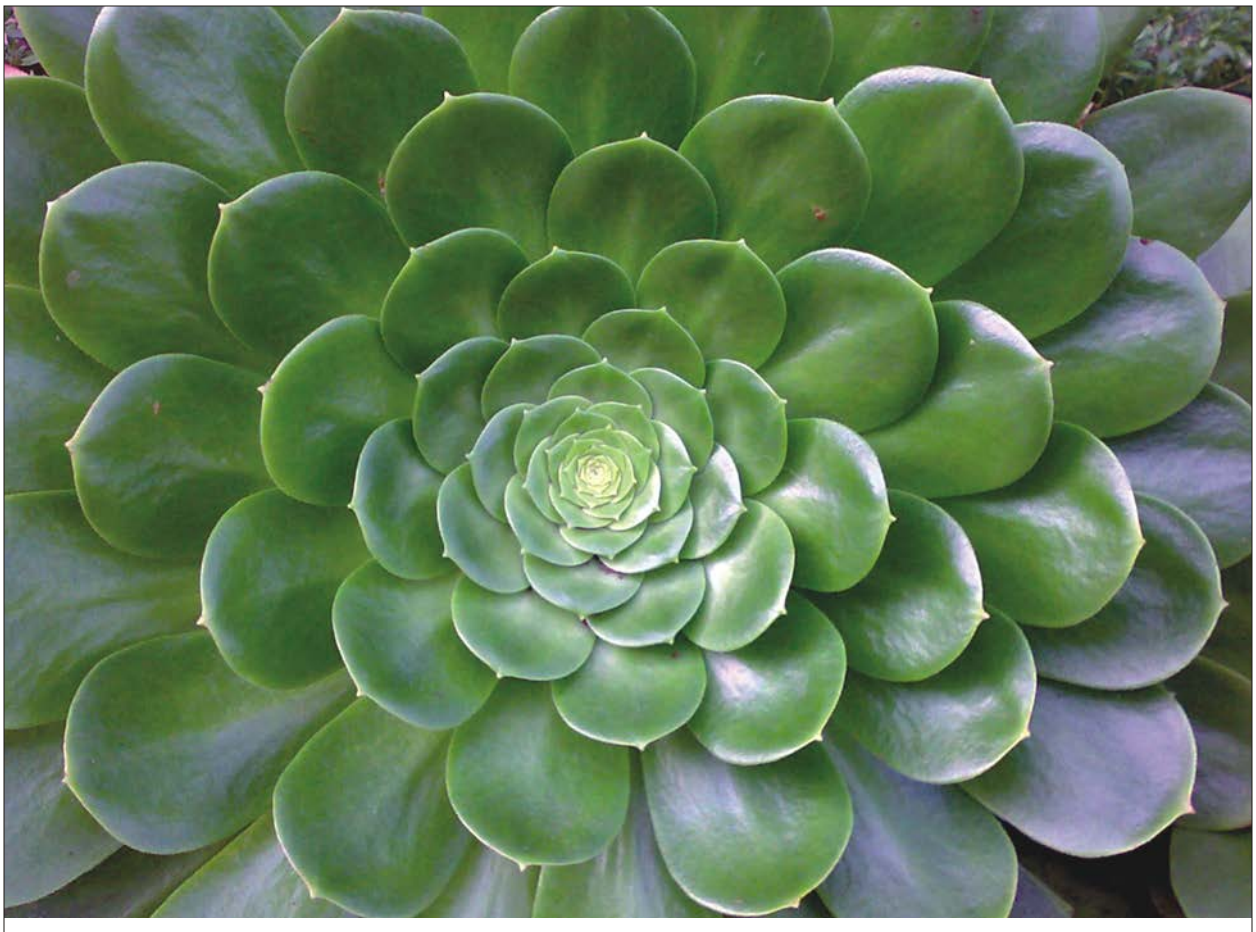

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Research Article
Improvement of Cucurbit Seed Health Status by Salicylic Acid and Fungicides

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Abstract

Background and Objectives: Infested seed can be the source of the pathogen for important diseases. Healthy or pathogen-free seeds are considered as the vital factor for the desired plant population and good harvest. The aim of the study was to examine the cucurbit seed associated fungi and efficacy of salicylic acid and some fungicides against these fungi. **Materials and Methods:** An investigation was carried out on the seed health status of 5 selected cucurbit seeds (Sweet gourd, *Cucurbita maxima*; Bottle gourd, *Lagenaria siceraria*; Cucumber, *Cucumis sativus*; Ridge gourd, *Luffa acutangula* and Snake gourd, *Trichosanthes cucumerina*) collected from 5 different sources of Bangladesh. Five identified (*Aspergillus flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.) and 2 unidentified fungi were isolated from cucurbit seeds and evaluate the efficacy of some selected chemicals (Dithane M-45 80 WP, Autostin 50 WDG, Tilt 250 EC and salicylic acid) against these isolated fungi. **Results:** The dry inspection revealed that the highest percentage of infected seeds found from snake gourd species and seeds collected from source 1. Blotter method stated that the maximum incidence found by *Aspergillus flavus* and *Rhizopus* sp. in all cucurbit species and seed sources. The seeds collected from source 1 and cucumber species showed the highest germination percentage. The efficacy of chemicals varied significantly in respect of isolated and identified fungi and the highest performance was shown by Tilt 250 EC. **Conclusion:** It was concluded that the seed health status of cucurbits seeds were not at a satisfactory level. Proper storage management with good chemicals can also give a satisfactory result.

Key words: Seed health, dry inspection method, blotter method, management, poisoned food technique

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the 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. However, seeds are an important factor to introduce plant pathogens into a new area and help to survive from one cropping season to another[1].

In the modern agriculture, seed health is a significant factor for plant growth and good harvest[2]. Seed is the main source of propagating materials for most of the food crops and its percentage is above ninety[3]. Pre and post-emergence death of grains and seedling vigor mainly affected by seed-borne fungi and thus hamper seed germination and plant morphology[4]. Seed borne pathogens are the initial factor to reduce crop yield by hampering seed quality and quantity[5]. In maximum countries, seed health testing routinely carried out to maintain seed quality and plant quarantine[6]. The first approach to manage seed borne diseases in plants is testing and detection of seed health[7]. The lack of high-quality seeds and the prevalence of seed-borne organisms are the main constraints in maintaining the crop production. Fakir[8] estimated that in Bangladesh more than 400 seed-borne diseases in 72 crops inflicting an estimated yield loss amounting to around 200 million US dollars annually.

Therefore, it is necessary to evaluate the health status of cucurbit seeds following different seed health testing method to maintain quality of seeds and needs to find out the effective technique to eliminate or destroy the seed borne pathogens to maintain economic value and growth of cucurbit plants. Chemical method is one the effective way to maintain seed borne pathogens. In plant disease resistance, salicylic acid (SA) may play a vital role during systemic acquired resistance (SAR)[9]. Amborabe *et al.*[10] found that mycelial growth of *Eutypa lata* inhibited by SA in both solid and liquid culture medium. Salicylic acid inhibited conidial germination and hyphae growth and development of *Sphaerotheca fuliginea*[11] and reduced spore viability of *Saccharomyces cerevisiae*[9]. Vega *et al.*[12] identified that, the germination of blastospores of *Paecilomyces fumosoroseus* inhibited by SA. Salicylic acid also affected on *Sclerotium rolfsii* and *Sclerotinia minor* by inhibiting sclerotial differentiation and growth[13]. Fungicides are chemicals used to kill, prevent or eradicate plants or seeds associated fungal infections[14]. Mancozeb inhibit spore germination[15] and interfere with different biochemical processes within the fungal cell cytoplasm and mitochondria[16] where, benzimidazole inhibits the synthesis of β-tubulin[17]. Triazole fungicide effect on sterol biosynthesis of fungi based on the inhibition of cytochrome P450-dependent enzyme activities[18].

Cucurbits are mostly grown in tropical and subtropical conditions but some of the species are grown in the temperate zone under artificial conditions like cucumber[19]. The Cucurbitaceae family ranks the highest among plant families for number and percentage of the species used as human food. These vegetables flourish under a temperature of about 18-30°C[20]. Cucurbits constitute a potent and important group of crops in Bangladesh. They are important for their low production cost, short duration of production and high nutritive value. Cucurbits are commonly cultivated vegetables in Bangladesh among the major vegetables and they play a prime role to supplement this shortage during the lean period. There are many factors responsible for low yield of these crops; disease and use of poor quality seeds play an important role. Richardson[21] reported that about 200 different seed-borne pathogens, including more than 100 fungi are responsible for causing diseases in different vegetable crops in the world. 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. For best crop, seed should be pure, viable and healthy. Keeping in view these facts, the present study was conducted to evaluate the cucurbit seed health status by properly detecting of seed borne pathogens and to find out the effective seed treating chemicals to regulate seed borne pathogens economically.

MATERIALS AND METHODS

Experimental period and sites: The investigation was conducted within one year during the period of July, 2016 to June, 2017 in the Seed Pathology Laboratory and Plant Disease Diagnostic Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh.

Collection of cucurbits seeds: Cucurbit seeds of 5 different species (Sweet gourd, *Cucurbita maxima*; Bottle gourd, *Lagenaria siceraria*; Cucumber, *Cucumis sativus*; Ridge gourd, *Luffa acutangula* and Snake gourd, *Trichosanthes cucumerina*) were collected from 5 different sources

(Siddik bazar, source 1; Mohammadpur bazar, source 2; Kochukhet bazar, source 3; Savar bazar, source 4 and BADC, source 5) of Bangladesh.

Seed health test

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

Inspection of incubated seed samples of blotter method: Seed samples consisting of 400 seeds were subjected to seed health analysis by blotter method following International Rules for Seed Health Testing[22]. In this method, 9 cm diameter plastic petri dish and Whatman No.1 filter paper was used. Four hundred seeds from each sample were taken randomly and placed on the moist filter paper in the petri dishes. In each petri dish 10 seeds were placed and altogether 40 petri dishes were needed for each seed sample. The petri dishes were incubated at 22±2°C for 7 days in the laboratory under the alternative cycle of 12 h NUV light and under 12 h of darkness for 7 days.

About 7 days after incubation the seeds were observed under a stereo microscope and the pathogens were identified following the key of Mathur and Kongsdal[23]. Appropriate keys[24] were consulted for identification of the microflora. The results were compiled as the incidence of the 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 a stereo microscope 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 a compound microscope at 100x and 400x for proper identification of fungi following the keys suggested by Chidambaram and Mathur[25]:

$$\text{Incidence of pathogen (\%)} = \frac{\text{No. of infected seeds associated with the seeds}}{\text{Total No. of seeds observed}} \times 100$$

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds observed}} \times 100$$

Isolation, purification and preservation of seed-borne fungal pathogens: The PDA medium was prepared as described by Mohsin *et al.*[26]. Isolation of the seed borne pathogens was carried out on PDA medium. The 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[27]. The pure cultures were also maintained on PDA slants kept at 5°C in a refrigerator for further studies.

Cultural and morphological characterization of isolated organisms: Cultures of all the isolates were studied for cultural and morphological variations. In terms of a number of conidia production and colony colour were observed in PDA medium. The conidia produced per unit surface area were estimated using haemacytometer, digital microscope (Model: Motic, BA-210) using the formula of Chauhan and Pandey[28] and modification of Mohsin *et al.*[29]:

$$\text{Conidia produced per unit surface} = \frac{\text{No. of conidia } (mL^{-1}) \times \text{Volume of water of suspension}}{\text{Total surface area of suspension}}$$

Management of isolated pathogens by poisoned food technique (cup method): Four different chemicals viz. Dithane M-45 80 WP, Autostin 50 WDG, Tilt 250 EC and salicylic acid (SA) were tested by following poisoned food technique *in vitro* condition to evaluate their efficacy on colony growth and mycelia formation of 5 different fungi viz. *Aspergillus flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. A 5 mL discs were scooped from the three places of the PDA medium by using a sterilized disc cutter maintaining the equal distance from the centre. An equal amount of chemicals was put into each hole of the medium and kept in refrigeration for 12 h to disperse the chemicals. Thereafter, one 5 mm block cut by sterilized block cutter from each fungal culture (7 days old) and placed at the centre of the petri plate, then kept at room temperature (25±2°C). Three days after inoculation, radial mycelial growths (cm) of all examined fungi in petri dishes were measured and recorded by taking an average of the colony diameters (length and breadth).

Statistical analysis: The investigation was conducted following completely randomized design (CRD) with 3 replications. Data collected during the experimental period were analyzed and tabulated following statistical

package STATISTIX-10. Treatment means were compared with least significant difference (LSD) test.

RESULTS

Seed health test of cucurbits by the dry inspection method: Significant variations were observed among the species and seed sources of cucurbits, in dry inspection method in respect of seed weight of the 400 seeds, apparently pure seed weight, infected seed weight and inert matter weight were observed (Table 1). The highest percent of infected seeds recorded from source 1 (among the seed sources) and snake gourd (among the cucurbit species), which were 33 and 34%, respectively. On the other hand, the lowest percent of infected seeds recorded from source 4 (22%) among the seed sources; which was statistically similar with source 2 (23%) and ridge gourd (25%) among the cucurbit species; which was statistically similar with sweet gourd (26%) and bottle gourd (27%), respectively (Fig. 1).

Seed health test of cucurbits by blotter method

Germination percentage of different species of cucurbits seeds collected from different seed sources: The highest seed germination percentages were found from source 1 (37%) among the seed sources and cucumber (42%) among the species. In contrary, the lowest seed germination percentages were recorded from source 4 (16%) among the seed sources and ridge gourd (7%) among the species (Fig. 2).

Prevalence of seed-borne pathogens in different species of cucurbits seeds collected from different seed sources: There are 5 identified fungi (*Aspergillus flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.), 2 unidentified fungi and 1 unidentified bacteria were observed from collecting cucurbit seeds of different species (Table 2, Fig. 3-7). The maximum incidence of *Aspergillus flavus* was recorded from source 1 (among the seed sources) and from ridge gourd (among the cucurbit species) which

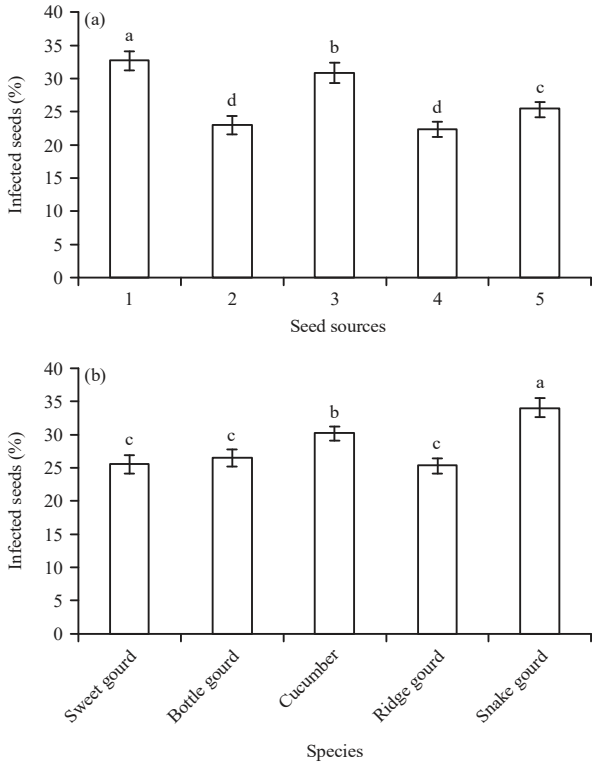

Fig. 1(a-b): Investigation of infection of seeds (%) by dry inspection method, (a) Seed sources and (b) Cucurbit species

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

Table 1: Seed health test of different species of cucurbits seeds by dry inspection method collected from different seed sources

Cucurbits seeds	Seed weight of 400 seeds (g)	Apparently pure seed weight (g)	Infected seed weight (g)	Inert matter weight (g)
Seed sources				
1	68.13^{a}	45.53^{c}	22.37^{a}	0.22^{b}
2	65.38^{b}	49.97^{a}	15.10^{d}	0.33^{ab}
3	65.45^{b}	44.32^{d}	20.27^{b}	0.85^{ab}
4	64.58^{b}	49.55^{a}	14.46^{d}	1.32^{a}
5	65.38^{b}	47.66^{b}	16.63^{c}	0.67^{ab}
Species				
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}
Level of significance	**	**	**	**

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

Table 2: Estimation of the percentage of pathogenic incidence of different species of cucurbit seeds by blotter seed health testing method collected from different seed sources

Cucurbit seeds	*Aspergillus flavus* (%)	*Aspergillus niger* (%)	*Rhizopus* sp. (%)	*Fusarium* sp. (%)	*Chaetomium* sp. (%)	Bacterial "ooze" (%)	Unknown 1 (%)	Unknown 2 (%)
Seed sources								
1	32.03^{a}	2.60^{c}	8.36^{d}	9.56^{a}	7.23^{b}	1.10^{a}	9.13^{e}	0.27^{ab}
2	20.40^{b}	13.30^{a}	5.73^{e}	3.46^{bc}	9.67^{a}	0.67^{ab}	59.37^{a}	0.47^{a}
3	22.06^{b}	13.46^{a}	39.06^{b}	3.83^{bc}	8.37^{ab}	0.03^{c}	38.63^{d}	0.00^{b}
4	16.63^{c}	1.47^{c}	22.47^{c}	4.60^{b}	6.93^{b}	0.00^{c}	43.60^{c}	0.00^{b}
5	20.40^{b}	7.43^{b}	43.50^{a}	2.83^{c}	0.50^{c}	0.30^{bc}	48.60^{b}	0.00^{b}
Species								
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}
Level of significance	**	**	**	**	**	**	**	**

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

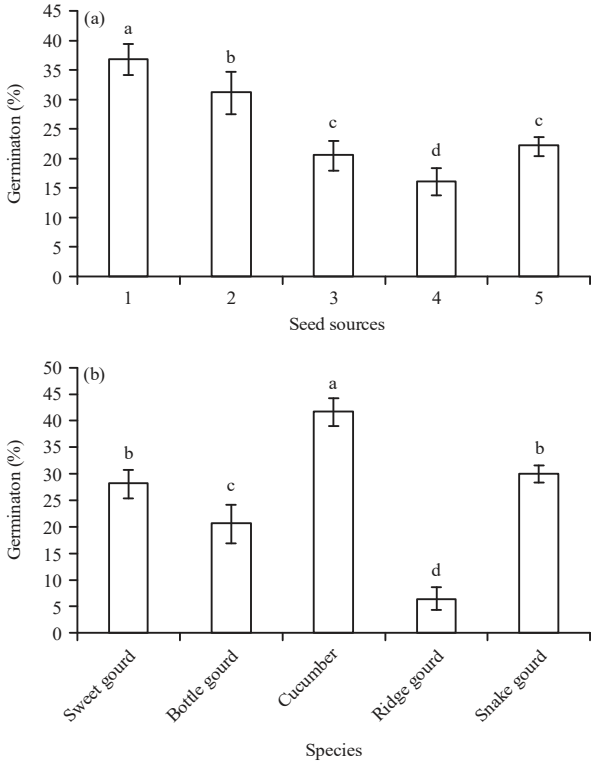

Fig. 2(a-b): Investigation of germination (%) of seeds by blotter method, (a) Seed sources and (b) Cucurbit species

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

were 32 and 42%, respectively. Contrary, the minimum incidence was recorded from source 4 (17%) among the seed sources and cucumber (7%) among the species; which was statistically similar with snack gourd (8%). *Aspergillus niger* incidence maximum found in the seeds collected from source 3 (13%) among the seed sources; which didn't statistically differ by source 2 (13%) and from sweet gourd (20%) among the species. On the other hand, the lowest incidence was recorded in seeds collected from source 4; which was statistically similar with source 1 and from snake gourd; which wasn't changed statistically with cucumber. Among the seed sources, the highest incidence of *Rhizopus* sp. was collected from source 5 seeds (43%) and among the species, the highest incidence found from sweet gourd (35%). In contrast, the lowest incidence was recorded from source 2 seeds (6%) and cucumber species (15%). The maximum incidence of *Fusarium* sp. was recorded in seeds collected from source 1 (10%) and snake gourd species (10%) whereas, the lowest incidence was found from source 5 seeds and bottle gourd species. Seeds collected from source 2 found the highest incidence (10%) of *Chaetomium* sp., which was statistically similar with source 3 (8%) whereas, the highest incidence of *Chaetomium* sp. was recorded from cucumber (11%) among the species. However, the lowest incidence was measured from source 5 seeds and bottle gourd species.

Bacterial incidence was the highest in source 1 seeds, which wasn't altered statistically with source 2. However, the highest bacterial incidence found from snake gourd which was statistically similar with ridge gourd. On the other hand, no incidence was found from source 4 seeds as well as the seeds of sweet gourd and bottle gourd species. The incidence of two unknown fungi also found in some species of cucurbit seeds.

Isolation, identification and characterization of isolated pathogens: About 7 different fungi were isolated and identified (Fig. 8). A number of spores and colony colour were

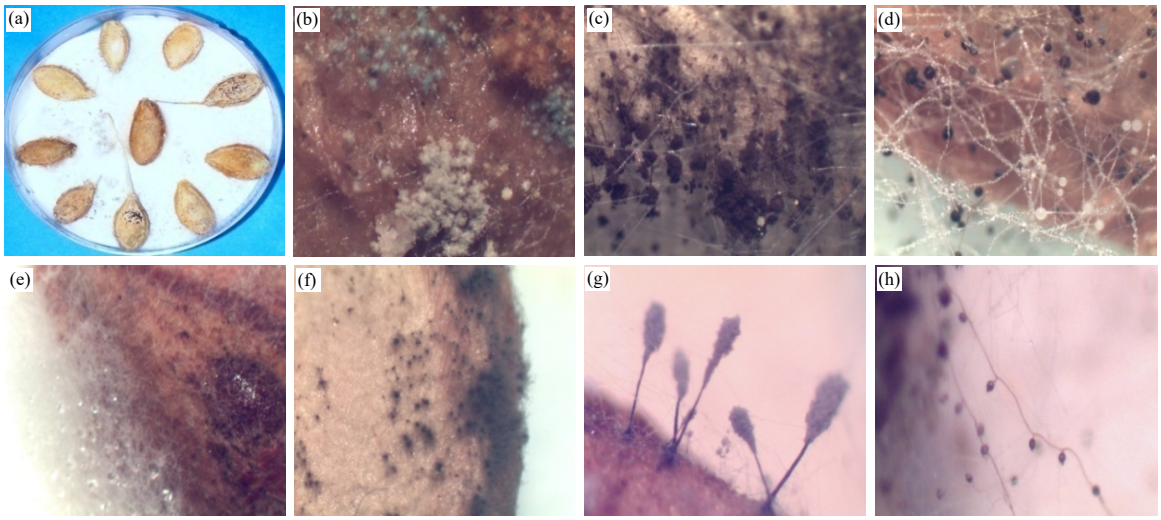

Fig. 3(a-h): Stereo microscopic view of pathogens on sweet gourd seed, (a) Sweet gourd seed in blotter paper, (b) *Aspergillus flavus*, (c) *Aspergillus niger*, (d) *Rhizopus* sp., (e) *Fusarium* sp., (f) *Chaetomium* sp., (g) Unknown-1 and (h) Unknown-2

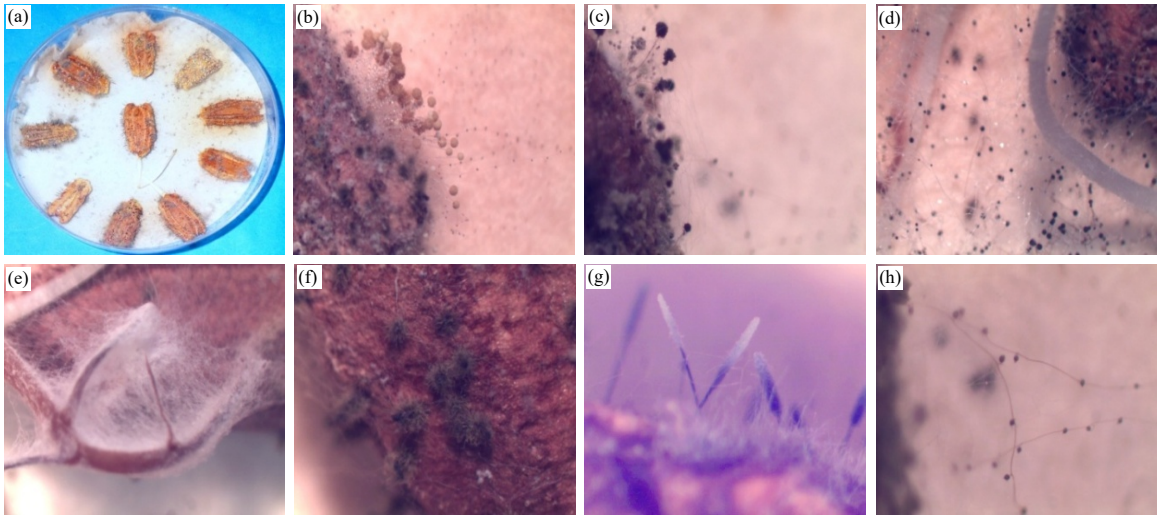

Fig. 4(a-h): Stereo microscopic view of pathogens on bottle gourd seed, (a) Bottle gourd seed in blotter paper, (b) *Aspergillus flavus*, (c) *Aspergillus niger*, (d) *Rhizopus* sp., (e) *Fusarium* sp., (f) *Chaetomium* sp., (g) Unknown-1 and (h) Unknown-2

observed at 5 days after inoculation (Table 3). From a pure culture medium number of spores per square millimetre in a petri dish were counted by using haemacytometer and digital microscope (Fig. 9). The highest number of spores (114.91×10^3 mm^{-2}) was found from *Aspergillus niger* and the lowest number of spores (3.11×10^3 mm^{-2}) was found in *Fusarium* sp., which was statistically similar with *Rhizopus* sp. (8.35×10^3 mm^{-2}). Different colony colour also observed and recorded from the pure culture of the pathogens.

***In vitro* evaluation of selected chemicals against isolated seed-borne fungi of cucurbits in poisoned food technique (cup method):** The results were compiled based on the inhibition of radial mycelium growth of every pathogen against 4 treatments (Dithane M-45 80 WP, Autostin 50 WDG, Tilt 250 EC and salicylic acid) along with control. Chemicals have a profound effect on reduction of radial mycelial growth of *Aspergillus flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp. and *Chaetomium* sp.

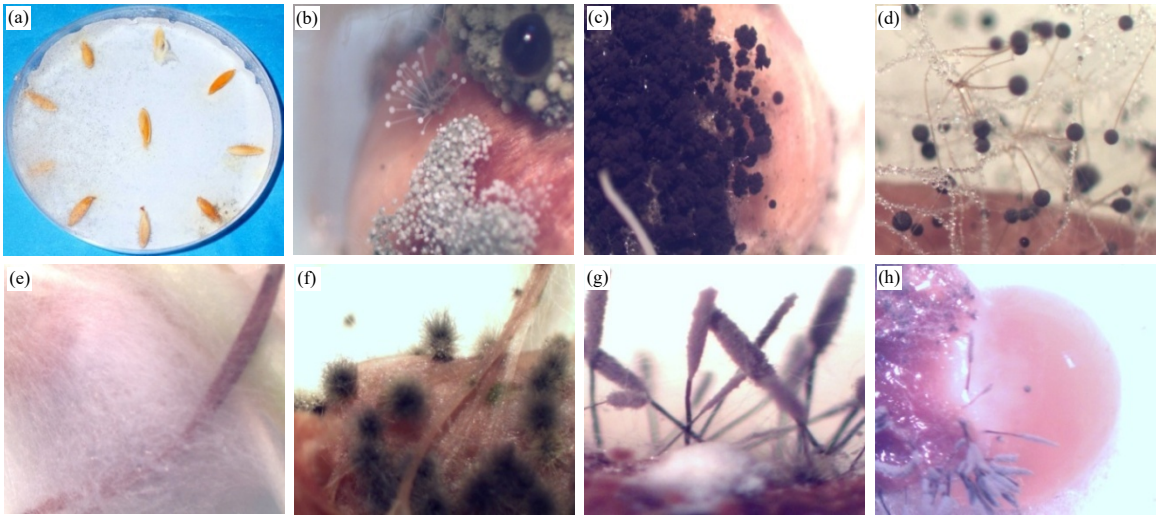

Fig. 5(a-h): Stereo microscopic view of pathogens on cucumber seed, (a) Cucumber seed in blotter paper, (b) *Aspergillus flavus*, (c) *Aspergillus niger*, (d) *Rhizopus* sp., (e) *Fusarium* sp., (f) *Chaetomium* sp., (g) Unknown-1 and (h) Bacterial "ooze"

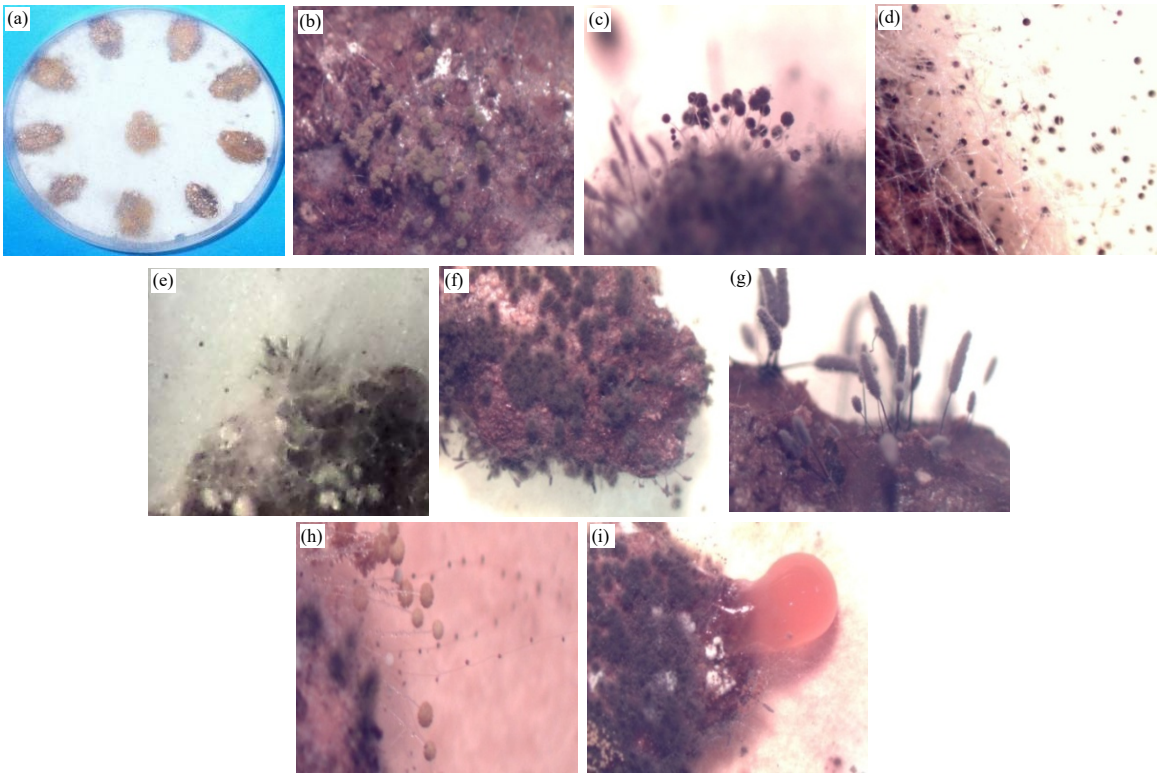

Fig. 6(a-i): Stereo microscopic view of pathogens on ridge gourd seed, (a) Ridge gourd seed in blotter paper, (b) *Aspergillus flavus*, (c) *Aspergillus niger*, (d) *Rhizopus* sp., (e) *Fusarium* sp., (f) *Chaetomium* sp., (g) Unknown-1, (h) Unknown-2 and (i) Bacterial "ooze"

(Fig. 10-14). The highest radial mycelial growth of *Aspergillus flavus* reduced by 90% compared with control, under the Tilt 250 EC, which was statistically similar with Autostin 50 WDG (80%). On the other hand, the lowest inhibition of mycelia growth found from Dithane M-45 80 WP (25%) compared with control followed by salicylic acid (47%) (Fig. 15a). Similarly, in the case of *Aspergillus niger* Tilt 250 EC inhibited the maximum radial mycelia growth (82%)

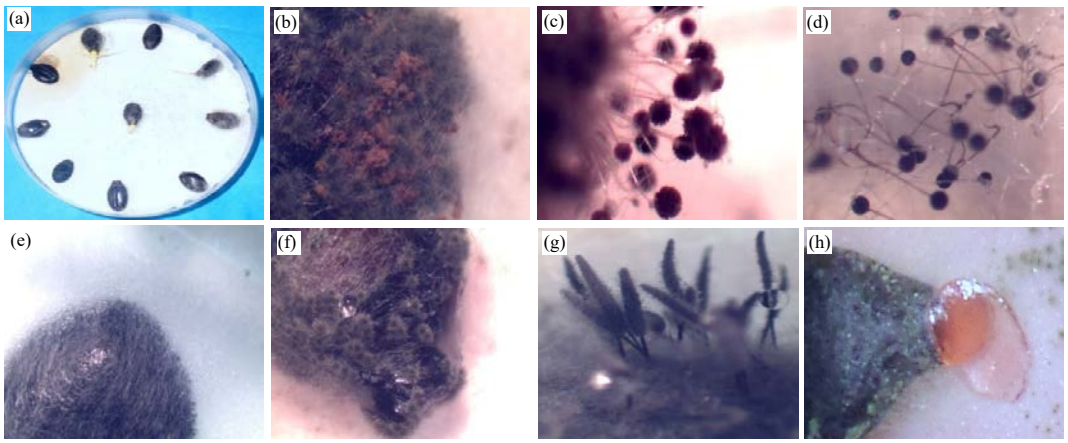

Fig. 7(a-h): Stereo microscopic view of pathogens on snake gourd seed, (a) Snake gourd seed in blotter paper, (b) *Aspergillus flavus*, (c) *Aspergillus niger*, (d) *Rhizopus* sp., (e) *Fusarium* sp., (f) *Chaetomium* sp., (g) Unknown-1 and (h) Bacterial "ooze"

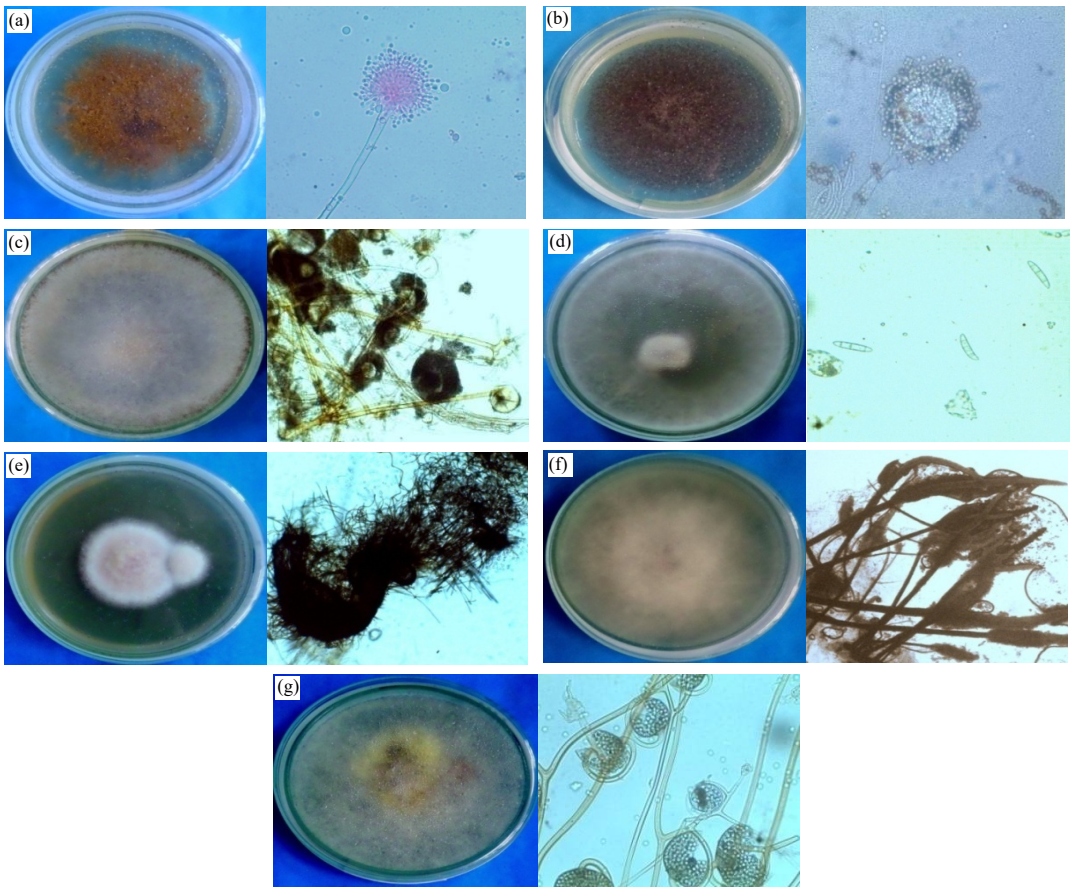

Fig. 8(a-g): Isolation of 7 pathogens in PDA media and identification under a compound microscope, (a) *Aspergillus flavus* (pure culture and compound microscopic view), (b) *Aspergillus niger* (pure culture and compound microscopic view), (c) *Rhizopus* sp. (pure culture and compound microscopic view), (d) *Fusarium* sp. (pure culture and compound microscopic view), (e) *Chaetomium* sp. (pure culture and compound microscopic view), (f) Unknown-1 (pure culture and compound microscopic view) and (g) Unknown-2 (pure culture and compound microscopic view)

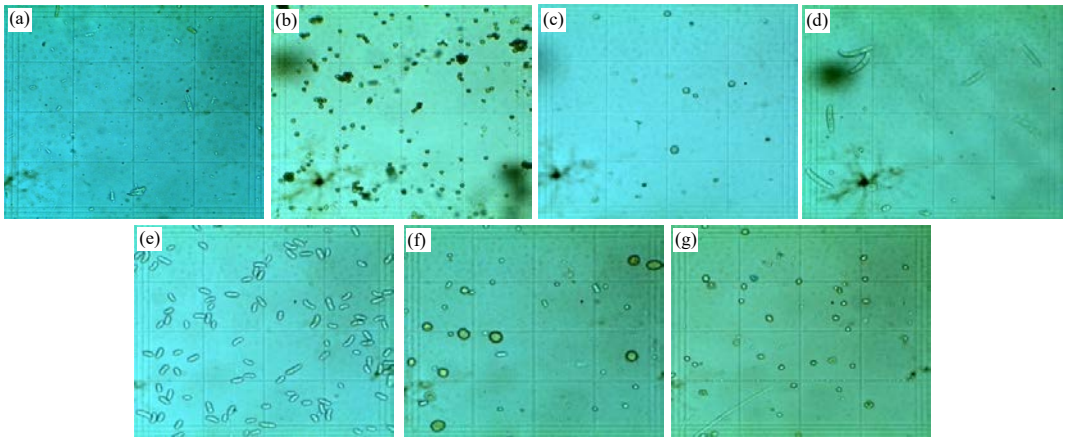

Fig. 9(a-g): Counting of 7 pathogen's conidia by using hemocytometer and digital microscope, (a) *Aspergillus flavus*, (b) *Aspergillus niger*, (c) *Rhizopus* sp., (d) *Fusarium* sp., (e) *Chaetomium* sp., (f) Unknown-1 and (g) Unknown-2

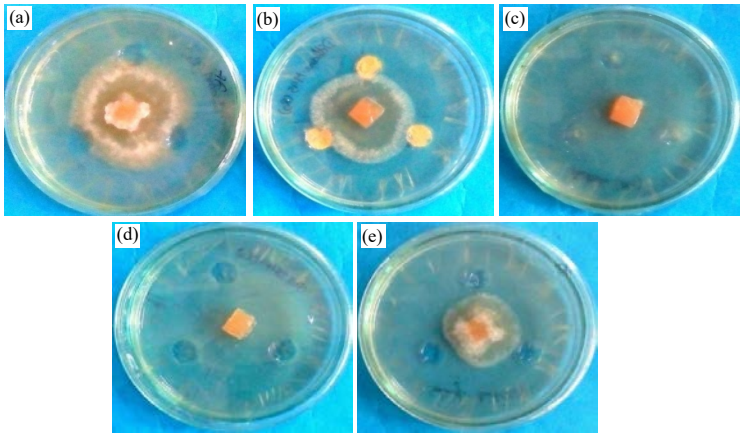

Fig. 10(a-e): Inhibition of radial mycelial growth of *Aspergillus flavus* by different chemicals at 3 days after inoculation, (a) Control, (b) Dithane M-45 80 WP, (c) Autostin 50 WDG, (d) Tilt 250 EC and (e) Salicylic acid

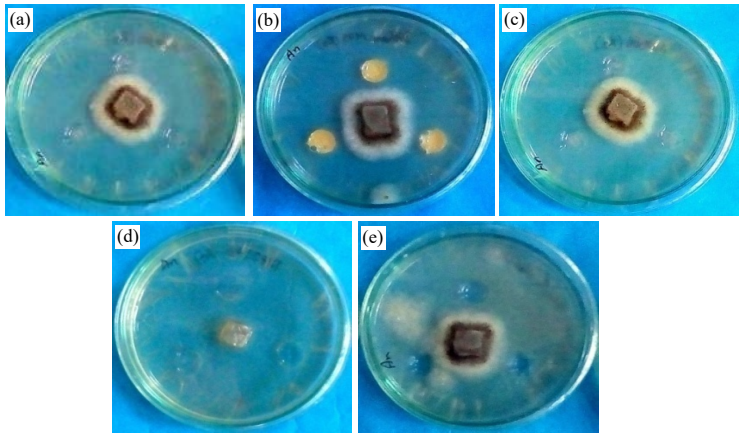

Fig. 11(a-e): Inhibition of radial mycelial growth of *Aspergillus niger* by different chemicals at 3 days after inoculation, (a) Control, (b) Dithane M-45 80 WP, (c) Autostin 50 WDG, (d) Tilt 250 EC and (e) Salicylic acid

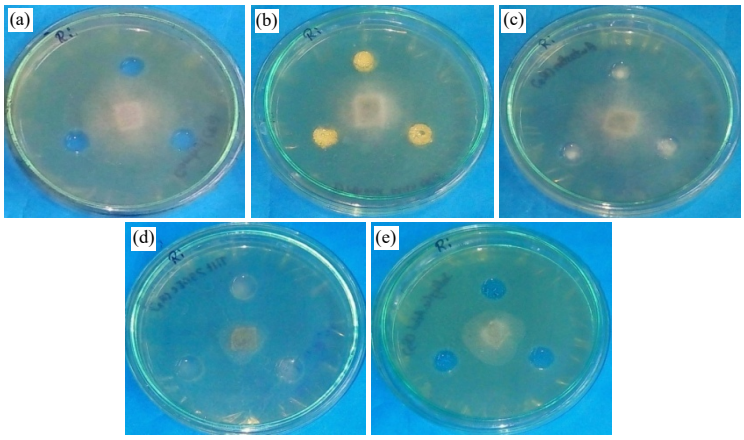

Fig. 12(a-e): Inhibition of radial mycelial growth of *Rhizopus* sp. by different chemicals at 3 days after inoculation, (a) Control, (b) Dithane M-45 80 WP, (c) Autostin 50 WDG, (d) Tilt 250 EC and (e) Salicylic acid

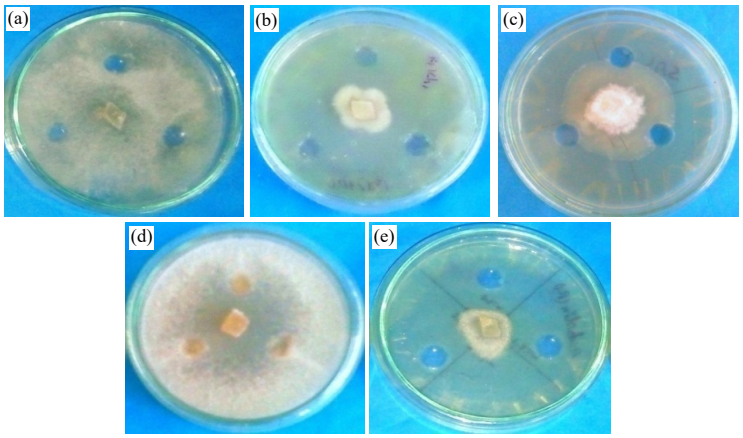

Fig. 13(a-e): Inhibition of radial mycelial growth of *Fusarium* sp. by different chemicals at 3 days after inoculation, (a) Control, (b) Dithane M-45 80 WP, (c) Autostin 50 WDG, (d) Tilt 250 EC and (e) Salicylic acid

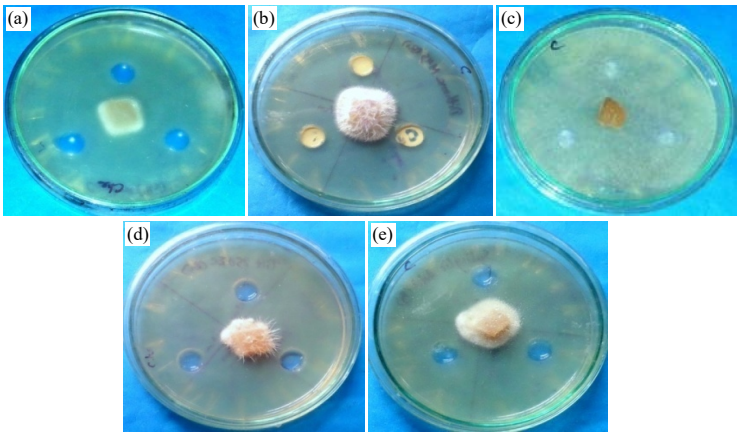

Fig. 14(a-e): Inhibition of radial mycelial growth of *Chaetomium* sp. by different chemicals at 3 days after inoculation, (a) Control, (b) Dithane M-45 80 WP, (c) Autostin 50 WDG, (d) Tilt 250 EC and (e) Salicylic acid

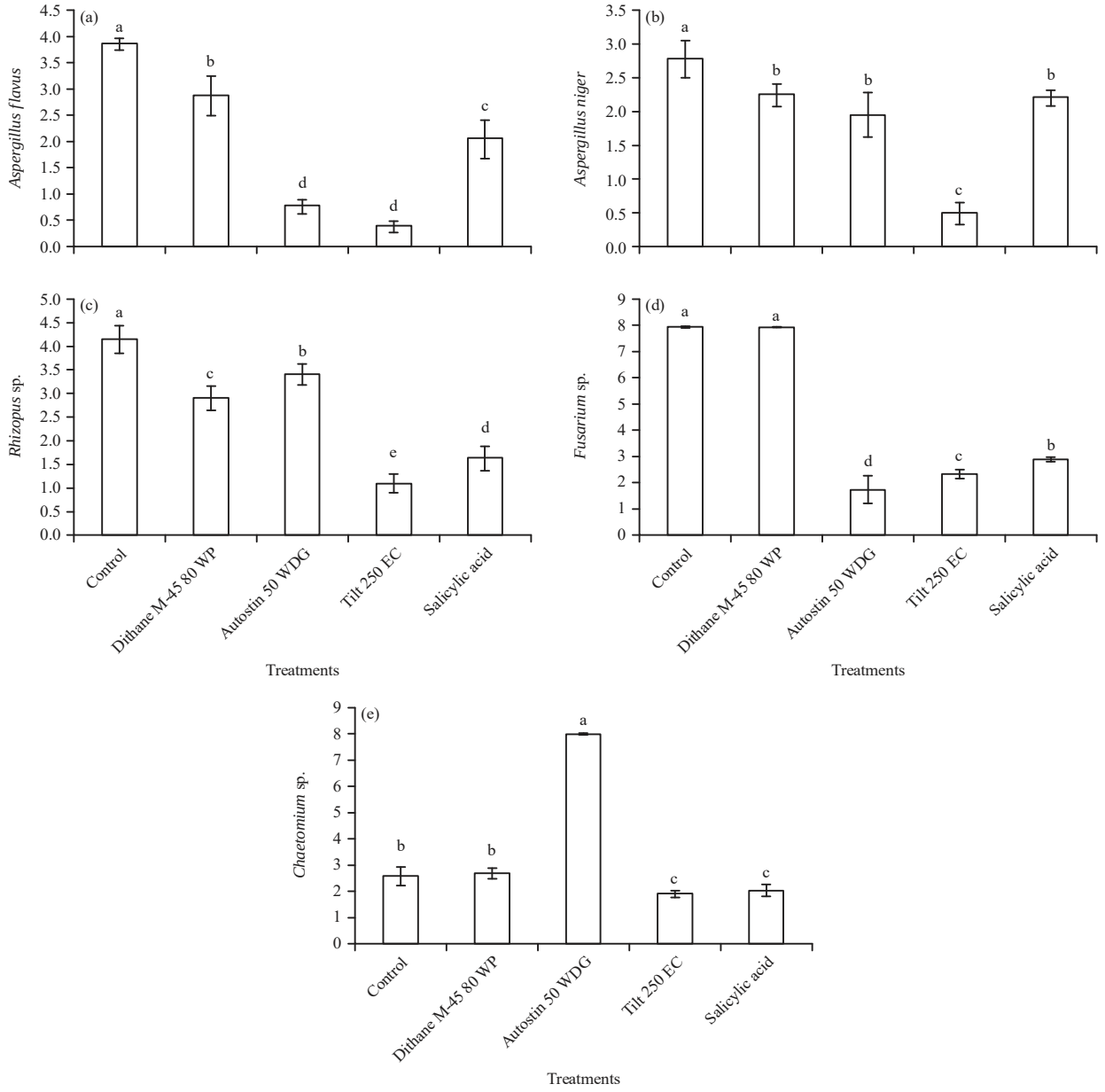

Fig. 15(a-e): Radial mycelial growth (cm) of isolated seed borne fungi of cucurbits at 3 days after application of different chemicals, (a) *Aspergillus flavus*, (b) *Aspergillus niger*, (c) *Rhizopus* sp., (d) *Fusarium* sp. and (e) *Chaetomium* sp.

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

Table 3: Cultural and morphological characterization of isolated seed borne fungi from cucurbits seeds

Pathogens	Number of spores mm^{-2} ($\times 10^3$)	Colony colour
Aspergillus flavus	50.95^{c}	Greenish yellow
Aspergillus niger	114.91^{a}	Black
Rhizopus sp.	8.35^{f}	Greyish white
Fusarium sp.	3.11^{f}	White
Chaetomium sp.	67.34^{b}	Brownish white
Unknown-1	20.56^{e}	White
Unknown-2	28.13^{d}	Yellowish white
Level of significance	**	

Means±SD were calculated from 3 replicates for each treatment, values with different letters are significantly different at $p \leq 0.05$ applying the Fisher's LSD test

compared with control. However, the minimum inhibition of radial mycelia growth found from Dithane M-45 80 WP (19%), which was statistically similar with salicylic acid (21%) and Autostin 50 WDG (30%), respectively (Fig. 15b). Compared with control, Tilt 250 EC reduced the maximum radial mycelial growth (74%) of *Rhizopus* sp., while the minimum radial mycelial growth reduced by Autostin 50 WDG (18%) (Fig. 15c). In case of *Fusarium* sp. the highest inhibition of radial mycelia growth found by Autostin 50 WDG (78%) compared with control whereas, the activity of Dithane M-45 80 WP was lowest and statistically similar with control (Fig. 15d). The highest inhibition of radial mycelia growth of *Chaetomium* sp. found from the Tilt 250 EC (26%) compared with the control which was statistically similar with salicylic acid (21%). In contrast, Autostin 50 WDG showed the lowest performance where fungus growth was more than the control (Fig. 15e).

DISCUSSION

Seed health testing is important to confirm the presence or absence of seed-borne pathogens and to detect seed-borne pathogens for crop disease management[30]. Seed health testing includes visual examination of seeds (externally or internally, macro or microscopically) for pathogen observation and seeds incubates on agar or blotter papers to identify pathogens[31]. Inspection of dry seed or direct examination is a semi-quantitative and qualitative method of seed health test where the fungi fruiting structure is observed under stereo microscope or fungal effect seen on the physical appearance of the seed[32]. It is possible to detect fungal spores, smut balls, sclerotia and other fruiting bodies (pycnidia, perithecia, etc.) by this method. In our study, we found maximum infected and deformed seeds from source 1 among the seed sources and snake gourd among the cucurbit species by observing fungal propagules and seed appearance. Agarwal and Sinclair[33] observed *Nigrospora* infected maize seeds having white streaks with black spore masses near the tips whereas, acremonium wilt infected sorghum seeds were completely deformed.

The blotter test is one of the most important techniques for seed-borne fungal pathogens in seed health testing[34]. Considerable amounts of seed-borne pathogenic fungi were detected by using blotter method. Among seven fungi five isolates were identified as *Aspergillus flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp. and two were unidentified during the investigation. Sultana and Ghaffar[35] identified 15 genera and 29 species of fungi from 10 bitter gourd seeds samples in Pakistan by ISTA techniques using the blotter and deep-freeze methods. Another report found that fungal species, including *Fusarium* sp., *Alternaria* sp., *Phoma* sp. and *Cladosporium* sp. were the most frequent on gourds, pumpkin and cucumber[36]. Earlier research reported that there were many seed-borne fungi found on cucurbits including *Alternaria alternata, Botryodiplodia theobromae, Chaetomium* sp., *Curvularia lunata, Drechslera tetramera, F. equiseti, F. moniliforme* and *F. solani* on gourd seeds[21]; on watermelon, squash, muskmelon, bitter gourd and cucumber[37,38]. In the present study, the incidence of *Aspergillus flavus*, *A. niger, Rhizopus* sp., *Fusarium* sp., *Chaetomium* sp. ranged from 7.40-42.10, 0.13-19.66, 15.13-34.76, 0.43-10.43 and 2.70-10.67%, respectively. This finding was partially supported by Hussain *et al.*[39], who evaluated the pathogenicity of two mostly prevailing fungal species *F. moniliforme* and *A. niger* on maize and found *F. moniliforme* was 50.2% pathogenicity on seeds and 6.55% in seedlings, whilst *A. niger* was 62.87% in seeds and 11.24% in seedlings. The present findings of the seed borne fungal organisms on seed germination were agreed with the information of the seed borne nature of the pathogen reported by Marley and Gbenga[40]. They conducted of pathogenicity test with 2 most frequently isolated fungi that are *F. moniliforme* and *A. niger* was carried out and showed pathogenic effects on seed 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 by Richardson[41] and Ahmad *et al.*[42]. *Fusarium moniliforme* was found to be highly infective by producing mycotoxins that are involved in retarding seed germination and seedling growth[43].

In this study, we observed the efficacy of chemicals (Dithane M-45 80WP, Autostin 50 WDG, Tilt 250 EC and salicylic acid) *in vitro* on the radial mycelial growth of the pathogens (*A. flavus*, *A. niger*, *Rhizophus* sp., *Fusarium* sp., *Chaetomium* sp.). It was revealed that all chemicals significantly inhibit the mycelial growth of the isolated fungi. Tilt 250 EC inhibited the maximum radial mycelia growth of *A. flavus*, *A. niger*, *Rhizophus* sp. and *Chaetomium* sp. whereas, Autostin 50 WDG reduced the maximum mycelial growth of *Fusarium* sp. An interesting result was found that salicylic acid (which is normally used in organic synthesis as a plant hormone) also significantly inhibited the growth all identified fungi and which result was better than most of the tested fungicides. Taskeen-Un-Nisa *et al.*[44] evaluated the effect of carbendazim, hexaconzol, bitertanol, myclobutanil, mancozeb, captan and zineb and extracts of *Allium sativum*, *Allium cepa* and *Mentha arvensis* on the inhibition of

mycelial growth and spore germination of *Fusarium oxysporum* and find that maximum inhibition in mycelial growth was observed in the hexaconozole. Similar finding was reported by Daradhiyar[45], Somner[46], Kalra and Sohi[47], Singh *et al.*[48], Patel *et al.*[49] and Banyal *et al.*[50] to other fungi.

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 a different percentage. So, the seed health status of cucurbits seeds needs to improve to reduce the introduction of a new pathogen. Farmers are therefore advised to collect the cucurbit seeds from reliable sources and should be treated before sowing.

SIGNIFICANCE STATEMENT

This study identified the pathogens associated with cucurbit seeds that will be helped to the farmer and researcher to know the present situation about the cucurbit seed borne pathogens, which will be helped the researcher to invent the proper management technique. We also observed that salicylic acid plays a vital role to regulate the growth of seed borne pathogens. So, it is recommended that the use of salicylic acid as a seed treating chemicals will be managed seed borne pathogens as well as improved plant growth by activating plant growth hormone.

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