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Soil Solarization Combined with Commercial Fungicides for Controlling of Onion and Garlic White Rot Disease

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Abstract

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are very important Egyptian and Chinese table vegetable crops as otherworld countries. But, these crops face a serious disease of white rot which is caused by the soil-borne fungus, *Sclerotium cepivora*. In this study, application of soil solarization technology with/without some commercial fungicides was tested for their ability to inhibit mycelial growth of *S. cepivora* at *in vitro* state. Complete inhibition was obtained with 5.0 ppm of Chipco 50 %, Actamyl M70 and Folicur 25 % and 10.0 ppm of Switch 62.5 %. Consequently, application of soil solarization combined with the used fungicides reduced garlic yield losses to about 7 % in treated fields, also with onion crop. Soil solarization combined with tebuconazole gave the best result (17.4 kg/plot). Application of soil solarization technique improved the ability of fungicides to control white rot disease and increase the final crop yield. Thus, the soil solarization technology may be developed to apply in Saharan and sub-Saharan countries like Egypt as a cheap and safe treatment for microbial plant diseases.

Keywords: Sclerotium cepivora; White rot disease; Garlic and Onion; Solarization; Fungicides.

1. Introduction

White rot disease is caused by *Stromatinia cepivora* (Berk.). Wetzel is the most significant disease affecting Allium production world-wide. In Egypt and China, this disease is one of the most important soil borne diseases of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.), mainly in furrow irrigated fields and in those with frequent monoculture of Allium crops or short rotations with other non-host crops [1]. These conditions results in drastic increases of pathogen populations (sclerotia), leading to high disease levels, therefore, considerable yield losses. The pathogen is spread through mycelia and sclerotia movement in the soil and on garlic seed, but not as airborne spores. Only one sclerotium per 10 kg of soil is enough to cause disease, and 10-20 sclerotia will cause upwards of 90% infestation. Generally, these levels of sclerotia in the soil can be reached in 2-4 cropping cycles of alliums grown under favorable conditions [2]. One of the primary reasons for this disease is a sclerotia production and staying in the soil, they can remain viable for up to 40 years [3].

Various control options have been effective in different locations of the world and under a variety of environmental conditions. A management approach which involves multiple strategies will likely be most effective. In Mediterranean climates, solarization technology has proven the most effective control for white rot [4]. Solarization technique is an economical and environment-friendly technique alternative to soil disinfection methyl bromide that was prohibited for using since 2005. It is conducted by covering irrigated soil with clear sheet of polyethylene through hot summer season to reach high soil temperature for disinfection of plant microbial pathogens [5]. The heated soil may make a great effect on soil pathogenic microorganisms. This technique caused significant

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reduction in recovery and viability of sclerotia at the topsoil [6]. Also, chemical protection of Allium plants from white rot infection can be achieved by sequential applications of different fungicides like vinclozolin, iprodione, procymidone and tebuconazole [7,8]. The modern agriculture technology leads to decrease the chemical fungicides application and turn to natural, biological and environment-safe methods for plant pathogens controlling and improve the quality of important crops [9-11]. Field application of anti-fungal agents in the soil opens the possibility of white rot disease control without using the chemicals and usually provides an environmentally control measure [12-14]. The combination effects of the soil solarization and chemical fungicides technology to control the white rot disease and yield quality haven't been attempted under Egyptian conditions. Therefore, the objective of this work was concentrated to evaluate the effectiveness of soil solarization either individually or in combination with chemical fungicides toward white rot disease in onion and garlic.

2. Materials and Methods

2.1. Fungal strains

Tow virulent pathogenic isolates (*S. cepivora* Sc2 isolated from infected onion plants and *S. cepivora* Sc8 isolated from infected garlic plants) were isolated, identified and studied in previous studies [15,16].

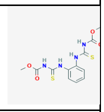
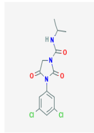
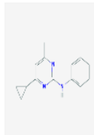
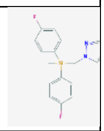
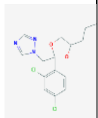
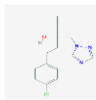
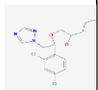
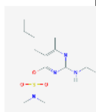
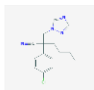
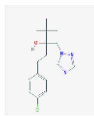
2.2. Fungicides

Ten fungicides *i.e.* tebuconazole (Folcure), Only one, Swetch, Chebco, Tomfix, Crown, Systhane, Actamyle, Tilt and Nimrod were obtained from the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt. All details about the tested fungicides were tabulated (**Table 1**).

2.3. Evaluation of different fungicides concentrations on the linear growth and number of sclerotia under in vitro condition

Ten fungicides with different concentrations were tested. Only one 40%, Tomfix 25%, Crown 25%, Systhane 24%, Tilt and Nimrod were applied at concentrations of 0.25, 0.5, 1.0, 2.0 and 3.0 ppm, in addition to Chipco 50%, Actamyl M70, Switch 62.5% and Folicur 25% were used by 10, 20, 30, 40 and 50 ppm concentrations. These commercial fungicides were evaluated for their capability to suppress S. cepivora growth. Certain volumes of each fungicide were added to PDA medium to obtain the proposed concentrations. Treated or untreated (control) medium were poured into 5 Petri dishes per each treatment.

Table 1. The details information related to the tested commercial fungicides

Commercial name	(Names and Identifiers)	Active ingredient	Molecular Formula	Chemical Structure
Actamyl 70%	Dimethyl 4,4 – (o-phenylene)bis (3-thioallophanate	Thioallophanate methyl	$C_{12}H_{14}N_4O_4S_2$	
Chipco 50%	3-(3,5-ichlorophenyl)-N-isoprpyl1-2,4-dioxoimidazolidine-1-carboxamide	Iprodione	$C_{13}H_{13}Cl_2N_3O_3$	
Switch 62.5 %	a- 4-cycloproply-6-methyl-N-phenylpyrim-idin-2-aminie. b- 4-(2,2-difluoro-1,3-benzodioxol-4-ylpyrrole-3-carbonitrile	a- Cyprodinil 37.5 % b- Fludioxonil 25 %	$C_{14}H_{15}N_3$	
Only one 40 %	Bis(4-fluorophenyl)(methyl)1H-1,2,4. Triazol-1-y1methyl)silane.	Flusilazole 40 %	$C_{16}H_{15}F_2N_3Si$	
Crown 25%	2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	Propiconazole 25 %	$C_{15}H_{17}Cl_2N_3O_2$	
Tomfix 25%	(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	Tebuconazole 25%	$C_{16}H_{22}ClN_3O$	
Tilt	2)-2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole propiconazole)	Propiconazole 25%	$C_{15}H_{17}Cl_2N_3O_2$	
Nimrod	5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulfamate	Bupirimate	$C_{13}H_{24}N_4O_3S$	
Systhane 24%	(4-chlorophenyl)-2-(1,2,4-triazol-1-ylmethyl) hexanenitrile	Myclobutanil %24	$C_{15}H_{17}ClN_4$	
Folicur 25%	(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol	Tebuconazole25%	$C_{16}H_{22}ClN_3O$	

After medium solidification, Petri dishes were inoculated with 5 mm discs of *S. cepivora*, 7 days old culture and then incubated at 20±1ºC for 5 days. Linear growth of *S. cepivora* was measured daily until to control plates are completely filled. The inhibition percent for mycelial growth of *S. cepivora* was calculated using the formula suggested by Elshahawy et al. [17] as follows:

$$Reduction\ \% = \frac{C - T}{C} \times 100$$

Where C = maximum linear growth of *S. cepivora* in control; T = maximum linear growth of *S. cepivora* treatment.

2.4. Assessment of sclerotia number

The sclerotia of *S. cepivora* were treated by fungicides, and then inoculated onto PDA plates to determine the number of viable fungal colony. *S. cepivora* isolate was grown on PDA medium and incubated at 20±1 ^{0}C for 14 days. Sclerotia numbers were counted and calculated as number of sclerotia in petri-dish according to Darwesh and Elshahawy [16].

2.5. Soil solarization

Field experiments (containing a natural infestation level of *S. cepivora* at 0.6 sclerotia g^{-1} of soil) were conducted during the summers of 2019 and 2020 in separate adjacent plots on a clay soil located at the El-Qalubia governorate in Egypt. Chemical fumigants have never been applied to soil in this experimental field. In each year, the experimental field was subdivided into six blocks each measuring 6.0 × 39.0 m with 1.0 m margins in between. Each block was divided lengthwise into two plots and solarization was carried out on one randomly chosen plot per block. Soil of plots to be solarized was thoroughly rotovated and irrigated to reach field capacity in the upper 30–40 cm layer/day before being covered with a 200 µm-thick transparent polyethylene sheet. The edges of the plastic sheet were buried in order to reduce evaporation and preserve the heat. Un-solarized soil of control plots was thoroughly rotovated and irrigated to reach field capacity. The solarization treatments were maintained for three weeks (from 1-August to 21-August).

2.6. Fungicides application

Five systemic fungicides *i.e.*, tebuconazole (Folcure) 1 mL/L, (Only one) 1 mL/L, (Swetch) 3g/L, (Chebco) 3g/L and (Actamyle) 3g/L, were applied. Treatments in field were dipping of garlic cloves and onion transplants in each fungicide for 5 min plus two times spraying stem bases of each crop with the same concentration of each fungicide at 6-weeks intervals from the date of sowing.

2.7. Planting after solarization

Field experiments were carried out during two successive season 2019/2020 and 2020/2021. To determine the effect of the treatments on white rot incidence, in 15-sepetember, plots were sown (at spacing 10 × 10 cm) with surface sterilized garlic bulb-seed cv. Chinase, which is highly susceptible to *S. cepivora*. Planting of onion transplants cultivar Giza red (at spacing 10 × 10 cm) was carried out on 1 December. Mineral fertilization, hand weeding, and irrigation (by furrows) were conducted according to standard practices. Depending on several sequential observations, the percentages of plant death due to white rot were made from planting until harvest. Bulbs of garlic or onion plants from each plot were harvested and weighted (kg/plot) for yield assessment. The following treatments were applied each season: (1): Un-treated plots (control), (2): tebuconazole, (3): Only one, (4): Swetch, (5): Chebco, (6): Actamyle, (7): Solarization only, (8): solarization +tebuconazole, (9): solarization + Only one, (10): solarization +Swetch, (11): solarization + Chebco, (12): solarization +Actamyle.

2.8. Statistical analysis

Prior to statistical analyses, data were checked for normality and homogeneity of variances. Percentages data were transformed to improve homogeneity of variances as arcsine square root transformation; however, untransformed data are presented. Data were first analyzed by analysis of variance (ANOVA) to test for possible interaction among the main effects, followed by the appropriate mean separation analysis using Duncan's multiple range test at $P < 0.05$. All analyses were performed with SPSS software version 14.0.

3. Results and Discussion

3.1. Evaluation the effect of some fungicides on growth of sclerotia at in vitro conditions

The present work aims to decrease application of chemical fungicides with highly controlling of white rot disease pathogenicity. Firstly, we targeted to find the lowest active concentration of the used commercial fungicides against *S. cepivora* sclerotia. To investigate this aim, ten commercial fungicides with different concentrations were tested for their inhibitory effect on linear growth and number of *S. cepivora* sclerotia. These fungicides are considered the main base of commercial fungicides in in Egyptian and Chinese market. The obtained results indicated that all fungicides had significantly reducing the linear growth of *S. cepivora*. Complete inhibition of linear growth was obtained by Chipco 50 %, Actamyl M70 and Folicur 25 % at concentration of 5.0 ppm, Switch 62.5 % (10.0 ppm) and Only one 40 % at conc. of 20.0 ppm, while the other fungicides gave the same result at higher concentrations (**Table 2&3**), then consequently the sclerotia not observed. Fungicides have been conventionally used for the soil at planting of crops, fertilizer treatments, post-planting as soil surface sprayers [18]. Unfortunately, the chemical fungicides have a lot of dangerous materials, thus they need to decrease their applications.

3.2. Effect of soil solarization combined with fungicides on final incidence of garlic and onion dead plants

The combination technology is noted as the one of modern agriculture branches, because it is complemented from benefits of two methods and decreased their disadvantages at the same time [19,20]. So, in the current study, we try to apply the combination technology between soil solarization and active fungicides for controlling the most harmful pathogenic fungi (*S. cepivora*). White rot disease on garlic plants as a main agricultural problem was studied during 2019& 2020 and how we can control it by using some control measures individually or combined with soil solarization technique. In case of un-solarized soil, the disease caused garlic yield losses up to 81% in the growing locations for control region and up to 78 % for the tested systemic fungicides. Garlic cloves were dipped for 5 min in individual treatments of systemic fungicides *i.e.*, tebuconazole at 1 ml/L, (Only one) at 1 mL/L, (Swetch) at 3g/L, (Shebico) at 3g/L and (Actamyle) at 3g/L, before planting. After planting, plant bases were treated with the same treatment three times intervals at 30, 60 and 90 days after planting. Solarization treatment was carried out by covering the irrigated soil with a 200 μm transparent plastic sheet for three weeks during the summer months. Values are means of three replications. Bars with the same letters within each growing season indicate that the means ± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsin $(\%)^{1/2}$ for analyses of variance, however untransformed data are presented. Application of soil solarization combined with the same applied fungicides reduced garlic yield losses to about 7 % in treated fields (**Fig. 1**). The most effective fungicide was tebuconazole (17.7 %). In general, rates of dead plants were greatly reduced in solarized plots. Soil solarization is an eco-friendly method for soil-disinfestation by its solar heating and it is suitable with organic and integrated crop management systems [21]. On reversa of chemical fungicides, soil solarization can will the best option for fungal pathogens control depend on its environmental-friendly properties.

Onion crop is one of the most important vegetables through world food tables, however, it is infected by the most harmful pathogenic fungi (*S. cepivora*). *S. cepivora* produces sclerotia as dangerous form of fungi growth. Thus, it is important to find eco-friendly technology to control this sclerotia and inhibit fungal growth [19]. In the present study, combination technology between soil solarization and fungicides was applied. The five fungicides individually or combined with soil solarization were evaluated *in vivo* against white rot disease on onion plants caused by *S. cepivora*. Onion transplants were dipped for 5 min in individual treatments of systemic fungicides *i.e.*, tebuconazole at 1 ml/L, (Only one) at 1 mL/L, (Swetch) at 3g/L, (Shebico) at 3g/L and (Actamyle) at 3g/L, before planting. After planting, plant bases were treated with the same treatment three times intervals at 30, 60 and 90 days after planting. Solarization treatment was carried out by covering the irrigated soil with a 200 μm transparent plastic sheet for three weeks during the summer months. Values are means of three replications. Bars with the same letters within each growing season indicate that the means ± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsin $(\%)^{1/2}$ for analyses of variance, however untransformed data are presented. The results presented in **Fig. (2)** indicated that with using fungicides individually showed reducing of dead plants comparing with control but with small ratios. While, in case of using solarized soil combined with the fungicides reduced onion yield losses to about 2 % in treated fields. The most effective fungicide was tebuconazole (8.3%). In general, rates of dead plants were greatly reduced in solarized plots.

Table 2. Evaluation of different concentrations of some fungicides on the average of linear growth and number of sclerotia of *Sclerotium cepivorum*

Treatments	Conc. (ppm)	Linear growth (cm^2)	Reduction (%)	No. of Sclerotia/cm^2	Reduction (%)
Only one 40%	10	0.59	99.10	0.00	100.0
	20	0.00	100.0	0.00	100.0
	30	0.00	100.0	0.00	100.0
	40	0.00	100.0	0.00	100.0
	50	0.00	100.0	0.00	100.0
Tomfix 25%	10	0.89	98.59	0.00	100.0
	20	0.68	98.92	0.00	100.0
	30	0.39	99.39	0.00	100.0
	40	0.20	99.69	0.00	100.0
	50	0.00	100.0	0.00	100.0
Crown 25%	10	3.30	94.81	211.33	7.41
	20	2.55	95.99	206.76	9.45
	30	0.85	98.66	0.00	100.0
	40	0.21	99.70	0.00	100.0
	50	0.00	100.0	0.00	100.0
Systhane 24%	10	2.18	96.57	201.0	11.94
	20	2.14	96.64	196.76	13.84
	30	1.22	98.10	0.00	100.0
	40	0.59	99.10	0.00	100.0
	50	0.22	99.65	0.00	100.0
Tilt	10	1.89	97.03	193.76	15.15
	20	0.32	99.50	0.00	100.0
	30	0.00	100.0	0.00	100.0
	40	0.00	100.0	0.00	100.0
	50	0.00	100.0	0.00	100.0
Nimrod	10	7.15	88.76	218.0	4.49
	20	3.14	95.06	215.33	5.66
	30	0.79	98.76	0.00	100.0
	40	0.35	99.45	0.00	100.0
	50	0.00	100.0	0.00	100.0
Control	0.0	63.64	0.00	228.25	0.00

Table 3. Evaluation of different concentrations of some fungicides on the average of linear growth and number of sclerotia of *Sclerotium cepivorum*

Treatments	Conc. (%)	Linear growth (cm^2)	Reduction (%)	No. of Sclerotia/cm^2	Reduction (%)
Chipco 50%	2.5	0.22	99.95	0.00	100.0
	5.0	0.00	100.0	0.00	100.0
	10	0.00	100.0	0.00	100.0
	20	0.00	100.0	0.00	100.0
	30	0.00	100.0	0.00	100.0
Actamyl M70	2.5	0.52	99.17	0.00	100.0
	5.0	0.00	100.0	0.00	100.0
	10	0.00	100.0	0.00	100.0
	20	0.00	100.0	0.00	100.0
	30	0.00	100.0	0.00	100.0
Switch 62.5%	2.5	2.55	96.0	207.76	9.02
	5.0	0.42	99.33	0.00	100.0
	10	0.00	100.0	0.00	100.0
	20	0.00	100.0	0.00	100.0
	30	0.00	100.0	0.00	100.0
Folicur 25%	2.5	1.54	97.58	195.33	14.42
	5.0	0.00	100.0	0.00	100.0
	10	0.00	100.0	0.00	100.0
	20	0.00	100.0	0.00	100.0
	30	0.00	100.0	0.00	100.0
Control	0.0	63.64	0.00	228.25	0.00

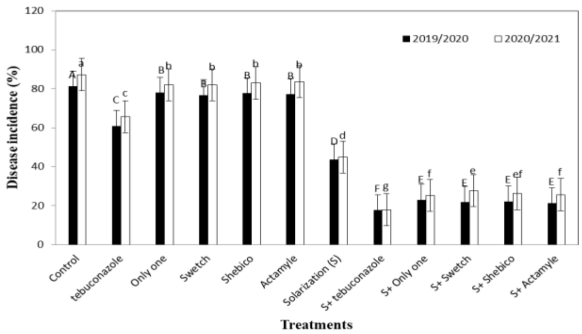


Fig. 1. Effect of soil solarization for 3 weeks combined with other chemical fungicides on final incidence of garlic dead plants due to white rot infection in field naturally infested with *S. cepivora*

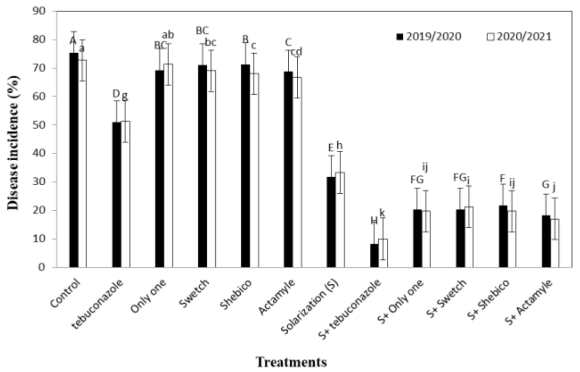


Fig. 2. Effect of soil solarization for three weeks combined with other chemical control measures on final incidence of onion dead plants due to white rot disease in field naturally infested with *S. cepivora*

3.3. Effect of soil solarization for four weeks combined with fungicides on garlic bulbs yield (kg/plot) in field naturally infested with Stromatinia cepivora

S. cepivora is one of the greatest negative diseases causing high loss in garlic and onion crops especially when the soil has been infested. Thus, it is vital to reduce the sclerotia in the soil, resulted to decrease the percentages of fungal biomass and plant disease [22]. Garlic cloves were dipped for 5 min in individual treatments of systemic fungicides i.e., tebuconazole at 1 ml/L, (Only one) at 1 mL/L, (Swetch) at 3g/L, (Shebico) at 3g/L and (Actamyle) at 3g/, before planting. After planting, plant bases were treated with the same treatment three times intervals at 30, 60 and 90 days after planting. Solarization treatment was carried out by covering the irrigated soil with a 200 µm transparent plastic sheet for three weeks during the summer months. Values are means of three replications. Bars with the same letters within each growing season indicate that the means ± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsin $(\%)^{1/2}$ for analyses of variance, however untransformed data are presented. Data in **Fig. (3)** indicated that treatment of garlic cloves and stem base to control white rot disease in field naturally infested with *Stromatinia cepivora* by some fungicides that cultivated in solarized or un-solarized soil, led to increase in Garlic bulbs yield. In the case of un-solarized soil, tebuconazole recorded the most result (11.4 kg/plot) followed by actamyle and only one for the tested fungicides. With respect to solarized soil combined with the same used fungicides, tebuconazole gave the best result (17.4 kg/plot) followed by only one (16.5 kg/plot).

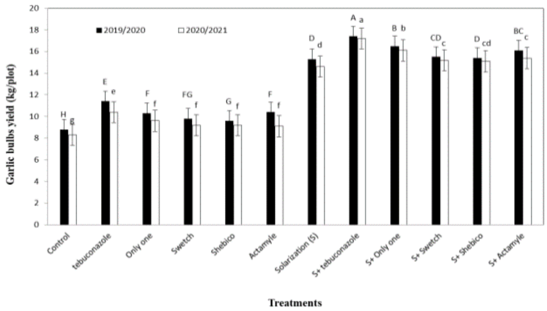


Fig. 3. Effect of soil solarization for three weeks combined with other chemical control measures on garlic bulbs yield (kg/plot) in field naturally infested with *Stromatinia cepivora*

3.4. Effect of soil solarization for four weeks combined with fungicides on onion bulbs yield (kg/plot) in field naturally infested with Stromatinia cepivora.

Onion transplants were dipped for 5 min in individual treatments of systemic fungicides i.e., tebuconazole at 1 ml/L, (Only one) at 1 mL/L, (Swetch) at 3g/L, (Shebico) at 3g/L and (Actamyle) at 3g/, before planting. After planting, plant bases were treated with the same treatment three times intervals at 30, 60 and 90 days after planting. Solarization treatment was carried out by covering the irrigated soil with a 200 μm transparent plastic sheet for three weeks during the summer months. Values are means of three replications. Bars with the same letters within each growing season indicate that the means ± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsin (%)1/2 for analyses of variance, however untransformed data are presented. Data in Fig. (4) showed treatment of onion transplants and stem base by some fungicides with solarized soil technology or un-solarized one to control white rot disease in field naturally infested with Stromatinia cepivora, led to achieve a significant increase in onion bulbs yield. In this regard, tebuconazole treatment in solarized soil was recorded the high result (20.7 kg/polt) followed by only one and actamyle. Also, the data stated that the solarization technology promoted controlling process of the dangerous Stromatinia cepivora. This may return to the effect of solarization on the soil microbes including Stromatinia cepivora especially with the optimum temperature of this fungus (20 ˚C) [16].

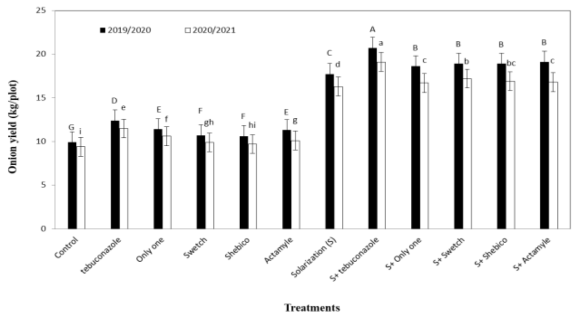


Fig. 4. Effect of soil solarization for four weeks combined with other chemical control measures on onion bulbs yield (kg/plot) in field naturally infested with *S. cepivora*

4. Conclusions

Onion and garlic as most important table vegetables are infected by dangerous pathogenic fungus (*S. cepivora*). To solve this problem, soil solarization technology for 4 weeks was applied to decrease the fungicides application and increase the antifungal activity of these fungicides against *S. cepivora*. Also, it is benefit to inhibit the final incidence of garlic and onion dead plants. Finally, the crops properties were improved with application the combination between soil solarization and fungicide application.

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6. Conflicts of Interest

The authors declare no conflict of interest.

7. References

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