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Chitosan and yeast elicitor in suppressing seed-borne fungi of cucurbitaceous vegetables

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Abstract

Experiments were conducted under laboratory condition to examine the efficacy of Chitosan and Yeast Elicitor to suppress the growth of seed-borne fungi of cucurbitaceous vegetables. Seeds of bottle gourd, sweet gourd, snake gourd, wax gourd and cucumber were collected from seed traders of Mymensingh districts and different seed borne fungi were isolated, purified and identified. Fourteen fungal species belonging to twelve genera consisting of *Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Fusarium moniliforme, Fusarium oxysporum, Phoma exigua, Rhizopus stolonifer*, *Macrophomina phaseolina, Penicillium spp.*, *Curvularia lunata, Chaetomium spp., Colletotrichum spp., Cercospora spp.* and *Alternaria alternata* were isolated and identified. Four concentrations of Chitosan and Yeast Elicitors solutions (200, 500, 1000 & 2000 ppm) including one positive control Vitavax-200 WP (0.35%) were evaluated for controlling seed-borne fungi. Among the seed treating agents Chitosan (2000 ppm) and Yeast Elicitor (2000 ppm) showed better performance in suppressing the seed-borne fungi. Chitosan (2000 ppm) showed superior performance than Yeast Elicitor (2000 ppm). Results from the present study revealed that application of elicitors as seed treatment is a potential alternative of chemical fungicide for selective vegetables.

Introduction

Cucurbits are important vegetable crops, not only in Bangladesh, but also in many other countries all over the world. Cucurbits belong to Cucurbitaceae family, which include bottle gourd *(Lagenarea siceraria L)*, sweet gourd (*Cucurbita moschata)*, snake gourd (*Trichosanthes cucumeria*), wax gourd (*Benincasa hispida)* and cucumber (*Cucumis sativus*) etc.

Cucurbits are commonly exposed to attack by many serious soil-borne and seed-borne pathogens. 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, seed borne fungi are responsible for poor quality seeds in many crops (Neergaard, 1979).

For suppressing seed-borne fungi various elicitors can be used. Yeast Elicitor and Chitosan (β-1,4 linked D-glucosamine) are two important bio-polymers, can be commercially derived from various crustaceans commonly from the exoskeleton of shrimps and crabs (Boonlertnirun *et al.,* 2008). These two products can modulate various cellular function including reactive oxygen production, ion channel activity through phosphorylation and dephosphorylation of target protein, stomatal movement, upregulation of pathogenesis related genes (Khokon *et al*. 2010). Both Yeast Elicitor and Chitosan can be used as seed treating agents and foliar application of these components can induce resistance to overcome the seedling diseases as well as final crop production (Mondal *et al.,* 2013). Properties of chitosan for inhibition of pathogenic bacteria and fungi in antimicrobial films and edible coatings are used. Antimicrobial activity of chitosan resulting from positively charged amino groups. This group responds to negatively charged cell membranes of microorganisms. This reaction leads to the leakage of intracellular protein components and other microorganism components (Yarahmadi *et al*. 2014).Yeast extract (YE) is an important elicitor and is found to be rich in vitamin B-complex. It also contains essential components like chitin, N-acetyl-glucosamine oligomers, glucan, glycopeptides and ergosterol (Boller, 1995); these compounds elicit plant defense responses by triggering metabolite synthesis (Putalun *et al.,* 2007; Cai *et al.,* 2012). Yeast Extract has successfully been used in culture and overproduction of important phytocompounds were observed in several studied of plant genera (Prakash and Srivastava, 2008; Zhao *et al.,* 2010; Cai *et al.,* 2012).

Therefore, the objective of the research was to know the potentiality of chitosan and yeast elicitor in suppressing seed-borne fungi isolated from bottle gourd, sweet gourd, snake gourd, wax gourd and cucumber.

Materials and Methods

The experiments were conducted in the Laboratory of Biosignaling, Bioactive Compounds and Bio

formulation, Plant Disease Diagnostic Clinic (PDDC), Department of Plant Pathology and Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensigh-2202 during the period from October, 2015 to November, 2016.

Seeds of bottle gourd, sweet gourd, snake gourd, wax gourd and cucumber were collected from the farmers of Mymensingh districts. These seeds were stored in zip-lock bags in refrigerator for further studies.

Blotter method was followed according to ISTA rules for seed health testing (ISTA, 2006) for detection of seed-borne fungi. After 7 days each individual incubated seed was observed under stereo-binocular microscope at 16x and 25x magnifications in order to record the incidence of seed borne fungi. For proper identification of fungi, temporary slides were prepared from the fungal colony and observed under compound microscope and identified with the help of keys suggested by Ellis (1971) and Neergard (1979).

For Chitosan (0.3% solution) preparation, 3g Chitosan was dissolved in concentrated (98%) acetic acid diluted by water to a volume of 1000 mL and 3000 ppm Chitosan stock solution was prepared. From the stock solution 200 ppm, 500 ppm, 1000 ppm & 2000 ppm chitosan solutions were prepared. For Yeast Elicitor (0.3% solution) preparation, Yeast *(Saccharomyces cerevisiae)* was cultured in YEPDA broth (Yeast extract 1%, Peptone 2% and Dextrose 2%). Erlenmeyer flask (250 mL) having all the ingredients was incubated with shaking on an orbital platform shaker at 30^{o}C and 140 rpm for 72 hrs for collecting filtrate from the yeast. After 72 hrs the broth was filtrated and filtrate were collected and mixed with ethanol solution following the key of Ari and Cakir (2009). By this procedure 3000 ppm Yeast Elicitor stock solution was prepared. From the stock solution 200 ppm, 500 ppm, 1000 ppm & 2000 ppm Yeast Elicitor solution were prepared.

Seed priming with Chitosan and Yeast Elicitor

Seeds were dipped in respected Chitosan and Yeast Elicitor solution for 2 hrs at room temperature and then seeds were placed in Blotting Paper following ISTA rules for seed testing (ISTA, 2006). As a positive control, seed treatment with Vitavax-200 WP was carried out following the method of Islam *et al.* (2001).

Statistical analysis

The data were analyzed following completely randomized design (CRD) with three replications by using the M-STAT C statistical software.

Results

Effect of Seed Priming with Chitosan and Yeast Elicitor on the Association of Seed-borne Fungi

Total twelve genera of seed borne fungi were observed associated with tested seeds of cucurbits. *Fusarium*, *Macrophomina*, *Colletotrichum*, *Aspergillus*, *Curvularia*, *Botrytis*, *Rhizopus*, *Phoma, Alternaria, Penicillium, Chaetomium* and *Cercospora* were predominantly associated at various intensity with most of the seed samples (Table 1–5).

In Bottle Gourd, eight fungi viz., *Aspergillu flavus, Botrytis cinerea*, *Aspergillus niger, Fusarium moniliforme*, *Fusarium oxysporum*, *Phoma exigua, Rhizopus stolonifer* and *Macrophomina phaseolina* were detected in T_0 (Control), while the least seed-borne fungal infections were recorded in T_4 (2000 ppm Chitosan), T_7 (1000 ppm Yeast Elicitor) and T_8 (2000 ppm Yeast Elicitor) followed by T_3 (1000 ppm Chitosan) and T_6 (500 ppm Yeast Elicitor). The prevalence of *Aspergillus flavus* (13%) was the most predominant fungus followed by *Botrytis cinerea* (9.6%), *Aspergillus niger* (8.2%), *Fusarium moniliforme* (7.7%) and *Fusarium oxysporum* (5.4%).

In Sweet Gourd, ten fungi viz., *Rhizopus stolonifer, Fusarium moniliforme, Aspergillus niger, Phoma exigua, Aspergillus flavus, Macrophomina phaseolina, Botrytis cinerea, Fusarium oxysporum, Penicillium spp.* and *Curvularia lunata* were recorderd in T_0 (Control), while the least seed-borne fungal infections were recorded in T_4 (2000 ppm Chitosan) followed by T_6 (500 ppm Yeast Elicitor), T_7 (1000 ppm Yeast Elicitor) and T_8 (2000 ppm Yeast Elicitor). The prevalence of *Rhizopus stolonifer* (28.1%) was the most predominant fungus followed by *Fusarium moniliforme* (12%), *Aspergillus niger* (7.4%), *Phoma exigua* (3.4%) and *Aspergillus flavus* (1.4%).

In Snake Gourd, eight fungi viz., *Fusarium moniliforme, Aspergillus flavus, Fusarium oxysporum, Chaetomium spp., Rhizopus stolonifer, Botrytis cinerea, Aspergillus niger* and *Macrophomina phaseolina* were recorded in T_0 (Control), while the least seed-borne fungal infections were recorded in T_4 (2000 ppm Chitosan) followed by T_5 (200 ppm Yeast Elicitor), T_6 (500 ppm Yeast Elicitor), T_7 (1000 ppm Yeast Elicitor) and T_8 (2000 ppm Yeast Elicitor). The prevalence of *Fusarium moniliforme* (35.6%) was the most predominant fungus followed by *Aspergillus flavus* (23.3%), *Fusarium oxysporum* (8.2%), *Chaetomium spp.* (7.4%) and *Rhizopus stolonifer* (5.4%).

In Wax Gourd, ten fungi viz., *Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus, Botrytis cinerea, Macrophomina phaseolina, Phoma exigua, Fusarium moniliforme, Colletotrichum spp.*, *Curvularia lunata* and *Fusarium oxysporum* were detected in T_0 (Control), while the least seed-borne fungal infections were recorded in T_4 (2000 ppm Chitosan) followed by T_3 (1000 ppm Chitosan) and T_8 (2000 ppm Yeast Elicitor). The prevalence of *Rhizopus stolonifer* (15.2%) was the most predominant fungus followed by *Aspergillus niger* (11.9%), *Aspergillus flavus* (9.3%) and *Botrytis cinerea* (6.9%).

In Cucumber, eleven fungi viz., *Fusarium moniliforme, Aspergillus flavus, Fusarium oxysporum, Botrytis cinerea, Rhizopus stolonifer, Macrophomina phaseolina, Penicillium spp., Phoma exigua, Colletotrichum spp., Cercospora spp.* and *Alternaria alternata* were observed in T_0 (Control), while the least seed-borne fungal infections were recorded in T_8 (2000 ppm Yeast Elicitor), followed by T_4 (2000 ppm Chitosan). The prevalence of *Fusarium moniliforme* (21.1%) was the most predominant fungus followed by *Aspergillus flavus* (20.9%), *Fusarium oxysporum* (7.6%) and *Botrytis cinerea* (6.0%).

Table 1. Effect of Chitosan and Yeast Elicitor on prevalence of seed-borne fungi of bottle gourd

Treatment	Prevalence of Seed-borne fungi (%)							
	Botrytis cinerea	*Fusarium moniliforme*	*Macrophomina phaseolina*	*Fusarium oxysporum*	*Phoma exigua*	*Rhizopus stolonifer*	*Aspergillus niger*	*Aspergillus flavus*
T_0 (Control)	66 a	30 a	4 a (11.54)	24 a	14 a (21.97)	6 a (14.18)	60 a	70 a (56.79)
T_1 (200ppm CS)	10 c (18.43)	15 b (22.79)	0 b (0.70)	15 b (22.70)	0 b (0.70)	4 b (11.54)	20 b (26.57)	25 b
T_2 (500ppm CS)	0 d (0.70)	12 c (20.27)	0 b (0.70)	0 e (0.70)	0 b (0.70)	3 c (9.97)	2 c (8.13)	10 c (18.43)
T_3 (1000ppm CS)	0 d (0.70)	10 d (18.43)	0 b (0.70)	0 e (0.70)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
T_4 (2000ppm CS)	0 d (0.70)	0 e (0.70)	0 b (0.70)	0 e (0.70)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
T_5 (200ppm YES)	20 b (26.57)	10 d (18.43)	0 b (0.70)	10 c (18.43)	0 b (0.70)	0 d (0.70)	0 d (0.70)	25 b
T_6 (500ppm YES)	0 d (0.70)	0 e (0.70)	0 b (0.70)	5 d (12.92)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
T_7 (1000ppm YES)	0 d (0.70)	0 e (0.70)	0 b (0.70)	0 e (0.70)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
T_8 (2000ppm YES)	0 d (0.70)	0 e (0.70)	0 b (0.70)	0 e (0.70)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
T_9 (Vitavax-200 WP)	0 d (0.70)	0 e (0.70)	0 b (0.70)	0 e (0.70)	0 b (0.70)	0 d (0.70)	0 d (0.70)	0 d (0.70)
$LSD_{0.05}$	1.66	1.53	0.290	0.880	0.541	0.546	0.572	0.795
CV (%)	8.41	7.95	9.73	6.29	11.24	7.92	3.22	3.61

Values within the same column having a common letter(s) do not differ significantly (P≥0.01)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figures in the parentheses are arcsine transformed values

Table 2. Effect of Chitosn and Yeast Elicitor on prevalence of seed-borne fungi of sweet gourd

Treatment	Prevalence of Seed-borne fungi (%)									
	Fusarium moniliforme	*Fusarium oxysporum*	*Curvularia lunata*	*Aspergillus niger*	*Aspergillus flavus*	*Macrophomina phaseolina*	*Rhizopus stolonifer*	*Phoma exigua*	*Penicillium spp*	*Botrytis cinerea*
T_0 (Control)	30a	4a (11.54)	2a (8.13)	34a	6a (14.18)	12a (20.27)	56a	34a	2a (8.13)	4a (11.54)
T_1 (200ppm CS)	22d	0b (0.7)	0b (0.7)	25b	4b (11.54)	0b (0.7)	40b	0b (0.7)	1b (5.74)	0c (0.7)
T_2 (500ppm CS)	21e	0b (0.7)	0b (0.7)	10c (18.43)	3c (9.97)	0b (0.7)	35c	0b (0.7)	0c (0.7)	0c (0.7)
T_3 (1000ppm CS)	20b (26.56)	0b (0.7)	0b (0.7)	5d (12.92)	0e (0.7)	0b (0.7)	30d	0b (0.7)	0c (0.7)	0c (0.7)
T_4 (2000ppm CS)	2g (8.13)	0b (0.7)	0b (0.7)	0e (0.7)	0e (0.7)	0b (0.7)	5g (12.92)	0b (0.7)	0c (0.7)	0c (0.7)
T_5 (200ppm YES)	20b (26.56)	0b (0.7)	0b (0.7)	0e (0.7)	3c (9.97)	0b (0.7)	30d	0b (0.7)	0c (0.7)	2b (8.13)
T_6 (500ppm YES)	15c (22.79)	0b (0.7)	0b (0.7)	0e (0.7)	0e (0.7)	0b (0.7)	25e	0b (0.7)	0c (0.7)	0c (0.7)
T_7 (1000ppm YES)	10f (18.43)	0b (0.7)	0b (0.7)	0e (0.7)	0e (0.7)	0b (0.7)	20e (26.56)	0b (0.7)	0c (0.7)	0c (0.7)
T_8 (2000ppm YES)	2g (8.13)	0b (0.7)	0b (0.7)	0e (0.7)	0e (0.7)	0b (0.7)	15f (22.79)	0b (0.7)	0c (0.7)	0c (0.7)
T_9 (Vitavax-200 WP)	0h (0.7)	0b (0.7)	0b (0.7)	0e (0.7)	1d (5.74)	0b (0.7)	25e	0b (0.7)	0c (0.7)	0c (0.7)
$LSD_{0.05}$	0.592	0.541	0.076	1.10	0.832	1.07	1.86	1.08	0.544	0.295
CV (%)	1.89	17.81	3.05	6.84	8.91	23.80	3.61	15.71	16.38	6.87

Values within the same column having a common letter(s) do not differ significantly (P≥0.01)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figures in the parentheses are arcsine transformed values

Table 3. Effect of Chitosn and Yeast Elicitor on prevalence of seed-borne fungi of snake gourd seeds

Treatment	Prevalence of Seed-borne fungi (%)							
	Aspergillus flavus	*Fusarium oxysporum*	*Fusarium moniliforme*	*Aspergillus niger*	*Rhizopus stolonifer*	*Macrophomina phaseolina*	*Botrytis cinerea*	*Chaetomium spp*
T_0 (Control)	68a	42a	76a (60.67)	18a (25.10)	8a (16.43)	2a (8.13)	42a	74a (59.34)
T_1 (200ppm CS)	45b	10c (18.43)	50b	15b (22.79)	8a (16.43)	0b (0.70)	0b (0.70)	0b (0.70)
T_2 (500ppm CS)	40c	0d (0.70)	45c	5c (12.92)	7a (15.34)	0b (0.70)	0b (0.70)	0b (0.70)
T_3 (1000ppm CS)	35d	0d (0.70)	15f (22.79)	0d (0.70)	3c (9.97)	0b (0.70)	0b (0.70)	0b (0.70)
T_4 (2000ppm CS)	0e (0.70)	0d (0.70)	0g (0.70)	0d (0.70)	0d (0.70)	0b (0.70)	0b (0.70)	0b (0.70)
T_5 (200ppm YES)	0e (0.70)	0d (0.70)	50b	0d (0.70)	8a (16.43)	0b (0.70)	0b (0.70)	0b (0.70)
T_6 (500ppm YES)	0e (0.70)	0d (0.70)	45c	0d (0.70)	7a (15.34)	0b (0.70)	0b (0.70)	0b (0.70)
T_7 (1000ppm YES)	0e (0.70)	0d (0.70)	40d	0d (0.70)	5b (12.92)	0b (0.70)	0b (0.70)	0b (0.70)
T_8 (2000ppm YES)	0e (0.70)	0d (0.70)	35e	0d (0.70)	4b (11.54)	0b (0.70)	0b (0.70)	0b (0.70)
T_9 (Vitavax-200 WP)	45b	30b	0g (0.70)	0d (0.70)	4b (11.54)	0b (0.70)	0b (0.70)	0b (0.70)
$LSD_{0.05}$	1.31	1.10	2.01	1.25	1.44	0.093	1.18	1.23
CV (%)	3.28	6.79	3.38	11.19	6.70	3.59	14.42	11.08

Values within the same column having a common letter(s) do not differ significantly (P≥0.01)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figures in the parentheses are arcsine transformed values

Table 4. Effect of Chitosn and Yeast Elicitor on prevalence of seed-borne fungi of wax gourd

Treatment	Percent Prevalence of Seed-borne fungi (%)									
	Fusarium oxysporum	*Macrophominaphaseolina*	*Aspergillus niger*	*Rhizopus stolonifer*	*Fusarium moniliforme*	*Curvularia lunata*	*Phoma exigua*	*Colletotrichum spp*	*Aspergillus flavus*	*Botrytis cinerea*
T_0 (Control)	12a (20.27)	40a	54a	52a	8a (16.43)	18a (25.10)	38a	24a	22a	24b
T_1 (200ppm CS)	0b (0.70)	0b (0.70)	40b	15d (22.79)	2e (8.13)	0b (0.70)	0b (0.70)	0b (0.70)	10c (18.43)	0e (0.70)
T_2 (500ppm CS)	0b (0.70)	0b (0.70)	20c (26.56)	10e (18.43)	1f (5.74)	0b (0.70)	0b (0.70)	0b (0.70)	8d (16.43)	0e (0.70)
T_3 (1000ppm CS)	0b (0.70)	0b (0.70)	5d (12.92)	0f (0.70)	0g (0.70)	0b (0.70)	0b (0.70)	0b (0.70)	6e (14.18)	0e (0.70)
T_4 (2000ppm CS)	0b (0.70)	0b (0.70)	0e (0.70)	0f (0.70)	0g (0.70)	0b (0.70)	0b (0.70)	0b (0.70)	5f (12.92)	0e (0.70)
T_5 (200ppm YES)	0b (0.70)	0b (0.70)	0e (0.70)	30b	8a (16.43)	0b (0.70)	0b (0.70)	0b (0.70)	15a (22.79)	20a (26.56)
T_6 (500ppm YES)	0b (0.70)	0b (0.70)	0e (0.70)	25c	7b (15.34)	0b (0.70)	0b (0.70)	0b (0.70)	12b ()20.27	15c (22.79)
T_7 (1000ppm YES)	0b (0.70)	0b (0.70)	0e (0.70)	20c (26.56)	6c (14.18)	0b (0.70)	0b (0.70)	0b (0.70)	10c (18.43)	10d (18.43)
T_8 (2000ppm YES)	0b (0.70)	0b (0.70)	0e (0.70)	0f (0.70)	3d (9.97)	0b (0.70)	0b (0.70)	0b (0.70)	5g	0e (0.70)
T_9 (Vitavax-200 WP)	0b (0.70)	0b (0.70)	0e (0.70)	0f (0.70)	0g (0.70)	0b (0.70)	0b (0.70)	0b (0.70)	0h (0.70)	0e (0.70)
$LSD_{0.05}$	0.685	1.08	1.24	1.63	0.807	0.594	1.35	1.29	1.12	1.14
CV (%)	15.16	13.73	5.29	5.40	5.37	11.12	17.86	25.13	4.36	6.99

Values within the same column having a common letter(s) do not differ significantly (P≥0.01)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figures in the parentheses are arcsine transformed values

Table 5. Effect of Chitosn and Yeast Elicitor on prevalence of seed-borne fungi of cucumber

Treatment	Percent Prevalence of Seed-borne fungi (%)										
	Botrytis cinerea	*Fusarium moniliforme*	*Macrophomi-napha seolina*	*Fusarium oxysporum*	*Aspergillus flavus*	*Phoma exigua*	*Colletotri-chum spp*	*Rhizopus stolonifer*	*Penicillium spp*	*Cercospora spp*	*Alternaria Alternata*
T_0 (Control)	40a	46a	12a (20.27)	56a	54a	6a (14.18)	6a (14.18)	4a (11.54)	8a (16.43)	4a (11.54)	2a (8.13)
T_1 (200ppm CS)	0c (0.7)	40b	0b (0.7)	20b (26.56)	40b	0b (0.7)	0b (0.7)	4a (11.54)	2b (8.13)	0b (0.7)	0b (0.7)
T_2 (500ppm CS)	0c (0.7)	10f (18.43)	0b (0.7)	0c (0.7)	35c	0b (0.7)	0b (0.7)	2c (8.13)	1c (5.74)	0b (0.7)	0b (0.7)
T_3 (1000ppm CS)	0c (0.7)	5g (12.92)	0b (0.7)	0c (0.7)	15e (22.79)	0b (0.7)	0b (0.7)	1d (5.74)	1c (5.74)	0b (0.7)	0b (0.7)
T_4 (2000ppm CS)	0c (0.7)	0h (0.7)	0b (0.7)	0c (0.7)	5f (12.92)	0b (0.7)	0b (0.7)	4a (11.54)	0d (0.7)	0b (0.7)	0b (0.7)
T_5 (200ppm YES)	20b (26.56)	40b	0b (0.7)	0c (0.7)	25d	0b (0.7)	0b (0.7)	3b (9.97)	0d (0.7)	0b (0.7)	0b (0.7)
T_6 (500ppm YES)	0c (0.7)	30c	0b (0.7)	0c (0.7)	20d (26.56)	0b (0.7)	0b (0.7)	3b (9.97)	0d (0.7)	0b (0.7)	0b (0.7)
T_7 (1000ppm YES)	0c (0.7)	25d	0b (0.7)	0c (0.7)	15e (22.79)	0b (0.7)	0b (0.7)	2c (8.13)	0d (0.7)	0b (0.7)	0b (0.7)
T_8 (2000ppm YES)	0c (0.7)	15e (22.79)	0b (0.7)	0c (0.7)	0g (0.7)	0b (0.7)	0b (0.7)	0e (0.7)	0d (0.7)	0b (0.7)	0b (0.7)
T_9 (Vitavax-200 WP)	0c (0.7)	0h (0.7)	0b (0.7)	0c (0.7)	0g (0.7)	0b (0.7)	0b (0.7)	0e (0.7)	0d (0.7)	0b (0.7)	0b (0.7)
$LSD_{0.05}$	1.12	1.87	0.178	1.52	1.61	0.357	0.107	0.544	0.240	0.274	0.178
CV (%)	9.10	4.67	4.00	10.15	3.95	10.15	3.18	4.09	3.53	9.04	7.23

Values within the same column having a common letter(s) do not differ significantly (P≥0.01)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figures in the parentheses are arcsine transformed values

Discussion

The present investigation has been carried out by seed priming of vegetable seeds with different elicitors such as Chitosan and Yeast Elicitor. Altogether fourteen fungi, representing twelve genera were recorded in the seeds of bottle gourd, sweet gourd, snake gourd, wax gourd and cucumber collected from seed traders of Mymensingh districts.

In Bottle gourd seeds, eight fungi were detected. Seed priming by Chitosan @ 2000 ppm and Yeast elicitor @ 1000 ppm & 2000 ppm and seed treatment with 0.35% Vitavax-200 WP significantly reduced seed-borne fungal pathogens followed by Chitosan @ 1000 ppm & 500 ppm. In Sweet gourd seeds, ten fungi were detected. Seed priming with Chitosan @ 2000 ppm, Yeast Elicitor @ 2000 ppm and Vitavax-200 WP significantly reduced seed-borne fungal pathogens followed by Yeast Elicitor @ 500 ppm, 1000 ppm & 2000 ppm. The present findings was supported by Begum and Momin (2000); Kamble *et al.* (1999) where they also reported the association of similar fungi viz. *Aspergillus flavus*, *Penicilium* spp., *Fusarium* spp., *Rhizopus* spp. with vegetable seeds. In Snake gourd seed, eight fungi were detected. Seed priming by Chitosan @ 2000 ppm significantly reduced seed-borne fungal pathogens followed by Yeast Elicitor Solution @ 200 ppm, 500 ppm, 1000 ppm & 2000 ppm. In Wax gourd seed, ten fungi were detected. Seed priming by Chitosan @ 2000 ppm significantly reduced seed-borne fungal pathogens followed by Chitosan @ 1000 ppm & Yeast Elicitor @ 2000 ppm. The present findings was supported by Begum and Momin (2000) where they also reported the association of similar fungi such as *Aspergillus flavus*, *Penicilium* spp., *Fusarium* spp., *Rhizopus* spp. with vegetable seeds. In Cucumber, eleven fungi were detected. Seed priming by Yeast Elicitor @ 2000 ppm significantly reduced seed-borne fungal pathogens followed by Chitosan @ 2000 ppm. The present findings was supported by Nasreen and Sultana (2000); Kamble *et al.* (1999) and Braccini and Dhingara (1996) where they also reported the association of similar fungi viz. *Rhizoctonia* spp., *Colletotrichum* sp., *Fusarium* spp., *Alternaria* spp., *Macrophomina phaseolina, Aspergillus niger, Penicillium* spp. and *Rhizopus* spp. with vegetable seeds.

Present investigation indicates that seed priming of cucurbits by elicitor can suppress the growth of seed-borne fungi as the elicitor may induce resistance against pathogens. All doses of Chitosan solution shown reduction in seed-borne fungal infection in bottle gourd, sweet gourd, snake gourd, wax gourd and cucumber. Chitosan (2000 ppm) significantly reduced the seed-borne fungal pathogens. The findings of the present investigation are in agreement with Zheng *et al*. (2012), who reported that, chitosan coating increased seed germination, plant growth and soybean yield efficiently. Tingda *et al.* (1994) reported that, 0.1% chitosan help for the growth stimulation of cotton and maize seeds. Alam *et al.* (2014) also reported that, 1% chiosan solution stimulate the germination percentage of chili seed and control seed-borne fungi associated with chilli seed. Similarly, seed priming with Yeast Elicitor solution showed that all doses of Yeast Elicitor solution reduced seed-borne fungal infection of cucurbits Yeast

Elicitor (2000 ppm) significantly reduced the seed-borne fungal pathogens which is statistically similar to the 0.35% Vitavax-200 WP. Al- Tawaha *et al.* (2011) reported that foliar application of yeast extract hold promises for increasing the seed yield and isoflavone content of soybean seeds. Moreover, due to natural sources both elicitors are environmentally safe.

Conclusion

Chitosan and Yeast Elicitor are potential compounds for priming of seed to control seed borne fungi of Cucurbits. Chitosan and yeast elicitor are bio-polymers and not harmful for ecosystem and completely safe for human health. Therefore, application of chitosan and yeast elicitor can be utilized as seed- treating bio-polymer in replace to chemical pesticides. The effectiveness of these products should also be assessed against seed-borne fungi of cucurbits for their commercial application.

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