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INTEGRATED USE OF FUNGICIDE, PLANT EXTRACT AND BIO-AGENT FOR MANAGEMENT OF *ALTERNARIA* BLIGHT DISEASE OF RADISH (*Raphanus sativus* L.) AND QUALITY SEED PRODUCTION

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Abstract. An attempt was taken to evaluate integrated management of *Alternaria* blight disease of radish variety BARI Mula-1 and quality seeds production in Bangladesh. Before setting the field experiment, *in-vitro* and *in-vivo* assays were laid out to select the virulent isolate AB-2 of *Alternaria brassicae*, antagonistic bio-agent *Trichoderma harzianum* isolate T-2, Neem (*Azadirachta indica*) leaf extract and Rovral 50WP (Iprodione) fungicide. The virulent isolateAB-2 of *A. brassicae* spore suspensions (5×10^5 spores ml^{-1}) was sprayed for inoculation purpose of radish field with micro-sprayer prior to flowering at 50 days after sowing (DAS). Bio-agent, *T. harzianum* isolate T-2 was used as soil amendment with wheat bran substrate (@ 90 gm^{-2}), seed treatment (@ 3 g Kg^{-1}) and also foliar spray (@ 5×10^5 spores ml^{-1}) at 3 times after 3 days of inoculation with *A. brassiceae*. Rovral 50WP was used as seed treatment @ 2.5 g Kg^{-1} seed. About 200 ppm conc. fungicide ((Iprodione) and 10% conc. Neem (*Azadirachta indica*) leaf extract was also used as foliar spry for 4 times at 10 days interval starting from flowering stage. The highest reduction of disease severity on leaf, siliqua and prevalence of *A. brassicae* on seeds (19.40, 15.40 and 10.00%) and increased seed yield and germination (160.96% and 85.45%) was observed in the treatment T_8 followed by T_7, T_5 and T_6, respectively. On the contrary, the lowest reduction of disease severity on leaf, siliqua and prevalence of *A. brassicae* on seeds (69.80, 62.20 and 43.22%) and decreased seed yield and germination (12.72% and 53.00%) was observed in the treatment T_2 followed by T_1. The intensification of seed yield was not only because of the reduction of diseases but also it might be due to addition of the bio-agent in the soil as a result, increases the number of soluble nutrients and provoke to secret the different growth promoting substances.

Keywords: *Alternaria blight, T. harzianum, Rovral 50WP, Neem leaf extract, Seed yield of Radish.*

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1. Introduction

Radish (*Raphanus sativus* L.) belongs to the family Brassicaceae is an important vegetable of the both tropical and temperate regions in the world. It fleshy edible root contains Ca, K, P and vitamin C (Srinivas & Naik, 1990). It is the 3rd major vegetable crop in term of both area and production in Bangladesh. In the 2015-16 year, the total radish cultivable land was 26.30 thousand hectares and yield production was 2.81 lac tons with an average yield 10.65 t ha^{-1} (Anon., 2017). Although many constrains are present for the low production of radish, the quality seed is important one. Seed quality is deteriorated by the plant diseases. The important diseases of radish are damping off, leaf spot, *Fusarium* rot, black rot, *Alternaria* blight etc. Among the many plant diseases, *Alternaria* blight caused by four species of *Alternaria* viz., *A. brassicae*, *A.*

brassicicola, *A. raphani* and *A. alternata*is the most devastating disease of radish crop in field condition. *A. brassicae* and *A. brassicicola* are seriously responsible for *Alternaria* blight disease of radish which can cause up to 30-40% yield loss in Bangladesh (Mondal *et al.*, 1989; Anon., 1997). *A. brassicae* and *A. brassicicola* appear on pod or siliqua as small, blackish, circular lesions which eventually cause the death of the pod or siliqua (Prasada *et al.*, 1970). The severe foliage infection causes reduction of flower which results in production of diseased pods and seeds (Chand & Jatian, 1969).

Control of *Alternaria* blight disease in Brassicaceae is very difficult due to wide host range. Several fungicides could be effective against the disease. Iprodione one of the fungicide which has been proved very effective in controlling the *Alternaria* blight of mustard in Bangladesh (Meah, 1992). In seed samples, Iprodione usually eliminated the fungus from the sample, but higher levels of infection required a larger dose of Iprodione. But excess use of chemical fungicide has favored the development of pathogen resistant and polluted the entire environment. Integrated use of fungicide, microbial biocontrol agent and plant extracts have been found effective against wide range of plant pathogens (Amadioha, 2003; Bowers & Locke, 2004).

Trichoderma harzianum is going to use as seed treating as well as foliar application in Bangladesh. This antagonistic fungus had a potential antifungal effect on *A. brassicae*, *A. brassicicola* and *A. alternata* as seed-borne as well as cause of leaf spot disease of radish (Bhuiyan *et al.*, 2006; Hossain, 2007; Sultana, 2007). It may be a possible source of non-phytotoxic, systemic and easily biodegradable alternative pesticides to control the disease economically and ecofriendly (Qasem & Abu-Blan, 1996; Mason & Mathew, 1996). According to the Kumar *et al.* (1979) and Naidu and John (1982), the most of aromatic and medicinal plants are the sources of many diverse nutrients and non-nutrient molecules. It has antioxidant and antimicrobial properties which can prevent the growth and development of the pathogens. Therefore, the current study was designed to integrated use of fungicide, plant extract and bio-agent for management of *Alternaria* blight disease of radish and quality seed production in Bangladesh.

2. Materials and Methods

2.1. Collection, Isolation and Preservation of Alternaria brassiceae

The *Alternaria brassiceae* isolates were collected from the infected field of different locations of Gazipur districts. The specimens which had typical symptoms of *Alternaria* blight was selected from infected radish fields. The fungal isolates were isolated by following standard method of Mian, 1995. The fungal colonies were grown on Potato Dextrose Agar (PDA) medium and identified by following standard key (Barneet & Hunter, 1972). The pure culture of *A. brassiceae* isolate was named individually with English capital letter and numerical number codes. The pathogenicity of the isolates was tested on a detached leaf of radish under moist petri dishes by spraying spore suspension. Among the many isolates, *A. brassicae* isolate AB-2 was produced maximum number of typical symptom on the detached radish leaves. Then, isolate AB-2 was preserved by using PDA slants at 10 ℃ in the refrigerator as a stock culture for further study.

2.2. Preparation of wheat grain colonized Trichoderma harzianum

Inoculum of the *T. harzianum* was prepared on autoclaved moist wheat grains in 500 ml Erlenmeyer flask. Before using, wheat grains were soaked in water for 12 hrs. After soaking excess water was drained out and water soaked grains were poured into 500 ml Erlenmeyer flask. Five-millimeter diameter mycelial discs were cut from the edge of three days old PDA cultures in petri dishes. Five to seven mycelial discs of fungi were added to autoclaved wheat grains in the flasks and incubated at 25 $°C$ for 21 days. It was shaken by hand at 2-3 days interval for proper colonization. The colonized wheat grains were air dried for 2 days and stored at 4 $°C$ for further work.

2.3. Inoculation of Alternaria brassiceae isolate AB-2

A. brassiceae spore suspensions (5×10^5 spores ml^{-1}) was prepared from 10 days old pure culture. Before spraying the inoculum into the field, Tween-20 @ 0.1 ml L^{-1} was added to the spore suspension for avoiding spores aggregation and proper attachment with the leaf surface. The prepared spore suspension was sprayed with micro-sprayer prior to flowering at 50 days after sowing (DAS).

2.4. Mixing and spraying of T. harzianum

Seeds were treated with *T. harzianum* @ 3 g Kg^{-1} seed. Tween-20 @ 0.1 ml/L was added for active attachment of conidia with seeds. On the other hand, an aqueous suspension of *T.harzianum* spore (5×10^5 spores ml^{-1}) was prepared from pure culture of *Trichoderma* isolate T-2. Before spraying the inoculum into the field, Tween-20 @ 0.1 ml L^{-1} was added to the spore suspension for avoiding spores aggregation and proper attachment with the leaf surface. The prepared spore suspension was sprayed with micro-sprayer at 3 times after 3 days of inoculation of *A. brassiceae* in the field, at siliqua forming to maturing stage.

2.5. Mixing and spraying of Rovral 50WP

Rovral 50WP was used as a seed treating fungicide @ 2.5 g Kg^{-1} seed. Seeds were taken into a beaker and shaken with fungicides for five minutes before sowing in the field. On the contrary, Rovral 50WP @ 200 ppm was also applied as foliar spray with a knapsack sprayer for 4 times at ten days interval from flowering stage.

2.6. Preparation and spraying of Neem leaf extract

Fresh parts of the test plant Neem (*Azadiracta indica*) was collected and washed thoroughly with distilled water. Hundred grams of washed sample was ground in mortar and pestle by adding an equal amount (100 ml) of sterilized distilled water (1:1 W/V) and boiled at 80 °C for 10 minutes in a hot water bath. The ground material was filtered through muslin cloth followed by filtering sterilized what man No. 1 filter paper and treated as a standard plant extract hundred percent. Then, the mixture was diluted 10% concentrations by adding proper volumes of sterilized water. Neem extract 10% conc. was sprayed 4 times at ten days interval from flowering stage.

2.7. Experimental design and Treatments

The field experiment was designed by using a Randomized Complete Block Design (RCBD) with eight treatments and four replications. The unit plot size was 3 m × 2 m where, row to row distance 40 cm. Seeds were sown @ 2.5 kg ha^{-1}.

The treatments are as follows:
T_1= Uninoculated field (Control-1)
T_2= Inoculated with *A. brassicae* isolate (IAI) AB-2 (Control-2)
T_3= *T. harzianum* treated seed (TTS) + IAIAB-2
T_4= Rovral 50WP treated seed (RTS) + IAIAB-2
T_5= TTS+ IAIAB-2+ Spraying Rovral 50WP (SR)
T_6= Spraying Neem leaf extract + IAIAB-2
T_7= Wheat grain inoculum of *T. harzianum* (WIT)+ RTS + IAI AB-2 + Spraying *T. harzianum*
T_8= WIT + IAIAB-2+ SR + Spraying Neem leaf extract + Spraying *T. harzianum*

2.8. Data collection

Data were recorded on yield and yield contributing characters of radish during the growing to harvesting period. Disease incidence and severity of *Alternaria* blight was recorded. Total seed yield was also recorded. Fifteen plants were randomly selected and tagged from in each individual plot, then checked individually for separation of healthy and infected plants or plant parts. The disease severity for *Alternaria* blight was rated based on a modified scale (0-5) by following standard method (Mian, 1995).

Rating	Observation
0	no infection
1	Small light brown spots scattered covering 1-10% of total leaf area and siliqua
2	Spots small, brown, with concentric rings, covering 11-20% of total leaf area and siliqua
3	Spots large, brown, irregular, with concentric rings, covering 21-30% of total leaf area and siliqua
4	Large, brown, irregular lesions with typical blight symptoms, covering 31-40% of total leaf area and siliqua
5	Large, brown, irregular lesions with typical blight symptoms, covering more than more than 40% of total leaf area and siliqua

Finally, the percent disease incidence and disease severity were calculated by following formula (Rahman *et al.,* 2013).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants/ tubers}}{\text{Total number of plants/ tubers assessed}} \text{ x } 100$$

$$\text{Percent disease index } = \frac{\text{Summation of all ratings}}{\text{Total number of rating x Maximum disease grade}} \text{ x } 100$$

2.9. Seed Health test

Seed quality in terms of seed grading, germination and prevalence of *Alternaria* spp. in seeds were determined and data recorded. Seeds were passed through 10, 12 and 16 mesh sieves for grading into three categories such as grade-1, grade-2 and grade-3, respectively. After grading, weight of seeds under each grade was measured. For seed health and germination test, four hundred seeds were taken randomly from each treatment plots. Then, 400 seeds were placed into sixteen replicated plates containing moist blotter paper by following International rules for seed health testing (ISTA, 1993). Seeds were placed on three plies moist blotter paper (Whatman No. 1) in sterilized 90 mm petri dishes. Twenty-five seeds were placed in each petri dish and maintained equal distances from seed to seed. After planting seeds, the petri dishes were incubated at room temperature (25 ± 2 °C) for seven days. Seven days after plating, number of germinated seeds and number of seeds *Alternaria* spp. infected un-germinated seeds were counted. Germination and prevalence of the pathogens were expressed in percentage based on total number of plated seeds.

3. Results

3.1. In-vitro evaluation of fungicide, bio-agent and plant leaf extract against inhibition of radial growth of A. brassiceae isolate AB-2

In-vitro evaluation of Rovral 50WP @ 200 ppm conc., *T. harzianum* and Neem leaf extract @ 10% conc. were conducted against the inhibition of radial growth of *A. brassiceae* isolate AB-2. About 86.89% inhibition of radial growth of *A. brassiceae* isolate AB-2 was appeared against Rovral 50WP @ 200 ppm conc. (Table 1). Antagonistic activity of *T. harzianum* against *A. brassiceae* isolate AB-2 was determined by dual culture method on PDA. The inhibition of radial growth against test pathogen was recorded at 79.66% (Table 1). The efficacy of neem leaf extract against *A. brassiceae* isolate AB-2 was also assayed *in-vitro*. The antimicrobial effect of the plant extract was observed and recoded 71.63% inhibition of radial growth of *A. brassiceae* isolate AB-2 (Table 1).

Table 1. *In-vitro* evaluation of fungicide, bio-agent and plant extract against inhibition of radial growth of *A. brassiceae* isolate AB-2

Treatments	Radial growth of *A. brassiceae* isolate AB-2 (mm)	% inhibition of radial growth of *A. brassiceae* isolate AB-2
Rovral 50WP (200 ppm)	11.80	86.89
T. harzianum	18.30	79.66
Neem leaf extract (10% conc.)	25.54	71.63
Control	90.00	-

3.2. Disease Development

Alternaria blight symptoms appeared on lower leaves at 25-30 days after sowing. Blight firstly appeared on leaves with typical concentric rings. After siliqua formation it was appeared on siliqua as small dark brown to black spots. Crush mounts were prepared using spotted tissues of leaves and siliqua and observed under compound microscope for confirmation of the causal agent of *A. brassicae* (Plate I).

A B C

Plate I. *Alternaria* blight symptom on the Leaf (A), Siliqua (B) and Conidia of *A. brassicae* (C)

3.3. Disease severity

The highest percent disease index (PDI) of *Alternaria* blight was observed in control-2 where plants were inoculated with the test pathogen. On the contrary, the lowest disease severity of *Alternaria* blight was observed in the treatment T_8 where integration of fungicide, plant extract and bio-agent were applied followed by the treatment T_7, T_4, T_5 and T_6, respectively (Table 2). Integration of *T. harzianum*, Rovral 50WP and neem leaf extract in the treatment T_8 was appeared identical with the treatment T_7 where Rovral 50WP and *T. harzianum* was applied in case of disease severity of both in the leaf and siliqua. The highest 72.2 and 75.28% reduction of *Alternaria* blight in leaf and siliqua was achieved with treatment T_8 while only *Trichoderma* treated seeds in the treatment T_3 and neem leaf extract spray in the treatment T_6 were appeared to be identical and most inferior in reducing the disease both in leaf and siliqua infections. Lesion area per leaf spot was highest in control plots which were observed 13.32 and 13.36 cm^2/leaf spot in T_1 and T_2, respectively. Lesion area per leaf spot was lowest (2.247 cm^2/leaf spot) in the treatment T_8 (Table 2). Similarly, numbers of spot/siliqua and lesion area per siliqua spot were also significantly higher in control plots in comparison to the treated plots. The results of the study suggested that Rovral 50WP alone reduced more than 50% *Alternaria* blight either applied as seed treating or applied as spraying fungicide. When Rovral 50WP was integrated with *T. harzianum* in the treatment T_7, PDI of *Alternaria* blight of radish was significantly increased compared to the individual application of fungicide, *T. harzianum* or neem leaf extract appeared to be identical in reducing disease severity with the treatment T_8. However, this study suggested that integration of fungicide, bio-agent and neem leaf extract in the treatment T_8 was found to be the best in controlling *Alternaria* blight of radish.

Table 2. Effect of integrated use of fungicide, plant extract and bio-agent on disease incidence and severity of *Alternaria* blight of radish

Treatments	*Alternaria* blight on leaf		*Alternaria* blight on siliqua		
	Lesion size (cm^2)	PDI	Number of spot/siliqua	Lesion size (cm^2)	PDI
T_1	13.32 ^{a}	63.40 ^{b}	28.55 ^{a}	13.83 ^{a}	56.80 ^{ab*}
T_2	13.36 ^{a}	69.80 ^{a}	31.83 ^{a}	14.05 ^{a}	62.20 ^{a}
T_3	8.88 ^{c}	58.00 ^{c}	17.77 ^{bc}	6.72 ^{c}	55.40 ^{b}
T_4	5.96 ^{d}	29.80 ^{d}	15.17 ^{c}	4.35 ^{d}	25.80 ^{c}
T_5	2.82 ^{ef}	32.60 ^{d}	9.92 ^{d}	1.95 ^{e}	28.20 ^{c}
T_6	9.85 ^{b}	58.80 ^{bc}	19.10 ^{b}	7.65 ^{b}	56.20 ^{ab}
T_7	3.15 ^{e}	23.10 ^{e}	9.90 ^{d}	2.07 ^{e}	17.60 ^{d}
T_8	2.24 ^{f}	19.40 ^{e}	9.67 ^{d}	1.72 ^{e}	15.40 ^{d}

*Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.[Where, T_1= Uninoculated field (Control-1), T_2= Inoculated with *A. brassicae* isolate (IAI) AB-2 (Control-2), T_3= *T. harzianum* treated seed (TTS) + IAI AB-2, T_4= Rovral 50WP treated seed (RTS) + IAI AB-2, T_5= TTS + IAI AB-2+ Spraying Rovral 50WP (SR), T_6= Spraying Neem leaf extract + IAI AB-2, T_7= Wheat grain Inoculum of *T. harzianum* (WIT) + RTS + IAI AB-2 + Spraying *T. harzianum*, T_8= WIT + IAI AB-2+ SR + Spraying Neem leaf extract + Spraying *T. harzianum*]

3.4. Yield and yield contributing characters

The effect of Rovral 50WP, neem extract and *T. harzianum* either alone or in combination on seed yield, seed weight of radish was observed in the inoculated field and the results are presented in the Table 3. All the treatment increased seed weight and yield significantly over the control-1 and 2. The highest seed weight and yield was observed in the treatment T_8 where *T. harzianum* was used as field spray and soil application, Rovral 50WP and neem extract applied as field spray followed by treatment T_5 where Rovral 50WP treated seeds were used following Rovral 50WP applied as field spray. Seed yield and seed weight was almost double in the treatment T_8 and T_5comparedthan control-2. In response to seed yield treatment T_7 was inferior to the treatment T_8 and T_5 but significantly superior to the all other treatments. *T. harzianum* treated seeds used in the treatment T_3 and Rovral 50WP treated seeds used in the treatment T_4 were found identical in increasing seed weight and seed yield of radish crop. Neem extract spray applied in the treatment T_6 was very effective in increasing seed yield and seed weight and significantly superior to the T_3 and T_4 but inferior to T_7 treatment. The lowest seed yield recorded in the control treatment T_2 where pathogen was inoculated without any other control measures but identical with the control treatment T_1 where only natural condition was maintained and untreated healthy seeds were sown without pathogen and application of any other treatments.

3.5. Effect of integrated treatment on seed quality

After harvest of the seeds, then it was graded into three categories: grade-1, 2 and 3 based on the seed size which was sorted by wire mesh 10, 12 and 16, respectively. The highest percent grade 1 seeds were produced by the treatment T_8 followed by the treatment T_5. The seed yield under grade 1, 2 and 3 were ranged from 5.20-18.69, 61.23-67.38 and 16-29.96%, respectively (Table 4). Among the total seeds around 25% seed yield was categorized as grade 3 in the treatment T_3, T_2 and T_1. On the contrary, highest seed germination was observed in the treatment T_8 followed by T_7 and T_5, respectively. Significantly, the lowest germination was observed in the control treatments T_1 and T_2. No significant difference was found in seed germination compared to the control treatments and T_6 treatment.

The prevalence of *Alternaria* spp. associated with harvested seeds under different treatments was tests in the blotter method. The results of the seed health tests are presented in the Table 4. Significantly, the highest percent *Alternaria* spp. was isolated from the seeds produced from the *Alternaria brassicae* sprayed control treatments T_2 followed by the control-1 treatment at the three grade of seeds. On the other hand, the lowest 9.00% *Alternaria* spp. was isolated from the grade 1 seeds of the treatment T_8 and T_5. The prevalence of *Alternaria* spp. in the grade 2 and 3 seeds of the T_8 treatment were also significantly lower in comparison to the other treatments. Prevalence of *Alternaria* spp. in the seeds yielded from neem extract sprayed plots in the treatment T_6 was significantly higher in comparison to all the treatments except the control treatments T_1 and T_2. Significantly, the highest 46.33% seeds yielding *Alternaria* spp. from the grade 3 seeds of the control T_2 treatment and identical with the grade 3 seeds of the control treatment T_1. The results of the present study demonstrated that, integrated use of *T. harzianum*, neem extracts and Rovral 50WP was appeared to be the best management package not only for controlling *Alternaria* blight disease of radish but also augmented the seed quantity and quality.

Table 3. Effect of integrated use of fungicide, plant extract and bio-agent on seed yield of radish

Treatments	Siliqua length (cm)	Siliqua No./Plant	1000 seed wt. (g)	Seed yield g/plot	Seed yield kg/ha	% Seed yield increase/ reduction (-) over control-1
T_1	5.86 ab	236.8 d	8.98 b	112.1	373.5 e	-
T_2	5.59 a-c	207.6 d	9.09 b	97.8	326.0 e	-12.72
T_3	5.90 ab	267.4 d	10.86 ab*	137.9	459.7d	23.08
T_4	4.98 c	261.9 d	9.46 ab	134.8	449.2 d	20.27
T_5	6.07 a	595.2 a	10.67 ab	281.2	937.4 a	150.98
T_6	5.67 a-c	428.3 c	9.48 ab	201.7	672.3 c	80.00
T_7	5.15 bc	520.7 b	11.07 a	244.6	815.3 b	118.29
T_8	5.51 a-c	621.1 a	11.18 a	292.4	974.7 a	160.96

*Means within same column followed by common letter(s) are not significantly different (P=0.05) by DMRT.

Table 4. Effect of integrated use of fungicide, plant extract and bioagent on seed germination and prevalence of *A. brassicae* on seeds

Treat-ments	% seeds grading			% seeds germination			Average % seeds germination	% prevalence of *A. brassicae* on seeds			Average % prevalence of *A.brassicae* on seeds
	G-1	G-2	G-3	G-1	G-2	G*-3		G-1	G-2	G-3	
T_1	5.56	64.48	29.96	52.33 ^{d}	59.33 ^{d}	54.33 ^{cd**}	55.33	37.33 ^{b}	37.67 ^{b}	46.00 ^{a}	40.33
T_2	5.20	65.50	29.30	51.33 ^{d}	58.33 ^{d}	53.00 ^{d}	54.22	42.00 ^{a}	41.33 ^{a}	46.33 ^{a}	43.22
T_3	9.35	61.23	29.42	73.00 ^{bc}	76.67 ^{c}	80.67 ^{a}	76.78	21.67 ^{d}	22.00 ^{c}	24.67 ^{d}	22.78
T_4	10.02	64.68	25.30	69.00 ^{c}	78.67 ^{bc}	72.00 ^{b}	73.22	19.33 ^{d}	21.67 ^{d}	28.67 ^{c}	23.22
T_5	15.38	67.38	17.24	79.00 ^{b}	85.00 ^{ab}	81.67 ^{a}	81.89	9.00 ^{f}	16.00 ^{e}	11.00 ^{e}	12.00
T_6	7.67	63.87	28.46	57.33 ^{d}	60.33 ^{d}	58.33 ^{c}	58.66	27.33 ^{c}	26.00 ^{c}	28.67 ^{c}	27.33
T_7	18.00	66.00	16.00	79.67 ^{b}	85.00 ^{ab}	78.67 ^{a}	81.11	13.00 ^{e}	15.00 ^{e}	12.00 ^{e}	13.33
T_8	18.69	62.38	18.93	87.00 ^{a}	88.67 ^{a}	80.67 ^{a}	85.45	9.00 ^{f}	10.00 ^{f}	11.00 ^{e}	10.00

* Grade, **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT. [Where, T_1= Uninoculated field (Control-1), T_2= Inoculated with *A. brassicae*isolate (IAI) AB-2 (Control-2), T_3= *T. harzianum* treated seed (TTS) + IAI AB-2, T_4= Rovral 50WP treated seed (RTS) + IAI AB-2, T_5= TTS + IAI AB-2+ Spraying Rovral 50WP (SR), T_6= Spraying Neem leaf extract + IAI AB-2, T_7= Wheat grain Inoculum of *T. harzianum* (WIT) + RTS + IAI AB-2 + Spraying *T. harzianum,* T_8= WIT + IAI AB-2+ SR + Spraying Neem leaf extract + Spraying *T. harzianum*]

4. Discussion

In Bangladesh, this is the first comprehensive study that aimed to develop a sustainable management package against *Alternaria* blight disease of radish and increase the seed yield and quality.

According to Kaushik *et al.* (1984) *Alternaria* blight of mustard caused by *A. brassicae* was one of the most severe yield destabilizing factors and resulted an average yield loss of 35-40%. The disease adversely affects seed quality by reducing seed size and seed colour. According to the Rashid and Hossain (2011), *Alternaria* blight symptoms appeared on lower leaves at 20-30days old transplanted stickling.

Management of the *Alternaria* blight in Crucifers is very difficult due to various sources of inoculum and wide range in conidial dispersal and uncertainty of the level of disease, whereas several protective fungicides could effectively control the disease (Howlider *et al.*, 1991; Meah *et al.*, 1992).

Many chemical fungicides have been evaluated for effectiveness in controlling *A. brassicae* and conclusions are often conflicting. Rovral 50WP (Iprodione) has been proved very effective in controlling the *Alternaria* blight of mustard (Meah, 1992) and early blight of tomato (Islam *et al*., 2012). In the present study, seed treatment and four times foliar spray at ten days interval from flowering stage to harvesting stage reduced maximum *Alternaria* blight infestation and simultaneously increased 1000-seed weight, seed yields per plant and per hectare. The efficacy of fungicidal effect on seed quality was also observed by the seed health test. Because the prevalence of *Alternaria* spp. were negligible in those plot where fungicide was applied. The results are supported with the findings of Ayub *et al.* (1996) and Singh and Singh (2006), who have successfully controlled the *Alternaria* blight of radish with three sprays of Rovral 50WP (Iprodione).

Trichoderma spp. are well known for their biocontrol activity against many soil-borne pathogens that cause major problems in the current agricultural scenario (Sharma *et al.*, 2011). The major biocontrol procedure involves antibiosis, providing plant nutrition and also mycoparasitism (Janisiewicz & Korsten, 2002). *Trichoderma* spp. are bio-fertilizers that induces plant defense responses and improved tolerance to abiotic stresses (Shoresh *et al.*, 2010; Hermosa *et al.*, 2012). Bio-agent *T. harzianum* produces a large number of chemicals substances such as β-1, 3-glucanases which can lyses the fungal cell wall and suppress the growth and development of the pathogens. On the contrary, some of the chemicals can solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+} and Cu^{2+} and increase iron availability and enhance iron uptake which might be contributed in increasing quantity and quality of radish seeds (Altomare *et al.*, 1999). Under the present study, it was observed that application of *T. harzianum* as seed treatment and foliar spry gave the positive result. Disease incidence and severity was reduced significantly and simultaneously increased the seed weight and quality. The findings of the current study are in agreement with several investigators who observed that integration of *Trichoderma* was superior in the management of *Alternaria* blight of crucifers as well as increased seed yield and improved seed quality (Meena *et al.*, 2003; Kumar *et al.*, 2008; Rathi & Singh, 2010; Rashid & Hossain, 2011). Hossain reported that, BAU-Biofungicide has a significant effect in reducing seed borne *Alternaria* leaf spot of radish in the field condition. Bhuiyan *et al.* (2006) reported that BAU-Biofungicide showed significant effect in reducing seed-borne Alternaria of radish with the luxuriant plant growth in the field. Sultana (2007) found that, remarkable reduction of leaf blight severity of wheat in foliar spray of BAU-Biofungicide (2.0%) in the field.

The neem plant present a potential for control of plant fungal diseases with chemical compound which are environment friendly. Neem plant extract (leaf, seed and bark) can be applies in many ways such as sprays, powders, drenches or diluents in irrigation water. One valuable property of the neem materials in plant disease control is that some of its compounds act as systemic agent in certain plant species (NRC, 1992). Neem products have been shown to be effective on a wide range of pests and diseases of many crops worldwide (Sexena, 1989). Neem crude extract is confirmed to have antifungal effect against *A. brassicae*. The present finding is in agreement will the work of Khare (1998), who also reported that leaf extract of neem distinctly reduced the growth of *A.brassicae*. The use of botanicals pesticides are of immense value in controlling crop diseases (Khare & Shukla, 1998). According to the Naziha *et al.*(2010) concentrations (5, 10 and 20%) of aqueous neem extract suppressed mycelial growth of pathogenic fungi and the degree of suppression gradually increased with increasing concentration. In the present study, foliar application of neem extract minimized the disease infestation in the field condition and also seed quality. Sanjeet *et al.* (2005) found that under both laboratory and field conditions *A. indica* extracts provided good control against the of leaf spot disease of faba beans which is caused by *A. alternata*. These findings are partially supported by Chaudhary *et al.* (2003), Chattopadhay *et al.* (2005), Sasode *et al.*(2012), Ramezani Abdollahi (2015) and Kakraliya *et al.*(2017), Thakur and Zacharia (2018).

5. Conclusion

The study revealed that, integrated use of Bio-agent (*T. harzianum,* isolate T-2), fungicide (Iprodione, Rovral 50WP^{R}) and plant leaf extract (*A. indica*) provided the best control measure against *Alternaria* blight diseases of radish caused by *A. brassicae* AB-2 isolate. It may be ecofriendly alternate way to suppress the growth and development of the hazardous seed-borne pathogens particularly *A. brassicae* and also directly or indirectly accelerate the growth and increase the seed yield as well as seed quality of radish.

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