YEAST ELICITOR AND CHITOSAN IN CONTROLLING SEED-BORNE FUNGI OF BEAN, OKRA AND RADISH

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ABSTRACT

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Experiment was conducted to assess suppression of seed-borne fungal pathogens of bean, okra and radish in the laboratory condition by yeast elicitor and chitosan. Eleven different fungal pathogens belonging to nine genera viz. *Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Fusarium moniliforme, Fusarium oxysporum, Phoma exigua, Rhizopus stolonifer*, *Macrophomina phaseolina, Penicillium spp., Curvularia lunata, Colletotrichum spp.* were isolated and identified from the seed samples. Four concentrations of Chitosan and Yeast Elicitors Solutions (200, 500, 1000 and 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 superior performance in suppressing the seed-borne fungi by blotter paper method. In *in-vitro* antagonism test different concentrations of Chitosan and Yeast elicitor (200, 500, 1000 and 2000 ppm) and Vitavax-200 WP (0.35%) were used for their efficacy in inhibiting mycelial growth of *Aspergillus flavus, Aspergillus niger, Penicillium spp., Fusarium moniliforme* and *Phoma exigua*. Both Chitosan and Yeast Elicitor at 1000 and 2000 ppm showed complete inhibition of mycelial growth of different fungi.

Keywords: Chitosan, Yeast Elicitor, Vegetables, Seed-borne fungi, Control

INTRODUCTION

Vegetables are important for their low production cost, short production and high nutritive value. Bean, okra and radish cover a large area considering all the vegetables in Bangladesh. During 2014-2015 about 49192, 28046 and 64091 acres of land were under bean, okra and radish cultivation in Bangladesh, respectively and produced 122091, 51885 and 270965 MT bean, okra and radish (BBS 2016). Quality seed is the prime consideration of better yield to gain maximum economic return. In Bangladesh high relative humidity and temperature causes seed deterioration. Vegetables suffer from a large number of diseases (Fakir *et al.* 1995). However, important diseases of these crops are caused by seed-borne fungi which cause damping off, foot and root rots, phomopsis blight, fruit rots, black leg, leaf spots, fusarium wilt, fusarium root rot, anthracnose and downy mildews disease. (Richardson 1990, Fakir *et al.* 1995).

Seed treatment is considered as the cheapest and safest method of plant disease control. It is unquestionable that proper seed treatment can substantially improve the quality of seed and seedling with satisfactory increase in the yield. Moreover, fungicide treatments are discouraged due to toxic residues and development of resistance in pathogens. Thus, there is urgent need for developing alternative method as effective, economic and ecological to control plant pathogen.

Elicitors in plant biology are extrinsic or foreign, molecules often associated with plant pests, diseases or synergistic organisms. Elicitor molecules can attach to special receptor proteins located on plant cell membranes (Bektas and Elugem 2015). These receptors are able to recognise the molecular pattern of elicitors and trigger intracellular defence signalling via the ocadecanoid pathway. This response results in the enhanced synthesis of metabolites which reduce damage and increase resistance to pest, disease or environmental stress (Bektas and Elugem 2015). 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).

Researchers in Bangladesh are trying to produce low-cost commercial chitosan production from shrimp by-product (Hossain and Iqbal 2014). The objectives of the work are identification of the seed-borne fungal pathogens associated with the selected vegetable seeds and examine the potentiality of chitosan and yeast elicitor for suppressing seed-borne fungal pathogens.

MATERIALS AND METHODS

The experiments were conducted in the Laboratory of Biosignaling, Bioactive Compounds and Bioformulation, Department of Plant Pathology, Plant Disease Diagnostic Clinic (PDDC), Department of Plant Pathology and Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensigh-2202. The experiments were conducted from October, 2015 to November, 2016.Seeds of okra, radish and bean were collected from the farmers of Netrokona 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 2001) for detection of seed-borne fungi. For proper identification of fungi, temporary slides were prepared from the fungal colony and identified with the help of keys of Ellis (1971) and Neergard (1979).

For chitosan (0.3% solution) preparation, 3g chitosan was dissolved in concentrated acetic acid then diluted by water to a volume of 1000 mL and 3000 ppm chitosan stock solution was prepared and then 200 ppm, 500 ppm, 1000 ppm and 2000 ppm chitosan solution was prepared. For Yeast Elicitor (0.3% solution) preparation, Yeast *(Saccharomyces cerevisiae)* was cultured in YEPDA broth (Yeast extract 1%, Peptone 2% and Dextrose 2%) and incubated using an orbital platform shaker at 30^0 C and 140 rpm for 72 hrs for collecting filtrate from the yeast. After 72 hrs the broth was filtrated and the filtrate was collected and mixed with ethanol solution following the key outlines by 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 and 2000 ppm Yeast Elicitor solution was prepared.

Seed Priming with Chitosan and Yeast Elicitor Solution

Seeds were dipped in respected chitosan and yeast elicitor solution for 2 hrs at room temperature. After draining the treated solution the seeds were placed in Blotting Paper following ISTA rules for seed testing (ISTA 2001). As a positive control, seed treatment with Vitavax-200 WP was carried out following the method of Islam *et al.* (2001). Vitavax-200 WP (0.35% of seed weight) was used for seed treatment.

Assessment of *In–Vitro* Antifungal Efficacy of Chitosan and Yeast Elicitor

To determine the growth inhibitory effects against the test fungi, chitosan and yeast elicitor were added to PDA medium. Different concentrations of Chitosan and Yeast Elicitor solution (200, 500, 1000 and 2000 ppm) were added to previously prepared Potato Dextrose Agar (PDA) culture medium. 1 mL of each concentration of chitosan and yeast elicitor solution was added in PDA plates and a 5 mm diameter fungal disc was placed in the center of each Petridish and incubated at a temperature of 24^0 C. As a positive control, assessment of *In–Vitro* Antifungal Potential of Vitavax-200 WP was carried out following the method of Bhuyian and Fakir (1985).

The collected data on different parameters were analyzed statistically by using MSTAT C package program. The means for all the treatments were compared by DMRT (Duncan Multiple Range Test).

RESULTS

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

Effect of different priming of seeds of bean, okra and radish with Chitosan and Yeast Elicitor on seed-borne fungi was recorded and presented in Table 1-3. In Bean, nine fungi in order of prevalence *Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer, Aspergillus niger, Penicillium* spp., *Curvularia lunata, Botrytis cinerea, Fusarium moniliforme, Colletotrichum* spp. and *Macrophomina phaseolina* were recorded in T_0 (Control), while the least seed-borne fungal infections were recorded in T_4 (2000 ppm Chitosan Solution) and T_8 (2000 ppm Yeast Elicitor Solution). *Aspergillus flavus* (22.6%) was the most predominant fungus followed by *Fusarium oxysporum* (8.2%), *Rhizopus stolonifer* (6.8%), *Aspergillus niger* (5.3%) and *Penicillium spp.* (5.3%).

In Okra, ten fungi in order of prevalence *Aspergillus flavus, Rhizopus stolonifer, Fusarium oxysporum,*

Botrytis cinerea, Fusarium moniliforme, Macrophomina phaseolina, Aspergillus niger, Penicillium spp., Phoma exigua and *Botryodiploidia theobromae* were recorded in T_0 (Control), while the least seed-borne fungal infections were recorded in T_9 (0.35% Vitavax-200 WP) followed by T_4 (2000 ppm Chitosan Solution) and T_8 (2000 ppm Yeast Elicitor Solution). *Aspergillus flavus* (25.5%) was the most predominant fungus followed by *Rhizopus stolonifer* (18.6%), *Fusarium oxysporum* (15.2%), *Botrytis cinerea* (11.1%) and *Fusarium moniliforme* (10.9%).

In Radish, eight fungi in order of prevalence *Aspergillus flavus, Fusarium moniliforme, Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Penicillium spp., Macrophomina phaseolina* and *Phoma exigua* were recorded in T_0 (Control), while the least total seed-borne fungal infections were recorded in T_9 (0.35% Vitavax-200 WP), T_8 (2000 ppm Yeast Elicitor Solution) and T_4 (2000 ppm Chitosan Solution). *Aspergillus flavus* (22.5%) was the most predominant fungus followed by *Fusarium moniliforme* (9.3%), *Aspergillus niger* (3.9%) and *Rhizopus stolonifer* (3.6%).

Table 1. Effect of chitosn and yeast elicitor on prevalence of seed-borne fungi of bean

Treatment	Prevalence of Seed-borne fungi (%)									
	Botrytis cinerea	*Curvularia lunata*	*Fusarium Oxysporum*	*Penicillium spp*	*Aspergillus flavus*	*Colletotrichum spp*	*Fusarium moniliforme*	*Rhizopus stolonifer*	*Aspergillus niger*	*Macrophomina Phaseolina*
T_0 (Control)	34 a	44 a	82 a (64.89)	28 a	34 a	8 a (16.43)	14 a (21.97)	24 b	30 a	4 a (11.54)
T_1 (200 ppm CS)	0 b (0.7)	0 b (0.7)	0 b (0.7)	15 b (22.79)	30 b	0 b (0.7)	10 b (18.43)	20 a (26.56)	20 b (26.56)	0 b (0.7)
T_2 (500 ppm CS)	0 b (0.7)	0 b (0.7)	0 b (0.7)	10 c (18.43)	20 cd (26.56)	0 b (0.7)	3 c (9.97)	8 d (16.43)	3 c (9.97)	0 b (0.7)
T_3 (1000 ppm CS)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	13 e (21.13)	0 b (0.7)	0 d (0.7)	6 e (14.18)	0 d (0.7)	0 b (0.7)
T_4 (2000 ppm CS)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 d (0.7)	0 b (0.7)
T_5 (200 ppm YES)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	30 b	0 b (0.7)	0 d (0.7)	10 c (18.43)	0 d (0.7)	0 b (0.7)
T_6 (500 ppm YES)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	27 c	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 d (0.7)	0 b (0.7)
T_7 (1000 ppm YES)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	25 d	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 d (0.7)	0 b (0.7)
T_8 (2000 ppm YES)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	20 cd (26.56)	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 d (0.7)	0 b (0.7)
T_9 (Vitavax-200 WP)	0 b (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	27 c	0 b (0.7)	0 d (0.7)	0 f (0.7)	0 d (0.7)	0 b (0.7)
$LSD_{0.05}$	0.541	0.544	1.08	1.22	1.72	0.258	0.587	1.10	1.07	0.108
CV (%)	7.89	6.32	8.93	9.71	4.10	6.65	6.23	6.30	8.86	3.55

Values within the same column having a common letter(s) do not differ significantly ($P \geq 0.01$)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figure in the parenthesis are arcsine transformed value

Table 2. Effect of chitosn and yeast elicitor on prevalence of seed-borne fungi of okra

Treatment	Prevalence of Seed-borne fungi (%)									
	Fusarium oxysporum	*Rhizopus stolonifer*	*Fusarium moniliforme*	*Macrophomina phaseolina*	*Aspergillus niger*	*Botrytis cinerea*	*Aspergillus Flavus*	Botryodiploidia theobromae	*Penicillium* spp	*Phoma Exigua*
T_0 (Control)	54 a	60 a	64 a	52 a	26 a	96 a	60 a	8 a (16.43)	16 a (23.58)	12 a (20.27)
T_1 (200 ppm CS)	5 f (12.92)	27 b	30 b	0 b (0.7)	20 a (26.56)	0 d (0.7)	40 b	0 b (0.7)	4 b (11.54)	0 b (0.7)
T_2 (500 ppm CS)	1 g (5.74)	13 d (21.13)	10 c (18.43)	0 b (0.7)	0 b (0.7)	0 d (0.7)	30 d	0 b (0.7)	1 c (5.74)	0 b (0.7)
T_3 (1000 ppm CS)	0 h (0.7)	10 e (18.43)	5 d (12.92)	0 b (0.7)	0 b (0.7)	0 d (0.7)	25 e	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_4 (2000 ppm CS)	0 h (0.7)	3 f (9.97)	0 e (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	10 f (18.43)	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_5 (200 ppm YES)	35 b	27 b	0 e (0.7)	0 b (0.7)	0 b (0.7)	10 b (18.43)	35 c	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_6 (500 ppm YES)	30 c	20 b (26.56)	0 e (0.7)	0 b (0.7)	0 b (0.7)	5 c (12.92)	25 e	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_7 (1000 ppm YES)	20 d (26.56)	16 c (23.58)	0 e (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	20 e (26.56)	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_8 (2000 ppm YES)	7 e (15.34)	10 e (18.43)	0 e (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	10 f (18.43)	0 b (0.7)	0 d (0.7)	0 b (0.7)
T_9(Vitavax-200 WP)	0 h (0.7)	0 g (0.7)	0 e (0.7)	0 b (0.7)	0 b (0.7)	0 d (0.7)	0 g (0.7)	0 b (0.7)	0 d (0.7)	0 b (0.7)
$LSD_{0.05}$	1.21	1.95	2.41	0.549	1.37	1.94	2.90	0.234	0.761	0.444
CV (%)	3.94	4.94	10.92	5.53	13.85	8.62	6.12	6.14	9.77	9.81

Values within the same column having a common letter(s) do not differ significantly ($P \geq 0.01$)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figure in the parenthesis are arcsine transformed value

Table 3. Effect of chitosn and yeast elicitor on prevalence of seed-borne fungi of radish

Treatment	Prevalence of Seed-borne fungi (%)							
	Fusarium oxysporum	*Fusarium Moniliforme*	*Rhizopus stolonifer*	*Aspergillus flavus*	*Penicillium spp*	*Aspergillus Niger*	*Phoma exigua*	*Macrophomina phaseolina*
T_0 (Control)	14 a (21.97)	20 a (26.56)	8 a (16.43)	66 a	16 a (23.58)	25 a	4 a (11.54)	15 a (22.79)
T_1 (200 ppm CS)	0 c (0.7)	17 b (24.35)	8 a (16.43)	42 b	0 b (0.7)	6 b (14.18)	0 b (0.7)	0 b (0.7)
T_2 (500 ppm CS)	0 c (0.7)	14 c (21.97)	6 b (14.18)	32 c	0 b (0.7)	4 c (11.54)	0 b (0.7)	0 b (0.7)
T_3 (1000 ppm CS)	0 c (0.7)	11 d (19.36)	2 d (8.13)	14 g (21.97)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
T_4 (2000 ppm CS)	0 c (0.7)	4 g (11.54)	0 e (0.7)	4 e (11.54)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
T_5 (200 ppm YES)	0 c (0.7)	13 c (21.13)	6 b (14.18)	32 c	0b (0.7)	4 c (11.54)	0 b (0.7)	0 b (0.70
T_6 (500 ppm YES)	0c (0.7)	8e (16.43)	4c (11.54)	20d (26.56)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
T_7 (1000 ppm YES)	0 c (0.7)	6 f (14.18)	2 d (8.13)	10 f (18.43)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
T_8 (2000 ppm YES)	0 c (0.7)	0 h (0.7)	0 e (0.7)	5 g (12.92)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
T_9 (Vitavax-200 WP)	4 b (11.54)	0 h (0.7)	0 e (0.7)	0 h (0.7)	0 b (0.7)	0 d (0.7)	0 b (0.7)	0 b (0.7)
$LSD_{0.05}$	0.614	1.32	0.650	1.87	0.380	0.761	0.170	0.186
CV (%)	9.22	4.94	4.19	4.18	7.48	6.73	5.61	3.44

Values within the same column having a common letter(s) do not differ significantly ($P \geq 0.01$)
CS indicates Chitosan Solution, YES indicates Yeast Elicitor Solution
Figure in the parenthesis are arcsine transformed value

Fungal Growth Suppression by Chitosan and Yeast Elicitor

Five fungi *Aspergillus flavus, Aspergillus niger, Penicillium* spp., *Fusarium moniliforme* and *Phoma exigua* collected from seeds of bean, radish and okra were considered for *in-vitro* antagonism assay. After 72 hrs of incubation, radial mycelial growth of each fungal colony was measured in response to 200, 500, ppm 1000 and 2000 ppm Chitosan solution and 200, 500, 1000 and 2000 ppm Yeast Elicitor solution and 0.35% Vitavax-200 WP solution and compared with control (only PDA medium).

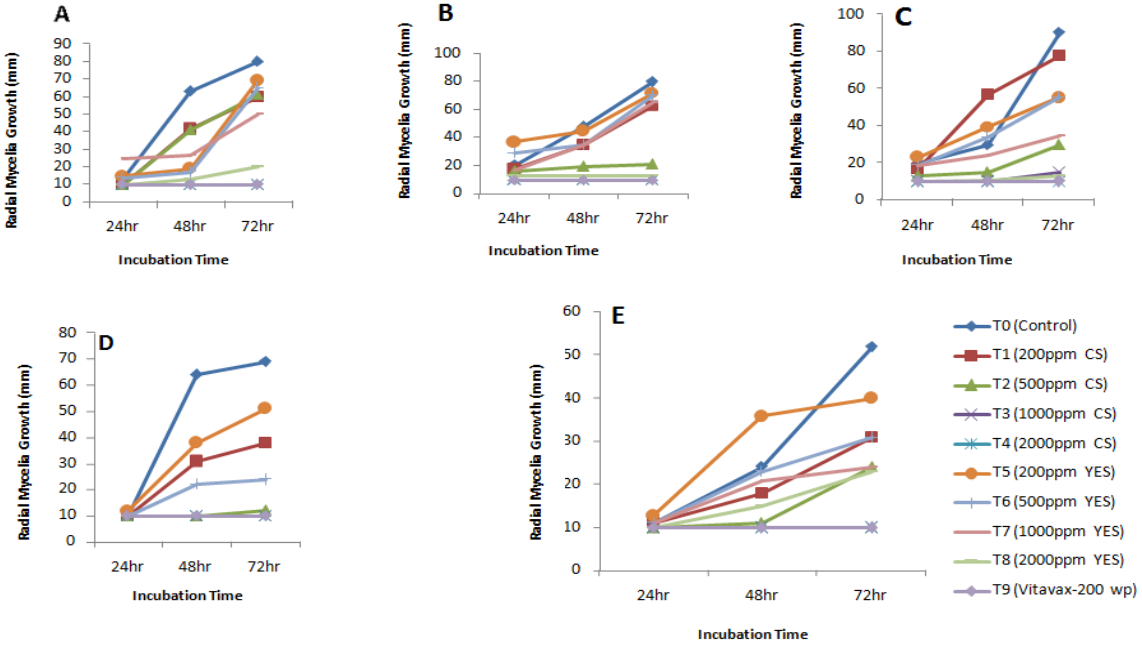

Figure 1. Effect of Chitosan and Yeast Elicitor on mycelia growth of A. *Aspergillus flavus* B. *Aspergillus niger* C. *Penicillium* spp D. *Fusarium moniliforme* and E. *Phoma exigua*

In case of *Aspergillus flavus, Aspergillus niger* and *Phoma exigua*, the radial mycelial growth was significantly reduced by 0.35% Vitavax-200 WP which was statistically similar to 1000 ppm and 2000 ppm Chitosan solution. But in case of *Penicillium spp.*, the radial mycelial growth was significantly reduced by 0.35% Vitavax-200 WP which was statistically similar to 2000 ppm Chitosan Solution. In case of *Fusarium moniliforme*, the radial mycelial growth was significantly reduced by 0.35% Vitavax-200 WP which was statistically similar to 1000 ppm and 2000 ppm Chitosan Solution and 1000 ppm and 2000 ppm Yeast Elicitor Solution. In all cases the maximum radial mycelial growth was found in control treatment.

DISCUSSION

Investigation has been carried out by seed priming of vegetable seeds with different elicitors such as Chitosan and Yeast Elicitor and Vitavax-200 WP. Antifungal ability of different elicitors against seed-borne fungal pathogens was also examined. Altogether eleven fungi, representing nine genera were recorded in the seeds of bean, okra and radish collected from the farmers. In Bean seeds, nine fungi were detected. Seed priming by Chitosan Solution @ 2000 ppm significantly reduced seed-borne fungal pathogens followed by 0.35% Vitavax-200 WP and Yeast Elicitor Solution @ 2000 ppm. In Okra seeds, ten fungi were detected. Seed treatment by Vitavax-200 WP (0.35%) significantly reduced seed-borne fungal pathogens. The present findings were supported by Fakir (2000); Moretto *et al.* (1997); Kassin (1996) and Majid (1996) where they also reported the association of similar fungi with vegetable seeds. In Radish seeds, eight fungi were detected. Application of Vitavax-200 WP (0.35%) significantly reduced seed-borne fungal pathogens followed by Yeast Elicitor Solution @ 2000 ppm and Chitosan Solution @ 2000 ppm. The present findings were supported by Fakir (2000) and Kassin (1996) where they reported the association of similar fungus with this vegetable seeds.

Present investigation indicates that vegetable seeds priming by elicitor can suppress the growth of seed-borne fungi as the elicitor may induce the defense genes which may show resistance against pathogens (Thakur and Sohal 2013). Seed priming by Chitosan Solution, all doses of Chitosan Solution shown

reduction in seed-borne fungal infection in bean, okra and radish. Chitosan Solution (2000 ppm) significantly reduced the seed-borne fungal pathogens. Alam *et al.* (2014) 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 vegetable crops. Yeast Elicitor Solution (2000 ppm) significantly reduced the seed-borne fungal pathogens which is statistically similar to the 0.35% Vitavax-200 WP. Moreover, due to organic organism both elicitors are environmentally safe (Deepmala *et al*. 2014)

To evaluate whether chitosan and yeast elicitor has any direct suppressing ability against seed-borne fungi, *in-vitro* antagonism tests revealed that chitosan and yeast elicitor can inhibit the mycelial growth of *Aspergillus flavus, Aspergillus niger, Penicillium* spp*., Fusarium moniliforme* and *Phoma exigua in-vitro.* The present findings were confirmed by the reports of Yarahmadi *et al* (2014); Wang Qing *et al.* (2014); EI Hadrami *et al*. (2010); and Xianghong *et al*. (2010). Yarahamadi *et al.* (2014) where they reported that Chitosan has antifungal activity against *Rhizopus stolonifer*. Kaur *et al.* (2012) reported that chitosan nano formulations (NFs) has antifungal activity against *Rhizoctonia solani, Aspergillus flavus* and *Alternaria alternata*. Xianghong *et al.* (2010) observed that both chitosan and oligochitosan strongly inhibit the spore germination and mycelial growth of *Alternaria kikuchiana* and *Physalospora piricola.*

Thus, it can be concluded that Chitosan and Yeast Elicitor are effective against *Aspergillus flavus, Aspergillus niger, Penicillium* spp*., Fusarium moniliforme* and *Phoma exigua* and can be utilized as an alternative to chemical fungicides.

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