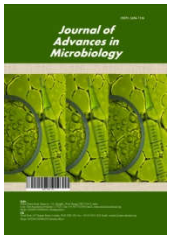

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Occurrence and Diversity of Soil Microflora in Potato Fields of Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAN conducted the research work. Author FMA collected soil samples from potato field of the study area in Bangladesh, designed and supervised the study and edited the manuscript. Author MR wrote the first draft of the manuscript. Author NS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Microflora from potato rhizosphere soil was isolated from different potato fields of Bangladesh. Seventeen soil samples were analyzed for the presence of microflora in selected potato field soils. Seven fungal species and one bacterium species were morphologically characterized using soil dilution and streak plate 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 Potato Dextrose Agar (PDA), V8 juice Agar and their presence in soil was compared in respect of different locations of potato fields. The occurrence of *Phytophthora* sp. was medium in Tongibari and lower in Singair Union, Sonargaon, Matlab Dakshin, Gobindaganj, Palashbari, Gopinathpur and Bagmara. The highest counts of *R. solanacearum* were found in Singair Union, Tongibari and Daudkandi and the lowest counts were made in Palashbari and Bagmara. This was the first reported examination of the microbial diversity of soil microflora in some selected potato fields of Bangladesh.

Keywords: Microflora; diversity; fungi; bacteria.

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1. INTRODUCTION

Soils are very composite systems, with many components playing diverse functions mainly due to the activity of soil organisms [1]. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth 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 microflora [3]. Continuous use of chemical fertilizers over a long period may cause imbalance in soil microflora and thereby indirectly affect biological properties of soil leading to soil degradation [4]. Fungi are 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 toxic chemicals 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 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 microflora 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].

The aim of the present investigation is to isolate and identify microflora 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, 2016 to April, 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 5^0C.

2.2 Preparation of Samples

Ten individual samples (100 g each) collected from a field 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 microflora.

2.3 Sterilization Technique

Petri plates, conical flasks, test-tubes and other glassware were sterilized in hot air oven at 165-170^0C 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 ease the count of colonies of microflora. At first, one gram of representative soil sample (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 into the sterilized glass Petri dish 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 for estimation of fungal population [14].

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). For *Phytophthora* sp. selective V8 Juice Agar was used for isolation. Fungi were identified following Watanabe [15].

2.6 Isolation of Bacteria

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. 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.

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 and a mucoid thread was formed. It means the test was positive and the bacterium was Gram negative [16]. Other than KOH solubility test gram staining test and Kovac's oxidase tests were also done that was positive for *R. solanacearum*.

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:

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

* CFU-Colony Forming Unit

3. RESULTS AND DISCUSSION

Soil microflora 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 microfloral 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 Identified Fungi Species

3.1.1 *Alternaria* sp.

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. Conidia were large with longitudinal and transverse septa and a short beak under motic microscope. *Alternaria* sp. was isolated and characterized from agricultural soil in India [17].

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

Districts	Upazilas	*Alternaria* 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	*Penicillium* 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	*Aspergillus* 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	*Rhizopus* 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

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

Districts	Upazilas	*Bipolaris* sp.(CFU/g soil)
Manikganj	Singair Union	13×10^3
Gaibandha	Gobindaganj	2×10^3
	Gaibandha Sadar	3×10^3
	Palashbari	2×10^3
Cumilla	Daudkandi	4×10^3
Rajshahi	Puthia	4×10^3

3.1.2 *Penicillium* sp.

The conidiophores are branched and are terminated by clusters of flask-shaped phialides. Conidia are round and unicellular. The conidia are produced in chains. *Penicillium* sp. was isolated and identified this soilborne fungi from the agricultural soil in Turkey [18]. *Penicillium* sp. fungus identified from the garden soil [19].The seasonal variation and percentage frequency of the microflora were statistically analyzed from India [20]. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as- From Kwara state [21].

3.1.3 *Aspergillus* sp.

The colony consists of mats of hyphae that make up a mycelium. The hyphae are septate and hyaline. Conidia are the asexual reproductive cells that are produced in specialized hyphae

called conidiophores. Conidia are globose and hyaline. This soilborne fungus was isolated from the agricultural soil in Turkey [18]. This fungal strain was isolated and characterized from agricultural soil in India [17]. *Aspergillus* sp. was identified from the garden soil [19]. *Aspergillus* sp. was previously cultured on potato dextrose and Sabouraud's dextrose agar media and microscopic method was used to identify the species [22]. The seasonal variation and percentage frequency of the microflora were statistically analyzed from India also [20]. 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. Sporangiophores are unbranched, long, and terminate in a columella and a dark round sporangium containing oval brown spores. Stolons bear large rhizoids which are found immediately adjacent to the sporangiophore in the nodal position. This fungal strain was isolated and characterized from agricultural soil in India also [17]. This fungus sp. was identified from the garden soil [19]. Potato dextrose and sabouraud's dextrose agar media and microscopic methods were used to identify microflora from soil previously [22]. Like our study the seasonal variation and percentage frequency of the microflora were statistically analyzed from India [20].

3.1.5 *Bipolaris* sp.

Colonies are moderately fast growing, effuse, grey 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, fusoid, rarely straight, 2–14 pseudoseptate. Isolation and characterization of *Bipolaris* sp. was previously done from gerbera field in Maharashtra [23].

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

Districts	Upazilas	*Phytophthora* sp. (CFU/g soil)
Manikganj	Singair Union	3×10^3
Gaibandha	Gobindaganj	4×10^3
	Gopinathpur	3×10^3
Rajshahi	Bagmara	3×10^3
Chandpur	Matlab Dakshin	4×10^3
Narayanganj	Sonargaon	3×10^3
Munshiganj	Tongibari	8×10^3

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

Districts	Upazilas	*Fusarium* sp. (CFU/g soil)
Manikganj	Singair	3×10^3
Gaibandha	Sadullapur	2×10^3
Cumilla	Daudkandi	1×10^3
Rajshahi	Bagmara	3×10^3
Chandpur	Matlab Uttar	3×10^3
Munshiganj	Tongibari	5×10^3

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

Districts	Upazilas	*Ralstonia solanacearum* (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

3.1.6 *Phytophthora* sp.

The pathogen produced hyaline lemon shaped sporangia arose straightly from the surface of the substrate. Asexual 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 [24]. The seasonal tracking of *Phytophthora* recovery was investigated from a variety of soil and forest types in northwestern California [25].

3.1.7 *Fusarium* sp.

Hyphae are hyaline are septate and showed divisions or walls within the hyphae. Conidiophores are short and non-septate. 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 observed. This fungal strain was isolated and characterized from agricultural soil in India [17]. The genus was previously identified and cultured on potato dextrose and Sabouraud's dextrose agar media [22]. The seasonal variation and percentage frequency of the microflora were statistically analyzed from India also [20]. The physicochemical characteristics of soil samples were found to affect the distribution and population of fungi such as reported from Kwara state [21].

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. The avirulent mutant colonies were butyrous, deep-red often with a bluish border. *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 [29].

3.3 Comparison of Fungi Species 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 soils 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.

3.3.2 *Penicillium sp.*

Occurrence of *Penicillium* sp. varied among the potato field soil of different potato growing regions. It was 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.

3.3.3 *Aspergillus* sp.

The variation of occurrence of *Aspergillus* sp. among potato field soil of different potato growing regions 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.

3.3.4 *Rhizopus* sp.

Rhizopus sp. showed a variation of soil colonization 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.

3.3.5 *Bipolaris* sp.

Soil colonization of *Bipolaris* sp. varied among the potato field soil of different potato growing regions. The variation was 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.

3.3.6 *Phytophthora* sp.

In this study, the distribution of *Phytophthora* sp. varied among the potato field soil of different potato growing regions that 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.

3.3.7 *Fusarium* sp.

Fusarium sp. showed a variation of soil colonization 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.

3.4 Comparison of Bacterium (*R. Solanacearum)* in Different Regions of Bangladesh

In this study, soil colonization of *R. solanacearum* varied among the potato field soil of different potato growing regions. It was 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.

3.5 Frequency of Microflora in the Potato Growing Regions of Bangladesh

To give a glance of presence of particular species present in seven (7) districts of Bangladesh, site wise results of numbers of species obtained during isolation is graphically shown in Figs. 1-4.

Among the microflora isolated from Singair upazilla of Manikganj district (Fig.1a), 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 (Fig. 1b), 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 (Fig. 1c), 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 upazila was 36.36%. But in the Sonargaon upazila of Narayanganj district (Fig. 1d), only two microflora 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%).

In the Tongibari upazila of Munshiganj district (Fig. 2a), 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 upazila of Chandpur district (Fig. 2b), 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 microflora isolated from Matlab Uttar upazila of Chandpur district (Fig. 2c), 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 microflora isolated from Daudkandi upazila of Cumilla district (Fig. 2d), 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 microflora isolated from Gobindaganj upazila of Gaibandha district (Fig. 3a), 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 microflora was isolated from Gaibandha sadar upazila of Gaibandha district (Fig. 3b), where the

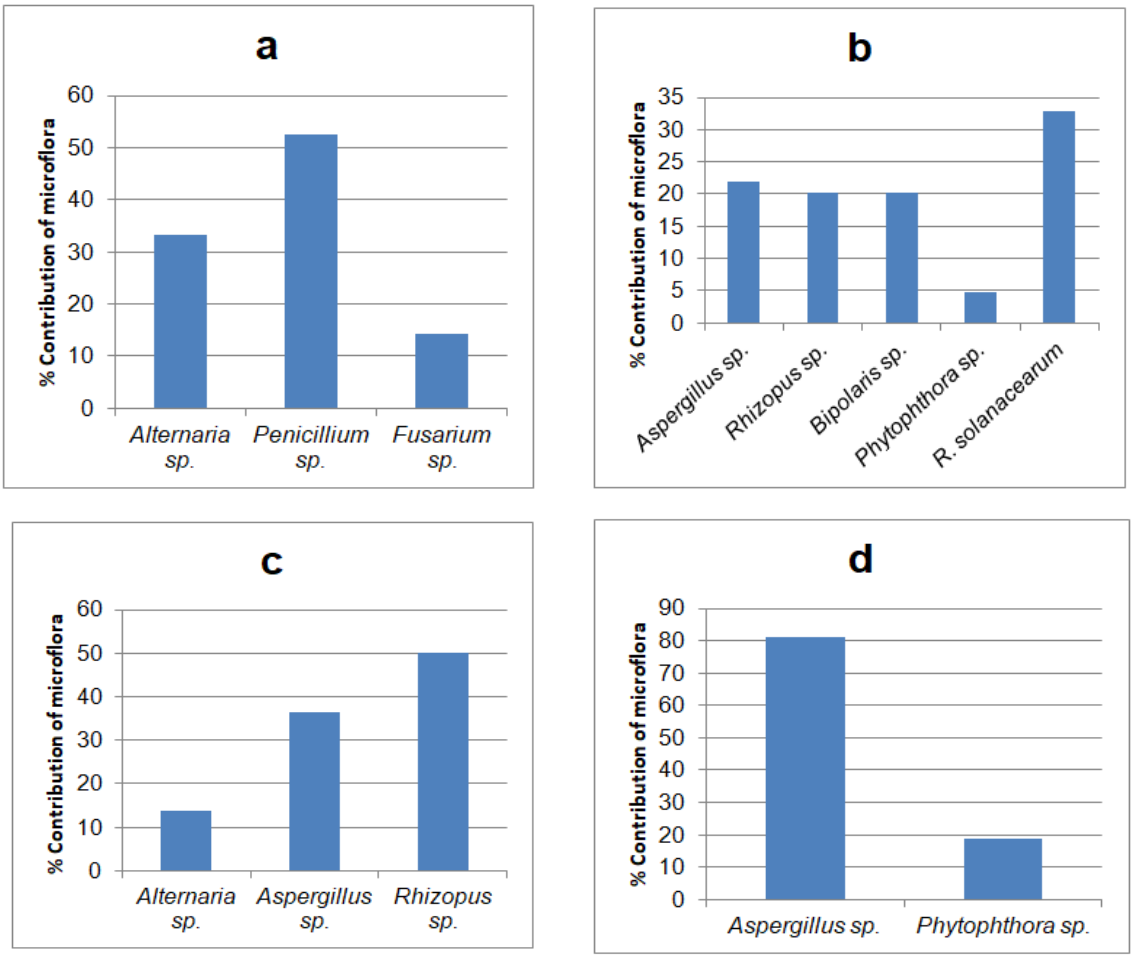

Fig. 1. Contribution (%) of microflora at; a. Singair Upazila of Manikganj district; b. Singair Union of Manikganj district; c. Bandar upazila of Narayanganj district; d. Sonargaon upazila of Narayanganj district potato field soil

highest contribution was found for *Rhizopus* sp. (78.57%) and the lowest contribution found for *Bipolaris* sp. (21.43%). Among the microflora isolated from Palashbari upazila of Gaibandha district (Fig. 3c), 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 microflora isolated from Sadullapur upazila of Gaibandha district (Fig. 3d), 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 microflora isolated from Gopinathpur of Gaibandha district (Fig 4a), 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 microflora isolated from Durgapur upazila of Rajshahi district (Fig. 4b), the highest contribution was found for *R. solanacearum* (75%) and the lowest contribution found for *Alternaria* sp. (25%). Among the microflora isolated from Puthia upazila of Rajshahi district (Fig. 4c), the highest contribution was found for *Rhizopus* sp. (50%) and the lowest contribution found for both *Penicillium* sp. and *Bipolaris* sp. (25%). Among the microflora isolated from Bagmara upazila of Rajshahi district (Fig. 4d), 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%.

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

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

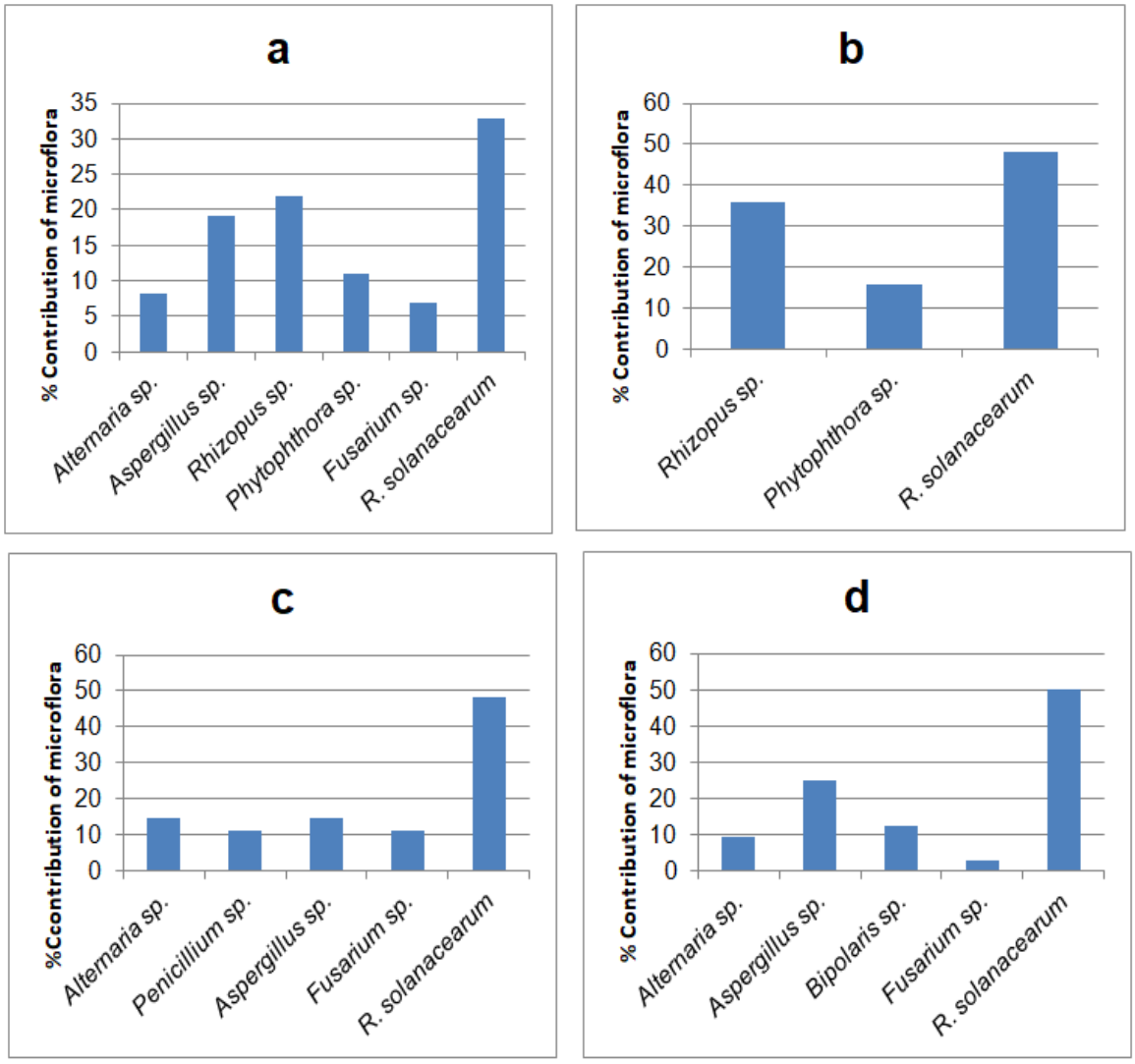

Fig. 2. Contribution (%) of microflora at; a. Tongibari upazila of Munshiganj District; b. Matlab Dakshin upazila of Chandpur District; c. Matlab Uttar upazila of Chandpur District; d. Daudkandi upazila of Cumilla District potato field soil

In the present study soil samples were analyzed with respect to different types of fungal and bacterial microflora 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. Probably, 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].

In present study, the total amount of *Penicillium* sp. was observed 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]. Gaddeyya *et al*., also found 1×10^3 CFU/g from agricultural fields in India [20]. 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

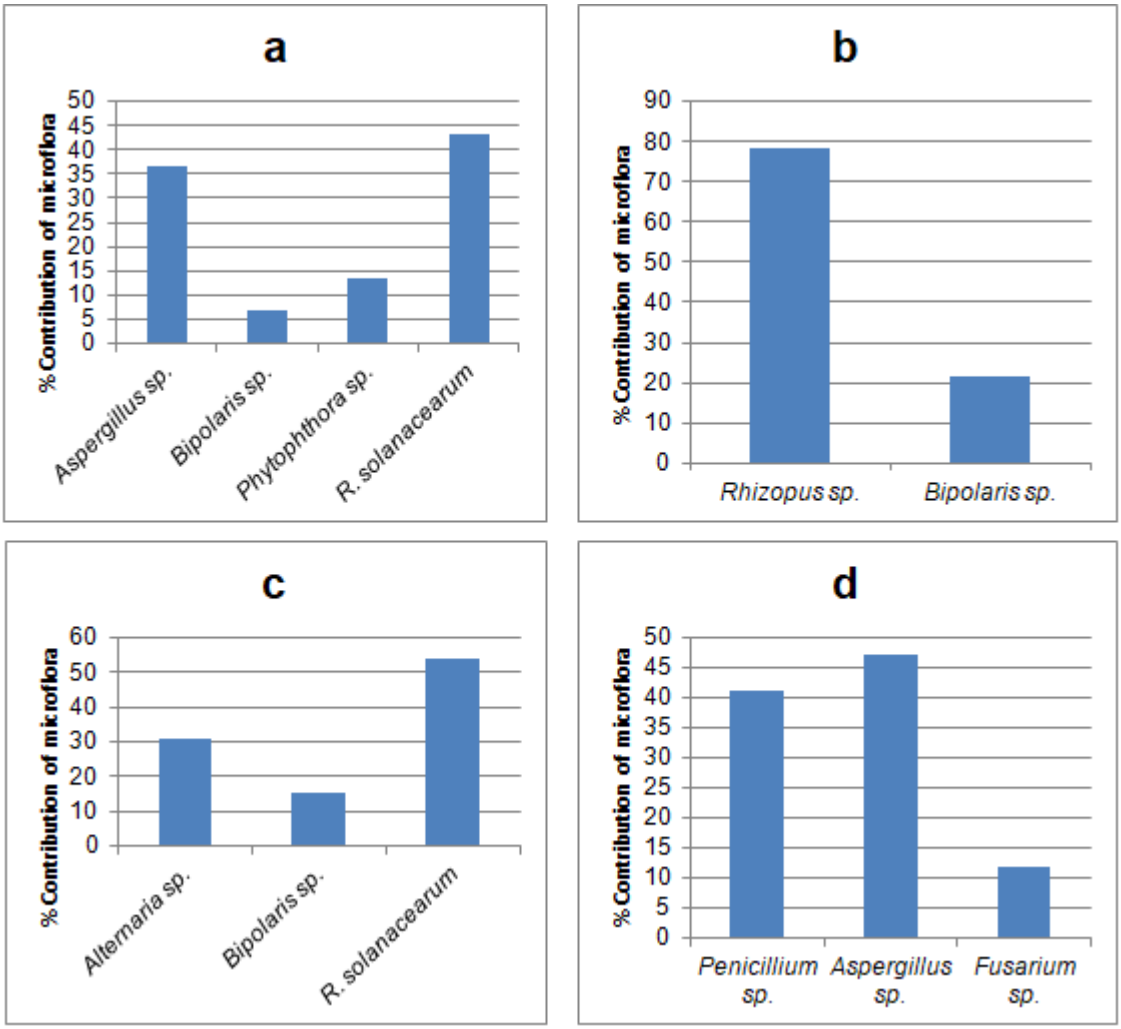

Fig. 3. Contribution (%) of microflora at; a. Gobindaganj upazila of Gaibandha district; b. Gaibandha Sadar upazila of Gaibandha district; c. Palashbari upazila of Gaibandha district; d. Sadullapur upazila of Gaibandha district potato field soil

the different potato fields in Bangladesh. But the total amount of *Rhizopus* sp. **(**115×10^3 CFU/g**)** was reported [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]. 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 [30,31]. *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]. The highest count (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 frequency of occurrence (2×10^3 CFU/g soil) of *Phytophthora* sp. was reported from agricultural fields in India [20]. Late blight of potato caused by *Phytphthora infestans* is a serious problem of potato in Bangladesh. Twenty races of *P. infestans* have been identified in the country.

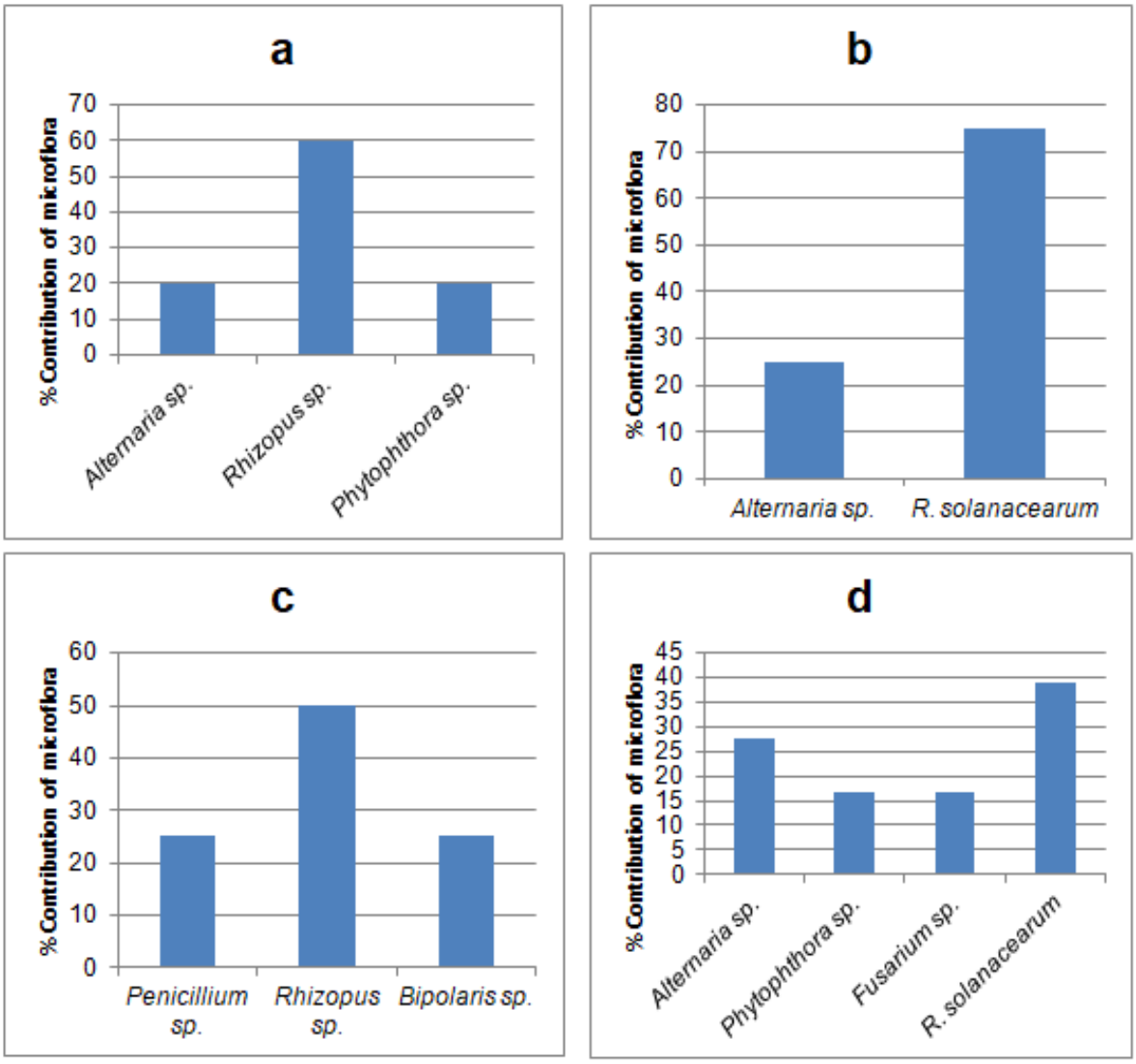

Fig. 4. Contribution (%) of microflora at; a. Gopinathpur of Gaibandha district; b. Durgapur upazila of Rajshahi district; c. Puthia upazila of Rajshahi district; d. Bagmara upazila of Rajshahi district potato field soil

Initially simple races were predominant but in last five years complex races have recorded. The complex races are predominant in the northern part of the country where winter sets earlier and continues for longer period [36]. On the other hand *Ralstonia solanacearum*, the cause of potato brown rot and wilt is other serious tuber quality limiting factor in the country hampering potato export in Europe and other country. The bacterium is soil borne and overwinters in soil and could be a primary source of infection in the next potato cultivation season. *Rhizoctonia solani* also causing foliar diseases is reported. Diversity study of these microfloras with other microorganisms is utmost important as they occur together and playing role to dominate on each other in the soil by colonization.

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 upazilas 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 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. Two genera *Phytophthora* and *Ralstonia* causing late blight and brown rot respectively, are the most damaging factors that reducing tuber yield and quality of potato tuber in Bangladesh. Frequency of occurrence of collected microflora varied among collection sites. The information resulted from the present study might be useful in integrated management of late blight, brown rot and other foliar diseases of potato in different potato growing regions of the country.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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