EFFECT OF BIOFUNGICIDE ON THE PRODUCTION OF HEALTHY AND QUALITY SEEDS OF *SALVIA HISPANICA* IN BANGLADESH

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ABSTRACT

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Experiments were conducted both in the field and laboratory to evaluate the roles of *Trichoderma*-based biofungicide for improving the health and quality of chia seeds. Four treatments viz. Control-untreated, Seeds treated with BAU-Biofungicide @ 2% of seed weight, Soil treated with IPM lab Biopesticide @ 64kg/ha and Seeds treated with BAU-Biofungicide @ 2% of seed weight + Soil treated with IPM-Biopesticide @ 64kg/ha were used in the field experiment. Among the treatments, BAU-Biofungicide showed better performance in terms of germination percentage, no. of branches, plant height, and seed yield. Both BAU-Biofungicide and BAU-Biofungicide + IPM lab Biopesticide performed better in terms of germination percentage on blotter paper. The identified seed borne fungi were *Fusarium oxysporum*, *Alternaria brassicae* and *Botrytis cinerea*. The prevalence of these fungi was lowest in the seeds treated with BAU-Biofungicide compared to control. Considering storage duration, freshly harvested seeds showed the highest prevalence of seed borne fungi. The quality of chia seeds treated with BAU-Biofungicide remained satisfactory up to six months compared to control. In *in-vitro* antagonism assay, *Trichoderma* based BAU-Biofungicide showed best performance to suppress the radial mycelial growth of *Fusarium oxysporum* and *Alternaria brassicae*. Application of *Trichoderma* based BAU-Biofungicide might be effective for growing healthy seeds of chia, as well as seeds can be stored up to six months.

Key words: *Salvia hispanica*, Seed-borne fungi, *Trichoderma harzianum,* BAU-Biofungicide, Biopesticide, Antagonism.

INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual herbaceous plant of Lamiaceae family. The cultivation of chia is gaining popularity across the world because it is considered as a good source of nutritional and healthy food (Ayerza and Coates 2011). Because of its nutritional value and stability, chia is already added to a range of foods (Ayerza and Coates 2002). Chia seed is consumed as a source of energy, composed of protein (15–25%), fats (30–33%), carbohydrates (26–41%), high dietary fiber (18–30%), ash (4-5%), minerals, vitamins, and dry matter (90–93%). It also contains a high amount of antioxidants (Ixtaina *et al*. 2008). The seed contains from 25% to 40% oil with 60% (omega) ω-3 alpha-linolenic acid and 20% of (omega) ω-6 linoleic acid of the oil contents (Ayerza and Coates 2011). These two essential fatty acids cannot be synthesized artificially. Both essential fatty acids are required by the human body for good health. However, feeding chia to chickens enriches their meat with omega-3 and to cattle, it enriches their milk with omega-3 (Ayerza and Coates 2002). Recently, chia has been reported as a promising health beneficiary new crop in Bangladesh (Hossain and Fakir 2014). Chia can also be added to commercially prepared infant formulas, baby foods, baked goods, nutrition bars, yogurt and other foods.

Like other plants, chia is also exposed to a number of plant pathogens. The previously reported organisms identified in chia seeds by moist blotter paper and agar plate method were *Fusarium solani*, *Pallidoroseum sp., Rhizopus sp., Cladosporium sp*. and the suspected disease is *Fusarium* wilt. This was evidenced that some plants were found wilted in the field plot (Yeboah *et al*. 2014).

Chia seeds are consumed freshly incorporating with yogurt and beverages. However, it is also known that essential oils in chia leaf have repellent properties against insects, making it suitable for organic cultivation. Therefore, organic production is necessary and will get more market value. Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Shalini and Kotasthane 2007; Eziashi *et al*. 2007; Tumpa *et al.* 2016). The potential of the antagonistic microorganisms in reducing the intensity of crop damage by the soil-borne plant pathogens has also been reported by Lewis and Larkin, (1997). Several strains of *Trichoderma spp.* have been found to be effective as biocontrol agents against various soil-borne plant pathogenic fungi such as *Rhizoctonia solani, Sclerotium rolfsii, Pythium aphanidermatium, Fusarium oxysporum, F. culmorum* and *Gaeumannomyces graminis* var. *tritici* and nematodes under greenhouse and field conditions (Cook and Baker 1983, Sivan and Chet 1993, Chet and Baker 1981, Papavizas 1985). *Trichoderma* strains isolated from Bangladesh and formulated as IPM Lab. Biopesticide showed a broad spectrum of antifungal action against *Fusarium oxysporum, Rhizoctonia solani, Fusarium circinatum, Phomopsis vexans, Sclerotium rolfsii* and *Pythium aphanidermatum* (Islam *et al*. 2016). Moreover, BAU-Biofungicide, a *Trichoderma*-based biofungicide has been reported for controlling seed-borne, soil-borne and air-borne diseases of different crop plants (Hossain and sultana 2011). *Trichoderma* inhibits the plant pathogens by a number of ways: mycoparasitism and antibiotic production, competition for nutrient and space with the plant pathogens, production of enzymes such as chitinases and/or glucanases that are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity, induction of defense response, and metabolism of germination stimulant (Howell 2003)

Still, chia is not cultivated in Bangladesh. But there is a very promising domestic and international market for this crop. Dr. Md. Alamgir Hossain, Professor of the Department of Crop Botany, Bangladesh Agricultural University introduced chia in Bangladesh in 2010. As it is newly introducing crop, the identification of pathogens associated with these seeds and their biological management is urgent for getting seed certification as well as spreading for cultivation in the whole country. Although experiments on plant characters, nutrient content, growth, and production of chia have been done worldwide, disease related to this crop has not been well investigated across the world. So, the present work was undertaken to investigate the effect of *Trichoderma* based bio-fungicide in improving seed health and quality of chia seeds.

MATERIALS AND METHODS

To investigate the effect of *Trichoderma* based biofungicide on the field performance of chia, seeds of chia were treated with BAU-Biofungicide @ 2% of seed weight, soil treated with IPM lab Biopesticide @ 64 kg/ha. All the necessary intercultural operations were done as and when necessary. After harvesting the seeds were used to investigate the association of seed borne fungi. To investigate the association of seed-borne fungi in `chia' seeds, seed health testing was done following the standard rules of ISTA (ISTA 2007). The associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Ramnath *et al*. (1970), and Khan and Islam (1975). For accurate identification of fungi, temporary slides were prepared from the fungal colony and observed under a compound microscope and identified with the help of keys suggested by Malone and Muskette (1964), Booth (1971), Ellis (1971), and Neergard (1979).The pure culture of the fungus was obtained by culturing the fungus on PDA medium and making the fresh culture from "hyphal tip" selected from the periphery of actively growing colony under aseptic conditions.

In-vitro antifungal bioassays with BAU Biofungicide and IPM lab Biopesticide were done following dual culture method. The biofungicides were evaluated against *Fusarium oxysporum*, and *Alternaria brassicae* by dual culture technique as described by Nafiza (2010).

Data were recorded on percent germination, no. of branches per plant, plant height, seed yield, percent moisture contents, and the prevalence of seed-borne fungi at different storage period and radial mycelial growth of the associated seed borne fungi in response to *Trichoderma* based bio-fungicides. The experiment was conducted with Completely Randomized Design (CRD) with three replications. Data were analyzed through a standard computer package statistical procedure MSTAT-C (Gomez and Gomez 1984).

RESULTS

Role of *Trichoderma*-based biofungicide on the field performance of chia

Seed treatment with *Trichoderma*-based BAU Biofungicide significantly increased percent germination, no. of branches per plant, plant height and seed yield compared to control (Table 1). Maximum germination (90.83%) was observed from BAU-Biofungicide treated seeds (T_1). Germination percentage in other treatments was statistically similar. The lowest germination was recorded in untreated control (T_0, 88.96%) Maximum no. of branches per plant (15.00) were recorded in T_1 treatments where seeds were treated with BAU-Biofungicide @ 2% of seed weight followed by 14.27, 14.14, 14.00 in T_3 (Seeds treated with BAU-Biofungicide @ 2%+ Soil treated with IPM lab Biopesticide @ 64 kg/ha), T2 (Soil treated with IPM lab Biopesticide @ 64 kg/ha) and T_0 (Control) treatments, respectively. The minimum no. of branches per plant was recorded in the untreated control treatment (T_0).The highest height (119.9 cm) was recorded in T_1 (Seeds were treated with BAU-Biofungicide @ 2% of seed weight) treatment. Statistically similar height 118.4 cm and 118.1 cm were observed in T_2 (Soil treated with IPM lab Biopesticide @ 64 kg/ha) and T_3 (Seeds treated with BAU-Biofungicide @ 2% of seed weight + Soil treated with IPM lab Biopesticide @ 64 kg/ha) treatment. The lowest height (111.3 cm) was recorded in untreated control (T0) treatment.The highest seed yield (1090 Kg) was recorded in T_1 (Seeds were treated with BAU- Biofungicide @ 2%) treatment. Comparatively the lowest seed yield (930 Kg) was found in untreated control (T_0) treatment. Higher seed yield (1050 kg and 1033 kg) results were also found from T_3 (Seeds treated with BAU-Biofungicide @ 2%+ Soil treated with IPM lab Biopesticide @ 64 kg/ha) and T_2 (Soil treated with IPM lab Biopesticide @ 64 kg/ha) treatments respectively (Table 1). Seed yield in all treatments except control is statistically similar.

Table 1: Effects of *Trichoderma*-based biofungicide on plant characters

Treatments	% Germination	No. of branches per plant	Plant Height (cm)	Seed Yield (kg/ha)
T_0	88.96 b	14.00 b	111.3 b	930 b
T_1	90.83 a	15.00 a	119.9 a	1090 a
T_2	89.17 b	14.14 b	118.4 a	1033 a
T_3	89.58 b	14.27 b	118.1 a	1050 a
$LSD_{0.05}$	1.16	0.603	6.08	95.64

T_0 = Control, T_1 = Seeds treated with BAU-Biofungicide @ 2% of seed weight, T_2 = Soil treated with IPM lab Biopesticide @ 64 kg/hectare (ha) and T_3 = Seeds treated with BAU-Biofungicide @ 2% of seed weight + Soil treated with IPM lab Biopesticide @ 64kg/ha

Role of *Trichoderma* based biofungicide on percent germination and moisture contents of seeds at different storage period

According to field experiment, the seeds were harvested and kept separately in plastic container for further study. In order to observe the storage ability of chia seeds, the seeds were preserved in plastic container and were kept in room condition for different storage duration. The seeds were taken out for health test time to time. Germination percentage of freshly harvested seeds did not vary in response to different *Trichoderma* based bio-fungicide. The range of germination percentage in freshly harvested seeds was 12.10 to 13.30. The results indicate the dormancy of freshly harvested seeds (Table 2). More than 80% germination of chia seeds were recorded after 2 month of storage. Among the treatments, the highest (85.90%) germination was found in T1 (Seeds treated with BAU-Biofungicide) and the lowest (80.20%) in control (T0) (Table 2). Germination of chia seeds in T1, T2 and T3 were statistically similar.

After four month of storage, germination of seeds significantly varied in different treatments ranged from 81.30% to 85.40%. Among the treatments, the highest (85.40%) germination was found in T1 (Seeds were treated with BAU- Biofungicide) and lowest (81.30%) in control (T_0) (Table 2). Germination of chia seeds of after four month of storage in T_1, T_2 and T_3 were statistically similar.

After six month of storage, germination of seeds significantly varied in different treatments ranged from 80.80% to 84.70%. Among the treatments, the highest (84.70%) germination was found in T_1 (Seeds were treated with BAU- Biofungicide) and the lowest (80.80%) in control (T_0) (Table 2). Germination of chia seeds of after six month of storage in T_0 and T_2 are statistically similar. Germination of chia seeds of after six month of storage in T_1 and T_3 were statistically similar.

Table 2: Effect of *Trichoderma* based biofungicide on germination percentage and % moisture content of seed at different storage period

Treatments	Freshly harvested		2 (MAS)		4 (MAS)		6 (MAS)	
	% germination	% moisture	% germination	% moisture	% germination	% moisture	% germination	% moisture
T_0	12.80 ab	20.13ab	80.20 b	11.23b	81.30b	14.80a	80.80 b	13.50 c
T_1	13.30 a	17.83 b	85.90 a	13.46a	85.40 a	14.20c	84.70 a	14.30 a
T_2	12.10 b	21.56 a	83.30 a	14.06a	84.20 a	14.70ab	82.50 ab	13.60 bc
T_3	12.20 b	21.42 a	84.20 a	14.01a	83.90 a	14.30bc	83.80 a	13.91b
$LSD_{0.05}$	0.831	2.67	2.61	1.69	2.57	0.453	2.43	0.376

MAS = Month After Storage

T_0 = Control, T_1 = Seeds treated with BAU-Biofungicide @ 2% of seed weight, T_2 = Soil treated with IPM lab Biopesticide @ 64 kg/ha and T_3 = Seeds treated with BAU-Biofungicide @ 2% of seed weight + Soil treated with IPM lab Biopesticide @ 64kg/ha

The moisture content of chia seeds was determined at different storage duration in response to different *Trichoderma* based biofungicide. In freshly harvested seeds, the moisture content is high which gradually decreased over the storage time. The moisture content of freshly harvested seeds significantly varied in different treatments ranged from 17.83% to 21.56%. Among the treatments, the highest (21.56%) moisture content was found in T2 (Soil treated with IPM lab Biopesticide @ 64kg/ha) and lowest (17.83%) in seeds treated with BAU-Biofungicide (Table 2). The moisture content of freshly harvested chia seeds in T_0 and T_1 was statistically similar.

After two months of storage, the moisture content of seeds significantly varied in different treatments ranged from 11.23% to 14.06%. Among the treatments, the highest (14.06%) moisture content was found in T_2 (Soil treated with IPM lab Biopesticide @ 64kg/ha) and lowest (11.23%) in control (T_0) (Table 2). The moisture content of chia seeds after two months of storage in T_1, T_2, and T_3 were statistically similar. After four months of storage, the moisture content of seeds significantly varied in different treatments ranged from 14.20% to 14.80%. Among the treatments, the highest (14.80%) moisture content was found on T0 (control) and the lowest (14.20%) in T_1 (Seeds treated with BAU-Biofungicide) (Table 2). The moisture content of chia seeds in T_2, T_3, and T_1, T_3 was statistically similar. After six months of storage, the moisture content of seeds significantly varied in different treatments ranged from 13.50% to 14.30%. Among the treatments, the highest (14.30%) moisture content was in T_1 (Seeds treated with BAU-Biofungicide) and the lowest (13.50%) in control (T_0) (Table 2). The moisture content of chia seeds in T_2 and T_3 was statistically similar.

Role of *Trichoderma* based biofungicide on percent seed borne infection

Prevalence of seed-borne fungi was recorded at different storage time. The identified fungi were *Fusarium oxysporum, Alternaria brassicae*, and *Botrytis cinerea*. The percent seed borne infection by *Fusarium oxysporum* and *Botrytis cinerea* was significantly decreased in freshly harvested seeds in response to different *Trichoderma* based biofungicide (Table 3). However, the percent seed borne infection by *Alternaria brassicae* was decreased in response *Trichoderma* based biofungicide but it was not significant statistically (Table 3).

Table 3: Role of *Trichoderma* based biofungicide on prevalence of seed-borne infection at different storage period

Treatments	% seed-borne infection											
	Fusarium oxysporum				*Alternaria brassicae*				*Botrytis cinerea*			
	0 MAS	2 MAS	4 MAS	6 MAS	0 MAS	2 MAS	4 MAS	6 MAS	0 MAS	2 MAS	4 MAS	6 MAS
T_0	3.200 a	3.100 a	2.980 a	2.580 a	5.87	6.100 a	5.790a	3.64	5.350 a	5.200a	5.280a	2.190a
T_1	1.600 c	1.700d	1.760 b	1.780 c	5.60	4.200 d	5.300b	3.43	4.270 b	3.960b	3.890b	1.960 b
T_2	2.400 b	2.600 b	2.700 a	2.320b	5.67	5.100 b	5.380b	3.48	5.330 a	5.110a	4.410b	2.120a
T_3	1.930 c	2.100 c	1.907 b	1.810 c	5.48	4.600 c	5.310b	3.45	4.710 b	4.210b	4.200b	1.990 b
$LSD_{0.05}$	0.461	0.326	0.297	0.188	0.465	0.342	0.336	0.429	0.453	0.291	0.505	0.103

T_0 = Control, T_1 = Seeds treated with BAU-Biofungicide @ 2% of seed weight, T_2 = Soil treated with IPM lab Biopesticide @ 64 kg/ha and T_3 = Seeds treated with BAU-Biofungicide @ 2% of seed weight + Soil treated with IPM lab Biopesticide @ 64kg/ha

After two, four and six months of storage the percent seed-borne infection by *Fusarium oxysporum*, *Alternaria brassicae* and *Botrytis cinerea* were significantly decreased in response to different *Trichoderma* based biofungicide (Table 3).

Antagonistic effect of *Trichoderma* based biofungicide against *Fusarium oxysporum* and *Alternaria brassicae*

The effect of *Trichoderma* based biofungicide on the radial mycelial growth of *Fusarium oxysporum* and *Alternaria brassicae* was recorded up to 72h (Table 4). The radial mycelial growth of *Fusarium oxysporum* was significantly decreased in response to *Trichoderma* at 24 hrs, 48 hrs, and 72 hrs. After 24h, the growth of all treatments showed a significant difference. At 24h of incubation, the highest mycelial growth was observed in T0 and T_2 treatment while the lowest was observed in T_1 and T_3 treatment (Table 4). At 48h, the highest mycelial growth was recorded in T_0 while the lowest growth was recorded in T_1 and T_3 treatments. At 72h, the highest mycelial growth was observed in T_0 while rest of the treatments showed lower mycelial growth (Table 4).

It was observed that all the treatments showed significant effects on mycelial growth of *Alternaria brassicae* at 24h, 48h, and 72h of an interval (Table 4). The growth of *Alternaria brasssicae* at the 24h interval against different treatments was significantly different (Table 4). Among the treatments, the highest mycelial growth (10.33mm) of *Alternaria brassicae* was observed in control treatment. The lowest growth (8.33mm) was observed in T_1. At 24h, the initial growth of this fungus is almost similar. The growth of *Alternaria brasssicae* at the 48h interval against different treatments was significantly different (Table 4). The highest mycelial growth of *Alternaria brassicae* (22.67mm) was observed in control treatment and the lowest growth (10.33mm) was observed in T_1. The growth of the fungus, *Alternaria brasssicae* at the 72h interval against different treatments was significantly different (Table 4). Among the treatments, the highest mycelial growth of *Alternaria brassicae* (30.67mm) was observed in T_0. Statistically similar growth of the fungus (15.67mm and 14.00mm) was observed in T_2 and T_3, respectively. The lowest growth (11.00mm) was observed in T_1 (Table 4). Among the treatments *Trichoderma* based BAU biofungicide showed the best performance.

Table 4: Effect of *Trichoderma* based biofungicide on inhibition of radial mycelial growth of *Fusarium oxysporum* and *Alternaria brassicae*

Treatments	Radial mycelial growth (mm)					
	24h		48h		72h	
	F. oxysporum	*A. brassicae*	*F. oxysporum*	*A. brassicae*	*F. oxysporum*	*A. brassicae*
T_0	9.670 a	10.330 a	14.67 a	22.67a	18.67 a	30.67a
T_1	8.330 b	8.330 c	11.33 c	10.33c	11.67 b	11.00 c
T_2	9.330 a	9.290 b	13.00 b	15.67b	12.33 b	15.67b
T_3	9.000 b	9.330b	11.67 c	15.33b	12.00 b	14.00b
$LSD_{0.05}$	0.665	0.854	1.09	1.06	1.11	2.13

T_0 (control= No *Trichoderma*), T_1 (*Trichoderma harzianum* of BAU-Biofungicide), T_2 (*Trichoderma harzianum* of IPM lab Biopesticide) and T_3 (*Trichoderma harzianum* of BAU-Biofungicide + *Trichoderma harzianum* of IPM lab Biopesticide).

DISCUSSION

Seed treatment and soil treatment with *Trichoderma*-based BAU Biofungicide significantly increased percent germination, no. of branches per plant, plant height and seed yield compared to control. Abd-El-Khair *et al*. (2010) reported that *Trichoderma spp.* improved the plant characters and decreased the damping off disease incidence of the bean. Alam *et al.* (2014) reported that seed treatment with *Trichoderma* based biofungicide decreased the prevalence of seed-borne fungi of chili. Sultana *et al*. (2009) and Hossain and Sultan (2011) reported that BAU-Biofungicide increased germination and seedling vigor of some vegetable seeds. Naznin and Hossain (2005) observed 50.80% higher germination over the control in cowpea by applying BAU-Biofungicide. *Trichoderma harzianum* treated seed of blackgram resulted up to 16.66% seed germination over control (Shamsuzzaman and Hossain (2003). These results indicate that *Trichoderma* might suppress the growth of soil-borne pathogens and stimulate the growth of plants.

In this experiment, the efficacy of bioagents against the isolated seed borne pathogens was assessed in lab condition. Trichoderma harzianum of BAU-Biofungicide showed better performance to suppress the growth of all isolated pathogens. Therefore this can be hypothesized that application of bioagents in the field suppressed both soil-inhabiting and seed borne fungi. Moreover, these bioagents may also activate some enzymes in the host plants which suppress the growth of pathogen as well as produce healthy seedlings. The findings of some researchers also support the present findings that *Trichoderma*-based bioagents can promote vegetative growth and increased yield of different crops. Harman (1990) reported that *Trichoderma harzianum* provided good control against a range of pathogens, including *Phytophthora*, *Pythium ultimum*, *Rhizoctonia solani*, *Fusarium spp.*, *Sclerotium rolfsii* and *Botrytis cinera*, if properly applied. Xu *et al.* (1993) found that mycelial growth of *Fusarium solani* and *F. oxysporum* was inhibited with *T. harzianum*. Michalikova and Michrina (1997) reported the greatest inhibition rate of the radial growth of *F. culmorum* (55-58%) with *T. harzianum*. Similar findings were obtained by Begum (1997), Sultana *et al*. (2001), and Kashem (2005). *Trichoderma* inhibit the plant pathogens by a number of ways: mycoparasitism and antibiotic production, competition for nutrient and space with the plant pathogens, production of enzymes such as chitinases and/or glucanases that are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity, induction of defense response, and metabolism of germination stimulant (Howell 2003).

CONCLUSIONS

Chia is a promising health beneficial new crop to be released in Bangladesh. The results of this research suggest that *Trichoderma* based biofungicide improves the field performance of chia, reduces the prevalence of major seed-borne fungi of chia, decreased the radial mycelial growth of seed-borne fungi in-vitro and improves the storability of chia seeds. Since *Trichoderma* based biofungicide promotes the production of healthy and quality seeds of chia, as well as chia has enormous beneficial effects on health; so it can be introduced in Bangladesh as a new crop. Therefore, Biofungicide can be recommended for better production of chia in Bangladesh.

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