

Occurrence and diversity of soil mycoflora in potato fields of Bangladesh

ABSTRACT

Mycoflora from potato rhizosphere soil was isolated from different potato fields of Bangladesh. Seventeen soil samples were analyzed for the presence of mycoflora in selected potato field soils. Seven (7) fungal species and one bacterium species were morphologically characterized using various isolation and identification methods. The predominant fungi isolated including *Alternaria* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Bipolaris* sp., *Phytophthora* sp., *Fusarium* sp. and one bacterium was identified as *Ralstonia solanacearum*. Individual colonies of fungi and bacteria were counted on PDA media and their presence in soil was compared in respect of different locations of potato fields. This was the first reported examination of the microbial diversity of soil mycoflora in some selected potato fields of Bangladesh.

Keywords: Mycoflora; diversity; fungi; bacteria.

1. INTRODUCTION

Soils are very composite systems, with many components playing diverse functions mainly due to the activity of soil organisms [1]. Soil mycoflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth [2] by biochemical transformation and mineralization activities in soils [2]. However, type of cultivation and crop management practices are found to have greater influence on the activity of soil mycoflora [3]. Continuous use of chemical fertilizers over a long period may cause imbalance in soil mycoflora and thereby indirectly affect biological properties of soil leading to soil degradation [4]. Fungi are an important component of the soil micro biota [5]. Micro fungi play a focal role in nutrient cycling by regulating soil biological activity [6]. Indirect accumulation of [????] 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 [7]. 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.

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 [8]. Soil borne diseases are considered as a limiting factor of many crops including potato. *Ralstonia solanacearum* [9,10] (formerly called *Pseudomonas solanacearum*) is a soil borne pathogen which generally occurs in lowlands in tropical or subtropical areas and is an extremely destructive potato pathogen, causing bacterial wilt or brown rot of potato in the highland tropics of Africa, Asia, and Latin America [11].

Comment [WU1]: Specify these standardized isolation and identification methods.

Comment [WU2]: First write PDA in full and then the acronym in brackets.

Comment [WU3]: Summarize here the findings of the comparison in Table 1. Which site has the highest and the least counts

Comment [WU4]: Use appropriate words to connect the various sentences to ensure a logical flow of your arguments.

Comment [WU5]: Of what??

Comment [WU6]: Rephrase this sentence to improve the flow.

The aim of the present investigation is to isolate and identify mycoflora from selected potato field soils in Bangladesh and to assess the contribution of the presence of different fungi and bacteria in the soil community of selected potato fields.

2. MATERIALS AND METHODS

2.1 Survey location and collection of samples

Soil samples were collected from different locations namely Manikganj, Gaibandha, Cumilla, Chandpur, Narayanganj, Rajshahi and Munshiganj of North-western regions of Bangladesh during November to April, 2016 to 2017 and put in a polyethylene bags [12]. Samples were brought to the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh and kept in refrigerator at 5°C.

2.2 Preparation of samples

Ten individual samples (100 g each) collected from a field ~~was~~ were mixed to prepare a composite sample (1000g). All sorts of debris and waste materials were removed from the collected samples and then soil was crushed to powder form so that the samples dissolve into water easily. Hundred gram (100 g) soil samples were taken from each composite sample to prepare working sample for isolation of mycoflora.

2.3 Sterilization technique

Petri plates, conical flasks, test-tubes and other glassware were sterilized in oven at 165-170°C for 3 hrs. Culture media were sterilized in autoclave at 15 PSI for 15 minutes at 121°C.

2.4 Dilution plate preparation

The purpose of preparing serial dilution was to ~~the~~ ease the count of colonies of mycoflora. At first, one gram of representative soil sample ~~from~~ (working sample) was taken from composite sample in a sterilized glass test tube containing nine milliliter (9 ml) of water and dissolved sufficiently to make stock solution. One ml of soil suspension was taken from stock solution and added in the sterilized glass test tube containing nine milliliter of sterilized water to make 1:10 dilution. To prepare 1:100 dilutions, one ml of soil suspension was carefully taken from 1:10 dilution into a sterilized glass tube containing nine milliliter of water and serial dilution was prepared accordingly. One milliliter of soil suspension was taken from each dilution ~~and placed e~~ into the sterilized glass petridish and 20 ml of PDA [13] was added to the plate. Soil suspension was mixed with culture media very carefully. Each dilution was replicated thrice. Then these plates were incubated at 28°C for 3 days and the colonies were counted [14]. Estimation of fungal population was done by standard spread plate dilution method described by [15].

2.5 Isolation and identification 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 plates were incubated for 3 days as described above. The fungal colonies from PDA plates were transferred by needle on PDA plate and incubated for 7 days. The fungi 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) [16].

2.6 Isolation of bacteria

Comment [WU7]:
Did you sample in November and April in both 2016 and 2017??

Rephrase the sentence to reflect these dates correctly

Bacterial colonies grown on PDA from diluted soil samples were transferred on nutrient agar by streaking method. A single colony from streaked plate was then transferred to a second plate, incubated for seven days to obtain pure culture.

2.6.1 Test and identification of bacterium (*R. solanacearum*)

KOH solubility test: A drop of potassium hydroxide (KOH) (3% aq. w/v) using Pasteur pipette was placed on a microscope slide. Then 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. Other than KOH solubility test gram staining test and Kovac's oxidase tests were also done that was positive for *R. solanacearum*. Then *R. solanacearum* was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 8).

2.7 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:

$$\frac{\text{Total number of CFU of an individual species}}{\text{Total number of CFU of all species}} \times 100$$

* CFU-Colony Forming Unit

???

3. RESULTS AND DISCUSSION

Soil mycoflora is a component of soil ecosystem that contributes to the nutrient cycle in rhizosphere and rhizoplane of many economically important agricultural crops. An attempt was made to determine the soil mycofloral diversity in some selected potato field soils of Bangladesh. The experiment was carried to study the presence of different fungi and bacteria into the rhizosphere soils of potato field collected from different locations of Bangladesh. The results are discussed and interpreted under the following subheads.

3.1 Characteristics of Identification of Fungi species

3.1.1 *Alternaria* sp.

The several temporary and semi-permanent slides were prepared to observe the micro-organisms under microscope. The *Alternaria* sp. was identified to the genus level on the basis of macromorphological characteristics by using suitable media and slide cultures during working in the laboratory (Table 1). Morphological observations of the fungus were recorded by adopting slide culture technique. The fungus produced profuse mycelial growth on PDA. 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* spp. under motic microscope. [17] isolated and characterized this fungal strain from agricultural soil in India.

3.1.2 *Penicillium* sp.

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Comment [WU8]: Rephrase

Comment [WU9]: Combine these short sentences!!

Comment [WU10]: Transfer this to the methods section

Comment [WU11]: You should also compare the fungi and bacteria in the different regions of Bangladesh using the most suitable statistics. Indicate which statistical packages you used for data analysis.

Comment [WU12]: Some of the highlighted sentences are part of the introduction and methods. Transfer them to the right sections.

Comment [WU13]: In this section, report the characteristics of the fungi that you observed during the experiment in case you did. You can support with literature information!!

Comment [WU14]: Transfer this to the methods section.

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. [18] isolated and identified this soilborne fungi from the agricultural soil in Turkey. [19] identified *Penicillium* sp. fungus from the garden soil. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as- From Kwara state [21].

Comment [WU15]: Report the characteristics of the fungi that you observed during the experiment in case you did not just giving the literature information!!

3.1.3 Aspergillus sp.

The colony consists of mats of hyphae that make up a mycelium. The hyphae are septate and hyaline. The spores produced called ascospore. Ascospores are the sexual reproductive cells that are produced in the cleistothesia. Conidia are the asexual reproductive cells that are produced in specialized hyphae called conidiophores. The produced ascus is known as Cleistothesia. [18] isolated and identified this soilborne fungi from the agricultural soil in Turkey. [17] isolated and characterized this fungal strain from agricultural soil in India. [19] identified *Aspergillus* sp. from the garden soil. [22] was carried out by culturing on potato dextrose and sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from india. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as- From Kwara state [21].

3.1.4 Rhizopus sp.

Colonies grow rapidly and resemble like as cotton candy. Colonies darken with age, becoming gray or yellow-brown. The reverse is white. Mycelia are marked by numerous stolons connecting groups of long sporangiophores. Sporangioophores 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. [17] isolated and characterized this fungal strain from agricultural soil in India. [19] identified sp. fungus from the garden soil. [22] was carried out by culturing on potato dextrose and sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India.

3.1.5 Bipolaris sp.

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 curved, canoe-shaped, fusoid or obclavate, rarely straight, 2–14 pseudoseptate (usually more than 6), germinating only from the ends (bipolar). A field research was conducted by [23] to isolate and characterize the *Bipolaris* sp. from gerbera plants in Maharashtra.

3.1.6 Phytophthora sp.

The pathogen produced hyaline lemon shaped sporangia arose straightly from the surface of the substrate. *Phytophthora* species may reproduce sexually or asexually. 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. Sporangioophores 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 [24]. [25] investigated the seasonal tracking of *Phytophthora* recovery from a variety of soil and forest types in northwestern California.

3.1.7 *Fusarium* sp.

Hyphae are hyaline, septate and showed divisions or walls within the hyphae. Conidiophores are short and usually non-septate when compared to other *Fusarium* species. The conidiophores have somewhat inflated appearance as their sides are not parallel but slightly in the middle and the pigmentations were pale brown to yellowish brown with a dark brown zonation. These conidiophores are produced singly as they extend from the aerial mycelium. The feature of conidiogenous cell with branched and long monophialides were commonly observed. [17] isolated and characterized this fungal strain from agricultural soil in India. [22] was carried out by culturing on potato dextrose and Sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India. The physicochemical characteristics of soil samples was found to affect the distribution and population of fungi such as- From Kwara state [21].

Comment [WU16]: Write separate paragraphs for identification of these fungi species under the various headings of the fungi species. Transfer the information on identification of the species from the results section here!!

3.2 Characteristics of Identified Bacterium species (*R. solanacearum*)

The virulent wild type colonies were large, elevated, fluidal and either entirely white or with a pale red center. 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 bacterial wilt pathogen *R. solanacearum* [26,27,28]. 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 [29].

3.3.1 Comparison of Fungi species *Alternaria* sp. in different regions of Bangladesh

3.3.1 *Alternaria* sp.

Soil colonization of *Alternaria* sp. varied among the potato field soil of different potato growing regions and ranged from 3×10^3 to 7×10^3 CFU/g soil where the highest colonization was recorded at Singair upazila of Manikganj district and the lowest colonization was recorded at Gopinathpur of Gaibandha, Daudkandi of Cumilla and Bandar of Narayanganj.

Comment [WU17]: Also carry out significant tests using the relevant statistics to find out if the fungi and bacteria are significantly different from one another and report the p values as well.

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3.2 Identification of *Penicillium* sp.

The several temporary and semi-permanent slides were prepared to observe the micro-organisms under microscope. The *Penicillium* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 2). Species of *Penicillium* are recognized by their dense brush-like spore-bearing structures called penicilli (sing.: penicillus). *Penicillium* spp. is 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. [18] isolated and identified this soilborne fungi from the agricultural soil in Turkey. [19] identified *Penicillium* sp. fungus from the garden soil. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as- From Kwara state [21].

Comment [WU18]: Delete as it is already in the methods section

3.3.2.1 Comparison of *Penicillium* sp. in different regions of Bangladesh

Soil colonization of *Penicillium* sp. varied among the potato field soil of different potato growing regions and ranged from 3×10^3 to 11×10^3 CFU/g soil where the highest colonization was recorded at Singair upazila of Manikganj district and the lowest colonization was recorded at Matlab Uttar upazila of Chandpur District.

Comment [WU19]: Vary the stem of the sentence to avoid monotony!!

3.3 Identification of *Aspergillus* sp.

~~The several temporary and semi permanent slides were prepared to observe the micro organisms under microscope. The *Aspergillus* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 3). The colony consists of mats of hyphae that make up a mycelium. The hyphae are septate and hyaline. The spores produced called asexual spores. Asexual spores are the asexual reproductive cells that are produced in the conidia. Conidia are the asexual reproductive cells that are produced in specialized hyphae called sterigmata. The produced asexual is known as Cleistothecia. [18] isolated and identified this soilborne fungi from the agricultural soil in Turkey. [17] isolated and characterized this fungal strain from agricultural soil in India. [10] identified *Aspergillus* sp. from the garden soil. [22] was carried out by culturing on potato dextrose and Sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as From Kwara state [21].~~

Comment [WU20]: Delete as it is already in the methods section

3.3.3.3.1 Comparison of *Aspergillus* sp. in different regions of Bangladesh

Soil colonization of *Aspergillus* sp. varied among the potato field soil of different potato growing regions and ranged from 4×10^3 to 14×10^3 CFU/g soil where the highest colonization was recorded at Singair union of Manikganj district and Tongibari of Munshiganj and the lowest colonization was recorded at Matlab Uttar upazila of Chandpur district.

Comment [WU21]: Vary the stem of the sentence to avoid monotony!!

3.4 Identification of *Rhizopus* sp.

~~The several temporary and semi permanent slides were prepared to observe the micro organisms under microscope. The *Rhizopus* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 4). Colonies grow rapidly and resemble like as cotton candy. Colonies darken with age, becoming gray or yellow-brown. The reverse is white. 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. [17] isolated and characterized this fungal strain from agricultural soil in India. [10] identified sp. fungus from the garden soil. [22] was carried out by culturing on potato dextrose and Sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India.~~

Comment [WU22]: Delete as it is already in the methods section

3.3.4.3.4.1 Comparison of *Rhizopus* sp. in different regions of Bangladesh

Soil colonization of *Rhizopus* sp. varied among the potato field soil of different potato growing regions and ranged from 8×10^3 to 16×10^3 CFU/g soil where the highest colonization was recorded at Tongibari upazila of Munshiganj district and the lowest colonization was recorded at Puthia upazila of Rajshahi.

Comment [WU23]: Vary the stem of the sentence to avoid monotony!!

3.5 Identification of *Bipolaris* sp.

~~The several temporary and semi permanent slides were prepared to observe the micro organisms under microscope. The *Bipolaris* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 5). Colonies are moderately fast growing, effuse, gray to blackish brown, suede like to floccose with a black reverse.~~

Comment [WU24]: Delete as it is already in the methods section

~~Microscopic morphology shows cymodial development of hyaline to deep olivaceous pigmented, pseudoseptate conidia on a geniculate or zig-zag rachis. Conidia mostly curved, canoe-shaped, fuscoid or obclavate, rarely straight, 2-14 pseudoseptate (usually more than 6), germinating only from the ends (bipolar). A field research was conducted by [22] to isolate and characterize the *Bipolaris* sp. from gerbera plants in Maharashtra.~~

3.3.53.5.4 Comparison of *Bipolaris* sp. in different regions of Bangladesh

Soil colonization of *Bipolaris* sp. varied among the potato field soil of different potato growing regions and ranged from 2×10^3 to 13×10^3 CFU/g soil where the highest colonization was recorded at Singair union of Manikganj district and the lowest colonization was recorded at Gobindaganj upazila and Palashbari upazila of Gaibandha district.

Comment [WU25]: Vary the stem of the sentence to avoid monotony!!

3.6 Identification of *Phytophthora* sp.

~~The several temporary and semi-permanent slides were prepared to observe the micro organisms under microscope. The *Phytophthora* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 6). The pathogen produced hyaline lemon-shaped sporangia arose straightly from the surface of the substrate. *Phytophthora* species may reproduce sexually or asexually. Asexual (mitotic) spore types are chlamydo-spores, and sporangia which produce zoospores. Also, sporangia may release zoospores, which have two unlike flagella which they use to swim towards a host plant. Sporangiphores were irregularly branched singly or in a loose cymodium 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 [24]. [25] investigated the seasonal tracking of *Phytophthora* recovery from a variety of soil and forest types in northwestern California.~~

3.3.63.6.4 Comparison of *Phytophthora* sp. in different regions of Bangladesh

Soil colonization of *Phytophthora* sp. varied among the potato field soil of different potato growing regions and ranged from 3×10^3 to 8×10^3 CFU/g soil where the highest colonization was recorded at Tongibari upazila of Munshiganj district and the lowest colonization was recorded at Singair union of Manikganj, Gopinathpur of Gaibandha, Bagmara of Rajshahi and Sonargaon of Narayanganj.

Comment [WU26]: Vary the stem of the sentence to avoid monotony!!

3.7 Identification of *Fusarium* sp.

~~The several temporary and semi-permanent slides were prepared to observe the micro organisms under microscope. The *Fusarium* sp. was identified to the genus level on the basis of macromorphological characteristics using suitable media and slide cultures during working in the laboratory (Table 7). Hyphae are hyaline are septate and showed divisions or walls within the hyphae. Conidiophores are short and usually non-septate when compared to other *Fusarium* species. The conidiophores have somewhat inflated appearance as their sides are not parallel but slightly in the middle and the pigmentations were pale brown to yellowish brown with a dark brown zonation. These conidiophores are produced singly as they extend from the aerial mycelium. The feature of conidiogenous cell with branched and long monophialides were commonly observed. [17] isolated and characterized this fungal strain from agricultural soil in India. [22] was carried out by culturing on potato dextrose and Sabouraud's dextrose agar media and microscopic method and identified sp. [20] analyzed the seasonal variation and percentage frequency of the mycoflora were statistically from India. The physicochemical characteristics of soil samples was found to affect the distribution and population of fungi such as From Kwara state [21].~~

Comment [WU27]: Delete as it is already in the methods section

3.3.73.7.4 Comparison of *Fusarium* sp. in different regions of Bangladesh

Soil colonization of *Fusarium* sp. varied among the potato field soil of different potato growing regions and ranged from 1×10^3 to 5×10^3 CFU/g soil where the highest colonization was recorded at Tongibari upazila of Munshiganj district and the lowest colonization was recorded at Daudkandi upazila of Cumilla district.

Comment [WU28]: Vary the stem of the sentence to avoid monotony!!

3.8 Identification of *R. solanacearum*

The virulent wild type colonies were large, elevated, fluidal and either entirely white or with a pale red center. And the avirulent mutant colonies were butyrous, deep red often with a bluish border. Even *R. solanacearum* colonies were white and fluidal with whorle characters. These are the typical characteristics of virulent isolates of the bacterial wilt pathogen *R. solanacearum*[26,27,28]. And these results indicated that the procedure of collecting virulent colonies of *R. solanacearum* based on cultural characteristics on TTC was appropriate as suggested by [20].

3.8.1 Comparison of *Bacterium (R. solanacearum)* in different regions of Bangladesh

Soil colonization of *R. solanacearum* varied among the potato field soil of different potato growing regions and ranged from 7×10^3 to 24×10^3 CFU/g soil where the highest colonization was recorded at Tongibari upazila of Munshiganj district and the lowest colonization was recorded at Palashbari of Gaibandha and Bagmara of Rajshahi.

Comment [WU29]: Vary the stem of the sentence to avoid monotony!!

3.9 Frequency of mycoflora in the regions of Bangladesh

~~The Soil pH, organic content and water are the main factors affecting the fungal population and diversity. During the investigation period 7 fungal species and one bacterium species were observed from the different upazilas.~~

~~The soil mycoflora in different potato fields soil from different locations of Bangladesh were observed.~~ To give a glance of presence of particular species present in seven (7) districts of Bangladesh, site wise results of numbers of species obtained on PDA media is graphically shown in Figure 1, Figure 2, Figure 3 and Figure 4.

Among the micoflora isolated from Singair upazilla of Manikganj district (Figure 1.a), the highest occurrence was found for *Penicillium* sp. (52.38%) and the lowest occurrence was found for *Fusarium* sp. (14.29%). *Alternaria* sp. resulted 33.33% contribution in microbial occurrence in Singair of manikganj. In the Singair union of Manikganj district (Figure 1.b), the highest contribution was found for *R. solanacearum* (32.81%) and the lowest contribution found for *Phytophthora* sp. (4.69%). And for the rest of fungi *Aspergillus* sp., *Rhizopus* sp. and *Bipolaris* sp. was contributed to 21.88%, 20.31% and 20.31% respectively for their occurrence in Singair union of Manikganj. Among the micoflora isolated from Bandar upazilla of Narayanganj district (Figure 1.c), the highest contribution was found for *Rhizopus* sp. (50%) and the lowest contribution found for *Alternaria* sp. (13.64%). The occurrence of *Aspergillus* sp. in Bandar upazilla was 36.36%. But in the Sonargaon upazila of Narayanganj district (Figure 1.d), only two mycoflora was found to be existed where the highest contribution was found for *Aspergillus* sp. (81.25%) and the lowest contribution found for *Phytophthora* sp. (18.75%).

Comment [WU30]: Discuss the findings in relation to other studies (literature) and cite the sources of literature.

Among the micoflora isolated from Tongibari upazilla of Munshiganj district (Figure 2.a), the highest contribution was found for *R. solanacearum* (32.87%) and the lowest contribution found for *Fusarium* sp. (6.85%). The occurrence of the rest of fungi *Alternaria* sp., *Aspergillus* sp., *Rhizopus* sp. and *Phytophthora* sp. was 8.22%, 19.18%, 21.92% and 10.96%, respectively. In the Matlab Dakshin upazilla of Chandpur district (Figure 2.b), the highest contribution was found for *R. solanacearum* (48%) and the lowest contribution found for *Phytophthora* sp. (16%). The occurrence of *Rhizopus* sp. was 36%. Among the micoflora isolated from Matlab Uttar upazilla of Chandpur district (Figure 2.c), the highest contribution was found for *R. solanacearum* (48.15%) and the lowest contribution found for both *Penicillium* sp. and *Fusarium* sp. was (11.11%). The rest of fungi *Alternaria* sp. and *Aspergillus* sp. contributed 14.81% and 14.81%, respectively. Among the micoflora isolated from Daudkandi upazilla of Cumilla district (Figure 2.d), the highest contribution was found for *R. solanacearum* (50%) and the lowest contribution found for

Fusarium sp. (3.12%). And for the rest of fungi *Alternaria* sp., *Aspergillus* sp. and *Bipolaris* sp. contributed 9.38%, 25.0% and 12.50%, respectively.

Among the micoflora isolated from Gobindaganj upazilla of Gaibandha district (Figure 3.a), the highest contribution was found for *R. solanacearum* (43.33%) and the lowest contribution found for *Bipolaris* sp. (6.67%). And for the rest of fungus *Aspergillus* sp. and *Phytophthora* sp. was (36.67%) and (13.33%) respectively. Only two mycoflora was isolated from Gaibandha sadar upazila of Gaibandha district (Figure 3.b), where the highest contribution was found for *Rhizopus* sp. (78.57%) and the lowest contribution found for *Bipolaris* sp. (21.43%). Among the mycoflora isolated from Palashbari upazilla of Gaibandha district (Figure 3.c), the highest contribution was found for *R. solanacearum* (53.85%) and the lowest contribution found for *Bipolaris* sp. (15.38%). The occurrence of *Alternaria* sp. was 30.77%. Among the mycoflora isolated from Sadullapur upazilla of Gaibandha district (Figure 3.d), the highest contribution was found for *Aspergillus* sp. (47.06%) and the lowest contribution found for *Fusarium* sp. (11.76%). *Penicillium* sp. was contributed to 41.18%.

Among the mycoflora isolated from Gopinathpur of Gaibandha district (Figure 4.a), the highest contribution was found for *Rhizopus* sp. (60%) and the lowest contribution found for both of *Alternaria* sp. and *Phytophthora* sp. was (20%). Between the two mycoflora isolated from Durgapur upazila of Rajshahi district (Figure 4.b), the highest contribution was found for *R. solanacearum* (75%) and the lowest contribution found for *Alternaria* sp. (25%). Among the mycoflora isolated from Puthia upazila of Rajshahi district (Figure 4.c), the highest contribution was found for *Rhizopus* sp. (50%) and the lowest contribution found for both *Penicillium* sp. and *Bipolaris* sp. (25%). Among the mycoflora isolated from Bagmara upazilla of Rajshahi district (Figure 4.d), the highest contribution was found for *R. solanacearum* (38.88%) and the lowest contribution found for both *Phytophthora* sp. and *Fusarium* sp. (16.67%). The occurrence of *Alternaria* sp. was 27.78%.

Comment [WU31]: Support these findings with previous studies (literature)

In the present study soil samples were analyzed with respect to different types of fungal and bacterial mycoflora and a distinct microbial occurrence and diversity was found in selected potato filed soil of Bangladesh where the occurrence of *Ralstonia solanacearum* was highest (24×10^3 CFU/g soil) in Tongibari of Munshiganj and the lowest occurrence (1×10^3 CFU/g soil) of *Fusarium* sp. was recorded in Daudkandi of Cumilla district. The variation of occurrence of *R. solanacearum* was due to environmental and soil factors. Like our study, variation in frequency of occurrence in *Alternaria* sp. was found in India where 20×10^3 CFU/g soil was found in sediment from the surface soil layer [30].

Comment [WU32]: You cannot be very sure as you did not analyze any of the soil parameters. Just speculate rather than being absolutely certain. Use speculative statements like 'probably', 'most likely' etc

In present study, the total amount of *Penicillium* sp. was observe as 25×10^3 CFU/g soil from the different potato fields of Bangladesh. But the amount (215×10^3 CFU/g) was found in Iraq by [31]. A frequency of 3×10^3 CFU/g [20] and 15×10^3 CFU/g [32] of *Penicillium* sp. was recorded from agricultural fields in India. *Aspergillus* sp. was isolated and counted 12×10^3 CFU/g soil from different crop fields in India by [32]. The total 539×10^3 CFU/g soil was observed from different areas in Erbil, Iraq by [31]. [20] also found 1×10^3 CFU/g from agricultural fields in India. The amount of *Rhizopus* sp. (11×10^3 CFU/g) was observed in soils sediment from the surface layer in India by [30]. In the present study, the total amount of *Rhizopus* sp. (77×10^3 CFU/g) was observed from the different potato fields in Bangladesh. But the total amount of *Rhizopus* sp. (115×10^3 CFU/g) was reported by [31].

Bipolaris sp. was observed as highest amount (13×10^3 CFU/g soil) from Singair union of Manikganj district. The nearest amount (4×10^3 CFU/g soil) was found from both Daudkandi upazila of Cumilla and Puthia upazila of Rajshahi, whereas, this pathogen was not identified from Chandpur, Narayanganj and Munshiganj districts. The average amount of *Bipolaris* sp. (8×10^3 CFU/g soil) was observed from the Kingdom of Saudi Arabia [33]. The highest amount (8×10^3 CFU/g soil) of *Phytophthora* sp. was observed from Munshiganj district (Tongibari upazila). The nearest amount (4×10^3 CFU/g soil) was found from Gaibandha (Gobindaganj upazila) and Chandpur (Matlab Dakshin upazila), whereas, this pathogen was not identified from Cumilla districts. The amount (2×10^3 CFU/g soil) was reported from agricultural fields in India by [20]. 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 [31,30]. *Fusarium* sp. was frequently identified from rhizosphere and rhizoplane of crop plants in several agricultural fields of Bangladesh [34]. Like this study *Aspergillus* sp.,

Penicillium sp., *Rhizopus* sp. and *Fusarium* sp. were previously identified from vegetable field soils of Bangladesh [35].

4. CONCLUSION

The aim of this study was isolation, identification and diversity of microorganisms which are present in potato field soil habitat of the investigated regions of Bangladesh. From the present investigation it is concluded that a total of seven (7) genera of fungi and one bacteria species were isolated and identified from 23 potato field soil samples collected from 14 upazillas under seven districts of potato growing 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 agricultural soils. The results obtained clearly indicates ~~that~~ the presence of *Alternaria* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Bipolaris* sp., *Phytophthora* sp., *Fusarium* sp. and one bacteria species *R. solanacearum* in the selected regions of Bangladesh. Frequency of occurrence of collected mycoflora varied among collection sites. 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.

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Comment [WU33]: The numbering the cited references intext will change as some were altered. Make sure you correct the references both intext and in the full reference!

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Table 1. Comparison of different potato field soil with the frequency of presence of *Alternaria* sp. (CFU/g soil)

Districts	Upazilas	<i>Alternaria</i> sp. (CFU/g soil)
Manikganj	Singair	7×10^3
Gaibandha	Palashbari	4×10^3
	Gopinathpur	3×10^3
Cumilla	Daudkandi	3×10^3
Chandpur	Matlab Uttar	4×10^3

Narayanganj	Bandar	3×10^3
Rajshahi	Durgapur	4×10^3
	Bagmara	5×10^3
Munshiganj	Tongibari	6×10^3

Table 2. Comparison of different potato field soil with the frequency of presence of *Penicillium* sp. (CFU/g soil)

Districts	Upazilas	<i>Penicillium</i> sp. (CFU/g soil)
Manikganj	Singair	11×10^3
Gaibandha	Sadullapur	7×10^3
Chandpur	Matlab Uttar	3×10^3
Rajshahi	Puthia	4×10^3

Table 3. Comparison of different potato field soil with the frequency of presence of *Aspergillus* sp. (CFU/g soil)

Districts	Upazilas	<i>Aspergillus</i> sp. (CFU/g soil)
Manikganj	Singair Union	14×10^3
Gaibandha	Gobindaganj	11×10^3
	Sadullapur	8×10^3
Cumilla	Daudkandi	8×10^3
Chandpur	Matlab Uttar	4×10^3
Narayanganj	Bandar	8×10^3
	Sonargaon	13×10^3
Munshiganj	Tongibari	14×10^3

Table 4. Comparison of different potato field soil with the frequency of presence of *Rhizopus* sp. (CFU/g soil)

Districts	Upazilas	<i>Rhizopus</i> sp. (CFU/g soil)
Manikganj	Singair Union	13×10^3
Gaibandha	Gaibandha Sadar	11×10^3
	Gopinathpur	9×10^3
Rajshahi	Puthia	8×10^3
Chandpur	Matlab Dakshin	9×10^3

Narayanganj

Bandar

11×10^3

Munshiganj

Tongibari

16×10^3

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Table 5. Comparison of different potato field soil with the frequency of presence of *Bipolaris* sp. (CFU/g soil)

Districts	Upazilas	<i>Bipolaris</i> sp.(CFU/g soil)
Manikganj	Singair Union	13x10 ³
Gaibandha	Gobindaganj	2x10 ³
	Gaibandha Sadar	3x10 ³
	Palashbari	2x10 ³
Cumilla	Daudkandi	4x10 ³
Rajshahi	Puthia	4x10 ³

Table 6. Comparison of different potato field soil with the frequency of presence of *Phytophthora* sp. (CFU/g soil)

Districts	Upazilas	<i>Phytophthora</i> sp.(CFU/g soil)
Manikganj	Singair Union	3x10 ³
Gaibandha	Gobindaganj	4x10 ³
	Gopinathpur	3x10 ³
Rajshahi	Bagmara	3x10 ³
Chandpur	Matlab Dakshin	4x10 ³
Narayanganj	Sonargaon	3x10 ³
Munshiganj	Tongibari	8x10 ³

Table 7. Comparison of different potato field soil with the frequency of presence of *Fusarium* sp. (CFU/g soil)

Districts	Upazilas	<i>Fusarium</i> sp.(CFU/g soil)
Manikganj	Singair	3x10 ³
Gaibandha	Sadullapur	2x10 ³
Cumilla	Daudkandi	1x10 ³
Rajshahi	Bagmara	3x10 ³
Chandpur	Matlab Uttar	3x10 ³
Munshiganj	Tongibari	5x10 ³

Table 8. Comparison of different potato field soil with the frequency of presence of *Ralstonia solanacearum* (CFU/g soil)

Districts	Upazilas	<i>Ralstonia solanacearum</i> (CFU/g soil)
Manikganj	Singair union	21×10 ³
Gaibandha	Gobindaganj	13×10 ³
	Palashbari	7×10 ³
Cumilla	Daudkandi	16×10 ³
Rajshahi	Durgapur	12×10 ³
	Bagmara	7×10 ³
Chandpur	Matlab Dakshin	12×10 ³
	Matlab Uttar	13×10 ³
Munshiganj	Tongibari	24×10 ³

Table 9. Comparative degree in occurrence of fungal and bacterial mycoflora in different potato field soils of Bangladesh

Degree in occurrence	CFU/g soil	Mycoflora detected in different potato fields															
		Singair	Singair Union	Bandar	Sonargaon	Tongibari	Matlab Dakshin	Matlab Uttar	Daudkandi	Gobindaganj	Gaibandha Sadar	Palashbari	Sadullapur	Gopinathpur	Durgapur	Puthia	Bagmara
High	16×10 ³ to 24×10 ³		<i>Ralstonia solanacearum</i>														
Medium	8×10 ³ to 15×10 ³	<i>Penicillium</i> sp.	<i>Aspergillus</i> sp., <i>Rhizopus</i> sp., <i>Bipolaris</i> sp.	<i>Aspergillus</i> sp., <i>Rhizopus</i> sp.	<i>Aspergillus</i> sp.	<i>Aspergillus</i> sp., <i>Phytophthora</i> sp.	<i>Rhizopus</i> sp., <i>Ralstonia solanacearum</i>	<i>Ralstonia solanacearum</i>	<i>Aspergillus</i> sp.	<i>Aspergillus</i> sp., <i>Ralstonia solanacearum</i>	<i>Rhizopus</i> sp.	<i>Aspergillus</i> sp.	<i>Rhizopus</i> sp.	<i>Ralstonia solanacearum</i>	<i>Rhizopus</i> sp.		
Low	1×10 ³ to 7×10 ³	<i>Alternaria</i> sp., <i>Fusarium</i> sp.	<i>Phytophthora</i> sp.	<i>Alternaria</i> sp.	<i>Phytophthora</i> sp.	<i>Alternaria</i> sp., <i>Fusarium</i> sp.	<i>Phytophthora</i> sp.	<i>Alternaria</i> sp., <i>Penicillium</i> sp., <i>Aspergillus</i> sp., <i>Fusarium</i> sp.	<i>Alternaria</i> sp., <i>Bipolaris</i> sp., <i>Fusarium</i> sp.	<i>Bipolaris</i> sp., <i>Phytophthora</i> sp.	<i>Bipolaris</i> sp.	<i>Alternaria</i> sp., <i>Bipolaris</i> sp., <i>Ralstonia solanacearum</i>	<i>Penicillium</i> sp., <i>Fusarium</i> sp.	<i>Alternaria</i> sp., <i>Phytophthora</i> sp.	<i>Alternaria</i> sp.	<i>Penicillium</i> sp., <i>Bipolaris</i> sp.	<i>Alternaria</i> sp., <i>Phytophthora</i> sp., <i>Fusarium</i> sp., <i>Ralstonia solanacearum</i>

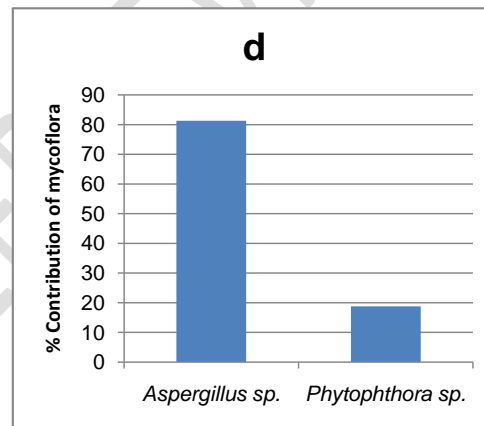
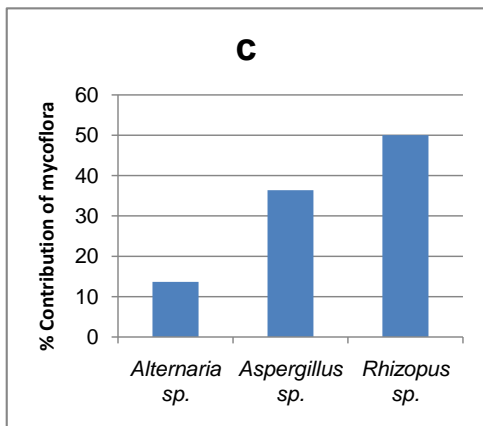
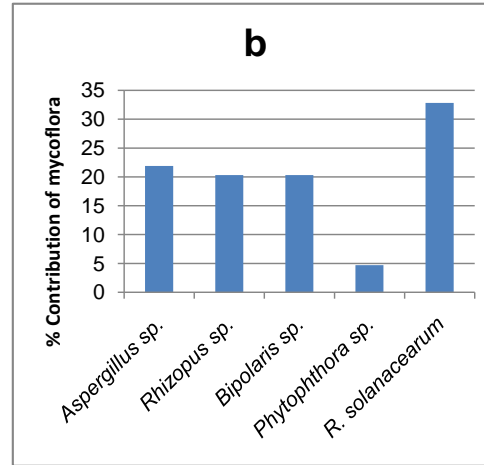
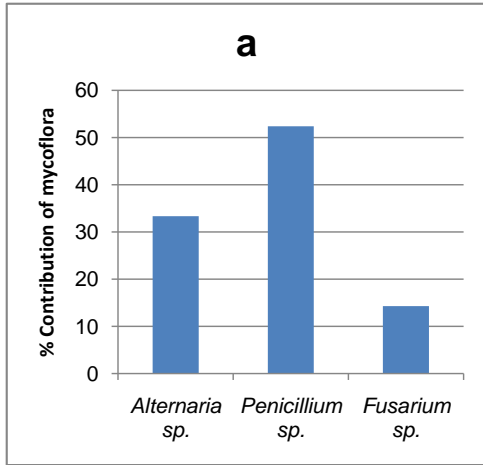


Fig. 1. Contribution (%) of mycoflora at; a. Singair Upazila of Manikganj district; b. Singair Union of Manikganj district; c. Bandar upazila of Narayanganj district; d. Sonargaon upazila of Narayanganj district potato field soil.

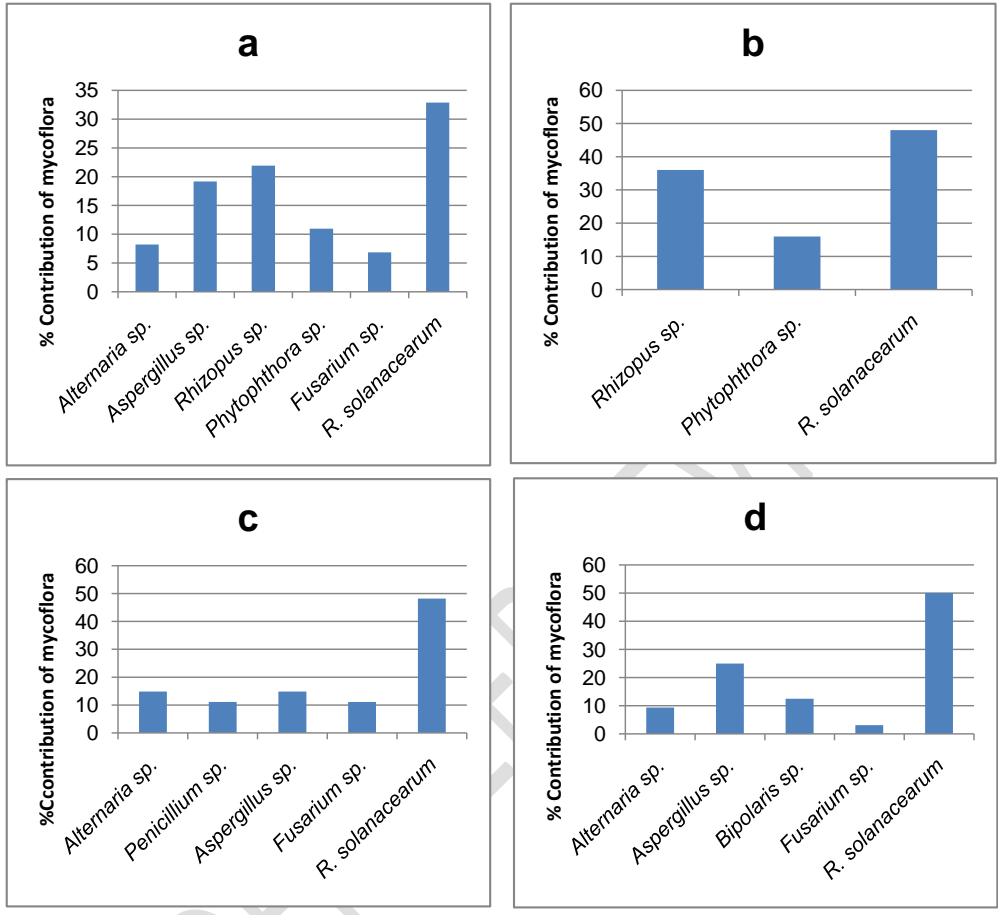


Fig. 2. Contribution (%) of mycoflora at; a. Tongibari upazila of Munshiganj district; b. Matlab Dakshin upazila of Chandpur district; c. Matlab Uttar upazila of Chandpur district; d. Daudkandi upazila of Cumilla district potato field soil.

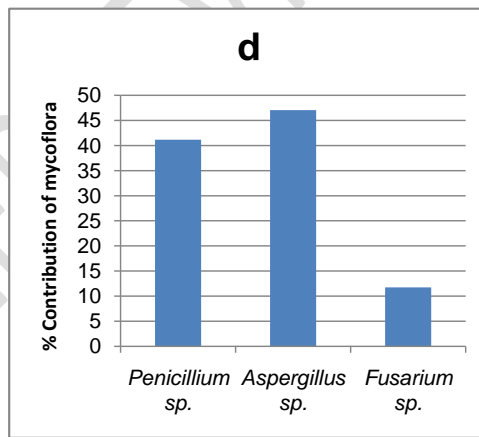
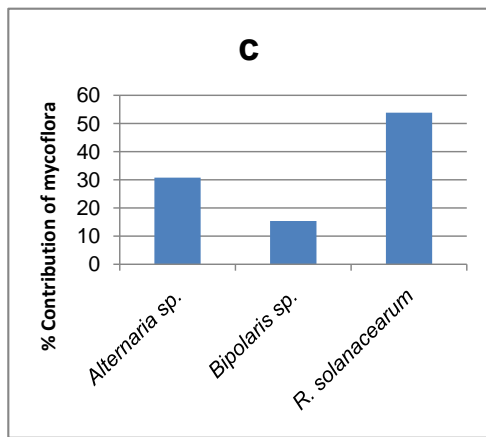
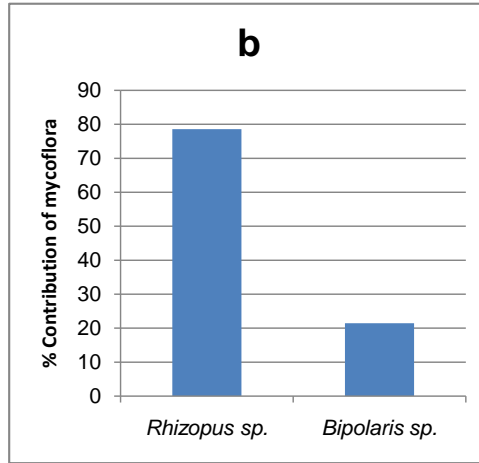
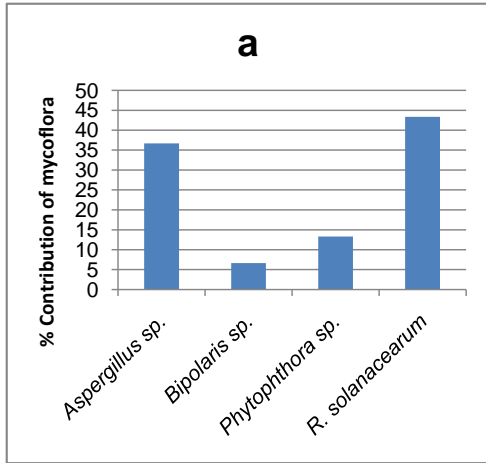


Fig 3. Contribution (%) of mycoflora at; a. Gobindaganj upazila of Gaibandha district; b. Gaibandha Sadar upazila of Gaibandha district; c. Palashbari upazila of Gaibandha district; d. Sadullapur upazila of Gaibandha district potato field soil.

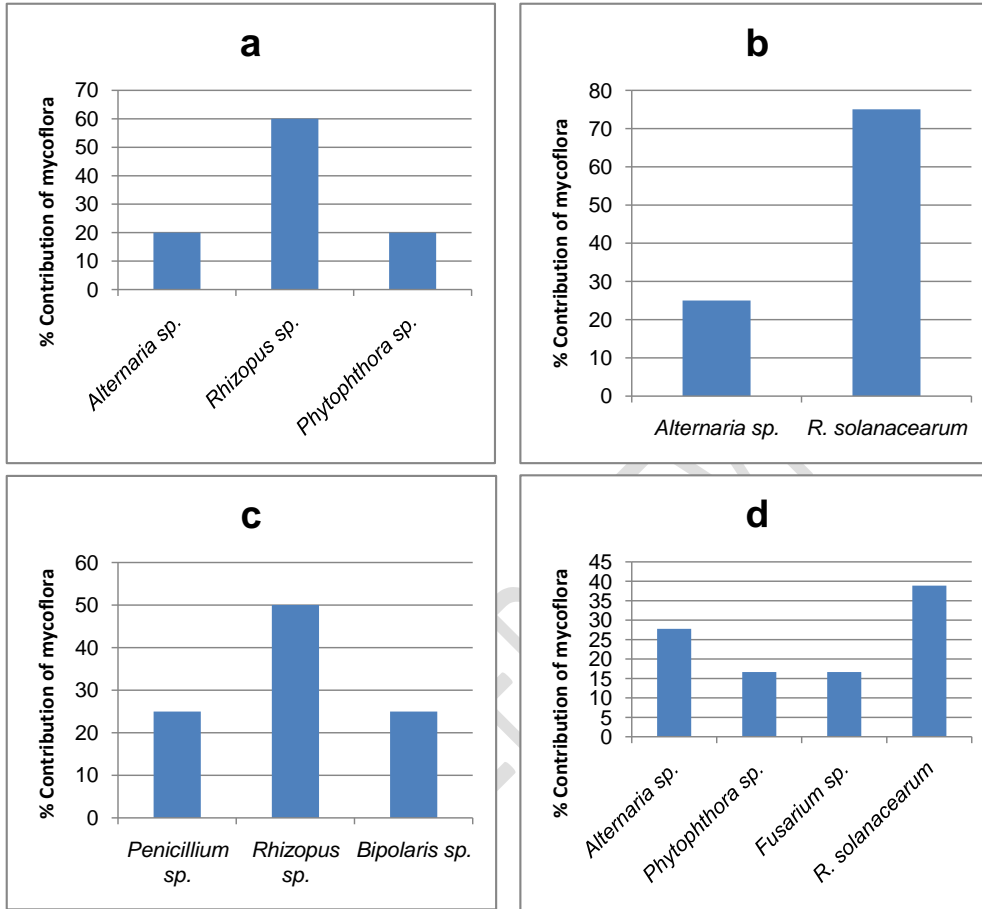


Fig 4. Contribution (%) of mycoflora at; a. Gopinathpur of Gaibandha district; b. Durgapur upazila of Rajshahi district; c. Puthia upazila of Rajshahi district; d. Bagmara upazila of Rajshahi district potato field soil.