EVALUATION OF HEALTH STATUS OF SELECTED VEGETABLES SEED COLLECTED FROM DIFFERENT DISTRICTS OF BANGLADESH

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*This is to certify that, the thesis entitled, "**EVALUATION OF HEALTH STATUS OF SELECTED VEGETABLES SEED COLLECTED FROM DIFFERENT DISTRICTS OF BANGLADESH**" submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY** embodies the result of a piece of bona fide research work carried out by **TARUN CHANDRO ROY**, Registration No.: **10-04133** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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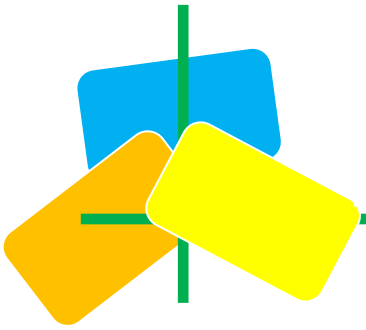

DEDICATED

TO MY PARENTS

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EVALUATION OF HEALTH STATUS OF SELECTED VEGETABLES SEED COLLECTED FROM DIFFERENT DISTRICTS OFBANGLADESH

ABSTRACT

Seed health status of stem amaranth (*Amaranthus lividus*), water spinach *(Ipomoea aquatica)*, Indian spinach (*Basella alba)*, sweet gourd *(Cucurbita moschata)*, snake gourd *(Trichosanthes cucumerina)*, ash gourd *(Benincasa hispida)*, country bean *(Phaseolus vulgaris)*, yard long bean *(Vigna unguiculata)*, radish *(Raphanus sativus)* and carrot *(Daucus carota)* was determined by inspection of dry seed and blotter seed health testing method prescribed by ISTA in Seed Pathology Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during January 2016 to December 2016. The laboratory experiment was conducted following Complete Randomized Design (CRD) with four replications. Untreated, unknown grower's/farmer's bulk vegetables loose seeds were considered for this experiment. Seeds were collected from five districts viz. Dhaka, Rangpur, Thakurgaon, Panchagarh and Dinajpur. For Dhaka, truthfully labeled seed of BADC (Bangladesh Agricultural Development Corporation) was considered to compare the health status of collected seeds. In dry inspection, fungal mycelium (white and cottony) and sclerotia were observed. No physical abnormalities were found in BADC's seeds, except sweet gourd, snake gourd, ash gourd. In most cases, the highest pure seeds were observed in BADC's seeds whereas the lowest was found seeds from Thakurgaon district. Eight fungi viz. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp., *Alternaria* spp. *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp. and *Cercospora* spp. were recorded from seeds by blotter method. Moreover one unidentified bacterium and one unknown fungus were also found in collected seed. In all tested seed sample, the common fungi were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp., *Chaetomium* spp., *Rhizopus* spp. However, *Alternaria* spp. were present in stem amaranth, country bean, radish seeds. *Curvularia* spp. were present in water spinach, Indian spinach, sweet gourd, snake gourd, yard long bean and carrot seeds. The prevalence of fungi varied significantly with respect to seed categories and seed sources. The lowest seed borne fungal infection were observed in BADC seeds followed by seeds of different districts. Considering the findings, farmers are advised to collect vegetables seeds from reliable sources and should check their seed health status before sowing. Farmers are also suggested to do seed treatment by recommended chemicals or botanicals before sowing to prevent seed borne diseases in the field.

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LIST OF ABBREVIATIONS

Full word	Abbreviations
Bangladesh Agricultural Research Council	BARC
Bangladesh Agricultural University	BAU
Bangladesh Agricultural Development Corporation	BADC
Bangladesh Bureau of Statistics	BBS
Centimeter	cm
Coefficient of Variance	CV
Degree Celsius	°C
Etcetera	etc.
Food and Agricultural Organization	FAO
And others	*et al.*
Least Significant Difference	LSD
Percentage	%
Ton per hectare	t/ha
Sher-e-Bangla Agricultural University	SAU
Videlicet (namely)	viz.
Truthfully Labeled Seed	TLS

CHAPTER I

INTRODUCTION

Vegetables and fruits play a major role in human nutrition, mainly as sources of vitamins (C, A, B_6, E, thiamine, niacin), minerals, and dietary fiber (Wargovich, 2000). Fruits and vegetables also supply 16% of magnesium, 19% of iron, and 9% of the calories. Vegetables included in daily schedule of diet viz. Sweet Pepper, Cauliflower, Carrot, Cabbage, Lettuce, Spinach, Tomato, Potato, Radish, and Bottle Gourd were analyzed for their proximate composition, vitamin and mineral contents to evaluate their importance in human nutrition (Hanif *et al.*,2006). According to BBS (2014), agriculture contribute about 16.33 percent of the country's GDP. In the year of 2014-15, vegetable cultivated area is 9,89,000 acres and 37,29,000 M. tons of production (BBS, 2015).

A world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera (Kays *et al.*1995, Kays, 2011). Leafy vegetables of which the leaves or young leafy shoots are consumed were the most often utilized (53% of the total), followed by vegetable fruits (15%), and vegetables with below ground edible organs comprised 17%. Many vegetable crops have more than one part used. Most of the vegetables are marketed fresh with only a small proportion processed because most vegetables are perishable.

Seed is essential for agriculture. Among the agricultural inputs, seed is the most important input for crop production. Quality and healthy seed is the crying need of the day. Healthy or pathogen free seeds of good quality are considering as the vital factor for desired plant population and good harvest. Health of seeds can be affected by direct infection of pathogens or through contamination of seeds by

pathogenic propagules as contamination in, on or with the seeds or as concomitant contamination (Fakir, 2000). Infection of seed by pathogenic organisms and presence of propagules of pathogen in a seed lot is really significant due to germination failure and subsequent infection to seedlings and growing plants. That's why good and healthy seed is considered as important factor for successful crop production. Seeds of vegetables are more vulnerable to attack by pathogens and quickly deteriorate in storage. Their inherent quality cannot be assessed easily just from their external appearances. Good seed is essential for good crop, which indicate that the seed should be pure, viable and healthy. Use of good seeds can contribute to increase vegetable yield as high as 30% remaining all other factors of production as content (Khanom, 2011).

The seed industry in Bangladesh comprises of both public and private sector initiatives. Government agencies involved in this sector include Bangladesh Agricultural Development Corporation (BADC), Bangladesh Agricultural Research institute (BARI), Bangladesh Rice Research Institute (BRRI), Bangladesh Jute Research Institute (BJRI), and Department of Agriculture Extension (DAE). The Government has newly given the seed sector a "primacy" status. However, in case of private sector, there are more than 100 companies involved, with over 5000 registered seed dealers operating across the country. The recent expansion of the private sector seed companies has resulted in the appointment of thousands of contract growers into the formal seed production chain, leading to improved livelihoods among the rural community. Bottle gourd, Snake gourd, Yard Long bean and Ridged gourd are commonly cultivated in the country. Among the various factors responsible for low yield of these crops, disease and use of poor quality seeds play an important role. Bottle gourd, Snake gourd, Yard Long bean, and Ridged gourd suffer from a large number of diseases

(Fakir *et al*., 2000). Coincidentally, important diseases of these crops are seed borne and caused by fungi. Common seed-borne fungal diseases occurring on vegetables are damping off, foot and root rots, phomopsis blight, fruit rots, black leg, leaf spots, fusarium wilt, fusarium root rot, anthracnose and downy mildews (Fakir *et al*., 2000).

About 200 different seed-borne pathogens including more than 100 fungi have been reported to cause diseases in different vegetable crops in the world. A total of 18 seed-borne fungal pathogens have been reported from the seeds of these four selected crops. Of these; 10, 4, 2 and 2 fungal pathogens have been found associated with the seeds of Bottle gourd, Snake gourd, Yard Long bean and Ridged gourd, respectively at home and abroad (Islam, 1990).

Considering the above facts, the present research was undertaken to assess the health and quality of vegetable seeds collected from different local market of Dhaka, Rangpur, Dinajpur, Thakurgaon and Panchagarh districts in Bangladesh. However, management of these seed-borne fungi is very important to produce vegetable successfully. In view of the above facts, the present study was undertaken to achieve the following objective:

- To assess the health status of selected vegetables seed viz. stem amaranth, water spinach, Indian spinach, sweet gourd, snake gourd, ash gourd, country bean, yard long bean, radish and carrot collected from different districts of Bangladesh.

CHAPTER II

REVIEW OF LITERATURE

Seed quality of vegetables is affected by different seed borne pathogens. Seed borne pathogens are reported to cause spotting or discolored of seeds. Seed borne fungi associated with vegetable seeds and thus deteriorate the seed quality. In this chapter, a review has been made on seed borne fungi of vegetables.

2.1 Fungal pathogens associated with leafy vegetables (Stem amaranth, Water spinach and Indian spinach)

Mohonto *et al.* (2015) reported that seeds of leafy vegetables viz. Amaranth and Indian spinach yielded six fungal species in blotter method. The identified fungi were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp., *Alternaria* spp., *Chaetomium* spp. and *Rhizopus* spp. Seed infection of an unidentified bacterium was also recorded. The highest total seed borne fungal infection was found in the seeds of New Bangla Seeds.

Begum (2012) collected leafy vegetables viz. Laffa, Mustard, Indian spinach, Jute, Red amaranth, Swamp cabbage, Spinach, Cabbage and Amaranth seeds from different shops of Saidpur. Different seed- borne fungi such as *Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Curvularia* spp., *Fusarium* spp., *Phoma* spp., *Penicillum* spp. and *Rhizopus* spp. were detected.

Khanom (2011) collected leafy vegetables viz. Cabbage, Indian spinach, Indian cabbage, Spinach and Red amaranth seeds from different seed shops of Mymensingh. Different seed borne fungi such as *Alternaria* spp., *Aspergillus* niger, *Curvularia* spp., *Fusarium* spp., *Phoma* spp., *Penicillium* spp. and *Rhizopus* spp. were detected.

Islam (2006) conducted an experiment to study the germination and health status of Indian spinach, red amaranth and spinach collected from BADC, Local Seed Company and Farmer. He observed nine fungi in Bottle gourd where seven fungi in Indian spinach, six fungi in red amaranth and six fungi in spinach. He found that the prevalence of fungi and percent germination varied significantly depending on the seed categories and seed source. He also found, only *Fusarium moniliforme* was capable of transmitting disease to growing seedlings.

Koike and Correll (1993) reported foliar disease symptoms on commercially produced spinach in the saline valley in Monterey country, California, USA. The causal organism was located and identified as *Colletotricum dematium* and its pathogenicity was confirmed.

Richardson (1990) reported that *Alternaria amaranthi* that carried through the seeds of Amaranthus spp. He listed four pathogenic fungi namely *Fusarium oxysporum* f. sp. *lagenariae*, *Lasiodipodia theobromae*, *Macrophomina phaseolina* and *Rhizoctonia solani* in seeds of Bottle gourd.

Wu *et al.* (2001) isolated *Colletotrichum dematium* from seeds of diseased amaranth. The amount of seed borne *C. dematium* was positively correlated with the amount of the abnormal seedlings and unmerging seeds of amaranth.

2.2 Pathogenic fungi associated with fruit vegetables seeds and their pathogenic effect (Sweet gourd, Snake gourd and Ash gourd)

Arfin *et al.* (2015) observed that in blotter seed health testing method, the predominant seed borne pathogens were *Aspergillus fiavus, Rhizopus* spp., and *Fusarium* spp. The total seed borne fungal infection of bottle gourd, sweet gourd, sponge gourd were ranged from 32.5-33.5%, 38-39% and 22-37% respectively. The highest total seed borne fungal infection (39%) was recorded in the seeds of sweet gourd collected from Alo Bij Vander. In overall observation, the seed health status of loose seeds of vegetables was not at satisfactory level.

Hossain *et al.* (2015) reported that 6 company's okra seeds collected from in Mymensingh district and dominance of seed borne fungi was studied by blotter method. Six predominant fungal genera were identified namely *Fusarium oxysporum* (5.08%), *Aspergillus flavus* (4.50%), *Aspergillus niger* (6.50%), *Colletotrichum dematium* (4.67%), *Rhizopus stolonifer* (3.33%) and *Penicillium* spp. (3%). They also conclude germination percentage and fungal association varied from company to company.

Hossain *et al.* (2014) reported that 10 fungi were found associated with the seeds which were *Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Chaetomium funicola, Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *Phoma* spp. and *Rhizopus* spp. The highest total seed-borne fungal infection was found in bottle gourd (15.5%) followed by sweet gourd (14.5%).

Aktar (2009) studied the health status of sweet gourd seeds collected from different villages of Madhupur upazilla of Tangail District. After incubation of sweet gourd

seeds on blotter six different fungi such as *Aspergillus flavus, Aspergillus niger, Penicillium* spp. and *Botrytis cineria* were recorded.

Sultana (2009) conducted an experiment to study the germination and health status of Truthfully Labeled Seed (TLS) of bottle gourd, sweet gourd, snake gourd, ridge gourd, cucumber, wax gourd and sponge gourd collected from BADC and other seed company. She observed eight fungi namely *Aspergillus* spp., *Botrytis* spp., *Curvularia* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Phomopsis* spp. *and Rhizopus* spp. She found only *Aspergillus* spp. was highly prevalent in all the crop seeds ranging from 1.6-14%.

Sultana and Ghaffar (2009) studied seed borne fungi of bottle gourd (*Lagenaria siceraria*). A total of 22 genera and 45 species of fungi were isolated of which 35 have to higher recorded from seeds of bottle gourd in Pakistan. Both blotter and deep-freezing methods yielded quantitatively as well as qualitatively more fungi than agar plate method *Lasiodiplodia theobromae, Fusarium semitectum, Macrophomina phaseolina* and *Fusarium oxysporum* were most frequently isolated from 3,39,150 and 66% seed samples of bottle gourd, respectively.

Anonymous (2003) reported that *Fusarium* spp. fungus can be seed borne in case of squash and pumpkin. They suggested paying special attention in using disease free seed.

Alimova *et al.* (2002) obtained different seed borne fungi and bacteria in cucumber and tomato seeds. They conclude that *Aspergillus* spp, and *Penicillium* spp. were predominant in tomato and cucumber seeds.

Shome (2002) studied seed quality of bringal, tomato and onion sees. He recorded *Aspergillus* spp., *Curvularia s*pp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. where maximum prevalence of *Aspergillus* spp. was (12%) in farmers seed.

Alam (2001 a) reported that by using blotter method, different pathogens for vegetables seeds were obtained. He recorded *Aspergillus, Penicillum, Curvularia, Fusarium, Rhizopus, Colletotrichum, Alternaria* and *Macrophomina* from okra seeds.

Alam (2001 b) studied the health of some vegetable seeds collected from different sources. He used the blotter method for detecting the seed-borne fungi. He found about six fungi in brinjal seeds such as *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Curvularia* spp., *Fusarium* spp. and *Rhizopus* spp. From the result of his experiment he found that the seed collected from Farmers and Local Seed Traders were infected by all most all fungi, which he detected. He also found that the higher prevalence, of all fungi was also in Farmer and Local Seed Trader seed.

Begum and Momin (2000) reported about the suitable detection techniques of associated seed borne fungi in laboratory conditions. Fifty four seed samples of three cucumber, namely sweet gourd (*Cucurbita moschata*), white gourd (*Benincasa hispida*) and bitter gourd (*Momordica charantia*) were collected from six different districts of Bangladesh, to find a suitable detection technique of associated seed borne fungi in laboratory conditions. Among the tested detection techniques, more infection rate were observed for *Aspergillus flavus* and *Penicillium* in all cucurbit seeds in blotter test method, were as higher infection

caused by *Fusarium* and *Rhizopus* were recorded in test tube seeding symptom test. The higher germination percentage of cucurbit seed was observed in blotter test.

Kamble *et al*. (1999) found eight different fungi *Aspergillus* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., *Rhizoctonia* spp., *Macrophomina phaseolina* and *Alternaria* spp. of pumpkin (cv. Local), cucumber (cv. Pnekhire), water melon (cv. Sugar baby) and muskmelon (cv. Punjab hybrid).

Lorenz and Maynard (1980) conducted an experiment to find out a minimum seed germination standard of vegetables for USA which was 50% for okra, 60% for brianjal, 70% for onion and 75% for tomato and radish while Nema (1986) determined that a minimum of 70% for tomato, radish, brinjal and onion and 65% for okra seed germination standard of certified vegetables seeds were obtained in India.

Akter (2008) found *Colletotrichum dematium*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Cercospora* spp., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. as seed borne fungi on okra seeds.

Sarker *et al*. (2006) studied the effect of seed-borne fungal pathogen on the planting value of brinjal (*Solanum melongena*) seeds collected from different sources of Bangladesh during March 2002-March 2003. *Apergillus flavus, Alternaria alternata, Colletotrichum dematium, Phomopsis* spp. *Curvularia lunatu* (*Cochliobolus lunatus*), *Fusarium moniliforme* (*Gibberella moniliformis*), *F. oxysporum, Penicillium* spp. and *Rhizopus* spp. were commonly found prevalent in all the seeds.

Thippeswamy *et al.* (2006) 145 brinjal seed samples were collected from different agro-climatic regions of Karnataka, India during 2001-03 and analysed for mycroflora. This crop is susceptible to phomopsis blight (*Phomopsis vexans*) and leaf spot (*Alternaria solani*) diseases. These diseases are seed borne fungal disease and reduce the yield up to 30-50 percent.

Alam (2004) studied the qualitative attributes of Brinjal (*Solanum melongena* L.) seeds obtained from different sources viz. BADC, BRAC, local Seed Trader, Local Seed Company and Farmers. *Aspergillus* spp., *Penicillium* spp., *Curvularia* spp., *Rhizopus* spp. and *Phomopsis* spp. were identified in the seeds of BADC. Among the studied fungi the highest (16%) prevalence of *Aspergillus* spp. was found jointly in the seeds of BADC and farmer followed by local seed company (12%) and BRAC (8%), while it was absent in local seed trader.

Fakir (2000) listed large number of seed borne fungi under 13 different genus of vegetables. It was 15 in brinjal, 14 in okra and 13 in chilli seeds. The genera were *Alternaria* spp., *Aspergillus* spp., *Colletotricum* spp., *Fusarium* spp., *Penicillium* spp., *Phomopsis* spp., *Pythium* spp., *Sclerotium* spp., *Cercospora* spp., *Macrophomina* spp., *Peronospora* spp. and *Rhizopus* spp.

2.3 Fungal pathogens associated with podded vegetables (country bean, yard long bean)

Alves *et al*. (2009) isolated *Collectotrichum gossypii* var. *cephalosporioides*, *Colletotrichum truncatum* and *Colletotrichum lindemuthianum* from common bean (*Phaseolus vulgaris* L.) by the water restriction technique. Considering the seeds submitted to the blotter test, it was possible to identify *Fusarium* spp., *C. gossypii*

var. *cephalosporiodes*, *C. truncatum* and *C. lindemuthianum* were observed in the surface of inoculated seeds.

Yesuf and Sangchote (2007) made surveys of major seed-borne fungi associated with seeds of common bean (*Phaseolus vulgaris* L.) in different bean-growing areas of Ethiopia. The major seed-borne fungi associated with common bean seeds were *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola* and *Ascochyta phaseolorum.* Bean anthracnose survived in infected seeds but not in the soil, and the primary source of bean anthracnose infection in the field was from infected seeds.

Domijan *et al.* (2003) identified seed-borne fungi on bean (*Phaseolus vulgaris* L.) crops grown in 13 countries of the Republic of Croatia. The most common fungi isolated were *Cladosporium* spp. (98%) *Alternaria* spp. (75%), *Aspergillus* spp. (73%), *Rhizopus* spp. (73%), *Penicillium* spp. (69%), *Fusarium* spp. (38%) and *Fusarium* spp. (38%).

Fakir (1980) reported that at least 2-3% of the total seeds were rotted in storage per annum due to various fungi and bacteria in Bangladesh and lost to the tons of approximately Taka 430 million.

2.4 Fungal pathogens associated with root vegetables (Radish and Carrot)

Hossain *et al.* (2014) reported that Seed quality and health status of 11 vegetable crop seeds of viz. Cabbage (*Brassica oleraceae* var. *capitata*), Indian cabbage (*Brassica oleraceae* var. *indica*), Indian spinach (*Basella alba*), Spinach (*Beta vulgaris* var. *bengalensis*), Red amaranth (*Amaranthus tricolor*), Bitter gourd

(*Momordica charantia*), Bottle gourd (*Lagenaria siceraria*), Sweet gourd (*Cucurbita moschata*), Carrot (*Daucus carrota* var. *sativa*), Radish (*Raphanus sativus*), and Turnip (*Brassica rapa*) were tested. Altogether the lowest infection was found in turnip seeds (6%). The maximum number of dead seeds was found in Indian spinach (89%) and no dead seed was found in Indian cabbage. Among the vegetables seed samples, seedling vigor ranged from 59 to 3083, where the highest seedling vigor was observed in sweet gourd (3083) and the lowest was in Indian spinach (59).

Rahman *et al.* (2012) health status of Bottle gourd, Snake gourd, Yard long bean and Ridged gourd were determined in the laboratory. Three fungi were recorded *Fusarium oxysporium*, *Aspergillus* spp., *Rhizopus* spp., *Fusarium moniliforme* on Bottle gourd, Snake gourd, Yard long bean and Ridge gourd seeds of different sources. More seed-borne fungi were present in Farmers' seed, followed by seeds of different companies and BADC. Per cent germination also varied significantly depending on the seed categories and source. There was a highly positive relationship between germination failure and total seed-borne fungal infection was observed. *F. moniliforme* was capable of transmitting disease to growing seedling. Seeds produced by BADC had the best health quality, followed by seed companies and farmers' source.

Groves and Skolko (2011) isolated seven species of *Alternaria* of which six were definitely pathogenic from seeds. They recorded *Alternaria brassicae, Alternaria oleraceae* and *Alternaria raphani* as pathogen to cruciferous plants. The latter had been isolated only from radish seeds. Two species occur on carrot were *Alternaria radicina* and *Alternaria dauci*.

Fakir (2000) listed 15, 14, 10, 8 and 7 seed-borne fungi on brinjal, okra, onion, radish and tomato seed, respectively. The genera of fungi were *Alternaria, Aspergillus, Colletotrichum, Fusarium, Peronospora* and *Rhizopus*.

Kassim (1996) isolated seed-borne fungi of locally cultivated okra, capsicum, radish and soybean seeds by moist blotter and agar plate method and studied their control by seed treatment. Fifteen species of fungi belonging to the genera *Alternaria, Aspergillus, Botrytis, Cladosporium, Colletotrichum, Curvularia, Epicoccum, Fusarium, Penicillium* and *Stemphylum* were isolated. The fungi were reported for the first time from tested seeds in Saudi Arabia.

Mallek (1995) assayed the seed samples of cabbage, cauliflower, radish and turnip collected from five localities in Egypt were for their fungal flora. He recorded highest fungi was in cabbage seed (75%), whereas the lowest was on turnip seeds (33%). Only five species viz. *Aspergillus flavus, Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium funiculosum* and *Rhizopus stolonifer* were found to be associated with seeds of five plants.

Fakir (1985) tested twenty seed sample of eight cultivars of radish following blotter method for the detection of the associated fungi in Bangladesh. Nineteen fungal species belonging to nine genera were detected. *Alternaria brassicae* and *Alternaria tenuis* were found to cause seed rot and seedling infection on blotter method. *Aspergillus* and *Penicillium* were mostly associated with in germinated seeds.

2.5 Bacteria associated with vegetable seeds

Seed Pathology Centre (SPC), BAU, Mymensingh carried out an experiment to identify bacterial infection of 16 vegetable crops viz. Bean, Bitter gourd, Bottle gourd, Brinjal, Cabbage, Carrot, Cauliflower, Indian cabbage, Indian spinach, Radish, Red amaranth, Spinach, Sweet gourd, Turnip and Yard long been collected from different shops of Mymensingh sadar. *Pseudomonas* spp. were found in association with the seeds of Bean, Bottle gourd, Indian spinach, Radish, Red amaranth, Sweet gourd and tomato. *Pseudomonas fluorescens* were found with the seeds of cabbage, carrot and yard long bean. But bacteria have not been found with the seeds of Bitter gourd by Hossain and Purnima, 2011.

Miklas *et al*. (2011) reported common bacterial blight (CBB; caused by *Xanthomonas oxonopodis* pv. *phaseoli*) a serious disease of dry and green beans (*Phaseolus vulgaris* L.,) in warm humid climates. Crop loss up to 40% has been reported due to this disease, which is most prominent east of the continental divide in the United States.

Warriner *et al.* (2005) evaluated the efficacy of seed decontamination to enhance the safety of salad vegetables and herbs. Seeds (celery, coriander, lettuce, spinach and watercress) were inoculated (as a level of 3-5 log efu g-l) with either *Escherichia coli* P36 or *Listeria monocytogenes* NCTC 7973 and decontaminated with ozone gas, acidified sodium chlorite (ASC) or quaternary ammonium salt preparation (QAS).

CHAPTER III
MATERIALS AND METHODS

3.1. Experimental site

The experiment was conducted in Seed Health Laboratory of the Department of Plant Pathology of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.2. Experimental Period

The experiments were conducted during the period from January, 2016 to December, 2016.

3.3. Collection of seeds

Untreated, unknown grower's/farmer's bulk vegetables loose seeds were used in this experiment. Ten different vegetables seeds were collected from five districts of Bangladesh namely Dhaka, Dinajpur, Thakurgaon, Panchagarh and Rangpur. In case of Dhaka district, truthfully labeled seed of BADC (Bangladesh Agricultural Development Corporation) was selected to compare the health status of collected seeds. The samples were taken to Seed Health Laboratory of Department of Plant Pathology and kept in air tight packed until condition used.

3.4. Vegetable species selected

Seeds of ten different categories of vegetables were studied for their health status.

3.5. Test materials

The categories of vegetables were:

I. Leafy vegetables viz. stem amaranth, water spinach and Indian spinach.

II. Fruit vegetables viz. sweet gourd, snake gourd, ash gourd.

III. Podded vegetables viz. country bean and yard long bean.

IV. Root vegetables viz. radish and carrot.

Bengali or common name, English name, scientific name and family of selected vegetable seeds are given in Table 1.

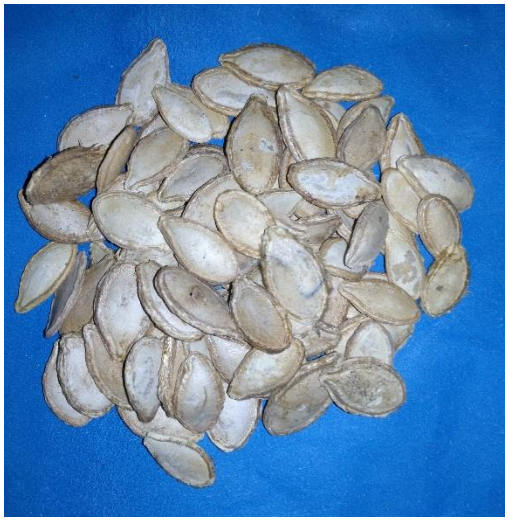
Table 1.The list of different vegetable seeds

Common Name	English name	Scientific name	Family
Leafy vegetables			
Danta	Stem amaranth	*Amaranthus lividus*	Amaranthaceae
Kolmi Shakh	Water spinach	*Ipomoea aquatica*	Convolvualceae
Pui Shakh	Indian spinach	*Basella alba*	Basellaceae
Fruit vegetables			
Misti kumra	Sweet gourd	*Cucurbita moschata*	Cucurbitaceae
Chichingga	Snake gourd	*Trichosanthes cucumerina*	Cucurbitaceae
Chal Kumra	Ash gourd	*Benincasa hispida*	Cucurbitaceae
Poded vegetables			
Shim	Country bean	*Phaseolus vulgaris*	Fabaceae
Borboti	Yard long bean	*Vigna unguiculata*	Fabaceae
Root vegetables			
Mula	Radish	*Raphanus sativus*	Cruciferae
Gajor	Carrot	*Daucus carota*	Umbelliferae

A. Stem amaranth seeds

B. Indian spinach seeds

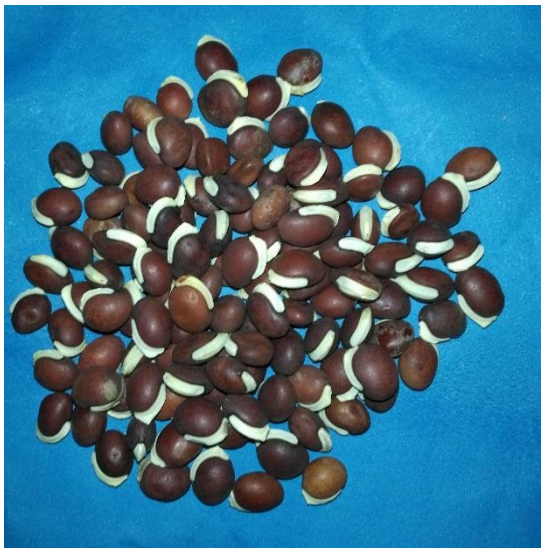
C. Water spinach seeds



D. Sweet gourd seeds

E. Snake gourd seeds

F. Ash gourd seeds



G. Country bean seeds

H. Yard long bean seeds

I. Radish seeds

J. Carrot seeds

Plate 1 (A-J): Working samples of loose vegetable seeds collected from five districts in Bangladesh

3.6. Laboratory experiment

A. Seed sampling: Three primary samples of each vegetable were collected from each sources. The composite sample was formed by combination and mixing all the primary samples taken from the lot or containers or bags.

B. Working samples: Working sample was obtained by following the rules of ISTA (1999).

3.6.1 Inspection of dry Seeds

The inspection of dry seeds is a method where the presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds, if any, are observed and recorded. The method provides quick information on insect and mechanical damage to the seeds as well as on seed treatment with pesticides so that the samples are handled with proper precautions. The fruiting structures of fungi can be in the form of pathogenically sclerotia on the seed surface or submerged in the seed coat. Physical abnormalities includes shriveled of seed coat, reduction or increase in seed size, discolored or spots in the seed coat. The inspection of dry seed is closely associated with the purity analysis as practiced at seed testing station (Mathur and Congsdal 2003).

Working procedure: For the inspection of dry seeds the working sample (2-80 g) various with their size and weight. It was advisable to use a sample of the same size as the one recommended for “purity analysis” for different crops by the International Rules for Seed Testing (1999). For the purity analysis weight the three components (pure seeds, inert matter and seeds of other crops) separated and

recorded. The pure seeds were examined by naked eye and under a stereomicroscope, visual inspection helps to separate the abnormalities, shrinkage, swelling of seeds and fruiting structure observed under microscope. After three purity test, results added and divided by three and then got an average purity test result.

3.6.2 Blotter method

The blotter method is one of the incubation methods where seeds are plated on well water soaked blotters (filter papers) and incubated usually for 7 days at 22^0 C under 12h alternating cycles of light and darkness. After incubation, fungi developed on each seed were examined under different magnifications of a stereomicroscope and identified. The identification of fungi was based on the way they grow on seeds "habit characters" and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope (Mathur and Congsdal 2003).

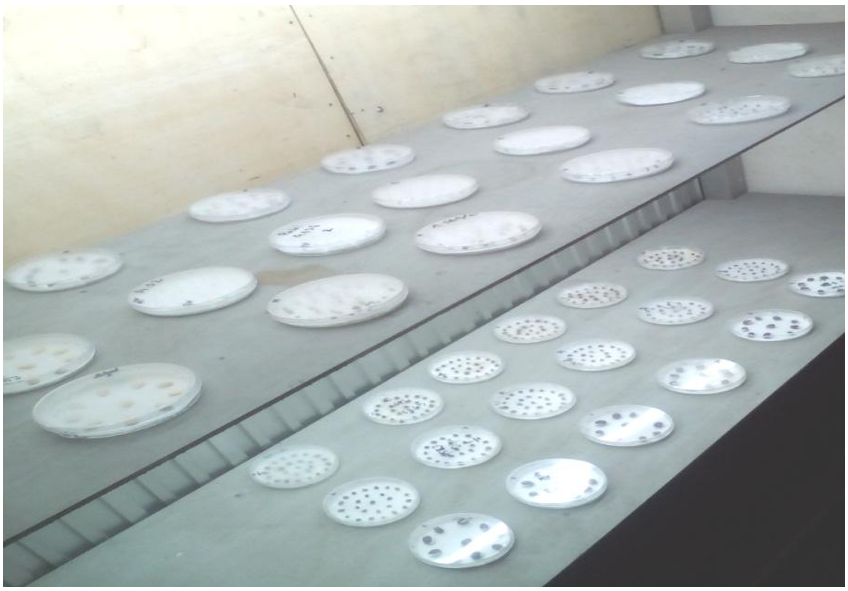


Plate 2: Incubation of seed

3.7. Design of experiments

The laboratory experiment was conducted following Complete Randomized Design (CRD) with four replications.

3.8. Analysis of data

The data obtained for different characters were statistically analyzed by "MSTAT-C" program to find out the significance of the difference levels of loose seeds collected from three seeds store. The mean values of all the characters were evaluated and analysis of variance was performing by the "F" (variance ratio) test. The significance of the difference among the treatment combinations means was estimated by the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS

4.1 Seed health status of leafy vegetables

Fungi detected in leafy vegetable viz. stem amaranth, water spinach and Indian spinach seeds that were collected from five districts like as Dhaka (BADC seeds), Dinajpur, Thakurgaon, Panchagarh and Rangpur presented in different table and figures. Prevalence of seed-borne fungal infections varied depending on the seeds of different vegetables.

4.1.1 Seed health study of stem amaranth (*Amaranthus lividus*)

4.1.1.1 Health status of stem amaranth seeds by dry inspection method

A. Physical appearance of seeds: Deformed and reduced seeds were observed in the seeds collected form Dinajpur and Panchagarh whereas shriveled and discolored of seeds were found in the seeds that collected from Thakurgaon and Rangpur. However, no physical abnormalities were observed in stem amaranth seeds that collected from Dhaka (BADC).

B. Presence of fruiting structures of fungi: No fungal fruiting structures were observed over the seed surface collected from Dhaka (BADC) whereas, sclerotia were observed in that seeds collected from Pachagarh, Rangpur and Thakurgaon but only mycelium was observed in the seeds that collected from Dinajpur.

C. Purity Analysis

In case of purity analysis, 40 g seeds of stem amaranth were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur

district seeds, 35.50 g (88.75 %) pure seeds, 4.0 g (10 %) inert matter and 0.5 g (1.25 %) other crop seeds were found. However, in Panchagarh district, pure seeds and inert matter other crop seeds were 35.85 g (89.62 %) and 4.15 g (10.38 %), respectively but there was no other crop seeds. Whereas, in seeds collected from Thakurgaon district, 36.1 g (90.25 %) pure seeds, 3.87 g (9.68 %) of inert matter and 0.03 g (0.08 %) other crop seeds were observed. Moreover, from Rangpur district seeds, the pure seeds 35.70 g (89.25 %), inert matter 4.25 g (10.63 %) and other crop seeds 4.25 g (10.63 %) were observed (Table 2).

4.1.1.2. Prevalence of seed-borne fungi of stem amaranth identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Six fungal pathogens viz. *Aspergillus flavus*, *Aspergillus niger, Fusarium* spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp., one unidentified bacterium and one unknown fungus were observed and recorded.

B. Incidence of seed borne pathogens

Fungal incidence in stem amaranth seeds collected from five different locations is presented in Table 3. Prevalence of total seed-borne fungal infections varied significant by depending on the seeds sources and seed categories. The highest total seed borne fungal infections were recorded in Panchagarh district's seeds (22.9 %), while the lowest infection was recorded from BADC seeds (8.61%). Incidence of *Aspergillus flavus, A. niger* were varied from 3.25 to 6.50% and 2.56

to 6.25%, respectively. Incidence of *Fusarium* spp. was varied from 2.85 to 3.3%. The lowest incidence was observed in BADC seeds (2.85%). Incidence of *Alternaria* spp. was varied from 1.70 – 3.50%, where the highest incidence was observed in Panchagarh and Thakurgaon district seeds (3.5 % and 3.4%). But the lowest was in BADC seeds (1.7%). *Chaetomium* spp. was varied from 2.05 – 5.20%. Of this *Chaetomium* spp. (2.05%) was the lowest predominant fungus. *Rhizopus* spp. was varied from 2.15 to 3.4 %. The lowest incidence was observed in BADC seeds accordingly. One unidentified fungus incidence was ranged from 1.05 to 2.25%. The lowest incidence was observed on Dhaka district (BADC) seeds (1.05%) where the highest was observed in those seeds that collected from Thakurgaon and Rangpur district.

Table 2. Health status of stem amaranth seeds by dry seed inspection method

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	35.50	88.75	4	10	0.5	1.25	Sclerotia	Deformed
Panchagarh	40	35.85	89.62	4.15	10.38	0	0	Sclerotia	Reduced
Thakurgaon	40	36.1	90.25	3.87	9.68	0.03	0.08	Sclerotia	Shriveled
Rangpur	40	35.70	89.25	4.25	10.63	0.05	0.12	Mycelium	Discolored

Table 3. Prevalence of seed-borne fungi of stem amaranth identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	3.25 c	2.56 c	2.85 c	1.70 d	2.05 c	2.15 c	1.05 c	8.61
Dinajpur	5.25 b	5.35 b	3.20 a	2.15 b	4.10 b	3.35 a	1.50 b	17.9
Panchagarh	6.50 a	6.25 a	3.30 a	3.50 a	5.20 a	3.40 a	1.75 b	22.9
Thakurgaon	5.15 b	5.55 b	2.75 b	3.40 a	4.50 b	2.90 b	2.25 a	19.5
Rangpur	6.00 a	6.10 a	3.15 a	2.00 b	5.10 a	3.00 b	2.25 a	20.6
LSD	1.07	0.943	0.616	0.665	0.743	0.653	0.360	
Level of significance	*	**	*	**	**	**	*	
CV %	7.41	7.53	10.47	10.16	7.78	11.25	12.86	

*= Significant at 5% level as per DMRT, ** = Significant at 1% level as per DMRT

4.1.2 Seed health study of water spinach (*Ipomoea aquatica*)

4.1.2.1 Health status of water spinach seeds by dry inspection method

A. Physical appearance of seeds: Deformed and shriveled seeds were observed in the seeds collected form Thakurgaon and Panchagarh district whereas reduced and discolored of seeds were found in the seeds that collected from Dinajpur and Rangpur district but no physical abnormalities were observed in water spinach seeds that collected from Dhaka district seeds (BADC) (Table 4).

B. Presence of pathogenic structures of fungi: Seeds collected from Dhaka district (BADC), no fungal fruiting structures were observed over the seed surface whereas, sclerotia were observed in that seeds collected from Pachagarh, Dinjpur and Thakurgaon district but mycelium was observed in the seeds that collected from Rangpur district.

C. Purity Analysis

In case of purity analysis, 40 g seeds of water spinach were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district seeds, 35.50 g (88.75 %) pure seeds, 4 g (10 %) inert matter and 0.5 g (1.25 %) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 35.74g (89.35%), 4.24g (10.60 %) and 0.02g (0.05 %), respectively. Whereas, in seeds collected from Thakurgaon district, 35.2g (88 %) pure seeds, 4.79g (11.98 %) of inert matter and 0.01g (0.02 %) other crop seeds were observed. Moreover, from Rangpur district, the pure seeds 36.40 g (91 %), inert matter 4.60 g (9.00 %) and other crop seeds 0 g (0%) were observed (Table 4).

4.1.2. 2. Prevalence of seed-borne fungi of water spinach identified by blotter method

The fungal incidence varied significantly among the samples for all pathogens at 5 % level of significance.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp., one unknown fungus and one unidentified bacterium were observed.

B. Incidence of seed borne pathogens

Prevalence of total seed-borne fungal infections varied significant depending on the seeds source and seed categories. In case of fungal infection, the highest fungal infection percentage on seed was recorded in Panchagarh district seeds (21.3%) and the lowest infection percentage in Dhaka district (BADC) seeds (7.1%) respectively. Incidence of *Aspergillus flavus* was varied from 3.05 to 6.15%. Where, the highest fungal infection was observed in the seeds of Thakurgaon district (6.15 %) that statistically similar with Rangpur district seeds. The lowest was in Dhaka district (BADC) seeds (3.05%). *A. niger* incidence were varied from 3.20 to 5.25%. The lowest prevalence was recorded in Dhaka district (BADC) seeds (3.20%), where the highest in Rangpur district seeds (5.25%) followed by Panchagarh district seeds (5.10%). Incidence of *Fusarium* spp. was varied from 1.85 to 3.5%. The lowest prevalence was recorded in Dhaka district (BADC) seeds (1.85%), where the highest in Rangpur and Panchagarh district seeds (3.50%). Incidence of *Curvularia* spp. was varied from 1.15 – 3.75%, where the lowest infection was observed in Dhaka district (BADC) seeds (1.15%).

Chaetomium spp. infection was varied from 2.45 to 5.50%. Dhaka district (BADC) seeds showed the lowest (2.45%) infection of that fungus. Infection of *Rhizopus* spp. was varied from 1.45 – 3.80%. The lowest infection was recorded in Dhaka district (BADC) seeds (1.45 %). In case of unidentified bacteria, infection ranged was varied from 0.95 to 2.25%. The lowest infection was recorded in Dhaka district (BADC) seeds (0.95 %), (Table 5).

Table 4. Health status of water spinach seeds by inspection of dry seed method

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	35.50	88.75	4	10	0.5	1.25	Sclerotia	Reduced
Panchagarh	40	35.74	89.35	4.24	10.60	0.02	0.05	Sclerotia	Shriveled
Thakurgaon	40	35.2	88.00	4.79	11.98	0.01	0.02	Sclerotia	Deformed
Rangpur	40	36.40	91.00	4.60	9.00	0	0	Mycelium	Discolored

Table 5. Prevalence of seed-borne fungi of water spinach identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	3.05 c	3.20 c	1.85 c	1.15 c	2.45 c	1.45 c	0.95 c	7.1
Dinajpur	4.84 b	4.45 b	2.85 b	2.50 b	3.95 b	3.15 b	1.65 b	16.39
Panchagarh	5.25 b	5.10 a	3.50 a	3.50 a	5.40 a	3.80 a	1.75 b	21.3
Thakurgaon	6.15 a	4.05 b	2.75 b	3.75 a	4.00 b	3.00 b	2.15 a	18.85
Rangpur	6.05 a	5.25 a	3.50 a	2.00 b	5.50 a	3.00 b	2.25 a	20.55
LSD	1.17	2.84	0.542	0.565	0.843	0.653	0.460	
Level of significance	**	**	*	**	**	**	*	
CV %	6.91	7.13	10.47	9.16	8.78	11.21	11.26	

*= Significant at 5% level as per DMRT, ** = Significant at 1% level as per DMRT

4.1.3 Seed health study of Indian spinach (*Basella alba*)

4.1.3.1 Health status of Indian spinach seeds by dry inspection method

A. Physical appearance of seeds: Except seeds collected from Dhaka district (BADC), reduced, shriveled and deformed seeds were observed from the seeds collected from other four districts for experiment (Table 6).

B. Presence of pathogenic structures of fungi: Only mycelium was observed over the seed surface but no pathogenic structure was observed over the Dhaka district seeds surface.

C. Purity Analysis

In case of purity analysis, 40 g seeds of Indian spinach were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district 36.25 g (90.63 %) pure seeds, 3.45 g (8.62%) inert matter and 0.3 g (0.75%) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 39 g (97.5 %), 1 g (2.5 %) and 0.02 g (0.05), respectively. Whereas, in seeds collected from Thakurgaon district, 37 g (92.5 %) pure seeds, 3 g (7.5 %) of inert matter and 0 g (0 %) other crop seeds were observed. Moreover, from Rangpur district, the pure seeds 37.2 g (93 %), inert matter 2.7 g (6.75 %) and other crop seeds 0.1 g (0.25%) were observed (Table 6).

4.1.3.2. Prevalence of seed-borne fungi of Indian spinach identified by blotter method

The fungal incidence varied significantly among the samples for all pathogens at 5 % level of significance.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Cercospora* spp., *Rhizopus* spp. and one unidentified bacterium were observed.

B. Incidence of seed borne pathogens

In case of fungal infection, the lowest percentage of seed infection (7.41%) was recorded in the seed collected from Dhaka district (BADC) and the highest (18.9%) in Thakurgaon district seeds. Incidence of *Aspergillusflavus*was varied from 2.5to 5.5% where, the highest (5.50 %) was observed in the seeds of Panchagarh district that followed by Rangpur district (5.20%). But the lowest (2.5%) was Dhaka district (BADC) seeds. The fungus *Aspergillus niger* incidence was varied from 1.45 to 5.45%. The lowest (1.45 %) infection was recorded in that seeds which collected from Dhaka district (BADC) and the highest in Rangpur district (5.45%). Incidence of *Fusarium* spp. was varied from 1.16 to 3.5%. The lowest (1.16 %) infection was recorded in that seeds which collected from Dhaka district (BADC) and the highest in Rangpur district (3.5%) followed by Panchagarh district (3.15 %). Incidence of *Curvularia* spp. was varied from 1.4 – 3%, where the lowest (1.4 %) incidence was in Dhaka district (BADC) . The infection of *Cercospora* spp. was varied from 2.14 to 5.05 %. Whereas the lowest infection was recorded in Dhaka district (BADC) seeds (2.14%).

Rhizopus spp. incidence was varied from 2.02 – 5.1%. Dhaka district seeds (BADC) showed the lowest infection of *Rhizopus* spp. (2.02%). Unidentified bacterial infection was varied from 1.75 to 3.45%. The lowest infection was recorded in Dhaka district seeds (BADC) (1.75 %) (Table 7).

Table 6. Health status of Indian spinach seeds by inspection of dry seed method

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	36.25	90.63	3.45	8.62	0.3	0.75	Mycelium	Reduced
Panchagarh	40	39.00	97.50	1	2.50	0.02	0.05	Mycelium	Shriveled
Thakurgaon	40	37.00	92.50	3	7.50	0	0	Mycelium	Deformed
Rangpur	40	37.20	93.00	2.7	6.75	0.1	0.25	Mycelium	Deformed

Table 7. Prevalence of seed-borne fungi of Indian spinach identified by blotter method

Source Name (District)	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Cercospora* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.5 d	1.45 c	1.16 c	1.40 c	2.14 c	2.02 c	1.75 c	7.41
Dinajpur	3.70 c	3.95 b	2.65 b	2.85 a	5.05 a	4.85 a	2.35 b	17.4
Panchagarh	5.50 a	4.65 b	3.15 a	2.50 a	-	5.10 a	2.15 b	15.05
Thakurgaon	4.45 b	5.25 a	2.55 b	3.00 a	4.10 b	4.30 b	3.25 a	18.9
Rangpur	5.20 a	5.45a	3.50 a	2.05 b	-	4.00 b	3.45 a	15.65
LSD	1.90	0.843	0.716	0.453	0.753	0.653	0.460	
Level of significance	*	**	*	**	**	**	*	
CV %	8.91	7.53	11.37	11.26	8.28	12.05	13.06	

*= Significant at 5% level as per DMRT, ** = Significant at 1% level as per DMRT

B. Stem amaranth seeds

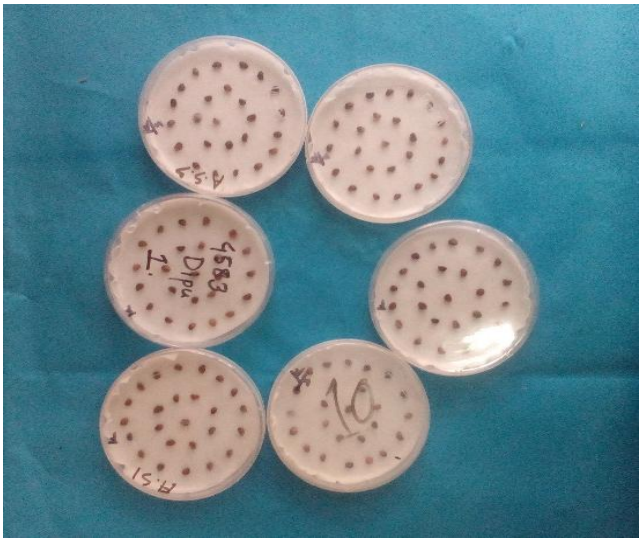
B. Indian spinach seeds

C. Water spinach seeds

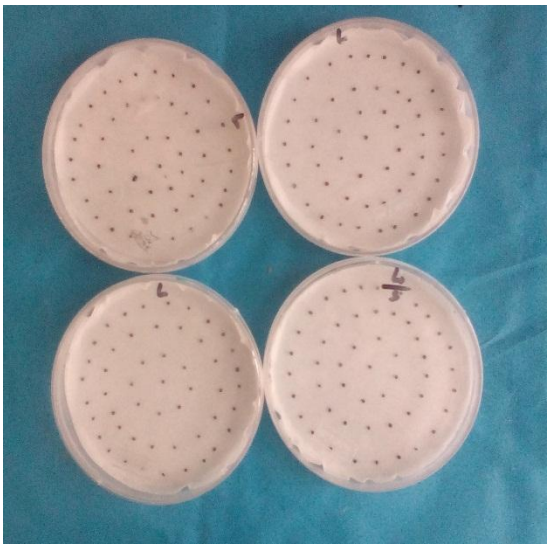
D. Inert matter

E. Other seeds

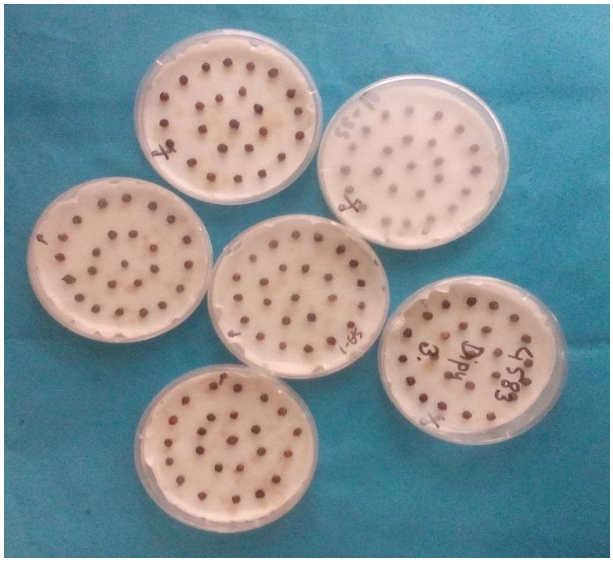
Plate 3: Pure seeds and inert matter observed by dry inspection method



A. Plating Seeds of water spinach by blotter method



B. Plating Seeds of stem amaranth by blotter method



C. Plating Seeds of Indian spinach by blotter method

Plate 4 (A-C): Seed health of stem amaranth, water spinach and Indian spinach by blotter method

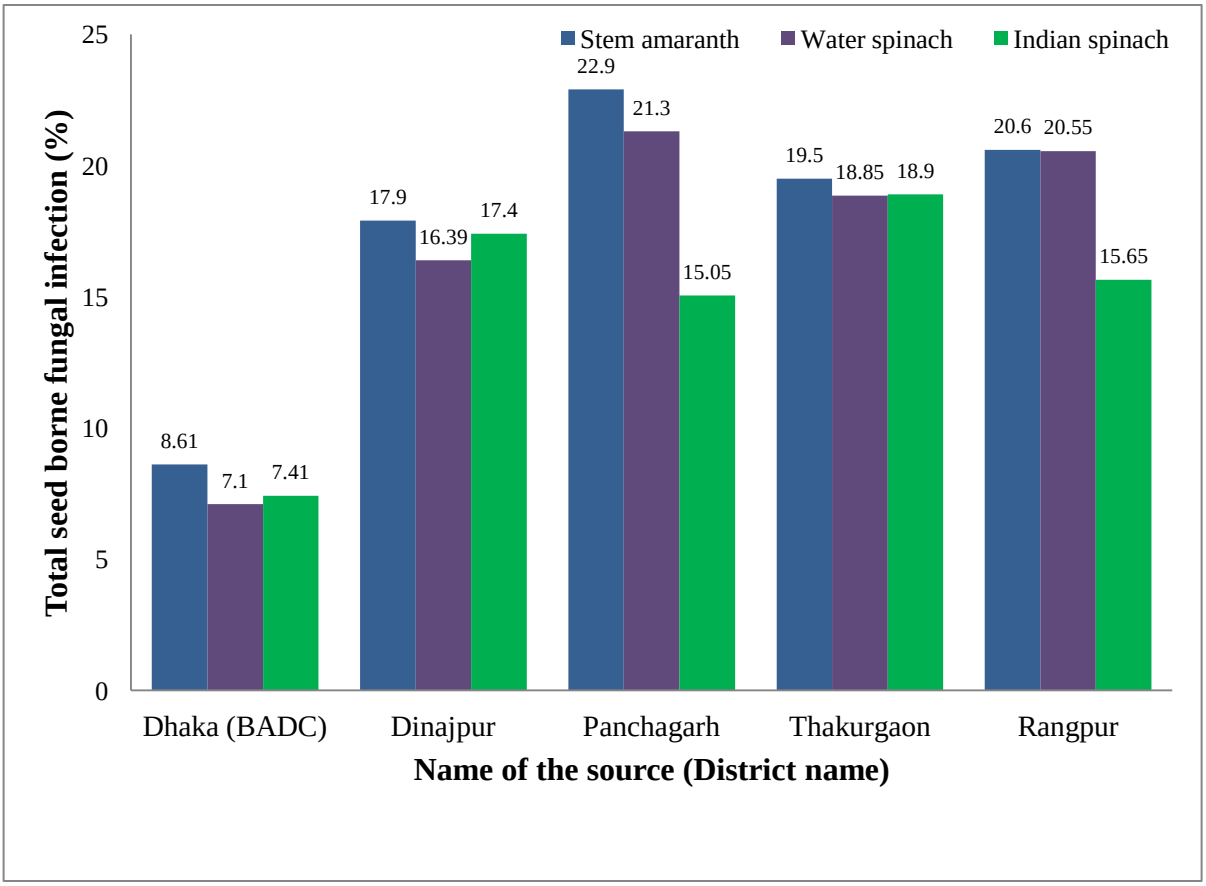
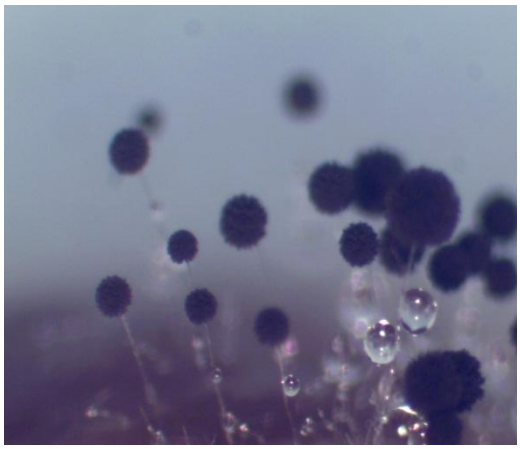


Figure 1: Total seed borne fungal infection (%) of leafy vegetables

The present study indicates that in seeds of Dhaka district (BADC) showed the lowest value of seed borne fungal infection for stem amaranth (8.61%), water spinach (7.1%), Indian spinach (7.41%). Whereas, three seeds of other four districts showed the highest value of seed borne fungal infection compared with BADC seeds.

A. Growth of *Alternaria* spp. on stem amaranth seedling

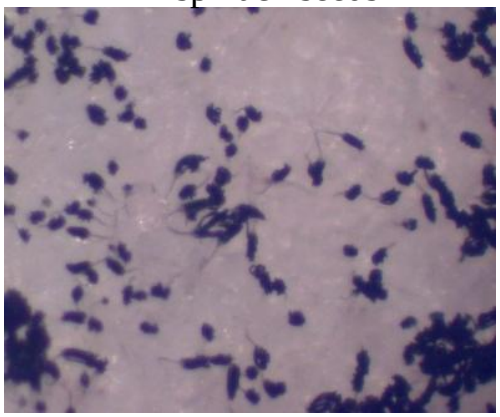


B. Growth of *Aspergillus niger* on water spinach seeds

C. Growth of *Curvularia* spp. on Indian spinach seeds

D. Growth of *Fusarium* spp. on water spinach seeds

E. Growth of *Rhizopus* spp. on blotting paper

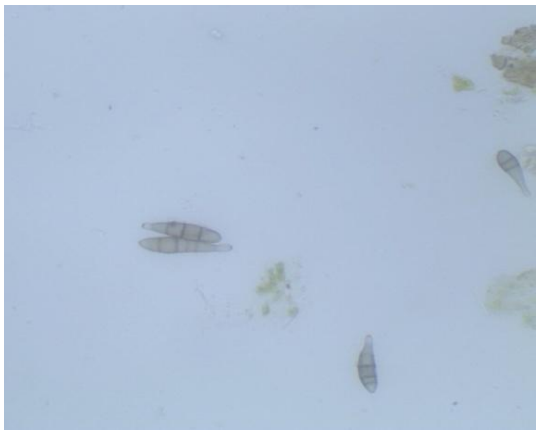


F. Growth of Unknown fungi on stem amaranth seeds

Plate 5(A-F): Stereomicroscopic view of different fungi on leafy vegetables

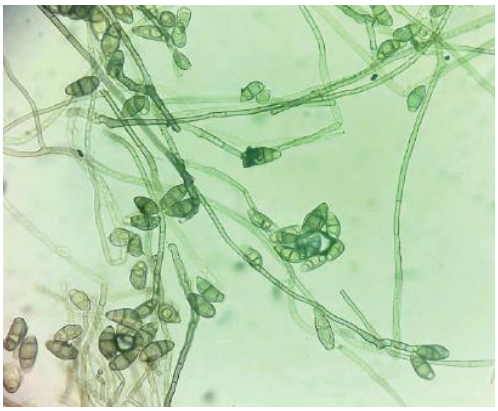
A. *Aspergillus niger* (40X)

B. *Rizopus* spp. (10X)



C. *Alternaria* spp. (40X)

D. *Cercospora* spp. (10X)



E. *Curvularia* spp. (40X)

F. *Fusarium* spp. (40X)

Plate 6(A-F): Compound microscopic view of different fungi identified from leafy vegetables

4.2 Seed health status of fruit vegetables

In fruit vegetable seeds namely sweet gourd, snake gourd and ash gourd seeds, fungi was detected that collected from different district like as Dhaka (BADC), Panchagarh, Thakurgaon, Dianjpur and Rangpur presented in table and figures. Incidence of seed-borne fungal infections varied depending on the seeds of different vegetable seed sources.

4.2.1 Seed health study of sweet gourd (*Cucurbita moschata*)

4.2.1.1 Health status of sweet gourd seeds by inspection of dry seed

A. Physical appearance of seeds

Discolored of seeds were observed in the seeds that collected from Dhaka (BADC) and whereas deformed size of seeds were observed that was collected from Rangpur, Dinajpur, Panchagarh and Thakurgaon district seeds respectively (Table 8).

B. Presence of pathogenic structures of fungi: Mycelium was observed in all seeds that collected from five districts (Table 8).

C. Purity analysis

In case of purity analysis of five district seeds, 40 g seeds of sweet gourd were seen in naked eye. Then pure seeds, inert matter and other crops seeds were separated manually. Out of 40 g, pure seeds 36.3 g (90.75 %), inert matter 1.5 g (3.75 %) and other crop seeds 2.2 g (5.5 %) were observed in Dhaka district (BADC) seeds. In Panchagarh district seeds pure seeds 37.05 g (92.62 %), inert matter 2.92 g (7.3 %) and other crop seeds 0.03 g (0.08 %) were observed. Pure seeds 36.85 g (92.13 %), inert matter 3 g (7.5 %) and 0.15 g (0.37 %) of other crop seeds were observed

which was collected from Thakurgaon district. In case of Dinajpur district, pure seeds were 36.65 g (91.62 %), inert matter 3.30g (8.25 %) and 0.05g (0.13%). Moreover, from Rangpur district, the pure seeds 37.04 g (92.6 %), inert matter 1.83 g (4.58 %) and other crop seeds 1.13 g (2.82%) were observed (Table 8).

4.2.1.2. Prevalence of seed-borne fungi of sweet gourd identified by blotter method

The fungal incidence varied significantly among the samples for all pathogens at 5 % level of significance.

A. Identified pathogen:*Aspergillus flavus, Aspergillus niger, Fusarium* spp., *Curvularia* spp., *Chaetomium* spp.,*Rhizopus* spp., one unidentified bacterium and one unknown fungus were observed.

B. Incidence of seed borne pathogens

The lowest fungal infestation percentage was observed on the seed collected from Dhaka district (BADC) (9.85%). However, the highest infection was recorded Thakurgaon district (20.9 %). Incidence of *Aspergillus flavus* were varied from 2.85 to 5.65 %.Where, the lowest incidence was observed in the seeds of Dhaka district seeds (BADC) (2.85%) and the highest was in Rangpur district seeds (5.65%) followed by Pachagarh and Dinajpur district seeds. Incidence of *A. niger* varied from 3.54 to 5.05%. Highest infection was observed in Thakurgaon district seeds (5.05%) that statistically similar with Pachagarh district seeds. Incidence of *Fusarium* spp. was varied from 2.05 to 4.50 %. Dhaka district seeds (BADC) was showed the lowest incidence (2.05%). Incidence of *Curvularia* spp. was varied from 2.35 – 4.5 %.Whereas, the lowest incidence was observed in Dhaka district seeds (BADC) (2.35%) and the highest was in Panchagarh district seeds (4.5%) that was statistically similar except Dhaka and Rangpur district seeds.

Chaetomium spp. infection ranged varied from 2.05 – 4.5%. The lowest incidence was observed in Dhaka district seeds (BADC) (2.05%). *Rhizopus* spp. infection ranged from 3.05 to 5.75% and the lowest infection was recorded in Dhaka district seeds (BADC) (3.05%). In case of unidentified bacterial infection, ranged was varied from 1.5 to 3.85%.The lowest infection was recorded in Dhaka district seeds (BADC) (1.05%) (Table 9).

Table 8. Health status of sweet gourd seeds by inspection of dry seed method

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	36.3	90.75	1.5	3.75	2.2	5.5	Mycelium	Discolored
Dinajpur	40	36.65	91.62	3.30	8.25	0.05	0.13	Mycelium	Deformed
Panchagarh	40	37.05	92.62	2.92	7.3	0.03	0.08	Mycelium	Deformed
Thakurgaon	40	36.85	92.13	3	7.5	0.15	0.37	Mycelium	Deformed
Rangpur	40	37.04	92.60	1.83	4.58	1.13	2.82	Mycelium	Reduced size

Table 9. Prevalence of seed-borne fungi of sweet gourd identified by blotter method

Source Name (District)	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.85 c	-	2.05 c	2.35 b	2.05 c	3.05 c	1.50 c	9.85
Dinajpur	5.20 a	3.54 b	4.00 a	4.25 a	4.15 a	-	3.85 a	17.99
Panchagarh	4.80 a	4.50 a	-	4.50 a	4.50 a	5.75 a	2.75 b	19.8
Thakurgaon	3.45 b	5.05 a	3.75 b	3.90 a	3.00 b	5.50 a	3.25 a	20.9
Rangpur	5.65 a	2.95 b	4.50 a	3.00 b	3.50 b	4.00 b	3.15 a	19.25
LSD	1.05	0.643	0.596	0.965	1.05	1.65	0.460	
Level of significance	*	*	*	**	**	**	*	
CV %	6.21	4.53	5.17	8.16	8.81	9.25	10.36	

* = Significant at 5 % level as per DMRT and ** = Significant at 1% level of as per DMRT

4.2.2 Seed health study of snake gourd (*Trichosanthes cucumerina*)

4.2.2.1 Health status of snake gourd seeds by dry inspection of seed

A. Physical appearance of seeds

Deformed seeds was observed in the seeds collected from Dhaka district (BADC),Dianjpur and Thakurgaon district seeds whereas shrinkage and discolored of seeds were observed in the seeds that collected from Panchagarh and Rangpur district respectively (Table 10).

B. Presence of pathogenic structures of fungi: White mycelium was observed seeds that collected from Dhaka (BADC) and Panchagarh district seeds. But white cottony mycelium was observed in both case of other three district seeds (Table 10).

C. Purity analysis

In case of purity analysis of five district seeds40 g seeds of snake gourd were seen in naked eye. Then determined by hand viz. pure seeds, inert matter and other crops seeds. Total seed 40 g, pure seeds 38 g (95%), inert matter 1.4 g (3.5 %) and other crop seeds 0.6 g (1.5 %) were observed in case of Dhaka district (BADC) seeds. But in Rangpur district seeds, pure seeds 39 g (97.5 %), inert matter 0.65 g (1.63 %) and other crop seeds 0.35 (0.87 %) were observed. Pure seeds 38.24 g (95.6 %), inert matter 1.75 g (4.38 %) and other crop seeds 0.01 (0.02 %) were observed in Panchagarh district seeds. However in Dinajpur district seeds, pure seeds 37.35 g (93.38 %), inert matter 2.55 g (6.37 %) and other crop seeds 0.1 (0.25 %) were observed. Whereas, in Thakurgaon district seeds, the pure seeds 40 g (100 %), but inert matter and other crop seeds were not found (Table 10).

4.2.2.2 Prevalence of seed-borne fungi of snake gourd identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp., one unidentified bacterium and one unknown fungus were recorded.

B. Incidence of seed borne pathogens

In case of fungal infection, the highest incidence was observed from the seed collected from Dinajpur district (20.85 %) and the lowest was in Dhaka district (BADC) seeds (8.15 %). Incidence of *Aspergillus flavus* was non-significant among five district seeds. *A. niger* were varied from 2.05 to 4.65 %. The lowest fungal infection was recorded in Dhaka district seeds (BADC) (2.05 %), where the highest in Dinajpur district seeds (4.65 %). Incidence of *Fusarium* spp. was also non-significant among the five district seeds. For *Curvularia* spp., ranged was varied from 2.04 to 4.05 %. Dhaka district seeds (BADC) was showed the lowest infection of fungus over the seed surface (2.04 %). In case of *Chaetomium* spp., incidence was varied from 2.40 to 4.5 %. No incidence was recorded in Dhaka district seeds (BADC), but the highest was in Rangpur district seeds (4.5%) that statistically similar with Dinajpur district seeds (4.35%). In case of *Rhizopus* spp. was varied from 1.96 to 3.85%. The lowest incidence was found in Dhaka district seeds (BADC) (1.96%). In case of unidentified bacterial infection ranged was varied from 1.50 to 3.25%. Dhaka district seeds (BADC) showed the lowest prevalence of fungal infection (1.50 %), respectively (Table 11).

Table 10. Health status of snake gourd seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	38	95	1.4	3.5	0.6	1.5	White mycelium	Deformed
Dinajpur	40	37.35	93.38	2.55	6.37	0.1	0.25	Cottony mycelium	Deformed
Panchagarh	40	38.24	95.6	1.75	4.38	0.01	0.02	White mycelium	Shrinkage
Thakurgaon	40	40	100	0	0	0	0	Cottony mycelium	Deformed
Rangpur	40	39	97.5	0.65	1.63	0.35	0.87	Cottony mycelium	Discolored

Table 11. Prevalence of seed-borne fungi of snake gourd identified by blotter method

Source Name (District name)	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	4.45 a	2.05 b	3.15 a	2.04 c	-	1.96 c	1.50 c	8.15
Dinajpur	5.05 a	4.65 a	3.65 a	3.75 a	435 a	3.85 a	2.55 b	20.85
Panchagarh	5.50 a	4.25 a	3.50 a	3.65 a	3.50 b	3.60 a	2.45 b	19.45
Thakurgaon	4.65 a	3.35 b	3.25 a	4.05 a	2.40 c	2.60 b	-	13.3
Rangpur	4.25 c	4.45 a	3.50 a	3.00 b	4.50 a	2.35 b	3.25 a	18.3
LSD	0.783	1.21	0.596	0.765	1.25	0.746	0.706	
Level of significance	ns	**	ns	**	**	**	*	
CV %	8.91	7.53	11.47	11.16	8.78	12.25	13.86	

ns = non-significant, *= Significant at 5% level as per DMRT,** = Significant at 1% level as per DMRT

4.2.3 Seed health study of ash gourd (*Benincasa hispida*)

4.2.3.1 Health status of ash gourd seeds by dry inspection of seed

A. Physical appearance of seeds

Shrinkage and discolor seeds were observed in the seeds collected from Dhaka (BADC) and Panchagarh district seeds, whereas shrinkage and deformed of seeds was observed in the seeds that collected from Thakurgaon, Dianjpur and Rangpur district (Table 12).

B. Presence of pathogenic structures of fungi: White cottony mycelium and mycelium were observed over the seed surface of all seeds that collected from five districts (Table 12).

C. Purity analysis

For purity analysis of five district seeds, 40 g seeds of ash gourd were seen in naked eye. Then determined in hand viz. pure seeds, inert matter and other crops seeds. Total seed 40 g, pure seeds 37 g (92.5 %), inert matter 1.9 g (4.75 % and other crop seeds 1.1 g (2.75%) were observed in case of Dhaka district (BADC) seeds. Whereas, in Panchagarh district seeds, pure seeds 38 g (95 %), inert matter 1.3 g (3.25 %) and other crop seeds were observed 0.7 g (1.75%). In case of, Thakurgaon district seeds, the pure seeds 39.1 g (97.75 %), inert matter 0.3 g (0.75 %) and other crop seeds 0.6 g (1.5 %) were observed. Pure seeds 36.74 g (91.85 %), inert matter 2.95 g (7.38 %) and other crop seeds 0.31 g (0.77 %) were observed in Dinajpur district seeds. Pure seeds 40 g (100 %) but no observation (0 %) of inert matter and other crop seeds which was collected from Rangpur district (Table 12).

4.2.3.2. Prevalence of seed-borne fungi of ash gourd identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus*s pp., unidentified bacteria and one unknown fungus were observed.

B. Incidence of seed borne pathogens

In case of fungal infection percentage over seed surface, the highest incidence was observed from the seed collected from Panchagarh district seeds (21.57 %). Where, the lowest in Dhaka district seeds (BADC) (8.99%). In case of *Aspergillus flavus* incidence was varied from 3.25 to 4.52 %. The highest was observed in the seeds of Pancahgarh district seeds (4.52%) which statistically similar with Rangur district seeds (4.25%). And the lowest was in Thakurgaon district seeds (3.25%). *A. niger* incidence statistically non-significant with each other district seeds. Incidence of *Fusarium* spp. was varied from 2.54 to 4.0 %. The lowest incidence was in Dhaka district seeds (BADC) (2.54%). Incidence of *Curvularia* spp. was varied from 1.05 to 3.70 %. In Dhaka district seeds (BADC), the lowest incidence was observed (1.05%). But in case of *Chaetomium* spp. infection was varied from 2.95 to 4.75%. The lowest fungal infection was observed in Dhaka district seeds (BADC) (2.95 %). *Rhizopus* spp. infection was non-significant and unidentified bacterial infestation range varied from 1.25 to 3.45%. The lowest incidence was observed in Dhaka district seeds (BADC) (1.25 %), respectively (Table 13).

Table 12. Health status of ash gourd seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	37	92.5	1.9	4.75	1.1	2.75	Mycelium	Shrinkage
Dinajpur	40	36.74	91.85	2.95	7.38	0.31	0.77	Cottony mycelium	Deformed
Panchagarh	40	38	95	1.3	3.25	0.7	1.75	Mycelium	Discolored
Thakurgaon	40	39.1	97.75	0.3	0.75	0.6	1.5	Cottony mycelium	Deformed
Rangpur	40	40	100	0	0	0	0	Cottony mycelium	Shrinkage

Table 13. Prevalence of seed-borne fungi of ash gourd identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	-	4.35 a	2.54 c	1.05 c	2.95 c	3.85 a	1.25 c	8.99
Dinajpur	3.55 b	4.95 a	4.00 a	2.60 b	4.75 a	4.25 a	3.45 a	20.55
Panchagarh	4.52 a	5.50 a	3.80 a	3.50 a	4.50 a	4.00 a	2.75 b	21.57
Thakurgaon	3.25 b	4.25 a	2.95 b	3.70 a	4.00 b	3.95 a	3.25 a	18.35
Rangpur	4.25 a	5.35 a	3.50 a	2.00 b	4.50 a	4.15 a	2.35 b	19.1
LSD	0.917	0.843	0.596	0.665	0.843	0.653	0.460	
Level of significance	*	ns	*	**	**	ns	*	
CV %	8.11	9.53	7.47	10.16	8.78	12.45	8.46	

ns = non-significant, *= Significant at 5% level as per DMRT,** = Significant at 1% level as per DMRT

A. Pure seeds of sweet gourd

B. Pure seeds of Snake gourd

C. Pure seeds of Ash gourd

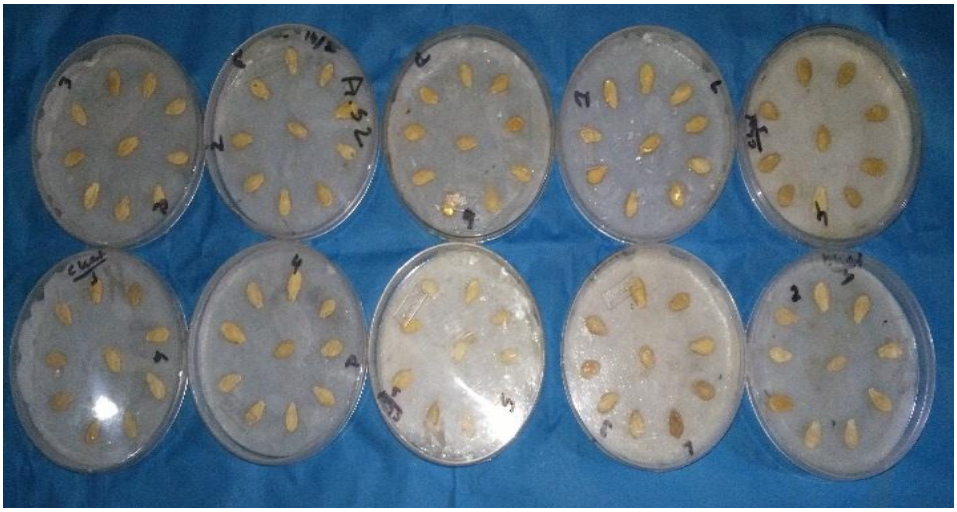
D. Other seeds

E. Inert matter

Plate 7 (A-E): Seed health of sweet gourd, snake gourd and ash gourd observed by dry inspection method

A. Plating of sweet gourd seeds by blotter method

B. Plating of snake gourd seeds by blotter method



C. Plating of ash gourd Seeds by blotter method

Plate 8 (A-C): Seed health of sweet gourd, snake gourd and ash gourd observed by blotter method

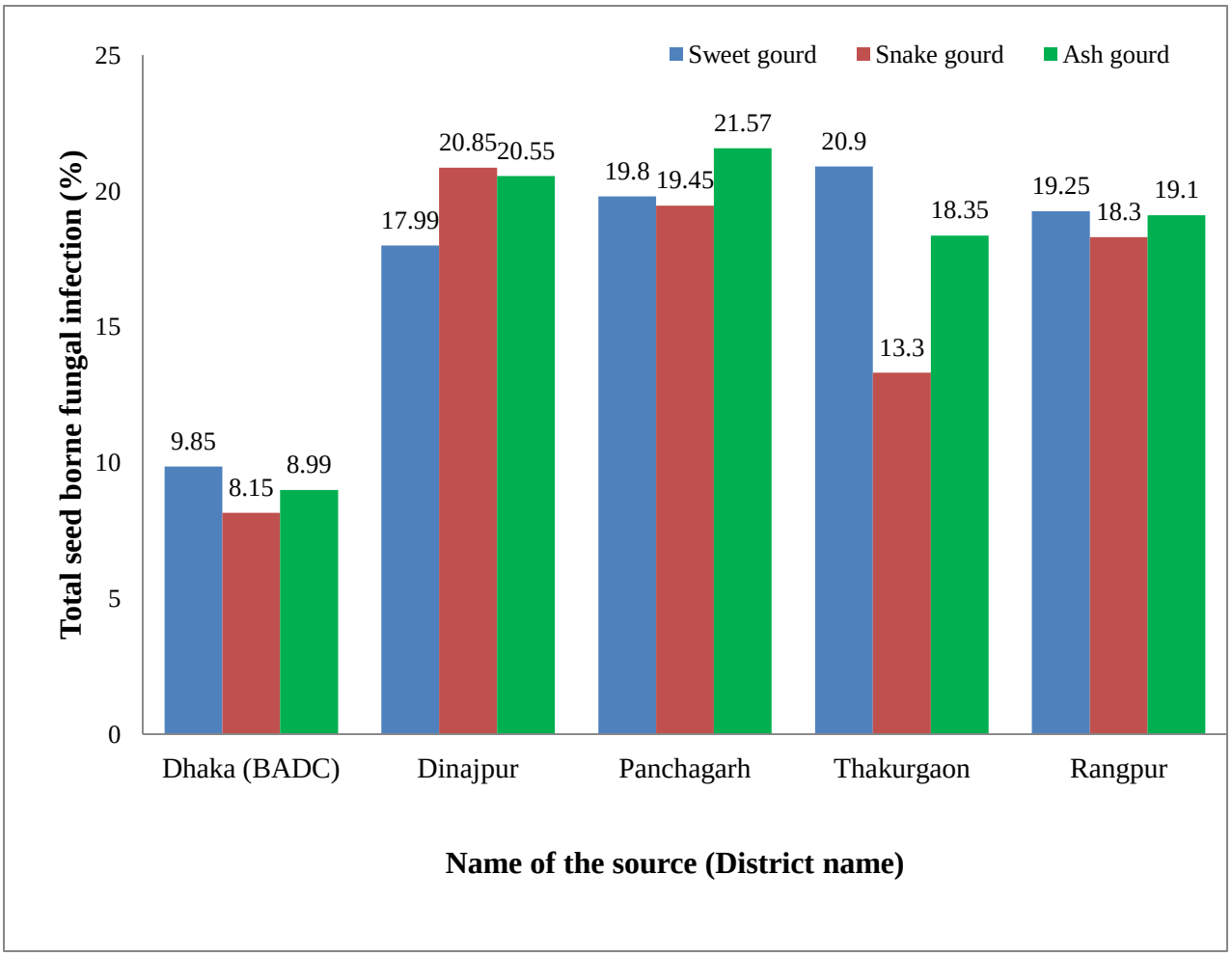
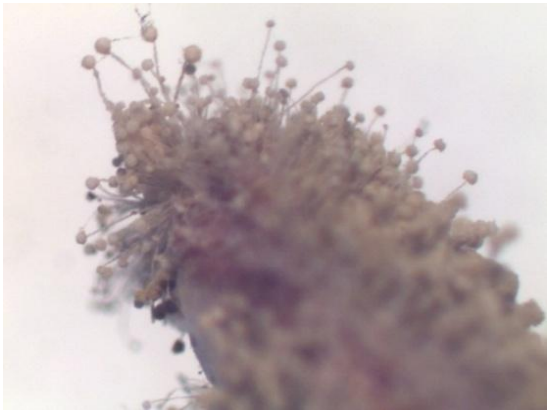
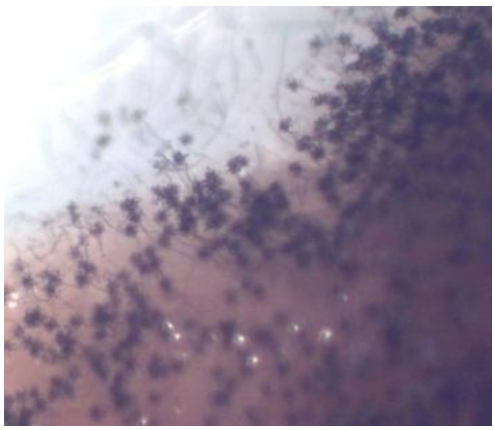


Figure 2: Total seed borne fungal infection (%) of fruit vegetables

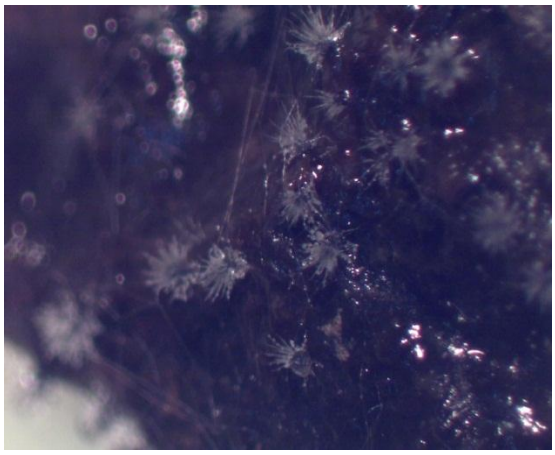
Figure 2 indicates that Dhaka seeds (BADC) showed the lowest seed borne fungal infection for sweet gourd (9.85%), snake gourd (8.15%) and ash gourd (8.99%). Whereas, these three seeds that collected from Thakurgaon, Dinajpur, Panchagarh and Rangpur district showed the highest fungal infection.



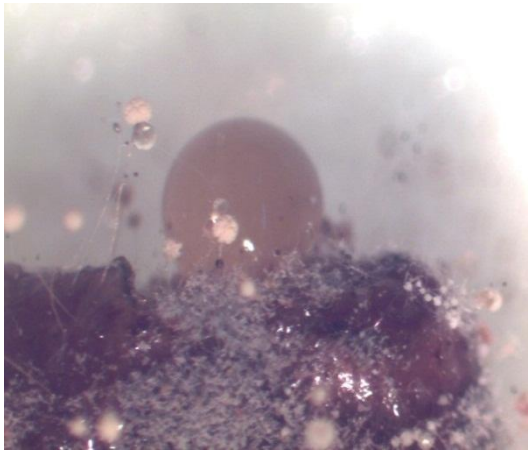
A. Growth of *Aspergillus flavus* on sweet gourd seeds



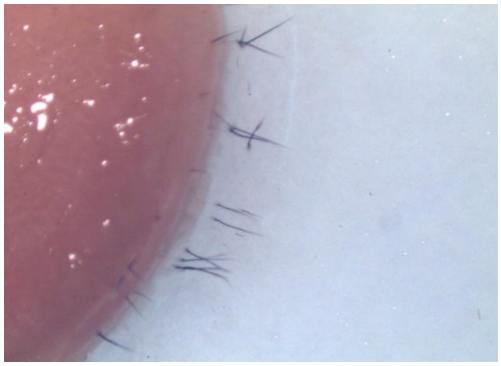
B. Growth of *Curvularia* spp. on ash gourd seeds



C. Growth of *Chaetomium* spp. on snake gourd seeds



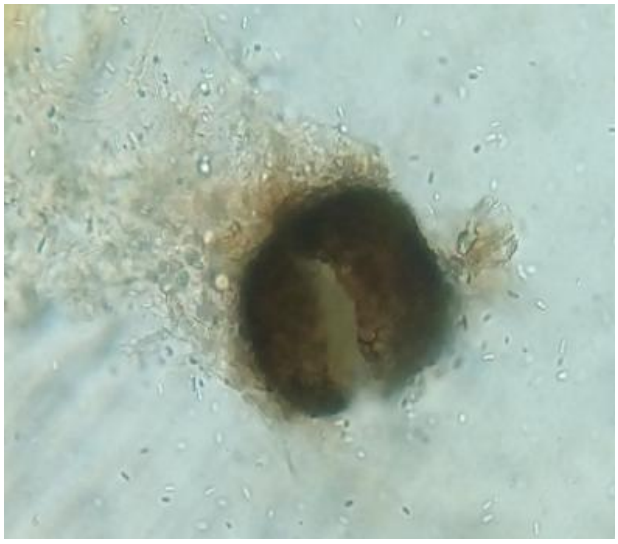
D. Growth of Bacterial ooze on sweet gourd seeds



E. Unknown fungi on ash gourd seeds

F. Growth of *Rhizopus* spp. on seedling

Plate 9 (A-F): Stereomicroscopic view of different fungi on fruit vegetables



A. *Chaetomium* spp. (40X)

B. Unknown fungi (40X)

Plate 10 (A-B): Compound microscopic view of different fungi on fruit vegetables

4.3 Seed health status of podded vegetables

Fungi detected in poded vegetables like as country bean and yard long bean seeds that were collected from different district like as Dhaka, Thakurgaon, Panchagarh, Dinajpur and Rangpur presented in table and figures. Prevalence of seed-borne fungal infections varied depending on the seeds of different vegetables.

4.3.1 Seed health study of country bean (*Phaseolus vulgaris*)

4.3.1.1 Health status of country bean seeds by dry inspection of seed

A. Physical appearance of seeds

No physical abnormalities of seeds were appeared over the seed surface of Dhaka district seeds (BADC). Deformed and black spot seeds were observed in the seeds collected from Dinajpur district seeds whereas, shrinkage and deformed of seeds were observed in the seeds that collected from Thakurgaon and Rangpur district seeds. But discolored seeds were observed which seeds that collected from Panchagarh district. (Table 14).

B. Presence of pathogenic structures of fungi: Cottony mycelium was observed over seeds surface that collected from Dinajpur, Thakurgaon and Rangpur district. However, mycelium was found in Panchagarh district seeds. No fungal structure was observed in Dhaka district seeds (BADC) (Table 14).

C. Purity Analysis

In case of purity analysis, 40 g seeds of country bean were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district

seeds, 38.10 g (95.25%) pure seeds, 1.65 g (4.13%) inert matter and 0.75 g (0.62%) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 37.54 g (93.85 %), 2.10 g (5.25 %) and 0.36 g (0.9%), respectively. Whereas, in seeds collected from Thakurgaon district, 38 g (95 %) pure seeds, 1.7 g (4.25%) of inert matter and 0.3 g (1.2%) other crop seeds were observed. Moreover, from Rangpur district seeds, the pure seeds 36.95 g (92.38 %), inert matter 1.85 g (4.62%) and other crop seeds 1.2 g (3%) were observed (Table 14).

4.3.1.2 Prevalence of seed-borne fungi of country bean identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Alternaria* spp., *Chaetomium* spp., *Rhizopus* spp. and unidentified bacteria were observed.

B. Incidence of seed borne pathogens

The highest incidence was observed from the seed collected from Dinajpur district in case of fungal infection percentage (20.2 %). However, the lowest fungal infection was recorded in Dhaka district seeds (BADC) (9.7%). Incidence of *Aspergillus flavus* was varied from 2.75 to 5.5%. Where, the highest was observed in the seeds of Rangpur district (5.5%). But the lowest was in Dhaka district seeds (BADC) (2.75%). *A. niger* incidence was varied from 2.05 to 5.50 %. The lowest infection was observed in Dhaka district seeds (BADC) (2.05 %). Incidence of

Fusarium spp. was varied 2.25 to5.75%. Whereas, the lowest incidence was observed in BADC seeds(2.25%) that was statistically similar with Dinajpur district seeds. *Alternaria* spp. incidence ranged was varied from 2.04 to 3.85 %. The lowest infection was recorded in Dhaka district seeds (BADC) (2.04%) and the highest was in Dinajpur district seeds (3.85 %). In case of *Chaetomium* spp. appearance ranged was varied from 3.00 to 4.9 %. The lowest infection was recorded in Thakurgaon district seeds (3.00%). *Rhizopus* spp. incidence ranged was varied from 1.97 – 4%. Dhaka district seeds (BADC) showed the lowest infection of that fungus (1.97%). Where in the unidentified bacterial infection, ranged was varied from 2.64 to 4.65%. The lowest incidence was recorded in Dhaka district seeds (BADC) (2.64%) and the highest in Thakurgaon district seeds (4.65%) respectively (Table 15).

Table 14. Health status of country bean seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	38.10	95.25	1.65	4.13	0.75	0.62	Cottony mycelium	Deformed
Panchagarh	40	37.54	93.85	2.10	5.25	0.36	0.9	Mycelium	Discolored
Thakurgaon	40	38	95.00	1.7	4.25	0.3	0.75	Cottony mycelium	Deformed
Rangpur	40	36.95	92.38	1.85	4.62	1.2	3.00	Cottony mycelium	Shrinkage

Table 15. Prevalence of seed-borne fungi of country bean identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.75 c	2.05 c	2.25 c	2.04 c	-	1.97 c	2.64 c	9.7
Dinajpur	4.85 b	5.05 a	3.01 c	3.85 b	4.85 a	3.85 a	3.74 b	20.2
Panchagarh	-	5.50 a	4.50 b	3.50 b	4.50 a	4.00 a	3.75 b	16.75
Thakurgaon	4.25 b	4.05 b	5.75 a	-	3.00 b	3.00 b	4.65 a	15.7
Rangpur	5.50 a	5.25 a	5.50 a	3.00 b	4.90 a	3.15 b	-	18.3
LSD	0.737	0.785	0.725	0.651	0.961	0.653	0.560	
Level of significance	*	*	*	**	**	**	**	
CV %	8.41	7.13	11.17	11.26	8.78	10.25	8.76	

* = Significant at 5% level as pre DMRT, ** = Significant at 1% level as per DMRT

4.3.2 Seed health study of yard long bean (*Vigna unguculata*)

4.3.2.1 Health status of yard long bean seeds by dry inspection of seed

A. Physical appearance of seeds

No physical abnormalities of seeds were found over the seed surface of Dhaka district seeds (BADC). Deformed seeds were observed in the seeds collected from Dinajpur and Thakurgaon district seeds whereas shrinkage of seeds was observed in Rangpur district seeds. But discolored seeds were observed which seeds that collected from Panchagarh district (Table 16).

B. Presence of pathogenic structures of fungi: Cottony mycelium was observed over seeds surface that collected from Dinajpur, Thakurgaon and Rangpur district. No fungal structure was observed in Dhaka district seeds (BADC) but mycelium was in Panchagarh district seeds (Table 16).

C. Purity Analysis

In case of purity analysis, 40 g seeds of long yard bean were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district seeds, 38.50 g (96.25%) pure seeds, 1.3 g (3.25%) inert matter and 0.2 g (0.5%) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 37.86 g (94.65 %), 2.11 g (5.28 %) and 0.03 g (0.07%), respectively. Whereas, in seeds collected from Thakurgaon district, 38.74 g (96.85 %) pure seeds, 1.16 g (2.9%) of inert matter and 0.1 g (0.25%) other crop seeds were observed. Moreover, from Rangpur district seeds, the pure seeds 36.85 g (92.13 %), inert matter 2.55 g (6.37%) and other crop seeds 0.6 g (1.5%) were observed (Table 16).

4.3.2.2 Prevalence of seed-borne fungi of yard long bean identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp. and unidentified bacteria were observed.

B. Incidence of seed borne pathogens

The highest fungal incidence was observed from the seeds collected from Rangpur (16.84%). Whereas, the lowest fungal infection was recorded in the seeds of BADC (10.71%). Incidence of *Aspergillus flavus* were varied from 2.05 to 4.95 %. Where, the highest was observed in the seeds of Dinajpur district (4.95%) that statistically similar with Rangpur district seeds. But the lowest was in Dhaka district seeds (BADC) (2.05%). *A. niger* incidence was varied from 2.56 to 4.57 %. The lowest infection was observed in Dhaka district seeds (BADC) (2.56 %). Incidence of *Fusarium* spp. was varied from 1.65 to 3.50%. Where the lowest incidence was observed in Dhaka district seeds (BADC) (4.01%). *Curvularia* spp. incidence ranged from 1.25 to 4.00%. The lowest infection was recorded in Dhaka district seeds (BADC) (1.25%) and the highest was in Thakurgaon district seeds (4 %) that was statistically similar with Panchagarh district seeds. In case of *Chaetomium* spp. appearance ranged from 2.05 to 4.59 %. The lowest infection was recorded in Dhaka district seeds (BADC) (2.05%). *Rhizopus* spp. incidence was varied from 2.15 – 4%. Dhaka district seeds (BADC) showed no infection of that fungus. Where the unidentified bacterial infection, varied from 1.15 to 3.75%.

The lowest incidence was recorded in Dhaka district seeds (BADC) (1.15%) and the highest in Panchagarh district seeds (3.75%) (Table 17).

Table 16. Health status of yard long bean seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	38.5	96.25	1.3	3.25	0.2	0.5	Cottony mycelium	Deformed
Panchagarh	40	37.86	94.65	2.11	5.28	0.03	0.07	Mycelium	Discolored
Thakurgaon	40	38.74	96.85	1.16	2.9	0.1	0.25	Cottony mycelium	Deformed
Rangpur	40	36.85	92.13	2.55	6.37	0.6	1.5	Cottony mycelium	Shrinkage

Table 17. Prevalence of seed-borne fungi of yard long bean identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.05 c	2.56 c	1.65 c	1.25 c	2.05 c	-	1.15 c	10.71
Dinajpur	4.95 a	3.00 b	3.35 a	2.45 b	3.25 b	2.15 c	2.40 b	14.55
Panchagarh	-	4.57 a	3.50 a	3.50 a	4.50 a	4.00 a	3.75 a	16.82
Thakurgaon	3.25 b	3.25 b	2.75 b	4.00 a	3.05 b	2.90 b	-	12.2
Rangpur	4.05 a	4.25 a	3.50 a	2.20 b	4.59 a	3.00 b	2.25 b	16.84
LSD	0.708	0.645	0.596	0.465	1.25	0.653	0.754	
Level of significance	*	**	*	**	**	*	**	
CV %	8.01	5.75	11.71	11.62	6.78	7.25	9.86	

*= Significant at 5% level as per DMRT,** = Significant at 1% level as per DMRT

A. Pure seeds of country bean

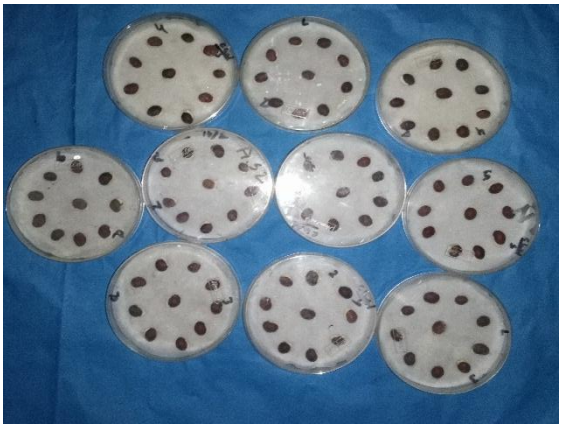
B. Pure seeds of yard long bean

C. Inert matter of country bean

D. Other seeds

Plate 11 (A-D): Seed health of country bean and yard long bean observed by dry inspection method

A. Plating of long yard bean seeds by blotter method



B. Plating of country bean seeds by blotter method

Plate 12 (A-B): Seed health of yard long bean and country bean observed by blotter method

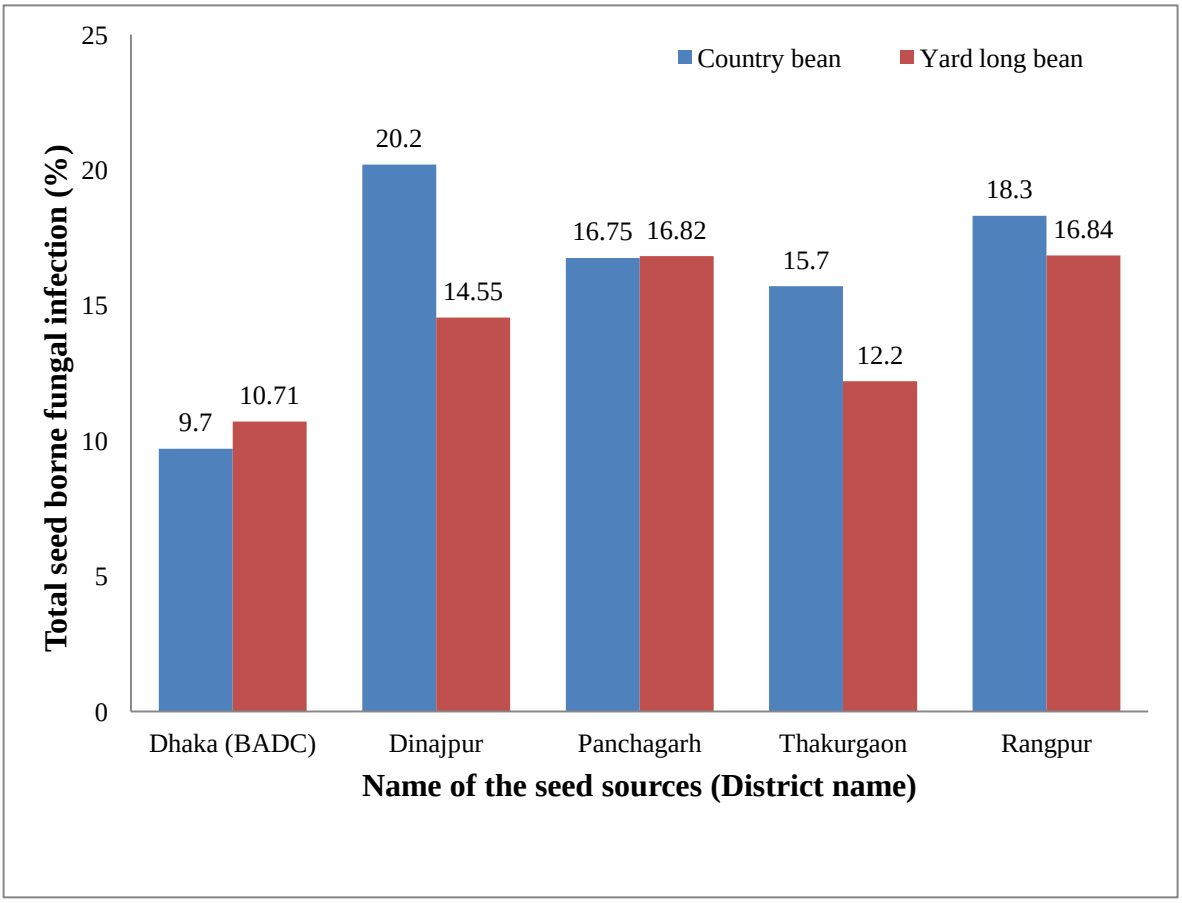
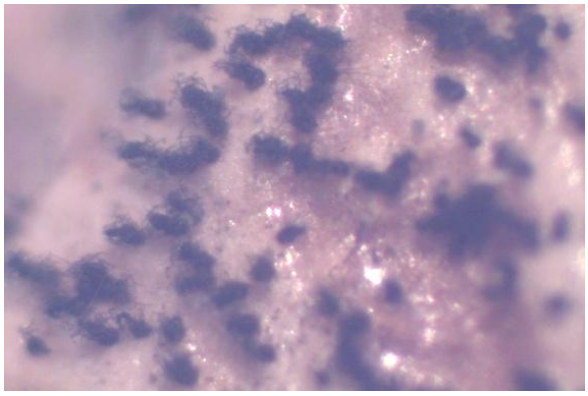
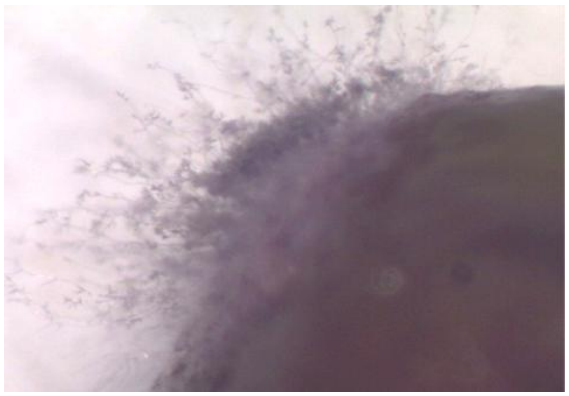


Figure 3: Total seed borne fungal infection (%) of podded vegetables

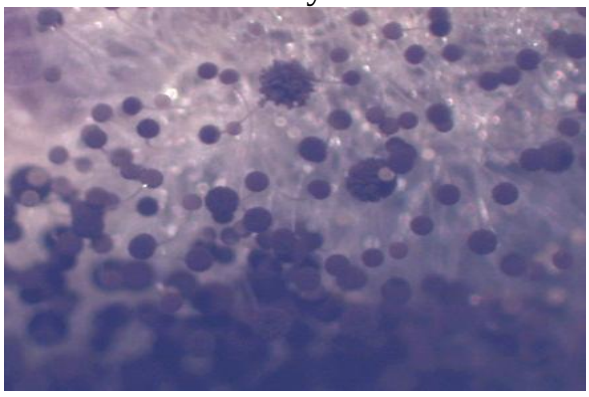
The present study showed that highest value of seed borne fungal infection was found in Dinajpur, Thakurgaon, Panchagarh and Rangpur district's seeds. Whereas, the BADC seeds that collected from Dhaka district showed the lowest fungal infection (9.7 % and 10.71%) in both podded vegetables seeds.



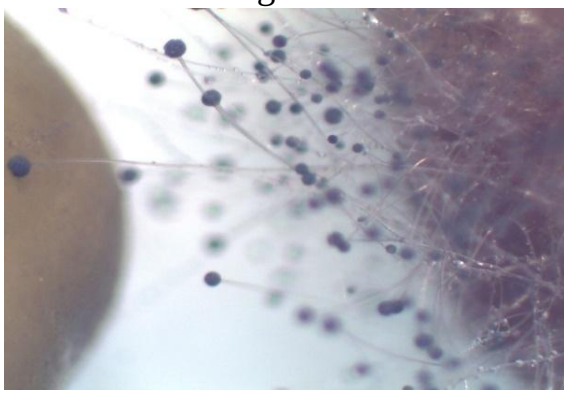
A. Growth of *Chaetomium* spp. on country bean seeds



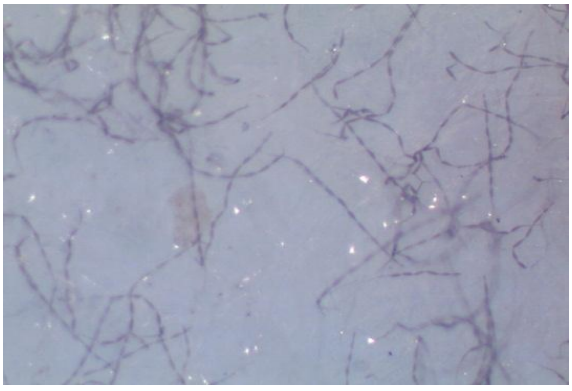
B. Growth of *Curvularia* spp. on yard long bean seeds



C. Growth of *Aspergillus niger* on country bean seeds



D. Growth of *Rhizopus* spp. on yard long bean seeds



E. Growth of *Alternaria* spp. on and around country bean seeds

F. Growth of *Fusarium* spp. on yard long bean seeds

Plate 13 (A-F): Stereomicroscopic view of different fungi on podded vegetables

4.4 Seed health status of root vegetables

Fungi detected in root vegetable seeds like radish and carrot that were collected from five district in Bangladesh presented in tables, plates and figures. Appearance of seed borne fungal infections varied depends on the seeds of different seed sources.

4.4.1 Seed health study of radish (*Raphanus sativus*)

4.4.1.1 Health status of radish seeds by dry inspection of seed

A. Physical appearance of seeds: Deformed seeds were observed in the seeds collected form Dinajpur and Thakurgaon district. Whereas, shrinkage and discolored of seeds were found in the seeds that collected from Rangpur and Panchagarh but no physical abnormalities were observed in radish seeds that collected from Dhaka district (BADC).

B. Presence of fruiting structures of fungi: No fungal fruiting structures were observed over the seed surface collected from Dhaka district (BADC) whereas cottony mycelium was observed in that seeds collected from Dinajpur, Rangpur and Thakurgaon district but only mycelium was observed in the seeds that collected from Panchagarh district.

C. Purity Analysis

In case of purity analysis, 40 g seeds of radish were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district seeds, 38.45 g (96.13 %) pure seeds, 1.4 g (3.5 %) inert matter and 0.15 g (0.37 %) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 37.55 g (93.88 %), 2.25 g (5.62 %) and 0.2 g (0.5%),

respectively. Whereas, in seeds collected from Thakurgaon district, 36.72 g (91.8 %) pure seeds, 3.13 g (7.82 %) of inert matter and 0.15 g (0.38 %) other crop seeds were observed. Moreover, from Rangpur district seeds, the pure seeds 39.1 g (97.75 %), inert matter 0.7 g (1.75 %) and other crop seeds 0.2 g (0.5 %) were observed (Table 18).

4.4.1.2 Prevalence of seed-borne fungi of radish identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Six fungal pathogens viz. *Aspergillus flavus*, *Aspergillus niger, Fusarium* spp., *Alternaria* spp., *Chaetomium* spp., *Rhizopus* spp., one unidentified bacterium and one unknown fungus were observed and recorded.

B. Incidence of seed borne pathogens

For fungi detection in radish seeds collected from five different location are presented in Table 19. Prevalence of total seed-borne fungal infections varied significant depending on the seeds sources and seed categories. The highest total seed borne fungal infections were recorded in Thakurgaon district seeds (19.5%), while the lowest infection was recorded on from Dhaka district (BADC) seeds (11.6%).Incidence of *Aspergillus flavus, A. niger* were varied from 2.15 to 5.25% and 2.45 to 5.0 % respectively. The highest fungal incidence was observed in the seeds of Thakurgaon district (5.25 % and 5.00%) that statistically similar with Panchagarh district (4.75%) and the lowest was in Dhaka district (BADC) (2.15 % and 2.45%). Incidence of *Fusarium* spp. was varied from 2.0 to 4.15%.

The lowest incidence observed in Dhaka district (BADC) seed (2.0%). Incidence of *Alteraria* spp. was varied from 2.90 – 4.5%, where the highest incidence was observed in Thakurgaon district seeds (4.5%) followed by Dinajpur district seeds (4.00 %) that statistically similar. But the lowest was in Rangpur district seed (2.9%). *Chaetomium* spp. was varied from 2.05 – 4.6%. Of this *Chaetomium* spp. (2.05%) was the lowest incidence of fungus found in Dhaka district (BADC) seed. *Rhizopus* spp. was varied from 2.30 to 4.1 %. The lowest incidence was observed in Dhaka district (BADC) seeds (2.30%). Unidentified fungus incidence was ranged from 1.65 to 3.85%. The lowest incidence was observed on Dhaka district (BADC) seeds (1.65%) where the highest was observed in those seeds that collected from Thakurgaon district seeds (3.35%) that statistically similar with Dinajpur district seeds (Table 19).

Table 18. Health status of radish seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	38.45	96.13	1.4	3.5	0.15	0.37	Cottony mycelium	Deformed
Panchagarh	40	37.55	93.88	2.25	5.62	0.2	0.5	Mycelium	Discolored
Thakurgaon	40	36.72	91.8	3.13	7.82	0.15	0.38	Cottony mycelium	Deformed
Rangpur	40	39.1	97.75	0.7	1.75	0.2	0.5	Cottony mycelium	Shrinkage

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Table 19. Prevalence of seed-borne fungi of radish identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.15 c	2.45 c	2.00 c	-	2.05 c	2.30 c	1.65 c	11.6
Dinajpur	3.35 b	3.25 b	2.75 b	4.00 a	3.50 b	3.00 b	3.25 a	16.1
Panchagarh	4.75 a	4.50 a	-	3.50 b	4.60 a	4.10 a	2.75 b	17.2
Thakurgaon	5.25 a	5.00 a	4.15 a	4.50 a	-	3.75 a	3.85 a	19.5
Rangpur	3.25 b	4.45 a	3.50 a	2.90 b	4.35 a	3.00 b	-	14.45
LSD	1.35	1.43	0.596	0.678	0.741	0.853	0.460	
Level of significance	*	**	*	**	**	**	*	
CV %	8.91	7.53	11.47	10.52	8.18	8.25	9.86	

*= Significant at 5% level as per DMRT,** = Significant at 1% level as per DMRT

4.4.2 Seed health study of carrot (*Daucus carrota*)

4.4.2.1 Health status of carrot seeds by dry inspection of seed

A. Physical appearance of seeds

Black spot seeds were observed in the seeds collected from Dinajpur district whereas shrinkage, deformed and discolored of seeds were observed in the seeds that collected from Rangpur, Thakurgaon and Panchagarh districts respectively. But no abnormalities were found in Dhaka district seeds (BADC) (Table 20).

B. Presence of pathogenic structures of fungi: Mycelium was observed on seeds that collected from Dinajpur, Panchagarh and Rangpur . But cottony mycelium was observed in Thakurgaon district seeds. In Dhaka district seeds (BADC), no fruiting structure was found (Table 20).

C. Purity analysis

In case of purity analysis, 40 g seeds of carrot were observed in naked eye. Then pure seeds, inert matter and other crops seeds were separated. No inert matter and other crop seeds were observed in BADC seeds. In case of Dinajpur district seeds, 38.2 g (95.5 %) pure seeds, 1.4 g (3.5 %) inert matter and 0.4 g (1 %) other crop seeds were found. However, in Panchagarh district, pure seeds, inert matter and other crop seeds were 36.74 g (91.85 %), 2.25 g (5.63 %) and 1.01 g (2.52 %), respectively. Whereas, in seeds collected from Thakurgaon district, 35.69 g (89.23 %) pure seeds, 3.14 g (7.85 %) of inert matter and 1.17 g (2.92 %) other crop seeds were observed. Moreover, from Rangpur district seeds, the pure seeds 39 g (97.75 %), inert matter 0.7 g (1.75 %) and other crop seeds0.3 g (0.75 %) were observed (Table 20).

4.4.2.2 Prevalence of seed-borne fungi of carrot identified by blotter method

At 5 % level of significance, the fungal prevalence varied significant among the samples for all pathogens.

A. Identified pathogen

Aspergillus flavus, Aspergillus niger, Fusarium spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp., one unidentified bacterium and one unknown fungus were observed.

B. Incidence of seed borne pathogens

The highest incidence of fungal infection(15.05 %) was observed from the seed collected from Rangpur district. However, the lowest fungal infection was recorded in Dhaka district seeds (BADC) (9.75 %). Incidence of *Aspergillus flavus* was varied from 2.25 to 5.10 %. Where, the highest was observed in the seeds of Dinajpur district (5.10%) that statistically similar with Rangpur and Panchagarh district seeds. But the lowest was in Dhaka district seeds (BADC) (2.25 %). *A. niger* incidence was varied from 1.95 to 5.0 %. The lowest infection was observed in Dhaka district seeds (BADC) (1.95 %). But the highest in Rangpur district seeds (5.0%) that was statistically similar with Panchagarh district seeds. Incidence of *Fusarium* spp. was varied 1.85 to 3.85%. Where the lowest incidence was observed in BADC seeds (1.85%). *Curvularia* spp. incidence was varied from 2.45 to 4.95 %. The lowest infection was recorded in Dhaka district seeds (BADC) (2.45%) and the highest was in Dinajpur district seeds (4.95%). In case of *Chaetomium* spp. appearance ranged was varied from 1.05 to 4.50 %. The lowest infection was recorded in Dhaka district seeds (BADC) (1.05%) *Rhizopus* spp. Incidence was varied from 3.80 – 4.50%.

Thakurgaon district seeds showed the lowest infection of that fungus (3.80%). Moreover, the unidentified bacterial infection was varied from 1.40 to 4.05%.The lowest incidence was recorded in Dhaka district seeds (BADC) (1.40%) and the highest in Dinajpur district seeds (4.05%) (Table 21).

Table 20. Health status of carrot seeds by dry inspection of seed

Seed Sources (District)	Total weight (g)	Pure seeds (g)	Pure seeds (%)	Inert matter (g)	Inert matter (%)	Other seeds (g)	Other seeds (%)	Fungal structures	Physical abnormalities of seed
Dhaka (BADC)	40	40	100	0	0	0	0	Absence	Absent
Dinajpur	40	38.2	95.5	1.4	3.5	0.4	1	Mycelium	Back point
Panchagarh	40	36.74	91.85	2.25	5.63	1.01	2.52	Mycelium	Discolored
Thakurgaon	40	35.69	89.23	3.14	7.85	1.17	2.92	Cottony mycelium	Deformed
Rangpur	40	39.00	97.5	0.7	1.75	0.3	0.75	Mycelium	Shrinkage

Table 21. Prevalence of seed-borne fungi of carrot identified by blotter method

Source Name	% Pathogen incidence							% Seed infection
	Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria	
Dhaka (BADC)	2.25 c	1.95 c	1.85 c	2.45 c	1.05 c	-	1.40 c	9.75
Dinajpur	5.10 a	3.35 b	-	4.95 a	4.10 a	4.10 a	4.05 a	14.55
Panchagarh	4.50 a	4.85 a	3.85 a	-	4.50 a	4.50 a	3.75 a	14.45
Thakurgaon	3.55 b	3.50 b	2.95 b	3.85 b	3.30 b	3.80 b	2.40 b	12.55
Rangpur	4.75 a	5.00 a	3.05 b	3.50 b	3.50 b	-	2.25 b	15.05
LSD	1.07	0.843	0.596	0.565	0.941	0.653	0.460	
Level of significance	*	**	*	**	**	*	*	
CV %	8.14	7.35	10.27	10.46	8.78	10.21	7.86	

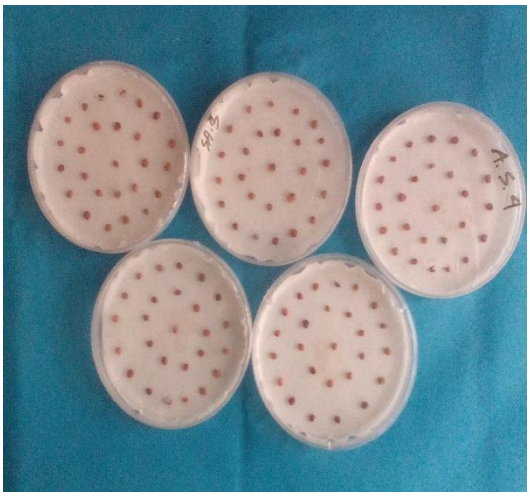
*= Significant at 5% level as per DMRT,** = Significant at 1% level as per DMRT

A. Pure seeds of radish

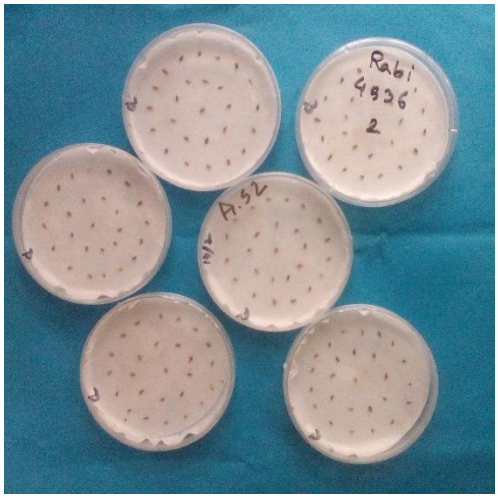
B. Pure seeds of carrot

C. Other seeds

Plate 14 (A-C): Seed health of radish and carrot observed by dry inspection method



A. Plating of radish seeds by blotter method



B. Plating of of carrot seeds by blotter method

Plate 15 (A-B): Seed health of radish and carrot observed by blotter method

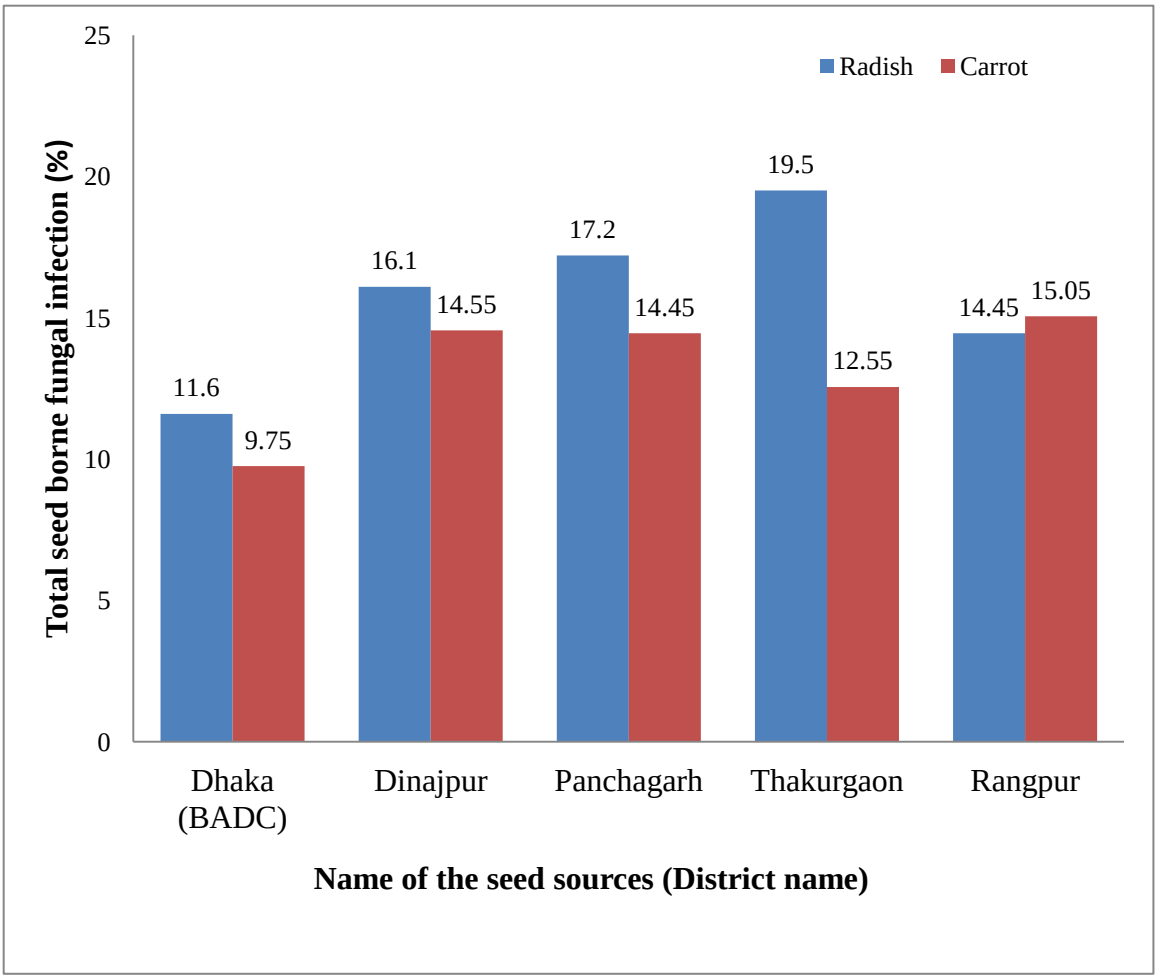
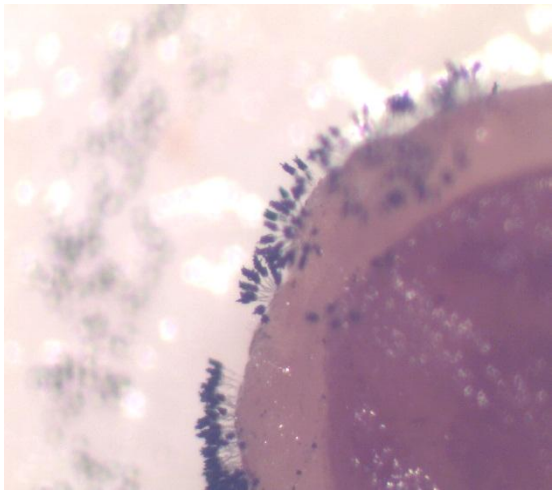
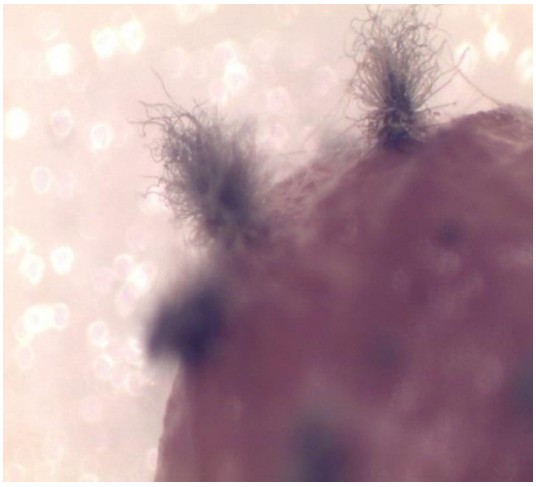


Figure 4: Total seed borne fungal infection (%) of root vegetables

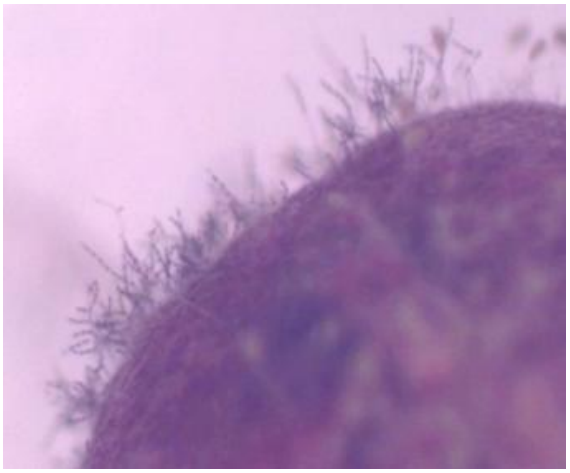
The present study showed that highest value of seed borne fungal infection was found in Dinajpur, Thakurgaon, Panchagarh and Rangpur district's seeds. Whereas, the BADC seeds that collected from Dhaka district showed the lowest fungal infection in radish (11.6 %) and in carrot (9.75%), respectively.



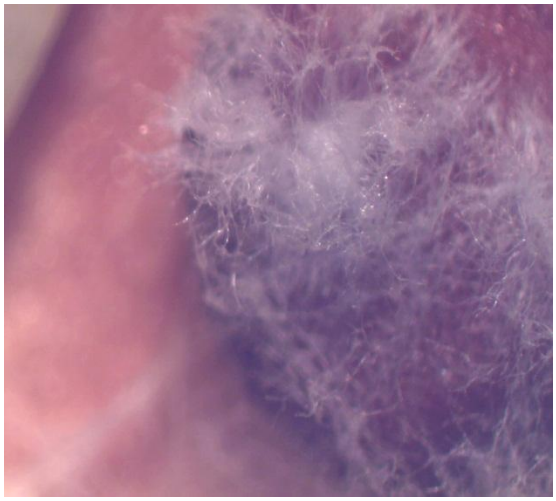
A. Unknown fungi on carrot seeds



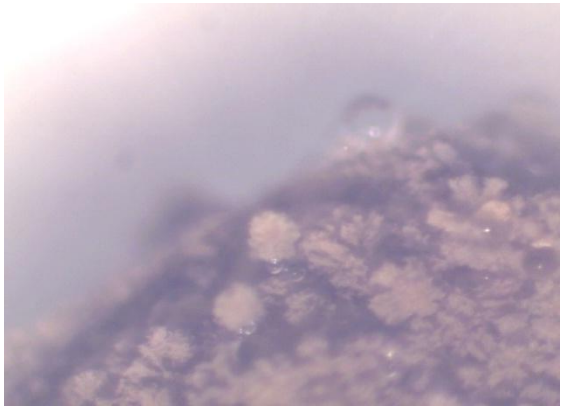
B. *Chaetomium* spp. on carrot seeds



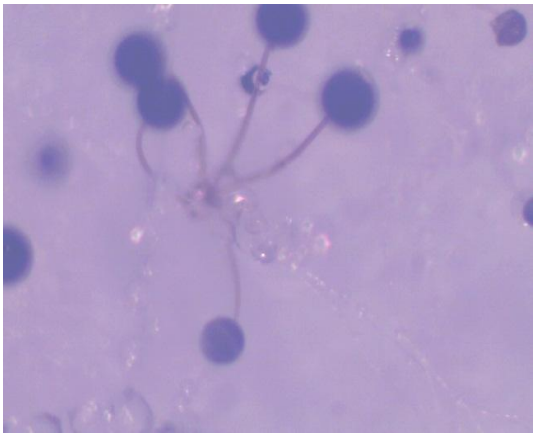
C. *Alternaria* spp. on radish seeds



D. *Fusarium* spp. on radish seeds



E. *Aspergillus flavus* on radish seeds



F. *Rhizopus* spp. around carrot seeds

Plate 16(A-F): Stereomicroscopic view of different fungi on root vegetables seeds

CHAPTER V

DISCUSSIONS

Fungi recorded in seeds of all the ten selected vegetable species under five districts revealed that BADC seeds from Dhaka yielded minimum seed-borne fungal infections followed by other district seeds. On the contrary, pure seeds recorded in seeds of ten selected vegetables show that the highest purity percentage was obtained in seeds of BADC followed by other district (Dinajpur, Thakurgaon, Panchagrah and Rangpur) seeds. This indicates that seeds of BADC are most superior than other seeds.

In Dry inspection method, white and cottony mycelium, sclerotia were identified.

In blotter method, seven seed borne fungal genera were identified namely *Fusarium, Aspergillus, Alternaria*, *Curvularia*, *Chaetomium, Rhizopus* and *Cercospora*.

In dry inspection of stem amaranth, highest pure seeds (100 %) were found in BADC seeds collected from Dhaka district. Seven pathogens were recorded on seeds collected from five districts. Pathogen that were counted namely *Aspergillus flavus, Aspergillus niger, Fusarium* spp., *Alternaria* spp. *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp. The lowest total seed borne pathogen infections (7.1%) were observed in Dhaka district seeds (BADC) and the highest (22.9 %) in Panchagarh district seeds. Regarding the above findings, the overall health status of BADC seeds of stem amaranth was up to the mark. However, the health status of others seeds were not satisfactory. This is because, saprophytic pathogenic

genera were found with the seed that may deteriorate the health status and quality of seed, seedling and finally yield.

On over the seed surface of stem amaranth, six different fungi viz. *Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Chaetomium* spp., *Fusarium* spp. and *Rhizopus* spp. were found during experiment running. Significant number of seed borne fungal pathogens belonging to the genera *Aspergillus, Curvularia, Fusarium a*nd *Penicillum* had been detected in Amaranth seeds reported by Begum (2012). The present findings clearly showed that *Alternaria* spp., *Aspergillus flavus, Aspergillus niger*, *Chaetomium* spp., *Fusarium* spp. and *Rhizopus* spp. were associated with the tested seed samples of amaranth significantly reduced percent germination. Similar result was reported by earlier worker (Islam, 2006).

In dry inspection of Indian spinach, the highest pure seeds (100%) was found in Dhaka district (BADC) seeds and the lowest (90.63%) was in Dinajpur district seeds. Five different fungi viz. *Curvularia, Aspergillus, Cercospora, Fusarium* and *Rhizopus* were found to be associated with Indian spinach seed surface. The lowest percent seed infection (7.41%) observed in Dhaka district (BADC) seeds and the highest (18.9 %) in Thakurgaon district seeds.

Significant number of seed borne fungal pathogens belonging to the genera *Alternaria, Curvularia, Fusarium* and *Penicillium* had been tracked out in Spinach seeds by many researchers (Begum, 2012; Khanom, 2011).

In water spinach, BADC seeds showed no presence of inert matter and other seeds whereas in Thakurgaon district seeds the lowest percent of pure seeds was found (88 %). Fungal structure like sclerotia was found over the seed surface. Reduce,

shriveled, deformed and discolored seeds also present in water spinach seeds. Percent of seed infection was observed the lowest (7.1%) in BADC seeds and the highest (20.55 %) in Rangpur district.

In dry seed inspection of sweet gourd, highest pure seeds were found (92.62 %) in Panchagar district seeds and lowest pure seeds were found BADC seeds (90.75 %). Whereas, discolored and deformed seeds were observed on seeds collected from five district. In case of sweet gourd seeds five different fungi viz. *Curvularia, Aspergillus, Chaetomium, Fusarium* and *Rhizopus* were found to be associated. The highest (20.9%) total seed borne pathogen infection was observed in Thakurgaon district seeds. Whereas, the lowest infection (9.85 %) observed in BADC seeds. Regarding the above findings, the total health status of loose seeds of sweet gourd was not up to the mark. Because of, few saprophytic pathogens were observed over the seed surface that may decline the health status and quality of seed and seedling.

After incubation of sweet gourd seeds on blotter method, four different fungi such as *Aspergillus niger, Aspergillus flavus, Penicillium* spp. and *Botrytis cineria* were recorded by Aktar (2009).

In case of snake gourd, during dry seed inspection the highest pure seeds (100%) was observed in Thakurgaon district seeds and the lowest in Dinajpur district seeds. Fungal infection in seeds was higher in Dinajpur district (20.85 %) and the lowest in BADC seeds (8.15%). Except unidentified bacteria all fungal pathogen were observed statistically the higher in Dinajpur district seeds.

In case of ash gourd seeds, observation of pure seeds was the highest (100%) in Rangpur district seeds. Whereas the lowest was in Dinajpur district seeds (91.85%). In blotter seed testing method, percentage of seed infection was observed highest in Panchagarh district seeds (21.57%) and the lowest in BADC seeds (8.99%).

Sultana (2009) reported that the germination and health status of Truthfully Labeled Seed (TLS) of bottle gourd, sweet gourd, snake gourd, Ridge gourd, cucumber, wax gourd and sponge gourd collected from BADC and other seed company. She observed eight fungi namely *Aspergillus* spp., *Botrytis* spp., *Curvularia* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Phomopsis* spp. *and Rhizopus* spp.. She found only *Aspergillus* spp. was highly prevalent in all the crop seeds ranging from 1.6-14%.

In dry inspection of country bean, 100% pure was observed in BADC seeds. The lowest pure seeds (92.38%) were observed in Rangpur district seeds. BADC seeds showed the lowest infection of seed (9.7%) whereas the Dinajpur district seeds showed the highest seed infection (20.2 %). Six fungal genera were identified in country bean seeds.

Domijan *et al.* (2003) reported that seed borne on bean (*Phaseolus vulgaris*) and most common fungi were isolated viz. *Alternaria* spp. (75 %), *Aspergillus* (73%), *Rhizopus* (73 %), *Cladosporium* spp. (98 %), *Penicillium* spp. (69%), *Fusarium* spp. (38 %).

Major seed borne fungi related with seeds of common bean (*Phaseolus vulgaris* L) different for bean growing areas of Ethiopia that survey conducted by Yesuf and Sangchote (2007). Major seed-borne fungi like *Collectotrichum lindemuthianum, Ascochyta phaseolorum* and *Phaeoisariopsis griseola* were associated with common bean.

100 % pure seeds of yard long bean were observed in BADC seeds whereas lowest (96.85 %) in Thakurgaon district seeds. Inert matter and other seeds were highest in Rangpur district seeds. Seeds infection percentage was the highest observed in Rangpur district seeds (16.84 %) and the lowest in BADC seeds (10.71%).

In dry inspection of radish seeds, pure seeds percentage was observed 100 % in Dhaka district (BADC) seeds whereas in Thakurgaon district seeds pure seeds percent was lowest (91.8%). Seed infection percentage was highest in Thakurgaon district seeds (19.5%) and the lowest infestation in BADC seeds (11.6%) compare to other district seeds.

Total seed borne fungal infection was highest (28%) in radish seeds. Main fungus was *Fusarium* spp. (5.8%) followed by *Alternaria* spp. (3.3%), *Penicillum* spp. (2%), *Aspergillus niger*(1.8%), *Aspergillus flavus* (1.7%) and *Curvularia* spp. (1.2%) reported by Hossain *et al.* (2014).

The lowest purity percentage of carrot seeds was observed in Thakurgaon district seeds (89.23%) and the highest in BADC seeds (100 %). In was observed in dry seed inspection method. In BADC seeds, the lowest seeds borne infection percentage was observed (9.95 %) and the highest in Rangpur district seeds (15.05%).

Aspergillus spp. infestation was highest most of the district seeds. *Aspergillus* spp. and *Fusarium* spp. infection percentage were 5.5 % and 4.5%. (Hossain *et al.* 2014).

CHAPTER VI

SUMMARY AND CONCLUSION

The main aim of this experiment was evaluation of health status of some selected vegetables seed collected from five districts of Bangladesh. The selected vegetables were stem amaranth, water spinach, Indian spinach, sweet gourd, snake gourd, ash gourd, country bean and yard long bean, radish and carrot were collected from five districts viz. Dhaka, Panchagarh, Thakurgaon, Dianjpur and Rangpur. The experiment was conducted in Seed health Laboratory (SHL), Sher-e-Bangla Agricultural University, Dhaka during January to December of 2016.

Two seed health testing methods viz. dry inspection of seeds and blotter method were followed as per ISTA (1999) rule. Prevalence of seed borne pathogen of ten vegetables recorded in both dry inspection of seed and blotter method varied significantly depending on seed sources and seed categories. Usually farmers get preliminary idea about seed quality of loose vegetable seeds by visual observation. Moreover, in blotter method most of the seed borne fungal pathogen can easily identified.

For dry inspection of seeds, present study mention that in every cases, seeds collected from BADC showed the highest percentages of pure seed expect sweet gourd, snake gourd and ash gourd seeds. Seeds collected from BADC were 100 % pure in most cases and the lowest pure seed (88 %) in water spinach was collected seeds from Thakurgaon district.

On seed health status analysis of ten vegetable seeds, eight fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp., *Curvularia* spp., *Chaetomium* spp., *Rhizopus* spp., *Cercospora* spp., *Alternaria* spp., unidentified bacteria and unknown fungi were identified by blotter testing method. The lowest prevalence of seed borne fungi was in BADC seeds in all cases of seeds varieties and the highest was in Panchagarh district in case of stem amaranth (22.9 %), water spinach (21.3 %), ash gourd (21.57%). In case of Indian spinach (18.9 %),radish (19.5 %) and sweet gourd (20.9 %) seeds showed the highest fungal infection in Thakurgaon district seeds. In case of, snake gourd (20.85 %) and country bean (20.2%)highest infection percentage was observed in Dinajpur district seeds. Rangpur district seeds showed highest infection percentage in yard long bean seed (16.84 %) and carrot (15.05 %).

In common, the lowest seed-borne fungal pathogens were recorded in BADC seed, followed by seeds of different districts viz. Dhaka, Panchagarh, Thakurgaon, Dianjpur and Rangpur. The total as well as the individual fungi recorded on each of the ten crops varied significantly differ with respect to seed categories and sources. Healthy and quality seed is important for higher crop production. Plant pathogen are easily carried out by seeds. So pathogen free seed is the most important input in agriculture. Untreated loose seeds of vegetables play a major role to carry pathogen within the country.

Seeds of the ten tested crops collected from BADC appear to be the best considering health and health status assessment compared to seeds of other four districts and had the highest pure seeds with the lowest seed-borne fungal infection. Farmers are finally advised to collect the seeds from reliable sources and check their seed health status before sowing in the field. Considering the observed

seed infection by different pathogens, farmers are also suggested to do seed treatment by recommended chemicals or botanicals before sowing to prevent seed borne diseases in the field.

CHAPTER VII

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CHAPTER VIII

APPENDICES

Appendix I: Analysis of variance of data on prevalence of seed borne fungi of stem amaranth identified by blotter method

Stem amaranth	Df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	1.65*	1.75**	0.750*	4.13**	3.10**	1.23**	0.343*
Within	15	0.349 ^{ns}	0.258 ^{ns}	0.139 ^{ns}	0.125 ^{ns}	0.247 ^{ns}	0.147^{ns}	0.073^{ns}
Total	19							

Appendix II: Analysis of variance of data on prevalence of seed borne fungi of water spinach identified by blotter method

Water spinach	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	3.10**	5.35**	0.376*	0.255**	1.38*	0.740**	1.48*
Within	15	0.601 ^{ns}	0.424 ^{ns}	0.205 ^{ns}	0.131 ^{ns}	0.326 ^{ns}	0.50 ^{ns}	0.131 ^{ns}
Total	19							

Appendix III: Analysis of variance of data on prevalence of seed borne fungi of indian spinach identified by blotter method

Indian spinach	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Cercospora* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	2.28*	0.323**	2.58*	0.353**	4.28**	1.40**	0.343*
Within	15	0.639 ns	0.212 ns	0.184 ns	0.177 ns	0.316 ns	0.250 ns	0.073 ns
Total	19							

Appendix IV: Analysis of variance of data on prevalence of seed borne fungi of sweet gourd identified by blotter method

Sweet gourd	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	4.23*	1.68*	1.59*	2.35**	1.74**	1.63**	20.13*
Within	15	0.546 ns	0.379 ns	0.131 ns	0.157 ns	0.232 ns	0.260 ns	0.157 ns
Total	19							

Appendix V: Analysis of variance of data on prevalence of seed borne fungi of snake gourd identified by blotter method

Brinjal	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Cuvilaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	1.55 ^{ns}	1.53**	0.069 ^{ns}	0.333**	3.28**	2.75**	0.343*
Within	15	0.278 ^{ns}	0.250 ^{ns}	0.250 ^{ns}	0.177 ^{ns}	0.361 ^{ns}	0.232 ^{ns}	0.073 ^{ns}
Total	19							

Appendix VI: Analysis of variance of data on prevalence of seed borne fungi of ash gourd identified by blotter method

Cucumber	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Cuvilaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	1.04*	0.35^{ns}	0.563*	0.573**	0.225**	3.33 ^{ns}	1.53*
Within	15	0.195 ^{ns}	0.139 ^{ns}	0.167 ^{ns}	0.163 ^{ns}	0.278 ^{ns}	0.306 ^{ns}	0.232 ^{ns}
Total	19							

Appendix VII: Analysis of variance of data on prevalence of seed borne fungi of country bean identified by blotter method

Bitter gourd	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	0.583*	1.75*****	0.563*	1.33******	5.35******	6.25******	0.753******
Within	15	0.306 ^{ns}	0.258^{ns}	0.141 ^{ns}	0.139 ^{ns}	0.528 ^{ns}	0.250 ^{ns}	0.184 ^{ns}
Total	19							

Appendix VIII: Analysis of variance of data on prevalence of seed borne fungi of yard long bean identified by blotter method

Sweet gourd	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	0.563*	0.750**	0.583*	0.250**	1.83*	1.55*	0.254**
Within	15	0.621 ^{ns}	0.194 ^{ns}	0.306 ^{ns}	0.333 ^{ns}	0.528 ^{ns}	0.611 ^{ns}	0.278 ^{ns}
Total	19							

Appendix IX: Analysis of variance of data on prevalence of seed borne fungi of radish identified by blotter method

Country Bean	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Alternaria* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	1.43*****	7.33******	0.373*****	2.08******	1.40******	1.68******	2.41*****
Within	15	0.730 ^{ns}	0.242 ^{ns}	0.250 ^{ns}	0.187 ^{ns}	0.341 ^{ns}	0.472 ^{ns}	0.164 ^{ns}
Total	19							

Appendix X: Analysis of variance of data on prevalence of seed borne fungi of carrot identified by blotter method

Okra	df	Seed borne infection (%)						
		Aspergillus flavus	*Aspergillus niger*	*Fusarium* spp.	*Curvularia* spp.	*Chaetomium* spp.	*Rhizopus* spp.	Unidentified bacteria
Between	4	2.73*	1.51**	1.08*	1.23**	6.58******	1.58*	1.08*****
Within	15	0.712^{ns}	0.447^{ns}	0.249^{ns}	0.245^{ns}	0.268 ^{ns}	0.298^{ns}	0.311^{ns}
Total	19							