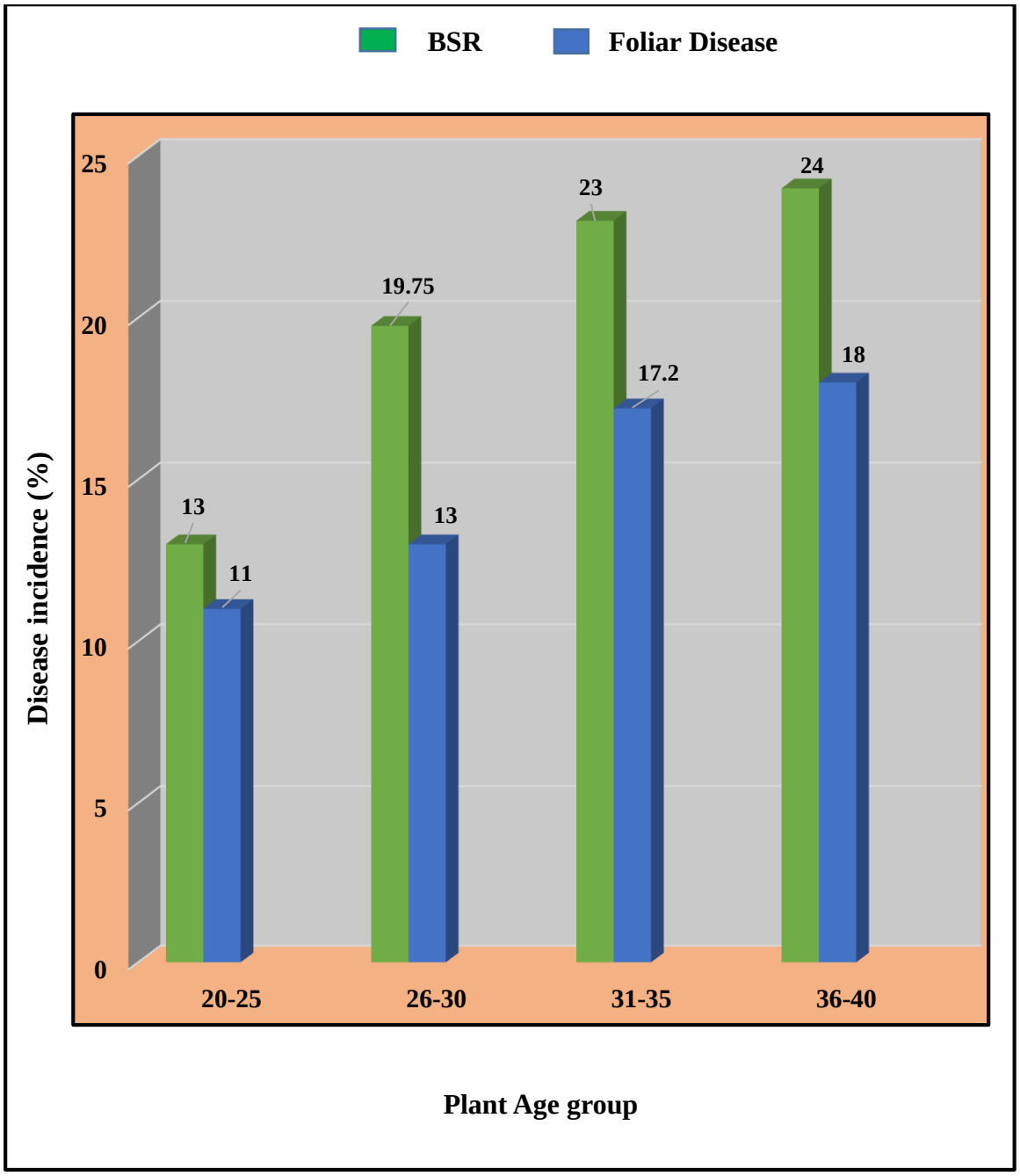


Figure 7. Disease incidence of basal stem rot and foliar diseases of coconut according to Plant age

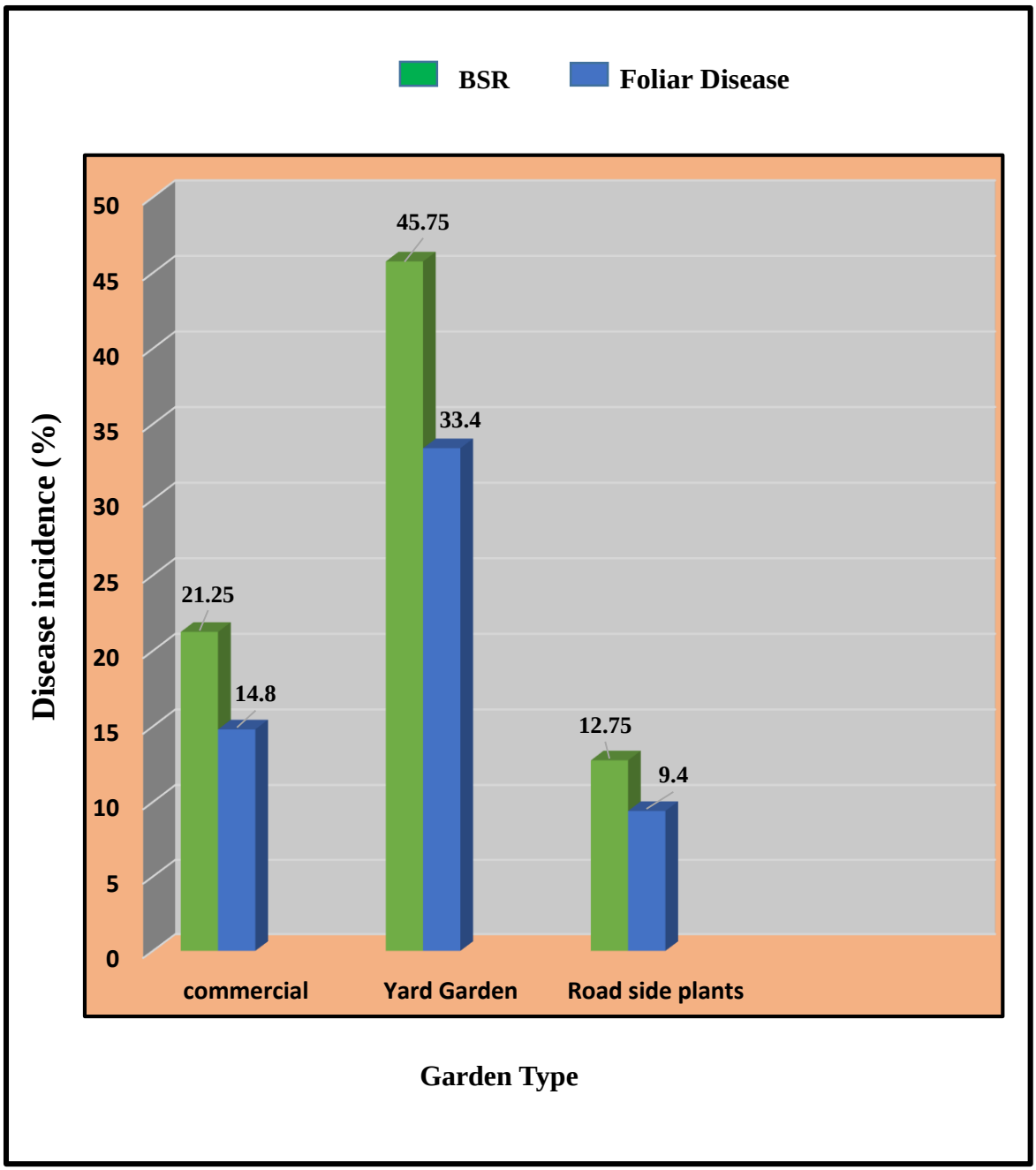


Figure 8. Disease incidence of basal stem rot and foliar diseases of coconut according to garden type

4.2 Isolation and designation of isolated causal organisms

Ganoderma sporophore and diseased root bits were found good source for aseptic isolation. About 39.17% isolates was obtained from root bits in coconut. Similarly, in case of three types of leaf disease pathogens were isolated from the leaf samples and percentage was higher (57.77%) than basal stem rot. In total 120 root samples were collected for *Ganoderma* isolation and pathogen association was found in 47 samples out of 120 samples. In case of leaf sample, pathogen association was found in 52 samples out of 90 samples (Figure 9).

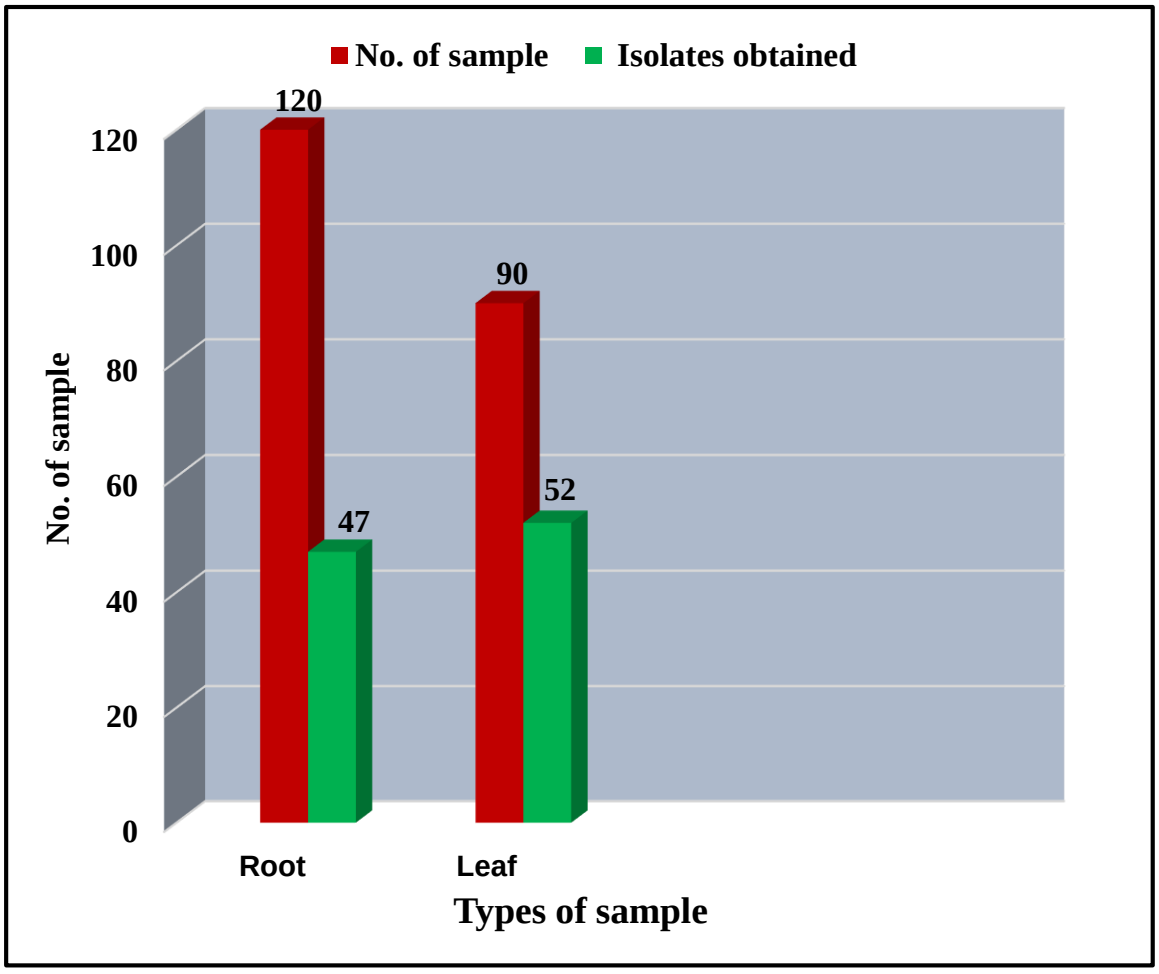


Figure 9. Number of samples and isolates obtained from the diseased samples

4.2.1 Isolation and study of cultural and morphological variability of *Ganoderma* isolates

Ganoderma was isolated from root samples and identified as *Ganoderma* sp. based on colony morphology and mycelia characteristics. To study the cultural and morphological variability in *Ganoderma* sp. different isolates were collected from different locations of coastal area of Bangladesh and SAU campus. They were subjected for the cultural and morphological characters viz., radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density on PDA and all these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates (Table 13, Figure 10-11). The results revealed that there were cultural and morphological variations among the isolates of *Ganoderma*. The colony diameter after 3, 6 and 9 days of inoculation was significantly varied, the radial mycelial growth ranged was 15.17 to 39.17 mm at 3 DAI,35.17-65.33mm at 6 DAI and 77.5-90mm at 9 DAI.The number of days taken to cover full plate was ranged from 9 to 15 days and most of the isolates covered entire plate in 9 days as noted were GNBg, GPDm, GPBl, GBBKr and GSAU. However, some of isolates taken <10 days to cover entire plate. Spore / µL of the isolates was ranged from 34-108. There were lot of variations were observed with respect to colony/ mycelial characteristics viz., reverse pigmentation, density of mycelium and colony margin. However, no too much variations were observed with respect to color and texture of the colony. After studying of microscopic view two types of Ganoderma species were identified. Viz., *Ganoderma lucidum* and *Ganoderma applanatum* (Appendix v).

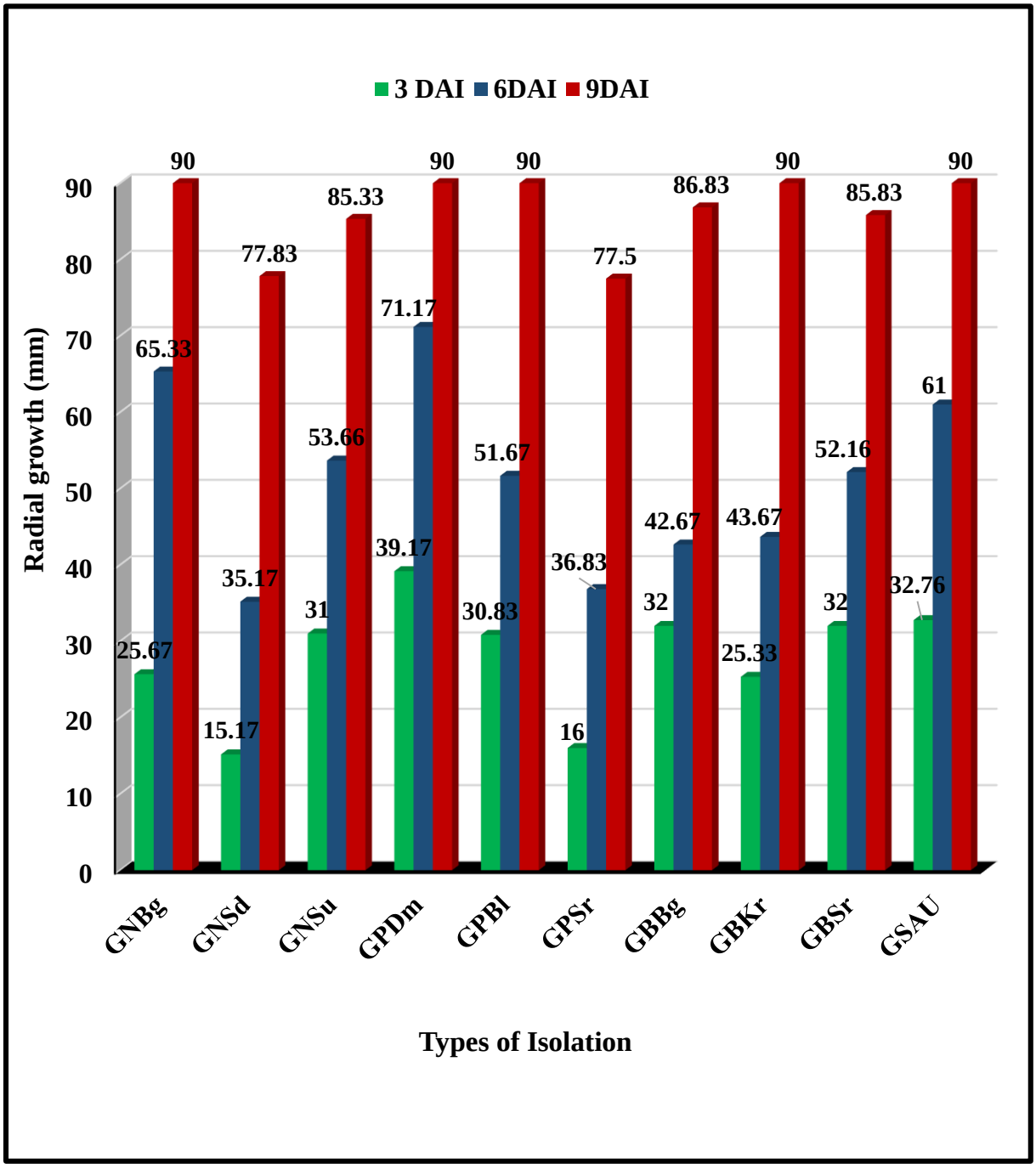


Figure 10. Radial growth of *Ganoderma* isolates on PDA media

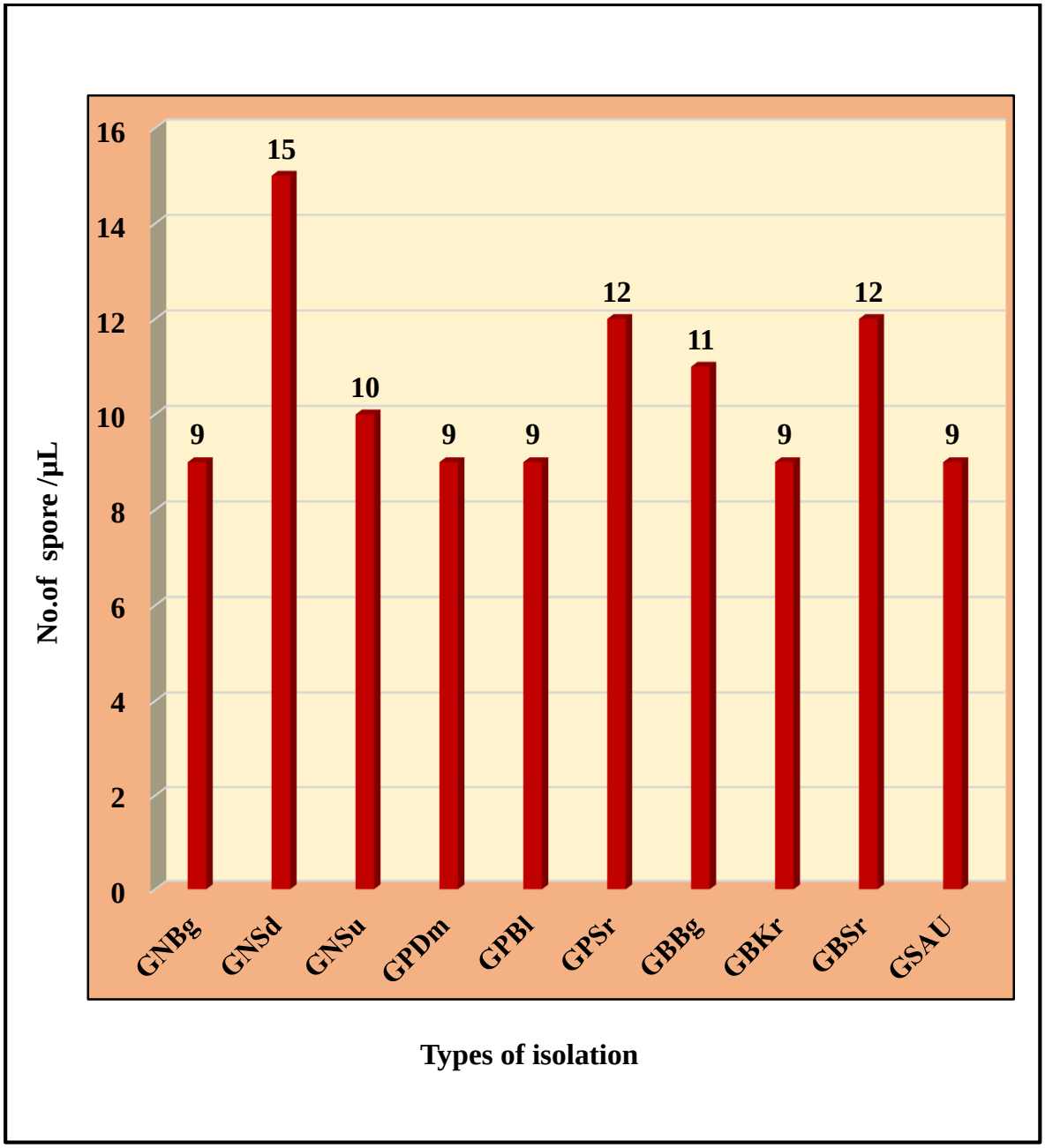


Figure 11. No. of Spore /µL of *Ganoderma* isolates

4.2.2 Isolation and study of cultural and morphological variability *Pestalotia* isolates

The *Pestalotia* was isolated from leaf samples and identified as *Peastalotia* sp. based on colony morphology and mycelia characteristics. To study the cultural and morphological variability in *Pestalotia* sp. different isolates were collected from different locations of coastal area of Bangladesh and SAU campus. They were subjected for the cultural and morphological characters viz., radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density on PSA and all these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates. (Table 14, Figure12-13). The results revealed that there were cultural and morphological variations among the isolates of *Pestalotia*. The colony diameter after 3, 6 and 9 days of inoculation was significantly varied, the radial mycelial growth ranged was 14.83 to 33.83 mm at 3 DAI, 42.17-64.83mm at 6 DAI and 82.67-90mm at 9 DAI. Spore / µL of the isolates was ranged from 5-11. The number of days taken to cover full plate was ranged from 9 to 15 days and most of the isolates covered entire plate in 9 days as noted were PNSd, PNSu, PPSr, PBKr, PBSr and PSAU. However, some of isolates taken <10 days to cover entire plate. There were lot of variations were observed with respect to colony/ mycelial characteristics viz., reverse pigmentation, density of mycelium and colony margin. However, no too much variations were observed with respect to color and texture of the colony (Appendix-v).

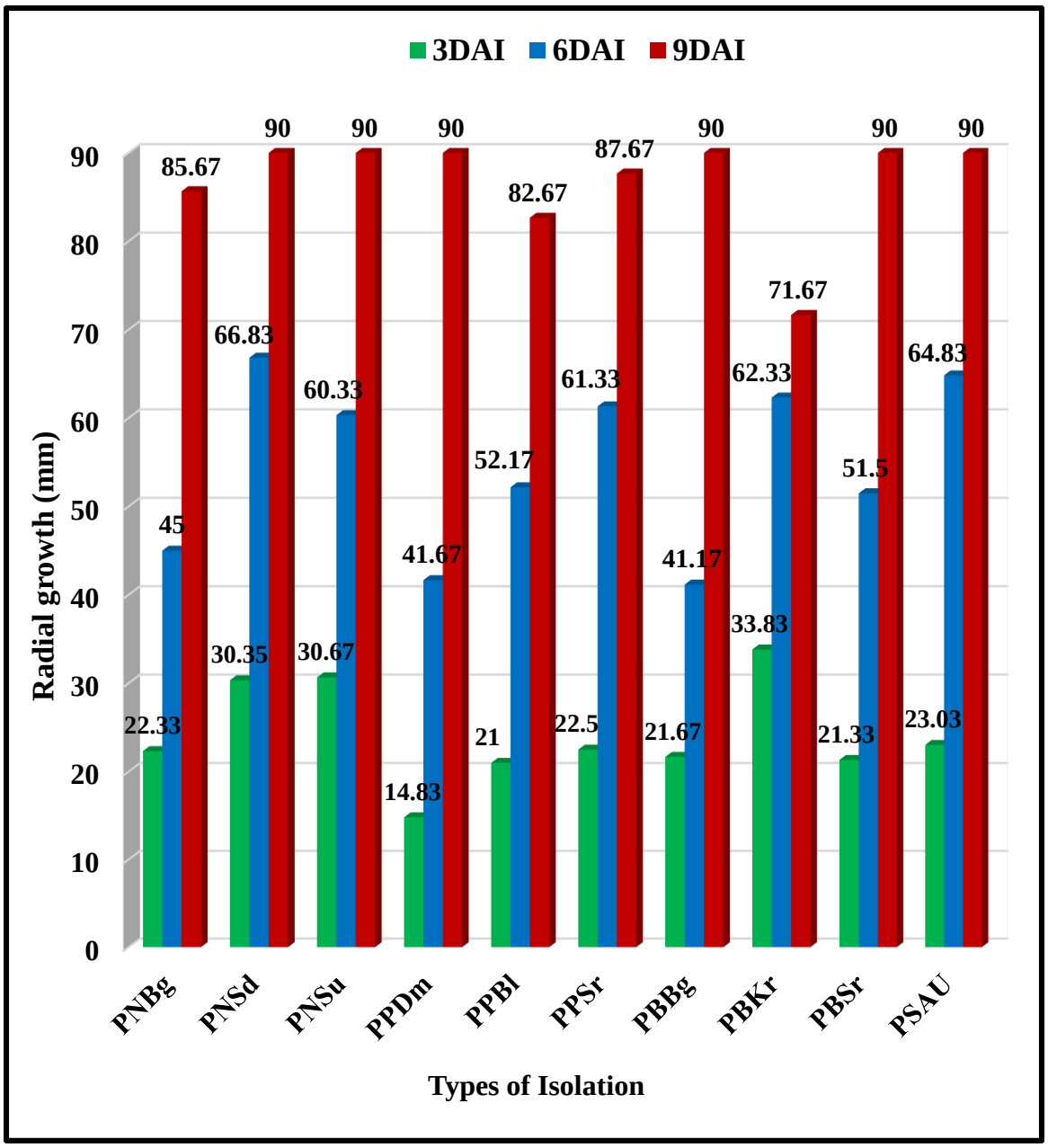


Figure 12. Radial growth of *Pestalotia* isolates on PSA media

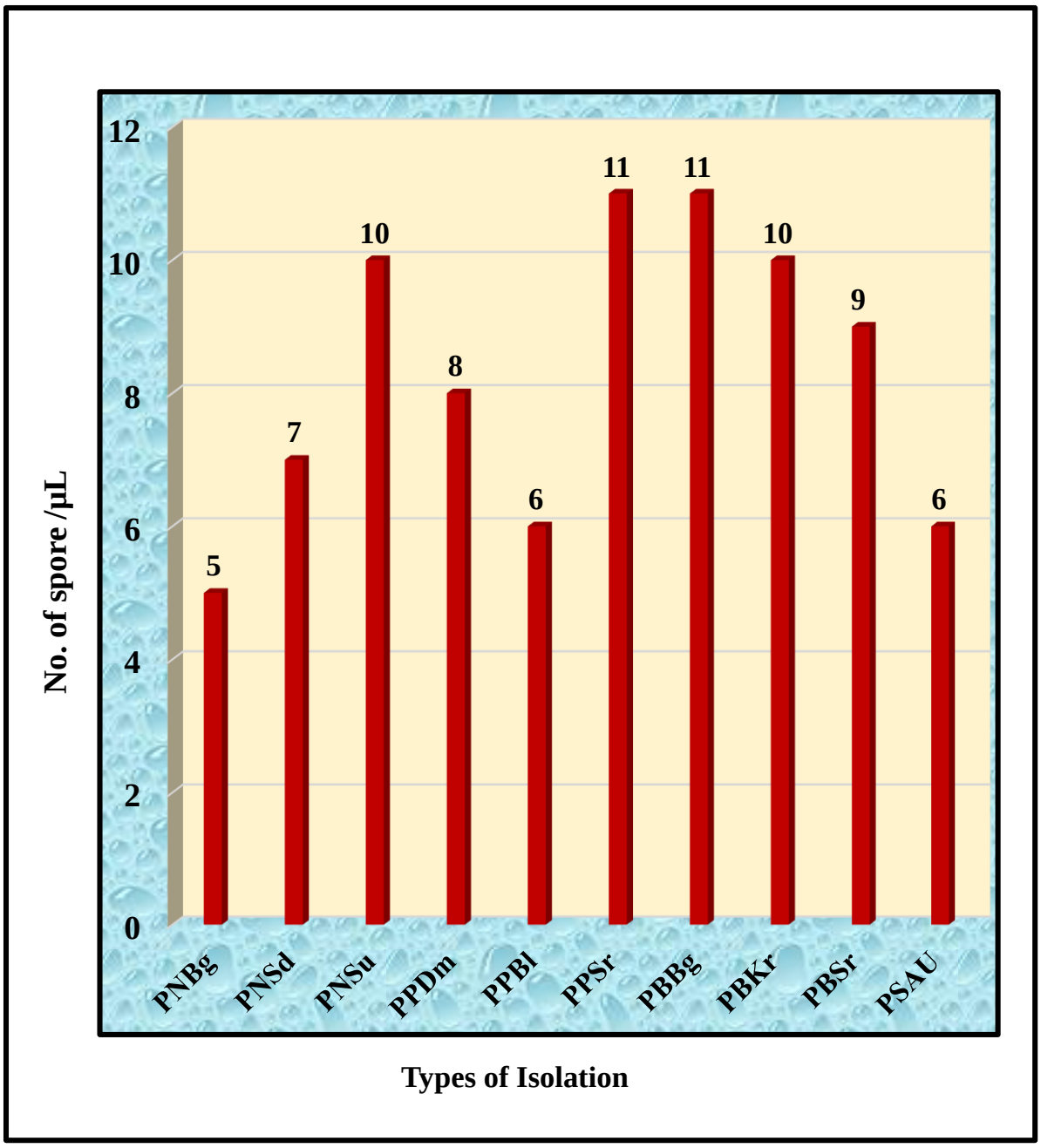


Figure 13. No. of spore /µL of *Pestalotia* isolates

4.2.3 Isolation and study of cultural and morphological variability *Curvularia* isolates

The *Curvularia* was isolated from leaf samples and identified as *Curvularia* sp. based on colony morphology and mycelia characteristics. To study the cultural and morphological variability in *Curvularia* sp. different isolates were collected from different locations of coastal area of Bangladesh and SAU campus. They were subjected for the cultural and morphological characters viz., radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density on PDA and all these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates (Table 15, Figure 14-15). The results revealed that there were cultural and morphological variations among the isolates of *Curvularia*. The colony diameter after 3, 6 and 9 days of inoculation was significantly varied, the radial mycelial growth ranged was 15.8 to 31.8 mm at 3 DAI,41.-63mm at 6 DAI and 72.73-90mm at 9 DAI. Spore / µL of the isolates was ranged from 8-15. The number of days taken to cover full plate was ranged from 9 to 15 days and most of the isolates covered entire plate in 9 days as noted were CNSd, CPDm, CBBg and CSAU. However, some of isolates taken <10 days to cover entire plate. There were lot of variations were observed with respect to colony/ mycelial characteristics viz., reverse pigmentation, density of mycelium and colony margin. However, no too much variations were observed with respect to color and texture of the colony (Appendix-v).

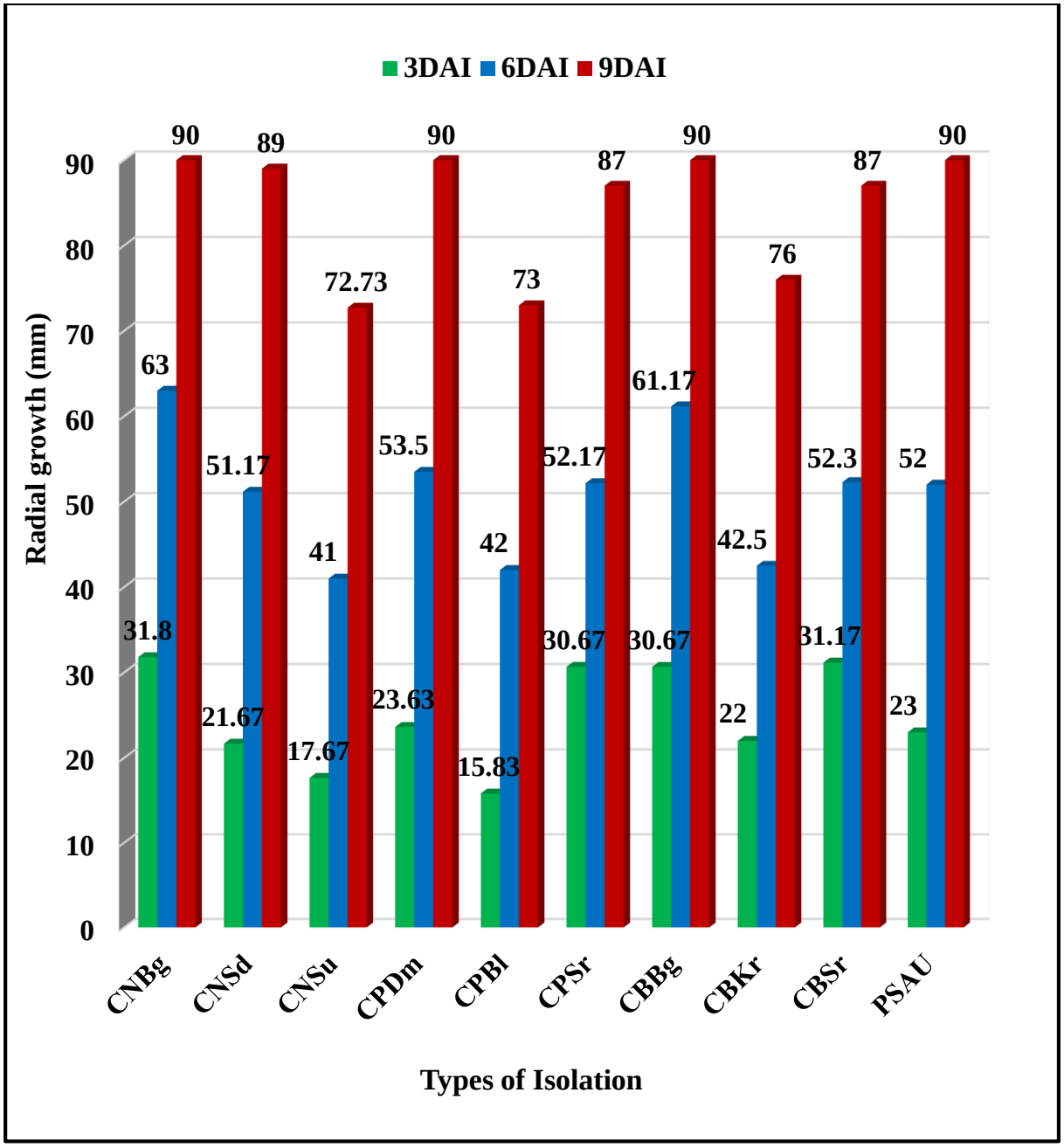


Figure 14. Radial growth of *Curvularia* isolates on PDA media

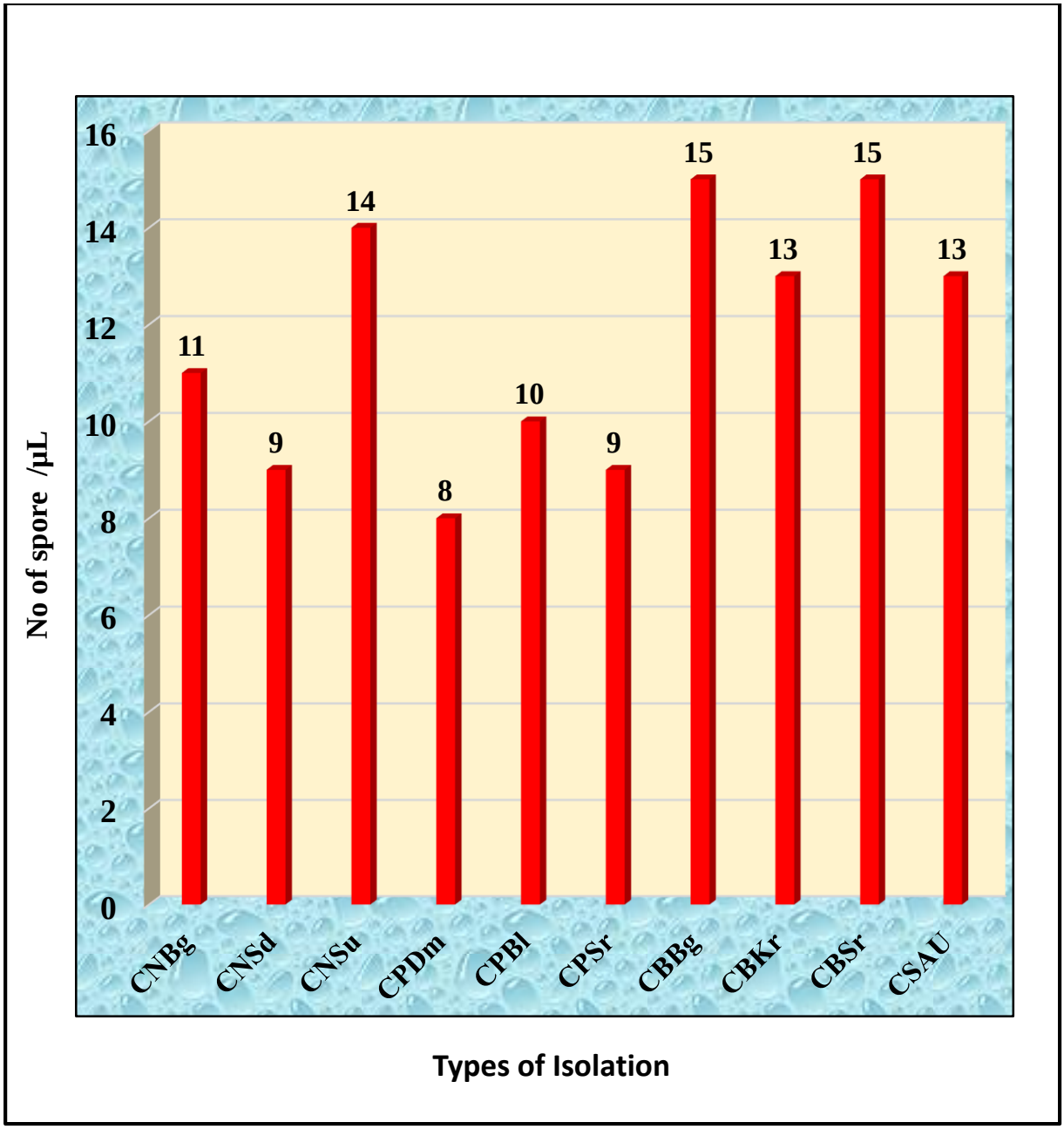


Figure 15. No. of spore /µL of *Curvularia* isolates

4.2.4 Isolation and study of cultural and morphological variability *Alternaria* isolates

The *Alternaria* was isolated from leaf samples and identified as *Alternaria* sp. based on colony morphology and mycelia characteristics. To study the cultural and morphological variability in *Alternaria* sp. different isolates were collected from different locations of coastal area of Bangladesh and SAU campus. They were subjected for the cultural and morphological characters viz., radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density on PDA and all these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates (Table 16, Figure 16-17). The results revealed that there were cultural and morphological variations among the isolates of *Alternaria*. The colony diameter after 3, 6 and 9 days of inoculation was significantly varied, the radial mycelial growth ranged was 18 to 31.33 mm at 3 DAI, 41.17-62.3mm at 6 DAI and 71.5-90mm at 9 DAI. Spore / µL of the isolates was ranged from 5-10. The number of days taken to cover full plate was ranged from 9 to 15 days and most of the isolates covered entire plate in 9 days as noted were ANSd, APDm, ABBg and ASAU. However, some of isolates taken <10 days to cover entire plate. There were lot of variations were observed with respect to colony/ mycelial characteristics viz., reverse pigmentation, density of mycelium and colony margin. However, no too much variations were observed with respect to color and texture of the colony (Appendix-v).

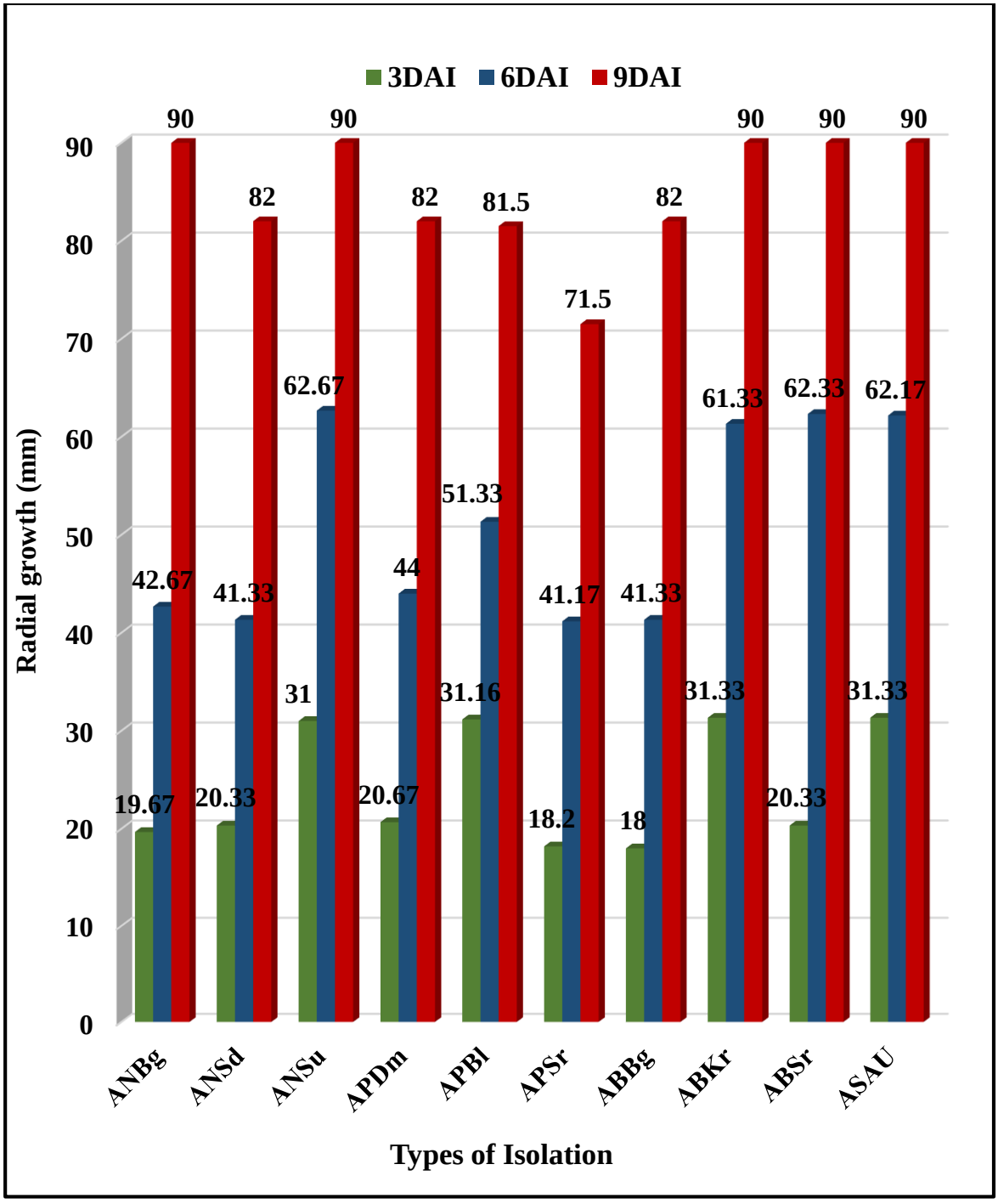


Figure 16. Radial growth of *Alternaria* isolates on PDA media

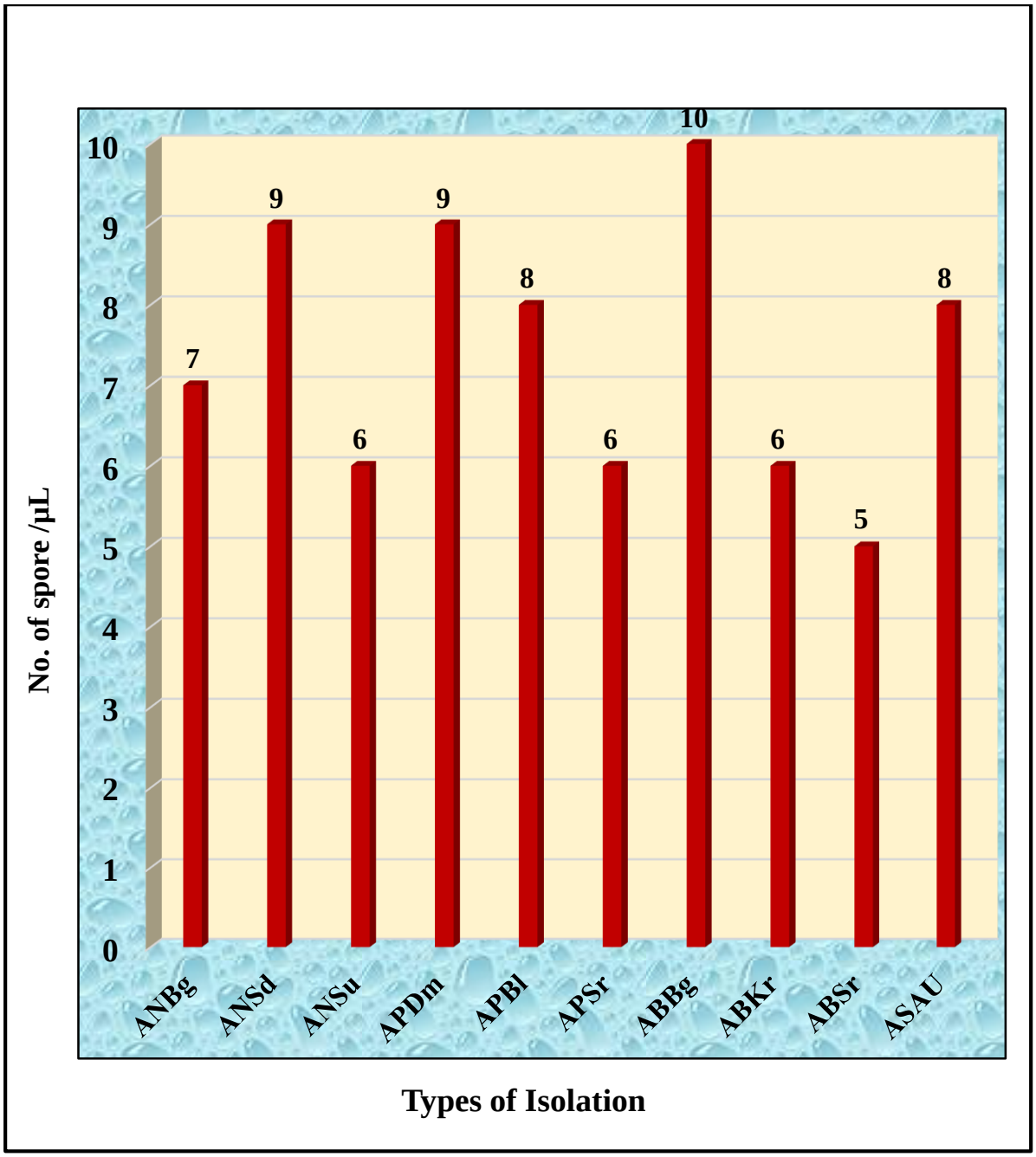


Figure 17. No of spore /µL of *Alternaria*

4.3 Biocontrol-Based Management Package for Basal Stem Rot of Coconut

The integrated disease management trial was laid out in SAU campus and 32 infected plants were selected for application the treatments. Bio-agent and botanicals combinations were used for the management of Basal stem rot of coconut. The treatments were imposed as explained in "Material and Methods". The observations were recorded as disease incidence and results are presented in Figure 18. The results of integrated disease management trial at SAU campus revealed that the disease spread was minimum in palms that received combined application of 5 kg mustard oilcake + 1kg grinded neemseeds enriched with 250 ml *T. viride* solution per plant. The result was so satisfactory, where disease incidence was estimated 65%, i.e., the disease incidence was reduced upto 35% over control. The treatment combination of 0.5L garlic extract + 5Kg mustard oilcake + 1kg grinded neemseeds per plant had 70% disease incidence, i.e., the disease incidence was reduced upto 30% over control. Cattle urine was given separately in the amount of 1L/plant. Cattle urine give good result against basal stem rot . (Figure 18).

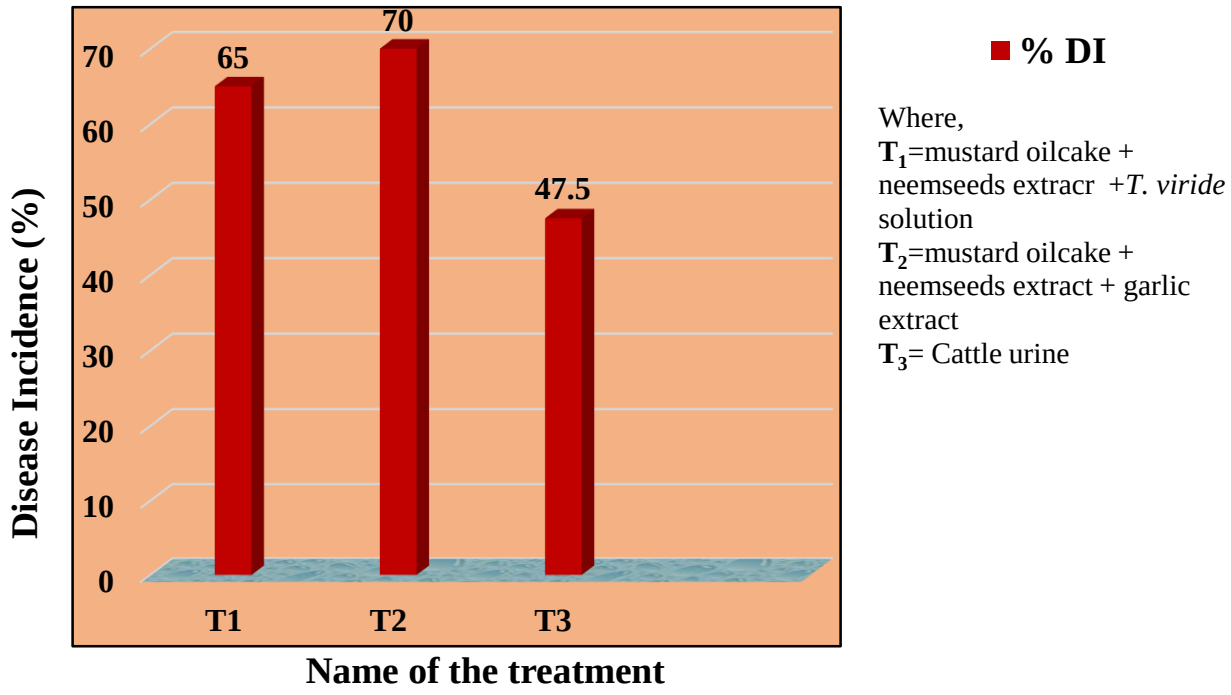


Figure 18. Effect of biocontrol - based management Package on Basal Stem Rot

Discussion

The present study was carried out to estimate the occurance and disease incidence of basal stem rot and foliar diseases of coconut in coastal zone of Bangladesh and SAU campus and study of a biocontrol-based management package against BSR in coconut. A structural questioner based survey was done in the selected location and disease incidence and severity was measured. A biocontrol-based management package was developed by using three types of treatment viz. mustard oilcake + neemseeds extract + *T. viride* solution, mustard oilcake + neemseeds extract + garlic extract and cattle urine. Among the treatments cattle urine gave satisfactory result.

4.4.1 Disease incidence and severity of Basal Stem Rot (BSR)

Among surveyed locations, Maximum disease incidence (70%) of BSR was recorded in Begumgong of Noakhali district and minimum disease incidence (20%) was recorded in Bakergong of Barisal district. Maximum disease severity (33.75%) of BSR was recorded in Begumgong of Noakhali district and minimum disease severity (22%) was recorded in Bauphal of Patuakhali district.

The BSR incidence with respect major soil type, soil pH and plant age were also recorded. The disease incidence with respect to soil types, maximum disease incidence (27.5%) was recorded in loamy soils while minimum incidence (11.25%) was found in clay soils. The disease incidence with respect to soil pH, maximum disease incidence (26.75%) was noticed in soil pH ranged 6-7 and minimum (20.5%) was in 7-8 ranged. The disease incidence with respect to plant age, maximum disease incidence (24%) was observed in age group of 36-40 years old plants and minimum disease incidence (13%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (45.75%) was found in yard garden while minimum disease incidence (12.75%) 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.* (2016), Naik *et al.* (2000).

4.4.2 Disease incidence and severity of foliar diseases

Among the surveyed areas, maximum disease incidence (88.33%) of foliar diseases was recorded in Begumgong of Noakhali district and minimum disease incidence (65%) in Dumki of Patuakhali district. The foliar diseases severity was also measured in surveyed locations. The maximum disease severity was found in Begumgong (45.92%) of Noakhali district and minimum disease severity (21.48%) was recorded in Dumki of Patuakhali district.

The foliar diseases incidence with respect major soil type, soil pH and plant age were also recorded. According to soil type, maximum foliar disease incidence (19.2%) was recorded in loamy soil and minimum foliar disease incidence (9%) was recorded in sandy soil. The disease incidence with respect to soil pH, maximum disease incidence was recorded (22.4%) in soil pH 5-6 and minimum (14.8%)was in soil pH 7-8. The disease incidence with respect to plant age, maximum disease incidence (18%) was observed in age group of 36-40 years old plants and minimum disease incidence (11%) was found in 20-25 years of aged plants. It was also observed that according to garden types, maximum disease incidence (33.4%) was found in yard garden while minimum disease incidence (9.4%) was recorded in road side plants. The investigation results regarding the percent disease incidence and severity of leaf diseases observed in the study is also recommended with previous study that reported by K. Athira (2017).

4.4.3 Isolation and identification of *Ganoderma* spp

In total 120 root samples were collected for *Ganoderma* isolation and pathogen association was found in 47 samples out of 120 samples. The *Ganoderma* was isolated from root samples and identified as *Ganoderma* sp. based on colony morphology and mycelia characteristics viz., reverse pigmentation, density of mycelium and colony margin. However, no too much variations were observed with respect to color and texture of the colony and all these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates. After studying of microscopic view of conidia two types of *Ganoderma* species were identified viz., *Ganoderma lucidum* and *Ganoderma applanatum*. The results recorded in this study regarding the isolation and identification is almost similar as

observed by Kandan *et al*. (2010), Naher *et al*. (2012), Vinayaka and Prathibha (2013), Palanna *et al.* (2016).

4.4.4 Isolation and identification of foliar diseases

About 90 leaf samples were collected and in total 90 samples pathogen association was found in 52 samples. Three types of pathogen viz, *Pestalotia, Curvularia, Alternaria* were isolated from leaf samples and identified based on colony morphology and mycelia characteristics viz., radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density on PSA and PDA.All these observations were recorded on the seventh day after inoculation and kept under observation to record number of days taken for maximum growth (9 cm) by different isolates. The results of this study regarding the isolation and identification of leaf diseases pathogens is almost similar as observed by Doraiswamy *et al*. (2003), M.A.H. Khan and I. Hossain (2013), Dr. Budhadev Mishra (2014) K. Athira. (2017).

4.4.5 Biocontrol-based management package against Basal stem rot

About 32 infected plants were selected for the treatment trial. Bio-agent and botanicals combinations were used for the management of basal stem rot in coconut. Three combinations viz, 5 kg mustard oilcake + 1kg grinded neemseeds enriched with 250 ml *T. viride* solution, 0.5L garlic extract + 5Kg mustard oilcake + 1kg grinded neemseeds, cattle urine was applied as treatment and five months later root sample were taken. After disease incidence measurement it was found, cattle urine gave 47.5% disease incidence which was 52.5% reduction over control. Mustard oilcake+ neemseeds+ *Trichoderma viridae* solution gave 65% disease incidence which was 35% reduction over control. These two treatments showed satisfactory results against basal stem rot. Bhaskaran (1990) reported that the soil application of *Trichoderma harzianum* at 500g inoculums along with various organic manures *viz.*, green leaves (50kg) or Farm yard manure (50kg) or neem cake (5kg) significantly reduces the BSR disease intensity. Kuberan *et al. (*2012) said garlic was found to be fungi toxic to a number of plant pathogen.

Summary and Conclusion

The study was conducted in Molecular Biology and Plant virology laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University. The study was performed based on two diseases of coconut, those are basal stem rot and foliar diseases of coconut. The objective of the study is measurement of disease incidence and severity of basal stem rot and foliar diseases and study of biocontrol-based management package against basal stem rot in coconut.

A survey was done in three coastal district (Noakhali, Patuakhali, and Barisal) of Bangladesh and SAU campus. The disease incidence of BSR was measured 60.83%, 64.44%, 49.17%, and 65% respectively in Noakhali, Barisal, Patuakhali and SAU. The disease severity was 33.88%, 24.58%, 26.67%, 30% respectively in Noakhali, Barisal, Patuakhali and SAU. In case of Leaf diseases incidence was 85%, 77.78%, 78.61%, 85% respectively in Noakhali, Barisal, Patuakhali and SAU. The disease severity was 39.13%, 24.13%, 33.56%, 28.33% respectively in Noakhali, Barisal, Patuakhali and SAU.

The disease incidence also measured in respect of soil type, soil pH, Garden type and age of the plant. The BSR (27.5%) and foliar disease (19.2%) incidence is more in loamy soil. Besides both diseases are high in the soil having pH 6-7 and the disease incidence were 26.75% and 22.45 respectively of BSR and foliar diseases. The study revealed that the older plant has the high disease incidence. The plant which were 36-40 years old showed high disease incidence rate of BSR (24%) and foliar diseases (18%).

Isolation of pathogens of BSR and foliar diseases was done from collected samples. For isolation 120 root samples and 90 leaf samples were used for aseptic isolation. From the sample 39.17% isolates from root and 57.77% isolates from leaves were isolated.

The isolated samples were identified based on colony morphology and mycelia characteristics viz. radial growth, colony texture, appearance of zone, reverse pigmentation colour, type of colony margin, mycelial density and conidial structure. All these isolates were observed for 7-15 days. The radial growth was taken at 3 days interval and spore/µL was counted. *Ganoderma* spp was isolated from root samples. Two species of *Ganoderma* was identified viz. *Ganoderma lucidum* and *Ganoderma applanatum.*

From leaf samples three pathogen was isolated i.e. *Pestalotia* sp., *Curvularia* sp., *Alternaria* sp. The data was analyzed by completely randomized design.

A biocontrol-based management package was given against Basal Stem Rot affected plants in SAU campus. About 32 plants were selected and treated with 5 kg mustard oilcake + 1kg neemseeds + 250ml *T. viridae* solution, 5 kg mustard oilcake + neemseeds + 500ml garlic extract + 1L water, 1L cattle urine + 1L water. After 5 months of treatment root samples were taken and disease incidence was estimated. Among the treatment mustard oilcake + neemseeds + *T. viridae* solution showed 65%, mustard oilcake + neemseeds + garlic extract showed 70% and cattle urine showed 47.5% disease incidence. Cattle urine found satisfactory among these three combination. BSR of coconut cannot be kept under control with just a single management strategy. In the present study an integrated approach disease management with mixtures of biocontrol agents and botanicals was found effective in the management of BSR of coconut. It can give a hopeful outcome in case of overcome the disease.

Finally, it may be concluded that lot of variability was observed with respect to cultural, morphological aspects of *Ganoderma* sp. and leaf diseases pathogens. The occurrence and distribution of the disease were measured by measuring the disease incidence and severity in the surveyed areas. In the study a correlation of soil type, soil pH, plant age and garden type with disease incidence is also presented. Adequate measurement should be taken from the early age of plants. Further, assessment of disease incidence with respect to different agronomic practices followed by farmers in disease hotspot areas of coastal areas and SAU campus, will be useful to take/design further remedial measures to combat the disease, thereby coconut production will be enhanced.

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APPENDICES

Appendix-I. Survey questioner

A. Structural questioner for survey study of *Ganoderma* wilt

Survey on coconut Diseases 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)

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		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
Total		

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

Cultural practices:

1. …..
2. …..
3. ……
4. …….

Use of fertilizer

1. **Chemical:**
   ……..
   ……..
   ……

2. **Biological:**
   ……..
   ……..
   ……..
   ……..

Other information:

Type of soil of the area:

Soil pH:

Relay crops with coconut:

Problems faced by the cultivar:

……
……
……

Comments after survey

……
……..

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B. Structural questioner for survey study of leaf diseases

Survey on coconut Diseases 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: Grade Description (% leaf area infected) (K. Athira, 2017).

0	No sign or symptoms
1	0-10 % infection
3	11-15% infection
5	16-25% infection
7	26-50% infection
9	>50%infection

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

***LDS = Leaf disease symptom**

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

Cultural practices:

1.
2.
3.

Use of fertilizer

1. **Chemical:**
   ……..
   ……..

2. **Biological:**
   ……..
   ……..
   ……..

Other information:

Type of soil of the area:

Soil pH:

Relay crops with coconut:

Problems faced by the cultivar:

Comments after survey

……
……..

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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

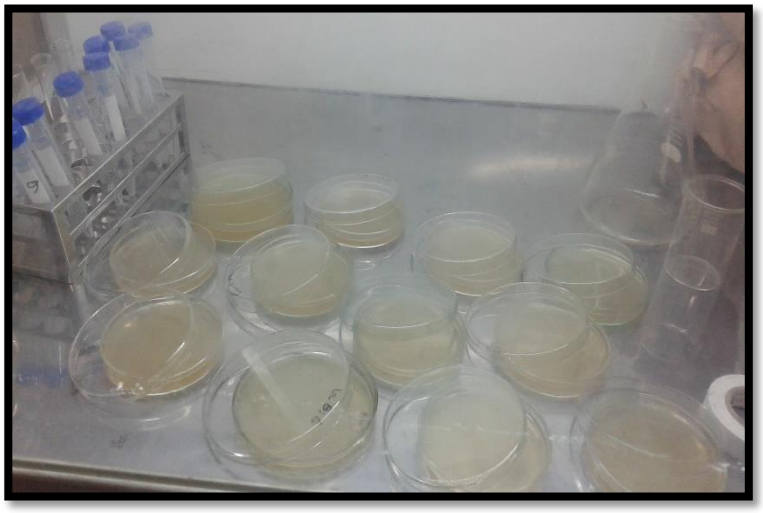
SI. No.	Area	Plants growing with coconut
01	Noakhali	Arecanut,palmyras,date,mango, Banana
02	Barisal	Banana, Rain tree, jujube
03	Patuakhali	Berry,Banana,Arecanut,Jackfruit

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	Noakhali	Cleaning,irregation,fertilization
02	Barisal	Cleaning
03	Patuakhali	Cleaning

Appendix-III. Inoculation of pathogens on PDA media



Photograph 1: Preparation of media for inoculation

Photograph 2: Inoculation of pathogen on PDA

Appendix- IV. *In-vitro* evaluation of garlic extract combination with *Trichoderma viridae*

For biocontrol-based management package against *Ganoderma* wilt *Trichoderma viridae* was cultured with garlic extract in food poisoning method. The garlic extract was taken at 5%,10%,15% concentration. The result showed that all the concentration totally inhibited the *Trichoderma* growth. So *Trichoderma viridae* + garlic extract combination was not taken.

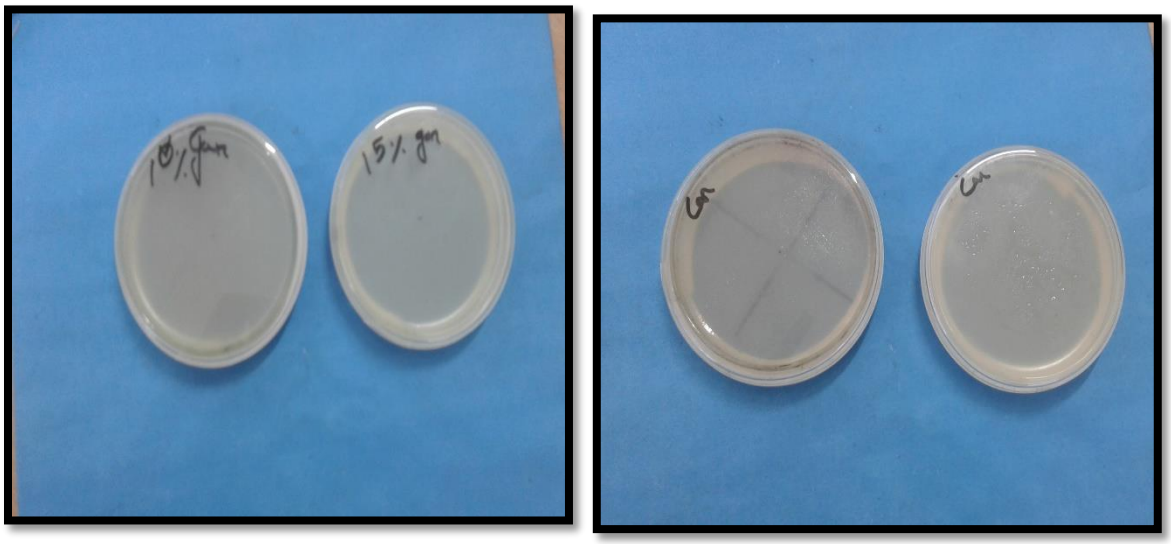


Figure 19. *T. viridae* cultured with garlic extract

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

Table 13: Cultural and morphological characteristics/variability of *Ganoderma* isolates of coconut

SL No	Isolates	Rdial growth(mm)			CFU / µL	Days Taken for full plate	Colony/ Mycelial characters		
		3DAI	6DAI	9DAI			Colony Color /Reverse pigmentation	Mycelia Texture/ Mycelia density	Margin
01	GNBg	25.67d	65.33e	90a	34c	9	White/ NP	Leathery/ Irregular	Filamentous
02	GNSd	15.17a	35.17a	77.83b	60a	15	Pale white/NP	Leathery/ Thin at centre and dense at centre	Even
03	GNSu	31c	53.66e	85.33b	63a	10	Pale white/NP	Fluffy/ Thin	Even
04	GPDm	39.17f	71.17e	90a	74b	9	Pale white/NP	Leathery /Thin	Undulate
05	GPBl	30.83e	51.67f	90a	88e	9	White/NP	Leathery/ dense	Filamentous
06	GPSr	16de	36.83d	77.5b	81e	12	Pale white/NP	Leathery/ Thin at centre and dense at centre	Even
07	GBBg	32e	42.67	86.83c	88e	11	White/pinkish	Leathery/ dense	Even
08	GBKr	25.33b	43.67bc	90a	108d	9	White/yellowish	Fluffy/ Dense	Undulate
09	GBSr	32e	52.16c	85.83c	103d	12	White/NP	Fluffy/ irregular	Undulate
10	GSAU	32.67b	61b	90a	92f	9	Pale white/NP	Leathery/ Thin at centre and dense at centre	Even
11	SEm	0.850	0.625	0.633	13.47				
12	LSD (0.05)	1.57	1.34	1.35	6.25				
13	CV(%)	3.30	1.54	0.92	4.63				

Note: DAI-Days After Inoculation

Table 14: Cultural and morphological characteristics/variability of *Pestalotia* isolates of coconut

SL No	Isolates	Radial growth(mm)			CFU /µL	Days Taken For full plate	Colony/ Mycelial characters		
		3DAI	6DAI	9DAI			Colony Color /Reverse Pigmentation	Mycelia Texture / Mycelia density	Margin
01	PNBg	24.33d	45e	85.67bc	5f	11	White/ NP	Leathery/ Irregular	Filamentous
02	PNSd	30.35a	66.83a	90a	7e	9	Pale white/NP	Leathery/ Dense at entre and Thin at corner	Even
03	PNSu	30.67c	60.33c	90a	10abc	9	white/ Yellowish	Leathery/ Dense	Undulate
04	PPDm	14.83g	41.67f	82.67c	8e	12	Pale white/ NP	Leathery/ Dense at centre	Undulate
05	PPBl	21f	52.17d	87.67c	6f	10	White/ NP	Leathery /Irregular	Filamentous
06	PPSr	22.5e	61.33c	90a	11ab	9	white/ NP	Leathery/ Dense	Undulate
07	PBBg	21.67ef	41.17f	71.67c	11c	15	White/ NP	Leathery /dense	Even
08	PBKr	33.83b	62.33c	90abc	10bc	9	Ash/ NP	Fluffy/ Dense	Undulate
09	PBSr	21.33ef	51.5d	90c	9d	9	Ash /NP	Fluffy/ irregular	Undulate
10	PSAU	23.03b	64.83b	90ab	6abc	9	Ash / NP	Leathery/ Thin at centre and dense at centre	Even
11	SEm	0.746	.463	8.483	0.342				
12	LSD (0.05)	1.84	1.45	2.72	1.25				
13	CV(%)	3.21	1.56	3.40	6.94				

Note: DAI-Days After Inoculation

Table 15: Cultural and morphological characteristics/variability of *Curvularia* isolates of coconut

SL No.	Isolates	Rdial growth(mm)			CFU /µL	Days Taken For full plate	Colony/ Mycelial characters		
		3DAI	6DAI	9DAI			Colony Color /Reverse pigmentation	Mycelia Texture/ Mycelia density	Margin
01	CNBg	31.8a	63a	90a	11cd	11	White/ NP	Leathery/ Irregular	Filamentous
02	CNSd	21.67a	51.17e	89a	9bcd	9	Pale white/NP	Leathery/ Dense at centre and Thin at corner	Even
03	CNSu	17.67a	41h	72.73d	14ab	15	Black/NP	Leathery/ Dense	Undulate
04	CPDm	23.63a	53.5c	90a	8a	9	Black/NP	Leathery/ Dense at centre	Undulate
05	CPBl	15.8bbc	42gh	73d	10cd	13	Black/NP	Leathery/ Dense	Even
06	CPSr	30.67bcd	52.17de	87b	9d	11	Ash/NP	Leathery/ Dense	Undulate
07	CBBg	30.67cd	61.17b	90a	15a	9	Black/NP	Leathery/ dense	Even
08	CBKr	22d	42.5f	76c	13bcd	12	Ash/NP	Leathery/ Dense	Undulate
09	CBSr	31.17e	52.3d	87b	15a	10	Black/NP	Leathery /Dense	Undulate
10	CSAU	23f	52de	90a	13abc	9	Black/NP	Leathery/ dense at centre	Even
11	SEm	0.475	0.445	1.01	2.622				
12	LSD (0.05)	1.47	1.42	2.15	3.46				
13	CV(%)	2.77	1.30	1.19	12.65				

Note: DAI-Days After Inoculation

Table 16: Cultural and morphological characteristics/variability of *Alternaria* isolates of coconut

SI No	Isolates	Rdial growth(mm)			CFU /µL	Days Taken For full plate	Colony/ Mycelial characters		
		3DAI	6DAI	9DAI			Colony Color /Reverse pigmentation	Mycelia Texture/ Mycelia density	Margin
01	ANBg	19.67a	42.67a	90a	7a	11	Light ash / NP	Leathery /Irregular	Filamentous
02	ANSd	20.33a	41.33a	82a	9a	9	Light ash /NP	Leathery / Dense at centre and Thin at corner	Even
03	ANSu	31a	62.67ab	90a	6a	15	Dark ash /NP	Leathery /Dense	Undulate
04	APDm	20.67a	44b	82a	9a	9	Light ash /NP	Leathery /Dense at centre	Undulate
05	APBl	31.16b	51.33c	81.5a	8b	13	Light ash /NP	Leathery /Dense	Even
06	APSr	18.2b	41.17d	71.5b	6b	11	Dark ash/NP	Leathery /Irregular	Undulate
07	ABBg	18b	41.33e	82b	10b	9	Dark ash /NP	Leathery /dense	Even
08	ABKr	31.33b	61.33f	90b	6b	12	Light ash /NP	Leathery /Dense	Undulate
09	ABSr	20.33c	62.33f	90b	5b	10	Light ash /NP	Leathery /Dense	Undulate
10	ASAU	31.33c	62.17f	90c	8b	9	Dark ash /NP	Leathery / dense at centre	Even
11	SEm	0.651	0.445	0.570	1.324				
12	LSD (0.05)	1.72	1.43	1.62	2.46				
13	CV	3.33	1.31	0.89	14.82				

Note: DAI-Days After Inoculation