Egypt. J. Phytopathol., Vol. **43**, No. 1-2, pp. 101-112 (2015)

Performance of some Environmental Safe Treatments on Controlling Onion White Rot

M.M. Amin and S.B.M. Fawaz
Plant Pathol. Res. Inst., A.R.C. Giza, Egypt.

Effects of sulphur and gypsum as soil treatment as well as clorox with water irrigation were tested to manage white rot of onion caused by *Sclerotium cepivorum* Berk. for two successive seasons (2012-2013 and 2013-2014) in greenhouse and open field in soil naturally infested with *S. cepivorum* at Mallawy Agric. Res. Sta., Menia Governorate. The recommended fungicide Folicure 25% EC (tebuconazole 25%) was used for comparison. In greenhouse, 1.2, 1.4 and 1.9 g/pot (25-cm-diam.) for sulphur and gypsum or 1.2, 1.4 and 1.9 ml/pot for clorox were used. While, under field conditions 100, 120 and 160 kg/fed for sulphur and gypsum or 100, 120 and 160 L/fed for clorox were tested. Under greenhouse conditions, all treatments reduced percentages of infection compared with non-treated plants. The reduction ranged between 4.2% with 1.2 g sulphur/pot. at soil preparation time and 66.7% with clorox treatments added to water irrigation four times at the rate of 1.4 or 1.9 ml/pot, while Folicure treatment gave 75% efficacy. In field experiments, clorox application four times with water irrigation at the rate of 160 L/fed led to 64.7% disease reduction and increased bulbs yield by 248.4% compared with 80.7% disease reduction and 119.4% increase in bulbs yield with Folicure fungicide treatment. Values for the efficiency of gypsum, sulfur and clorox in controlling the disease were somewhat similar to that found with Folicure fungicide.

Keywords: Clorox, gypsum, onion, *Sclerotium cepivorum*, sulphur and white rot.

Onion (*Allium cepa* L.) is one of the most important vegetable crops grown in Egypt, not only for local consumption but also for exportation. Onion white rot disease was recorded for first time in Egypt at Maghagha County, Minia Governorate by Nattrass (1931). Many studies have been conducted to manage the disease biologically (Abd El-Moity and Shatla, 1981; Abd El-Moity *et al*., 1982 and Amin, 2003), chemically (El-Shehaby *et al*. 1992; Kay and Stewart, 1994 and Khaled *et al*., 1997), with solarization (Porter and Merriman, 1983 and 1985; Satour *et al*., 1989 and 1991; Basallote-Ureba and Merlero-Vara, 1993 and Amin, 2003), as well as with soil amendments and agricultural practices (Rushdi *et al*., 1974; Utkhede, 1982; Yehia and Khater, 1986 and Salama *et al*., 1988).

Sulphur is the fourth major plant nutrient after nitrogen, phosphorus, and potassium and it is essential for the plant growth (Havlin *et al*., 2004). It plays an important role for plant disease management (Bell, 1981; Jaggi and Dixit, 1999 and Morsy, 2012). Also, the effects of gypsum on plant diseases control and to improve plant growth was mentioned by several researchers (Chen and Hung, 1992; Nadph and Reddy, 1996 and Morsy, 2012).

Sodium hypochlorite solution (clorox) was used for a long time as surface sterilizer for plant disease (Somerville and Hall, 1987; Brix and Zinkernagel, 1992 and Shiver and Beasley, 2005). Therefore, disinfection efficacy of sodium hypochlorite solution on vegetables and food industry have been reported and reviewed by Koseki *et al*. (2001) and Huang *et al*. (2008).

The aim of this investigation was to study the effect of sulphur and gypsum as fertilizers and clorox as disinfectant to manage onion white rot and to improve onion yield compared to the recommended fungicide Folicure.

Materials and Methods

Source of Sclerotium cepivorum isolate:

Sclerotium cepivorum isolate used in this investigation was previously isolated from infected onion plants collected from Mallawy Province, Menia Governorate. The fungal isolate was identified in Onion, Garlic and Oil Crops Dept., Plant Pathol. Res. Inst., ARC, based on its morphological characteristics according to Mordue (1976).

Treatments tested:

Different treatments of sulphur, gypsum and clorox were applied in the presence of recommended fungicide Folicure 25% EC (tebuconazole 25%) as comparison to study their effect on white rot of onion incidence and onion bulbs yield.

Greenhouse experiments:

Pot experiments were carried out in the greenhouse of Onion, Garlic and Oil Crops, Plant Pathol. Res. Inst., ARC, during 2012-2013 and 2013-2014 growing seasons to evaluate white rot of onion incidence under different treatments.

Disks (5-mm-diam.) of *S. cepivorum* grown on PDA medium for 7 days were used to inoculate sterilized barley seeds medium (Van der Meer *et al*., 1983) in glass bottles (500 ml). The inoculated bottles were incubated at 20^{o} C for 3 weeks. Plastic pots (25-cm-diam.), filled with sterilized sand-clay soil (1:1 v/v) were infested with the prepared inoculum at the rate of 2 % w/w, 7 days before transplanting. Four pots were used as replicates for each treatment and the check (infested pots without treatment). Five seedlings of cv. Giza 6 (60-day-old) were transplanted in each pot in November and irrigated when necessary. Percentage of infection (treatment-check/check X100) was estimated at the end of each season during April.

Sulphur treatment:

Agricultural sulphur (95.0%) was applied as soil treatment at the rate of 1.2, 1.4 and 1.9 g/pot. Eight pots were used for each concentration at soil preparation, then divided into two groups; first group (A) was left till harvest, and the second group (B) was treated by another dose after 8 weeks from transplanting.

Gypsum treatment:

Gypsum (Calcium sulphate dihydrate 79.1%) was applied as soil treatment at the same rate of sulphur (1.2, 1.4 and 1.9 g/pot). Eight pots were used as replicates for each concentration as soil drenching just before transplanting, then divided into two groups; first group (C) was left till harvest, and the second group (D) was treated by another dose after 8 weeks from transplanting.

Clorox treatment:

Clorox (Sodium hypochlorite 5%) was applied with water irrigation at the rate of 1.2, 1.4 and 1.9 ml/pot. Twelve pots were treated for each concentration during transplanting, then divided into three groups; first group (E) was treated by another dose at 4 weeks from transplanting, second group (F) was treated by another dose at 4 and 8 weeks from transplanting and the third group (G) was treated by another dose 4, 8 and 12 weeks after transplanting.

Both sulphur and gypsum were obtained from Mallawy Agric. Res. Station, Menia Governorate, while clorox (manufactured in The Egyptian Company for Household Cleaners - Tenth of Ramadan City) was obtained from the local markets.

Folicure treatment:

The recommended fungicide Folicure 25% EC (tebuconazole 25%) was used in this investigation as a comparison with the treatments tested. Seedlings were dipped for 10 min. in Folicure 25% (25 ml/l water) just before transplanting then the grown plants were sprayed at 6 and 12 weeks after transplanting (187.5 ml/100 l water).

Field experiments:

These experiments were carried out during 2012-2013 and 2013-2014 seasons in soil with a back history of white rot incidence at Mallawy Agric. Res. Sta., Menia Governorate, to study the effect of the tested treatments on the infection (%) and onion bulbs yield at harvest.

Sixty days old onion transplants (cv. Giza 6), were transplanted in November. Experiments were designed as complete randomized blocks. All treatments under field conditions were conducted as described in greenhouse experiments at the rate of 100, 120 and 160 kg/fed for sulphur and gypsum or 100, 120 and 160 l/fed for clorox. Four replicate plots were used for each treatment and the check. The area for each plot was 10.5 m^2 (3.0 X 3.5 m). All treatments received the same normal agricultural practice till harvest in April.

Statistical analysis:

Collected data were statistically analyzed and significance among means was assessed by least significant difference (LSD) at 5% probability level using SAS ANOVA program Ver. 9 (Anonymous, 2014).

Results

Greenhouse experiments:

Data presented in Table (1) show that, on the average of the two tested seasons, all treatments reduced the infection (%) compared to the check. The efficacy ranged between 4.2% for sulphur (A) at 1.2 g/pot to 66.7% for clorox (G) at 1.4 and 1.9 ml/pot.

In case of sulphur treatment, the best results in reducing the disease infection (%) were recorded in (B) at the rate of 1.4 and 1.9 g/pot that gave the same mean values (45.0%), followed by treatment (A) at the rate of 1.4 g/pot (50.0%). As for gypsum application, treatment (D) at 1.4 and 1.9 g/pot showed the best levels of white rot reduction in the two tested seasons (being 37.5%).

Table 1. Effect of sulphur, gypsum, clorox and Folicure (25%) treatments on infection (%) of onion white rot disease under greenhouse conditions during 2012-2013 and 2013-2014 growing seasons

Treatment	Dose		Application	Infection (%)			Efficacy*** (%)
				2013	2014	Mean	
Sulphur	1.2	g/pot	A *	60.0	55.0	57.5	4.2
			B	55.0	50.0	52.5	12.5
	1.4		A	50.0	50.0	50.0	16.7
			B	45.0	45.0	45.0	25.0
	1.9		A	50.0	55.0	52.5	12.5
			B	45.0	45.0	45.0	25.0
Gypsum	1.2	g/pot	C	50.0	45.0	47.5	20.8
			D	40.0	45.0	42.5	29.2
	1.4		C	40.0	45.0	42.5	29.2
			D	35.0	40.0	37.5	37.5
	1.9		C	40.0	45.0	42.5	29.2
			D	35.0	40.0	37.5	37.5
Clorox	1.2	ml/pot	E	40.0	40.0	40.0	33.3
			F	40.0	40.0	40.0	33.3
			G	40.0	40.0	40.0	33.3
	1.4		E	40.0	40.0	40.0	33.3
			F	30.0	30.0	30.0	50.0
			G	20.0	20.0	20.0	66.7
	1.9		E	30.0	30.0	30.0	50.0
			F	20.0	30.0	25.0	58.3
			G	20.0	20.0	20.0	66.7
Folicure **				20.0	10.0	15.0	75.0
Check				60.0	60.0	60.0	-
L.S.D. at 0.05%				10.1	11.8	-	-

* A= Sulphur added to soil at preparation time, B= Sulphur added to soil at preparation time and 8 weeks after transplanting, C= Gypsum added to soil then drenched before transplanting, D= Gypsum added to soil then drenched before transplanting and 8 weeks after transplanting, E= clorox added to soil at transplanting and 4 weeks after transplanting with water irrigation, F= clorox added to soil at transplanting, 4 and 8 weeks after transplanting with water irrigation G= clorox added to soil at transplanting, 4, 8 and 12 weeks after transplanting with water irrigation.

**Fungicide Folicure 25%: Transplants dipped for 10 minutes in the fungicide (25 ml/l) just before transplanting and the grown plants were sprayed after 6 and 12 weeks from transplanting by the fungicide (187.5 ml/100 l).

***Efficacy (%)= Value relative to the check.

Meanwhile, all clorox treatments significantly reduced the percentages of infection. In the two seasons, treatment (G) at the rate of 1.4 and 1.9 ml/pot gave the same percent of infection just like Folicure at the first season (20%), compared with the check of non-treated plants (60% infection).

Field experiments:

Data in Table (2) show that all treatments reduced the percentages of infection compared to non-treated plants with percentages of efficacy ranged between 23.5%-64.7% with sulphur treatment (A); 100 kg/fed. and clorox treatment (G); 160 l/fed., respectively.

Table 2. Effect of sulphur, gypsum, clorox and Folicure (25%) treatments on infection (%) of onion white rot disease under naturally infested soil with *S. cepivorum* located at Mallawi Agric. Res. Station, Menia Governorate, during 2012-2013 and 2013-2014 growing seasons

Treatment	Dose		Application	Infection (%)			Efficacy*** (%)
				2013	2014	Mean	
Sulphur	100	kg/fed	A*	44.0	45.6	44.8	23.5
			B	38.1	40.3	39.2	33.1
	120		A	31.3	31.8	31.6	46.1
			B	26.3	27.0	26.7	54.4
	160		A	31.3	30.6	31.0	47.1
			B	25.0	25.6	25.3	56.8
Gypsum	100	kg/fed	C	32.3	31.7	32.0	44.9
			D	28.0	32.3	30.2	52.2
	120		C	26.0	26.1	26.1	55.6
			D	23.0	24.4	23.7	60.8
	160		C	23.8	24.3	24.0	59.5
			D	22.5	24.4	23.4	61.6
Clorox	100	l/fed	E	40.7	40.9	40.8	30.4
			F	40.0	39.8	39.9	31.9
			G	38.7	37.4	38.1	35.0
	120		E	38.1	39.5	38.8	33.8
			F	26.3	29.1	27.7	52.7
			G	20.1	21.6	20.9	64.3
	160		E	25.0	25.3	25.2	57.0
			F	23.1	24.0	23.6	59.7
			G	20.0	21.3	20.7	64.7
Folicure**				11.0	11.5	11.3	80.7
Check				56.5	60.6	58.6	-
L.S.D. at 0.05%				2.2	1.4	-	-

*; **; *** As described in foonote of Table (1).

In case of sulphur, the best results were recorded in (B) at the rate of 160 and 120 kg/fed which gave 25.3 and 26.7% infection respectively. Gypsum treatment (D) at the rate of 160 and 120 kg/fed yielded 23.4 and 23.7% infection, respectively. On the other hand, clorox treatment (G) at 160 and 120 l/fed gave 20.7% and 20.9% infection respectively. While, treatment with Folicure fungicide only gave 11.3% infection compared to non-treated plants (58.6%).

Concerning to effect of treatments used on bulb yield of onion in field experiments, data in Table (3) clearly indicate that these treatments caused significance increases in bulb yield/plot. These increases recorded 141.9% with treatment (C); 160 kg gypsum/fed and 248.4% for clorox treatment (G); 160 l/fed, while Folicure fungicide treatment increased bulb yield by 119.4 % compared to non-treated plants. Therefore, sulphur treatment (B) and gypsum treatment (D) at 160 kg/fed gave mean value 9.0 kg/plot compared to non-treated plants that gave only 3.1 kg bulbs/plot.

Table 3. Effect of sulphur, gypsum, clorox and Folicure (25%) treatments on bulbs yield of onion under naturally infested soil with *S. cepivorum* located at Mallawi Agric. Res. Sta., Menia Governorate, during 2012-2013 and 2013-2014 growing seasons

Treatment	Dose		Application	Bulbs yield (kg/pot)			Increase*** (%)
				2013	2014	Mean	
Sulphur	100	kg/fed	A *	7.9	7.8	7.9	153.2
			B	8.2	8.1	8.2	162.9
	120		A	7.6	7.5	7.6	143.5
			B	8.1	8.6	8.4	169.4
	160		A	8.3	8.1	8.2	164.5
			B	9.1	8.8	9.0	188.7
Gypsum	100	kg/fed	C	8.1	7.9	8.0	158.1
			D	8.9	8.5	8.7	180.6
	120		C	7.8	8.1	8.0	156.5
			D	8.9	8.2	8.6	175.8
	160		C	7.8	7.2	7.5	141.9
			D	9.5	8.4	9.0	188.7
Clorox	100	l/fed	E	9.6	9.8	9.7	212.9
			F	10.5	10.9	10.7	245.2
			G	10.2	10.9	10.6	240.3
	120		E	8.5	9.4	9.0	188.7
			F	9.4	10.2	9.8	216.1
			G	10.2	10.6	10.4	235.5
	160		E	7.1	8.8	8.0	156.5
			F	10.1	10.3	10.2	229.0
			G	11.2	10.4	10.8	248.4
Folicure**				6.8	6.8	6.8	119.4
Check				3.1	3.1	3.1	-
L.S.D. at 0.05%				0.5	0.7	-	-

*; **; *** As described in foonote of Table (1).

Clorox treatment (G) at the rate of 160 l/fed gave the highest mean yield of bulbs weight/plot (10.8 kg), followed by treatment (F) and (G) at the rate of 100 l/fed which gave 10.7 and 10.6 kg/plot, respectively.

According to the presented results, the application of clorox at 160 l/fed three (F) or four (G) times during season significantly decreased the incidence of white rot and increased bulbs yield compared to non-treated plants and very close from fungicide treatment.

Discussion

Considerable values of sulphur, gypsum and clorox (5% sodium hypochlorite) as effective control measures against soil borne diseases of various crops or improve growth were confirmed, using different application methods and doses (Somerville and Hall, 1987; Brix and Zinkernagel, 1992; Hussein *et al*., 2000; Sahu *et al.*, 2001; Shiver and Beasley, 2005 and Sammour *et al.*, 2011).

The present study show that sulphur treatments significantly decreased the percentages of infection and increased bulbs yield compared with non-treated plants at all applied concentrations. The obtained results are in a harmony with those of Sammour *et al.* (2011), who stated that using sulphur powder in field trials proved to be efficient agent for onion white rot control. Also, Morsy (2012) stated that sulphur as soil treatments before or during sowing sunflower seeds caused significant decrease in infection percentage of damping-off and/or charcoal rot disease(s) and increased seed yield and oil content. On the other hand, it caused a significant increase in plant height gradually with increasing the levels of the added sulphur (Vachhani and Patel, 1993; Alam, 1995; Jalil, 1998 and Havlin *et al.*, 2004). Sulphur application increased the garlic yield and enhanced the uptake of N, P, K, and Ca (Ahmed *et al*., 1988 and Hossain, 1997). Plant height, number of leaves/plant, cloves/bulb, diameter and weight of bulb and bulb yield were increased with the application of sulphur as reported by Alam (1995) and Nasrin *et al.* (2007). However, sulphur play an important role in reducing soil pH, which occur when sulphur is oxidized and that causes suppression to many soilborne pathogens (Adams, 1975; Ries *et al*., 1982 and Ibrahim, 2006). On the other hand, sulphur is the fourth major plant nutrient after nitrogen, phosphorus, and potassium and is essential for the synthesis of amino acids like cystine, cysteine, and methionine, a component of vitamin A and activates certain enzyme systems in plants (Havlin *et al*., 2004).

Gypsum (Ca $SO_4.2H_2O$) applications as soil treatment decreased percentages of infection and increased bulbs yield. Recently, several publications have been concerned with the relationship between calcium application and the disease incidence. Calcium dosage found to have different effects, partly it can shift the soil pH, affect plant metabolism, and also increase disease resistance in host (Osman *et al*., 2002). Calcium application usually leads to an increase in apoplastic Ca concentration (Poovaiah, 1979), and that may affect the structure and functions of cell walls and membranes and certain aspects of cell metabolism (Glenn *et al*., 1988). Nadph and Reddy (1996) demonstrated that calcium role in building cell walls of the plant tissues through the formation of calcium pectate, which is more resistant to pectic enzymes that play an important role in the pathogenesis. On other hand, the oxidized part (SO_4) of gypsum may be play a role in reducing soil pH, and that causing suppression to many soilborne pathogens and change the solubility of many elements in soil. Chen and Hung (1992) stated that application of gypsum reduced pod-rots and improved the appearance and quality of peanut pods. Moreover, Nadph and Reddy (1996) reported that application of gypsum reduced the incidence of groundnut dry root-rot caused by *Macrophomina phaseolina*, especially when combined with NPK and Zn. Also, Mahmoud (2004) found that application of gypsum at 500 kg/fed reduced peanut pod rots caused by *Rhizoctonia. solani*, *Macrophomina phaseaolina*, *Fusarium* spp., *Sclerotium rolfsii* and *Aspergillus* spp., as well as the colonization of pods by aflatoxigenic fungi.

Results of clorox application showed that all treatments significantly reduced percentages of infection either in greenhouse or in open field with a reverse relationship between percentages of infection and clorox concentration and frequency of application. On the other hand, clorox treatments under open field

conditions increased bulbs yield. This result was similar to those found in many studies which stated the disinfection efficacy of sodium hypochlorite on food, veterinary and plant pathogens with the recommended concentration (Somerville and Hall, 1987; Brix and Zinkernagel, 1992; Koseki *et al*., 2001; Shiver and Beasley, 2005 and Huang *et al*., 2008). Clorox totally eradicate the growth of sclerotia of *S. cepivorum in vitro*, while treating infested soil with clorox and that lead to complete inhibition for either the pathogen vegetative form or sclerotia. In field experiments when clorox is added to water, underchloric acids formed, depending on the pH value, underchloric acid partly expires to hypochlorite ions. Underchloric acids (HOCl, which is electrically neutral) and hypochlorite ions (OCl^- electrically negative) will form free chlorine when bound together. These results were very important in disinfection. Both substances have very distinctive behavior. Underchloric acid is more reactive and is a stronger disinfectant than hypochlorite. Underchloric acid is split into hydrochloric acid (HCl) and atom air oxygen (O). The oxygen atom is a powerful disinfectant. The disinfecting properties of chlorine in water are based on the oxidizing power of the free oxygen atoms and on chlorine substitution reaction (Sammour *et al*, 2011).

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(Received 10/06/2015;
in revised form 19/07/2015)

فعالية بعض المعاملات الآمنة على البيئة فى مقاومة مرض العفن الأبيض فى البصل

محسن محمدى أمين ، سيد بدوى مصطفى فوّاز
معهد بحوث أمراض النباتات - مركز البحوث الزراعية – الجيزة.

تم أختبار الكبريت والجبس كمعاملة تربة والكلوروكس مع ماء الرى فى مقاومة مرض العفن الأبيض على البصل المتسبب عن الفطر *Sclerotium cepivorum* Berk. تحت ظروف الصوبة والحقل لموسمين متتاليين (٢٠١٢-٢٠١٣ و ٢٠١٣-٢٠١٤) مع المقارنة بمبيد فوليكيور الموصى به لمقاومة المرض ، وقد تم استخدام تركيز ١.٢، ١.٤ و ١.٩ جم/اصيص مع الكبريت و الجبس أو ١.٢، ١.٤ و ١.٩ مل/اصيص مع الكلوروكس تحت ظروف الصوبة، فى حين تم استخدام تركيز ١٠٠، ١٢٠ و ١٩٠ كجم/فدان مع الكبريت والجبس أو ١٠٠، ١٢٠ و ١٩٠ لتر/فدان مع الكلوروكس تحت ظروف الحقل.

خفضت جميع المعاملات تحت ظروف الصوبة نسبة الأصابة بالمرض مقارنة بغير المعامل. تراوحت نسبة كفاءة أختزال المرض بين ٤.٢% عند معاملة التربة بالكبريت بمعدل ١.٢جم/اصيص و ٦٦.٧% عند المعاملة بالكلوروكس أربع مرات مع ماء الرى بتركيز ١.٤ و ١.٩ مل/اصيص. فى حين أعطت المعاملة بالفوليكيور ٧٥% كفاءة في مقاومة المرض.

أعطت معاملة الكلوروكس بأضافتة أربع مرات مع ماء الرى تحت ظروف الحقل بمعدل ١٦٠ لتر/فدان أفضل نسبة خفض للمرض حيث بلغت ٦٤.٧% مع زيادة للمحصول ٢٤٨.٤%، بينما بلغت الكفاءة فى خفض نسبة الأصابة ٨٠.٧% و زيادة المحصول ١١٩.٤ عند المعاملة بالفولكيور. ولحسن الحظ فإن قيم فعالية الجبس والكبريت والكلوروكس في مقاومة المرض هي نفسها تقريباً المسجلة بأستخدام مبيد الفوليكيور.