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EVALUATION OF DIFFERENT FIELD TREATMENTS ON SEED AND SEEDLING QUALITY OF ONION AGAINST PURPLE BLOTCH DISEASE

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Abstract

An investigation was carried out at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to evaluate the effect of some field treatments' on seed and seedling quality of onion. From the 16 treatment integrations, the harvested seeds were significantly varied in terms of seed germination, seedling vigor, Zinc & Boron content, and the presence of pathogenic microorganism in onion seed. Treatment T_{11} (Score 250 EC+Poultry waste) and T_8 (Rovral+Poultry waste) showed significantly the highest seed germination of 99.50%, 99% and vigor index of 1592 & 1438; respectively. The Zn and B contents in seed were found to be increased in the seed produced from the integrated treatment where micronutrient was applied. The T_9 treatment (Rovral + Zn+B) and T_{12} (Score 250 EC + Zn+B) showed the best result in respect of Zn and B contents. No pathogenic microorganisms were found in treatment T_{11} (Score 250 EC+Poultry waste) and T_8 (Rovral+Poultry waste) which was statistically significant over T_1 (Rovral 50 WP), T_2 (Score 250 EC), T_3 (Alamanda), T_7 (Rovral + Bioagent) and T_9 (Rovral + Zn+B).

Keywords: Boron**,** Chemical fungicides, Seed health, Vigor index, Zinc

Introduction

Among the main spice crops, onion (*Allium cepa*) is one of the most essential spiecs in Bangladesh. It is used as a salad while it's stalk becomes green as well. The leaves of an onion and it's stem are full of Vitamin C and Calcium. Onion is mainly grown in Rabi (winter) season and harvested in the spring or early summer. China is the largest onion producer in the world with 26% and India produces 21% while Bangladesh produces 2% of world production yearly (Quaiyum, 2020). Bangladesh made a place on the list of top ten onion-producing countries in the world four years ago. Farmers use their own produced seed, local quality seeds, improved seed, and HYV and Hybrid seeds returning the different size of the onion bulb. Seeds were required about 1245 MT to cultivate 4,40,000 acres of land in 2018-19 (Quaiyum, 2020). It is known that 90% seeds are produced by the farmers and the rest by BADC along with other Private

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Companies (Quaiyum, 2020). Thus, a deficit of onion bulbs for consumption and a huge amount of onion seed for bulb production per annum creates a market crisis among onion farmers in the growing season in Bangladesh. Practically, onion plants raised from the bulb for seed production need to be kept in the field for a month-long period as a standing crop compared to bulb crops. Lower seed production makes the seed market vulnerable and affects the onion growers and overall onion production in the country. Application of Zn and B is required for achieving a higher bulb yield of onion. Zinc (Zn) and Boron (B) can positively influence the yield and quality of onion seeds (Sarker, 2011; Rafique *et al.*, 2011). Zinc-enriched seeds can perform better with respect to seed germination, seedling health, crop growth, and finally yield advantage (Cakmak *et al.*, 1996; Alam *et al.*, 2010). For the formation of plant auxin, zinc has a significant role. During water absorption, the necessity of zinc is indispensable. Boron is necessary for cell division, nitrogen and carbohydrate metabolism, salt absorption, and water relation in the plant. Studies demonstrated that boron application had remarkable effects on the production of leaf, plant height, root numbers, seed yield, the weight of 1000 seeds, germination percentage, and quality of onion seed (Quddus *et al*., 2014). Usually, dicots have higher boron requirements than monocots. Micronutrients are also important for seed formation and seed quality (Jahiruddin *et al*., 1992; Yilmaz *et al*., 1998, Ahmed *et al*., 2007). Baktear *et al*., (2001); reported that there was a significant positive effect of micronutrients on the yield of T. aman rice. Boron deficiency may induce grain sterility in crops. Onion also suffers from different diseases by various pathogens causing substantial losses every year in Bangladesh. Among the diseases, a purple blotch of onion caused by *Alternaria porri* and white blotch of onion caused by *Stemphylium vesicarium* is presently considered the most damaging diseases. This disease is most devastating affecting both bulb yield and seed production all over the world including Bangladesh (Mishra and Gupta, 2012; Rahman *et al.*, 1988). Lower seed production makes the seed market vulnerable and affects the onion growers as well as onion production in the country. Based on the facts the present investigation was undertaken to evaluate the different treatments that affect seed germination, seed vigor, the presence of pathogenic microorganisms (*Alternaria porri* and *Stemphylium vesicarium*), and Zn+B contents in onion seed production in the field.

Materials and Methods

The experiments were conducted at the Seed Health Laboratory of the Department of Plant Pathology and Genetics and Plant Breeding Lab of Sher-e-Bangla Agricultural University (SAU), Dhaka. Test for the presence of Zn and B was done at Soil Resource Development Institute (SRDI), Khamarbari, Dhaka. The experiments were conducted during 2012-13 by using a Completely Randomized Design (CRD) with 4 replications for Laboratory experiments and a Randomized Complete Block Design (RCBD) with 4 replications for field experiments. Duncan's Multiple Range Test (DMRT) was explored for comparison of means (Gomez and Gomez, 1983). The complete package program MSTAT-C was used for the analysis of the experimental data.

Treatments

In the field, the following IDM Components were used as a treatment for the management of the purple blotch complex disease of onion. The treatments were T_0 = Control (Bulb treatment + foliar spraying with plain water); T_1 = Bulb treatment + Foliar spraying with Rovral 50 WP (Ipridione) @ 0.2%; T_2 = Bulb treatment + Foliar spraying with Score 250 EC (Difenoconazole) @ 0.1%; T_3 = Bulb treatment and Foliar spraying with Alamanda leaf extract (*Allamanda cathartica*) @ 1:2 (w/v); T_4= Soil amendment with Trico-compost (*Trichoderma sp*.) @ 5 t/ha; T_5 = Soil amendment by fully decomposed Poultry waste @ 5 t/ha; T_6 = Soil amendment by $ZnSO_4$ (Zn) @ 5 kg/ha and Borax (B) @ 5 kg/ha; T_7= T_1+T_4; T_8= T_1+T_5; T_9= T_1+T_6; T_{10}= T_2+T_4; T_{11}= T_2+T_5; T_{12}= T_2+T_6; T_{13}= T_3+T_4; T_{14}= T_3+T_5; and T_{15}= T_3+T_6. Seeds were collected from each treatment and later seed health study was conducted to evaluate the treatment effect on seed germination, seed vigor, and the presence of the pathogenic organism, *Alternaria porri,* and *Stemphylium vesicarium* in germinating seedlings.

Field study

The selected IDM components were integrated for their combined performance in controlling the purple blotch complex of onion for seed production. The popular and widely cultivated local variety of onion 'Taherpuri' was used in this experiment. Before plantation, the onion bulbs were treated with the respective solutions of plant extracts and fungicides by dipping the bulbs for 15 minutes. The treated bulbs were then shade dried and sown in the field without delay. For the control treatment, the bulbs were treated with plain water. Inoculation was done with a spore suspension of *A. porri* and *S. vesicarium* 21 days after planting (DAP). Spraying of fungicides, plant extracts, and tri-compost was started 36 days after bulb planting and 10 sprayings were done at 7 days intervals by a hand sprayer. One liter suspension of each fungicide and plant extract was used to spray the plants under each treatment. To avoid the drifting of the fungicides during the application, temporary fencing was made with a polyethylene sheet surrounding the unit plot. A control treatment was maintained in each block where spraying was done with plain water only. Irrigation, weeding, and mulching were done as per requirement of the plots at regular intervals.

Seed health study

For the seed health study, 400 seeds from each treatment were tested by following the standard procedure of the blotter method of seed health testing (ISTA, 2000). Sixteen petridishes were used for each treatment and 25 seeds were placed in each petridish in equidistance. All the plates with seeds were kept at room temperature for germination. After 12 days, seed germination was counted and expressed in percentage for each treatment (Plate 1-A, B). For detecting the incidence of *A. porri* and *S. vasicarium*, all the plates with seeds were incubated at room temperature ($25^0 \pm 2^0$C). After 14 days of incubation, each seed was observed under the stereo-binocular microscope to detect the presence of *A. porri* and/or *S. vasicarium* (Fig. 1-E, F).

Seedling vigor test

For the seedling vigor test, 400 seeds from each treatment were tested in a tray filled with soil so that the root and shoot can develop in a natural situation. Four trays were used for each treatment and 100 seeds were placed in each tray randomly. On the 15^{th} day of seed plating, 10 normal seedlings were selected randomly in each treatment from all the replications (Plate 1-C, D). The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean shoot length was expressed in cm. The root length was measured from the tip of the primary root to the base of hypocotyls and the mean root length was expressed in cm. The vigor index (VI) was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in numbers.

Vigor index = Germination (%) × (Shoot length+ Root length) cm

The harvested seed samples were tested for the presence of micronutrients (% Zn and % B) by the help of Soil Resource Development Institute (SRDI), Khamarbari, Dhaka.

Zinc content of seed

The seed sample was oven dried and analyzed for Zn by using an Absorption Spectrophotometer (AAS) according to Rahayu *et al*., (2001).

Boron content of seed

Boron content was determined by the Curcumin method (Hunter, 1980). A 0.25 ml seed filtrate was taken in a plastic bottle; 2.0 ml of curcumin in HOAC was added and thoroughly mixed. A 0.5 ml of conc. H_2SO_4 was added and thoroughly mixed and was allowed to stand for at least 35 minutes for cooling. Then, 15 ml of methanol solution (3: 2) was added, and mixed thoroughly. After 15 minutes, the spectrophotometer reading was recorded at 555 nm. The boron value (ppm) was determined by the following equation:

Boron (ppm) = Y x Abs (X) x f

Where, Y = df = 100/0.25 x (1) x ml /0.5 = 800

Where, 100 = Sample volume up to 100 ml

F = 6.25 (From standard curve)

0.25 ml = filtrate taken

0.5 g = seed sample

Results and Discussion

Seed germination

The effect of different integrated treatments was recorded in respect of seed germination percent (Table 1). Treatments showed significant variation in respect of seed germination. At 15 days of incubation treatment T_{11} (Score 250 EC + Poultry waste) showed 99.5% germination followed by T_8 (Rovral+Poultry waste) 99%, T_7 (Rovral + Bioagent) 97%, and T_1 (Control) 96%, however their performances were statistically indifferent. Most of the treatments showed above 80% germination except T_4 (Soil amendment with Bioagent) 63.3% and control (56.8%).

Table 1. Effect of different integrated treatments on seed health against purple blotch complex of onion

Treatments		% Germination	Seeding Vigor Index
T_0	Control	56.8 g	438.5 g
T_1	Rovral 50 WP	96.5 a	1274.0 b
T_2	Score 250 EC	92.0 bc	1170.0 bc
T_3	Alamanda	83.3 de	976.8 de
T_4	Soil amendment with Bioagent	63.3 f	669.0 f
T_5	Soil amendment with Poultry waste	80.0 e	846.4 e
T_6	Micronutrient (Zinc+Boron)	56.8 g	438.5 g
T_7	Rovral + Bioagent	97.0 a	1312.0 b
T_8	Rovral + Poultry waste	99.0 a	1438.0 a
T_9	Rovral + Micronutrient	92.3 bc	1173.0 bc
T_{10}	Score 250 EC + Bioagent	96.0 b	1270.0 b
T_{11}	Score 250 EC + Poultry waste	99.5 a	1592.0 a
T_{12}	Score 250 EC + Micronutrient	84.5 d	981.1 de
T_{13}	Alamanda + Bioagent	89.0 c	1092.0 cd
T_{14}	Alamanda + Poultry waste	89.5 c	1098.0 cd
T_{15}	Alamanda + Micronutrient	83.5 de	982.3 de
CV (%)		2.51%	8.11%

Values in a column with the same letter (s) do not differ significantly (p=0.01)

Seed vigor

Seedling vigor was evaluated by a seed germination test in a plastic tray (Table 1). On the 15th day of seed plating the highest vigor index was estimated on treatment T_{11} (Score 250 EC + Poultry waste) 1592 which was statistically similar to treatment T_8 (Rovral+Poultry waste) 1438. Statistically, the 2nd highest vigor index was estimated in T_7 (Rovral + Bioagent) 1312 which was statistically indifferent with T_{10} (Score 250 EC + Bioagent) 1270 and T_1 (Rovral 50 WP) 1274. The lowest vigor index was recorded on treatment T_0 (Control) at 438.50.

Zinc content of seed

The seeds produced from integrated treatment were subjected to estimate the micronutrient (% Zn and % B) shown in table 2. The amount of Zn differed significantly among the treatments. The seed content of Zn was found to be higher in the treatments where Zn is applied as a micronutrient during soil amendment. The treatments where poultry waste is used for soil amendment also enrich the Zn content of the seed. Statistically, the highest Zn content was found in T_9 (Rovral + Micronutrient) 37.78 ppm which is statistically similar to T_{12} (Score 250 EC + Micronutrient) 36.46 ppm followed by T_{15} (Alamanda + Micronutrient) 33.15 ppm, T_6 (Micronutrient) 31.96 ppm, T_8 (Rovral + Poultry waste) 28.89 ppm and T_{11} (Score 250 EC + Poultry waste) 27.36 ppm. The lowest Zn content was estimated at 8.36 ppm in control treatment T_0.

Table 2. Zinc and Boron content of the harvested onion seeds from different treatments applied plots for the management of purple blotch disease

Treatment		Zn (ppm)	B (ppm)
T_0	Control	8.36 m	10.60 hi
T_1	Rovral 50 WP	13.79 jk	18.64 ef
T_2	Score 250 EC	13.36 kl	14.27 gh
T_3	Alamanda	9.57 lm	8.80 i
T_4	Soil amendment with Bioagent	11.79 k-m	15.69 fg
T_5	Soil amendment with Poultry waste	24.73 fg	22.23 de
T_6	Micronutrient (Zinc+Boran)	31.96 cd	39.71 a
T_7	Rovral + Bioagent	17.35 ij	16.32 fg
T_8	Rovral + Poultry waste	28.89 de	23.40 d
T_9	Rovral + Micronutrient	37.78 a	41.63 a
T_{10}	Score 250 EC + Bioagent	20.36 hi	17.02 fg
T_{11}	Score 250 EC + Poultry waste	27.36 ef	29.06 c
T_{12}	Score 250 EC + Micronutrient	36.46 ab	32.56 bc
T_{13}	Alamanda + Bioagent	18.18 i	16.55 fg
T_{14}	Alamanda + Poultry waste	22.40 gh	21.48 de
T_{15}	Alamanda + Micronutrient	33.15 bc	35.46 b
CV (%)		8.71	8.74

Source: Test done at Soil Resource Development Institute (SRDI), Khamarbari, Dhaka

Boron content of seed

The amount of Boron differed significantly between the treatments (Table 2). The seed content of Boron was found to be higher in the treatments where Boron is applied as a micronutrient during soil amendment. The treatments where poultry waste was used for soil amendment also enrich the Boron content of the seed. Statistically, the highest Boron content was found in T_9 (Rovral + Micronutrient) 41.6 ppm which is statistically similar to T_6 (Micronutrient) 39.7 ppm followed by T_{15} (Alamanda + Micronutrient) 35.46 ppm, T_{12} (Score 250 EC + Micronutrient) 32.6 ppm and T_{11} (Score 250 EC + Poultry waste) 29.1 ppm. The lowest Boron content was estimated in treatment T_3 (Alamanda) at 8.80 ppm which is statistically similar to control treatment T_0 (10.6 ppm).

Incidence of pathogenic organism

In the present studies, different integrated treatments showed significant variation in respect of the percent of pathogenic organism presence in germinating seedlings. On the 18th day of seed plating the highest incidence of *A. porri* and *S. vesicarium* was recorded on treatment T_0 (Control) 2.75% which was statistically similar to treatment T_6 (Micronutrient)) 2.25%, T_{13} (Alamanda + Bioagent) 2.25%, T_{14} (Alamanda + Poultry waste) 2.25% and T_{15} (Alamanda + Micronutrient) 2.25%. No pathogenic incidence was noticed on treatment T_8 (Rovral + Poultry waste) and T_{11}(Score 250 EC + Poultry waste). The treatments T_1 (Rovral 50 WP), T_2 (Score 250 EC), T_3 (Alamanda), T_7 (Rovral + Bioagent), and T_9 (Rovral + Micronutrient) showed a statistically indifferent effect with respect to pathogenic incidence (Fig.1).

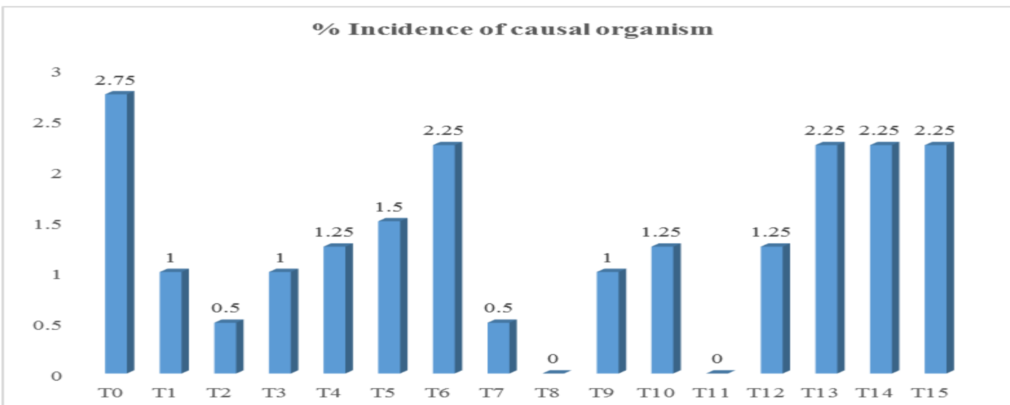

Fig. 1. Percentage of Incidence for *Alternaria porri* & *Stemphylium vesicarium* of purple blotch disease in onion seeds harvested from different integrated treatment applied plots.

Pathogens *A. porri* and *S. vesicarium* were isolated and identified by observing the key characteristics under a stereo and compound microscope. The quality of onion seed produced from the 15 integrated treatments was analyzed based on seed germination, seed vigor, presence of pathogenic microorganisms, micronutrient (Zinc+Boron) contents, and BCR. All the parameters significantly varied as per the treatments applied. The seed germination varied from 56.8% -99.5% where control scored the lowest germination and the treatment that comprised poultry waste for soil amendment and fungicide Score 250EC for bulb treatment and foliar spraying. The use of Rovral 50 WP in the place of Score 250EC in combination with poultry waste for soil amendment also scored statistically similar seed germination (99.0%). The seedling vigor ranged from 438.50-1592.00 owing to the application of different treatments. The vigor index was found to be higher where Rovral 50WP or Score 250EC were integrated with soil amendment with poultry waste or bioagent or micronutrient (Zn+B). The incidence of microorganisms on seed also varied statistically in response to the variation of treatments applied and the highest incidence (2.75%) was noticed in the case of control while no incidence of pathogenic microorganisms was found in the case of application of

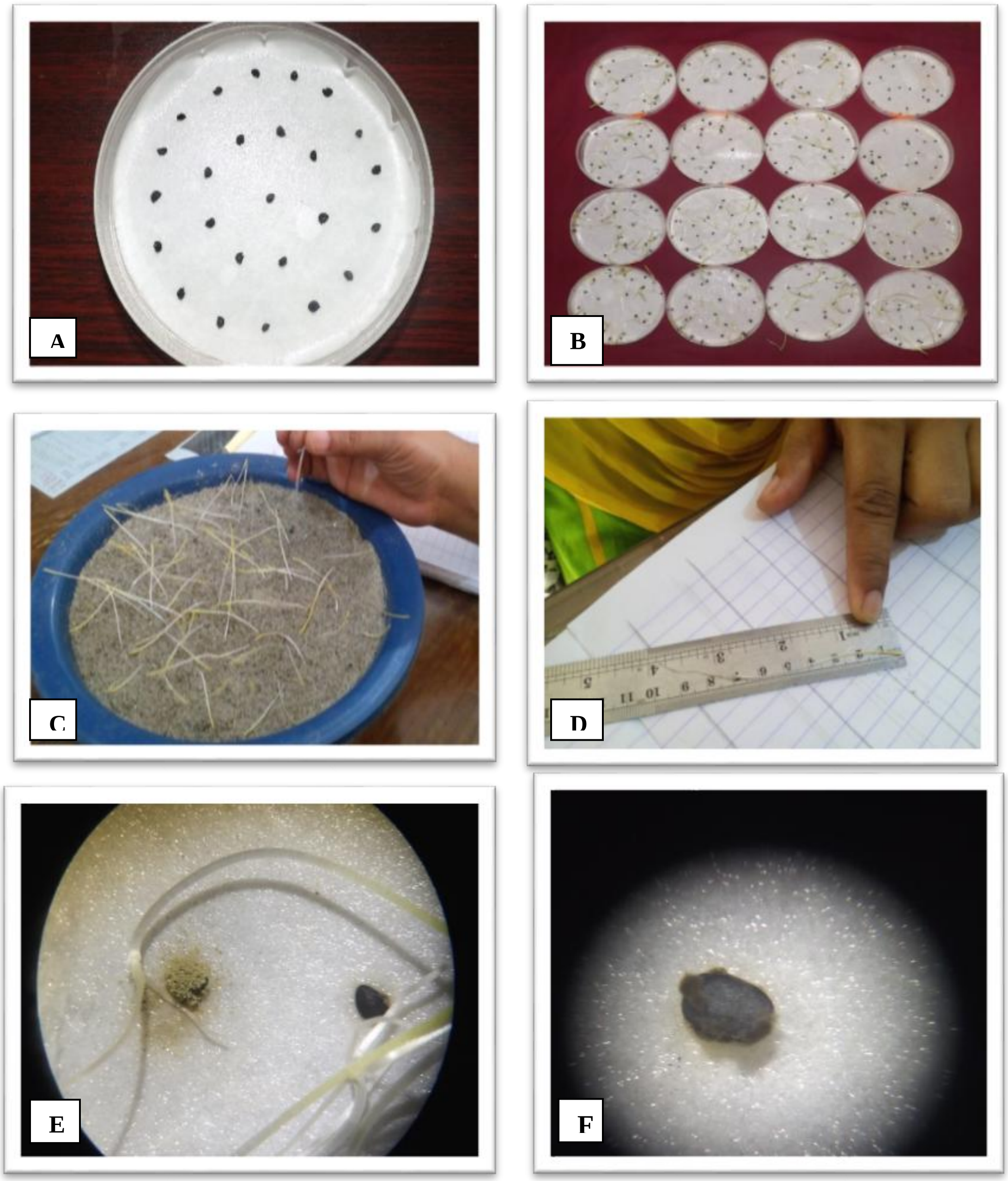

Fig. 2. Testing of seed quality of harvested seed of onion; A). Plotting of Onion Seed on blotter paper; B). Germinating seedlings at 7 DAS; C). Seed sowing on sand media; D) Measuring of shoot and root length; E). Observing seedlings under Compound Microscope; F). Non-germinated infected seed.

Score 250EC with poultry waste and Rovral 50WP with poultry waste. Among the other treatments, the pathogenic incidence was lower where Rovral 50WP or Score

250EC was applied either alone or in combination with other soil amendment options. For the case of micronutrient (Zn+B) analysis it was observed that the Zn and Boron contents of the seed varied significantly. The higher amount of Zn and B content were recorded where micronutrient was provided as a component of integrated treatments. The highest content of Zn (37.8 ppm) and B (41.6 ppm) was recorded in the treatment where micronutrient was applied in combination with Rovral 50WP.

The lowest content of Zn (8.26 ppm) was recorded in the control while the B (8.80 ppm) was noted in the seed sample harvested from Alamanda treated plot. The health quality of the seed regarding seed germination, seedling vigor, and the absence of pathogenic microorganisms was found to be improved significantly due to the application of different treatment combinations. The treatment combinations that had remarkable contributions in reducing the disease incidence and severity of the purple blotch complex of onion in field conditions had contributed the lowest incidence of pathogenic microorganisms in the corresponding seeds which subsequently improved the seed quality regarding seed germination and seed vigor. Patil *et al.*, (2006) reported that Zn had a favorable effect on seed formation and subsequent seed development which might be the reason for increasing the seedling vigor. Sarker (2011) also reported that there have positive effects of zinc and boron on the highest seed yield and germination quality of onion. Rafique *et al*., (2011) stated that Zn concentration in mature onion seeds also appeared to be a good indicator of soil Zn availability status. Haque *et al*., (2014) reported that Zinc (Zn) and boron (B) positively influence the yield and quality of onion seed, the response is genotype dependent and the interaction of both elements is rarely studied. A similar result was reported by Begum *et al*., (2015).

Conclusion

The quality of onion seed produced due to the integrated treatments was evaluated in terms of seed germination, seed vigor, presence of pathogenic microorganisms, and micronutrient (Zn+B). The treatment T_{11} (Score 250 EC + Poultry waste) and T_8 (Rovral + Poultry waste) showed significantly the highest seed germination (99.50%, 99.00%) and vigor index (1592, 1438) and the lowest (0.0%) presence of pathogenic microorganisms, respectively. The micronutrient (Zn+B) contents of the seed were found to be increased in the seed produced from the integrated treatment where micronutrient was combined which influenced the seedlings' vigor. A significant amount of Zn and B was found in T_{11} and T_8 where poultry waste was combined. Results showed that the presence of Zn and B in seed influence of the highest seed germination, vigor index, and the lowest presence of pathogenic microorganisms.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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