EFFECT OF FUNGICIDES ON MANAGEMENT OF FUNGAL BULB ROT IN CLUSTER ONION

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ABSTRACT

Onion (*Allium cepa* L.) is one of the important condiment and cash crop grown in Sri Lanka. The average yield of cluster onion in Sri Lanka ranged from 11 to 15 t/ha. It has been reported that there are several factors affecting the yield of cluster onion. Among them, occurrence of fungal bulb rot is the key factor that effect on yield losses of cluster onion in the field. Currently, management of fungal blub rot of onion relies mainly on soil drenching, blub treatment of fungicides and the use of integrated disease management (IDM) package. There are recommended fungicide for the controlling fungal bulb rot diseases in Sri Lanka. However, growers claim that available fungicides in the country do not effectively control bulb rot of onion. Therefore, identification of more effective fungicides is necessary to control the bulb rot. The present study was undertaken to investigate the efficacy of three recommended fungicides and two fungicides which are not recommended for controlling fungal bulb rot, by *in vitro* and field screening.

The main causal agents of onion bulb rot in DL2b region i.e. *Fusarium* spp.

and *Sclerotium rolfsii* were isolated from soil of onion cultivations. Results of in vitro study revealed that 1g/l and 1.8g/l concentrations of Captan 50WP and Homai 80 WP were significantly reduced the growth of *Fusarium* spp. and *Sclerotium rolfsii*, respectively. Bulb treatment i.e. dipping bulbs in 1g/l concentrations of Captan 50WP for one hour and 1.8g/l concentration of Homai 80 WP significantly reduced the incidence of cluster onion bulb rot while increased the bulb yield under field condition compared to other tested fungicides. Bulb treatment with Fluazinam 500SC and Tebuconazole 250EC had no effect on control of cluster onion bulb rot.

Keywords: Bulbrot, Cluster onion, Fungicides

INTRODUCTION

Cluster onion is mainly grown in northern region of Sri Lanka. It is estimated to expand cluster onion cultivation up to 5,000 ha in Mannar, Hambantota, Rathnapura, Batticoloa and Ampara under the National Food Production Programme of the Ministry of Agriculture.

Cook (1986) has proved that maintaining root health is a critical condition for high yields in crops. Production of cluster onion in Sri Lanka has reduced due to infection of fungal and bacterial root diseases. Among them fungal bulb rot severely affects the cluster onion cultivation especially under stormy weather conditions. The disease is caused by several soil borne pathogens such as *Fusarium* spp. (Lewis and Papacizas, 1977; Szczech, 1999), *Pythium* spp. (McKellar and Nelson, 2003; Veeken *et al.*, 2005), *Rhizoctonia solani* (Papavizas and Davey, 1960; Diab *et al.*, 2003) and *Sclerotium* spp. (Coventry *et al.*, 2005). Basal rot is caused by the soil-borne fungus *Fusarium* spp. in onion. It generally occurs when soil temperatures are very warm (optimum 29 °C). In *Ontario,* fungal bulb rot is mostly controlled by using the IDM methods. Make use of Homai (Thiophanate methyl 50% + Thiram 30% WP) at the rate of 18 g/10 l or Thiram 80% WP at the rate of 15 g/10 l water for 30 min or Thiophanate-methyl 70% WP at the rate of 20 g/10 l water for 30 min as a bulb treatment at planting is recommended by the Department of Agriculture (Pesticide Recommendations, 2015). Meanwhile, adding cow-dung at the rate of 15- 20 t/ha at land preparation showed the low fungal bulb rot incidences in DL2b agro ecological zone in Sri Lanka (Fernando *et al.*, 2013).

In some countries, farmers plant only disease-free seeds or bulbs; treat seed with fungicide; rotate with non-susceptible crops such as cereals or grasses to reduce levels of pathogen in soil at least 4 years, steam treatment or fumigation of soil can help to reduce levels of *Fusarium* spp. in the soil and plant resistant onion varieties.

Some farmers in Sri Lanka are not satisfied with the existing recommendation probably due to low efficacy or shortage of recommended agrochemicals in some areas of the country. Hence, this study was conducted to identify suitable fungicides and dipping time of bulbs in fungicide solution for controlling fungal bulb rot in different treatment durations which give the lowest disease incidence with high yields in cluster onion. Field study was conducted at the Regional Agriculture Research and Development Center (RARDC), Aralaganwila and Agriculture Research Station (ARS), Thirunelvely, two of the main cluster onion growing areas of Sri Lanka.

MATERIALS AND METHODS

Isolation of pathogens

A study was conducted in *Yala* 2012 season as a laboratory screening. The main pathogens which are common in research fields were *Fusarium* spp. and *Sclerotium rolfsii.* Therefore, isolates of *Fusarium* spp. and *Sclerotium rolfsii* from soils of research fields were used for the experiment.

In-vitro study for fungicides screening

Five fungicides which are commonly used in cluster onion cultivations were tested with three replicates in a Complete Random Design (CRD). Treatments arrangements are shown in the Table 1.

Table 1. Treatments of the in-vitro experiment

Treatment	Fungicide and the concentration
T1	Captan 50% WP, 10g/10l
T2 (Positive control)	Thiram 80%WP, 15g/10l
T3	Tizca (Fluazinam 500g/l SC), 5ml/10l
T4 (Positive control)	Homai (Thiophanate methyl 50% + Thiram 30% WP), 18g/10l
T5	Orius (Tebuconazole 250 ml/l), 3.5 ml/10l

Among the selected fungicides, Captan WP, Thiram WP and Homai WP are recommended fungicides for bulb treatments and others i.e. Fluazinam SC and Tebuconazole are recommended for foliar diseases. Fungicide screening test (*in-vitro*) was conducted by following food poison technique. Cultures were incubated at 26^{0}C +/- 2^{0}C for 7 days. The diameters of colonies were measured after 3, 5, and 7 days of the experiment. Then, the mean growth rates of each fungus were calculated with the following formula.

$$\text{Mean growth rate} = \frac{\text{Colony diameter (cm)}}{\text{Age of the culture (Days)}}$$

Antifungal Activity Calculation were done with five mm disc from an actively growing culture of each fungi separately placed in the center of Potato Dextrose Agar (PDA) plate mixed with fungicides. Three replicate plates were used per treatment. Fungi were also grown on fungicide free PDA as a control. Cultures were incubated at 26^{0}C +/- 2^{0}C for 7 days. Relative inhibition of colony growth (%) was calculated for each isolate by using the fungal growth diameter values measured after seven days on control plates and plates amended with fungicides (Ivic *et al.,*2011). Percentage of inhibition was calculated as per the following formula (Barrata *et al.*, 1998; and Janssen *et al.*, 1986);

Field trials at Aralaganwila and Thirunelvely

Studies were conducted during *Yala* 2012 and *Maha* 2012/13 seasons as a field trail at the RARC Aralaganwila (DL_{2b} agro-ecological region) and ARS Thirunelvely (DL_3 agro-ecological region) to find out the appropriate combination of fungicides and the bulb treatment time to reduce the fungal bulb rot disease in onion. Variety *Vethalan* was used for all studies in raised beds. The experiment plots at Aralaganwila were at a site of the regular cultivation of cluster onion and inoculated crop debris of infected plants, crop residue were also added (Sick plot). Twenty five treatments were tested as combination treatments including five fungicides and five bulb treatment times (Table 2). Treatments were replicated three times and arranged as a Randomized Complete Block Design (RCBD) at each location and each growing season. Except the treatment, all the other recommended agronomic practices were followed for all plots (Anonymous, 1990). Goal 2 XL (Oxyfluorfen 240 g/l EC) application was done 1 day after planting as a post-emergent weedicide to control weeds (Pesticide Recommendations, 2010). Data were recorded on disease incidence (%) by counting total number of plants and diseased plants at weekly intervals since planting up to harvesting. Percent disease incidence (DI %) was calculated using the following formula.

$$\text{Disease incidence (DI \%)} = \frac{\text{Number of diseased plants} \times 100}{\text{Total Number of assessed plants}}$$

Fungicides	Bulbs treatment duration				
	30 min	1 hr	3 hr	6 hr	24 hr
Captan 50%wp	T1	T2	T3	T4	T5
Thiram 80%wp	T6	T7	T8	T9	T10
Tizca	T11	T12	T13	T14	T15
Homai	T16	T17	T18	T19	T20
Orius	T21	T22	T23	T24	T25

RESULTS AND DISCUSSION

Effect of fungicide on colony growth

Effect of different fungicides on the growth of *Fusarium* spp. and *Sclerotium rolfsii* during the incubation period is shown in Figure 1 and 2. *In vitro* screening revealed that there is a significant difference among the fungicides tested ($p < 0.0001$). The lowest growth rate was shown in culture plates treated with Captan 10g/ 101 for both *Fusarium* spp. and *Sclerotium rolfsii* (Figure 1 and 2).

Homai also showed a similar growth rate as with Captan (Figure 1 and 2). Homai is widely used recommended fungicide to the fungal bulb rot in cluster onion cultivations in Sri Lanka. Thiophanate – methyl is highly effective on *Fusarium* spp. and *Sclerotium rolfsii* (Pesticide Recommendations, 2015). Homai contains Thiophanate – methyl than Thiram. Thus, it could be the most probable reason for showing significantly low Fungal growth rate with Homai than Thiram (Figure 1 and 2).

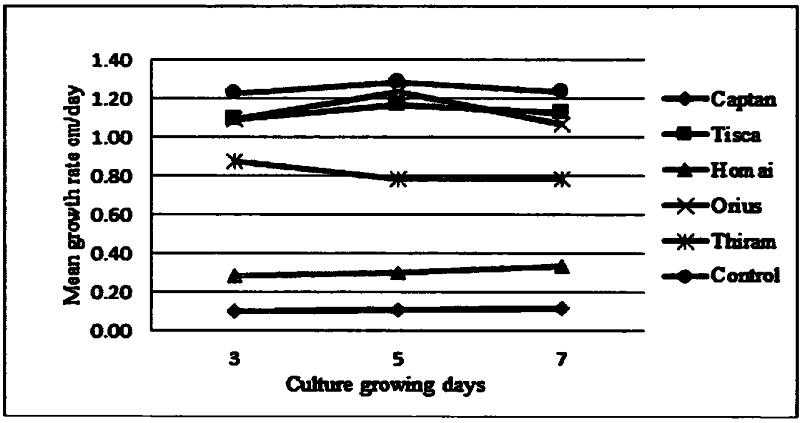

Figure 1. Mean growth rate of *Sclerotium rolfsii* under *in-vitro* conditions

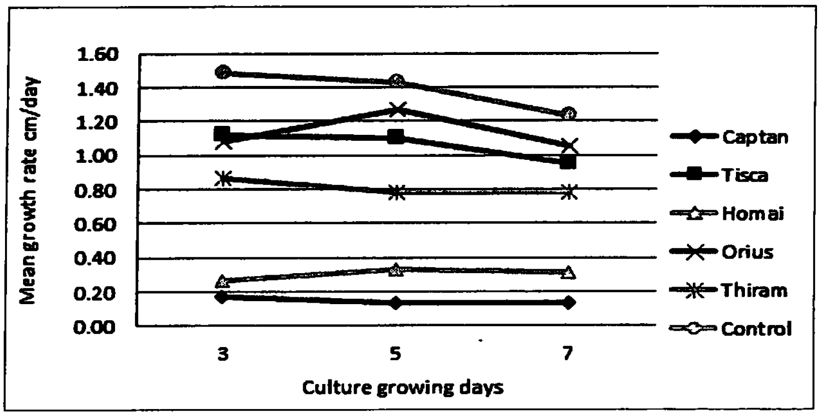

Figure 2. Mean growth rate of *Fusarium* spp. under *in-vitro* conditions

However, Tizca and Orius did not show effective control on tested fungi (Figure 1 and 2) and it may be due to that they are mainly meant for controlling foliar diseases.

Relative inhibition of colony growth (%)

The percentage reduction in growth of the tested pathogens due to the effect of different fungicides was calculated and is shown in Table 3. Normality test was run for relative inhibition of colony growth (%) and according to the Shapiro-Wilk normality test. The p-value was 0.9672 for both fungi. Therefore, parametric test was done using Analysis of variance and mean separation was done by using Least Significant Difference Test (LSD). Results showed that both fungi have significantly better colony growth inhibition in Captan 50% WP and Homai (Thiophanate methyl 50% + Thiram 30% WP) (Table 3) compared to other tested fungicides. Captan 50% WP, a more efficient fungicide resulted in 89.4 % and 90.7% reduction of *Fusarium* spp. and *Sclerotium rolfsii* growth, respectively. Homai also showed a similar efficiency with 72.7% and 74.9% reduction with respective fungi. (Table 3).

Table 3. Relative inhibition of colony growth (%) of *Fusarium* spp. and *Sclerotium rolfsii* by different fungicides 7 days after culturing

Treatments	Relative inhibition of colony growth (%)	
	Sclerotium rolfsii	***Fusarium* spp.**
Captan 50% WP	90.72 a	89.36a
Tizca (Fluazinam 500g/l SC)	20.12b	23.02b
Homai (Thiophanate methyl 50% + Thiram 30% WP)	72.73a	74.85a
Orius (Tebuconazole 250 ml/l)	13.35c	14.31c
Thiram 80%WP	37.72b	36.75b

Means followed by the same letters in each column are not significantly different.

Field screening of fungicides and dipping time for bulb treatment

With respective F value and P value it clearly reveals that effect of fungicides varies depending on the dipping time. Statistical analysis also shows that the both main effects, namely, fungicides and dipping time are evident. Normality test was run for disease index (%) and it was found that all data did not follow a normal distribution. Thus, non-parametric test was done using CATMOD for combination treatments of both fungicide and dipping time. There are significant differences in Homai (Thiophanate methyl 50% + Thiram 30% WP) and Captan 50% WP with Thiram in In- vitro and In-vivo experiments.

In both seasons at Thirunelvely there were less than 5% disease incidence level may be due to low pathogen density in that soil under natural infection conditions. It shows that the fungal bulb rot was not a serious disease under DL3 climate conditions in both seasons tested. But, in Aralaganwila the disease incidences were comparatively high. It is logical to assume that disease presence and its potential impact on crop would be greater in Aralaganwila than Thirunelvely as the latter does not maintain a sick plot for the disease. Thus, more cluster onion crop residues on soil of sick plot is likely to act as an inoculums source to potentially contribute to high disease infection at Aralaganwila. At the end of the growing season, there were more than 75% and 35% cumulative disease incidences at Aralaganwila during 2012 *Yala* season and 2012/13 *Maha* season with some treatments. It hints that the sick plot is rich with fungal bulb rot pathogens.

Table 4. Incidences of fungal bulb rot (%) of onion in different treatments during 2012 *Yala* season, Aralaganwila.

Trt	Disease incidence (DI %) at weekly intervals										
	1	2	3	4	5	6	7	8	9	10	11
T1	2.09 d	4.17 cde	4.17 cde	4.17 de	4.17 de	4.17 efg	4.17 fg	4.69 e	5.21 efgh	7.29 cdef	9.37 cdef
T2	0.52 d	0.52 e	0.52 e	1.04 e	1.56 e	1.56 g	2.08 g	2.60 e	2.60 gh	3.56 f	3.56 f
T3	3.13 cd	3.65 cde	4.69 cde	4.69 cde	4.69 de	4.69 defg	5.73 defg	6.25 de	7.81 defg	7.81 cdef	7.81 cdef
T4	1.04 d	1.56 de	3.13 cde	3.13 de	3.65 e	4.17 efg	6.25 defg	6.25 de	7.81 defg	8.85 cdef	8.85 cdef
T5	2.60 d	8.86 c	9.90 c	11.46 c	11.46 cd	11.98 cde	11.98 def	11.98 d	11.98 d	11.98 c	11.98 cd
T6	0.52 d	1.04 e	1.56 de	2.08 de	3.13 e	3.13 fg	3.65 g	4.69 de	5.73 defgh	6.25 cdef	6.25 cdef
T7	1.56 d	3.12 cde	3.65 cde	3.65 de	4.17 de	4.17 efg	5.21 efg	5.21 de	5.73 defgh	6.25 cdef	6.25 cdef
T8	2.61 d	3.12 cde	5.21 cde	5.21 cde	5.21 de	5.73 defg	5.73 defg	5.73 de	6.77 defgh	7.29 cdef	7.29 cdef
T9	1.04 d	1.04 e	1.56 de	2.08 de	2.60 e	2.60 fg	2.60 g	3.65 e	4.17 fgh	4.69 ef	4.69 ef
T10	4.69 cd	5.73 cde	6.25 cde	6.77 cde	11.46 cd	12.50 cd	13.02 de	14.06 de	14.58 def	15.10 cdef	15.10 cdef
T11	1.04 d	2.08 cde	2.08 de	2.08 de	2.60 e	2.60 fg	2.60 g	3.12 e	3.12 gh	4.17 f	4.17 f
T12	2.60 d	3.65 cde	4.16 cde	4.69 cde	6.77 de	9.90 def	13.54 d	14.58 de	15.63 defg	16.67 cde	17.71 c
T13	1.04 d	1.56 de	2.08 de	2.08 de	2.08 e	2.61 fg	3.13 g	3.13 e	3.13 h	3.65 f	4.65 ef
T14	2.08 d	2.6 cde	3.65 cde	3.65 de	5.21 de	5.73 defg	8.34 defg	8.86 de	9.90 defgh	9.90 cdef	10.42 cdef
T15	19.79 b	24.48 b	26.04 b	29.69 b	32.81 b	34.38 b	35.42 b	35.94 b	36.46 b	37.50 b	37.50 b
T16	2.08 d	2.08 cde	2.08 de	2.08 de	2.08 e	2.08 fg	2.60 g	3.12 e	3.12 efgh	3.12 ef	3.62 f
T17	1.56 d	2.08 cde	2.08 de	2.08 de	2.08 e	2.61 fg	4.69 fg	4.69 de	5.21 defgh	5.73 cdef	6.25 cdef

Table 4. Cont..

T18	4.69 cd	4.69 cde	4.69 cde	4.69 cde	5.21 de	5.73 defg	5.73 defg	6.25 de	6.25 defgh	6.25 cdef	6.25 cdef
T19	3.65 cd	4.69 cde	6.25 cde	6.25 cde	6.77 de	8.86 defg	10.42 defg	11.46 de	11.98 defgh	13.54 cdef	14.06 cdef
T20	0.52 d	2.09 cde	2.09 de	2.61 de	4.17 de	4.69 defg	5.21 efg	5.21 e	5.21 fgh	5.73 def	5.73 ef
T21	1.56 d	3.12 cde	5.73 cde	6.25 cde	15.63 c	18.75 c	21.88 c	25.00 c	27.61 c	30.73 b	32.82 b
T22	7.81 c	8.86 c	8.86 cd	8.86 cd	8.86 cde	8.86 defg	9.38 defg	9.90 de	10.94 de	11.46 cd	11.46 cde
T23	18.75 b	29.17 b	31.25 b	31.25 b	32.81 b	32.81 b	32.81 b	32.81 b	32.81 b	34.37 b	34.37 b
T24	7.81 c	8.33 cd	8.33 cd	8.33 cd	8.33 de	8.33 defg	8.33 defg	8.33 de	8.85 defgh	8.85 cdef	9.38 cdef
T25	65.10 a	68.75 a	68.75 a	70.31 a	70.83 a	70.83 a	71.88 a	72.92 a	74.48 a	74.48 a	75.52 a

Means followed by the same letters in each column are not significantly different.

Table 5. Incidences of fungal bulb rot (%) of onion in different treatments during *Maha* season 2012/13 at Aralaganwila.

Trt	Disease incidence (DI %) at weekly intervals									
	1	2	3	4	5	6	7	8	9	10
T1	1.56 de	3.13 cde	4.17 cd	5.73 def	7.29 def	8.34 ef	9.38 ef	10.94 ef	10.94 de	12.50 cd
T2	0.52 e	0.52 e	1.56 d	2.60 f	4.17 f	5.21 f	5.73 f	6.77 f	7.29 e	8.33 d
T3	0.52 e	1.56 de	3.13 cd	4.69 ef	6.25 ef	7.81 ef	9.38 ef	10.42 ef	10.94 de	10.94 d
T4	0.00 e	1.56 de	2.61 cd	4.17 ef	5.73 ef	7.29 ef	8.34 ef	8.34 f	8.86 e	10.42 d
T5	3.13 bcde	6.25 bcde	9.38 bcd	9.90 cdef	11.46 def	12.50 def	13.54 def	14.58 def	15.63 cde	16.15 cd
T6	5.21 abcd	8.85 abcd	12.50 abcd	16.67 abcde	19.27 abcde	21.88 abcde	24.48 abcde	26.04 abcde	26.56 abcd	28.12 abc

Table 5. Cont..

T7	3.64 bcde	7.29 abcde	10.94 abcd	13.54 abcdef	16.15 abcdef	18.23 bcdef	19.79 bcdef	21.87 bcdef	22.40 abcde	22.92 abcd
T8	6.77 ab	11.98 ab	17.19 ab	22.40 ab	26.56 ab	30.21 ab	34.38 ab	36.46 ab	36.46 a	36.98 a
T9	2.61bcde	5.21 bcde	7.81 bcd	10.42 bcdef	12.50 cdef	15.10 cdef	17.19 cdef	19.27 cdef	19.27 bcde	20.84 bcd
T10	0.52 e	1.56 de	3.13 cd	4.17 ef	6.25 ef	8.33 ef	10.42 def	11.46 def	12.50 cde	13.02 cd
T11	3.65 bcde	5.73 bcde	8.33 bcd	10.94 bcdef	13.54 bcdef	15.62 bcdef	17.19 cdef	19.27 cdef	19.79 bcde	20.31 bcd
T12	1.56 de	3.12 cde	4.69 cd	6.77 def	8.34 def	10.94 def	12.50 def	14.58 def	15.11 cde	16.15 cd
T13	3.13 bcde	5.73 bcde	8.85 bcd	12.50 bcdef	14.59 bcdef	16.67 bcdef	18.23 cdef	19.27 cdef	19.27 bcde	20.32 bcd
T14	1.56 de	3.12 cde	4.69 cd	6.77 def	9.90 def	12.50 def	14.58 def	15.63 def	16.15 cde	17.19 cd
T15	0.00 e	1.04 de	2.09 cd	2.61 f	4.17 f	5.21 f	6.25 f	6.77 f	7.29 e	7.81 d
T16	0.52 e	1.56 de	2.60 cd	4.17 ef	5.21 ef	6.25 f	6.77 f	7.29 f	7.29 e	8.34 d
T17	6.25 abc	10.94 abc	15.63 ab	21.35 abc	25.52 abc	29.17 abc	32.81 abc	34.38 abc	34.38 ab	35.42 abc
T18	2.08 cde	3.64 cde	4.69 cd	6.25 def	7.81 def	9.37 def	10.94 def	11.98 def	12.50 cde	12.50 cd
T19	0.52 e	1.56 de	2.61 cd	4.17 ef	6.25 ef	8.85 ef	10.94 def	11.98 def	12.50 cde	13.54 cd
T20	5.21 abcd	8.85 abcd	13.02 abc	17.71 abcd	21.36 abcd	24.48 abcd	26.56 abcd	28.13 abcd	28.13 abc	28.65 abc
T21	8.33 a	14.58 a	20.31 a	25.00 a	28.65 a	32.81 a	36.46 a	38.02 a	38.02 a	38.54 a
T22	0.00 e	1.04 de	1.56 d	3.65 f	6.25 ef	9.38 def	11.98 def	13.55 def	14.07 cde	15.11 cd
T23	3.13 bcde	5.21 bcde	7.29 bcd	9.37 cdef	11.46 def	13.02 def	15.11 def	16.15 def	16.67 cde	17.19 cd
T24	3.64 bcde	6.25 bcde	7.81 bcd	9.38 cdef	13.02 bcdef	16.15 bcdef	17.71 def	18.75 cdef	19.27 bcde	19.79 bcd
T25	0.00 e	1.04 de	3.65 cd	5.73 def	7.81 def	8.86 ef	10.42 def	10.94 ef	11.46 cde	12.50 cd

Means followed by the same letters in each column are not significantly different.

Orius dipped in 24hrs (T25) showed the significant highest DI of >75% in 2012 *Yala* season. The lowest cumulative DI % of approximately 3.5% reported at the T16 and T2 in 2012 *Yala* season and approximately 8% at the 2012/13 *Maha* season. In both seasons current recommendation (T16) and T2 gave the similar lower DI value. T2 had maintained continuously lowest DI value throughout the season. Results of the experiment repeated in 2012/13 *Maha* season also confirmed the results obtained in 2012 *Yala*. Results of field trail was confirmed as Tizca and Orius did not show effective control on tested fungi (Table 4 and 5) may be due to mode of action of fungicides. Captan is a broad-spectrum, non-systemic fungicide effective against various seed decay and damping-off fungi, such as *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizoctonia*. Thiophanate methyl is a systemic fungicide labeled for a wide range of control seed, bulb, and tuber decay, and damping-off.

CONCLUSIONS

Both treatments of bulbs dipped for 1 hr in Captan 50% WP at the rate of 10g/10l and Homai (Thiophanate methyl 50% + Thiram 30% WP), 18g/10l in 30 min application had shown as the best treatment among the tested treatments for cluster onion in DL_{2b} agro ecological zone for fungal bulb rot management in field conditions. *In - vitro* testing also confirmed that the Captan 50% WP and Homai at the same rate are the better fungicides to control *Fusarium* spp and *Sclerotium rolfsii,* two main pathogenic fungi causing fungal bulb rot in cluster onion. Therefore, both Captan 50% WP at the rate of 10g/10l and Homai (Thiophanate methyl 50% + Thiram 30% WP), 18g/10l in 30 min can be recommended as an effective fungicide to control fungal bulb rot in cluster onion.

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