

Evaluation of Combined Effect of Micronutrients (ZnSO₄ + Borax) and Fungicides to Control the Purple Blotch Complex of Onion (*Allium cepa*)

Hasan Ali, Hosna Ara Chowdhury Nisha, Md. Belal Hossain*, Md. Rafiqul Islam

Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh
Email: mbhossainsau@yahoo.com, dr.mbhossain@sau.edu.bd

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Abstract

Ten different treatments *viz.* T₀ = Control, T₁ = Micronutrients (ZnSO₄ + Borax), T₂ = Rovral 50 WP + Micronutrients, T₃ = Dithane M-45 + Micronutrients, T₄ = Ridomil Gold + Micronutrients, T₅ = Cupravit + Micronutrients, T₆ = Rovral 50 WP, T₇ = Dithane M-45, T₈ = Ridomil Gold and T₉ = Cupravit were evaluated against the purple blotch complex disease of onion caused by *Alternaria porri* and *Stemphylium vesicarium* in field condition for bulb yield. In lab condition, all the tested chemical fungicides (Rovral 50 WP, Dithane M-45, Ridomil Gold MZ-72 and Cupravit 50 WP) significantly reduced mycelial growth of both pathogens. Among the fungicides, Rovral 50 WP @ 0.2% reduced the highest radial mycelial growth followed by Dithane M-45 @ 0.45% and Ridomil Gold MZ-72 @ 0.2% compared to control. In field experiment, application of Rovral 50 WP @ 0.2% along with micronutrients showed significant effect in reducing disease incidence and severity which significantly increased bulb yield and yield contributing characters.

Keywords

Onion, Purple Blotch Complex, Micronutrients, Fungicides

1. Introduction

Onion (*Allium cepa*) is one of the most important and familiar spices crops throughout the world. Out of 15 important vegetables and spice crops listed by FAO, onion stands second in terms of annual world production [1].

*Corresponding author.

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In Bangladesh onion covers 36% areas under spices and it ranks first in production of 889,000 MT and second in area of 1,25,101 hectare of land [2]. Among the onion producing countries China tops the list with 205.08 million tons followed by India 133.72 million tons, USA 33.21 million tons and Iran 19.23 million tons whereas the production of onion in Bangladesh is only 11.59 million tons [3] which is much lower compared to other onion growing countries in the world. Onions are attacked by ten diseases caused by various pathogens [4] [5]. Among them, *Stemphylium vesicarium*, the causal agent of white blotch of onion is considered as the pathogen that initiates the infection on onion, which facilitates the subsequent infection of *Alternaria porri* causing purple blotch and hence presently the disease is named as Purple Blotch Complex of onion. This disease is very important as it reduces the bulb yield which directly affects seed yield and quality. As a result the disease is also a threat to seed production of onion [6]. Gupta *et al.* (2013) stated that more than 25% yield losses occur in India due to foliar diseases, especially stemphylium blight (*S. vesicarium*) and purple blotch (*Alternaria porri*) [7]. Nowadays farmers are not interested in onion cultivation due to yield loss which reduces the national production, making the country demand for importing enormous quantity of onion bulb every year at the cost of huge foreign exchange. Unstable price of onion in the local market, especially in the month of Ramadan is mainly due to the shortage of onion production. Proper disease control measures can improve the quality of onion bulbs and significantly increase the yield. In Bangladesh, limited attempts have been made to find out the suitable control measures of this disease for bulb and seed production [8]. Rahman *et al.* (1989) found Rovral 50 WP and Dithane M-45 fungicides effective against the disease [9]. Uddin *et al.* (2006) also found Dithane M-45 (0.45%) and Rovral 50 WP (0.2%) effective in controlling the disease incidence and severity of purple blotch complex of onion [10]. In Bangladesh production of good quality onion bulbs is an important target by onion growers who have inadequate knowledge about the beneficial role of micronutrients in increasing yield and quality of onion for local and foreign markets. Nutrients are important for growth and development of plants and also microorganisms as they are important factors in disease control [11]. All the essential nutrients can affect disease severity and some showed high sensitivity in onion production *viz.* Zn, B, Mn and Mo [12] [13]. Application of zinc and boron through soil or foliar or in combination had a beneficial effect on the growth of onion regardless of stages [14]. Zinc and boron play an essential role in improving plant growth, through the biosynthesis of endogenous hormones which is responsible for promotion of plant growth [15] [16]. Increase in the number of leaves per plant may also be attributed due to the role of micronutrients (Zn, B) in cell division, meristematic activity of plant tissue and expansion of cells [17]. Considering the above factors the present study was undertaken to study the combined effect of micronutrients (ZnSO₄ + Borax) and selected fungicides to control the purple blotch complex of onion.

2. Materials and Methods

2.1. Experimental Site

The laboratory experiment was conducted at the Seed Health Laboratory and field experiment was conducted at the experimental farm of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka during November, 2014 to April, 2015.

2.2. Laboratory Experiment

2.2.1. Isolation, Purification and Preservation of Pathogens

Alternaria porri and *Stemphylium vesicarium* were isolated by using tissue planting methods and purification was carried out by transferring conidia of pathogens into the potato dextrose agar (PDA) medium separately and incubated at 25°C ± 1°C maintaining alternating dark and light period. The pure cultures of pathogens were preserved in refrigerator at 4°C for further use. Before preservation at 4°C, pathogenic structures were also studied through slide culture by using compound microscope. Infected plant tissues, pure cultures and pathogenic structures of the organisms are presented in **Figure 1**.

2.2.2. Preparation of Fungicidal Suspension

In total four (4) fungicides were used in the experiment *viz.*; Rovral 50 WP (a.i. Iprodione 50%), Dithane M-45 (a.i. Mancozeb 80% + manganese ethylene), Ridomil Gold MZ-72 (a.i. Metalaxyl 67% + Mancozeb 6%) and Cupravit 50 WP (a.i. Copper oxychloride 50%). Fungicidal solutions were prepared according recommended doses of selected fungicides. Requisite quantity of fungicides was mixed thoroughly in distilled water. It was

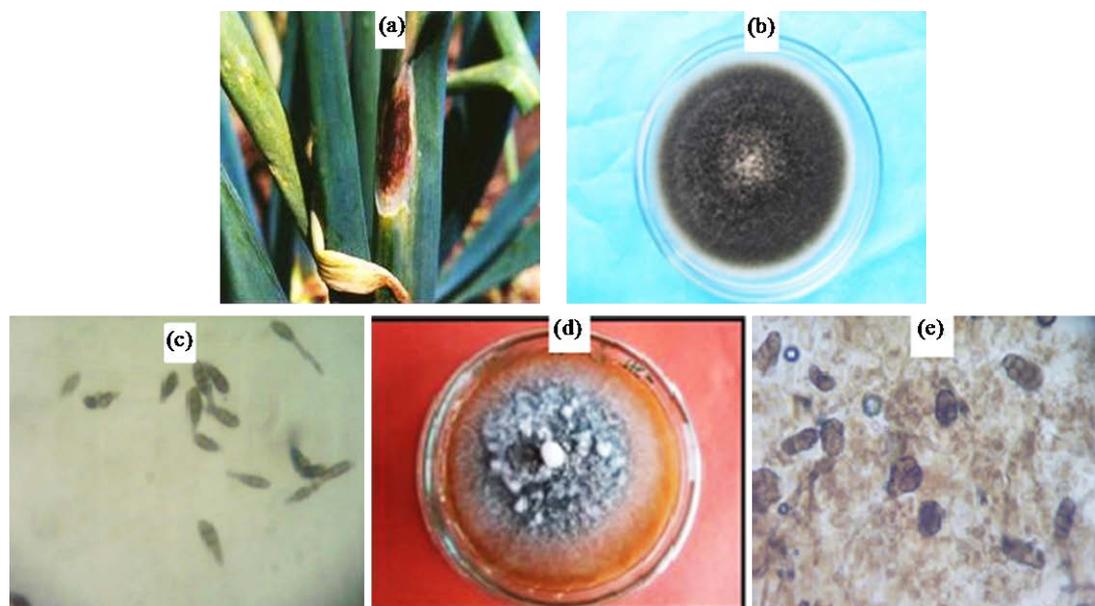


Figure 1. Isolation of *Alternaria porri* and *Stemphylium vesicarium* from infected stems of onion. (a) Showing purple blotch complex, (b) Pure culture of *Alternaria porri*, (c) Conidia of *Alternaria porri* under compound microscope ($\times 40$), (d) Pure culture of *Stemphylium vesicarium* and (e) Conidia of *Stemphylium vesicarium* under compound microscope ($\times 40$).

required 3 gm/liter of Cupravit 50 WP, 2 gm/liter of Rovral 50 WP, 4.5 gm/liter of Dithane M-45, 2 gm/liter of Ridomil Gold (MZ-72) for preparation of solution for recommended concentration.

2.2.3. Bioassay of Fungicides through Cup/Groove Method

Cup or groove method was used for bio-assay of fungicides against the *Alternaria porri* and *Stemphylium vesicarium*. Each treatment was replicated thrice in number, and only sterile water was used for control treatment. The linear mycelia growth (cm) was recorded from seven days of after inoculation and continuously observed with 3 days interval until the control plates were completely filled with mycelium of the pathogens.

2.3. Field Experiment

2.3.1. Experimental Design and Seedling Transplantation

The experimental plots were arranged in Randomized Complete Block Design (RCBD) with three (3) replications. Before transplanting the seedlings in the experiment field, land was prepared by maintaining proper agronomic practices and required fertilizers. Gap filling, irrigation, weeding and mulching were done by following production technology of onion. Forty five days old seedlings of BARI Piaz-1 variety were used which was collected from “Spices Research Center”, Bangladesh Agricultural Research Institute (BARI), Shibganj, Bogra, Bangladesh.

2.3.2. Treatments of Experiment

Total ten treatments were applied into the assigned plots as per design of the experiment. T_0 = Control, T_1 = Micronutrients ($ZnSO_4$ + Borax), T_2 = Rovral 50 WP + Micronutrients, T_3 = Dithane M-45 + Micronutrients, T_4 = Ridomil Gold + Micronutrients, T_5 = Cupravit + Micronutrients, T_6 = Rovral 50 WP, T_7 = Dithane M-45, T_8 = Ridomil Gold and T_9 = Cupravit 50 WP.

2.3.3. Application of Fungicides

Fungicidal solution that prepared according to recommended doses was sprayed in the field. Spraying was started from one month after transplanting. Total five spraying were done at 7 days intervals with a hand sprayer. A control treatment was maintained in each block where spraying was done with normal water only.

2.4. Data Collection

Ten plants were randomly selected for each unit plot and tagged for data collection. Data collection was started after the onset of the disease symptoms and continued up to maturity with 7 days interval. The data were collected on the following parameters; number of total plants at different vegetative growth stages, number of healthy plants at different vegetative growth stages, number of leaves per plants from randomly selected plants from each plot, number of infected plants under each treatment, number of leaf infected per plant.

2.5. Calculation of Disease Incidence for Different Treatment

The disease incidence was calculated using the following formula

$$\% \text{ leaf infection} = \frac{\text{Number of infected leaf}}{\text{Total number of inspected leaf}} \times 100$$

2.6. Leaf Area Diseased (LAD)/Plant in Different Treatment

Leaf area diseased of the ten selected plants in each plot against each treatment were measured and recorded by conversion to percentage. Mean percentage of leaf area diseased was calculated by dividing number of total observation and used for PDI (percent disease index) estimation.

2.7. Estimation of PDI (Percent Disease Index)

The following diseased scoring scale 0 - 5 scale was used to estimate the disease severity (PDI) of purple blotch of onion for each unit plot under each treatment. 0 = no disease symptoms, 1 = a few spots towards the tip, covering less than 10% leaf area, 2 = several dark purplish brown patches covering 10% to less than 20% leaf area, 3 = several patches with paler outer zone, covering 20% to 40% leaf area, 4 = long streaks covering 40% to 75% leaf area or bricking of leaves/stems from the center, 5 = complete drying of the leaves/stems or breaking of the leaves/stems from the base. The percent disease index (PDI) was calculated using the following formula [18].

$$\text{PDI(Leaf/Stalk)} = \frac{\text{Total sum of numerical ratings}}{\text{No. of observation} \times \text{Maximum disease rating in the scale}} \times 100$$

2.8. Statistical Analysis

Data were analyzed statistically using MSTAT-C computer Program. Data were transformed, whenever necessary, following Arcsine transformation. Means of treatment were separated using Duncan's Multiple Range Test (DMRT), [19].

3. Results

3.1. Bioassay of Fungicides against *Alternaria porri* and *Stemphylium vesicarium*

Data on radial mycelial growth (cm) of *Alternaria porri* and *Stemphylium vesicarium* against fungicides was recorded at 6, 9, 12 and 15 days after incubation (DAI). The fungicide Rovral 50 WP (*Alternaria porri* reduced 69% and *Stemphylium vesicarium* reduced 76.56%) and Dithane M-45 (*Alternaria porri* reduced 65% and *Stemphylium vesicarium* reduced 71.87%) appeared better in controlling the radial mycelial growth of both pathogens at different DAI. Results are clearly depicted in **Figure 2** and also presented in **Table 1**.

3.2. Effect of Different Treatments against Disease Incidence and Severity of Purple Blotch Complex of Onion

The lowest plant infection (15.00%) was observed in the treatment T₂ (Rovral 50 WP + Micronutrients) where T₀ (Control) treatment showed the highest plant infection (25.50%). All the treatments were significantly differed in controlling percent plant infection. Results obtained from the effect of spraying different fungicides along with micronutrients in controlling leaf infection are presented in **Figure 3**. Different treatments showed

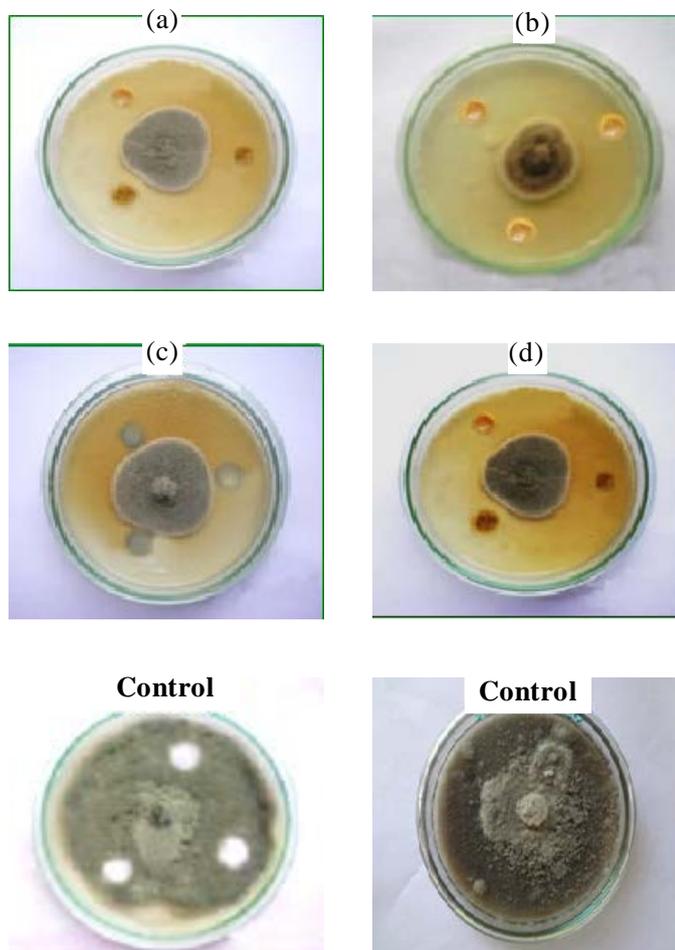


Figure 2. Bioassay of fungicides through cup/groove method against *Alternaria porri* and *Stemphylium vesicarium*. (a) (c) Treated with fungicide Rovral 50 WP against *Alternaria porri*; and (b) (d) Treated with fungicide Dithane M-45 against *Stemphylium vesicarium*.

Table 1. Effect of different fungicides on mycelial growth of *Alternaria porri* and *Stemphylium vesicarium* at different days after inoculation (DAI).

Fungicides	Radial mycelial growth (cm) of <i>Alternaria porri</i>				% Reduction in mycelial growth at 15 DAI	Radial mycelial growth (cm) of <i>Stemphylium vesicarium</i>				% Reduction in mycelial growth at 15 DAI
	6 DAI	9 DAI	12 DAI	15 DAI		6 DAI	9 DAI	12 DAI	15 DAI	
Rovral 50 WP	1.06 d	1.24 e	1.48 e	1.86 e	69.00	0.85 e	1.00 d	1.20 e	1.50 e	76.56
Dithane M-45	1.40 c	1.60 d	1.90 d	2.10 d	65.00	1.00 d	1.30 cd	1.60 d	1.80 d	71.87
Ridomil Gold	1.80 b	2.00 c	2.40 c	2.80 b	53.34	1.30 c	1.60 bc	1.90 c	2.25 c	64.84
Cupravit	2.20 a	2.50 b	2.60 b	2.70 c	55.00	1.40 b	2.10 b	2.80 b	3.50 b	45.31
Control	2.50 a	4.00 a	5.50 a	6.00 a	-	1.80 a	4.00 a	5.10 a	6.40 a	-
CV (%)	2.88	1.37	2.23	1.50		2.40	2.60	1.92	1.25	
LSD (P = 0.05)	0.3151	0.05753	0.08136	0.1151		0.05954	0.4573	0.08420	0.05954	

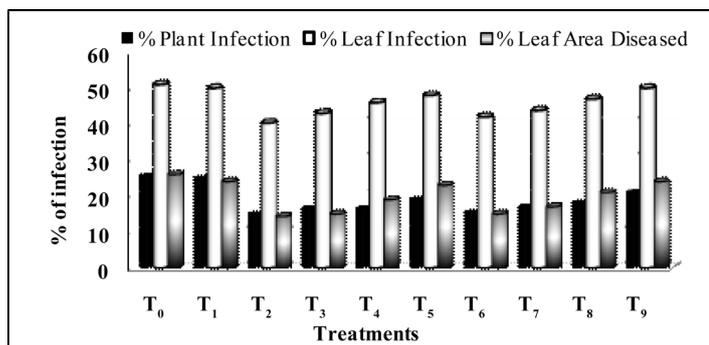


Figure 3. Effect of different treatments against disease incidence (plant infection and leaf infection) and severity (% leaf area diseased) of purple blotch complex of onion (T₀ = Control, T₁ = Micronutrients (ZnSO₄ + Borax), T₂ = Rovral 50 WP + Micronutrients, T₃ = Dithane M-45 + Micronutrients, T₄ = Ridomil Gold + Micronutrients, T₅ = Cupravit + Micronutrients, T₆ = Rovral 50 WP, T₇ = Dithane M-45, T₈ = Ridomil Gold, T₉ = Cupravit 50 WP).

statistically significant variation where the lowest infection (40.33%) was observed in the treatment of T₂ (Rovral 50 WP + Micronutrients). The selected treatments showed statistically significant variation in respect of percent leaf area diseased (%LAD) where the treatment T₂ gave the best performance (14.33%) in minimizing percent leaf area disease. The highest %LAD was observed in T₀ treatment (26.00%), where only water was sprayed.

3.3. Effect of Different Treatments on Growth Parameters of Onion Plants

Plant height was increased from 30.00 cm to 50.00 cm due to the effect of micronutrients and fungicides. The highest plant height (50.33 cm) was recorded in the treatment of T₂ and the lowest plant height (30.00 cm) was recorded in the treatment T₀. The effect of micronutrients and fungicides on dry matter of plant differed significantly where T₂ gave the best performance (10.33 gm) in getting dry matter. The root length was influenced by the application of different treatments which was varied significantly among each other. The treatment T₂ gave the best performance (8.10 cm) in increasing root length where the lowest growth of root (4.00 cm) was recorded in the treatment T₀. Root weight showed significant variation due to the effect of treatments. The treatment T₂ gave the best performance (8.63 gm) in increasing the root where the lowest root weight (3.50 gm) was recorded in the treatment T₀. These results are presented in **Table 2**.

3.4. Effect of Different Treatments on Yield Parameters of Onion Plants

The bulb yield was found to increase from 11.67 t/ha (T₀) to 18.49 t/ha (T₂) due to application of fungicides and Micronutrients. The combination of Rovral + Micronutrients, Rovral alone, Dithane M-45 + Micronutrients and Dithane M-45 alone gave promising bulb yield (18.49 t/ha, 18.34 t/ha, 18.00 t/ha and 17.67 t/ha, respectively) in comparison to other treatments which were significantly similar as clearly present in **Figure 4**.

4. Discussion

In *in-vitro* test, about four fungicides were tested to identify the fungicide which would be the best for retarding the radial mycelial growth of both pathogens. All the chemical fungicides significantly retarded the radial mycelial growth of both pathogens over control. Among the fungicides Rovral 50 WP was found best in reducing the mycelial growth of *Alternaria porri* (69%) and *Stemphylium vesicarium* (76.56%) at 15 DAI. The present finding was supported by the results obtained from the previous researchers [20]-[22] who reported that Rovral totally inhibited the mycelial growth of *Alternaria porri*. Datar (1996) reported that iprodione (Rovral) at 500 ppm significantly reduced the mycelial growth of *Alternaria porri*. Rovral 50 WP was effective to inhibit the mycelial growth of *Stemphylium vesicarium* that observed in another *in-vitro* study [23]. In the field experiment, the effect of treatments in controlling purple blotch complex of onion was assessed on the basis of percent leaf

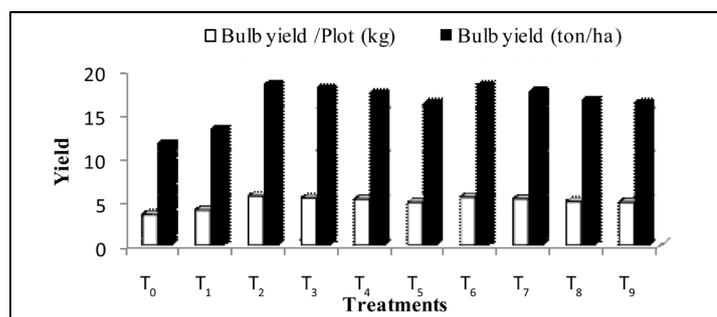


Figure 4. Effect of different treatments on bulb yield of onion (T₀ = Control, T₁ = Micronutrients (ZnSO₄ + Borax), T₂ = Rovral 50 WP + Micronutrients, T₃ = Dithane M-45 + Micronutrients, T₄ = Ridomil Gold + Micronutrients, T₅ = Cupravit + Micronutrients, T₆ = Rovral 50 WP, T₇ = Dithane M-45, T₈ = Ridomil Gold, T₉ = Cupravit 50 WP).

Table 2. Effect of different treatments and treatment combinations on growth parameters of onion.

Treatments	Plant height (cm)	Dry matter (gm)	Root length (cm)	Root weight (gm)
T ₀	30.00 i	5.00 h	4.00 d	3.50 h
T ₁	35.00 h	7.00 g	7.50 b	5.50 e
T ₂	50.33 a	10.33 a	8.10 a	8.63 a
T ₃	44.00 b	9.00 b	8.00 a	7.50 b
T ₄	48.00 e	8.00 d	7.50 b	5.50 e
T ₅	40.00 g	7.00 g	7.00 bc	6.50 c
T ₆	46.00 d	9.00 b	7.00 bc	6.50 c
T ₇	44.00 b	8.50 c	5.50 cd	4.50 g
T ₈	45.00 f	7.50 e	6.00 c	4.00 h
T ₉	35.00 h	7.00 g	6.00 c	5.25 ef
CV (%)	2.41	1.15	2.86	1.28
LSD (P = 0.05)	0.1715	0.3431	0.0214	0.4131

infection, percent plant infection, percent leaf area diseased (% LAD), growth parameters and finally yields of onion. The effect of Rovral 50 WP (0.2%) along with micronutrients on those parameters was found promising. The highest reduction of plant infection (41.18%), leaf infection (20.92%), leaf area diseased (44.88%) was recorded in case of Rovral 50 WP + Micronutrients. The results showed that application of Micronutrients in combination with fungicides had contributory effect in reducing the disease incidence and disease severity. The present findings were supported by the reports of the previous researches. Rahman (2004) screened 6 fungicides among them Rovral 50 WP significantly reduced the disease severity of purple blotch of onion [24]. Sugha (1995) reported that Iprodione (0.2%) was highly effective against purple blotch of onion resulting 79.6% - 84.9% control of the disease [25]. Khatun (2007) reported that 6 foliar spraying of Rovral 50 WP (0.2%) or Dithane M-45 (0.45%) at 10 days interval starting from 20 DAP successfully minimized disease incidence and severity of stemphylium blight of onion caused by *Stemphylium vesicarium* [26]. Hoque (2008) also reported that the bulb treatment with Rovral 50 WP (0.2%) followed by foliar spraying with Rovral 50 WP at 7 days interval starting from onset of the disease minimized disease incidence and severity [23].

There were significant differences among the effect of fungicides and micronutrients on the yield and yield contributing characters of onion. Rovral 50 WP (0.2%) and Rovral 50 WP @ 0.2% + micronutrients showed the best performances in controlling purple blotch complex of onion and in increasing yield. This finding was well supported by Barnoczki-Stoilova *et al.* (1989) and Georgy *et al.* (1986) [27] [28]. Alam *et al.* (2010) expressed the response of micronutrients for onion growth and yield in calcareous soils as the following orders (Zn + B) >

Zn > B > Mo [29]. Hoque (2008) also reported that micronutrients alone without spraying of fungicides had significant effect compared to control. Barnoczki-Stoilova *et al.* (1989) sprayed Rovral 50 WP (Iprodione) and Ridomil plus 50 WP (Methyl + Copper oxychloride) fungicides at different blooming stages of flowers and reported that both fungicides showed less harmful and effective in controlling disease in onion seed production. Georgy *et al.* (1986) also reported that the Iprodione group and Ridomil MZ (Metalaxyl + Mancozeb) proved most effective in reducing the disease severity and increasing bulb and seed yield. Hoque (2008) reported that Rovral 50 WP effectively controlled stemphylium blight of onion.

5. Conclusion

On the basis of present findings it may be concluded that the onion growers may be suggested to apply Rovral 50 WP (0.2%) along with micronutrients in controlling purple blotch complex of onion for increasing bulb and seed production in their field.

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