ISOLATION AND IDENTIFICATION OF SOIL MICROORGANISMS IN SELECTED POTATO FIELDS OF BANGLADESH

MUSFIKA AHMED NITU

DEPARTMENT OF PLANT PATHOLOGY

FACULTY OF AGRICULTURE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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BY

MUSFIKA AHMED NITU

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APPROVED BY :

_____	_____
(Dr. F. M. Aminuzzaman)	**(Dr. Nazneen Sultana)**
Professor	**Professor**
Department of Plant Pathology	**Department of Plant Pathology**
Supervisor	**Co-Supervisor**

_____

(Dr. Md. Belal Hossain)
Associate Professor
Chairman
Examination Committee
Department of Plant Pathology

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ABSTRACT

Microorganisms from the rhizoosphere soil was isolated from different potato fields of Bangladesh. Seventeen soil samples were analyzed for the incidence of microorganisms from potato field soils. A total of seven fungal species, one bacterium species and one nematode species were characterized using various isolation and identification methods. The predominant fungi isolated include among others; *Alternaria solani*, *Aspergillus niger*, *Penicillium* sp., *Rhizopus stolonifer*, *Bipolaris* sp., *Phytophthora infestans* , *Fusarium oxysporum*, *Ralstonia solanacearum* and *Meloidogyne* sp.. And individual colonies of fungi, bacteria and nematode were counted from media and compared their presence in soil in respect of different locations of potato fields. The present study aimed to find out the fungal diversity and this was the first reported examination of the microbiological diversity of soil microorganisms of some selected potato fields in Bangladesh.

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CHAPTER I

INTRODUCTION

Soils are very composite systems, with many components playing diverse functions mainly due to the activity of soil organisms (Chiang and Saudi , 1994). Soil microorganisms plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Singh *et al.*, 1999) by biochemical transformation and mineralization activities in soils. Type of cultivation and crop management practices found to have greater influence on the activity of soil microorganisms (Mc.Gill *et al.*, 1980). Continuous use of chemical fertilizers over a long period may cause imbalance in soil microorganisms and thereby indirectly affect biological properties of soil leading to soil degradation (Manickam and Venkataraman, 1972). Fungi are an important component of the soil micro biota (Ainsworth and Bisby, 1995). Micro fungi play a focal role in nutrient cycling by regulating soil biological activity (Barbhuiya *et al.*, 2004). Indirect accumulation in higher trophic level organisms, such as mammals, may cause health problems over time because of the increasing levels of toxic compounds within the body. There are two main reasons that these compounds persist in nature. First, the conditions necessary for their biodegradation are not present. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site. If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavorable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation (Field *et al.*, 1993). The quantities of organic and inorganic materials present in the soil have a direct effect on the fungal population of the soil. In addition to chemical fertilizers and wide range of pesticides

shows adverse effect on mycoflora the members and kinds of micro organisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration, pH and temperature etc.

Moreover, Nematodes are an evolutionarily successful group of organisms and represent an important part of the soil microfauna that affect the soil microflora as they occupy positions at primary, secondary and/or tertiary consumer level in soil food webs (Moore and de Ruiter, 1991). The accurate assessment of all members of the nematode community is essential for obtaining a clear impression of community composition. Nematodes can be free-living or parasitic to plant and animal. Substrates such as litter, moss or compost often contain high numbers of non-parasitic (Saprophagous) nematodes of plant. For estimation of nematode feeding types and calculation of their contribution to energy and nutrient uses in soil ecosystems, it is necessary to obtain a reliable quantification of nematode numbers in soil. This also holds for population studies that need reliable estimates of age-structure and sex-ratio (Ferris, 1987; Schouten and Arp, 1991). Plant parasitic nematodes cause significant economic reduction to crops. Before giving appropriate recommendations, the specific types and numbers of nematodes present in the field must be determined (Mai, 1985; Bezooijen, 2006). While a wide variety of methods for extracting nematodes from soil are available, all are imperfect and have various degrees of inefficiency (Barker *and Campbell*, 1981; Hooper, 1986; McSorley and Frederick, 2004).

Potato (*Solanum tuberosum* L.) is the world's fourth largest and third largest food crop in Bangladesh and has recently occupied an important place in the list of major food and cash crops in Bangladesh (Ali and Haque, 2011). Soil borne diseases are considered as a limiting factor of many crops including potato. *Ralstonia solanacearum* (Smith, 1896; Yabuuchi *et al*., 1995) (formerly called *Pseudomonas solanacearum*) is a soil borne pathogen generally occurs in lowlands in tropical or subtropical areas, is an extremely destructive potato pathogen, causing bacterial wilt or brown rot of potato in the highland tropics of Africa, Asia and Latin America (Elphinstone, 2005).

The aim of the present investigation is to isolate mycoflora from different fields, and to observe the percentage contribution of different fungi by soil dilution method with the following objectives:

- To isolate and identify different fungi, bacteria and nematodes from the selected potato fields of Bangladesh.
- To assess the presence of fungi, bacteria and nematode in the soil community of selected potato fields.

CHAPTER II
REVIEW OF LITERATURE

A brief of the relevant works on the isolation of fungi, bacteria and extraction of nematodes performed in the past are presented in this chapter.

2.1 Isolation of fungi from the soils of different regions

Solomon and Odelade (2016) conducted a research to determine the microbes, soil and water samples were collected from petrochemical industry, Eleme, Port-Harcourt, Rivers State, Nigeria, for microbiological analysis. This was carried out by the isolation, assessment and characterization of the isolated organisms. The highest bacterial counts was determined in soil sample 1 (SS1) and water sample 4 (WS4) with microbial loads of 1.48 x 106 cfu/mL and 9.40 x 105 cfu/mL and the lowest count was found in soil sample 2 (SS2) and water sample 2 (WS2) with microbial load 2.90 x 105 cfu/mL and 3.67 x 104 cfu/mL. The highest fungal counts was determined in soil sample 2 (SS2) and water sample1 (WS1) with microbial loads of 1.76 x 106 cfu/mL and 2.17 x 106 cfu/mL and the least colonies was in soil sample 1 (SS1) and water sample 2 (WS2) with microbial counts of 1.75 x 105 cfu/mL and 4.30 x 104 cfu/ml. The results present that the presence of these microbes can be linked to the prehistory of the effects or contamination of surface and underground water in this region and could leads to water-borne diseases.

Meszka and Michalecka (2016) conducted a research with 45 isolates of *Phytophthora* spp. from plants of cultivars Elsanta, Honeoye, Florence, Camarosa, Roxana, Onebor, Alba, Elegance, Albion, Senga Sengana and Malwina originating from crowns (20 isolates) and fruits (25 isolates) as well as 25 soil samples from the same fruiting strawberry plantations located in central, north-eastern, and south-eastern regions of Poland were identified. Among them, 44 isolates from plant organs and eight from soil samples were identified as *Phytophthora cactorum* and from one plant and one soil sample as *P. citricola*; of those remaining from the soil, one was identified as *P. citrophthora* and one as *P. cryptogea*. Identifications were based on morphological characteristics and DNA analyses. Species-specific polymerase chain reaction confirmed the presence of *Phytophthora* spp. in all positive examined plant and soil samples and confirmed that *P. cactorum* is predominantly responsible for crown and leather rot diseases of strawberry in monitored regions of Poland. Pathogenicity tests showed that all isolates of *Phytophthora* spp. originating from soil derived from strawberry plantations were pathogenic both to strawberry and to raspberry.

Kumar *et al.* (2015) performed a research work with the seventeen species belonging to thirteen genera of fungi were isolated from soil samples collected from diverse part of city Bareilly during November 2012 to March 2013 at three intervals. The mycoflora were isolated on Yeast powder soluble starch (YpSs) agar added with antibacterial substances streptomycin. Identification and characterization of the mycoflora were made with the help of available literature *and* photomicrograph. The most common fungal forms were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Trichoderma viride, Rhizopus*

oryzae and Botrytis cineria while Curvularia clavata, Fusarium oxysporum, Fusarium solani, Curvularia lunata were unusual frequency of occurrence of the isolates was also evaluated.

Kumar *et al.* (2015) collected soil samples to determine the fungus colonies and a total of 168 colonies were isolated. About 18 species belonging to 6 genera of fungi were isolated and identified while 20 strains, respectively were left unknown. Identification and characterization of the soil mycoflora were made with the help of authentic manuals of soil fungi. Maximum number of fungal colonies belonged to deuteromycotina (143) and few to zygomycotina (5). Among the isolates *Aspergillus flavus, A.fumigatus, A.nidulans, A.niger, A.terreus, Penicillium chrysogenum, P.frequentens* were predominant.

Soni and Sharma (2014) studied with the mycoflora from the soil to investigate at some different locations from February to June, 2003. The fungi were isolated by using soil dilution and soil plate method. On the basis of cultural and microscopic characteristics, the isolated strains were identified as *Trichoderma viride, Aspergillus niger, Aspergillus fumigatus, Curvularia* sp., *Fusarium* sp. etc. Total 14 species were obtained from 20 soil samples. *Aspergillus niger* showed highest percentage contribution.

Nagi *et al.* (2013) isolated fungus for the polymerase chain reaction method used to detect soil-borne plant pathogens such as *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* in the soil was developed and used with a range of soil textures. A direct method for the extraction of DNA from soil samples, which can be used for PCR-mediated diagnostics

without a need for further DNA purification, was developed. The developed protocol seemed adequate to the range of soil textures that were artificially infested by a variety of soil-borne pathogens.

Magnet *et al.* (2013) collected different kinds of soil sample from various industry sides in Dhaka city. The bacteria identified in the soil sample are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp. whereas in road side soil *Aspergillus niger* and *Penicillium* spp and in garden soil *Mucor* sp., *Rhizopus* sp. and *Penicillium* sp. were identified as fungus.

Malek *et al.* (2013) found 75 isolates of aerobic *Actinomycetes* were detected that *Actinomadura madurae* and *Nocardia asteroides* were the most prevalent strains, with 14.66 and 28% prevalence respectively. *Microsporum gypseum* was more frequent than other keratinophilic fungi (22.96%) and *Aspergillus* spp. was the most species of saprophyte fungi (15.92%).

Jasuja *et al.* (2013) isolated several bacteria and fungi from polyhouse soil, using serial dilution method. These bacterial isolates were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Shigella* sp., *Proteus mirabilis, Bacillus anthracis, Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis* species which were further identified on the basis of colony morphology, Gram staining, biochemical tests and using selective and differential media. Identification of fungi such as *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Trichoderma* sp. and *Rhizopus* sp. was carried out by culturing on potato dextrose and sabouraud's dextrose agar media and microscopic method.

Microorganisms play an important function in biodegradation of solid agriculture waste and also help in the crop production.

Gaddeyya *et al.* (2012) observed total of 15 species belonging to 6 genera of fungi were isolated from agricultural fields at Salur Mandal during March 2011 to November 2011 in three intervals. The mycoflora were isolated by using soil dilution technique and soil plate technique on Potato Dextrose Agar and Czapek,s Dox Agar medium supplemented by suitable antibiotics such as penicillin and streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. The most common among them *viz*; *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium funiculosum, Trichoderma viride, Trichoderma harzianum, Fusarium oxysporum, Fusarium solani, Curvularia clavata, Curvularia lunata, and Rhizopus stolanifer* were isolated and characterized. The seasonal variation and percentage frequency of the mycoflora were statistically analyzed.

Bi *et al.* (2012) evaluated several strains of fungi were isolated and identified from Scandinavian soil using agar plates with lignin as a carbon source. The strains grew significantly faster on this medium than on control plates without lignin. Different types of technical lignins were used, some of which contained trace amounts of sugars, even if the increased growth rate seemed not related to the sugar content. Some strains were cultivated in shaking flask cultures with lignin as a carbon source, with lignin apparently consumed by microbes – while accumulation of the microorganism biomass occurred. The cellfree filtrates of these cultures could reduce the apparent

molecular weights of lignosulphonates, while the culture filtrate of one strain could cleave the β-O-4 bond in a lignin model compound.

Tkaczuk *et al.* (2012) observed total of six fungal species representing the anamorphs of Hypocreales (Ascomycota) were isolated from the soil collected from mid-field woodlots: *B. bassiana, B. brongniartii, M. anisopliae, M. flavoviride, I. farinosa* and *I. fumosorosea*. The presence of only three species was reported in the farmland soil: *B. bassiana, M. anisopliae* and *I. fumosorosea.* This fact confirms the important role of semi-natural habitats as a source of biodiversity of entomopathogenic fungi in agricultural landscape. It was found that entomopathogenic fungi together formed more colony-forming units in the soil from arable fields than that of neighbouring mid-field woodlots. *B. bassiana* was the species of fungus which infected more bait insect larvae and formed significantly more colony-forming units (CFU) in the soil from mid-field woodlots than that of farmland in the localities studied, whereas the trend was the opposite in the case of *I. fumosorosea* and *M. anisopliae*. Given the presence of entomopathogenic fungi in the farmland soil in the three test places together, it was found that *I. fumosorosea* was dominant in the soil from the two arable fields, where this fungus infected more *G. mellonella* larvae and formed significantly more CFUs than the other species of fungi. *M. anisopliae* was the second most frequently isolated farmland species.

Rohilla and Salar (2012) studied twenty three soil samples and characterized for the incidence of fungal strains from pesticides contaminated agricultural soils. A total of 59 fungal strains were isolated and 33 fungi were characterized using various isolation and identification

methods. Soil samples were also characterized for physiochemical properties. The isolated fungal strains were successfully identified belonging to the phylum ascomycota (7 genera), deuteromycota (2) and zygomycota (1). *Alternaria, Aspergillus, Drechslera* and *Fusarium* were predominant genera. *Curvularia, Exserohilum, Humicola, Rhizopus* and *Torula* were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used incubation conditions. Such strains were designated as Mycelia sterilia. Further, these species will be used in biodegradation of commonly used pesticides.

Wang *et al.* (2011) isolated from soil samples and collected around a *C. rutilus* colony in the Beijing region. Of these, 22 bacterial and 14 fungal isolates were selected for sequencing and phylogenetic analysis, based on their growth characteristics and colony morphology. Using 16S rRNA gene se-quence analysis, the bacterial isolates were divided into two monophyletic clusters which had significant hits to the genera *Bacillus* and *Pseudomonas*, respectively. Using internal transcribed spacer (ITS) sequence analysis, fungal isolates were divided into four monophyletic clusters: *Penicillium, Trichoderma, Mortierella,* and *Bionectria*. Moreover, the phylogenetic diversity of these isolates was analysed. The results indicated that numerous microorganisms were present in *C. rutilus* habitat. This was the first reported examination of the microbiological ecology of *C. rutilus*.

Safavi (2010) isolated a new isolate (BEH) of entomopathogenic fungus, *Beauveria bassiana* was isolated from soil using DOC2 selective medium. This isolate was characterized by conidiophores consisting of whorls and dense clusters of short conidiophorous cells with one-celled spherical conidia. Colonies on SDYA medium were normally white to pale yellow and sometimes red pigmented in reverse. Because of importance of this pathogen in biocontrol programs around the world and difficulties with morphological identification, a molecular technique was developed to assist complementary identification of the fungus. Pr1, a pathogenicity-related alkaline cuticle-degrading serine protease, with defined sequence in *B. bassiana* was amplified using PCR technique. The presence of this gene in isolated fungus (BEH) with 744 bp sequence length, as visualized on agarose gel affirmed the data from morphological studies that the new isolate (BEH) pertained to entomopathogenic fungus, *B. bassiana*. Pathogenicity of new isolate against *Tenebrio molitor* and its recovering was the other confirmation that the isolated fungus belonged to *B. bassiana*, using further light microscope studies.

Durowade *et al* .(2008) carried out on the ascomycetous fungi present in six different but carefully selected sites on the University of Ilorin permanent site soil. Fungi isolation was done by the soil dilution method incubated at 27°C for 72 hours. The predominant *Ascomycetes* fungi isolated include among others; *Aspergillus niger, Fusarium solani, Fusarium oxysporum, Penicillium italicum, Fusarium acuminatum, Fusarium culmorum, Candida albicans, Botrytis cinerea, Geotrichum candidum, Trichoderma viride, Verticillium lateritum, Curvularia palescens, Penicillium griseofulvum, Penicillium janthinellum, Penicillium chrysogenum, Aspergillus terreus,*

Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus clavatus, Cladosporium resinae, Alternaria alternata, Trichothecium roseum, Phialophora fastigiata, Aspergillus nidulans, Aspergillus wentii, Humicola grisea, Trichophyton rubrum, Helminthosporium cynodontis, Penicillium funiculosum, Penicillium purpurogenum, Saccharomyces cerevisiae, Trichoderma harzianum, Scopulariopsis candida.

Kurek *et al.* (2007) isolated saprotrophic filamentous microfungi by means of the soil dilution method from soil samples collected from four locations in the Bellsund region of Spits− bergen ($77^0$33'N, $14^0$31'E) representing the following forms of surface micro−relief: an old stormbank, a sorted circle, a frost fissure between tundra polygons, and the central part of a tundra polygon. The fungal isolates were identified and screened for their ability to grow at low temperatures. The oligotrophy of psychrophilic and psychrotrophic strains was then determined as the ability of growth on silica gel without a C source added. Differences in some physico−chemical properties were found between the soils sampled from the four sites. A total of 89 taxa from 17 genera were isolated. Most of the isolates were species of *Mortierella*, *Penicillium*, *Chrysosporium* and *Phialophora*, and half of them were psychro− philes. Fungal communities isolated from a frost fissure between tundra polygons (site 3) and from the central part of a tundra polygon (site 4) were dominated by psychrophiles but those isolated from an old stormbank (site 1) and a sorted circle (site 2) were predominantly psychrotrophic. Oligopsychrophilic taxa accounted for 27% and oligopsychrotrophic for 20% of all the isolated taxa but only from 0.7% to 11.7% and from 1.2% to 6.3% of the total number of cfu (colony forming unit) isolated from an individual site, respectively.

Anastasi *et al.* (2005) illustrated the qualitative and quantitative composition of the mycoflora of both a green compost (thermophilically produced from plant debris) and a vermicompost (mesophilically produced by the action of earthworms on plant and animal wastes after thermophilic preconditioning). Fungi were isolated using three media (PDA, CMC, PDA plus cycloheximide), incubated at three temperatures (24, 37 and 45 C). Substantial qualiquantitative differences in the species composition of the two composts were observed. The total fungal load was up to 8.2 3 105 CFU/g dwt in compost and 4.0 3 105 CFU/g dwt in vermicompost. A total of 194 entities were isolated: 118 from green compost, 142 from vermicompost; 66 were common to both. Structural characterization of this kind is necessary to determine the most appropriate application of a compost and its hygienic quality.

Azaz (2003) described the microfungal flora of field soils irrigated by the South-eastern Anatolia Project (GAP: G.neydoÛu Anadolu Projesi) in Harran Plain were investigated in terms of quality and quantity, using the soil dilution plate and soil washing methods. A total of 1690 microfungi were isolated from 105 soil samples. With the identification of these isolates, 109 species plus 16 different sterile fungi were identified. Sixty-two of these taxa were isolated through the soil dilution plate method, seven through the soil washing method, and 40 through both methods. The results indicate that ten of these species belong to Mucorales, four to Sphaeriales, one to Coelomycetes and 94 to Hyphomycetes. The most widespread genera were *Penicillium* Link ex Gray (24 species), *Aspergillus* Mich. ex Fr. (20 species), and *Acremonium* Link ex Fr. with the soil dilution plate method.

The most common species were *Aspergillus niger* Tiegh. (284 colonies), *Penicillium lanosum* Westling (238 colonies), *Penicillium canescens* Sopp. (170 colonies), *Penicillium brevicompactum* Dierckx (174 colonies) and *Penicillium clavigerum* Demelius (146 colonies). The results obtained from the soil dilution plate method show that fresh soil bulk equivalent to 1 g of oven-dried soil contains on average 72 487 propagules.

Rabeendran *et al.* (1998) carried out to identify fungi with biocontrol activity against *Sclerotinia sclerotiorum* on cabbage. Sixty three fungal isolates from 10 vegetable sites in Canterbury, and four isolates with known biocontrol activity from culture collections, were evaluated using a cabbage petiole bioassay for their ability to inhibit colonisation of host tissue by *S. sclerotiorum*. Twenty seven isolates reduced infection of cabbage petioles and subsequent sclerotial production by *S. sclerotiorum* compared to the pathogen control. Eight isolates (five *Trichoderma* sp., two *Gliocladium virens* and one *G. roseum*) reduced petiole infection by more than 75% and completely prevented sclerotial production.

Henderson (1961) performed research to isolate from soil under several vegetational types by an enrichment technique with vanillin or p-hydroxybenzaldehyde as sole source of carbon. Although similar morphologically, the isolates obtained are classified in two separate groups, yeasts and hyphomycetes. A study was made of the growth in pure culture of representative species, namely, *Pullularia pullulans*, *Margarinomyces heteromorpha* and *M . mutabilis* on several aromatic compounds related to lignin.

2.2 Extraction of nematode from the soils of different regions

Hossain *et al.* (2016) conducted three experiments to find out the feeding type and age structure of nematodes in different soil habitats; to know the efficiency of different extraction techniques of nematodes and to observe the hatching behavior of *Meloidogyne* juvenile in a Baermann device. Bacterivore, Omnivore, Predator, Fungivore and Plant Parasitic nematodes were recorded in the soil of flower, vegetable, moss, grass, and corn and compost habitats. Bacterivore was the highest feeder (67.5%) in the soil habitats. In comparison of nematode extraction methods, Seinhorst method extracted more cysts (188), but less juvenile (34) and eggs (18) from cysts than stirring (145, 87 and 50, respectively) while AZC was found superior (936) over Cobb method (204) in extracting nematodes. The AZC method also extracted huge juvenile number from roots which was higher than Baermann funnel method. In Baermann funnel, juvenile hatched more in favorable condition than unfavorable condition which led to an increasing and decreasing pattern of hatching.

Erbaş *et al.* (2014) investigated an experiment for entomopathogenic nematodes in various agricultural fields in the Eastern Black Sea region of Turkey. A total of 77 soil samples were collected from 15 distinct geographic areas during 2010–2011. Seven entomopathogenic nematode isolates (ZET02, ZET04, ZET09, ZET28, ZET31, ZET35, and ZET76) were detected from the soil samples (9.1% positive) using the Galleria baiting technique. Morphological and molecular characterizations of the isolates were performed for species identification. Five isolates were identified as *Heterorhabditis bacteriophora* (ZET02, ZET04, ZET09, ZET28, and ZET35) and 2 isolates were identified as *Steinernema feltiae* (ZET31 and

ZET76). The efficacy of all isolates was tested on *Melolontha melolontha* larvae in plastic boxes and pot experiments. Different concentrations of nematodes at 0, 500, 1000, or 2000 infective juveniles (IJs)/mL and 2 different temperature regimes (15 and 25°C) were used. One hundred percent mortality was obtained from the ZET09 and ZET35 isolates at a concentration of 2000 IJs/mL at 25°C. The same isolates also provided 100% protection with 100 IJs/cm2 in strawberry planted pot experiments.

Mondino *et al.* (2014) examined four strategies (Tris/EDTA, sodium dodecyl sulfate, Chelex 100 resin and cetyltrimethylammonium bromide -CTAB-) for extracting nucleic acid (DNA) from communities of nematodes. Nematodes were isolated from an agricultural area under different management of long-term crop rotation experiment from Argentina during three seasons. After DNA extraction, Polymerase Chain Reaction-amplifications were performed and considered as indicators of successful DNA extraction. The CTAB combined with proteinase K and phenol-chloroform-isoamyl alcohol was the unique successful method because positive amplifications were obtained by using both eukaryotic and nematode specific primers. This work could contribute to biodiversity studies of nematodes on agroecosystems.

Laura (2013) isolated nematodes from seventeen towns within the Central Massachusetts region, from five separate sub-regions. It was found that nematodes could, in fact, be isolated from each of the seventeen towns through one or both isolation methods, but abundance of nematodes and time to isolate the nematodes varied from location to location.

Environmental factors from isolation sites were also noted. The most attention was given to soil pH conditions for each town. It was found that there is a certain pH range most optimal for nematode isolation falling within 4.51-5.00. After isolation, PCR was completed on single worms to amplify the DNA using the Small Subunit rRNA molecular marker (SSU). Sequencing was then done on the amplified nematode DNA samples from Hopedale and two *Pristionchus* species were found: *P. marianneae* and *P. pauli*.

Kumar *et al.* (2012) stated that nematodes cause lot of damage to crops, in order to identify and control them the proper quantification of its population in the soil and/or plant should be known through the use of different extraction methods. These extraction methods were used and compared for suitability in terms of the life stages of nematodes extracted from soil and plant material. Seinhorst method is found to be the most appropriate technique for extraction of cyst nematode, while the Automatic Zonal Centrifugation was the most suitable in extracting mostly all stages of the nematodes from both soil and plant materials. Different techniques have its own advantages and disadvantages in which the choice of the desired technique depends mainly on the target stage of the nematode that an individual will be working with or to the purpose of extraction.

Deng *et al.* (2008) established an new technique to extract nematode, which is based on an iso-osmotic densitygradient medium (OptiPrepTM). This technique resulted in significantly higher numbers of clean eggs and vermiform nematodes that retain higher viability (48.6%) than samples processed with the sucrose method (28.7%). Nematodes survived exposure

to OptiPrepTM for 22 hours without significant mortality whereas all nematodes died in the sucrose medium. OptiPrepTM provided a suitable, non-toxic alternative to the traditional density gradient material for the isolation of nematodes. This technique is convenient and relatively simple, with the added benefit of yielding cleaner samples compared to traditional isolation techniques.

Sturhan and Mráček (2000) performed a study with the forty soil samples from forests and other biotopes in Germany and the Czech Republic for the presence of entomopathogenic nematodes using the *Galleria* bait method at the same time as a sieving-decanting method for direct extraction of infective-stage juveniles. All five species were recovered with both methods, but the baiting technique was generally less effective and mixtures of species were frequently undetected. The direct extraction method provided quantitative estimates of infective-stage juvenile density but no information on their infectivity or on morphological characters of adults, and nematode cultures could not be established.

CHAPTER III
MATERIALS AND METHODS

3.1 Survey location and collection of samples

Soil samples were collected from different locations (Manikgonj, Gaibanda, Comilla, Chandpur, Narayangonj, Rajshahi and Munshigonj) of North-western regions of Bangladesh in 2016 to 2017 (November to April), and put in a polyethylene bags (Malik *et al.,* 2005). Samples were brought to the laboratory of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.2 Preparation of samples

The collected samples were taken to the central laboratory of Plant Pathology of Sher-e-Bangla Agricultural University for the preparation of soil to complete the dilution procedure. First, the all sorts of debris and waste materials were removed from the samples and then crushed soil or made it as like as powder to dissolve into water easily.

3.3 Sterilization technique

Petri plates, conical flasks, test-tubes and other glassware were sterilized in oven at 165-170^{0}C for 2-3 hrs. autoclave. For sterilization purpose all apparatus were autoclaved for 30 minutes at 121°C. After autoclaving all sterilized material was dried in an oven at 90°C.

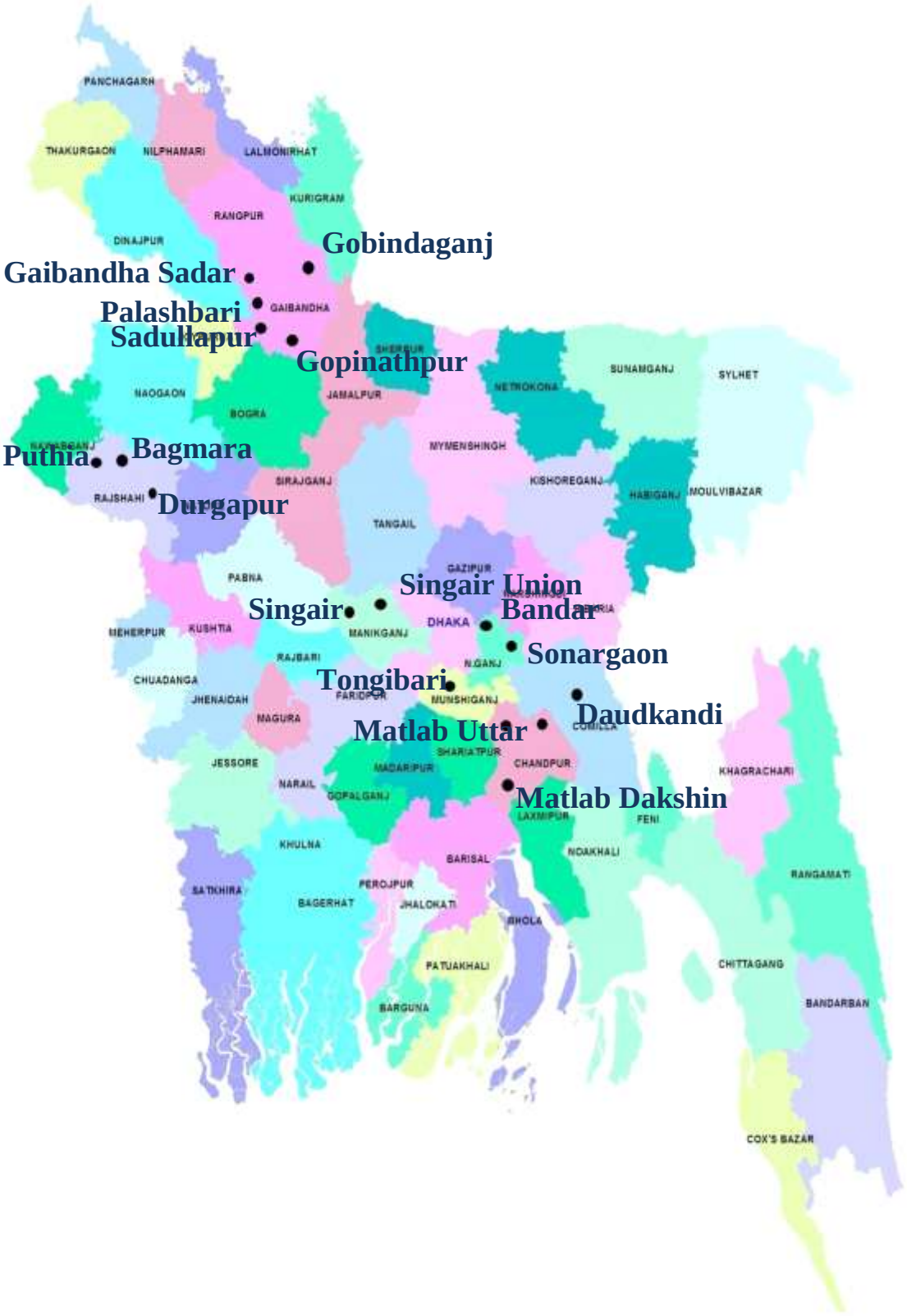

Fig. 1. Survey upazillas of different districts in Bangladesh.

3.4 Dilution preparation

The purpose of serial dilution was to the colonies of fungi. One ml of sewage water was taken from each sample. Serial dilution was set up by carefully taking the 10 ml of distilled water in glass test tubes.

Then these test tubes were autoclaved for 30 minutes at 121°C. From the sample of sewage water 1 ml was dissolved in 10 ml of sterile distilled water in test tubes to give (1:10) and shaked well. The test tubes 2 was inoculated with 1 ml from bottle 1 to give 1:100 dilutions. Test tubes 2 was also shaked well. Test tubes 3 was inoculated with 1 ml from bottle 2 to give 1:1000 dilutions. Test tubes 4 was inoculated with 1 ml from bottle 3 to give 1:10000 dilutions. To complete the serial dilution micropipette was used with sterilized tips. Estimation of fungal population was done by standard spread plate dilution method described by Seeley and Van (1981) in triplicates.

3.5 Media preparation

Potato Dextrose Agar (PDA) media was used for fungal cultures growth (Razak *et al.*, 1999). Two hundred grams of potato were peeled, sliced, boiled and then sieved through a clean Muslin cloth to get a broth in which agar and glucose were added. The media was then autoclaved for 30 minutes at 121°C.

3.6 Preparation of soil dilution plate

A sample from an accurate dilution of microbes/sample is pipetted onto a Petri - dish, then agar medium is poured over the liquid and mixed.

3.7 Isolation of fungi

Spread plate technique was used for enumeration of fungi from given samples. From each test tube 0.5 ml of sample was taken separately with the

help of micropipette along with sterilized tips. Then these diluted samples were inoculated on sterile PDA plates with the help of micropipette and L shape rod was used to spread the diluted sample on the PDA plate. The same step was repeated with all other sewage water samples. Then these plates were incubated at 30°C for 3 days and then the colonies were counted (Adesemoye *et al.,* 2006).

3.8 Identification of fungi

The cultures were identified at genus level on the basis of macroscopic (colonial morphology, color, texture, shape and appearance of morphology) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia) (Zafar *et al.,* 2006).

3.9 Extraction of nematode

The extraction of nematodes from soil was done by using a Whitehead and Hemming tray method (1965) as follows: Soil was mixed thoroughly and different samples of 100 g soil was weighted and put it on the sieve that was on a bowl filled with water. The upper portion of sieve was lined with three layers of kitchen tissue paper. After 5 days the nematode suspension was collected in a beaker and left for a day, excess water was discarded leaving 100 ml su*sp*ension and 5 ml sub sample was taken and put into a counting dish. Juveniles counting were done by using a compound microscope.

3.10 Isolation of bacteria

Potato tuber are surface sterilize by dipping. 5% sodium hypochlorite for 2-5 minutes. After surface sterilization the material should be rinsed few times in sterile distill water to remove all traces of disinfectant. Washing the material in running tap water and left to dry before isolation.

3.11 Test of bacteria

Preparation of Triphenyl Tetrazolium Chloride (TTC) Medium

TTC is the standard selective media for the proper growth of *R. solanacearum* (Kelman, 1954 and Schaad, 1988). The medium was prepared by dissolving peptone 10gm, glucose 10gm, casamino acid (i.e., casein hydrolysate) 1gm and agar 18gm in 1000 ml of distilled water. The p^H of the medium was adjusted to 7.0 using 0.1N KOH and cooked on hot plate. After cooking, the medium was autoclaved for 20 minutes at 121^0c under 1.1kg/cm^2 pressure. The aqueous solution of Triphenyl Tetrazolium Chloride (TTC) agar was prepared by dissolving 1gm of chemical in 100 ml (i.e. 1% w/v) distilled water in Erlenmeyer flask. The TTC solution was separately sterilized in an autoclave at 121^0c under 1.1kg/cm^2 pressure for 20 minutes. The sterilized TTC solution was poured into the sterilized medium at the rate of 5ml/1000ml and mixed thoroughly. The medium was poured in petridishes at the rate of 20ml/plate. One loopful of the suspension was streaked in each plate on the surface of the solidified selective TTC medium and incubated at 30^0c for 48 hours.

3.12 Statistical analysis

The theory behind the technique of CFU establishes that a single microbe can grow and become a colony via division. These colonies are clearly different from each other, both microscopically and macroscopically. The number of colonies per plate in 1g of soil was calculated. The percent contribution of each isolate was calculated by using the following formula:

$$\% \text{ Contribution} = \frac{\text{Total no. of CFU of an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

* CFU-Colony Forming Unit

CHAPTER IV

RESULTS AND DISCUSSION

An experiment was carried out in the laboratory of the Department of Plant Pathology in Sher-e-Bangla Agricultural University (SAU) to study the presence of different fungi, bacteria and nematode into the rhizosphere soil of potato collected from different potato growing locations of Bangladesh. The results are discussed and interpreted under the following subheads.

4.1 Identification of *Alternaria solani*

The several temporary and semi permanent slides were prepared to observe the micro organisms under microscope. The *Alternaria solani* was identified to the genus level on the basis of macromorphological characteristics by using PDA media and slide cultures during working in the laboratory**.** The structures of *Alternaria solani* was observed under compound microscope (Motic,BA210)(Fig.2).

Morphological observations of the fungus were recorded by adopting slide culture technique. Initially, the mycelium was hyaline that turned to grey-brownish, multicelled, septate and irregularly branched. Fungus colonies were dark to grey-black and conidiophores arising singly or in small groups produced spores in chains. Conidiospores were large with longitudinal and transverse septa and a short beak typical for *Alternaria solani* under compound microscope. The results of the present study were in accordance with the results of Rohilla and Salar (2012). They found conidia of *Alternaria solani* as colonized, obclavate and multicelled to septate.

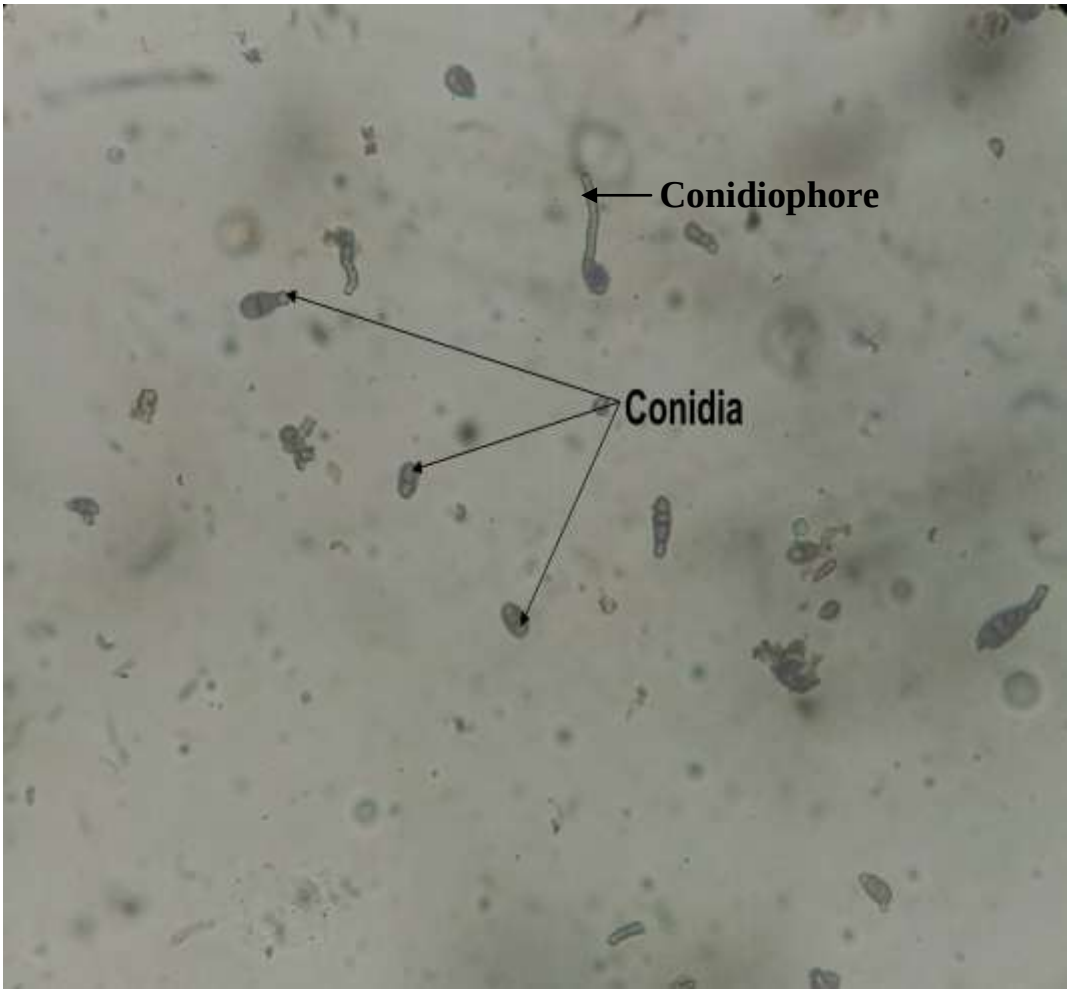

Fig. 2. Conidiophore and conidia of *Alternaria solani* under compound microscope (40x).

4.1.1 Comparison of CFU/g soil of *Alternaria solani* in different potato growing regions of Bangladesh

The maximum CFU/g soil (7×10^3) of *Alternaria solani* was observed from the Manikgonj upazilla, whereas the minimum (3×10^3) in Gopinathpur, Daudkandi and Bandar upazillas(Table.1).

Table 1. Comparison of different locations with the frequency of *Alternaria solani* isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair	7×10^3
Gaibanda	Palashbari	4×10^3
	Gopinathpur	3×10^3
Comilla	Daudkandi	3×10^3
Chandpur	Matlab Uttar	4×10^3
Narayangonj	Bandar	3×10^3
Rajshahi	Durgapur	4×10^3
	Bagmara	5×10^3
Munshigonj	Tongibari	6×10^3

4.2 Identification of *Penicillium* sp.

The *Penicillium* sp. was isolated on PDA and identified to the genus level on the basis of macromorphological characteristics by preparing semi permanent slide cultures during working in the laboratory. The structures of *Penicillium* sp. was observed under compound microscope (Motic,BA210) (Fig.3).

Species of *Penicillium* are identified by their brush-like spore-bearing structures called penicilli (sing.: penicillus). *Penicillum* spp. are filamentous fungi. The conidiophores are simple or branched and are terminated by clusters of flask-shaped phialides. Conidia are round and unicellular. Hyphae may contain internal crosswalls, called septa, that divide the hyphae into separate cells. The spores (conidia) are produced in dry chains from the tips of the phialides, with the youngest spore at the base of the chain, and are nearly always green. The results of the present study were in accordance with the results of Azaz (2003), Magnet et al (2013). They observed *Penicillium* sp. as the brush-like spores and filamentous fungi and identified *Penicillium* sp. fungus from the garden soil and described the isolated *Penicillium* sp. *as* flask-shaped phialides.

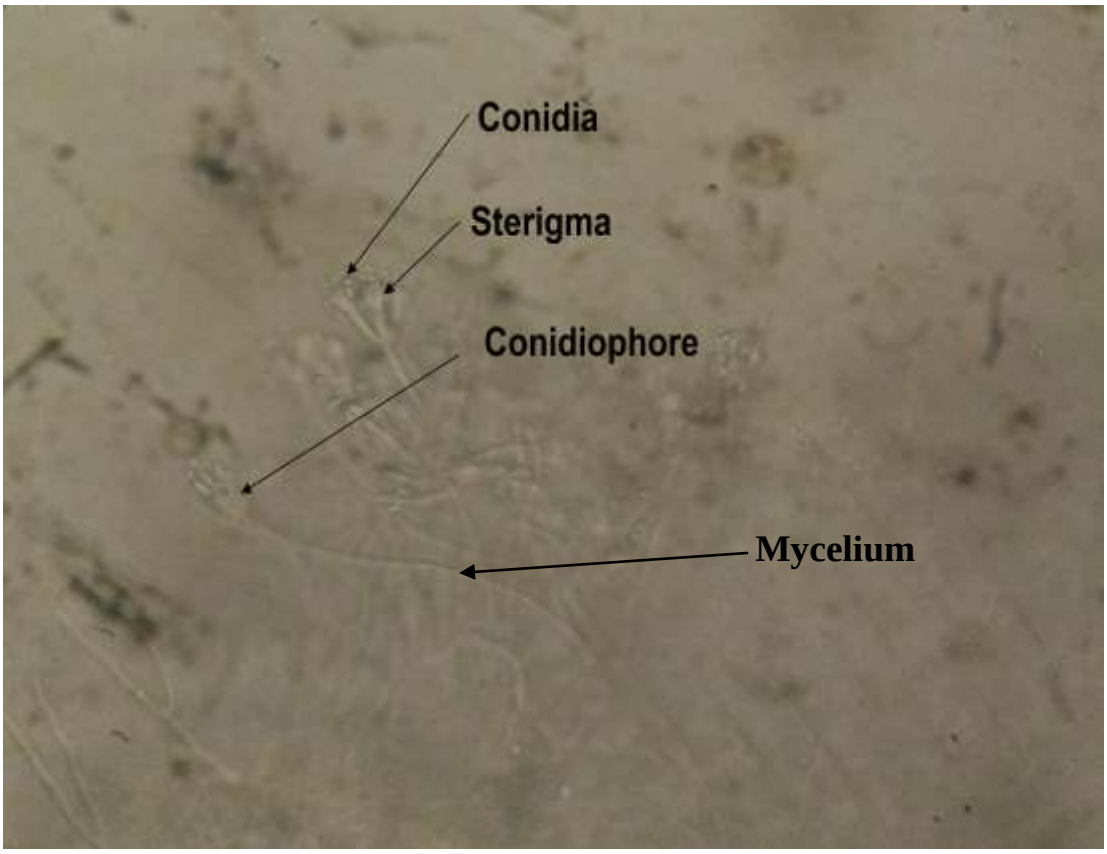

Fig. 3. Mycelia, coniodiophore and conidia of *Penicillium* sp. under compound microscope (40x).

4.2.1 Comparison of CFU/g soil of *Penicillium* sp. in different potato growing regions of Bangladesh

The maximum CFU/g soil (11×10^3) and the minimum (3×10^3) of *Penicillium* sp. were observed in Singair and Matlab Uttar upazilla, respectively (Table.2).

Table 2. Comparison of different locations with the frequency of *Penicillium* sp. isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair	11×10^3
Gaibanda	Sadullapur	7×10^3
Chandpur	Matlab Uttar	3×10^3
Rajshahi	Puthia	4×10^3

4.3 Identification of *Aspergillus niger*

The *Aspergillus niger* was identified to the species level on the basis of macromorphological characteristics using PDA media and slide cultures during working in the laboratory. The structures of *Aspergillus niger* were observed under compound microscope (Motic,BA210) and were shown in (Fig.4).

The colony consists of mats of hyphae that make up a mycelium. The hyphae are septate and hyaline. Conidia produced on specialized hyphae called condiophores. The results of the present study were in accordance with the results of Azaz (2003), Durowade *et al.* (2008), Jasuja *et al.* (2013).They reported this soilborne fungi from the agricultural soil in Turkey and characterized the hyphae are septate and hyaline and carried out by culturing on potato dextrose agar media and identified the ascospores of *Aspergillus niger.*

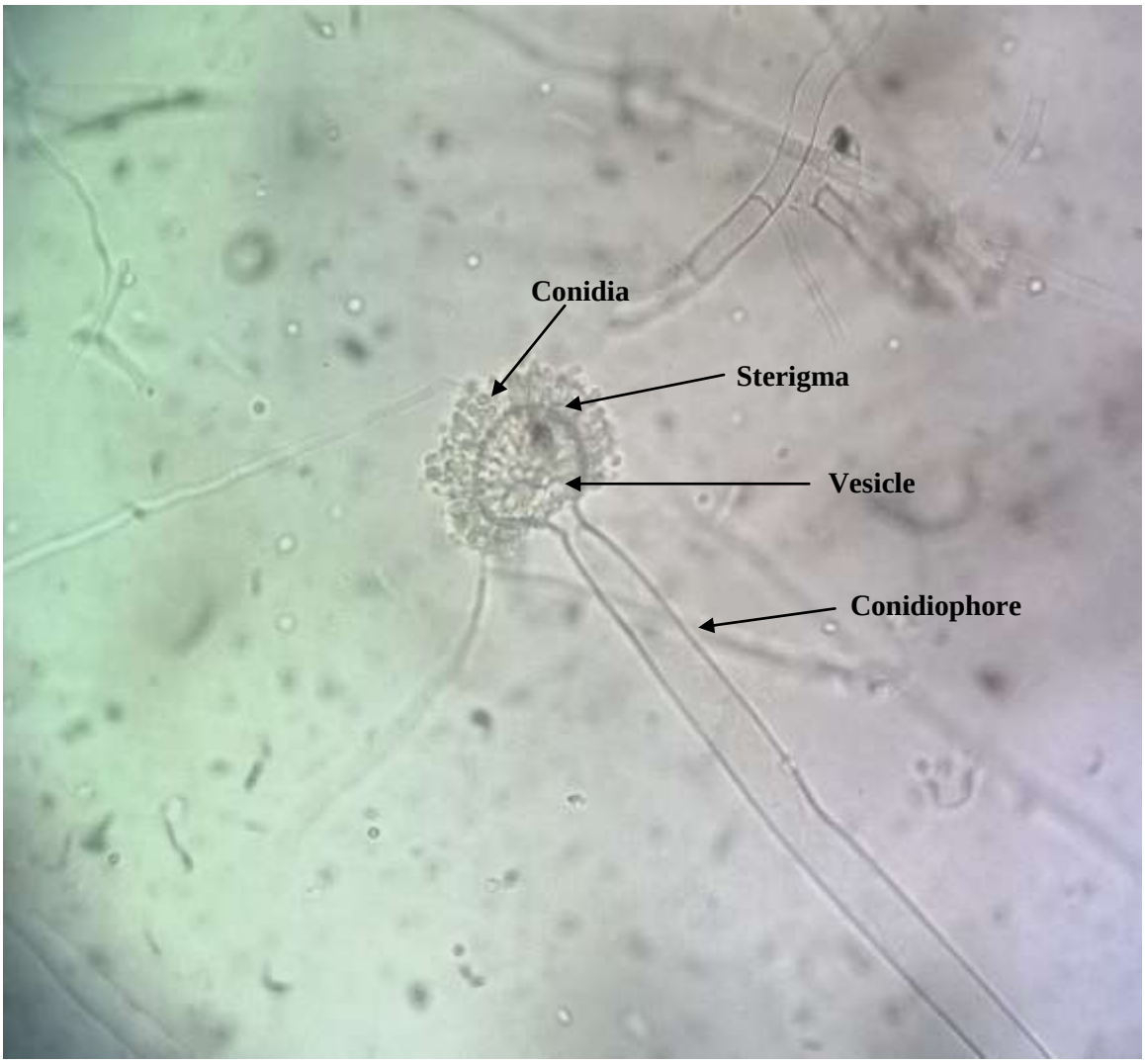

Fig. 4. Conidiophore and conidia of *Aspergillus niger* under compound microscope (40x).

4.3.1 Comparison of CFU/g soil of *Aspergillus niger* in different potato growing regions of Bangladesh

The maximum CFU/g soil (14×10^3) was obserfved from the upazillas of Tongibari and Singair. The minimum (4×10^3) of *Aspergillus niger* was found in Singair upazilla (Table 3).

Table 3. Comparison of different locations with the frequency of *Aspergillus niger* isolation (CFU/g soil)

Districts	**Upazillas**	**CFU/g soil**
Manikgonj	Singair	14×10^3
Gaibanda	Gobindaganj	11×10^3
	Sadullapur	8×10^3
Comilla	Daudkandi	8×10^3
Chandpur	Matlab Uttar	4×10^3
Narayangonj	Bandar	8×10^3
	Sonargaon	13×10^3
Munshigonj	Tongibari	14×10^3

4.4 Identification of *Rhizopus stolonifer*

The *Rhizopus stolonifer* was identified to the species level on the basis of macromorphological characteristics using PDA media and slide cultures during working in the laboratory. The structures of *Rhizopus stolonifer* were observed under compound microscope (Motic,BA210) and were shown in (Fig 4).

Colonies grow rapidly and resemble like as cotton candy. Colonies were gray and darken with age. Mycelia are marked by numerous stolons connecting groups of long sporangiophores. Sporangiophores are usually unbranched, long, and terminate in a columella and a dark round sporangium containing oval colorless to brown spores. Stolons bear large rhizoids which are found immediately adjacent to the sporangiophore in the nodal position. Columella and sporangium collapse easily after discharging spores. The results of the present study were in accordance with the results of Gaddeyya *et al.* (2012) , Jasuja *et al.* (2013).They carried out their study by culturing on potato dextrose and microscopic method and analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India.

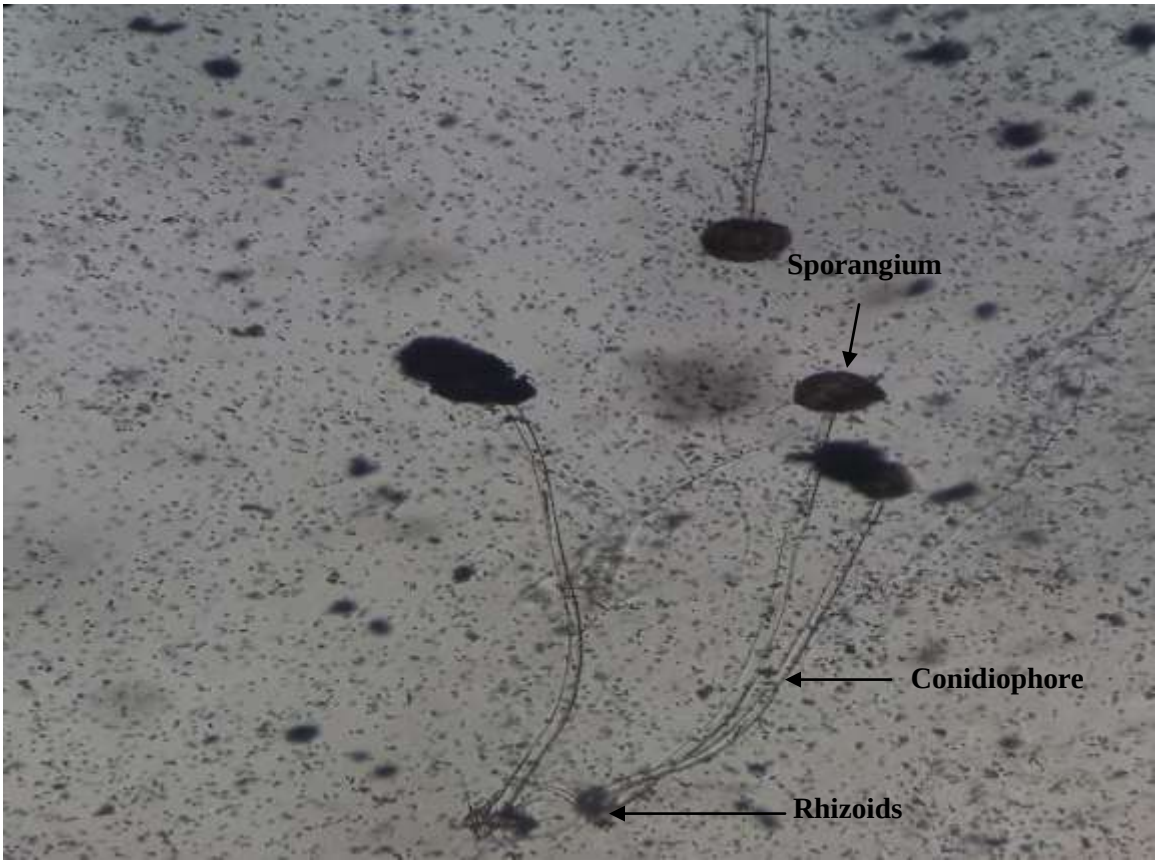

Fig. 5. Structures of *Rhizopus stolonifer* under compound microscope (40x).

4.4.1 Comparison of CFU/g soil of *Rhizopus stolonifer* in different potato growing regions of Bangladesh

The maximum CFU/g soil (16×10^3) and the minimum (8×10^3) of *Rhizopus stolonifer* were found in Tongibari and Puthia upazillas, respectively (Table .4).

Table 4. Comparison of different locations with the frequency of *Rhizopus stolonifer* isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair Union	13×10^3
Gaibanda	Gaibandha Sadar	11×10^3
	Gopinathpur	9×10^3
Rajshahi	Puthia	8×10^3
Chandpur	Matlab Dakshin	9×10^3
Narayangonj	Bandar	11×10^3
Munshigonj	Tongibari	16×10^3

4.5 Identification of *Bipolaris* sp.

Semi permanent slides were prepared to observe *Bipolaris* sp. under compound microscope. The *Bipolaris* sp. was identified to the genus level on the basis of macromorphological characteristics isolated on PDA media and slide cultures during working in the laboratory. The morphology of *Bipolaris* sp. were shown in Fig. 6.

Colonies are moderately fast growing, effuse, grey to blackish brown, suede-like to floccose with a black reverse. Microscopic morphology shows sympodial development of hyaline to deep olivaceous pigmented, pseudoseptate conidia on a geniculate or zig-zag rachis. Conidia mostly straight, elliptical, 2–14 distoseptate (usually more than 6), germinating only from the ends (bipolar). A field research was conducted by Nagrale *et al*. (2013) to isolate and characterize the *Bipolaris* sp. from gerbera plants in Maharastra. He found the conidia on a geniculate or zig-zag rachis and mostly straight and ellipsoidal .

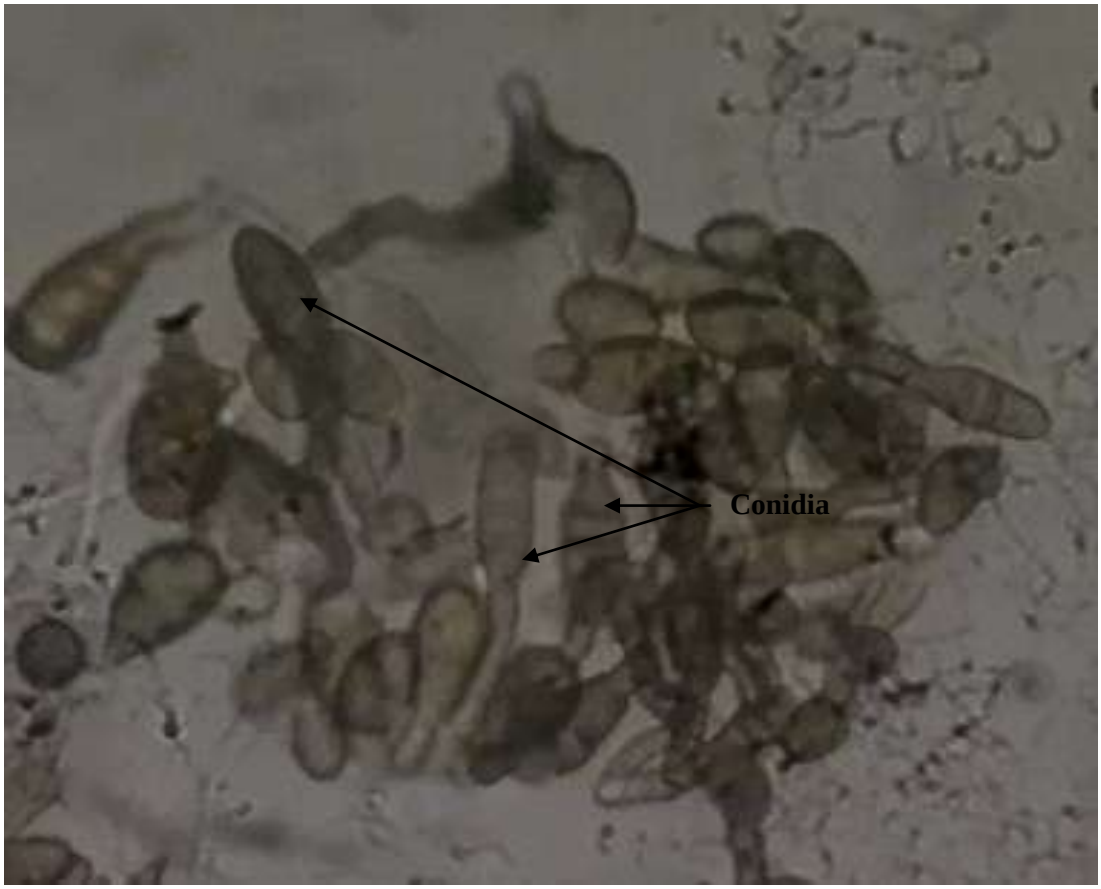

Fig. 6. Conidia of *Bipolaris* sp. under compound microscope (40x).

4.5.1 Comparison of CFU/g soil of *Bipolaris* sp. in different potato growing regions of Bangladesh

Bipolaris sp. was observed with the highest CFU/g soil (13×10^3) from Singair Union and lowest (2×10^3) CFU/g soil in Gobindaganj and Palashbari upazilla, respectively(Table 5).

Table 5. Comparison of different locations with the frequency of *Bipolaris* sp. isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair Union	13×10^3
Gaibanda	Gobindaganj	2×10^3
	Gaibandha Sadar	3×10^3
	Palashbari	2×10^3
Comilla	Daudkandi	4×10^3
Rajshahi	Puthia	4×10^3

4.6 Identification of *Phytophthora infestans*

The several semi permanent slides were prepared to observe the micro organisms under compound microscope (Motic,BA210). The *Phytophthora infestans* was identified to the genus level on the basis of macromorphological characteristics using PDA media and slide cultures during working in the laboratory. The morphology of *Phytophthora infestans* were shown in (Fig.7).

The pathogen produced hyaline lemon shaped sporangia arose straightly from the surface of the substrate. Asexual (mitotic) spore types are chlamydospores, and sporangia which produce zoospores. Also, sporangia may release zoospores, which have two unlike flagella which they use to swim towards a host plant. Sporangiophores were irregularly branched singly or in a loose sympodium with a swelling at the point of branching.

Direct isolation from soil on many of the common agar media used for isolating soil fungi has long been unsuccessful for species of *Phytophthora*, one of the most destructive fungal pathogens (Tsao & Ocana, 1969). The identified the hyaline lemon shaped sporangia arose straightly. And Edinger-Marshall (2016) investigated the seasonal tracking of *Phytophthora* recovery from a variety of soil and forest types in northwestern California. He found that the sporangia release zoospores, which have two unlike flagella.

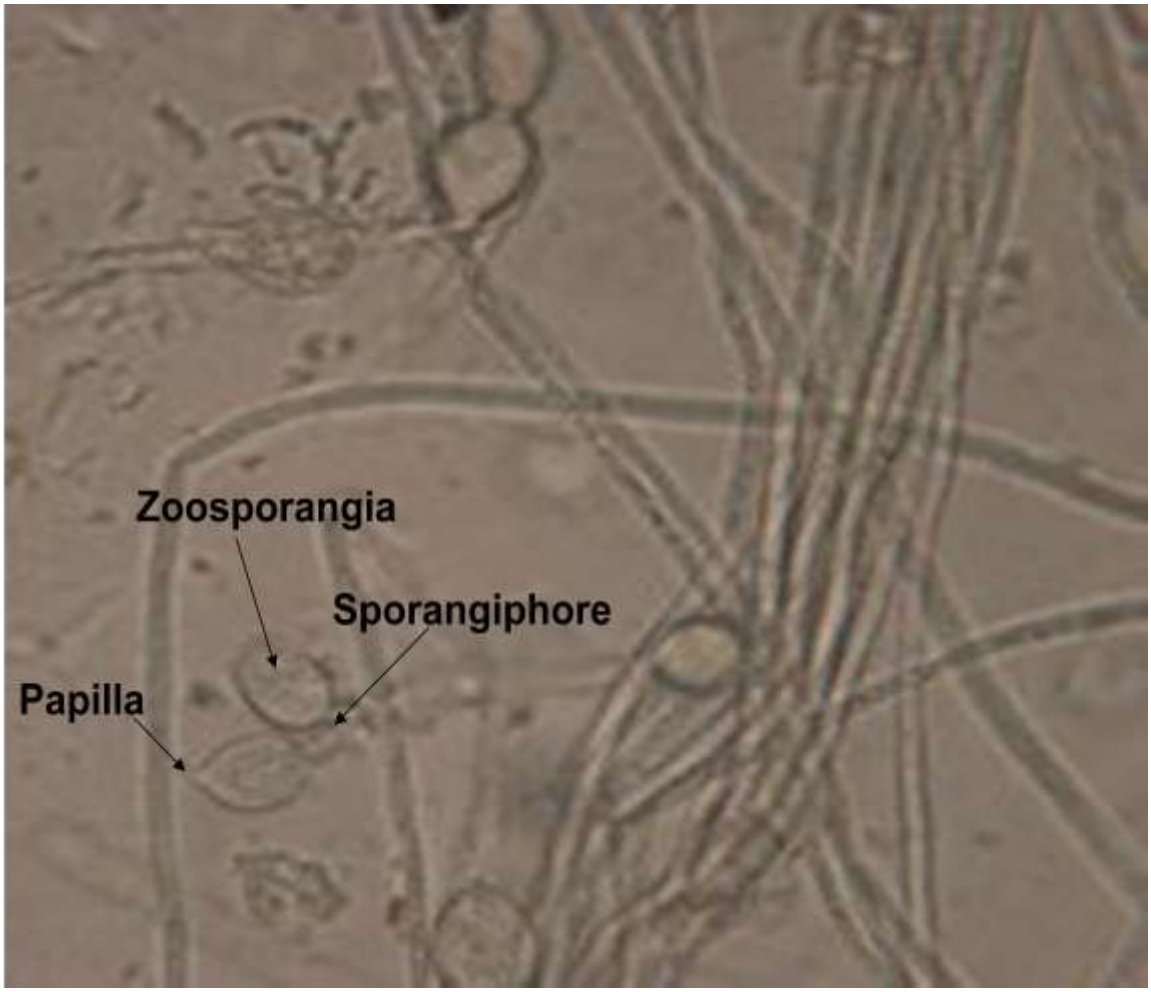

Fig. 7. Morphology of *Phytophthora infestans* under compound microscope (40x).

4.6.1 Comparison of CFU/g soil of *Phytophthora infestans* in different potato growing regions of Bangladesh

The maximum CFU/g soil (8×10^3) from Tongibari and the minimum (3×10^3) of *Phytophthora infestans* were observed in Singair, Gopinathpur and Sonargaon upazilla, respectively(Table 6).

Table 6. Comparison of different locations with the frequency of *Phytophthora infestans* isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair Union	3×10^3
Gaibanda	Gobindaganj	4×10^3
	Gopinathpur	3×10^3
Rajshahi	Bagmara	3×10^3
Chandpur	Matlab Dakshin	4×10^3
Narayangonj	Sonargaon	3×10^3
Munshigoj	Tongibari	8×10^3

4.7 Identification of *Fusarium oxysporum*

The several semi permanent slides were prepared to observe the *Fusarium oxysporum* under microscope (Motic,BA210). The *Fusarium oxysporum* was isolated on PDA and identified to the species level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory. The morphology of *Fusarium oxysporum* were shown in Fig.8.

Hyphae are hyaline are septate and showed divisions or walls within the hyphae. Conidiophores are short and usually non-septate and are produced singly as they extend from the aerial mycelium. The feature of conidiogenous cell with branched and long monophialides were commonly observed. The results of the present study were in accordance with the results of Rohilla and Salar (2012) and Jasuja *et al.* (2013). They isolated and characterized *Fusarium oxysporum* from agricultural soil in India. They described the morphology of *Fusarium oxysporum* as hyaline, branched, septate hyphae having short conidiophores by culturing on potato dextrose agar.

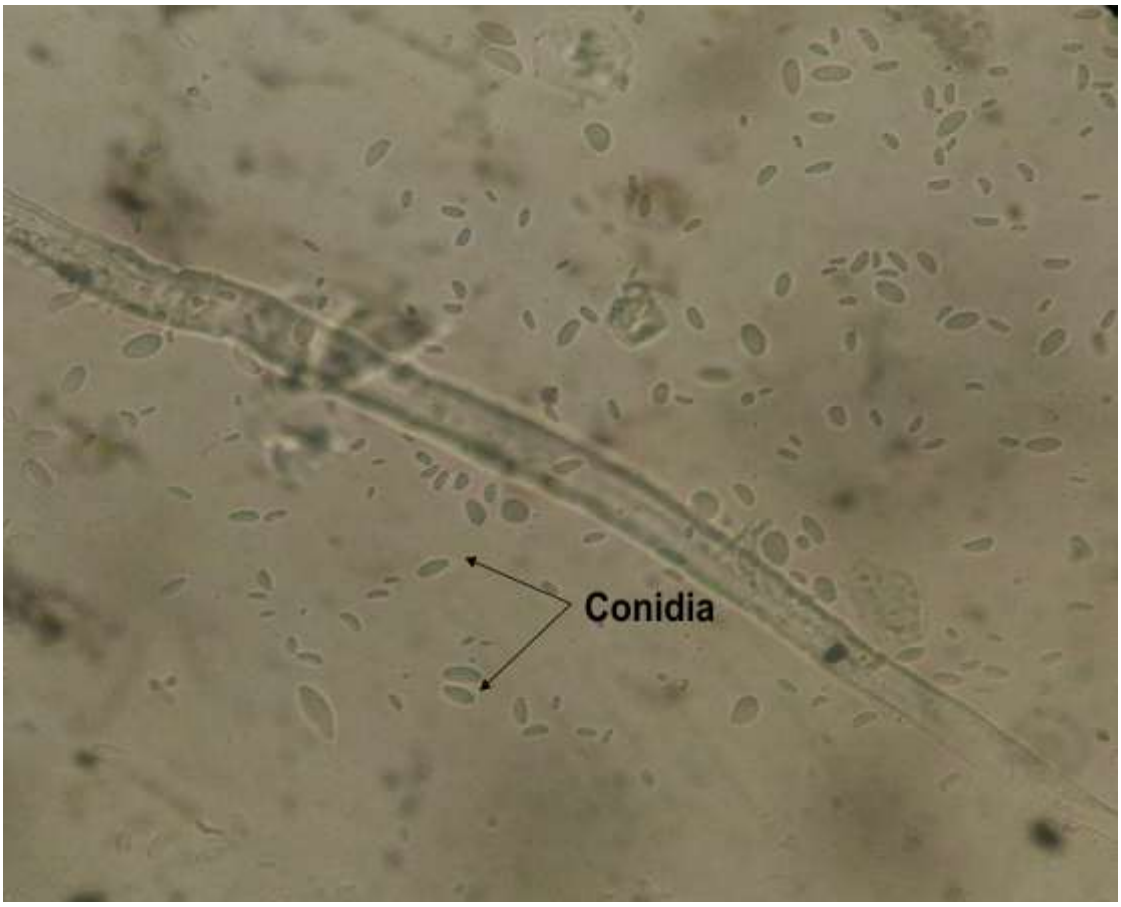

Fig. 8. Conidia of *Fusarium oxysporum* under compound microscope (40x).

4.7.1 Comparison of CFU/g soil of *Fusarium oxysporum* in different potato growing regions of Bangladesh

The highest CFU/g soil (5×10^3) and the lowest (1×10^3) were found *Fusarium oxysporum* in Tongibari and Daudkandi upazilla, respectively (Table 7).

Table 7. Comparison of different locations with the frequency of *Fusarium oxysporum* isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair	3×10^3
Gaibanda	Sadullapur	2×10^3
Comilla	Daudkandi	1×10^3
Rajshahi	Bagmara	3×10^3
Chandpur	Matlab Uttar	3×10^3
Munshigonj	Tongibari	5×10^3

4.8 Identification of *Ralstonia solanacearum*

Ralstonia solanacearum was isolated on TTC media. A part of the single colony was removed by using sterile loop from agar medium and mixed bacteria into KOH solution until an even suspension was obtained. After mixing the loop was lifted slowly from the slide. A mucoid thread was formed. It means the test was positive and the bacterium was Gram negative.The colony of *R. solanacearum* was shown in Fig. 9 .

The virulent wild type colonies were large, elevated, fluidal and either entirely white or with a pale red center on TTC media. And the avirulent mutant colonies were butyrous, deep-red often with a bluish border. Even *R. solanacearum* colonies were white and fluidal with whorls characters. These are the typical characteristics of virulent isolates of the *R. solanacearum* (Hayward, 1964; Hayward, 1976; Mehan and McDonald, 1995). And these results indicated that the procedure of selecting virulent colonies of *R. solanacearum* based on cultural characteristics on TTC was appropriate as suggested by Kelman (1954).

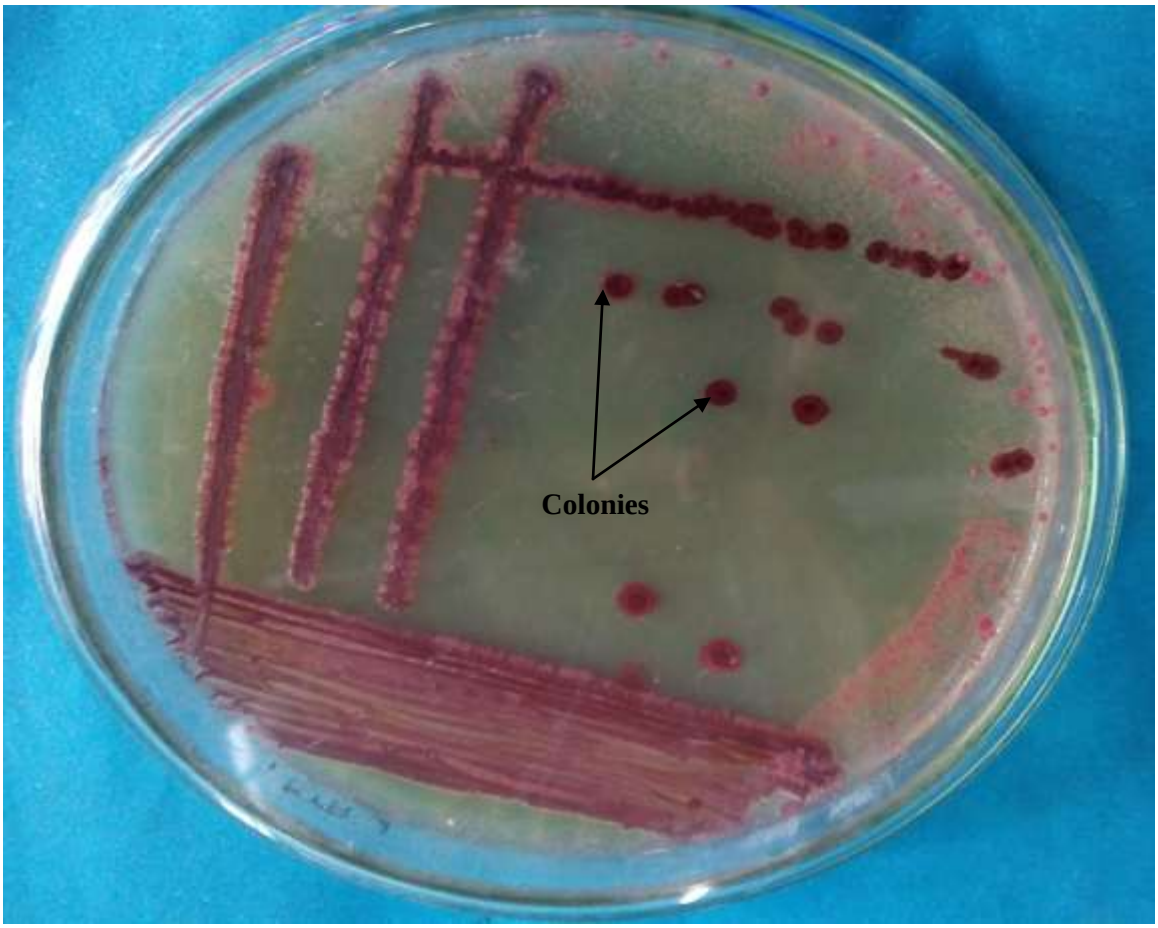

Fig. 9. Colonies of *Ralstonia solanacearum* on TTC media

4.8.1 Comparison of CFU/g soil of *R. solanacearum* in different potato growing regions of Bangladesh

The maximum CFU/g soil (24×10^3) was observed from the upazilla of Tongibari upazilla and the minimum (7×10^3) in Bagmara upazilla, respectively (Table 8).

Table 8. Comparison of different locations with the frequency of *R. solanacearum* isolation (CFU/g soil)

Districts	Upazillas	CFU/g soil
Manikgonj	Singair Union	21×10^3
Gaibanda	Gobindaganj	13×10^3
	Palashbari	7×10^3
Comilla	Daudkandi	16×10^3
Rajshahi	Durgapur	12×10^3
	Bagmara	7×10^3
Chandpur	Matlab Dakshin	12×10^3
	Matlab Uttar	13×10^3
Munshigonj	Tongibari	24×10^3

4.9 Identification of *Meloidogyne* sp.

The nematode was extracted using modified white head tray method. The Nematode was identified to the genus level on the basis of morphological characteristics as observed under stereo microscope. Nematode juvenile is shown in Fig. 10 .

Nematodes are typically elongate, tapered at both ends, and bilaterally symmetrical, 1 mm long. They have cylindrical bodies with no trace of segmentation. The outer elastic cuticles is shed times during the life of the worms. The mouth is at or near the anterior end, and the gut is a straight non-muscular tube with an anus at or near the posterior end. A muscular pharynx with a bulbous swelling towards the end is observable in the microscopic forms. Mondino *et al.* (2014) collected soils samples from the different locations of agricultural land in Brasil and identified the nematode species. The found the *Meloidogyne* sp. and described the morphology as elongate, non-segment and symmetrically bilateral. Sturhan and Mráček (2000) conducted a research with forty soil samples to isolate and identify the harmful *Meloidogyne* sp. from forests. They found the mouth was near the anterior end and the gut was a straight non-muscular tube.

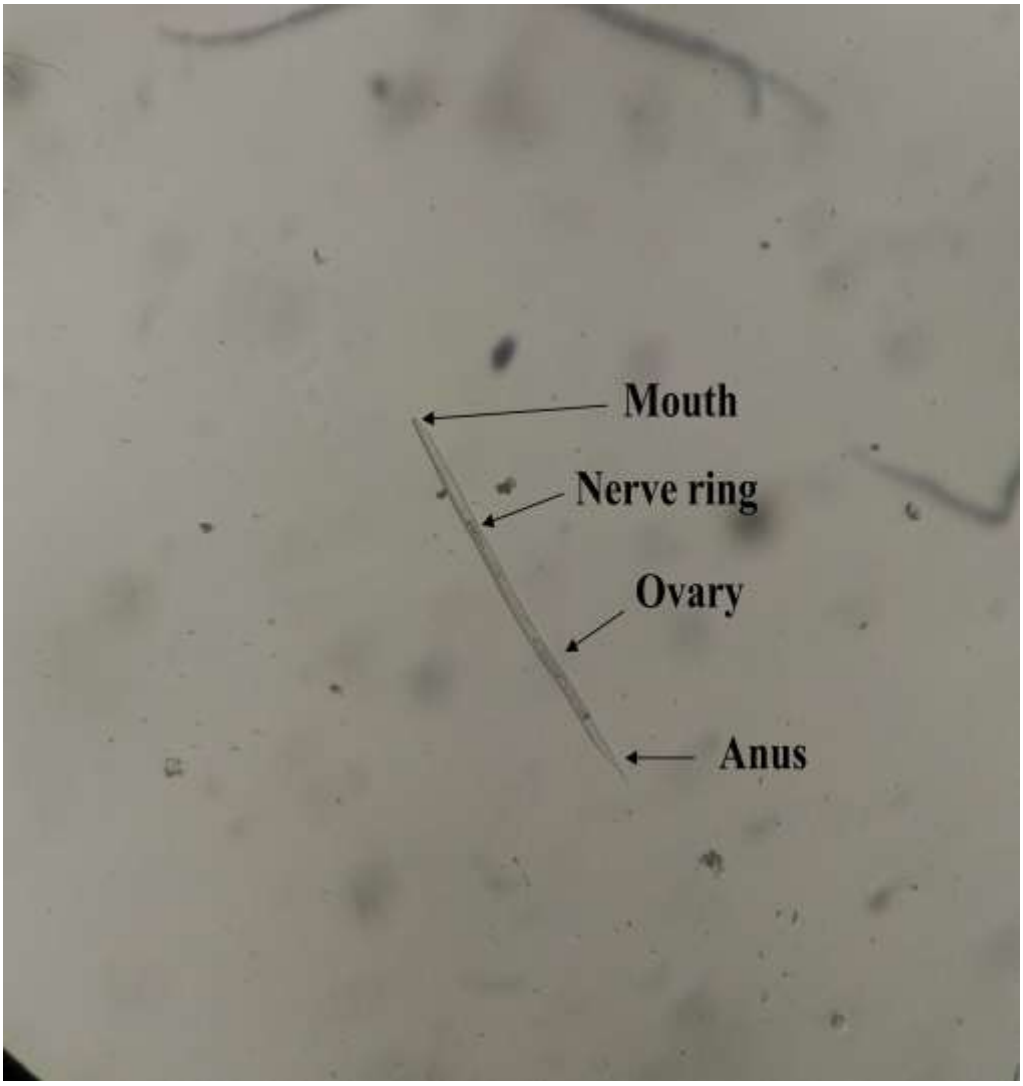

Fig. 10. Morphology of nematode juvenile (*Meloidogyne* sp.) under stereo microscope (40x).

4.9.1 Number of nematode juvenile / g soil in Tangibari

Nematode (*Meloidogyne* sp.) was found in Tongibari with the number of juvenile/g soil was 20 (Table 9).

Table 9. Number of nematode juvenile / g soil in Tangibari

Districts	**Upazilas**	**No.of juvenile/g soil**
Munshigonj	Tongibari	20

4.10 Frequency of mycoflora

The Soil pH, organic content and water are the main factors affecting the fungal population and diversity. During the investigation period 423 fungal colonies of 7 fungal species, one bacterium and one nematode species were observed from the different upazillas (Table 11). In our findings, the maximum (77.92%) frequency of *Alternaria solani* was observed from the Tongibari upazilla, whereas the minimum frequency (13.36%) in Bandar upazilla. In case of *Aspergillus niger*, the maximum (81.25%) and the minimum frequency (14.18%) were found in Sonargaon and Singair Union upazilla, respectively.

The maximum (25%) and the minimum frequency (11.11%) of *Penicillium* sp. were observed in Puthia and Matlab Uttar upazilla. The maximum (75%) and the minimum (20.78%) frequencies of *Rhizopus stolonifer* were found in Gaibandha Sadar and Tongibari upazillas, respectively during investigation. In case of *R. Solanacearum*, the maximum (75%) frequency was observed from the upazilla of Durgapur and the minimum frequency (31.17%) in Tongibari upazilla. The *Bipolaris* sp. was observed with the frequency (25%) in Palashbari upazilla (Table 11).

Moreover, the maximum (18.75%) and the minimum frequency (10.39%) of *Phytophthora infestans* were observed in Sonargaon and Tongibari upazilla. In case of *Fusarium oxysporum*, the highest (16.67%) and the lowest frequency (6.49%) were found in Bagmara and Tongibari upazilla, respectively. *Meloidogyne* sp. was found in Tongibari with the frequency (21.50%).

Table 10. Frequency of mycoflora in different potato growing regions of Bangladesh

Upazilla	**No. of Colonies/dilution**	**No. of individual colonies / nematode**								
		Alternaria solani	*Aspergillus niger*	*Penicillium* sp.	*Rhizopus stolonifer*	*Ralstonia solanacearum*	*Bipolaris* sp.	*Fusarium oxysporum*	*Phytophthora infestans*	*Meloidogyne* sp.
Singair	21	7	-	11	-	-	-	3	-	-
Singair Union	64	-	14	-	13	21	13	-	3	-
Gobindaganj	30	-	11	-	-	13	2	-	4	-
Gaibandha Sadar	14	-	-	-	11	-	3	-	-	-
Palashbari	11	4	-	-	-	7	-	-	-	-
Sadullapur	17	-	8	7	-	-	-	2	-	-
Gopinathpur	15	3	-	-	9	-	-	-	3	-
Daudkandi	32	3	8	-	-	16	4	1	-	-
Durgapur	16	4	-	-	-	12	-	-	-	-
Puthia	16	-	-	4	8	-	4	-	-	-
Bagmara	18	5	-	-	-	7	-	3	3	-
Matlab Dakshin	25	-	-	-	9	12	-	-	4	-
Matlab Uttar	27	4	4	3	-	13	-	3	-	-
Bandar	22	3	8	-	11	-	-	-	-	-
Sonargaon	16	-	13	-	-	-	-	-	3	-
Tongibari	77	6	14	-	16	24	-	5	8	20

Table 11. Contribution (%) of micro-organisms in different potato growing regions of Bangladesh

Upazilla	Contribution (%) of fungi , bactaria and nematode								
	Alternaria solani	*Aspergillus niger*	*Penicillium sp.*	**Rhizopus *stolonifer***	*Ralstonia solanacearum*	*Bipolaris* sp.	*Fusarium oxysporum*	*Phytophthora infestans*	*Meloidogyne* sp.
Singair	33.33	-	*52.38*	-	-	-	14.28	-	-
Singair Union	-	21.87	-	20.31	32.81	20.31	-	4.68	-
Gobindaganj	-	36.67	-	-	43.33	6.66	-	13.33	-
Gaibandha Sadar	-	-	-	78.57	-	21.42	-	-	-
Palashbari	36.36	-	-	-	**63.63**	-	-	-	-
Sadullapur	-	47.05	41.18	-	-	-	11.76	-	-
Gopinathpur	20	-	-	60	-	-	-	20	-
Daudkandi	9.37	25	-	-	50	12.5	3.13	-	-
Durgapur	25	-	-	-	75	-	-	-	-
Puthia	-	-	25	50	-	25	-	-	-
Bagmara	27.78	-	-	-	38.89	-	16.67	16.67	-
Matlab Dakshin	-	-	-	36	48	-	-	16	-
Matlab Uttar	14.81	14.81	11.11	-	48.15	-	11.11	-	-
Bandar	13.36	36.36	-	50	-	-	-	-	-
Sonargaon	-	81.25	-	-	-	-	-	18.75	-
Tongibari	77.92	18.18	-	20.78	31.17	-	6.49	10.39	21.50

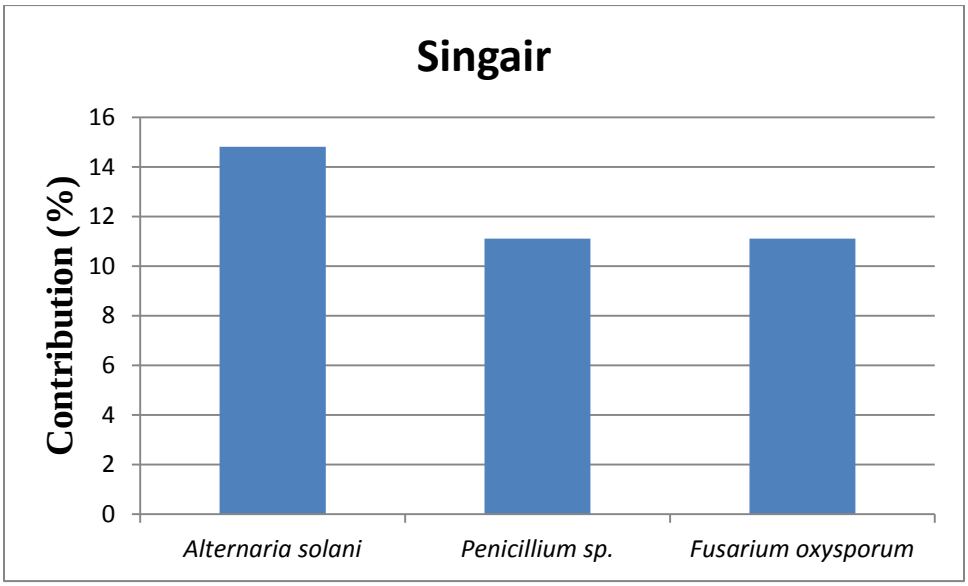

Fig. 11. Contribution (%) of microorganisms at Singair Upazilla

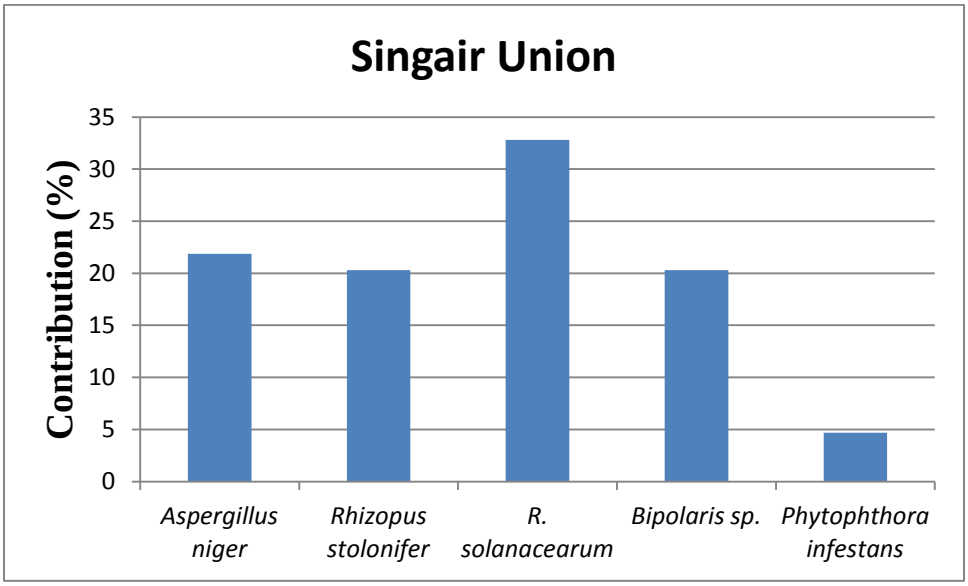

Fig. 12. Contribution (%) of microorganisms at Singair Union

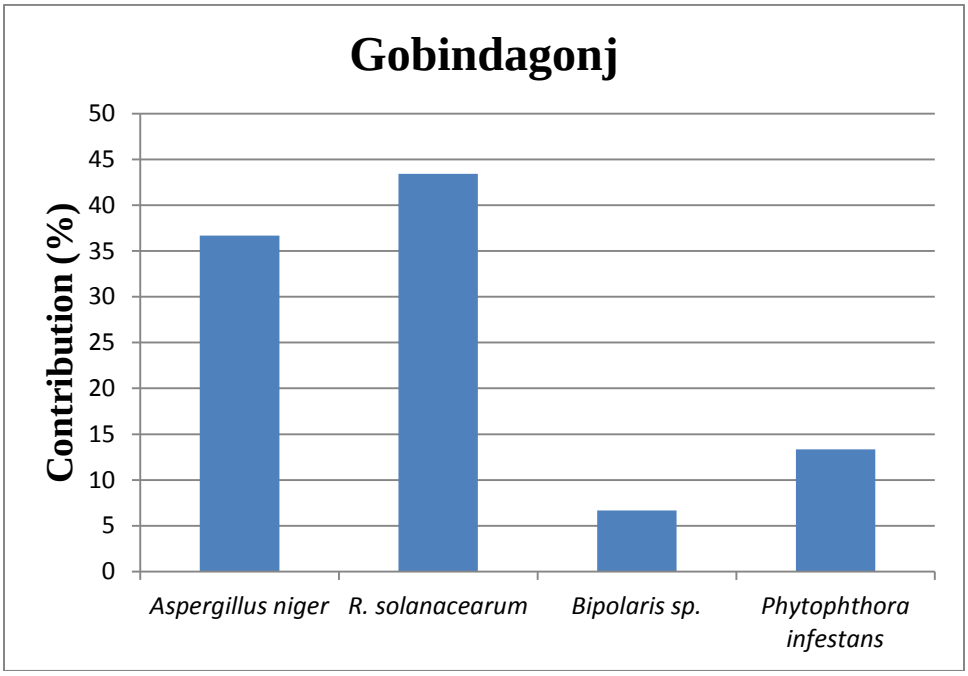

Fig. 13. Contribution (%) of microorganisms at Gobindagonj Upazilla

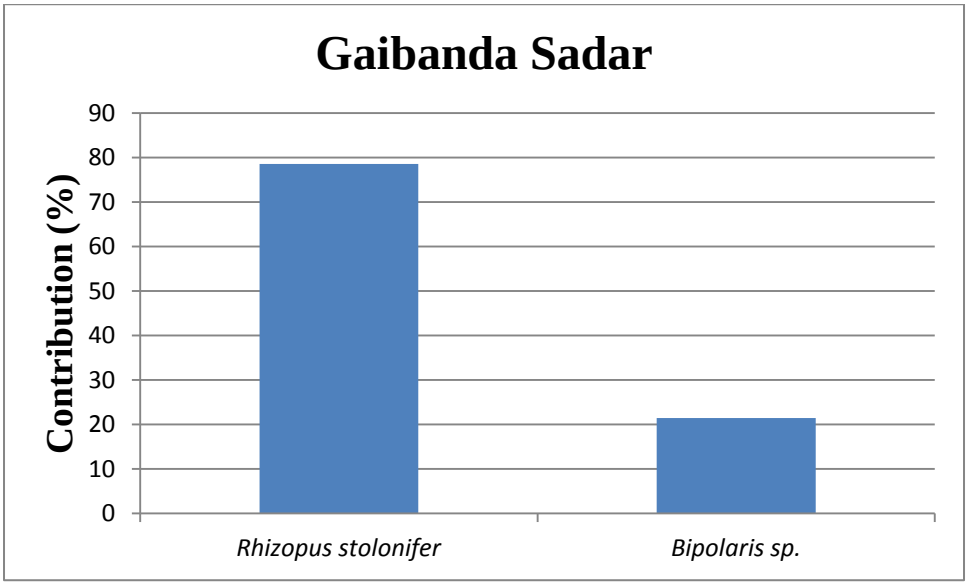

Fig. 14. Contribution (%) of microorganisms at Gaibandha sadar Upazilla

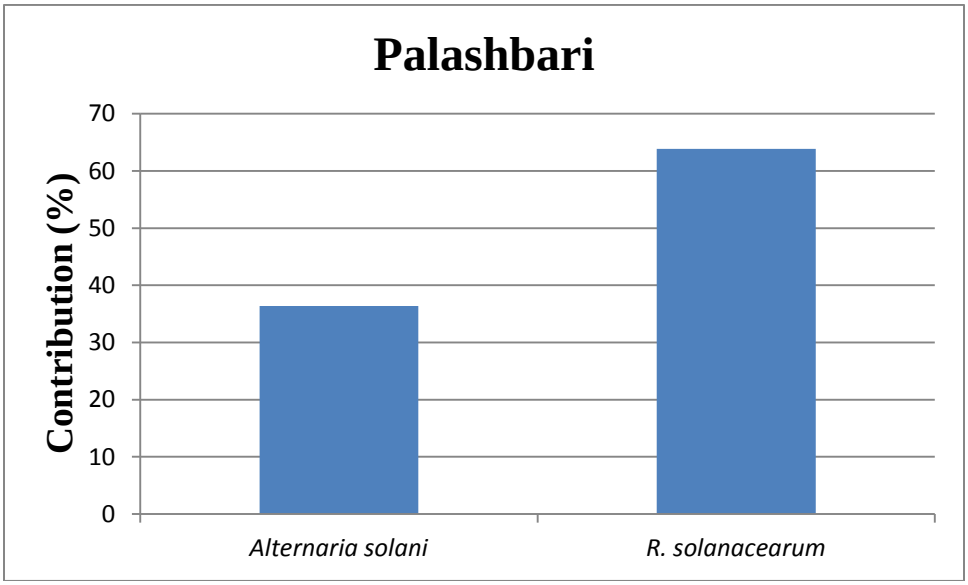

Fig. 15. Contribution (%) of microorganisms at Palashbari Upazilla

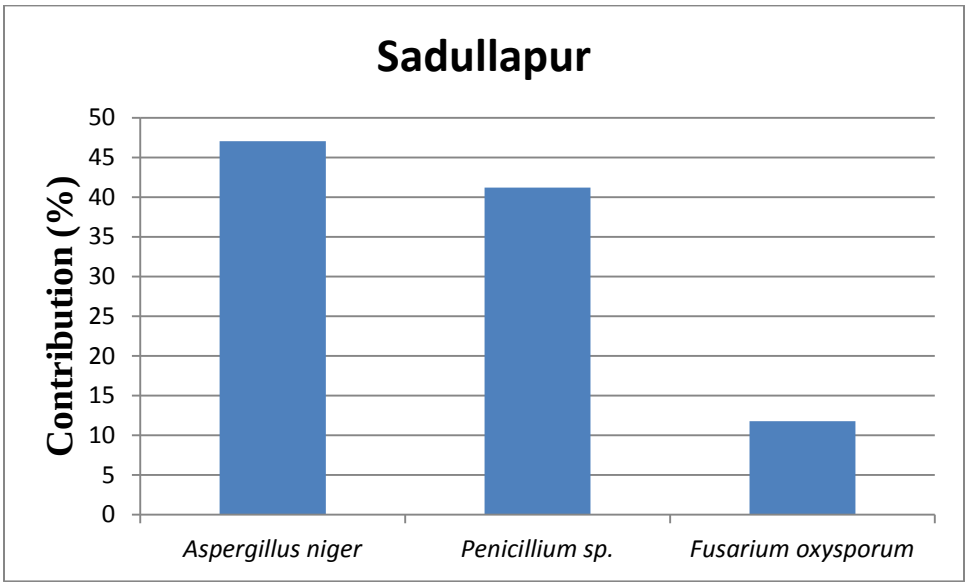

Fig. 16. Contribution (%) of microorganisms at Sadullapur Upazilla

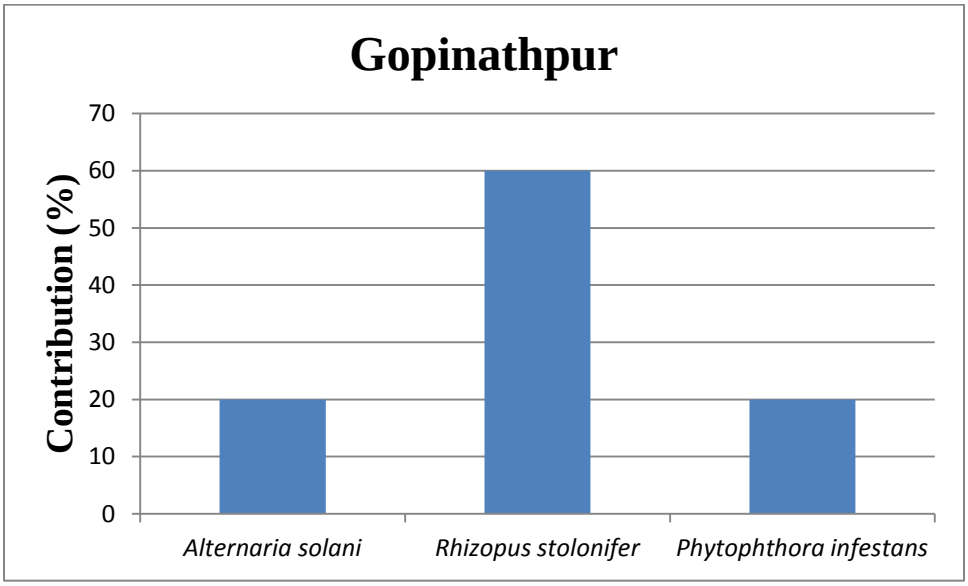

Fig. 17. Contribution (%) of microorganisms at Gopinathpur Upazilla

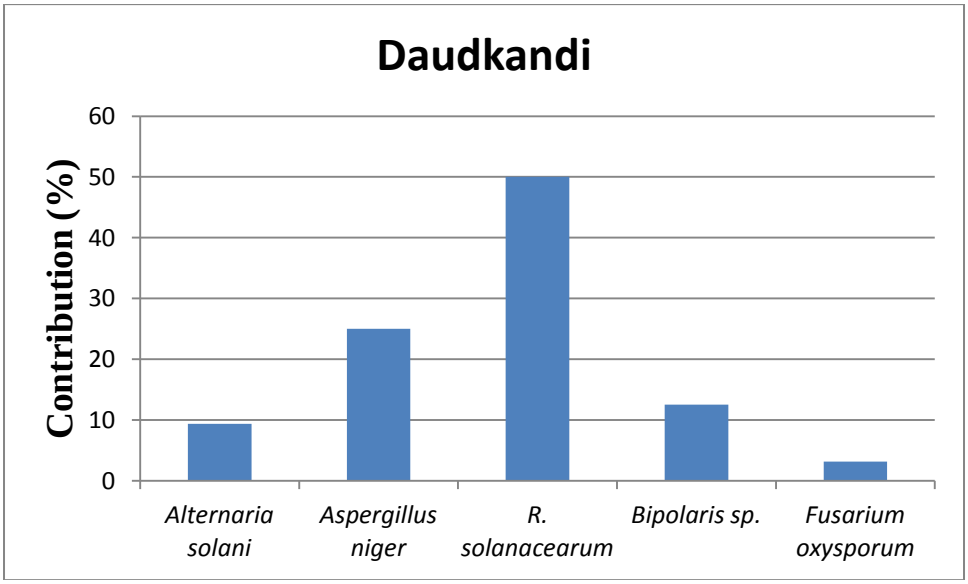

Fig. 18. Contribution (%) of microorganisms at Daudkandi Upazilla

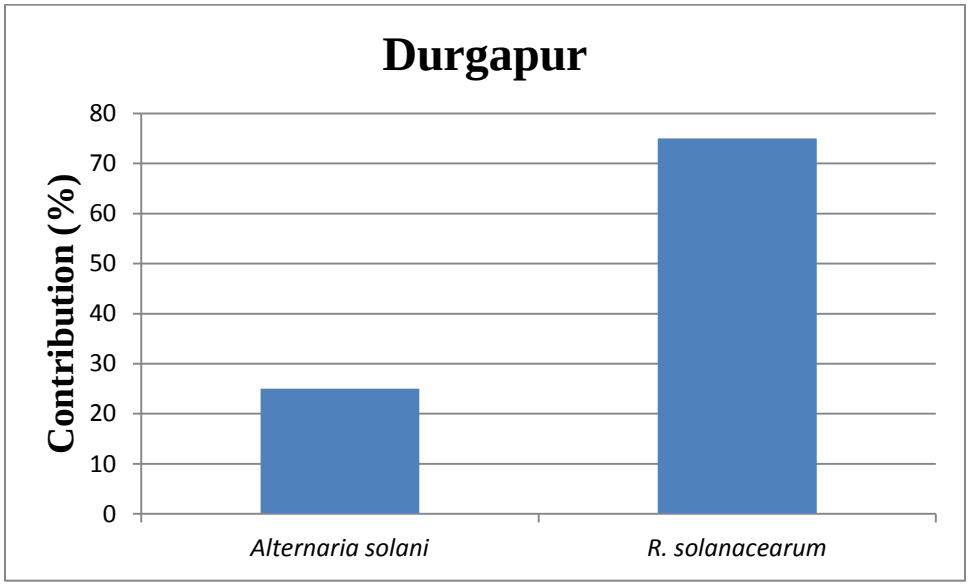

Fig. 19. Contribution (%) of microorganisms at Duragapur Upazilla

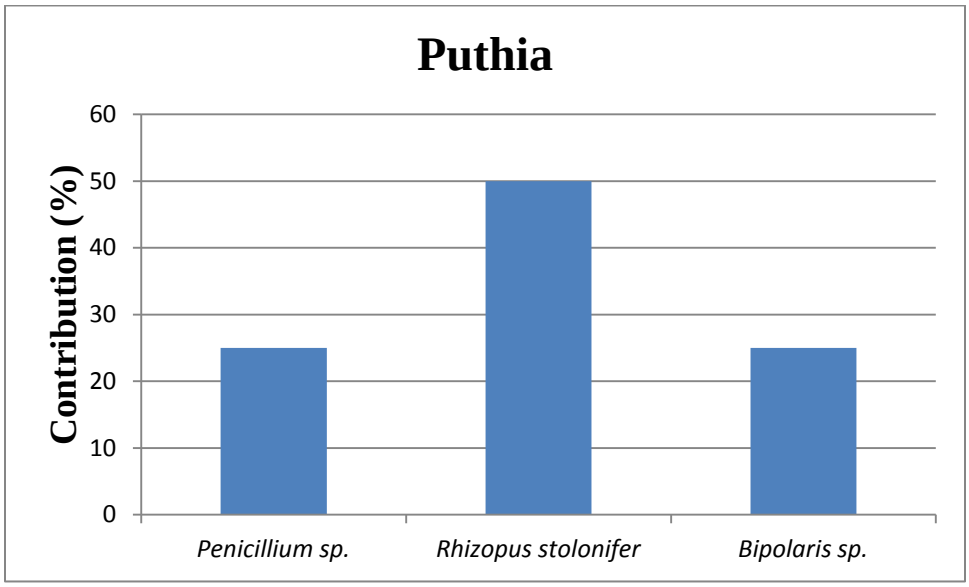

Fig. 20. Contribution (%) of microorganisms at Puthia Upazilla

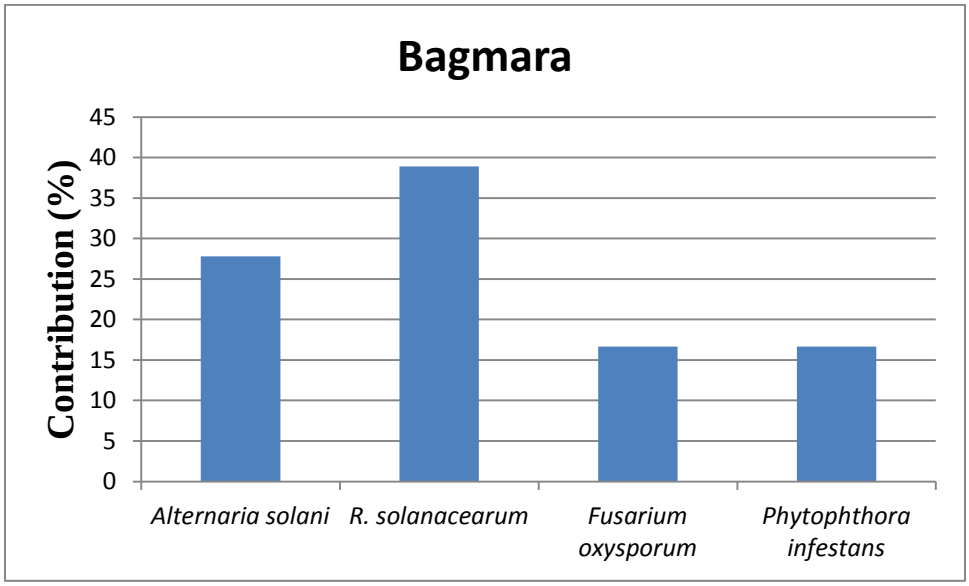

Fig. 21. Contribution (%) of microorganisms at Bagmara Upazilla

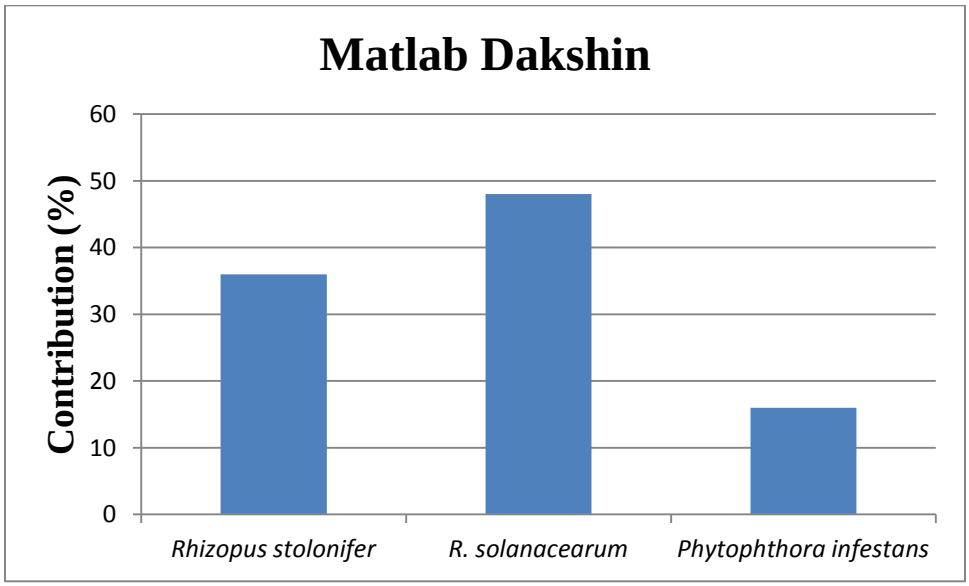

Fig. 22. Contribution (%) of microorganisms at Matlab Dakshin Upazilla

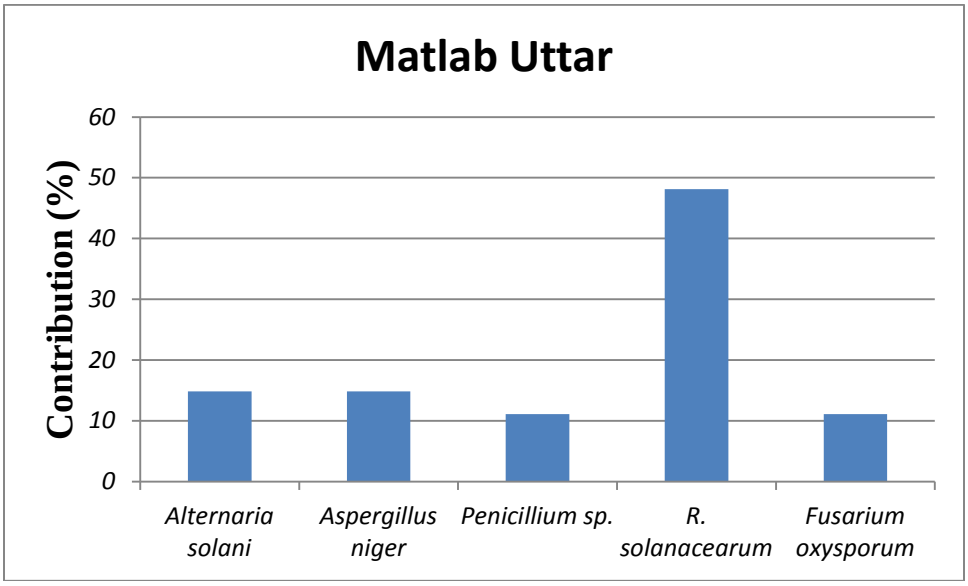

Fig. 23. Contribution (%) of microorganisms at Matlab Uttar Upazilla

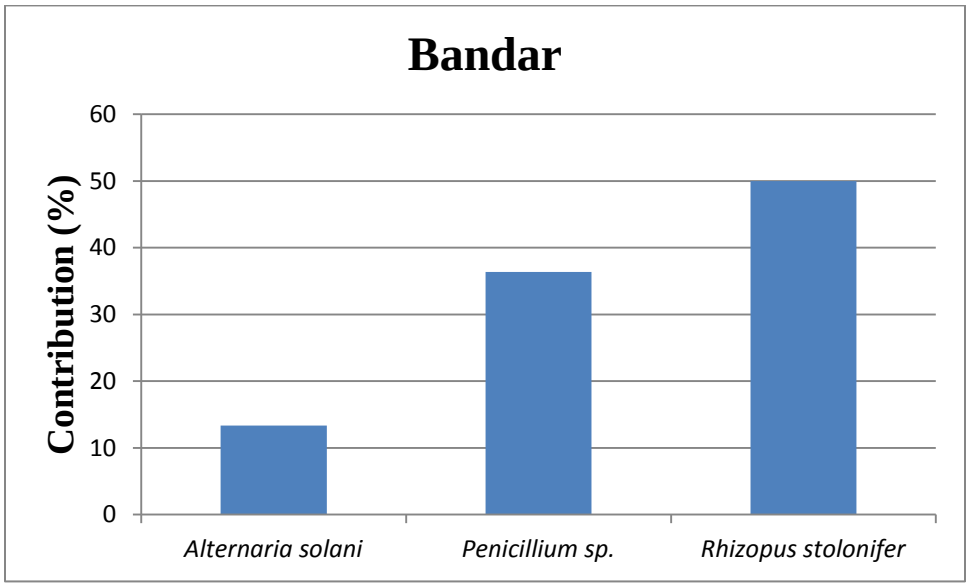

Fig. 24. Contribution (%) of microorganisms at Bandar Upazilla

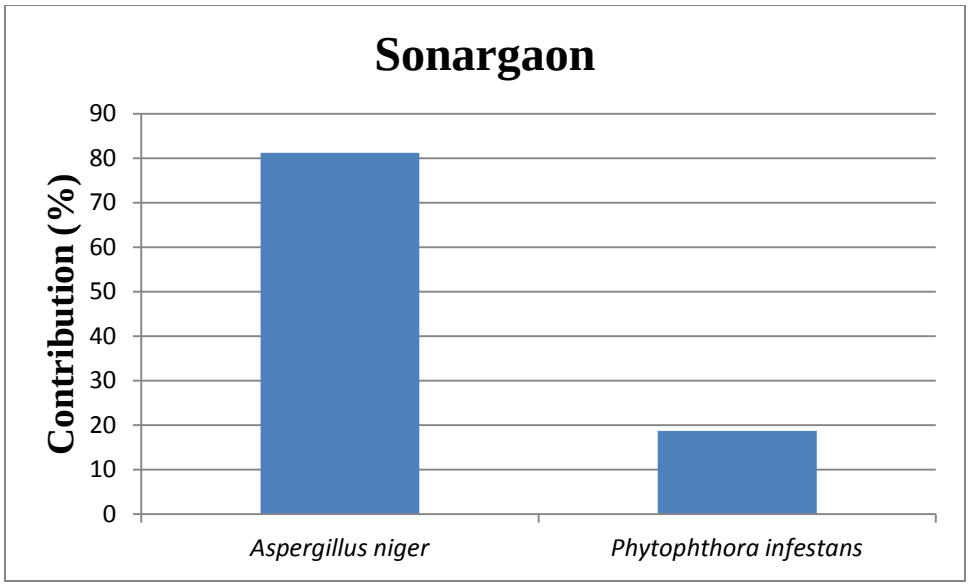

Fig. 25. Contribution (%) of microorganisms at Sonargaon Upazilla

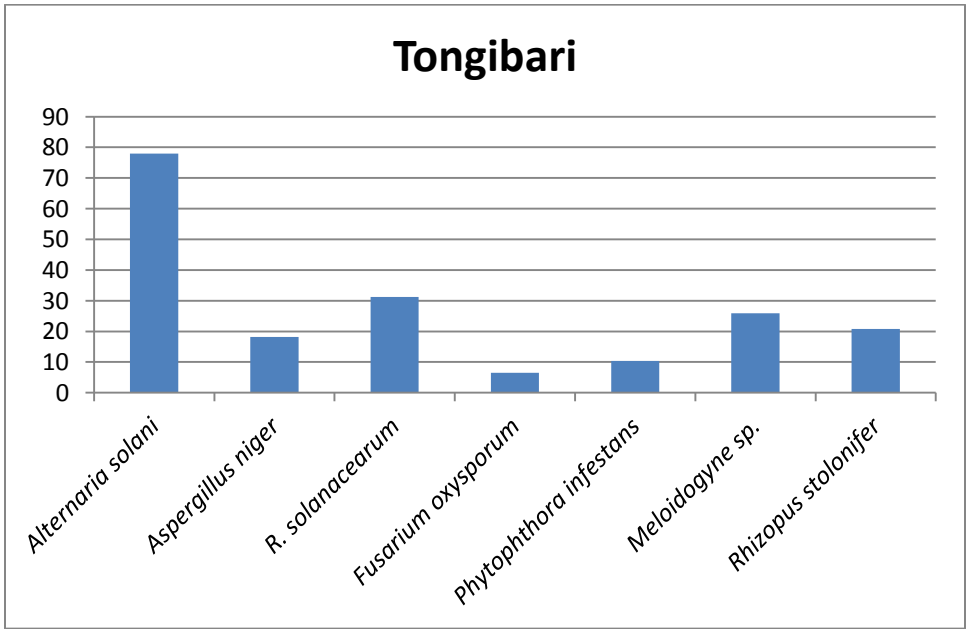

Fig. 26. Contribution (%) of microorganisms at Tongibari Upazilla

The soil samples were analyzed with respect to different types of microorganisms (Fungi, Bacteria and Nematode). The soil samples were analysed and indentified different type of fungi such as- *Alternaria solani, Penicillium* sp., *Rhizopus stolonifer, Aspergillus niger, Fusarium oxysporum*, *Phytophthora infestans*, *Bipolaris* sp. and the most common bacteria *R. solanacearum* and one nematode *Meloidogyne* sp. were recorded from the selected regions.

During the investigation, nine microorganisms were indentified and counted the colony forming unit per gm soil. The highest amount (7×10^3 CFU/g soil) of *Alternaria solani* was observed from the upazilla of Singair, whereas the lowest amount (3×10^3 CFU/g soil) was found from the Gopinathpur, Daudkandi and Bandar (Table 10). Sharma and Tiwari (2015) isolated *Alternaria* sp. and found 20×10^3 CFU/g in soils sediment from the surface layer in India.

The highest amount (11×10^3 CFU/g soil) of *Penicillium* sp. was observed from the district of Manikgonj (Singair) followed by Gaibanda (Sadullapur) (7×10^3 CFU/g soil). During the investigation, *Penicillium* sp. was not recorded in Comilla, Rajshahi, Chandpur, Narayangonj and Munshiganj districts (Table 10). The amount (16×10^3CFU/g soil) was found in soils sediment from the surface layer in India by Sharma and Tiwari (2015). In present study, the total amount of *Penicillium* sp. was observed as 25×10^3CFU/g soil from the different potato fields of Bangladesh. But the amount (215×10^3CFU/g) was found in Iraq by Toma and Abdulla (2012). Gaddeyya *et al.* (2012) and Niharika *et al.* (2013) also found 3×10^3CFU/g and 15×10^3CFU/g from agricultural fields in India, respectively.

The maximum amount (14×10^3 CFU/g soil) of *Aspergillus niger* was counted from the Munshiganj district (Tongibari), whereas, Chandpur district was free from *Aspergillus niger* during survey. *Aspergillus* sp. was isolated and counted (12×10^3 CFU/g) soil from different crop fields in India by Niharika *et al.* (2013). The total 539×10^3 CFU/g soil was observed from different areas in Erbil, Iraq (Toma and Abdulla , 2012).

The highest amount (16×10^3 CFU/g soil) of *Rhizopus stolonifer* was observed from the Munshiganj district (Tongibari), whereas the nearest amount (13×10^3 CFU/g soil) was found from Manikgonj (sSingair Union). *Rhizopus stolonifer* could not identify from the Comilla district. Gaddeyya *et al.* (2012) also found 1×10^3CFU/g of *Rhizopus stolonifer* from agricultural fields in India. The amount of *Rhizopus* sp. (11×10^3CFU/g) was observed in soils sediment from the surface layer in India Sharma and Tiwari (2015). In the present study, the total amount of *Rhizopus* sp. (142×10^3CFU/g) was observed from the different potato fields in Bangladesh. But the total amount of *Rhizopus* sp. (115×10^3CFU/g) was reported by Toma and Abdulla (2012). *Bipolaris* sp. was observed as highest amount (4×10^3 CFU/g soil) from Comilla and Rajshahi district. The nearest amount (3×10^3 CFU/g soil) was found from Gaibandha, whereas, this pathogen was not identified from Manikgonj, Chandpur, Narayangonj and Munshiganj districts potato field soil. The average amount of *Bipolaris* sp. (8×10^3 CFU/g soil) was observed from the Kingdom of Saudi Arabia (Vijayakumar *et al*., 2017). The highest amount (8×10^3 CFU/g soil) of *Phytophthora* sp. was observed from Munshiganj district (Tongibari)**.** The nearest amount (4×10^3 CFU/g soil) was found from Gaibandha, Chandpur and Narayangonj districts, whereas, this pathogen was not identified from Manikgonj, Comilla and Rajshahi districts.

The maximum amount (5×10^3 CFU/g soil) of *Fusarium oxysporum* was counted from the Munshiganj district (Tongibari). The minimum amount (1×10^3 CFU/g soil) was calculated from Comilla, Narayangonj district potato rhizosphere was free from *Fusarium oxysporum* .The amount (2×10^3 CFU/g soil) *Fusarium oxysporum* was previously reported from agricultural fields in India by Gaddeyya *et al.* (2012). And the amounts of *Fusarium* sp. (47×10^3 CFU/g soil and 22×10^3 CFU/g soil) from different crop fields in Iraq and India was recorded by Toma and Abdulla, 2012 and Sharma and *Tiwari,* 2015, respectively.

R. solanacearum was observed as highest amount (24×10^3 CFU/g soil) from Munshiganj district (Tongibari)**.** The nearest amount (21×10^3 CFU/g soil), were found from Manikgonj (Singair Union). The ranges of CFU/g soil (12×10^3 to 16×10^3) were observed from the rest of the districts. Nematode (*Meloidogyne* sp.) was observed from the Munshiganj district (Tongibari) and the number of juvenile/g soil (20) was counted from the soil samples. The rest upazillas of the districts were free from nematode during investigation.

The maximum frequencies of mycoflora as *Alternara solani* (66.67%), *Aspergillus niger* (81.25%), *Penicillium* sp. (61.11%), *Rhizopus stolonifer* (78.57%), *R. Solanacearum* (75%), *Bipolaris* sp. (50%), *Fusarium oxysporum* (16%), *Phytophthora infestans* (36.67%) and *Meloidogyne* sp. (21.50%) were the dominant species from the different upazillas in Bangladesh (Table 11).

CHAPTER V

SUMMARY AND CONCLUSION

From the present investigation it is concluded that a total of seven genera of fungi, one bacteria species and one genus of nematode were isolated and identified from potato field soils of selected regions of Bangladesh. Most of the fungal species were able to grow efficiently and appear concurrently which means these indigenous fungi have the capacity to adapt in of agricultural soils.

In the present study 23 soil samples from seven districts (16 upazillas) were studied for screening and detection of fungi, bacteria and nematode. The results obtained clearly indicates that the presence of *Alternaria solani*, *Penicillium* sp., *Aspergillus niger*, *Rhizopus stolonifer*, *Bipolaris* sp., *Phytophthora infestans*, *Fusarium oxysporum*, *R. solanacearum* and *Meloidogyne* sp. in the selected regions of Bangladesh. The highest amount (7×10^3 CFU/g soil) of *Alternaria solani* was observed from the district of Manikgonj (Singair) and the lowest amount (3×10^3 CFU/g soil) was found from the Narayangonj (Bandar) and (Comilla) Daudkandi. The highest amount (11×10^3 CFU/g soil) of *Penicillium* sp. was observed from the district of Manikgonj (Singair_) and the nearest amount (7×10^3 CFU/g soil) was found from the *Gaibanda* (Sadullapur).

The maximum amount (14×10^3 CFU/g soil) of *Aspergillus niger* was counted from the district of Munshiganj district (Tongibari and Singair Union) but Chandpur district was free from *Aspergillus niger* during investigation. The highest amount (16×10^3 CFU/g soil) of *Rhizopus stolonifer* was observed from the district of Munshiganj district (Tongibari)

and the nearest amount (13×10^3 CFU/g soil) was found from Manikgonj (Singair).

Bipolaris sp. was observed as highest amount (4×10^3 CFU/g soil) from Comilla and Rajshahi and the nearest amount (3×10^3 CFU/g soil) was found from Gaibandha, whereas, this pathogen was not identified from Manikgonj, Chandpur, Narayangonj and Munshiganj districts.

The maximum amount (5×10^3 CFU/g soil) of *Fusarium oxysporum* was counted from Tongibari upazilla. The minimum amount (1×10^3 CFU/gm soil) was calculated from Comilla and Narayangonj district was free from *Fusarium oxysporum, R. solanacearum* was observed as highest amount (24×10^3 CFU/g soil) from Tongibari upazilla and the nearest amount (21×10^3 CFU/g soil), (17×10^3 CFU/g soil) were found from Singair Union and Bandar upazillas, respectively. *Meloidogyne* sp. was observed from Tongibari upazilla and the CFU/g soil (4×10^3) was counted from the soil samples. The rest upazillas of the districts were free from nematode during investigation.

Finally, the environment where we live is the habitat for various microorganisms; mostly fungi and bacteria which are harmful for our agricultural production. The beneficial micro-organisms play an important role in composting of organic waste and can be an important contributor to optimal agricultural waste. This study revealed the isolation and identification of diversity of micro-organisms which are present in potato field soil habitat of the investigated regions of Bangladesh.

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