INVESTIGATION ON SEED HEALTH STATUS OF CUCURBITS FOR STORAGE MANAGEMENT

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CERTIFICATE

*This is to certify that the thesis entitled, "**INVESTIGATION ON SEED HEALTH STATUS OF CUCURBITS FOR STORAGE MANAGEMENT**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN PLANT PATHOLOGY, embodies the result of a piece of bona fide research work carried out by **HASIBUR RAHMAN**, bearing Registration No. **10-03819** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma and anywhere in the country or abroad.*

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

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The Author

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ABSTRACT

An investigation was carried out on the seed health status of selected Cucurbits seed and evaluate the efficacy of some selected chemicals against the seed borne pathogens. The experiments were carried out in the seed health laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University during the period from July 2016 to June 2017. Five selected cucurbit seeds namely Sweet gourd, Bottle gourd, Cucumber, Ridge gourd and Snake gourd were collected from five different locations of Dhaka city viz. Siddik Bazar, Mohammadpur Bazar, Kochukhet Bazar, Savar Bazar and BADC seed store (Gabtoli). Four different chemicals viz. Dithane M-45, Autostin 50 WDG, Tilt 250 EC and Salicylic acid were assayed in the experiment. During the investigation, five fungi were identified as *Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. and two unidentified fungi were also isolated from seed. Dry inspection revealed that the cucurbit seeds collected from Siddik Bazar yielded significantly the highest 400-seed wt. (68.19 g), the highest infected seed wt. (22.37 g) and the lowest inert matter wt. (0.22 g) while the Ridge gourd showed the highest 400-seed wt. (110.27 g) and the Cucumber yield the lowest infected seed wt. (3.71 g) and the lowest wt. (0.27 g) of inert matter. The interaction of cucurbits seed and sources of collection showed that the highest seed germination was recorded in Sweet gourd seed collected from Siddik Bazar (L_1S_1) while the lowest seed germination was recorded in Ridge gourd seed from Mohammadpur Bazar (L_2S_4). The incidence of seed borne pathogen isolated and identified from the seed varied significantly in respect of collected areas and cucurbit species. As per the efficacy of the chemicals evaluated in the experiment, the highest performance was showed by Tilt 250 EC followed by Autostin 50 WDG against isolated fungi.

TABLE OF CONTENTS

CHAPTER	TITLE		PAGE
	ACKNOWLEDGEMENT		i-iii
	TABLE OF CONTENTS		iv-vi
	LIST OF TABLES		vii-xii
	LIST OF ABBREVIATED TERMS		xiii
I	**INTRODUCTION**		1-4
II	**REVIEW OF LITERATURE**		5-13
	2.1.	Origin of Cucurbits	5
	2.2.	Importance of cucurbits	7
	2.3.	Diseases of cucurbits	9
	2.4.	Seed health test by dry seed observation	10
	2.5.	Seed health test by blotter method	10
	2.6.	Isolation and Identification of pathogens	11
	2.7.	Management	12
III	**MATERIALS AND METHODS**		14-27
	3.1.	Experimental sites	14
	3.2.	Experimental period	14
	3.3.	Collection of different cucurbits seeds	14
	3.4.	Locations of seed sources	14
	3.5.	Cucurbits species	14
	3.6.	Laboratory experiment	14
	3.6.1.	Inspection of dry seeds	15
	3.6.2.	Blotter method	17
	3.6.2.1.	Inspection of incubated seed samples	17
	3.6.3.	Preparation of Potato Dextrose Agar Media (PDA media)	20

	3.6.4.	Isolation, purification and preservation of seed borne fungal pathogens	21
	3.6.5.	Cultural and morphological characterization of isolated pathogens	21
	3.7.	Management of Storage Pathogens	23
	3.7.1.	Poisoned food technique (Cup method)	25
	3.7.2.	Measurement of radial growth (cm) and determination of percent inhibition	27
	3.8.	Design of Experiment	27
IV	**RESULTS**		28-57
	4.1.	Seed health test of cucurbits	28
	4.1.1.	Seed health test of cucurbits by dry inspection method	28
	4.1.2.	Seed health test of cucurbits by blotter method	32
	4.1.2.1.	Germination and pathogenic incidence of cucurbits seeds collected from different local markets	32
	4.1.2.2.	Germination and pathogenic incidence of different species of cucurbits seeds	34
	4.1.2.3.	Interaction effect on germination and pathogenic incidence of cucurbits seeds	39
	4.1.3.	Cultural and morphological characterization of isolated pathogens	48
	4.1.3.1.	Radial mycelial growth	48
	4.1.3.2.	Number of spores	52
	4.2.	*In vitro* evaluation of selected chemicals against isolated seed borne fungi of cucurbits	54

	4.2.1. *In vitro* efficacy of fungicides in inhibition of mycelial growth of fungal pathogens in poisoned food technique (Cup method)	54
V	**DISCUSSION**	61-63
VI	**SUMMARY AND CONCLUSION**	64-65
VII	**REFERENCES**	66-73
VIII	**APPENDICES**	74-75

LIST OF TABLES

SL. NO.	TITLE	PAGE
1.	Chemicals used for evaluation against storage pathogens of cucurbits	23
2.	Seed health test of cucurbits seeds by dry inspection method collected from different local markets of Dhaka	29
3.	Seed health test of different species of cucurbit seeds by dry inspection method	30
4..	Estimation of percentage of pathogenic incidence of cucurbits seeds by Blotter seed health testing method collected from different local markets	37
5.	Estimation of percentage of pathogenic incidence of different species of cucurbits seeds by blotter seed health testing method	38
6.	Interaction effect on percent pathogenic incidence of cucurbits seeds by Blotter method	42
7.	Cultural and morphological characterization of isolated seed borne fungi from cucurbits seeds	49
8.	Interaction effect of different isolated pathogen and chemicals on radial mycelial growth	57

LIST OF GRAPHS

SL. NO.	TITLE	PAGE
1.	Comparative study of germination (%) in blotter method for different places	32
2.	Comparative study of germination (%) in blotter method for different cucurbits seeds	34
3.	Comparative study of germination (%) interaction between places and cucurbits seed in blotter method	39
4.	Radial mycelial growth of different identified pathogens isolated from cucurbits seeds after application of different chemicals	55
5.	Effect of different chemicals on radial mycelial growth of different identified pathogens isolated from cucurbits seeds	56

LIST OF PLATES

SL. NO.	TITLE	PAGE
1.	Seed sample (400 seeds) of different cucurbits for the investigation of seed health status	15
2.	Seed health test of five different cucurbits seeds by blotter paper method	19
3.	Chemicals used for evaluation against storage pathogens	23
4.	Cup method used for in vitro management of different pathogens (Poisoned food technique)	26
5.	Sweet gourd seeds (pure seeds, infected seeds, inert matter)	30
6.	Bottle gourd seeds (pure seeds, infected seeds, inert matter)	30
7.	Cucumber seeds (pure seeds, infected seeds, inert matter)	31
8.	Ridge gourd seeds (pure seeds, infected seeds, inert matter)	31
9.	Snake gourd seeds (pure seeds, infected seeds, inert matter)	31
10.	Steriomicroscopic view of pathogens on Sweet gourd seeds	43
11.	Steriomicroscopic view of pathogens on Bottle gourd seeds	44
12.	Steriomicroscopic view of pathogens on Cucumber seeds	45
13.	Steriomicroscopic view of pathogens on Ridge gourd seeds	46
14.	Steriomicroscopic view of pathogens on Snake gourd seeds	47
15.	*Aspergillus flavus* (Pure culture and compound microscopic view)	50
16.	*Aspergillus niger* (Pure culture and compound microscopic view)	50
17.	*Rhizopus* sp. (Pure culture and compound microscopic view)	50
18.	*Fusarium* sp. (Pure culture and compound microscopic	51

	view)	
19.	*Chaetomium* sp. (Pure culture and compound microscopic view)	51
20.	Unknown-1 (Pure culture and compound microscopic view)	51
21.	Unknown-2 (Pure culture and compound microscopic view)	52
22.	Number of spore of different fungal pathogens using haemocytometer	52
23.	Mycelial growth of *Aspergillus flavus* against different chemicals at 3DAI	58
24.	Mycelial growth of *Aspergillus niger* against different chemicals at 3DAI	58
25.	Mycelial growth of *Rhizopus* sp. against different chemicals at 3DAI	59
26.	Mycelial growth of *Fusarium* sp. against different chemicals at 3DAI	59
27.	Mycelial growth of *Chaetomium* sp. against different chemicals at 3DAI	60

LIST OF PHOTOGRAPHS

SL. NO.	TITLE	PAGE
1.	Steriomicroscope	18
2.	250 ml PDA medium	20
3.	PDA media in sterile petriplates	21
4.	Digital microscope (Model: Motic, BA-210)	22
5.	Haemacytometer used in counting spore	22
6.	Measurement of radial mycelial growth (cm)	27
7.	Sweet gourd seeds (Blotter method)	43
8.	Bottle gourd seeds (Blotter method)	44
9.	Cucumber seeds (Blotter method)	45
10.	Ridge gourd seeds (Blotter method)	46
11.	Snake gourd seeds (Blotter method)	47

LIST OF APPENDICES

SL. NO.	TITLE	PAGE
1.	Effect of different chemicals on radial mycelial growth of different identified pathogens isolated from cucurbits seeds	74
2.	Radial mycelial growth of different identified pathogens isolated from cucurbits seeds after application of different chemicals	74
3.	Composition of media (For 250ml)	75

LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
et al.	And others
wt.	Wt.
^{0}C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
g	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
%	Percent
LSD	Least Significant Difference
CRD	Completely Randomized Design
Res.	Research
Viz.	Namely
Var.	Variety
sp.	Species
SAU	Sher-e-Bangla Agricultural University
BBS	Bangladesh Bureau of Statistics
BADC	Bangladesh Agricultural Development Corporation
BARI	Bangladesh Agricultural Research Institute
FAO	Food and Agriculture Organization
ISTA	International Seed Testing Association

CHAPTER I
INTRODUCTION

The Cucurbitaceae commonly known as cucurbits and the gourd family. This plant family is consisting of about 825 species and around 118 genera (Rai, 2008). Cucurbits are mostly grown in tropical and sub-tropical conditions but some of the species are grown in temperate zone under artificial conditions like cucumber (Dhaliwal, 2008). The Cucurbitaceae family ranks the highest among plant families for number and percentage of species used as human food. Most of the cucurbits are annual and grown through the seeds by direct sowing in the field but some cucurbits are perennial. Cucurbit vegetables are grown in warm weather and these vegetables cannot grow under the cold conditions. Cucurbitaceous vegetables are tender annual vegetables and are grown only for their fruits. These vegetables flourish under temperature of about 18 to 30 ^{o}C (68-85 ^{o}F) (Saljoqi and Khan, 2007).

In Bangladesh, agriculture accounts for 532,032 million taka of its gross domestic product (BBS 2013). The consumption of vegetables in Bangladesh is about 50g per day per person which is the lowest amongst the countries of South Asia and South Africa (Rekhi, 1997). However, dietitian recommended a daily allowance of 285 gm vegetables for an adult person for a balance diet (Ramphall and Gill, 1990). In general, per hectare yield of these crops are low. The cultivable area of Bangladesh is 0.46 million acres in summer and 0.53 million acres in winter season while total annual vegetable production of Bangladesh is 1.58 million metric tons in summer season and 2.24 million metric tons in winter season (BBS, 2016). As a result, chronic malnutrition is often seen in Bangladesh. Of the total production, less than 25% is produced during *Kharif* season and more than 75% is in the *Rabi* season (Anon, 1993). In Bangladesh context, reducing of cultivable land, high population, low fertility, flood, drought, scanty of irrigation water and low yield are unavoidable hazards. Therefore, the yield of the crops is to be

increased to feed the hungry people of the country. The successful crop production for ensuring food and eliminating poverty is essential. The bumper crop production and use of healthy seed is unquestionably the most important basic input. The farmers must be aware of the consequences of the crop losses with low quality and unhealthy seeds. Although Bangladesh is an agro-based country, only 18% certified seeds are produced by different seed organizations. The rest 82% seeds are produced by the farmers which are uncertified with unknown quality and out of the supervision of the Seed Certification Agency. In fact, this is actually an alarming situation in the country. The lack of high quality seeds and the prevalence of seed borne organisms are the main constraints in maintaining the crop production. (Fakir, 2000) estimated more than 400 seed borne diseases in 72 crops inflicting an estimated yield loss amounting to around Tk. 1000 million, i.e. 200 million US dollars annually. The per capita consumption of vegetables in Bangladesh is only 112g which is far below from the daily requirement of 400g/head (FAO, 2012). The lack of high quality healthy seeds and the prevalence of seed borne diseases are among the main constraints for Bangladesh in maintaining the sustainability of vegetable crop production and per capita consumption.

Among the major vegetables, cucurbits are commonly cultivated vegetables in Bangladesh and they play a prime role to supplement this shortage during the lean period (Rashid, 1993). Among the various factors responsible for low yield of these crops, disease and use of poor quality seeds play an important role. About 200 different seed-borne pathogens including more than 100 fungi have been reported to cause diseases in different vegetable crops in the world (Richardson, 1990). Considerable amount of works have been done on the seed health particularly, about the transmission of fungi through the seeds of a number of vegetable crops like okra, tomato, chilli, radish, cucumber, bitter gourd, sweet gourd, brinjal and cabbage in the country. But, very limited study has been carried out on seed-borne fungi or seed health of cucurbitaceous vegetables.

For crop production among the agricultural inputs, seed is the most important input. Seeds are also the important carrier of pathogens. Coincidentally, devastating and important diseases of crops are seed borne and mostly affected by several fungi. 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. The research work need to be conducted to evaluate seed health status of cucurbits loose seeds and to find out the different pathogens associated with seeds. Cucurbits constitute a potential and important group of crops in Bangladesh. They are important for their low production cost, short duration of production and high nutritive value. Seed-borne fungi create a great threat to the production of these crops in Bangladesh. Healthy or pathogen free seeds are considering as the vital factor for desired plant population and good harvest. Infection of seed by pathogenic organisms and presence of propagules of pathogen in a seed lot is important because of 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. 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 (Rashid and Fakir, 2000). For good crop, 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.

Considering the above facts, the present research is undertaken to find out the following objectives:

1. To assess seed health status of cucurbits seed collected from different local markets of Dhaka city;
2. To isolate and identify the seed borne micoflora from collected cucurbits seed; and
3. To evaluate the efficacy of selected seed treating chemicals against identified seed borne fungi.

CHAPTER II

REVIEW OF LITERATURE

2.1. Origin of Cucurbits

Cucurbits belong to the family Cucurbitaceae and consist of about 118 genera and 825 species, according to the last taxonomic treatment (Jeffrey, 1990).

Cucurbita or yellow flowered cucurbit is considered to be one of the most morphologicaly variable genera in the entire plant kingdom (Robinson *et al.*, 1997).

Archaeological records of the New World suggest that *Cucurbita* was one of the first plant to be domesticated (Nee, 1990).

The first species to be domesticated in the New World was *C. pepo*. Cultivated by the inhabitants of Guila Naquitz cave dated between 10,000 to 8,000 before present (BP), predating corn and beans by more than 4,000 years (Smith, 1997).

The origin and early spread of all *Cucurbita* species was in the Americas. *Cucurbita ficifolia* was the most widespread cultivated species with a native range in the mountains from Mexico to northern Chile and Argentina (Wilson *et al.,* 1992).

Cucurbits are divided into five sub-families: Fevilleae, Melothrieae, Cucurbitaceae, Sicyoideae, and Cyclanthereae. The most important cultivated genera are *Cucurbita* L., *Cucumis* L., *Citrullus* L., *Lagenaria* L., and *Luffa* L., found in the sub-family Cucurbitaceae, and *Sechium* L., found in the sub-family Sicyoideae (Whitaker and Davis, 1962).

The cultivated species of *Cucurbita* can be divided into mesophytic annuals (*C. maxima, C. argyrosperma, C. moschata,* and *C. pepo*) or mesophytic perennial (*C. ficifolia*) (Whitaker and Devis, 1962).

Among the cucurbits, watermelon is the most popular in the world. The United Nations' Food and Agriculture Organization (FAO) estimated an average annual area of cultivation of 2.5 million ha and an annual production of 46.6 million tons of watermelon fruits between 1996 and 1998. Next in total world production were cucumber, melon, squash and pumpkins. In terms of countries, China is the leading producer of major cucurbit crops followed by Turkey, Iran and Ukraine. In the Americas, Argentina is an important producer of squash and pumpkins and the United States is an important producer of cucumber, melon and watermelon (FAO, 1998).

The genus *Cucumis* includes 32 annual and perennial species divided in to two very distinct groups defined by geographic origin and chromosome number (African 2n = 24 and Asiatic group 2n = 14 chromosomes). The African group includes melon (*C. melo*) and the Asiatic group includes cucumber (*C. sativus*) and its probable ancestor *C. sativus* var. *hardwickii* (Royle) or simply *C. hardwickii* (Perl-treves and Galun, 1985).

Bottle gourd varieties are primarily identified based on fruit shape and recognized 15 fruit shapes as just the most common ones. Fruit shape and size in bottle gourd is the most variable among cucurbits (Heiser, 1979)

A total of six species have been recognized as belonging to the genus Lagenaria or white flowered gourds. One is the domesticated monoecious species L. siceraria while five of them are wild perennial, dioecious forms from Africa and Madagascar. Tropical Africa remains as the primary gene pool for this species (Singh, 1990).

At the present time, it is cultivated throughout the tropical and subtropical regions of the world for food and useful gourds (Whitaker and Davis, 1962).

2.2. Importance of cucurbits

Cucurbita is prized for their edible seed, shell and rind. Selection for large seed may have resulted in large fruit. Immature fruit were selected for non-bitter flesh and mature fruit for non-bitter and starchy flesh and non-lignified rinds (Paris, 1989).

Domestication was characterized by the selection for shape, less bitter flesh, larger and fewer seeds, and larger fruit. Selection for non-bitter fruit was a key step in squash domestication. Seed was probably the first part used as food, since generally bitter fruit had non-bitter seeds (Robinson and Decker-walters, 1997).

The domestication selection in other cucurbits was also for fruit characteristics. In cucumber, the spiny character and bitterness in fruit have decreased or disappeared (Mallick and Masui, 1986).

In watermelon, domestication selection was for fruit size and quality from wild progenitors with bitterness fruit (Singh, 1990).

Cucurbits have one of the most variable and complex sex expression systems, which is regulated by both genetic and environmental factors. Sex expression has a direct effect on breeding and seed production. Most cucurbit species are monoecious and dioecious evolved more recently in the family. Sex expression is either controlled by a single gene (*Cucurbita pepo*) or two or more genes (*Cucumis melo* and *C. sativus*) with three or more alleles for each gene (*Luffa* ssp.) (Robinson and Decker-walters, 1997).

Cucurbit seeds can be classified as oil seeds because decorticated seeds contain by wt. 50% oil and 35% protein. The oil is unsaturated and generally edible; however, the contents of conjugated trienoic fatty acids in the oil of a few species preclude edibility but increase industrial values as drying oils. Proteins of cucurbit seeds appear edible and supplementation with certain amino acids increases the

nutritional value of the protein. However, the possibility that a meal or protein from a given species might be inedible, e.g., through presence of a toxic compound in the seed, must be determined by appropriate feeding tests with the seed products. Thus cucurbits are potentially valuable oilseed crops. Of special interest are certain xerophilous species that grow particularly well in desert regions. Propagation of these species on currently unproductive wastelands could provide an additional source of oil and protein for industrial and edible purposes (Jacks *et al*., 1972).

Crystalline globulins from squash and pumpkin seed yielded two distinct electrophoretic components at pH 4.8 but those from cucumber and watermelon seed yielded only one. Each yielded a single component at pH 4.6 but at pH values below 3.5 there was a tendency for a second component to appear. At pH 2.3 all showed two components although separation was not complete nor was it uniform among species. There was a tendency for two components to appear at pH values above the isoelectric range. The results support the conclusion that the globulins from closely related species differ less than those from more distantly related species. They suggest that the crystalline globulins are not each a single homogeneous protein (Anderson *et al*., 1960).

The entire range of fatty acid composition is found for seeds of self-pollinated fruit from 22 individual plants representing 17 named species of *Cucurbita*. It appears that some varieties merit interest as a source of drying oil and edible oils. Xerophytes that are genetically related have similar types of unsaturation and molecular-wt. distribution (Bemis *et al*., 1967).

The seeds of cucurbits contain lots of oils and proteins which making different human foods such as pasta and also animal food (Nerson, 2007).

There are medical usages of cucurbits i.e. medicinal applications and human health to treat many diseases such as eating disorder or constipation and also to

purify blood and cure nerve diseases. Furthermore, the vegetables can be used in musical instruments, utensils, fuels, sponges and boxes (Rahman *et al*., 2008).

2.3. Diseases of cucurbits

Cucurbit plants are commonly exposed to attack by many serious soil-borne and seed-borne pathogens, i.e. *Pythium debaryanum, P. ultimum, P. dissotocum, P. oligandrum, P. violae, Rhizoctonia solani, Fusarium solani, F. semitectum, Aphanomyces euteiches, Sclerotinia sclerotiorum* and many species of *Verticillium* and *Cladosporium*. Most of them cause damping-off and root rot diseases, leading to great economic losses in crop yield and quality. Seed is the most important input for crop production. Pathogen free healthy seeds are essential for desired plant populations and a good harvest. Of the 16% annual crop losses due to plant diseases, at least 10% loss occurs due to seed-borne diseases (Fakir, 1983).

Coincidentally important or devastating crop diseases are seed-borne and caused by fungi. In addition, it has demonstrated that seed borne fungi are responsible for poor quality seeds in many crops (Neergaard, 1979).

Fusarium sp. can produce a number of toxic compounds including fusaric acid, fusarins, moniliformin and fumonisins (Marasas *et al*., 1981)

The seed borne fungal diseases are transmitted by seeds, where the fungi can survive as conidia or mycelia on the seed coat or surface (Blancart *et al*., 1991; Champion, 1997; Gargouri *et al*., 2000; Martyn and Bruton, 1989).

Penicillium spp., *Rhizopus* spp., *Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp. and *Fusarium* spp. were the main seed-borne fungi on the surface of ten major cucumber varieties from Beijing and Jilin provinces in China (Bi *et al*., 2007).

2.4. Seed health test by dry seed observation

Seed health refers to presence or absence of disease causing organisms like fungi, bacteria and viruses, animal pests such as eelworms and insects as well physiological conditions such as trace element deficiency (ISTA, 2003).

Seed samples are subjected to dry seed examination and based on the visual examination of the seeds; they are classified into infected or healthy. This method is very useful in detecting the con-contaminant contamination i.e., those seeds that are transformed as smut sori, bunt, gall or sclerotia. The working sample, usually recommended for dry seed examination, are carefully observed under bright light on a purity board and represented as number of contaminants per gram or per 1000 units (Prakash, 2001).

2.5. Seed health test by blotter method

One hundred seeds were picked up at random from each sample, surface sterilized by dipping in 3% sodium hypochlorite for three minutes, and then rinsed thoroughly in distilled water. The seeds were left to dry in the laminar flow hood, then mounted on three layers of sterilized wet filter papers in glass Petri dishes (9 cm in diameter) to provide enough moisture during the period of the test. Five seeds were placed in each dish contained blotter and then incubated for 7 days at 18°C and 25°C under fluorescent light (light / darkness rotations of 12/12 hours), as described by (Neergaard, 1979).

Generally the total number of isolates recovered from the plate agar isolation technique was higher than that of the blotter technique. This was in difference with the previous study that found the blotter method was the most suitable technique for detection of fungi in bitter gourd, which yielded maximum number of the fungal genera (Sultana and Ghaffar, 2007).

Blotter method was found to be good for the isolation of *Chaetomium* species. Most of the *Chaetomium* species are cellulose decomposing fungi causing soft rot, decay and decomposition of wide variety of hosts besides being food for mites (Domsch *et al*., 1980).

It has been revealed that blotter method was useful for detection of most infectious fungi of cucurbits (Begum and Momin 2000; Avinash and Ravishankar Rai, 2013).

Significant Differences of fungal isolations among the tested cucurbitaceous crops and also among the chosen locations were observed using both blotter and plate agar isolation techniques. This was consistent with earlier observations that exhibited fungi on the surface of different cucumber seeds from China, and these were significantly different from each other using the petridish testing method (Bi *et al*., 2007).

2.6. Isolation and Identification of pathogens

In order to purify the isolated fungi, single spore technique was used to purify the spore forming fungi and hyphal tip technique was used for non-spore forming fungi (Hansen, 1926).

Pure cultures of the obtained isolates were identified in laboratory on the basis of cultural and microscopical characteristics according to (Talbot, 1971; Subramanian, 1971; Alexopoulos and Mims, 1979; Domsch *et al*., 1980; Barnett and Hunter, 1998).

The aim of this investigation was to isolate and identify seed-borne fungi associated with cucurbit seeds. Isolation was made from seed coats and inner seed parts, applying both blotter and agar-plate techniques described in detail in the section of "Materials and Methods". The obtained fungal isolates were, then purified and identified. Isolation processes yielded a number of fungal isolates

related to the genera *Fusarium, Macrophomina, Alternaria, Aspergillus, Penicillium, Sclerotinia, Cladosporium,* and *Rhizopus*. Recovered isolates were preliminary identified in the Laboratory of Plant Pathology, Dept. of Microbiology, Faculty of Science, Misurata University. Preliminary identification was carried out according to cultural and morphological characteristics (Gilman, 1957; Ellis, 1971; Booth, 1971; Alexopoulos and Mims, 1979).

Isolation and identification studies revealed many fungal isolates namely, *F. oxysporum, F. semitectum, F. moniliforme, F. solani, M. phaseolina, R. solani, A. alternata, S. sclerotiorum, Stemphylium* sp.*, Nigrospora* sp.*, P. ultimum, Aspergillus* sp.*, A. flavus, A. niger, A. ochracus* and *Penicillium* sp. (Abushaala, 2008).

A total of 26 genera and 39 species of seed borne fungi were isolated from 19 different seed samples of cucurbitaceae including gourds, pumpkin and cucumber using the standard blotter and deep-freeze methods and identified by morphological characteristics. Fungal species including *Fusarium* spp., *Alternaria* spp., *Phoma* spp. and *Cladosporium* spp. were the most frequent on all the tested cucurbit seeds (Avinash and Ravishankar Rai, 2013).

Several pathogenic fungal isolates were isolated from cucumber seed samples collected from commercial markets in Egypt. *Fusarium oxysporum* and *Fusarium solani* were the common fungi isolated from cucumber seeds followed by *Alternaria* sp, *Rhizoctonia solani*, *Helminthosporium* sp. and *Penicillium* spp. (Farrag *et al.,* 2013).

2.7. Management

Seed borne diseases are controlled by seed treatment practices. Seed treatment is the oldest practice in plant protection. Its origins can be traced to the 18th century with use of brine for the control of cereal smuts. The modern era of seed

treatments began with the introduction of organo-mercury fungicides in 1912 which were widely used for several decades (McGee, 1995).

Plants sprayed with Carbendazim (1ml/litre of water) immediately after the appearance of the disease were found effective against powdery mildew of bitter gourd. Two to three sprays were taken at an interval of 15 days (Mane, 2015).

The bad effect of seed-borne pathogens of cucurbits not only include reduction of yield but also concerns with the transmission of the pathogens from a season to another and from one field in a country to other fields in other countries. Analysis of seed infection level is a valid investigation tool to foresee the disease development transmitted by seeds (Taylor *et al*., 2001).

Eight fungicides were used viz. Mycosulf 80 WP (0.2%), Censor MZ 72 (0.25%), Insuf 80 WP (0.3%), Sulphochem 80 WP (0.25%), Carbozim (0.1%), Haydazim (0.2%) %), Carbendazim (0.1%) Thiovit (0.2%) effectively controlled powdery mildew in sweet gourd (Yasmin *et al*. 2008)

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental sites

The investigation was conducted in the Seed Pathology Laboratory and Plant Disease Diagnostic Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka.

3.2. Experimental period

The investigation was conducted during the period of July 2016 to June 2017.

3.3. Collection of different cucurbits seeds

Altogether cucurbits seeds of five different species were collected from five different locations of Dhaka district.

3.4. Locations of seed sources

a. Siddik Bazar (L_1)
b. Mohammadpur Bazar (L_2)
c. Kochukhet bazaar (L_3)
d. Savar bazaar (L_4)
e. BADC (Gabtoli) (L_5)

3.5. Cucurbit species

a. Sweet gourd (*Cucurbita maxima*) (S_1)
b. Bottle gourd (*Lagenaria siceraria*) (S_2)
c. Cucumber (*Cucumis sativus*) (S_3)
d. Ridge gourd (*Luffa acutangula*) (S_4)
e. Snake gourd (*Trichosanthes cucumerina*) (S_5)

3.6. Laboratory experiment

Two seed health testing methods viz. Dry seed inspection method and Blotter method were used for assessment of seed health status.

3.6.1. Inspection of dry seeds

The sample seeds consisting 400 seeds of cucurbits collected from different location were investigated by Dry inspection method (Plate 1). Apparently pure seeds, infected seeds and inert matter were sorted out by observing the physical appearance, presence of fruiting structures and discoloration of seeds.

Sweet gourd seeds

Bottle gourd seeds

Cucumber seeds

Ridge gourd seeds

Snake gourd seeds

Plate 1: Seed sample (400 seeds) of different cucurbits for the investigation of seed health status

3.6.2. Blotter method

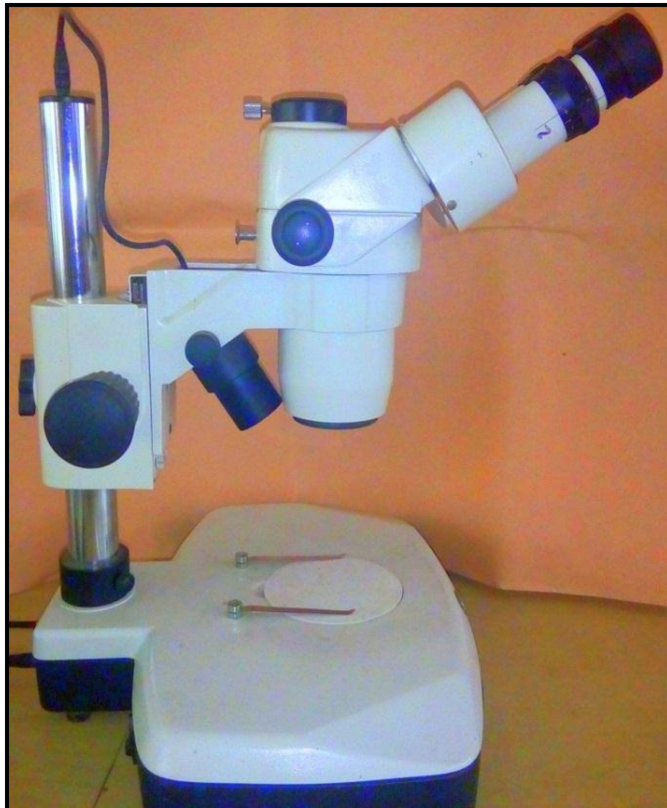
Seed samples consisting of 400 seeds were subjected to seed health analysis by blotter method following International Rules for Seed Health Testing (ISTA, 1996). In this method 9 cm diameter plastic petridish and Whatman no.1 filter paper was used. Four hundred seeds from each sample was taken randomly and placed on the moist filter paper in the petridishes. Ten seeds were placed in each petridish and altogether 40 petridishes were needed for each seed samples (Plate 2). The petridishes were incubated at 22 ± 2 ^{o}C for seven days in the laboratory under the alternative cycle of 12 hours NUV light and under 12 hours darkness for 7 days.

3.6.2.1. Inspection of incubated seed samples

After 7 days incubation the seeds were observed under stereomicroscope and the pathogens were identified following the key of Mathur and Kongsdal (2003). Appropriate keys (Booth, 1971; Misra *et al*., 1994 and Malone and Muskette, 1964) were consulted for identification of the microflora. The results were compiled as incidence of individual organism and germination percentage of the seeds were also observed and recorded. In order to record the incidence of seed borne fungi individual incubated seed was observed under stereomicroscope (Photograph 1) at 16x and 25x magnification. Most of the associated fungi were detected by observing their growth characters on the incubated seeds on blotter paper. Temporary slides were prepared from the fungal colony and observed under compound microscope at 100x and 400x for proper identification of fungi following the keys suggested by (Malone and Muskette, 1964; Booth, 1971; Ellis, 1971; Chidambaram and Mathur, 1975). The fungi from the incubated seeds were also transferred to PDA for pure culture. The pure cultures were kept at 25±2 ^{o}C for future use.

$$\text{\% Incidence of Pathogen} = \frac{\text{Number of infected seeds associated with the seeds}}{\text{Total number of seeds observed}} \times 100$$

$$\text{\% Germination} = \frac{\text{Number of germinated seed}}{\text{Total number of seeds observed}} \times 100$$

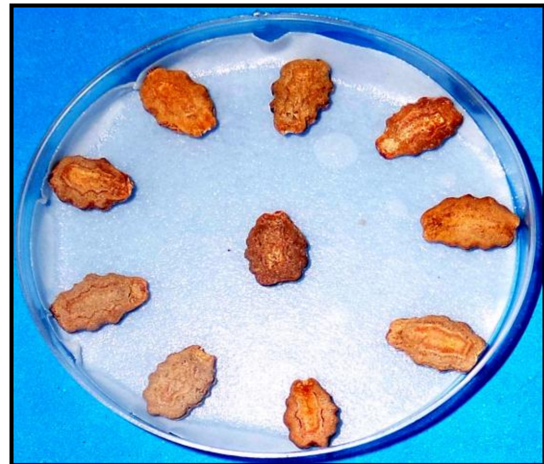


Photograph 1: Steriomicroscope used in investigation

Sweet gourd

Bottle gourd

Cucumber



Ridge gourd

Snake gourd

Plate 2: Seed health test of five different cucurbits seeds by blotter paper

3.6.3. Preparation of Potato Dextrose Agar Media (PDA media)

PDA medium (Appendix-III) was prepared as described by Islam (2009). For this, reason 200 g of peeled and sliced potato was boiled in 1000 ml of water in an aluminium bowl for about 30 minutes. Then the boiled extract of the potato was filtered through cheese cloth. The other two ingredients viz. 17 g agar and 20 g dextrose were added in the extract and the volume was made up to 1000 ml mark. Then the prepared standard 1000 ml PDA medium was poured in 1000 ml conical flask and sterilized (121°C temperature, 15 psi pressure for 15 min) in an autoclave machine. After autoclaving the media was kept few minutes for cooling and then 25-30 drops of lactic acid was added to the media.

Photograph 2: PDA medium solution

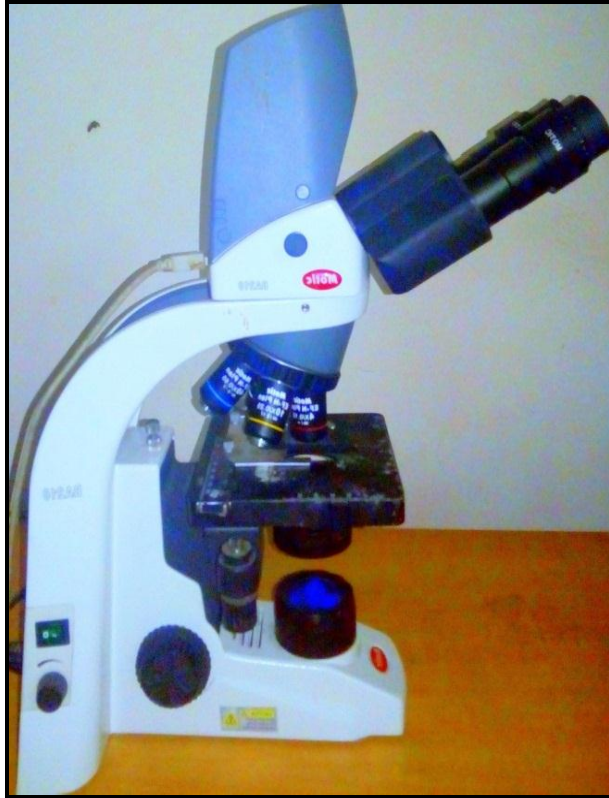
Photograph 3: PDA medium in sterile petridishes

3.6.4. Isolation, purification and preservation of seed borne fungal pathogens

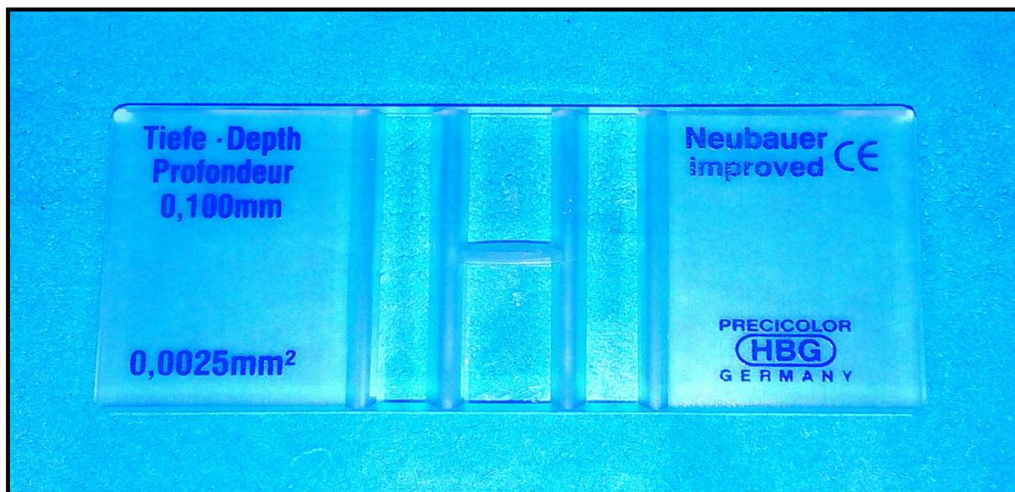
Isolation of the seed borne pathogens were carried out on PDA medium. PDA plates were inoculated by taking a bit of mycelia from the incubated seed surface and transferred on PDA plates. The fungi were isolated, purified by using the hyphal tip culture technique. Purification was done by reculturing of the isolated organisms. Identification was done following the keys of (Barnett and Hunter 1992). The pure cultures were also maintained on PDA slants kept at 5°C in refrigerator for further studies.

3.6.5. Cultural and morphological characterization of isolated pathogens

Cultures of all the isolates were studied for morphological variations. In terms of conidia color, shape, size, septation, time of sporulation and number of conidia production were observed on PDA medium. Five recordings per replication (3 replications for each organism) were made for the purpose. The conidia produced per unit surface area were estimated using haemacytometer, digital microscope (Model: Motic, BA-210) and motic software (Photograph 4 and 5).



Photograph 4: Digital microscope (Model: Motic, BA-210)



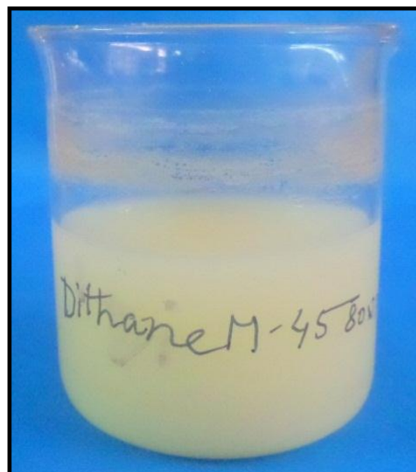
Photograph 5: Haemacytometer used in counting spore

3.7. Management of Storage Pathogens

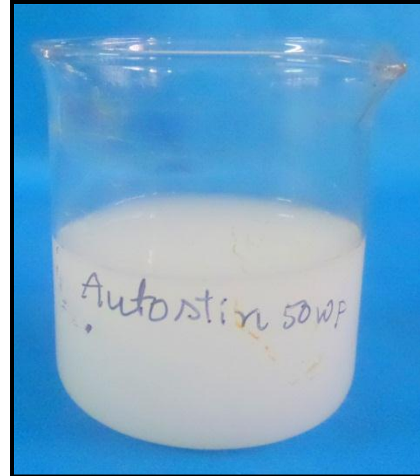
Four different chemicals viz, Dithane M-45, Autostin 50 WDG, Tilt 250 EC and Salicylic acid were tested by following poisoned food technique *in vitro* condition to evaluate their efficacy on colony growth and mycelia formation of five different fungus viz, *Aspergillus flavus, Aspergillus niger, Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. The details of the fungicides are presented in Table 1.

Table 1: Chemicals used for evaluation against storage pathogens of cucurbits

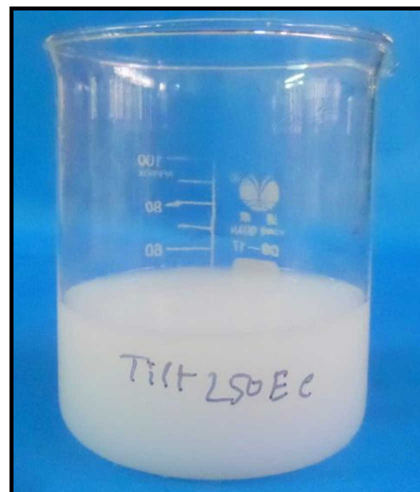
Trade Name	Chemical Name	Active Ingredient	Conc. Used (1000 ppm)
Dithane M-45	Manganous ethylene bisdithiocarbamate-ion	80% Mancozeb	1.25 g/L
Autostin 50 WDG	Mythyl-Benzimidazole Carbamate	50% Carbendazim	2.0 g/L
Tilt 250 EC	1-[2-(2,4-Dichlorophenyl) 4-propyle-1,3-dioxalane-2	25% Propiconazole	4.0 ml/L
Salicylic Acid (Powder)	2-Hydroxybenzoic Acid	Benzoic Acid	5.6 g/L



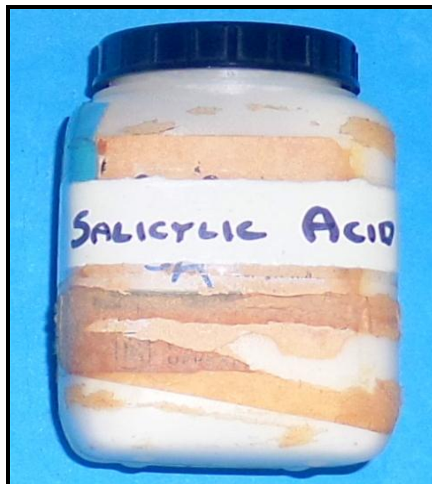
Dithane M-45

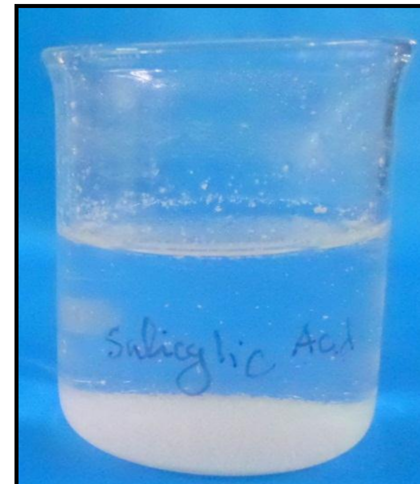


Autostin 50 WDG



Tilt 250 EC





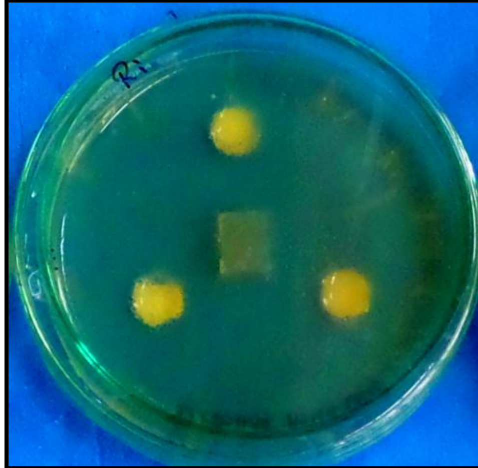
Salicylic Acid

Control

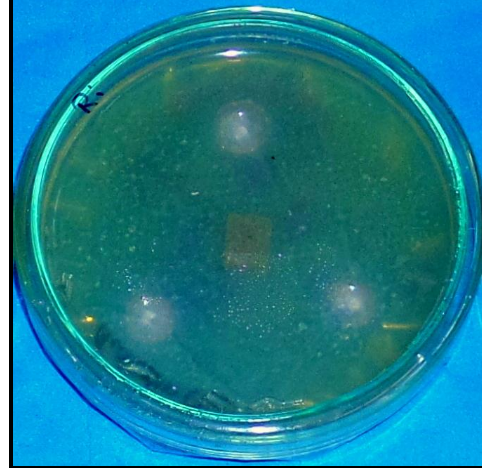
Plate 3: Chemicals used for evaluation against storage pathogens

3.7.1. Poisoned food technique (Cup method)

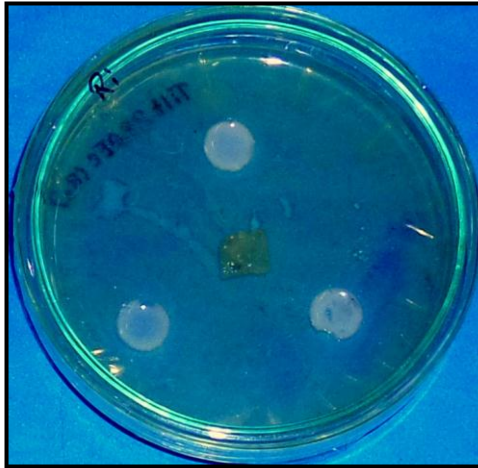
From a PDA plate three 5mm discs of the medium were scooped from three places by using a sterilized disc cutter maintaining the equal distance from the center (Plate 4). 1-2 drops of chemicals were put into each hole and kept in refrigeration 12 hrs. for dispersion of the chemicals. Then one 5 mm block of 7 days old fungal culture (pathogen) cut by sterilized block cutter and was placed at the center of the petri plate and were kept in room temperature 25±2 ^{o}C for 7 days. The linear growth (cm) of mycelium of five different fungus viz, *Aspergillus flavus, Aspergillus niger, Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. were recorded at 24 hrs. interval till fifth days (Nene and Thapliyal, 1979).



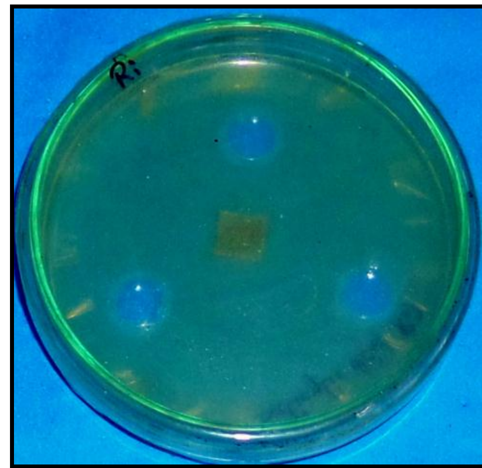
Dithane M-45



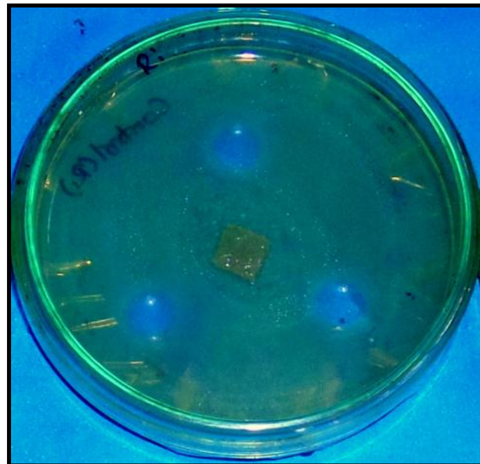
Autostin 50WDG



Tilt 250 EC



Salicylic Acid

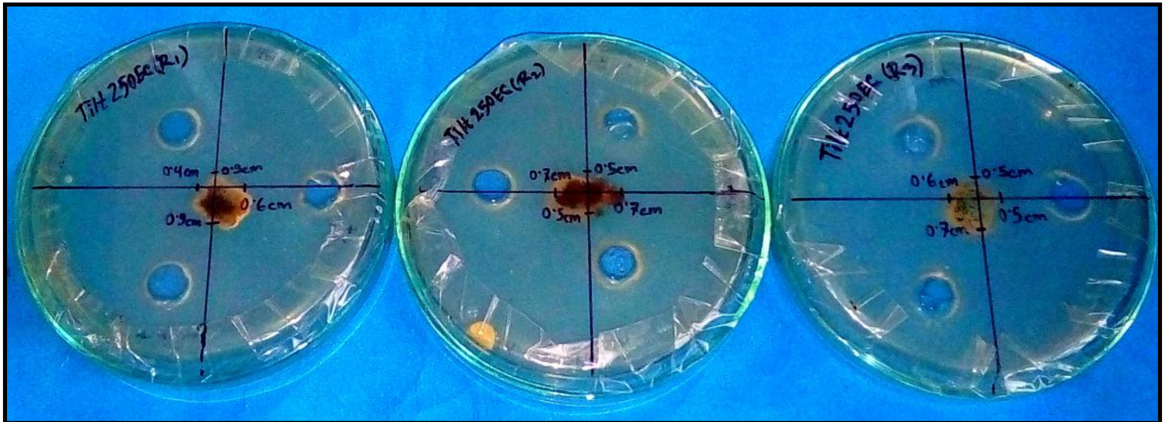


Control

Plate 4: Cup method used for *in vitro* management of different pathogens

3.7.2. Measurement of radial growth (cm) and determination of percent inhibition

After 48 hours, radial mycelial growth (cm) of all examined fungi (*Aspergillus flavus, Aspergillus niger, Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.) in petridishes were recorded. The radial growth (cm) of mycelium of each plate was measured by taking average of the diameters (Length and Breadth).



Photograph 6: Measurement of radial mycelial growth (cm)

3.8. Design of Experiment

The investigation was conducted following Completely Randomized Design (CRD) with three replications. Data collected during experimental period were analyzed and tabulated following Statistical package STATISTIX-10. Treatment means were compared with Least Significant Difference (LSD) Test.

CHAPTER IV

RESULTS

4.1. Seed health test of cucurbits

4.1.1. Seed health test of cucurbits by dry inspection method

Among the species and seed sources of cucurbits, variations were observed in dry inspection method and place in respect of seed wt. of 400 seeds, apparently pure seed wt., infected seed wt. and inert matter wt. were observed (Table- 2, 3).

In case of place, significant variation observed among the places. The highest seed wt. of 400 seeds was recorded in the seeds collected from Siddik Bazar (68.13 g) followed by Kochukhet Bazar (65.45 g) and the lowest seed wt. of 400 seeds was recorded in the seeds collected from Savar Bazar (64.58 g). The highest apparently pure seed wt. was recorded in the seeds collected from Mohammadpur Bazar (49.97 g) followed by Savar Bazar (49.55 g) and the lowest apparently pure seed wt. was recorded in the seeds collected from Kochukhet Bazar (44.32 g). The highest infected seed wt. was recorded in the seeds collected from Siddik Bazar (22.37 g) followed by Kochukhet Bazar (20.27 g) and the lowest infected seed wt. was recorded in the seeds collected from Savar Bazar (14.46 g). The highest inert matter wt. was recorded in the seeds collected from Savar Bazar (1.32 g) followed by Kochukhet Bazar (0.85 g) and the lowest inert matter wt. was recorded in the seeds collected from Siddik Bazar (0.22 g).

Table 2: Seed health test of cucurbits seeds by dry inspection method collected from different local markets of Dhaka

Local markets	Seed weight of 400 seeds (g)	Apparently pure seed weight (g)	Infected seed weight (g)	Inert matter weight (g)
Siddik Bazar	68.13 a	45.53 c	22.37 a	0.22 b
Mohammadpur Bazar	65.38 b	49.97 a	15.10 d	0.33 ab
Kochukhet Bazar	65.45 b	44.32 d	20.27 b	0.85 ab
Savar Bazar	64.58 b	49.55 a	14.46 d	1.32 a
BADC	65.38 b	47.66 b	16.63 c	0.67 ab
CV (%)	2.56	3.46	5.39	2.25
LSD	1.23	1.20	0.70	1.01
Level of Significance	**	**	**	**

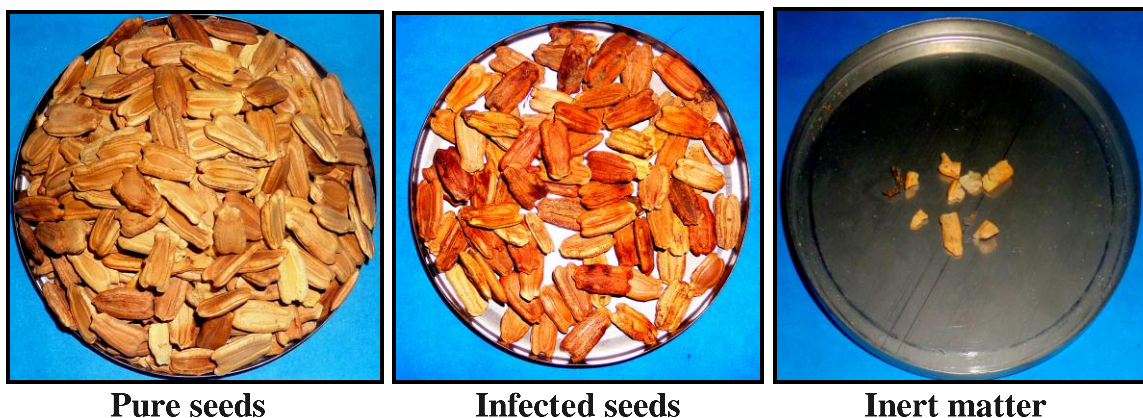
In case of cucurbits, significant variation observed among the seeds. The highest seed wt. of 400 seeds was recorded in the seeds of Ridge gourd (110.27 g) followed by Bottle gourd (87.97 g) and the lowest seed wt. of 400 seeds was recorded in the seeds of Cucumber (12.27 g). The highest apparently pure seed wt. was recorded in the seeds of Ridge gourd (81.94 g) followed by Bottle gourd (63.81 g) and the lowest apparently pure seed wt. was recorded in the seeds of Cucumber (8.28 g). The highest infected seed wt. was recorded in the seeds of Ridge gourd (27.96 g) followed by Bottle gourd (23.33 g) and the lowest infected seed wt. was recorded in the seeds of Cucumber (3.71 g). The highest inert matter wt. was recorded in the seeds of Sweet gourd (1.34 g) followed by Bottle gourd (0.85 g) and the lowest inert matter wt. were recorded in the seeds of Cucumber (0.27 g).

Table 3: Seed health test of different species of cucurbit seeds by dry inspection method

Species	Seed weight of 400 seeds (g)	Apparently pure seed weight (g)	Infected seed weight (g)	Inert matter weight (g)
Sweet gourd	76.94 c	56.68 c	19.67 c	1.34 a
Bottle gourd	87.97 b	63.81 b	23.33 b	0.85 ab
Cucumber	12.27 e	8.28 e	3.71 e	0.27 b
Ridge gourd	110.27 a	81.94 a	27.96 a	0.36 ab
Snake gourd	41.46 d	26.32 d	14.16 d	0.58 ab
CV (%)	2.56	3.46	5.39	2.25
LSD	1.23	1.20	0.70	1.01
Level of Significance	**	**	**	**

Pure seeds **Infected seeds** **Inert matter**

Plate 5: Sweet gourd seeds (pure seeds, infected seeds and inert matter)



Pure seeds **Infected seeds** **Inert matter**

Plate 6: Bottle gourd seeds (pure seeds, infected seeds and inert matter)

Pure seeds **Infected seeds** **Inert matter**

Plate 7: Cucumber seeds (pure seeds, infected seeds and inert matter)

Pure seeds **Infected seeds** **Inert matter**

Plate 8: Ridge gourd seed (pure seed, infected seed and inert matter)

Pure seeds **Infected seeds** **Inert matter**

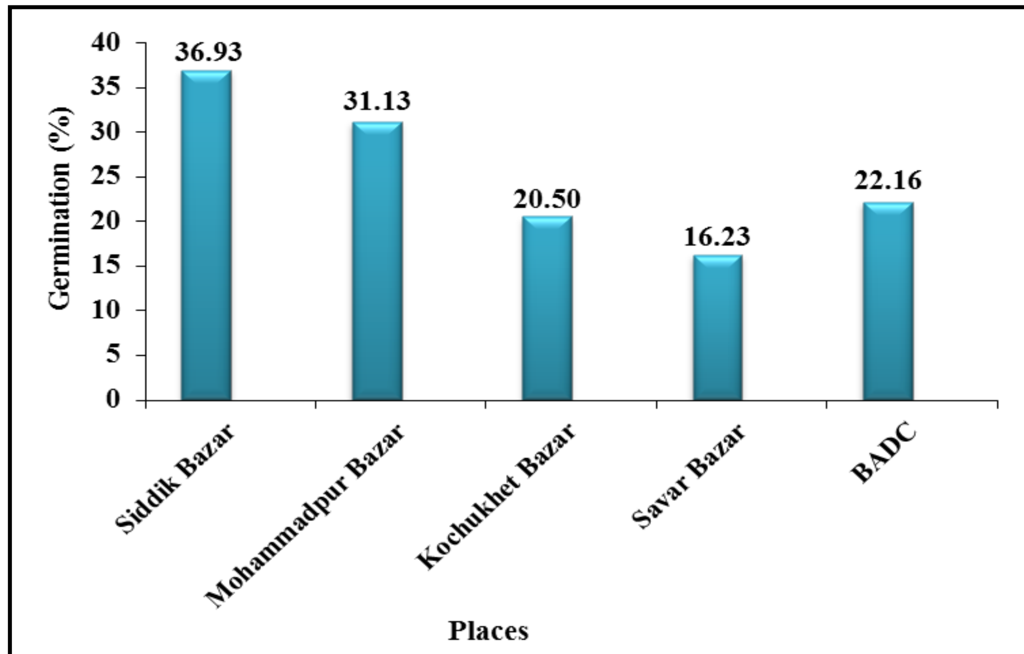
Plate 9: Snake gourd seeds (pure seeds, infected seeds and inert matter)

4.1.2. Seed health test of cucurbits by blotter method

Germination percentage and prevalence of seed borne pathogens were recorded from different cucurbits seeds by blotter paper methods. Significant variations among the places, seeds and place seed interaction in respect of percent seed germination and prevalence of seed borne pathogens were observed. Among 7 pathogens 5 fungal pathogens were identified (*Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.) and 2 fungal pathogens were unidentified (Unknown-1 and Unknown-2) and bacterial ooze were observed (Table 4, 5, 6).

4.1.2.1. Germination and pathogenic incidence of cucurbits seeds collected from different local markets

In case of places, the highest seed germination percentage was found in seeds collected from Siddik Bazar (36.93%) followed by Mohammadpur Bazar (31.13%). The lowest germination (16.23%) was recorded in seeds collected from Savar Bazar (Graph.1).



Graph 1: Comparative study of germination (%) in blotter method for different places

The incidence of *Aspergillus flavus* ranged from 16.63% to 32.03% where the highest incidence of *Aspergillus flavus* was recorded in seeds collected from Siddik Bazar (32.03%) followed by Kochukhet Bazar (22.06%). The lowest incidence was recorded in seeds collected from Savar Bazar (16.63%).

The incidence of *Aspergillus niger* ranged from 1.47% to 13.46% where the highest incidence of *Aspergillus niger* was recorded in seeds collected from Kochukhet Bazar (13.46%) followed by Mohammadpur Bazar (13.30%). The lowest incidence was recorded in seeds collected from Savar Bazar (1.47%).

The incidence of *Rhizopus* sp. ranged from 1.47% to 13.46% where the highest incidence of *Rhizopus* sp. was recorded in seeds collected from BADC (43.50%) followed by Kochukhet Bazar (39.06%). The lowest incidence was recorded in seeds collected from Mohammadpur Bazar (5.73%).

The incidence of *Fusarium* sp. ranged from 2.83% to 9.56% where the highest incidence of *Fusarium* sp. was recorded in seeds collected from Siddik Bazar (9.56%) followed by Savar Bazar (4.60%). The lowest incidence was recorded in seeds collected from BADC (2.83%).

The incidence of *Chaetomium* sp. ranged from 0.50% to 9.67% where the highest incidence of *Chaetomium* sp. was recorded in seeds collected from Mohammadpur Bazar (9.67%) followed by Kochukhet Bazar (8.37%). The lowest incidence was recorded in seeds collected from BADC (0.50%).

The incidence of Bacterial ooze ranged from 0.00% to 1.10% where the highest incidence of Bacterial ooze was recorded in seeds collected from Siddik Bazar (1.10%) followed by Mohammadpur Bazar (0.67%). No incidence was recorded in seeds collected from Savar Bazar (0.00%).

The incidence of unknown-1 pathogen ranged from 9.13% to 59.37% where the highest incidence of unknown-1 pathogen was recorded in seeds collected from

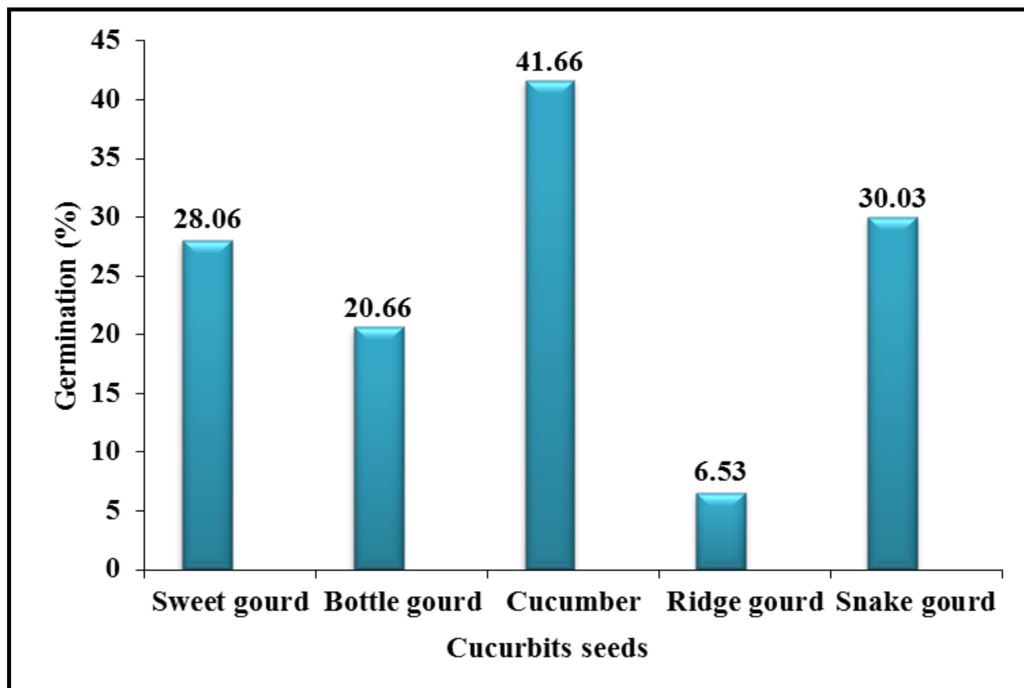
Mohammadpur Bazar (59.37%) followed by BADC (48.60%). The lowest incidence was recorded in seeds collected from Siddik Bazar (9.13%).

The incidence of unknown-2 pathogen ranged from 0.00% to 0.47% where the highest incidence of unknown-2 pathogen was recorded in seeds collected from Mohammadpur Bazar (0.47%) followed by Siddik Bazar (0.27%). No incidences were recorded in seeds collected from Kochukhet Bazar, Savar Bazar and BADC (0.00%).

These results have been presented in table 4.

4.1.2.2. Germination and pathogenic incidence of different species of cucurbits seeds

In case of cucurbits seeds, the highest seed germination percentage was found in Cucumber seeds (41.66%) followed by Snake gourd seeds (30.03%). The lowest germination (6.53%) was recorded in Ridge gourd seeds (Graph 2).



Graph 2: Comparative study of germination (%) in blotter method for different cucurbits seeds

The incidence of *Aspergillus flavus* ranged from 42.10% to 7.40% where the highest incidence of *Aspergillus flavus* was recorded in Ridge gourd seeds (42.10%) followed by Sweet gourd seeds (26.96%). The lowest incidence was recorded in Cucumber seeds (7.40%).

The incidence of *Aspergillus niger* ranged from 0.13% to 19.66% where the highest incidence of *Aspergillus niger* was recorded in Sweet gourd seeds (19.66%) followed by Bottle gourd seeds (14.47%). The lowest incidence was recorded in Snake gourd seeds (0.13%).

The incidence of *Rhizopus* sp. ranged from 34.76% to 15.13% where the highest incidence of *Rhizopus* sp. was recorded in Sweet gourd seeds (34.76%) followed by Ridge gourd seeds (29.87%). The lowest incidence was recorded in Cucumber seeds (15.13%).

The incidence of *Fusarium* sp. ranged from 0.43% to 10.43% where the highest incidence of *Fusarium* sp. was recorded in Snake gourd seeds (10.43%) followed by Sweet gourd seeds (5.86%). The lowest incidence was recorded in Bottle gourd seeds (0.43%).

The incidence of *Chaetomium* sp. ranged from 10.67% to 2.70% where the highest incidence of *Chaetomium* sp. was recorded in Cucumber seeds (10.67%) followed by Snake gourd seeds (7.30%). The lowest incidence was recorded in Bottle gourd seeds (2.70%).

The incidence of Bacterial ooze ranged from 0.00% to 0.90% where the highest incidence of Bacterial ooze was recorded in Snake gourd seeds (0.90%) followed by Ridge gourd seeds (0.80%). No incidences were recorded in Sweet gourd seeds and Bottle gourd seeds (0.00%).

The incidence of unknown-1 pathogen ranged from 28.40% to 47.77% where the highest incidence of unknown-1 pathogen was recorded in Ridge gourd seeds

(47.77%) followed by Bottle gourd seeds (44.30%). The lowest incidence was recorded in Sweet gourd seeds (28.40%).

The incidence of unknown-2 pathogen ranged from 0.00% to 0.37% where the highest incidence of unknown-2 pathogen was recorded in Bottle gourd seeds (0.37%) followed by Sweet gourd seeds (0.23%). No incidences were recorded in Cucumber seeds and Snake gourd seeds (0.00%).

These results have been presented in table 5.

Table 4: Estimation of percentage of pathogenic incidence of cucurbits seeds by blotter seed health testing method collected from different local markets of Dhaka

Local markets	*Aspergillus flavus* (%)	*Aspergillus niger* (%)	*Rhizopus* sp. (%)	*Fusarium* sp. (%)	*Chaetomium* sp. (%)	Bacterial ooze (%)	Unknown 1 (%)	Unknown 2 (%)
Siddik Bazar	32.03 a	2.60 c	8.36 d	9.56 a	7.23 b	1.10 a	9.13 e	0.27 ab
Mohammadpur Bazar	20.40 b	13.30 a	5.73 e	3.46 bc	9.67 a	0.67 ab	59.37 a	0.47 a
Kochukhet Bazar	22.06 b	13.46 a	39.06 b	3.83 bc	8.37 ab	0.03 c	38.63 d	0.00 b
Savar Bazar	16.63 c	1.47 c	22.47 c	4.60 b	6.93 b	0.00 c	43.60 c	0.00 b
BADC	20.40 b	7.43 b	43.50 a	2.83 c	0.50 c	0.30 bc	48.60 b	0.00 b
CV (%)	14.80	26.66	12.51	14.93	34.73	15.8	10.34	9.94
LSD	2.44	1.50	2.19	1.49	1.67	1.04	3.02	0.31
Level of Significance	**	**	**	**	**	**	**	**

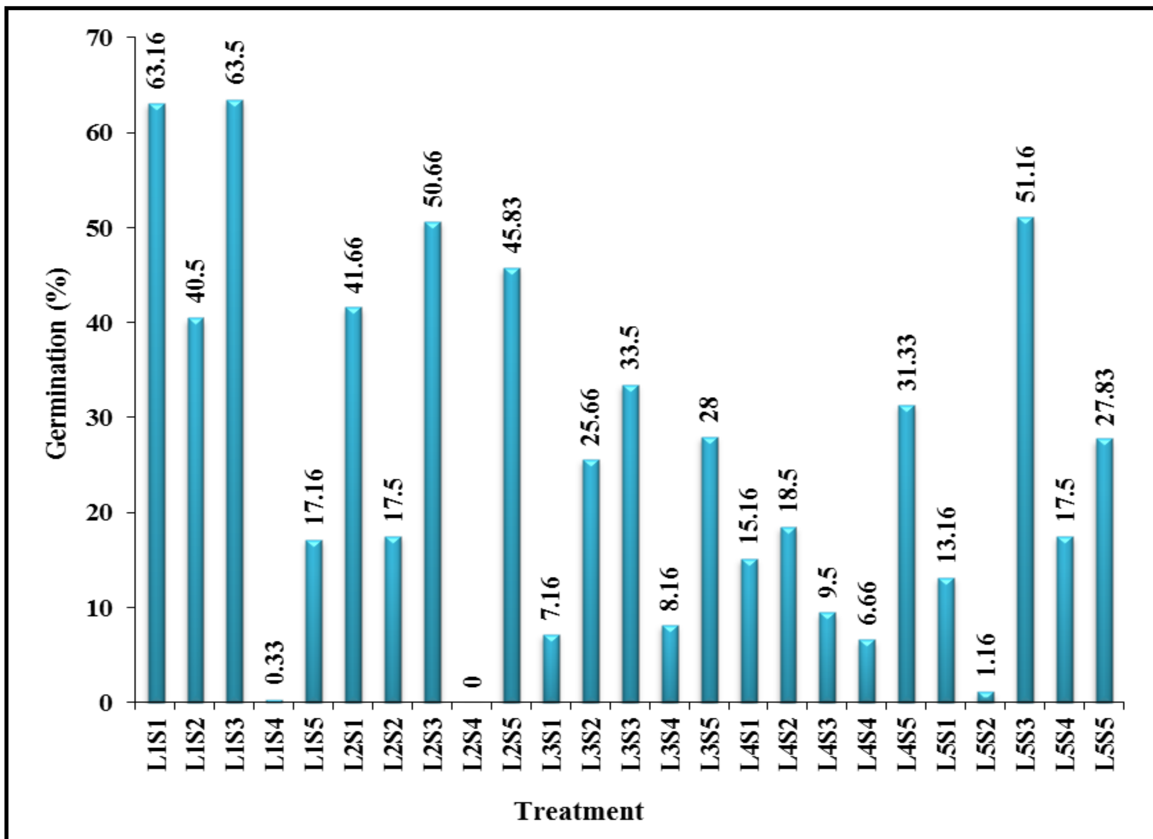
Table 5: Estimation of percentage of pathogenic incidence of different species of cucurbits seeds by blotter seed health testing method

Species	*Aspergillus flavus* (%)	*Aspergillus niger* (%)	*Rhizopus* sp. (%)	*Fusarium* sp. (%)	*Chaetomium* sp. (%)	Bacterial ooze (%)	Unknown 1 (%)	Unknown 2 (%)
Sweet gourd	26.96 b	19.66 a	34.76 a	5.86 b	4.83 c	0.00 c	28.40 d	0.23 ab
Bottle gourd	26.93 b	14.47 b	19.93 c	0.43 d	2.70 d	0.00 c	44.30 b	0.37 a
Cucumber	7.40 c	0.70 d	15.13 d	2.47 c	10.67 a	0.40 bc	37.93 c	0.00 b
Ridge gourd	42.10 a	3.30 c	29.87 b	5.10 b	7.20 b	0.80 ab	47.77 a	0.13 ab
Snake gourd	8.13 c	0.13 d	19.43 c	10.43 a	7.30 b	0.90 a	40.93 c	0.00 b
CV (%)	14.89	26.66	12.51	14.93	34.73	15.83	10.34	9.94
LSD	2.44	1.50	2.19	1.49	1.67	1.04	3.02	0.31
Level of Significance	**	**	**	**	**	**	**	**

4.1.2.3. Interaction effect on germination and pathogenic incidence of cucurbits seeds

In case of interaction between places (L) and crops (S), significant variation observed among the interaction of places and seeds.

The seed germination interaction ranged from 0.00% to 63.50% where the highest seed germination interaction percentage was found in L_1S_3 (63.50%) followed by L_1S_1 (63.16%). No germination interaction percentage was recorded in L_2S_4 (0.00%) shown in graph 3.



Graph 3: Comparative study of germination (%) interaction between places (L) and cucurbits seed (S) in blotter method

L_1= Siddik Bazar, L_2= Mohammadpur Bazar, L_3= Kochukhet Bazar, L_4= Savar Bazar, L_5= BADC S_1= Sweetgourd, S_2= Bottle gourd, S_3= Cucumber, S_4= Ridge gourd, S_5= Snake gourd

The pathogen incidence interaction of *Aspergillus flavus* ranged from 0.00% to 52.83% where the highest incidence of *Aspergillus flavus* was recorded in L_1S_4 (52.83%) followed by L_2S_4 (50.50%). No incidences were recorded in L_4S_3 and L_5S_3 (0.00%).

The pathogen incidence interaction of *Aspergillus niger* ranged from 0.00% to 34.16% where the highest incidence of *Aspergillus niger* was recorded in L_2S_1 (34.16%) followed by L_3S_1 (33.33%). No incidences were recorded in L_1S_1, L_1S_3, L_1S_4, L_1S_5, L_3S_5, L_4S_3, L_4S_4, L_4S_5 and L_5S_5 (0.00%).

The pathogen incidence interaction of *Rhizopus* sp. ranged from 0.00% to 54.17% where the highest incidence of *Rhizopus* sp. was recorded in L_5S_4 (54.17%) followed by L_5S_5 (52.17%). No incidences were recorded in L_1S_3, L_1S_5 and L_2S_3 (0.00%).

The pathogen incidence interaction of *Fusarium* sp. ranged from 0.00% to 25.33% where the highest incidence of *Fusarium* sp. was recorded in L_1S_1 (25.33%) followed by L_1S_5 (19.33%). No incidences were recorded in L_3S_2, L_4S_2, L_5S_1 and L_5S_3 (0.00%).

The pathogen incidence interaction of *Chaetomium* sp. ranged from 0.00% to 22.17% where the highest incidence of *Chaetomium* sp. was recorded in L_2S_3 (22.17%) followed by L_2S_4 (17.67%). No incidences were recorded in L_4S_1, L_4S_2, L_5S_1, L_5S_2 and L_5S_3 (0.00%).

The pathogen incidence interaction of Bacterial ooze ranged from 0.00% to 4.00% where the highest incidence of Bacterial ooze was recorded in L_1S_5 (4.00%) followed by L_2S_4 (2.50%). No incidence were recorded in L_1S_1, L_2S_2, L_2S_1, L_2S_2, L_2S_5, L_3S_1, L_3S_2, L_3S_4, L_3S_5, L_4S_1, L_4S_2, L_4S_3, L_4S_5, L_5S_1, L_5S_2 and L_5S_3 (0.00%).

The pathogen incidence interaction of unknown-1 pathogen ranged from 0.00% to 70.17% where the highest incidence of unknown-1 pathogen was recorded in L_2S_2

(70.17%) followed by L_2S_4 (66.50%). No incidences were recorded in L_1S_1 and L_1S_3 (0.00%).

The pathogen incidence interaction of unknown-2 pathogen ranged from 0.00% to 1.17% where the highest incidence of unknown-2 pathogen was recorded in L_2S_1 and L_2S_2 (1.17%) followed by L_1S_2 and L_1S_4 (0.67%). No incidence were recorded in L_1S_1, L_1S_3, L_1S_5, L_2S_3, L_2S_4, L_2S_5, L_3S_1, L_3S_2, L_3S_3, L_3S_4, L_3S_5, L_4S_1, L_4S_2, L_4S_3, L_4S_4 L_4S_5, L_5S_1, L_5S_2, L_5S_3, L_5S_4 and L_5S_5 (0.00%)

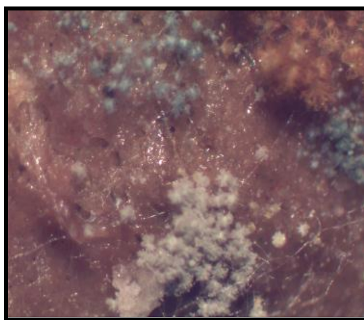
These results have been presented in table 6.

Table 6: Interaction effect on percent pathogenic incidences of cucurbits seeds by blotter method

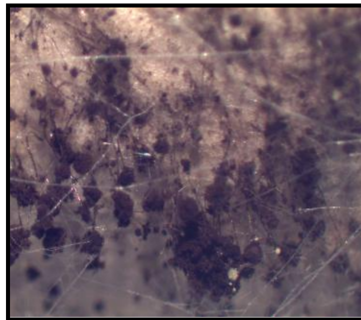
Interaction	*Aspergillus flavus* (%)	*Aspergillus niger* (%)	*Rhizopus* sp. (%)	*Fusarium* sp. (%)	*Chaetomium* sp. (%)	Bacterial ooze (%)	Unknown 1 (%)	Unknown 2 (%)
L_1S_1	38.00 bc	0.00 h	23.33 ef	25.33 a	8.83 cdef	0.00 c	0.00 k	0.00 b
L_1S_2	39.16 b	13.00 e	6.167 hi	1.00 gh	10.67 cde	0.00 c	0.83 k	0.67 ab
L_1S_3	28.00 e	0.00 h	0.00 j	1.50 gh	7.83 def	1.00 c	0.00 k	0.00 b
L_1S_4	52.83 a	0.00 h	12.33 g	0.67 gh	0.50 g	0.50 c	35.33 h	0.67 ab
L_1S_5	2.16 l	0.00 h	0.00 j	19.33 b	8.33 cdef	4.00 a	9.50 j	0.00 b
L_2S_1	25.50 ef	34.16 a	19.17 f	1.33 gh	7.33 ef	0.00 c	34.83 h	1.17 a
L_2S_2	21.83 fg	29.50 b	1.17 j	0.33 h	0.67 g	0.00 c	70.17 a	1.17 a
L_2S_3	1.16 l	0.33 h	0.00 j	3.83 efg	22.17 a	0.83 c	63.17 b	0.00 b
L_2S_4	50.50 a	1.83 h	4.83 ij	6.00 de	17.67 b	2.50 b	66.50 ab	0.00 b
L_2S_5	3.00 kl	0.66 h	3.50 ij	5.833 def	0.50 g	0.00 c	62.17 b	0.00 b
L_3S_1	28.50 de	33.33 a	28.67 d	0.17 h	8.00 def	0.00 c	21.50 i	0.00 b
L_3S_2	33.66 cd	19.50 d	38.83 c	0.00 h	2.16 g	0.00 c	54.17 cd	0.00 b
L_3S_3	7.83 jk	2.33 gh	45.83 b	0.17 h	11.83 c	0.17 c	32.67 h	0.00 b
L_3S_4	30.33 de	12.16 e	52.50 a	12.16 c	10.33 cdef	0.00 c	47.33 ef	0.00 b
L_3S_5	10.00 ij	0.00 h	29.50 d	6.67 de	9.50 cdef	0.00 c	37.50 gh	0.00 b
L_4S_1	13.33 hi	5.33 fg	51.00 a	2.50 fgh	0.00 g	0.00 c	33.50 h	0.00 b
L_4S_2	17.83 gh	2.00 gh	13.83 g	0.00 h	0.00 g	0.00 c	34.83 h	0.00 b
L_4S_3	0.00 l	0.00 h	10.00 gh	6.83 de	11.50 cd	0.00 c	60.67 bc	0.00 b
L_4S_4	38.00 bc	0.00 h	25.50 de	5.50 def	6.83 f	0.00 c	45.50 ef	0.00 b
L_4S_5	14.00 hi	0.00 h	12.00 g	8.17 d	16.33 b	0.00 c	43.50 fg	0.00 b
L_5S_1	29.50 de	25.50 c	51.67 a	0.00 h	0.00 g	0.00 c	52.16 de	0.00 b
L_5S_2	22.16 fg	8.33 f	39.67 c	0.83 gh	0.00 g	0.00 c	61.50 b	0.00 b
L_5S_3	0.00 l	0.83 h	19.83 f	0.00 h	0.00 g	0.00 c	33.17 h	0.00 b
L_5S_4	38.83 bc	2.50 gh	54.17 a	1.17 gh	0.67 g	1.00 c	44.17 fg	0.00 b
L_5S_5	11.50 ij	0.00 h	52.17 a	12.17 c	1.83 g	0.50 c	52.00 de	0.00 b
CV (%)	14.89	26.66	12.51	14.93	34.73	15.83	10.34	9.94
LSD	5.44	3.35	4.89	3.34	3.73	1.04	6.76	0.70
Level of Significance	**	**	**	**	**	**	**	**

L_1= Siddik Bazar, L_2= Mohammadpur Bazar, L_3= Kochukhet Bazar, L_4= Savar Bazar, L_5= BADC
S_1= Sweetgourd, S_2= Bottle gourd, S_3= Cucumber, S_4= Ridge gourd, S_5= Snake gourd

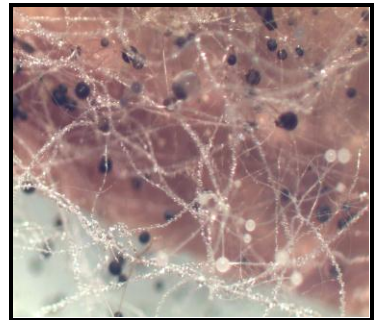
Photograph 7: Sweet gourd seeds (Blotter method)



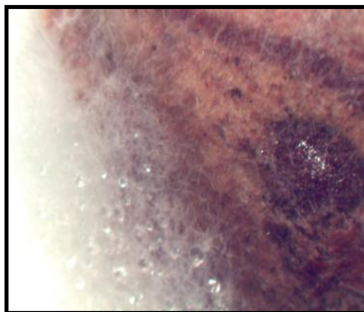
Aspergillus flavus



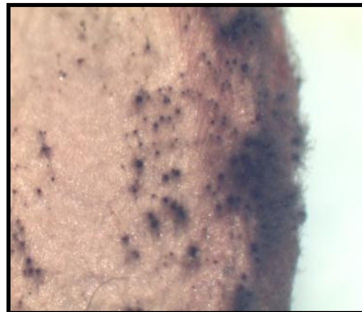
Aspergillus niger



***Rhizopus* sp.**

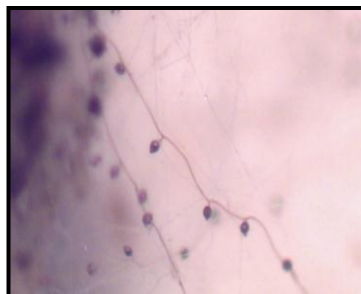


***Fusarium* sp.**



***Chaetomium* sp.**

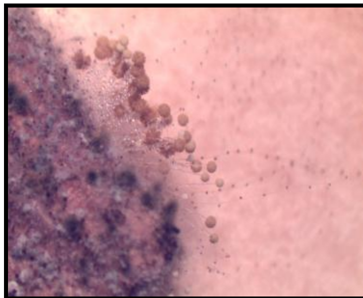
Unknown-1



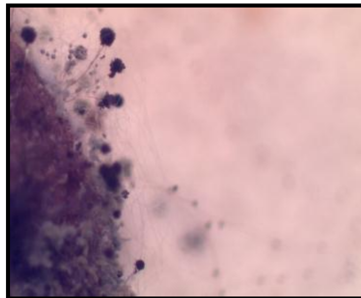
Unknown-2

Plate 10: Steriomicroscopic view of pathogens on Sweet gourd seeds

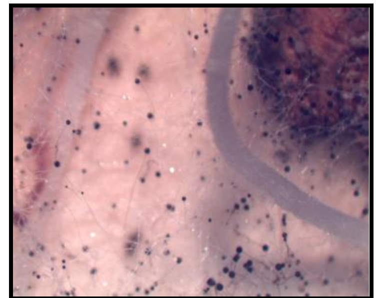
Photograph 8: Bottle gourd seeds (Blotter method)



Aspergillus flavus



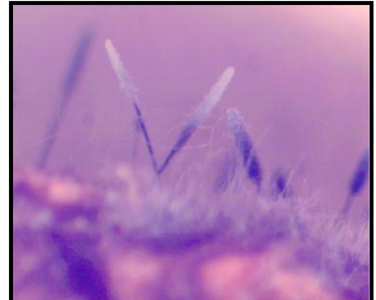
Aspergillus niger



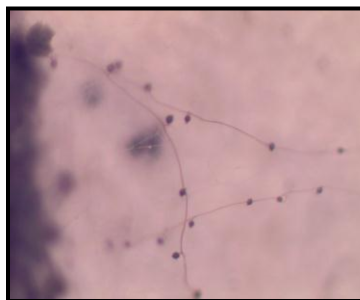
***Rhizopus* sp.**

***Fusarium* sp.**

***Chaetomium* sp.**



Unknown-1

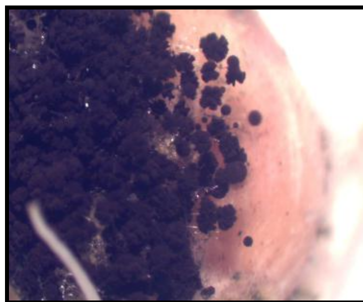


Unknown-2

Plate 11: Steriomicroscopic view of pathogens on Bottle gourd seeds

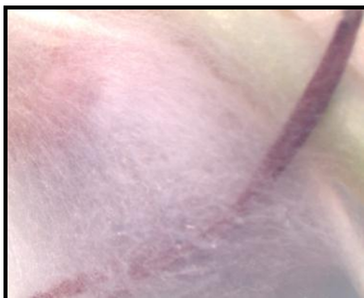
Photograph 9: Cucumber seeds (Blotter method)

Aspergillus flavus



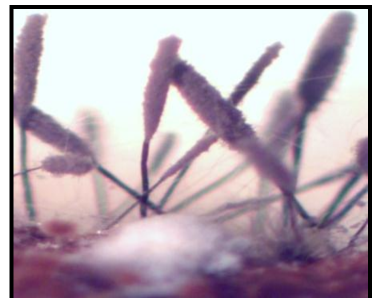
Aspergillus niger

Rhizopus sp.

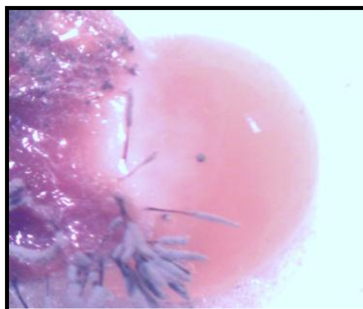


Fusarium sp.

Chaetomium sp.



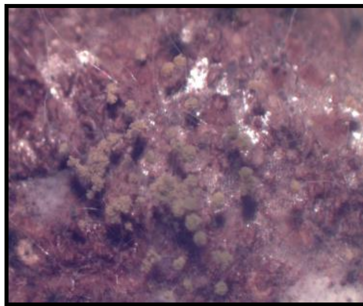
Unknown-1



Bacterial ooze

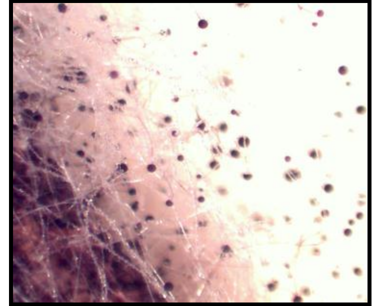
Plate 12: Steriomicroscopic view of pathogens on Cucumber seeds

Photograph 10: Ridge gourd seeds (Blotter method)



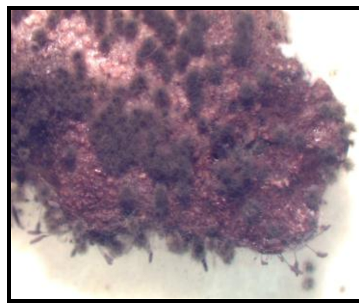
Aspergillus flavus

Aspergillus niger

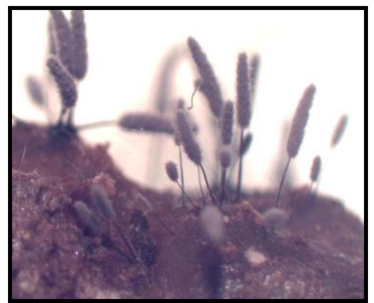


***Rhizopus* sp.**

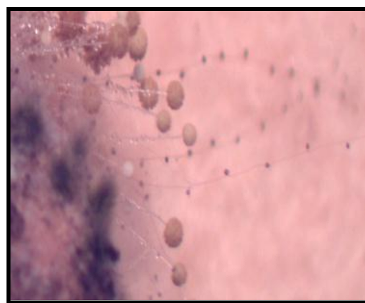
***Fusarium* sp.**



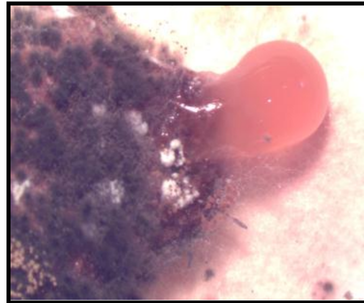
***Chaetomium* sp.**



Unknown-1



Unknown-2

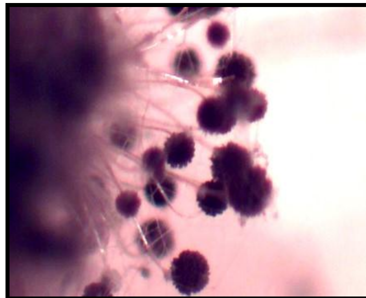


Bacterial ooze

Plate 13: Steriomicroscopic view of pathogens on Ridge gourd seeds

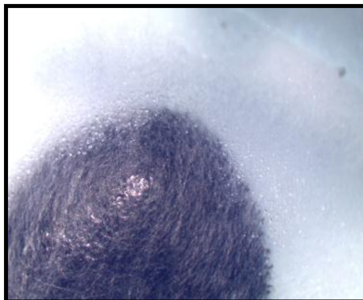
Photograph 11: Snake gourd seeds (Blotter method)

Aspergillus flavus

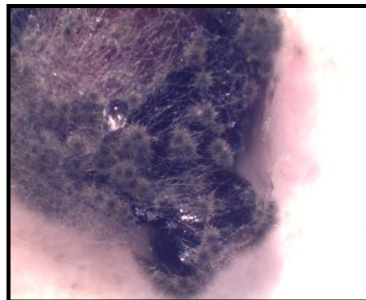


Aspergillus niger

***Rhizopus* sp.**

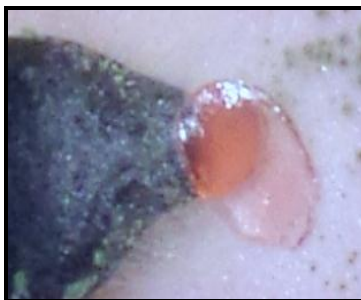


***Fusarium* sp.**



***Chaetomium* sp.**

Unknown-1



Bacterial ooze

Plate 14: Steriomicroscopic view of pathogens on Snake gourd seeds

4.1.3. Cultural and morphological characterization of isolated pathogens

4.1.3.1. Radial mycelial growth

The radial mycelial growth of seven different fungal pathogens *in vitro* are shown in (Table 7). Radial mycelial growth for all the fungal pathogens ranged from 0.78 cm to 8.00 cm recorded from 2 days after inoculation to 5 days after inoculation. The lowest radial mycelial growth was found in *Aspergillus flavus* (1.27 cm) 5 days after inoculation and highest mycelial growth were found in *Rhizophus sp.*, *Fusarium sp.*, Unknown-1, Unknown-2 (8.00 cm) 5days after inoculation. The pathogens were confirmed by observing under compound microscope with a 10X optical view.

Table 7: Cultural and morphological characterization of isolated seed borne fungi from cucurbits seeds

Pathogens	Radial mycelial growth (cm)				Number of spore/mm^2 ($\times 10^3$)	Colony color
	2 DAI	3 DAI	4 DAI	5 DAI		
Aspergillus flavus	0.78 d	0.98 d	1.10 c	1.27 c	50.95 c	Greenish yellow
Aspergillus niger	7.18 b	7.53 b	7.70 a	7.78 a	114.91 a	Black
Rhizopus sp.	8.00 a	8.00 a	8.00 a	8.00 a	8.35 f	Grayish white
Fusarium sp.	8.00 a	8.00 a	8.00 a	8.00 a	3.11 f	White
Chaetomium sp.	4.68 c	5.00 c	5.23 b	5.55 b	67.34 b	Brownish white
Unknown-1	8.00 a	8.00 a	8.00 a	8.00 a	20.56 e	White
Unknown-2	8.00 a	8.00 a	8.00 a	8.00 a	28.13 d	Yellowish white
CV (%)	3.43	3.18	3.38	4.07	8.98	
LSD	0.38	0.36	0.39	0.47	6.59	
Level of significance	**	**	**	**	**	

*In a column, DAI = Days after inoculation

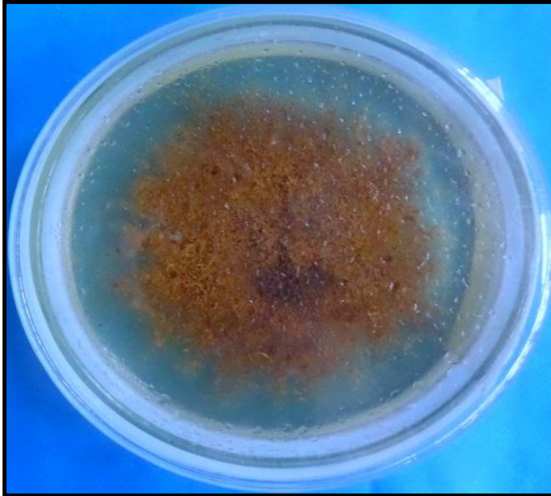
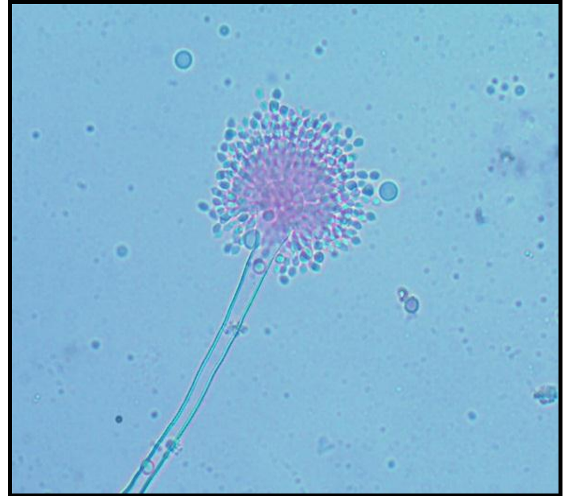



Plate 15: ***Aspergillus flavus*** **(Pure culture and compound microscopic view)**

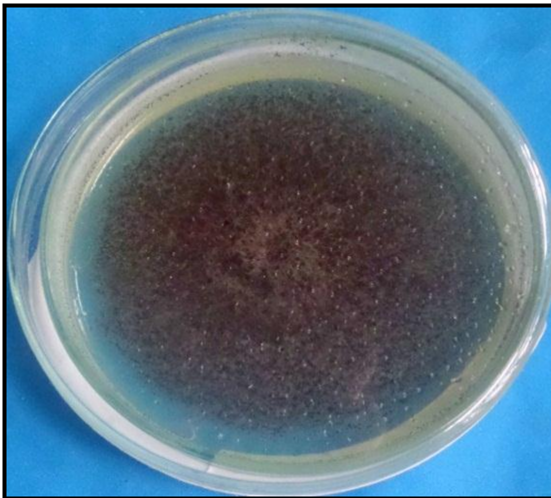
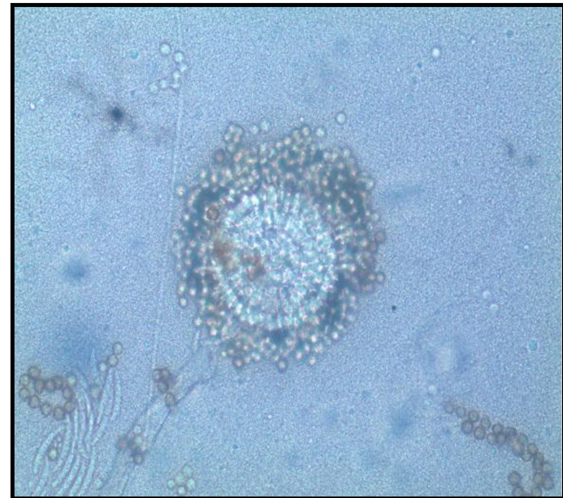



Plate 16: ***Aspergillus niger*** **(Pure culture and compound microscopic view)**

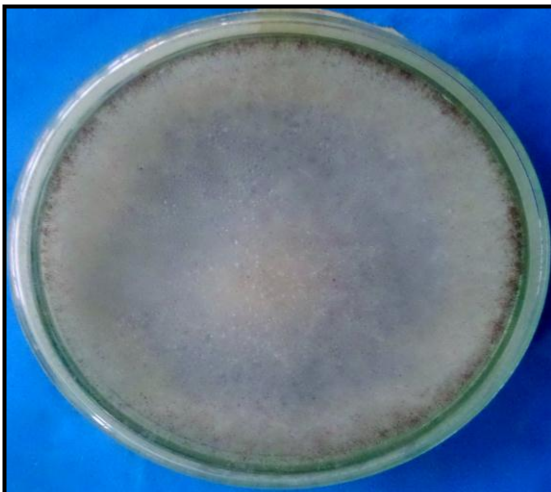
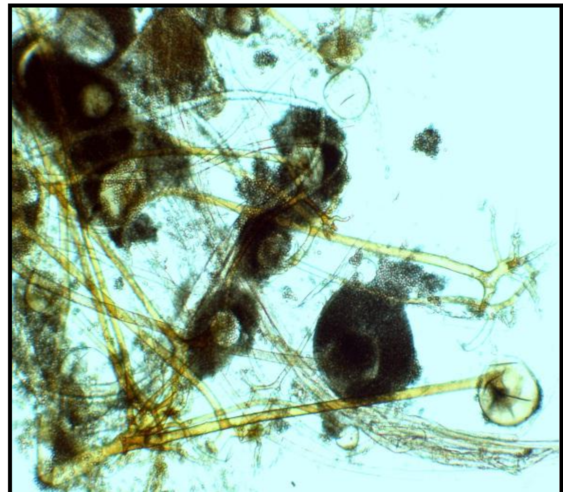



Plate 17: ***Rhizopus*** **sp. (Pure culture and compound microscopic view)**

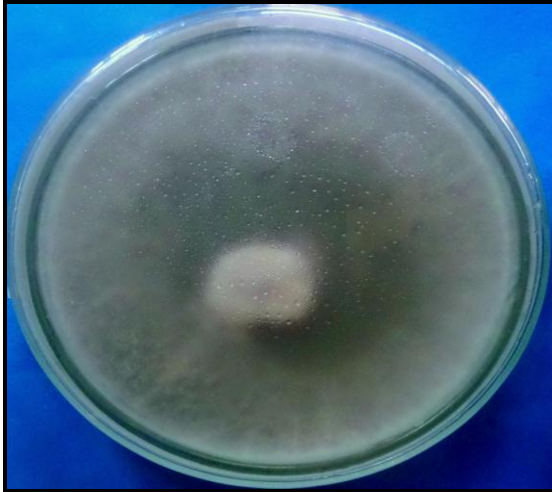
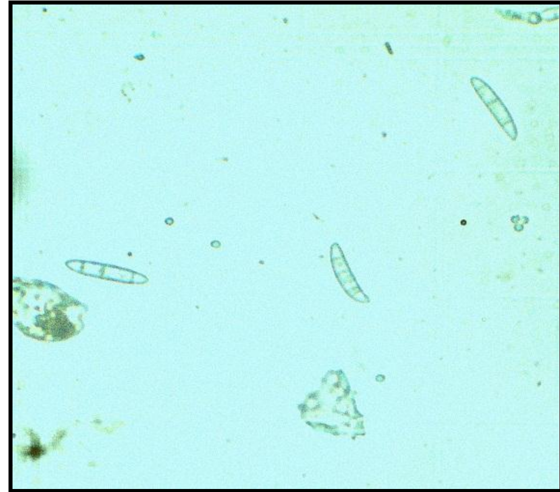



Plate 18: *Fusarium* sp. (Pure culture and compound microscopic view)

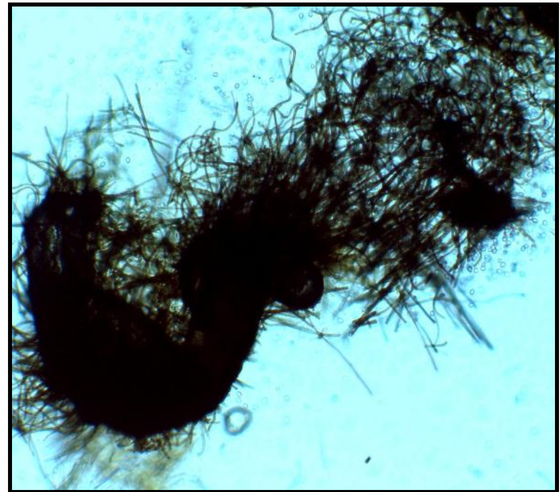


Plate 19: *Chaetomium* sp. (Pure culture and compound microscopic view)

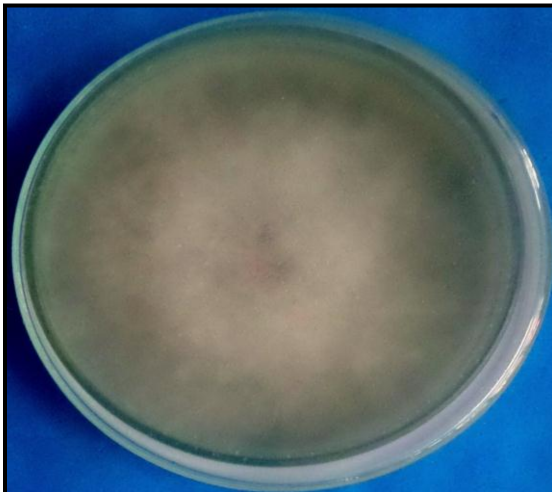
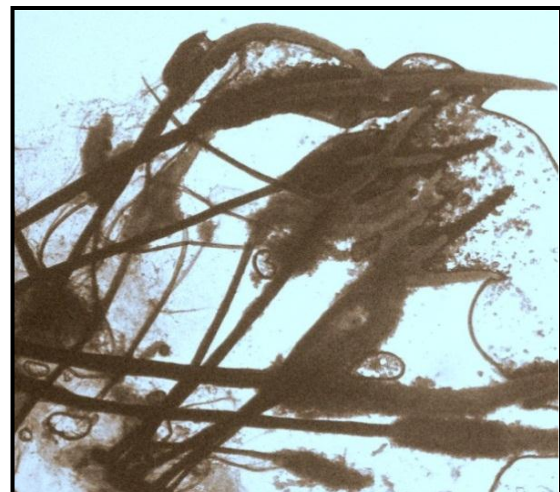



Plate 20: Unknown-1 (Pure culture and compound microscopic view)

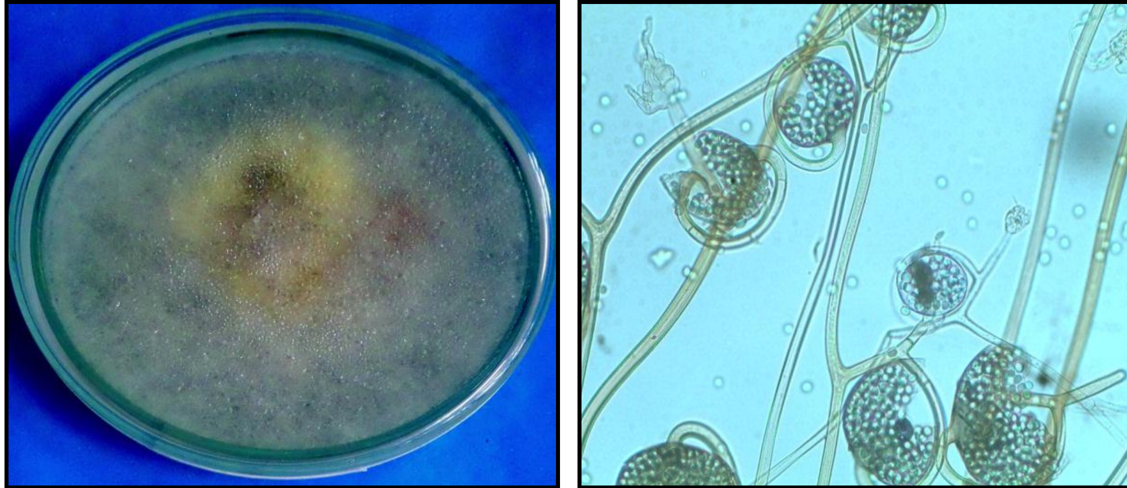
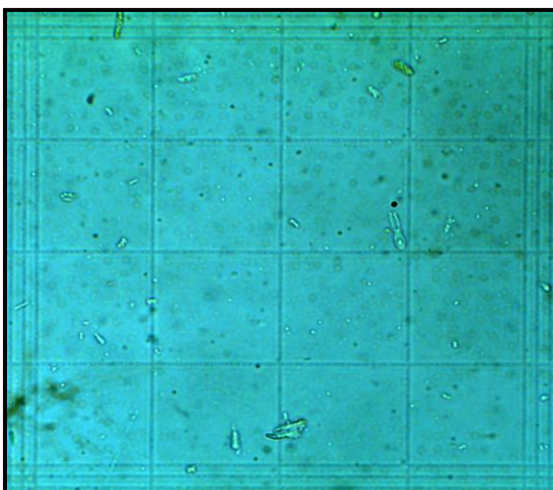


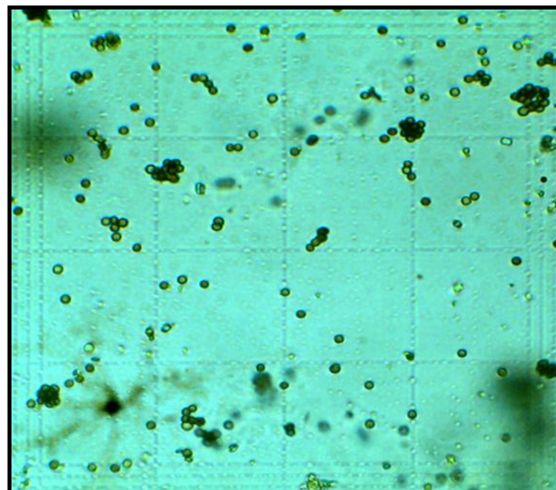
Plate 21: Unknown-2 (Pure culture and compound microscopic view)

4.1.3.2. Number of spores

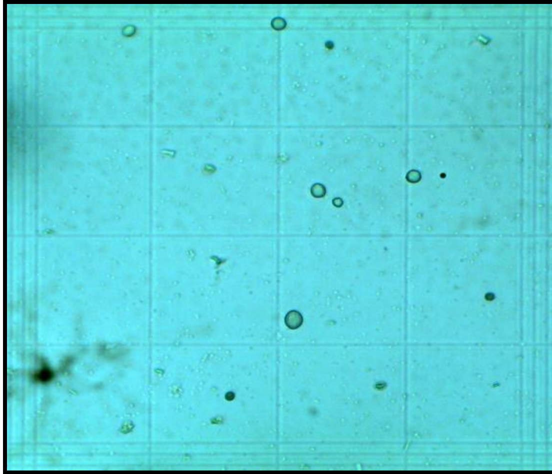
From pure culture medium number of spore per square millimeter in a petridish was counted by using haemacytometer and digital microscope. The number ranged from 3.11($\times10^3$) to 114.91 ($\times10^3$). The highest number of spore was found in *Aspergillus niger* 114.91 ($\times10^3$) followed by *Chaetomium* sp. 67.34 ($\times10^3$) and lowest number of spore was found in *Fusarium* sp. 3.11($\times10^3$). Different colony color also observed and recoded from the pure culture of the pathogens (Table 7).



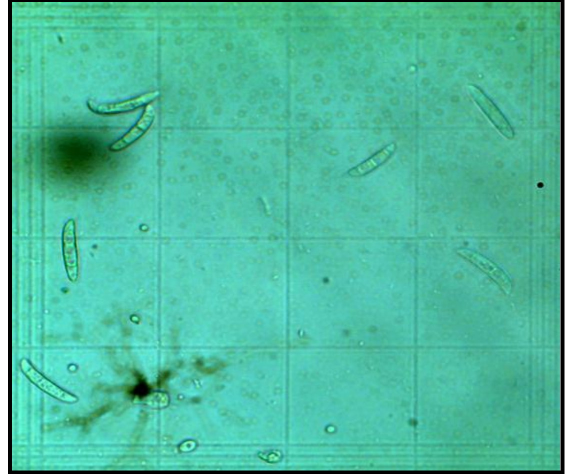
Aspergillus flavus



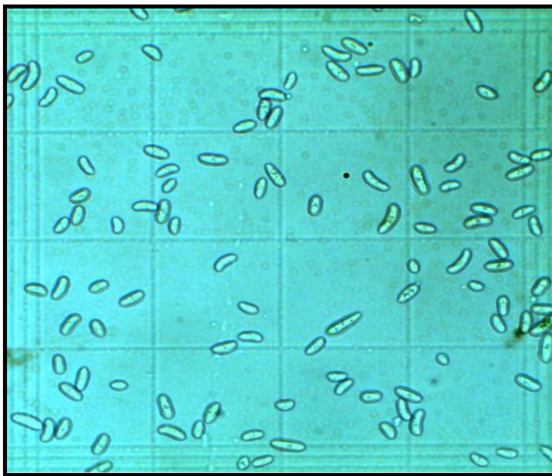
Aspergillus niger



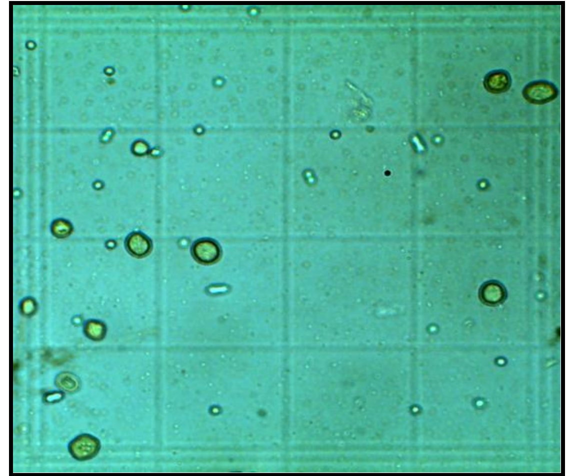
***Rhizopus* sp.**



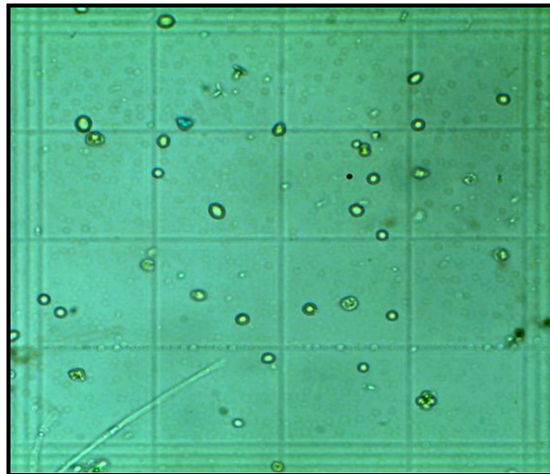
***Fusarium* sp.**



***Chaetomium* sp.**



Unknown-1



Unknown-2

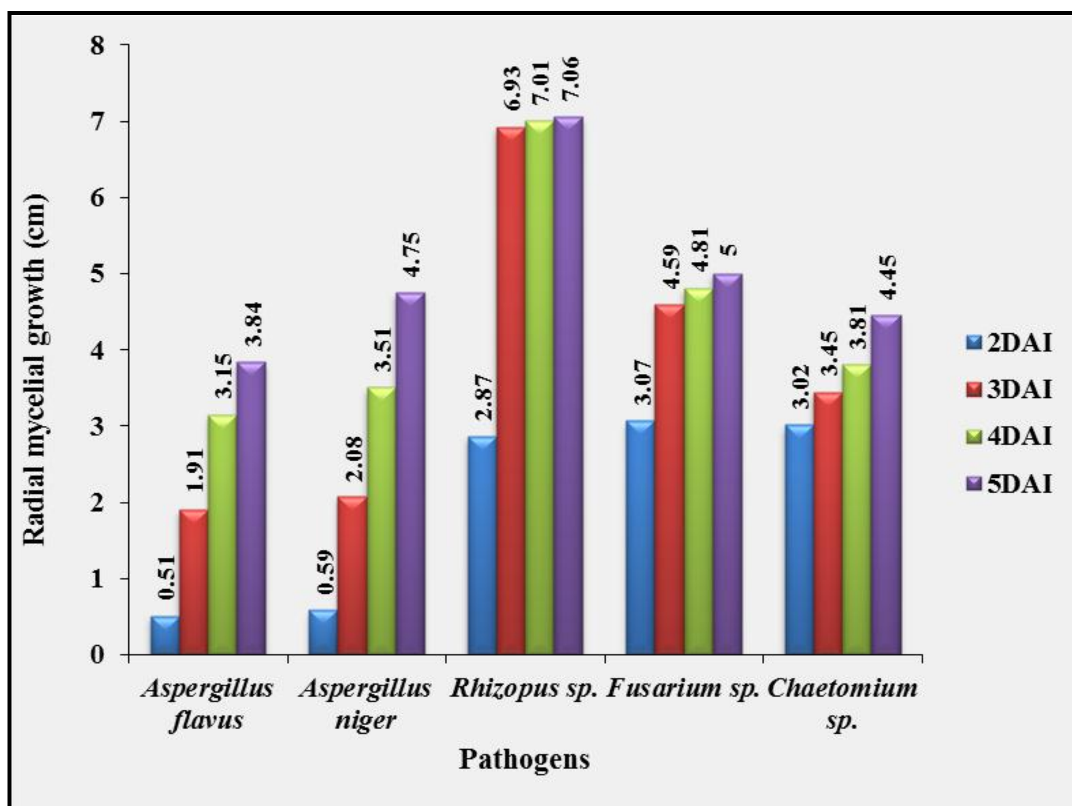
Plate 22: Number of spore of different fungal pathogens using haemocytometer

4.2. In vitro evaluation of selected chemicals against isolated seed borne fungi of cucurbits

Effect of the treatments in controlling storage pathogen (*Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.) was assessed *in vitro*. The results were compiled based on the inhibition of radial mycelium growth of every pathogen against four treatments (Dithane M-45, Autostin 50 WDG, Tilt 250 EC and Salicylic acid) along with control.

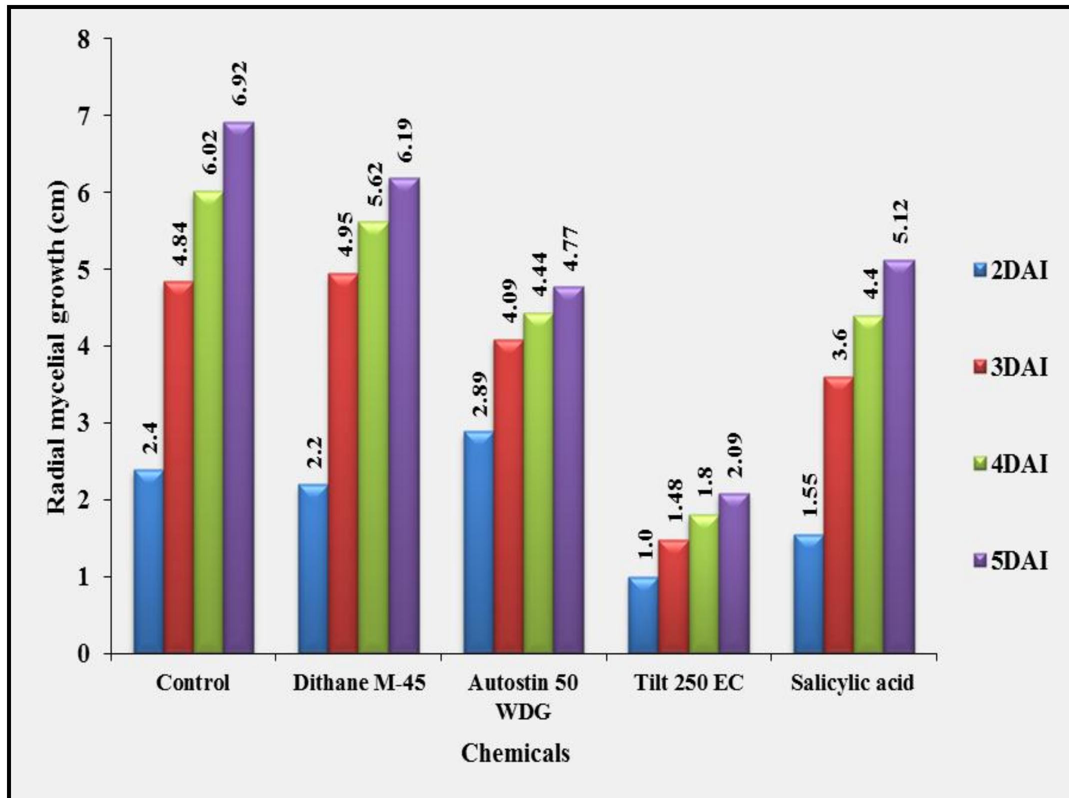
4.2.1. *In vitro* efficacy of fungicides in inhibition of mycelial growth of fungal pathogens in poisoned food technique (Cup method)

In case of fungal pathogens, the efficacy of chemicals on radial mycelial growth of *Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.*in vitro* shown in Graph 4. Chemicals have profound effect on reduction of radial mycelial growth of the fungus. Radial mycelial growth for all the tested pathogens ranged from 0.51 cm to 8.00 cm recorded after inoculation of 5 days. The lowest radial mycelial growth (0.51 cm, 1.91 cm, 3.15 cm, 3.84 cm) was recorded in *Aspergillus flavus* at 2 days, 3 days, 4 days, 5 days after inoculation respectively. The highest radial mycelial growth (2.87 cm, 6.93 cm, 7.01 cm, 7.06 cm) was recorded in *Rhizopus* sp. Followed by *Fusarium* sp., *Aspergillus niger, Chaetomium* sp. at 2 days, 3 days, 4 days, 5 days after inoculation respectively.



Graph 4: Radial mycelial growth of different identified pathogens isolated from cucurbits seeds after application of different chemicals

In case of chemicals, the efficacy of chemicals (Dithane M-45, Autostin 50 WDG, Tilt 250 EC and Salicylic acid) and control on radial mycelial growth of pathogens *in vitro* shown in Graph 5. All the tested chemicals significantly reduced radial mycelial growth of the fungus. The lowest radial mycelial growth (1.00 cm, 1.48 cm, 1.80 cm, 2.09 cm) was recorded for Tilt 250 EC at 2 days, 3 days, 4 days, 5 days after inoculation respectively. The highest radial mycelial growth (2.40 cm, 4.84 cm, 6.02 cm, 6.92 cm) was recorded for Control followed by Dithane M-45, Salicylic acid, Autostin 50WDG at 2 days, 3 days, 4 days, 5 days after inoculation respectively.



Graph 5: Effect of different chemicals on radial mycelial growth of different identified pathogens isolated from cucurbits seeds

In case of pathogens (P) and chemicals (C) interaction, radial mycelial growth ranged from 0.00 cm to 8.00 cm. The lowest radial mycelial growth (0.00 cm, 0.00 cm, 0.00 cm, 0.00 cm) was recorded in P_1C_4 at 2 days, 3 days, 4 days, 5 days after inoculation respectively. The highest radial mycelial growth (8.00 cm, 8.00 cm, 8.00 cm, 8.00 cm) was recorded in P_5C_3 at 2 days, 3 days, 4 days, 5 days after inoculation respectively (Table 8).

Table 8: Interaction effect of different isolated pathogens and chemicals on radial mycelial growth

Treatment	Radial mycelial growth (cm)							
	2 DAI		3 DAI		4 DAI		5 DAI	
$P_1 C_1$	0.52	lm	3.03	c	5.50	b	6.85	bc
$P_1 C_2$	0.85	l	3.68	b	5.77	b	6.52	c
$P_1 C_3$	0.30	mn	0.77	j	1.03	hi	1.25	g
$P_1 C_4$	0.00	n	0.00	k	0.00	j	0.00	h
$P_1 C_5$	0.87	l	2.07	fghi	3.45	d	4.57	de
$P_2 C_1$	1.48	jk	2.38	efgh	5.17	bc	7.30	ab
$P_2 C_2$	0.80	l	2.55	cdef	3.43	d	4.27	de
$P_2 C_3$	0.28	mn	1.95	hi	3.45	d	4.83	d
$P_2 C_4$	0.25	mn	0.50	jk	0.80	i	1.15	g
$P_2 C_5$	0.13	mn	3.02	cd	4.68	c	6.23	c
$P_3 C_1$	2.77	de	8.00	a	8.00	a	8.00	a
$P_3 C_2$	2.45	ef	8.00	a	8.00	a	8.00	a
$P_3 C_3$	4.18	c	8.00	a	8.00	a	8.00	a
$P_3 C_4$	1.80	ghij	2.67	cde	3.07	de	3.32	f
$P_3 C_5$	3.13	d	8.00	a	8.00	a	8.00	a
$P_4 C_1$	5.25	b	8.00	a	8.00	a	8.00	a
$P_4 C_2$	4.98	b	8.00	a	8.00	a	8.00	a
$P_4 C_3$	1.77	ghijk	1.75	i	1.75	gh	1.77	g
$P_4 C_4$	1.33	k	2.33	efgh	2.83	def	3.20	f
$P_4 C_5$	2.07	fg	2.90	cd	3.47	d	4.03	e
$P_5 C_1$	2.00	gh	2.78	cde	3.45	d	4.47	de
$P_5 C_2$	1.97	ghi	2.52	defg	2.90	def	4.20	de
$P_5 C_3$	8.00	a	8.00	a	8.00	a	8.00	a
$P_5 C_4$	1.67	hijk	1.90	hi	2.32	fg	2.82	f
$P_5 C_5$	1.50	ijk	2.03	ghi	2.40	efg	2.77	f
CV (%)	12.84		8.09		9.80		8.64	
LSD	0.42		0.50		0.72		0.71	
Level of significance	**		**		**		**	

P_1= *Aspergillus flavus*, P_2= *Aspergillus niger*, P_3= *Rhizopus* sp., P_4= *Fusarium* sp., P_5= *Chaetomium* sp.

C_1= Control, C_2= Dithane M-45, C_3= Autostin 50 WDG, C_4= Tilt 250 EC, C_5= Salicylic acid

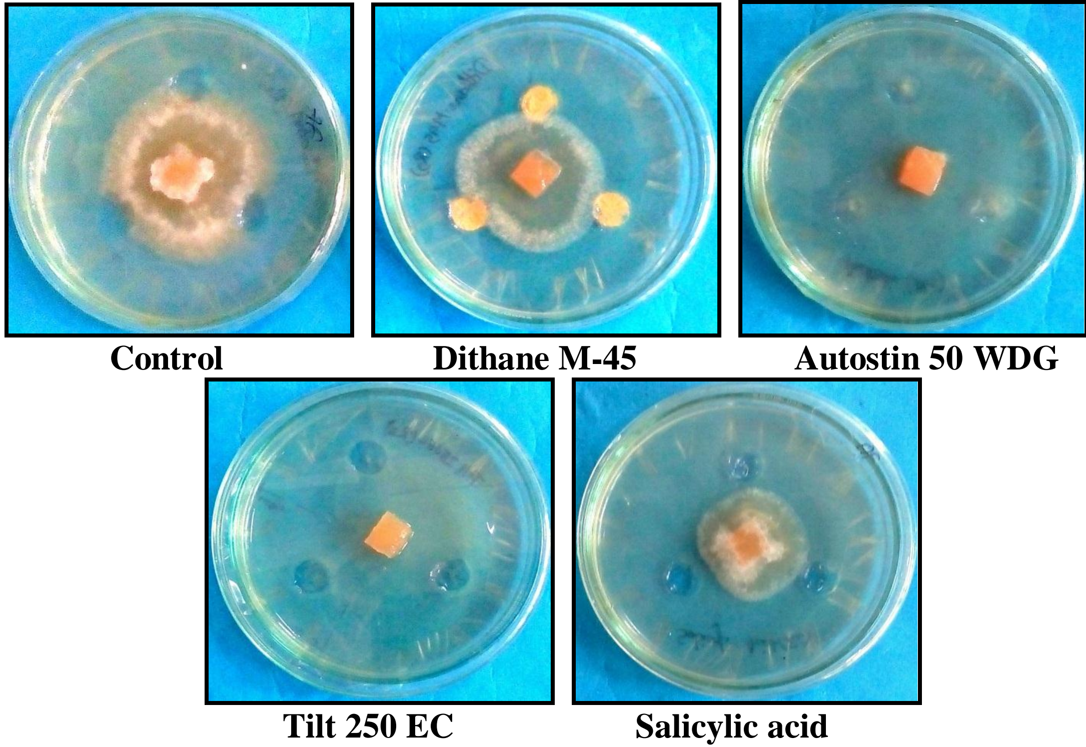


Plate 24: Mycelial growth of *Aspergillus flavus* against different chemicals at 3DAI

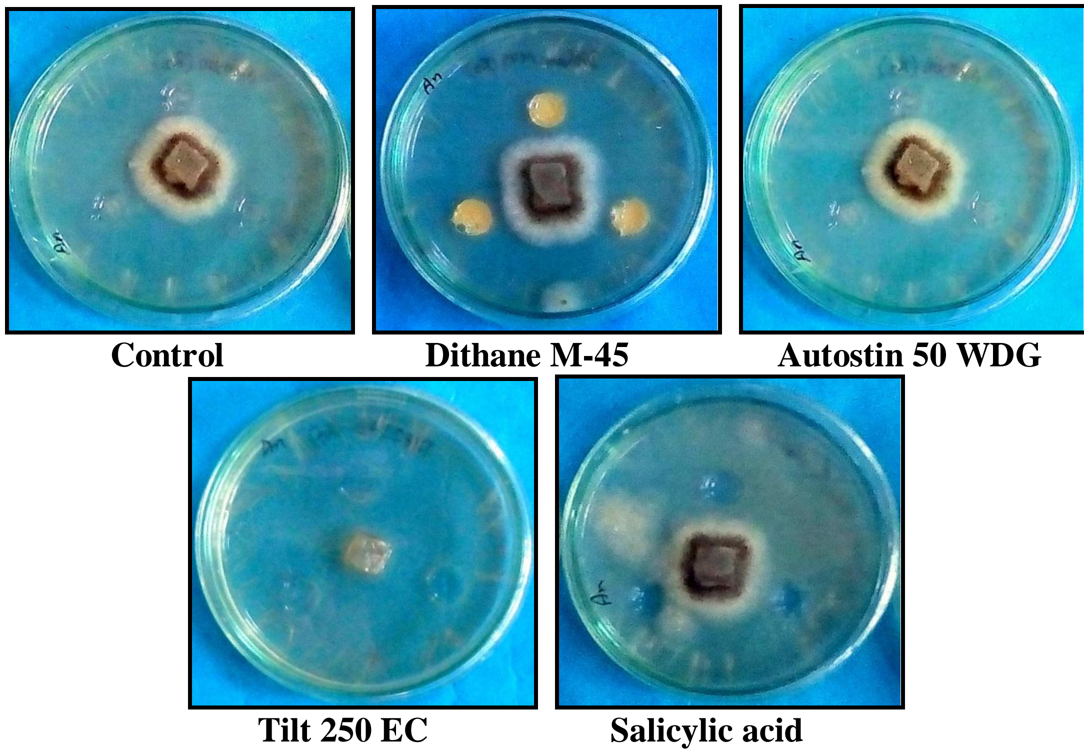
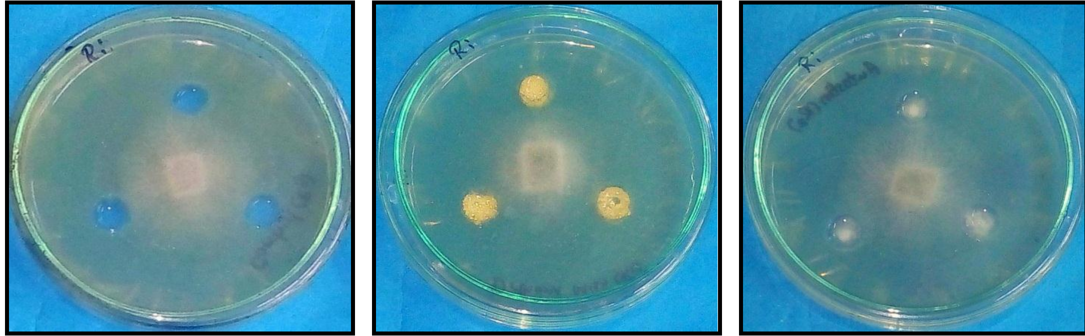
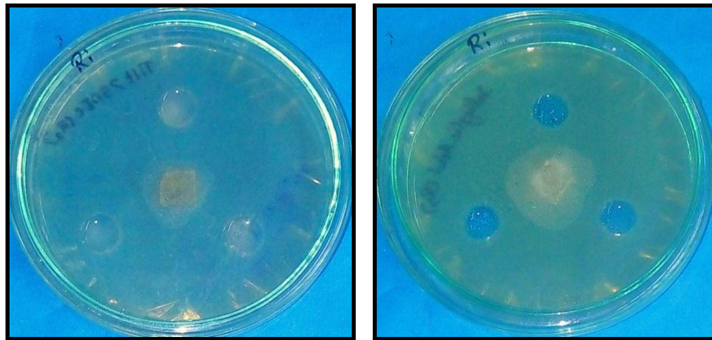


Plate 25: Mycelial growth of *Aspergillus niger* against different chemicals at 3DAI

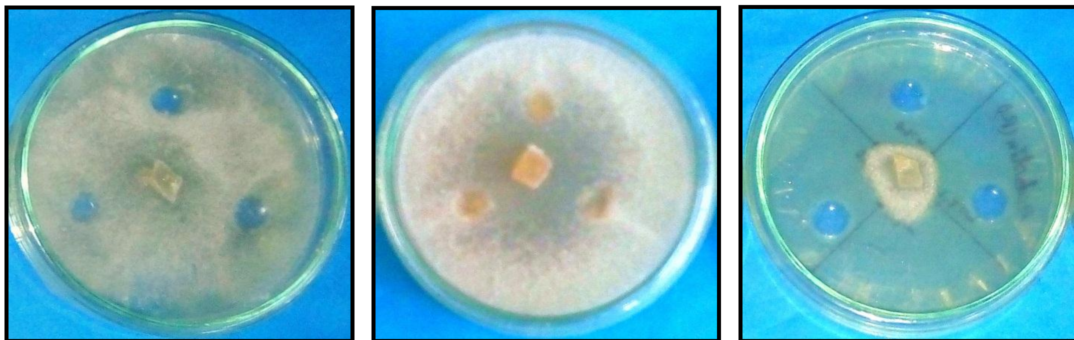


. Control Dithane M-45 Autostin 50 WDG

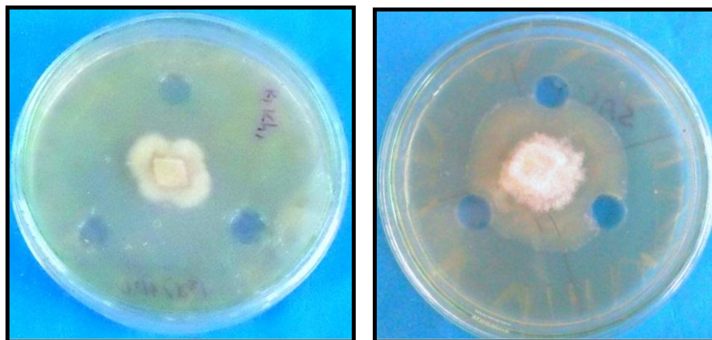


Tilt 250 EC Salicylic acid

Plate 26: Mycelial growth of *Rhizopus* sp. against different chemicals at 3DAI



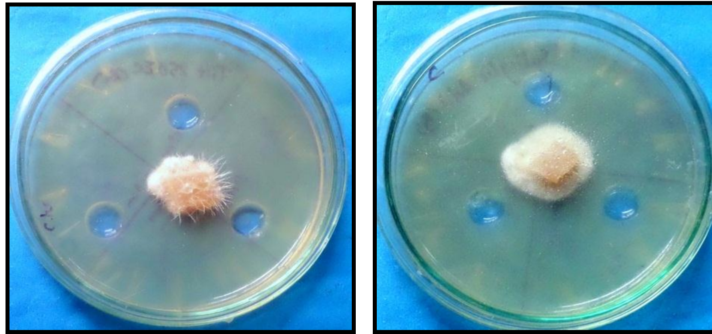
. Control Dithane M-45 Autostin 50 WDG



Tilt 250 EC Salicylic acid

Plate 27: Mycelial growth of *Fusarium* sp. against different chemicals at 3DAI

Control **Dithane M-45** **Autostin 50 WDG**



Tilt 250 EC **Salicylic acid**

Plate 28: Mycelial growth of *Chaetomium* sp. against different chemicals at 3DAI

CHAPTER V
DISCUSSION

The experiment was conducted in Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The investigation was conducted to assess the seed health status of five selected cucurbit seeds namely Sweet gourd (L_1), Bottle gourd (L_2), Cucumber (L_3), Ridge gourd (L_4), Snake gourd (L_5) collected from five different location of Dhaka city namely Siddik Bazar (S_1), Mohammadpur Bazar (S_2), Kochukhet Bazar (S_3), Savar Bazar (S_4), BADC seed store (Gabtoli) (S_5). The result of the investigation revealed that seed borne fungi were present on most of the cucurbits seeds.

Seed samples were subjected to dry seed examination and based on the visual examination of the seeds. The seed sample varied in respect of sample seed weight, apparently pure seed, infected seed and inert matter both in location wise and species wise.

Considerable amount of seed borne pathogenic fungi were detected by using blotter method. Among seven fungi five isolates were identified as *Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp., and two were unidentified during the investigation. It was reported that a total of 15 genera and 29 species of fungi were isolated from 10 samples of bitter gourd seeds in Pakistan using ISTA techniques using the standard blotter and deep-freeze methods and identified by morphological characteristics (Sultana and Ghaffar, 2007). Another report said that fungal species including *Fusarium* spp., *Alternaria* spp., *Phoma* spp. and *Cladosporium* spp. were the most frequent on gourds, pumpkin and cucumber (Avinash and Ravishankar Rai, 2013). Earlier research reported that there were many seed borne fungi found on cucurbits including: *Alternaria alternata, Botryodiplodia theobromae, Chaetomium* spp., *Curvularialunata, Drechslera tetramera, Fusarium equiseti, F. moniliforme* and *F. solani* on gourd seeds (Richardson, 1990); on watermelon, squash, muskmelon,

bittergourd and cucumber (Nair, 1982; Mathur, 1990). In the present study, the incidence of *Aspergillus flavus*, *Aspergillus niger, Rhizopus* sp., *Fusarium* sp., *Chaetomium* sp. Ranged from 7.40% to 42.10%, 0.13% to 19.66%, 15.13% to 34.76, 0.43% to 10.43%, 2.70 to 10.67 respectively. This findings were partially supported by (Hussain *et al.* 2013) who evaluated the pathogenicity of two mostly prevailing fungal species *F. moniliforme* and *A. niger* on maize and found *F. moniliforme* had 50.2% pathogenicity on seeds and 6.55% on seedlings, whilst *A. niger* had 62.87% on seeds and 11.24% on seedlings. The present findings of the seed borne fungal organisms were in agreement with the information of seed borne nature of the pathogen reported by (Marley and Gbenga 2004). They conducted of pathogenicity test with two most frequently isolated fungi that is *F. moniliforme* and *A. niger* was carried out and showed pathogenic effects on seeds germination. These are highly pathogenic seeds-borne fungi that were frequently recorded almost with all samples from different localities and were also reported as pathogenic by several other studies (Richardson, 1979; Fakhrunnisa and Hashmi, 1992, Ahmad *et al.*, 1993). *F. moniliforme* was found to be highly infective by producing mycotoxins that are involved in retarding seed germination and seedlings growth as also reported by (Yates *et al.*, 1997).

The efficacy of chemicals (Dithane M-45, Autostin 50 WDG, Tilt 250 EC and Salicylic acid) on radial mycelial growth of pathogens *in vitro* showed that the lowest radial mycelial growth (1.00 cm, 1.48 cm, 1.80 cm, 2.09 cm) was recorded for Tilt 250 EC at 2 days, 3 days, 4 days, 5 days after inoculation, respectively. The highest radial mycelial growth (2.40 cm, 4.84 cm, 6.02 cm, 6.92 cm) was recorded for control followed by Dithane M-45, Salicylic acid, Autostin 50 WDG at 2 days, 3 days, 4 days, 5 days after inoculation, respectively. The efficacy of chemicals on radial mycelial growth of *Aspergillus flavus*, *Aspergillus niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. *in vitro* showed that chemicals have profound effect on reduction of radial mycelial growth of the fungus. Radial mycelial growth for all the tested pathogens ranged from 0.51 cm to 8.00 cm

recorded after inoculation of 5 days. The lowest radial mycelial growth (0.51 cm, 1.91 cm, 3.15 cm, 3.84 cm) was recorded in *Aspergillus flavus* at 2 days, 3 days, 4 days, 5 days after inoculation, respectively. The highest radial mycelial growth (2.87 cm, 6.93 cm, 7.01 cm, 7.06 cm) was recorded in *Rhizopus* sp. followed by *Fusarium* sp., *Aspergillus niger, Chaetomium* sp. at 2 days, 3 days, 4 days, 5 days after inoculation, respectively. The present findings partially reported by (Macedo *et al.,* 2002) who reported that the incidence of *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. Increased during storage for six months and last long up to 12 months.

CHAPTER VI
SUMMARY AND CONCLUSION

The results of the present study revealed that seed borne pathogens were present on most of the cucurbits seeds in Bangladesh. Although in certain instances they occurred in trace but they may create the disease in epidemic level. Pathogen free seed is the important input in agriculture and it was observed that all pathogens were found in all cucurbits seeds at different percentage. So the seed health status of cucurbits seeds need to improve for introduction of new pathogen.

In dry seed observation method, significant variation observed among the places and seeds. The highest seed wt. of 400 seeds was recorded in Ridge gourd seed (110.27 g) and seeds collected from Siddik Bazar (68.19 g). The highest apparently pure seed wt. was recorded in Ridge gourd seed (81.94 g) and seeds collected from Mohammadpur Bazar (49.97 g). The lowest infected seed wt. was recorded in Cucumber seed (3.71 g) and seeds collected from Savar Bazar. The highest inert matter wt. was recorded in Sweet gourd (1.34 g) and seeds collected from Savar Bazar (1.32).

In blotter paper method, significant variation observed among the interaction of places (L) and seeds (S). The highest incidence of *Aspergillus flavus* was recorded in L_1 (Siddik Bazar) S_4 (Ridge gourd) 52.83%. The highest incidence of *Aspergillus niger* was recorded in L_2 (Mohammadpur Bazar) S_1 (Sweet gourd) 34.16%. The highest incidence of *Rhizopus* sp. was recorded in L_5 (BADC) S_4 (Ridge gourd) 54.17%. The highest incidence of *Fusarium* sp. was recorded in L_1 (Siddik Bazar) S_1 (Sweet gourd) 25.33%. The highest incidence of *Chaetomium* sp. was recorded in L_2 (Mohammadpur Bazar) S_3 (Cucumber) 22.17%. The highest incidence of Bacterial ooze was recorded in L_1 (Siddik Bazar) S_5 (Snake gourd) 4.00%. The highest incidence of Unknown-1 pathogen was recorded in L_2

(Mohammadpur Bazar) S_2 (Bottle gourd) 70.17%. The highest incidence of Unknown-2 pathogen were recorded in L_2 (Mohammadpur Bazar) S_1 (Sweet gourd) and L_2 (Mohammadpur Bazar) S_2 (Bottle gourd) 1.17%.

In management, significant variation observed among the interaction of pathogens (P) and chemicals (C). The lowest radial mycelial growth (0.00 cm, 0.00 cm, 0.00 cm, 0.00 cm) was recorded in P_1 (*Aspergillus flavus*) C_4 (Tilt 250 EC) at 2 days, 3 days, 4 days, 5 days after inoculation respectively. The highest radial mycelial growth (8.00 cm, 8.00 cm, 8.00 cm, 8.00 cm) was recorded in P_5 (*Chaetomium* sp.) C_3 (Autostin 50 WDG) at 2 days, 3 days, 4 days, 5 days after inoculation, respectively.

High quality cucurbits seeds are not only important for increasing crop production but also proper establishment of good seed industries in the country. Seed is a common carrier of plant pathogens. Pathogen free seed is the important input material in agriculture. The present experiment showed that many seed borne pathogens were associated with cucurbits seeds. Seed borne pathogens appeared maybe due to improper management of cucurbits seeds in storage condition. From the findings of the study, it is very clear that the seed health status of cucurbits seeds were not at satisfactory level. Farmers are therefore advised to collect the seeds from reliable sources and cucurbits seed should be treated before sowing. Proper storage management with good chemicals can also give satisfactory result.

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APPENDICES

Appendix 1: Effect of different chemicals on radial mycelial growth of different identified pathogens isolated from cucurbits seeds

Chemicals	Radial mycelial growth			
	2DAI	**3DAI**	**4DAI**	**5DAI**
Control	2.40 b	4.84 a	6.02 a	6.92 a
Dithane M-45	2.20 c	4.95 a	5.62 b	6.19 b
Autostin 50 WDG	2.89 a	4.09 b	4.44 c	4.77 d
Tilt 250 EC	1.00 e	1.48 d	1.80 d	2.09 e
Salicylic acid	1.55 d	3.60 c	4.40 c	5.12 c
CV (%)	12.84	8.09	9.80	8.64
LSD	0.18	0.22	0.32	0.31
Level of significance	**	**	**	**

Appendix 2: Radial mycelial growth of different identified pathogens isolated from cucurbits seeds after application of different chemicals

Pathogens	Radial mycelial growth			
	2 DAI	**3 DAI**	**4 DAI**	**5 DAI**
Aspergillus flavus	0.51 c	1.91 d	3.15 d	3.84 d
Aspergillus niger	0.59 c	2.08 d	3.51 c	4.75 bc
Rhizopus sp.	2.87 b	6.93 a	7.01 a	7.06 a
Fusarium sp.	3.07 a	4.59 b	4.81 b	5.00 b
Chaetomium sp.	3.02 ab	3.45 c	3.81 c	4.45 c
CV (%)	12.84	8.09	9.80	8.64
LSD	0.19	0.23	0.32	0.31
Level of significance	**	**	**	**

Appendix 3: Composition of media (For 250ml)

The compositions of the media used in this thesis work are given below:

Unless otherwise mentioned all media were autoclaved at 121^0c for 15 minutes at 15 psi pressure.

Potato Dextrose Agar (PDA)- 250ml	
Peeled Potato	200 g
Dextrose	20g
Agar	17g
Water	1000 ml