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# Control of Mycoflora of Farmer's Stored Seeds of Mungbean

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Abstract: The prevalence of fungi and bacteria associated with mungbean (Vigna radiata) seeds and their control were studied during the month of January-November, 2004. Twenty seed samples of mungbean were collected from two villages of Mymensingh district. The germination percentage of all samples of mungbean remarkably varied. Hot water treatment at 53°C for 15 min showed comparatively better performance than Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> in case of germination. Seed-borne fungi were recorded and detected by using blotter method. Four different fungal pathogens were identified, viz. Aspergillus flavus, Aspergillus niger, Fusarium spp. (F. oxysporum, F. moniliforme, F. semitectum) and Penicillium spp. Two groups of bacteria were identified on un-germinated seeds and emerging radicles. Yellow and creamy colored bacterial colonies were found in seed surface during blotter incubation test. It was also observed that liquid assay was better than the washing test and blotter method for detecting pathogenic bacteria. Xanthomonas campestris pv. vignicola and Pseudomonas syringae pv. syringae were identified. Efficacy of two seed treating methods viz. Vitavax-200 and hot water were tested in the laboratory. Mungbean seeds treated with Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> showed highest control of seed-borne fungi than hot water treatment. Hot water treatment at 55°C temperature for 15 min also effective to reduction of seed-borne fungi and bacterial infections. But at 55°C of hot water treatment hampered the germination percentage of seeds. Hot water treatment at 53°C temperature for 15 min was effective for controlling seed-borne mycoflora next to Vitavax-200at the rate of 2.5 g kg<sup>-1</sup>.

Key words: Mungbean, fungi and bacteria association, control

# INTRODUCTION

Pulse is considered as one of the important staple food item for the people of Bangladesh. Pulses form an important constituent of the daily diet and main source of protein for both human beings and animals (Sattar *et al.*, 1996). Legumes are cheap protein source and considered as poor man's meat (Kaul and Gouwda, 1982). Pulses are grown in an area of about 453036 ha. and production is about 346000 metric tons i.e., 0.76 ton/ha in Bangladesh (BBS, June, 2004). Moreover, pulses have the remarkable quality of helping the symbiotic root rhizobia to fix atmospheric nitrogen and thus improving soil fertility. Pulses also supply food and fodder to the domestic birds and animals. The soil, climate and land topography of Bangladesh are suitable for growing pulses.

Mungbean is considered as fifth major pulse of Bangladesh. About 44129 ha of land is under its cultivation with a production of about 30000 m. tons i.e., 0.68 ton ha<sup>-1</sup> which contributes 6.75% of total pulse production in the country (BBS, June, 2004). But the yield of mungbean in Bangladesh is lower than that of the other

Asian and Latin American countries. The per unit production has been declining in the past few years due to many seed-borne diseases.

The climate and other agro-ecological conditions of Bangladesh are highly favorable for plant disease development caused by fungi, bacteria, viruses and nematodes for which pulses suffer from a number of diseases. As many as 17 and 16 diseases have been reported on mungbean. Many of these diseases have been reported as seed-borne (Fakir, 1983).

Fourteen seed-borne mycoflora were recorded in mungbean among them Botryodiplodia palmarum, Cercospora kikuchii, Colletotrichum traneatum, Diaporthe phaseolorum var. sojae, Fusarium equiseti, Fusarium moniliforme, Macrophomina phaseolina and Myrothecium roridum which caused seed rot, leaf spots, seedling blight or collar rot in the artificial inoculation tests (Ramnath et al., 1970). Besides those, members of the genera Aspergillus, Penicillium and Rhizopus remain associated with mungbean seeds. These fungi deteriorate the seeds in storage and cause germination failure (BARI, 1984).

The most common seed-borne bacterial diseases of pulse crops are leaf spot of mungbean (*X. campestris* pv. *vignicola*) and pea blight (*P. syringae* pv. *pisi*). The prevalence and severity of these diseases vary considerably from year to year depending on weather conditions. Leaf spot of mungbean and bacterial blight of pea caused by *Xanthomonas campestris* pv. *vignicola* and *Pseudomonas syringae* pv. *pisi* were also reported by Patel and Jindal (1972) and Hagedorn (1984), respectively as a seed-borne diseases.

Mungbean is known to suffer from different diseases most of which are seed-borne. It has been observed that only few seedlings emerge and grow as healthy. Many studies were done in the Plant Pathology division of BARI and BAU to investigate the prevalence of the fungi in seeds. Four fungi were detected in mungbean. They are rust, leaf spot and seed rot of mungbean. But very limited studies mainly on association of fungus and bacteria with mungbean seeds and their control have done in Bangladesh. Preliminary observations have revealed that detection process of bacteria from seeds has yet not been developed in our country. So, it is a crying need to detect seed-borne pathogenic bacteria from seeds in a simple, suitable and cheapest way.

The seed-borne disease infection can be effectively reduced if the seeds are treated before sowing is necessary for direct disease control. Work on Vitavax-200 has been done to control seed-borne pathogens of mungbean seeds by seed treatment which is an agreement with Jain and Kahare (1972), Shanmugan and Govindaswamy (1973), Rodriguez (1984), Singh and Singh (1986), Mortuza and Bhuiya (1988) and Shah *et al.* (1992a, b).

Eradicate the pathogen from seeds by hot water treatment is essential as a part of integrated pest management. Hot water seed treatment has drawn a very urgent attention in Bangladesh for obtaining good plant seeds, it can be useful in reducing the amount of pesticides required to manage a disease. Erdey et al. (1997) observed that hot water treatment at 55°C for 15 min helped in reducing 85% Fusarium moniliforme infection in maize seeds. Nega et al. (2003) used hot water treatment against most important seed-borne pathogens of five important vegetable crops and found no infected seeds from those vegetables.

Keeping these views in mind the present research work has been undertaken to record the prevalence of mycoflora of farmer's stored mungbean seeds and to determine the efficacy of seed treating methods against the major seed borne pathogens of mungbean.

# MATERIALS AND METHODS

The research activities were conducted in the Seed Pathology Centre (SPC), MS laboratory, Department of Plant Pathology, IPM Laboratory and Department of Veterinary Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh during January to November, 2004.

The experiment was laid out in two villages' randomly selected farmers around BAU campus for this experiment namely Suthiakhali and Chariswardi. A total of 20 working samples of local variety mungbean seeds five samples were collected from five randomly selected farmers of each village (Table 1).

Each sample was about 300 g. The samples were enclosed in polythene bags with proper labeling, brought directly to SPC Laboratory and kept in the refrigerator at 5±1°C until used for subsequent studies.

For detection of seed-borne pathogens associated with mungbean seed samples were detected by blotter test (ISTA, 1976) for fungal pathogens.

Blotter incubation test, washing test, Liquid assay test were conducted for pure colony of bacteria and NA media bacterial slant was then prepared in NA slant for future identification (Carmen Nieves Mortensen, 1997) bacterial pathogens. Future Identification and confirmatory test of bacteria were:

# Physiological test

- Gram staining test
- Potassium hydroxide solubility test
- Potato soft rot test,
- Temperature test (Mortensen, 1997).

**Biochemical test:** Kovac's oxidase test (Kovac's, 1956.; Hildebrand and Schroth, 1972).

Host tests: Hypersensitivity test (Lelliot and Stead, 1987)

**Pathogenicity test of isolated bacteria:** Seedling leaf inoculation (Lelliot and Stead, 1987).

 $\underline{\textbf{Table 1: Sources of seed collection of mungbean}}$ 

	Place of seed	
Crops	collection	Name of farmers
Mungbean (Vigna radiata)	Sutiakhali	M. Mohsin Miah (F <sub>1</sub> )
	Sutiakhali	M. Idris Molla (F2)
	Sutiakhali	M. Iasin uddin (F <sub>3</sub> )
	Sutiakhali	M. Mobinul Haque (F4)
	Sutiakhali	M. Abser Miah (F <sub>5</sub> )
	Chariswardi	M. Abu Ishaque (F <sub>6</sub> )
	Chariswardi	M. Kasem Ali (F <sub>7</sub> )
	Chariswardi	Lal Miah (F <sub>8</sub> )
	Chariswardi	M. Ziauddin (F <sub>9</sub> )
	Chariswardi	M. Shahin Ali (F <sub>10</sub> )

 $F_{1-10}$  = Ten farmers in two villages

**Control of mycoflora of mungbean seed:** The efficacies of seed treating methods against the major seed borne pathogens of mungbean were then tested by Seed treatment with Vitavax-200 and hot water.

Vitavax-200 (5, 6 dehydro-2-Methyl-1, 4-Oxathin-3-carboxilide) was used in this study. The seeds were treated with Vitavax-200 at the rate of 0.5, 1.0, 1.5, 2.0 and 2.5 g kg<sup>-1</sup> of seed weight. Twenty grams of seeds was taken in a 250 mL conical flask and required amount of fungicide was added to it. The mixture was then shaken for 15 min for uniform coating of fungicides on the seeds. In case of control, seeds were not treated with Vitavax-200.

For hot water treatment, the vegetable seed treating device was used in the IPM Laboratory. The vegetable seed treating Plant made up of locally available materials works automatically controlling temperature with time. Firstly seeds were soaked in normal water for 2-3 h in a cotton fabric bag. Then pouring 2 L water in the device below the red marking, the device was connected with electricity with the help of heater coil the device was heated. With the time and thermostat bulb the device was regulated to the desired temperatures such as 45, 48, 50, 53 and 55°C for 15 min. The desired temperature and time were controlled with the help of thermometer and stop watch. After the treatment, seeds were taken out of the tank, drained off and spread on a piece of brown paper and shade dried. Then the seeds were plated for test. In case of control, seeds were presoaked as above but were not treated in hot water.

A Completely Randomized Design (CRD) having four replications for each treatment were used to analyze the data of the study on the efficacy of seed treating methods in controlling seed-borne mycoflora mungbean seeds. The data were statistically analyzed using analysis of variance (ANOVA) to find out the variation resulting from experimental treatments. Treatment means were compared by DMRT (Duncan's Multiple Range Test)

# RESULTS AND DISCUSSION

Detection, identification and prevalence of seed-borne fungi associated with mungbean seeds: Seed-borne mycoflora is one of the major factors of substantial damages mungbean crops. A total of four fungi representing 3 genera were recorded from mungbean seed samples. The fungi were Aspergillus flavus, Aspergillus niger, Fusarium spp. (Fusarium oxysporum, F. moniliforme, F. semitectum) and Penicillium spp. The percentage of Aspergillus flavus ranged from 7.00 to 17.50% as shown in Table 2. Incidence of Aspergillus niger ranged from 4.00 to 11.50%, incidence of Fusarium spp. ranged from 7.25 to 19.50% and incidence of Penicillium spp. ranged from 4.00 to 8.50%. The germination percentage of mungbean seeds ranged from 65.50 to 78.50% as shown in Table 2.

Analysis of location effect indicated that incidence of *Aspergillus flavus* was found in Sutiakhali (13.80%) and in Chariswardi (9.30%) that was statistically significant as shown in Table 3. Incidence of *Aspergillus niger* was found in Sutiakhali (7.90%) and in Chariswardi (6.50%) that was statistically significant. Incidence of *Fusarium* spp. was found in Sutiakhali (14.30%) and in Chariswardi (13.10%) that was statistically significant. Incidence of *Penicillium* spp. was found in Sutiakhali (6.60%) and in Chariswardi (5.70%) that was statistically significant. The germination percentage was found in Chariswardi (74.90%) and in Sutiakhali (70.30%) that was statistically dissimilar as shown in Table 3.

Prevalence of seed-borne bacterial colony associated with mungbean seeds: The present study also elucidated that mungbean seeds harbour important bacterial pathogens. During standard blotter incubation test, yellow and creamy color bacterial colony (ooze) were found on non-germinating seeds and emerging radicles. The percentage of yellow color colony ranged from 2.50 to

Table 2: Interaction effect between village and sample on germination and percent incidence of seed-borne mycoflora of farmer's stored mungbean seeds (Blotter method)

		Incidence of pathogen (%)				
Village	Sample No.	Germination (%)	Aspergillus flavus	Aspergillus niger	Fusarium spp.	Penicillium spp.
Sutiakhali	1	65.50d	15.00b	7.50d	14.50d	7.50b
	2	70.00c	13.50b	6.00e	17.00bc	5.00cd
	3	75.50ab	14.00b	11.50a	13.50d	7.50b
	4	71.00c	9.00de	9.50b	7.25f	8.50a
	5	69.50c	17.50a	5.00f	19.50a	4.50cd
Chariswardi	6	70.50c	8.50ef	8.50c	15.00cd	7.50b
	7	77.50a	10.50cd	5.00f	17.50ab	4.00d
	8	73.00bc	9.00de	7.50d	8.50f	5.00cd
	9	75.00ab	11.50c	4.00g	13.50d	5.50c
	10	78.50a	7.00f	7.50d	11.00e	6.50b
LSD $(p = 0.05)$		3.57	1.58	0.89	2.28	0.95
CV %)		4.79	9.48	8.60	11.50	10.70

Fusarium spp. = Fusarium oxysporum, Fusarium moniliforme, Fusarium semitectum, Four hundred seeds were tested, Column having the same letter(s) are statistically identical

Table 3: Effect of village on germination and percent incidence of seed-borne mycoflora of farmer's stored mungbean seeds (Blotter method)

		Incidence of pathoge	n (%)		
Village	Germination (%)	Aspergillus flavus	Aspergillus niger	Fusarium spp.	Penicillium spp.
Sutiakhali	70.30b	13.80a	7.90a	14.30a	6.60a
Chariswardi	74.90a	9.30b	6.50b	13.10b	5.70b
LSD $(p = 0.05)$	1.60	0.71	0.40	1.02	0.42
CV (%)	4.79	9.48	8.60	11.50	10.70

The figures having common letter(s) within a column do not differ significantly at 5% level of significance as per DMRT, Fusarium spp. = F. oxysporum, Fusarium moniliforme, Fusarium semitectum, Four hundred seeds were tested, Column having the same letter(s) are statistically identical

Table 4: Effect of village on percent incidence of bacterial colony of farmer's stored munchean seeds (Blotter method)

	Incidence of bacterial	colony (%)
Village	Yellow color	Creamy color
Sutiakhali	7.20a	2.80a
Chariswardi	6.35b	2.60b
LSD $(p = 0.05)$	0.37	0.12
CV (%)	8.52	6.96

Column having the same letter(s) are statistically identical

10.50% as shown in Table 4 and incidence of cream color colony ranged from 1.00 to 3.75%. Weller and Saettler (1980) discussed that in addition to contamination from mechanical threshing, seed surfaces might become contaminated during the pod infection. He detected infestations, which appeared as yellow bacterial ooze on the seed coat of navy bean seeds. Incidence of yellow color colony was found in Sutiakhali (7.20%) and in Chariswardi (6.35%) that was statistically significant. Incidence of cream color colony was found in Sutiakhali (2.80%) and in Chariswardi (2.60%) that was statistically significant (Table 4). The highest percentage of yellow color colony was found in sample No. 1 of Sutiakhali (10.50%) that was statistically significant to other samples and lowest in sample No. 10 of Chariswardi (2.50%) which was statistically similar to sample No. 4 of Sutiakhali (3.00%). Highest infection of cream color colony was found in sample No. 2 of Sutiakhali (3.75%) that was statistically similar to sample No. 3, 6 and sample No. 8. Lowest incidence of cream color colony in sample No. 9 of Chariswardi (1.00%) that was statistically significant to other sample (Table 5).

Standard methods for the identification of phytopathogenic bacteria to genus and species in diagnostic laboratories rely on physiological and biochemical tests. As blotter method is not suitable for isolating bacteria, so washing test and liquid assay were performed. Out of 12 bacterial strains, strain No. 1, 2, 3, 8, 9 and 10 were identified as *Xanthomonas campestris* pv. *vignicola* and strain No. 4, 5, 6, 7, 11 and 12 were identified as *Pseudomonas syringae* pv. *syringae* by different morphological, physiological, biochemical and pathogenicity test (Table 6 and 7).

Table 5: Interaction effect between village and sample on per cent incidence of bacterial colony of farmer's stored mungbean seeds (Blotter poetbod)

		Incidence of bacterial colony (%)		
Village	Sample No.	Yellow color	Creamy color	
Sutiakhali	1	10.50a	2.00c	
	2	5.00e	3.75a	
	3	8.25c	3.50a	
	4	3.00f	1.75c	
	5	9.25b	3.00b	
Chariswardi	6	9.00bc	3.50a	
	7	6.25d	2.00c	
	8	9.00bc	3.50a	
	9	5.00e	1.00d	
	10	2.50f	3.00b	
LSD $(p = 0.05)$		0.83	0.27	
CV (%)		8.52	6.96	

Column having the same letter(s) are statistically identical

Effect of Vitavax-200 and hot water treatments in controlling seed-borne fungi of mungbean seeds: Five doses of Vitavax-200 at the rate of 0.5, 1.0, 1.5, 2.0 and 2.5 g kg<sup>-1</sup> of seed weight and five temperatures of hot water viz., 45, 48, 50, 53 and 55°C for 15 min were used in this experiment. Results on the effect of Vitavax-200 and hot water in controlling seed-borne infections of mungbean are presented in Table 8. Both Vitavax-200 and hot water treatments were effective in controlling seed-borne fungi of the crop compared to control (untreated). In general higher doses of Vitavax-200 were more effective than lower doses. Seed treatment with hot water reduced seed-borne infection with the entire temperatures regime and increased the germination of seeds.

Effects of treatments in controlling seed-borne fungi were statistically significant. *Aspergillus flavus* the highest infection was found (18.50%) in control. *Aspergillus flavus* was found to be controlled most effectively when seeds were treated with Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> (0.75% infection) and hot water at 55°C (1.0% infection). The secondly lowest infection of *Aspergillus flavus* was found (1.50%) in Vitavax-200 at the rate of 2.0 g kg<sup>-1</sup> that was statistically similar to Vitavax-200 at the rate of 1.5 g kg<sup>-1</sup> (2.75%) and hot water at 53°C (2.50%) as shown in Table 8.

Table 6: Occurrence of bacterial colonies in the farmer's stored seeds of mungbean

			Washing test (dilution 10 <sup>-3</sup> )		Liquid assay test (dilution 10 <sup>-3</sup> )	
Village	Farmer	Sample No.	Yellow color	Creamy color	Yellow color	Creamy color
Sutiakhali	$F_1$	1	+	-	-	+
	$F_2$	2	+	+	+	-
	$\mathbf{F}_{3}$	3	-	+	+	+
	$F_4$	4	-	-	+	+
	$\mathbf{F}_5$	5	+	-	+	-
Chariswardi	$F_6$	6	+	+	+	+
	$\mathbf{F}_{7}$	7	-	+	-	-
	$\mathbf{F}_8$	8	+	-	-	-
	$\mathbf{F}_{9}$	9	-	+	+	+
	$\mathbf{F}_{10}$	10	-	-	+	+

<sup>+ =</sup> Indicates presence of bacteria, = Indicates absence of bacteria F<sub>1</sub>- F<sub>10</sub> = Ten farmers in two villages

Table 7: Bacterial strain obtained by washing test and liquid assay from mungbean seed samples

	Washing test (dilution	10 <sup>-3</sup> )	Liquid assay (dilution 1	0-3)
	Strain No.	Strain No.	Strain No.	Strain No.
Sample No.	Yellow color	Creamy color	Yellow color	Creamy color
1	Strain-1	-	-	Strain-7
2	-	Strain-4	Strain-8	-
5	Strain-2	-	-	-
6	-	Strain- 5	Strain- 9	Strain- 11
8	Strain-3	-	-	-
9	-	Strain-6	Strain-10	Strain-12

Table 8: Efficiency of Vitavax-200 and hot water treatment in controlling seed-borne fungi of mungbean seeds in blotter method

			Seed-borne infect	ion (%)		
Treatments	Doses <sup>1</sup> /Temperatures <sup>2</sup>	Germination (%)	Ocd         13.00b         9.00b           Obc         8.00c         5.25c           Oab         2.75e         2.25d           5a         1.50ef         1.25ef           Oa         0.75f         0.50g           5cd         13.50b         9.50b           5ab         8.75c         5.75c           Oa         4.00d         2.75d           5a         2.50e         1.50e           Oab         1.00f         0.75fg	Fusarium spp.	Penicillium spp.	
Vitavax-200	0.5	76.50cd	13.00b	9.00b	16.25b	7.00b
	1.0	84.50bc	8.00c	5.25c	10.50c	3.25d
	1.5	91.50ab	2.75e	2.25d	4.50e	0.75ef
	2.0	93.75a	1.50ef	1.25ef	1.75f	0.25f
	2.5	94.50a	0.75f	0.50g	0.50g	0.00f
Hot water (15 min)	45°C	77.25cd	13.50b	9.50b	15.50b	7.50b
	48°C	88.25ab	8.75c	5.75c	10.25c	4.25c
	50°C	94.00a	4.00d	2.75d	6.00d	2.50d
	53°C	95.25a	2.50e	1.50e	4.25e	1.50e
	55°C	89.50ab	1.00f	0.75fg	1.75f	1.25e
Control	0.0	69.25d	18.50a	12.50a	19.50a	8.50a
LSD $(p = 0.05)$		8.066	1.204	0.588	1.19	0.99
CV (%)		6.50	12.62	8.99	9.96	11.42

<sup>1 =</sup> g kg<sup>-1</sup> of seed weight, 2 = temperatures for 15 min, Four hundred seeds were tested, Column having the same letter (s) are statistically identical

Highest infection of *A. niger* was recorded (12.50%) in control. The lowest infection of *Aspergillus niger* was found (0.50%) in Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> that was statistically similar effect to hot water at 55°C (0.75%). In controlling *Aspergillus niger* second better performance was found (1.25% infection) at the rate of 2.0 g kg<sup>-1</sup> of Vitavax-200 that was statistically identical to hot water at 53°C (1.50%).

Fusarium spp. was found to be controlled most effectively when seeds were treated with Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> (0.5% infection). The second better performance in controlling Fusarium spp. showed Vitavax-200 at the rate of 2.0 g kg<sup>-1</sup> and 55°C of hot water. Penicillium spp. was totally eliminated when seeds were treated with Vitavax-200 at the rate of 2.5 g kg<sup>-1</sup> (Table 8).

The highest germination percentage was found (95.25%) at 53°C of hot water that was statistically identical to Vitavax-200 at the rate of 2.0 g kg<sup>-1</sup> (93.75%),  $2.5 \,\mathrm{g \, kg^{-1}}$  (94.50%) and 50°C (94.00%) of hot water. The lowest germination percentage was recorded (69.25%) in control (untreated). In both Vitavax-200 and hot water treatments increased the germination percentage gradually with the increasing doses of Vitavax-200 and temperatures of hot water. But at 55°C of hot water decreased the germination percentage of mungbean seeds (Table 8). Results also showed that treatment of seeds at 53°C for 15 min reduced greatly the seed-borne infections as well as increased germination. Hot water treatment was also proved to be effective in the elimination of seedborne infection of some other crop seeds (Raychuduri and Lele, 1966; Tenente et al., 1993; Erdey et al., 1997;

Table 9: Efficiency of Vitavax-200 and hot water treatment in controlling bacterial colony of mungbean seeds in Blotter method

		Seed-borne infe	ction (%)
	$Doses^{1}/$		
Temperatures	temperatures <sup>2</sup>	Yellow color	Creamy color
Vitavax-200	0.5	10.00ab	3.25a
	1.0	8.50c	2.50b
	1.5	6.25d	1.75cd
	2.0	3.25e	1.25d
	2.5	3.00e	1.00d
Hot water			
(15 min)	45°C	9.00bc	3.25a
	48°C	7.75c	2.25bc
	50°C	5.50d	1.50d
	53°C	1.25f	0.50e
	55°C	0.00f	0.00e
Control	0.0	10.50a	3.50a
LSD $(p = 0.05)$		1.37	0.55
CV (%)		14.95	14.65

 $1=g~kg^{-1}$  of seed weight, 2= temperatures for 15 min, Four hundred seeds were tested, Column having the same letter(s) are statistically identical

Hermansen et al., 1999; Gaur, 2003; Nega et al., 2003). These findings are closely supported by Meah (2004). Meah also found that 53-55°C for 15 min as the best suited temperature for controlling seed-borne pathogens in different vegetable crops.

# Effect of Vitavax-200 and hot water treatments in controlling bacterial colony of mungbean seeds: Mungbean seeds produced yellow and cream color bacterial colony on seed surface. The highest percentage of yellow color colony was found (10.50%) in control that was statistically identical to Vitavax-200 0.5 g kg<sup>-1</sup>. Yellow color colony was totally controlled at 55°C that was statistically similar to the effect of hot water at 53°C (1.25% infection). In general, it was found that higher temperatures of hot water were more effective than the higher doses of Vitavax-200. (Table 9).

Cream color bacterial colony infection was highest (3.50%) in control that was statistically similar to treatment of Vitavax-200 at the rate of 0.5 g kg<sup>-1</sup> (3.25%) and treatment of hot water at 45°C (3.25%). Cream color colony was totally eliminated at 55°C of hot water that was statistically identical to 53°C of hot water (0.50% infection). From Table 9, it was found that in controlling creamy color bacterial colony the higher temperatures of hot water were more effective than the higher doses of Vitavax-200. This finding was in close agreement with Borah, *et al.* (1993), Little *et al.* (1997) and Nega *et al.* (2003). The researchers have given their verdict that hot water treatment is effective for reducing bacterial infection in various crops.

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