



Biological and Physical Control Measures to Manage White Rot Disease Incidence in Onion and Garlic under Natural Field Conditions

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ABSTRACT

White rot caused by the soil borne fungus *Stromatinia cepivora* (Berk.) Whetzel, is the most destructive disease affecting onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) crops in Egypt and worldwide. Therefore, the present investigation aimed to evaluate the efficacy of soil solarization alone or integrated with bio-control agents, in controlling white rot disease incidence in onion and garlic crops as well as their resulting yield. The data obtained revealed significant reduction and increase in yield for garlic and onion crops when cultivated using treated seeds or transplants with biocontrol agents, followed by planting in solarized soil and subsequent cultivation in solarized soil alone. The garlic bulb-seeds immersed in growth suspension of *P. oxalicum*; *B. pumilus* followed by *B. subtilis* revealed the lowest disease incidence. Meanwhile, high records of disease incidence were recorded in un-solarized soil. Furthermore, the treatment of bio-agents combined with soil solarization resulted in a significant increase in garlic bulb yield compared with plants grown in un-solarized soil. Onion plants showed similar results concerning disease incidence and harvested yield. The current study aims to utilize the hot weather months in Egypt to apply soil mulching technique for controlling serious soilborne plant pathogens. This can be done either alone or in conjunction with biological agents as part of an integrated pest management program.

Keywords: biocontrol agents, garlic, onion, soil mulching, yield, white rot

1. Introduction

White rot caused by the soil borne fungus *Stromatinia cepivora* (Berk.) Whetzel is a significant disease affecting commercial onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) cultivation in Egypt and worldwide (Elshahawy *et al.*, 2018a; Elshahawy *et al.*, 2019). Crop rotation, a method used to manage *S. cepivora*, is ineffective due to the formation of abundant sclerotia that can remain dormant for several years (Crowe *et al.*, 1993). Additionally, host resistance alone is not sufficient for commercially acceptable control (Earnshaw *et al.*, 2000). Soil bio-solarization, a recent alternative physical control technique that combining soil bio-fumigation with soil solarization, has been successfully used to manage soil borne fungi (Domínguez *et al.*, 2014). The solarization process traps solar radiation, causing the soil to heat up to a temperature that is fatal to pests. Bio-solarization involves adding organic additives to the soil before the solarization process, which increases soil temperature by promoting microbial activity (Simmons *et al.*, 2013; Zveibil *et al.*, 2012; Elshahawy *et al.*, 2024; Saied, 2024 and Lessmann and Rudolph, 2026)

Chamorro *et al.* (2015) reported that bio-solarization with fresh chicken manure was a promising and sustainable option for strawberry crops. The term solarization refers to the solar heating of moist soil by covering it with clear plastic sheets during periods of high solar radiation. This technique has been found to significantly reduce the recovery and viability of sclerotia in the topsoil (McLean *et al.*

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2001). Field application of fungal and bacterial bio-control agents to the soil offers the possibility of controlling white rot disease without the use of chemicals, and typically provides an environmentally friendly control measure (Elshahawy *et al.*, 2017 and Castellano-Hinojosa *et al.*, 2022).

The main goal of this study is to evaluate the effectiveness of soil solarization either alone or in combination with bio-control agents, in controlling white rot disease in onion and garlic. Additionally, we will assess the effects of these treatments on crop yields.

2. Materials and Methods

2.1. Field study

Field experiments were conducted during the summer months in separate plots containing clay soil located in the El-Qalubia governorate of Egypt. The soil in the field study was characterized by natural infestation, as referenced previous study by Elshahawy *et al.* (2018b) with a level of *S. cepivora* at a rate of 0.6 sclerotia per gram of soil.

2.2. Origins and production of bio-control agents

Several antagonistic fungal and bacterial bio-control isolates were graciously provided by the Culture Collection Unit, in the Plant Pathology Department National Research Centre, in Egypt. These isolates have demonstrated their high antagonistic capabilities against various soil-borne fungi in previous studies conducted at the Phytopathology Department. The isolates include *Trichoderma koningii*, *T. viride*, *T. virens*, *Penicillium oxalicum* (Po9), *Pseudomonas fluorescens*, *Bacillus subtilis* and *B. pumilus*. They were obtained from an onion field infested with *S. cepivora* in El-Deer village, El-Qalubia governorate, Egypt. Each isolate was cultured on PDA medium for 7 days at 25±1°C, and a growth suspension was then prepared.

2.3. Soil solarization

The experimental field was plowed and leveled at a rate of 12 row/7.0m, then irrigated to its holding field capacity and left to settle. The experimental field was divided into 90 plots each 3.0×3.5m with 0.5m in all sides as margins. Afterward, the half plots (48 plot) were randomly covered using 40μ polyethylene sheets and maintained for a period of 6 weeks from July¹⁵ to August³⁰. During the period of soil mulching, soil temperatures as minimum temp. (at 7am) and maximum temp. (at 4pm) were measured every day at three soil depths of 0-10; 11-20 and 21-30cm using soil thermometer in both solarized and un-solarized plots. All previous procedures were followed at the same field for two successive seasons.

2.4. Planting and application of biocontrol agents after solarization (Bio-solarization)

To assess the impact of the biocontrol treatments in solarized soil against white rot incidence of garlic and onion, highly susceptible varieties of garlic and onion to white rot disease (to *Stromatinia cepivora*) were used in this experiment. After removal the polyethylene sheets, surface sterilized garlic bulb-seed cv. Chinase were cultivated with a spacing of 10cm x 10cm on September 15th. Meanwhile planting of onion transplants cv. Giza red was carried out on December 1st, also with the same used spaces. Both garlic bulb-seed and transplants of onion were immersed in fungal or bacterial spore suspension for 5 minutes before cultivation.

The following treatments were applied in each season:

- | | |
|---|--|
| (1) Un-treated plots (control) | (9) Soil solarization only |
| (2) <i>Trichoderma koningii</i> (cfu 1x01 ⁶) | (10) Soil solarization + <i>Trichoderma koningii</i> (cfu 1x10 ⁶) |
| (3) <i>Trichoderma viride</i> (cfu 1x01 ⁶) | (11) Soil solarization + <i>Trichoderma viride</i> (cfu 1x10 ⁶) |
| (4) <i>Trichoderma virens</i> (cfu 1x10 ⁶) | (12) Soil solarization + <i>Trichoderma virens</i> (cfu 1x10 ⁶) |
| (5) <i>Penicillium oxalicum</i> (cfu 1x10 ⁶) | (13) Soil solarization + <i>Penicillium oxalicum</i> (cfu 1x10 ⁶) |
| (6) <i>Pseudomonas fluorescens</i> (cfu 1x10 ⁸) | (14) Soil solarization + <i>Pseudomonas fluorescens</i> (cfu 1x10 ⁶) |
| (7) <i>Bacillus subtilis</i> (cfu 1x10 ⁸) | (15) Soil solarization + <i>Bacillus subtilis</i> (cfu 1x10 ⁶) |
| (8) <i>Bacillus pumilus</i> (cfu 1x10 ⁸) | (16) Soil solarization + <i>Bacillus pumilus</i> (cfu 1x10 ⁶) |

White rot disease incidence in both garlic and onion was calculated 100 days after transplanting as follows: % disease incidence = (number of infected plants/total number of plants) × 100. Three replicates (plots) for each particular treatment were used. The harvested garlic and onion bulbs for each applied treatments was determined.

2.5. Statistical analysis

Prior to conducting statistical analyses, the data were examined for normality and homogeneity of variances. In order to improve the homogeneity of variances, the percentage data were transformed using the arcsine square root transformation. However, for presentation purposes, the untransformed data are shown. The data were initially analyzed using analysis of variance (ANOVA) to investigate any potential interactions between the main effects. This was followed by conducting the appropriate mean separation analysis using Duncan's multiple range tests at a significant level of $P < 0.05$. All statistical analysis was performed using SPSS software version 14.0.

3. Results

The data presented in Table (1) shows that in solarized soil, the average temperature at the surface layers of 0-10cm, 11-20cm, 21-30cm were recorded as 54°C, 49°C, 44°C and 41°C, 39°C, 37°C as maximum and minimum temp. in solarized soil, in respective order to soil depths. As for soil temp. in un-solarized soil the recorded soil temp. were 40°C, 38°C, 36°C and 33°C, 31°C, 29°C at the three soil depths in relevant respective order. During the second summer season, a similar trend of soil temperatures. was observed, with soil temperatures. in mulched soil being higher than those in un-mulched soil. Additionally, the measured soil temperatures decreased with by increasing soil depths. In current study seven fungal bio-agents were applied in solarized soil to determine their effectiveness against white rot disease incidence caused by *S. cepivora*.

Table 1: Average soil temperatures at three soil depths during six weeks in solarized and un-solarized soli

Soil category	Soil depth (cm)	First summer season		Second summer season	
		Maximum temp. (°C)	Minimum temp. (°C)	Maximum temp. (°C)	Minimum temp. (°C)
Solarized soil	0-10	54	41	50	39
	11-20	49	39	48	37
	21-30	44	37	41	35
Un-solarized soil	0-10	40	33	38	31
	11-20	38	31	36	30
	21-30	36	29	34	28

Figures with the same letter in a column are not significantly different ($P \leq 0.05$)

Table 2. Effect of soil solarization for six weeks combined with bioagents for controlling white rot disease in garlic under natural infested field with *Stromatinia cepivora*

Treatment	Garlic diseased plants (%)			
	1 st season	2 nd season	1 st season	2 nd season
	Un-solarized soil		Solarized soil	
Control	81.3±0.8 a	87.3±0.6 a	43.7±0.8 f	45.0±0.5 e
<i>T. koningii</i>	76.3±0.3 bc	80.0±0.5 bc	12.3±0.3 gh	13.3±0.3 f
<i>T. viride</i>	76.3±0.6 bc	79.0±0.5 cd	11.0±0.5 gh	12.7±0.8 fg
<i>T. virens</i>	77.0±0.5 b	79.0±0.0 cd	11.7±0.3 gh	12.3±0.3 fg
<i>P. oxalicum</i>	69.7±0.3 e	77.3±0.3 d	6.7±0.8 i	8.3±0.3 h
<i>P. fluorescens</i>	76.3±0.3 bc	81.7±0.8 b	13.0±0.0 g	14.0±0.5 f
<i>B. subtilis</i>	74.7±1.8 cd	78.3±0.8 cd	11.7±0.3 gh	13.7±0.3 f
<i>B. pumilus</i>	72.7±0.6 d	79.0±0.5 cd	10.3±1.2 h	11.3±0.6 g

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05$

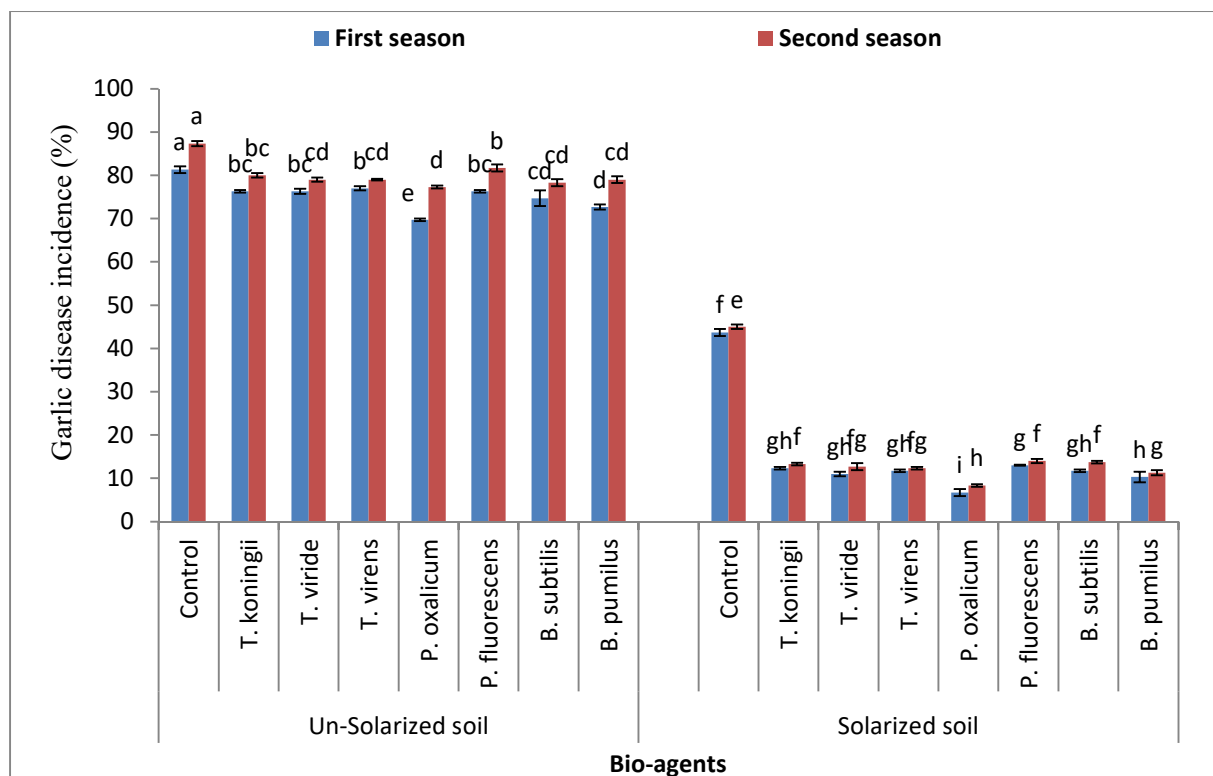


Fig. 1: Effect of soil solarization combined with biocontrol agents on the percentage of white rot infection on garlic plants grown in field naturally infested with *Stromatinia cepivora*.

For garlic plants, the results presented in Table (2) and Fig. (1) showed that using bioagents in un-solarized soil resulted in lower diseased plants compared to the control. The garlic bulb-seeds immersed in growth suspension of *P. oxalicum*; *B. pumilus* followed by *B. subtilis* revealed the lowest disease incidence recorded as 69.7% - 77.3%; 72.7% - 79.0% and 7.7% - 78.3% for the first and second season, respectively. A moderate effect was observed in *T. virens* treatment causing 77.0% - 79.0%, disease incidence throughout the two seasons. Meanwhile, the other tested bioagents revealed rot infection estimated by (76.3%-79.0%), (76.3%-80.0%) and (76.3%-81.7%) for applied *T. viride*; *T. koningii* and *P. fluorescens*, in respectively. High records of disease incidence were recorded as 81.3% and 87.3% in un-solarized soil. Another promising feature against white rot incidence was observed in solarized soil. Lower records of diseased garlic plants were observed in solarized soil with all utilized treatments. Treatment of *P. oxalicum* recorded the lowest white rot incidence at 6.7% and 8.3% in the first and second growing seasons. The bacterial isolates showed disease percentages ranged from 10.3% to 14.0%, meanwhile disease percentages ranging between 11.7% to 13.3% were recorded with the fungal isolates throughout the two growing seasons. In the control treatment, in solarized soil only white rot disease percentages of 43.7% and 45.0% were recorded in the first and second seasons.

Furthermore, the data presented in Table (3) and Fig. (2) indicated that the treatment of bio-agents combined with soil solarization resulted in a significant increase in garlic bulb yield. In the case of un-solarized soil, throughout the two growing seasons, the harvested garlic bulb yield (Kg/plot) ranged from 9.0 -9.9 Kg/plot for all applied bioagents treatment and 8.3-8.8Kg/plot for the control treatment. Meanwhile a significant increase in the harvested yield was recorded in all bio agent's treatments cultivated in solarized soil, with a yield amount ranging between 15.2 and to 20.2Kg/plot compared to 14.6-15.3Kg/plot in only solarized soil throughout the two seasons.

Table 3: Effect of soil solarization for six weeks combined with bioagents on garlic bulbs yield (kg/plot) grown in field naturally infested with *Stromatinia cepivora*.

Treatment	Garlic bulbs yield (kg/plot)			
	1 st season	2 nd season	1 st season	2 nd season
	Un-solarized soil		Solarized soil	
Control	8.8±0.41 h	8.3±0.13 j	15.3±0.33 f	14.6±0.05 g
<i>T. koningii</i>	9.4±0.05 g	8.9±0.05 i	18.5±0.14 b	17.3±0.08 b
<i>T. viride</i>	9.4±0.03 g	8.8±0.06 i	17.5±0.05 d	16.8±0.1 d
<i>T. virens</i>	9.4±0.0 g	8.8±0.06 i	17.4±0.10 d	16.2±0.14 e
<i>P. oxalicum</i>	9.9±0.06 g	9.5±0.17 h	20.2±0.28 a	18.4±0.12 a
<i>P. fluorescens</i>	9.3±0.06 gh	9.1±0.06 i	16.5±0.0 e	15.2±0.08 f
<i>B. subtilis</i>	9.4±0.06 g	9.0±0.11 i	18.2±0.35 bc	17.2±0.11 bc
<i>B. pumilus</i>	9.5±0.03 g	8.9±0.05 i	17.7±0.05 cd	16.9±0.15 cd

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05

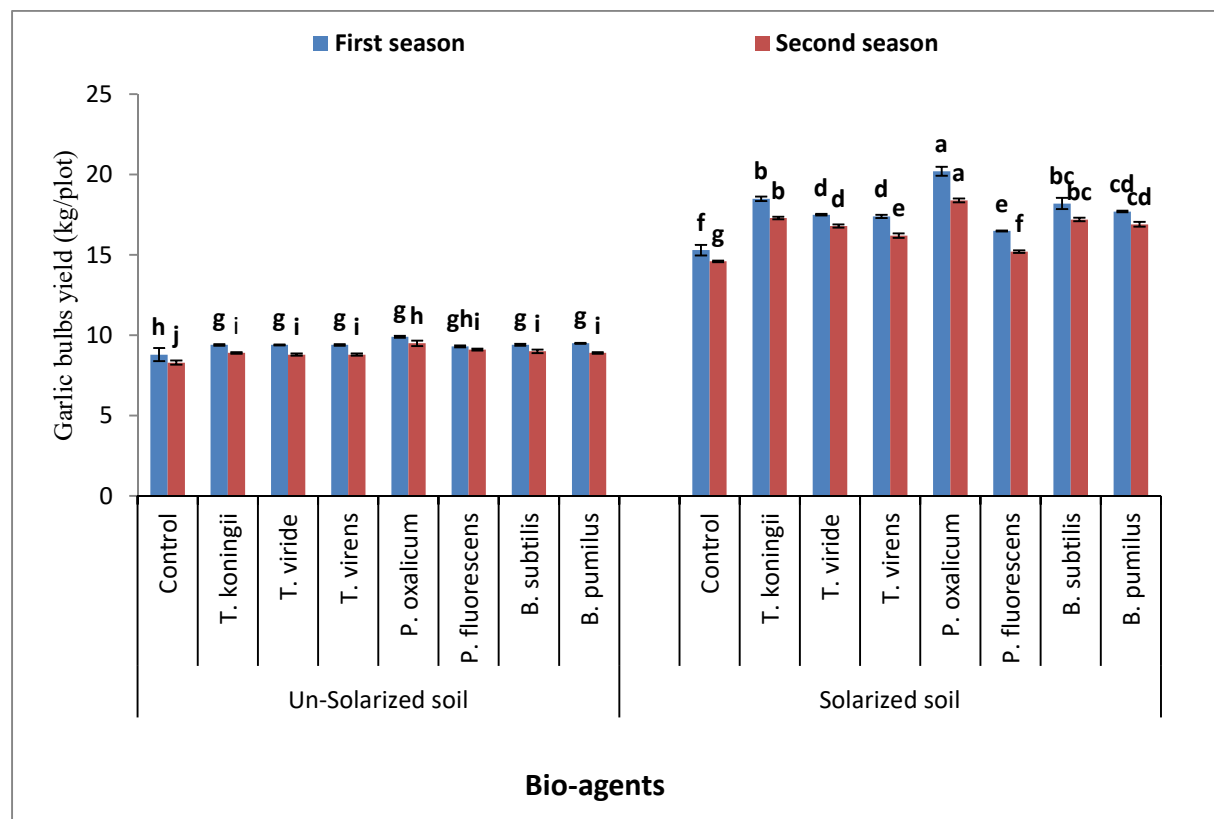


Fig. 2: Yield estimation of garlic plants treated with biocontrol agents and grown in solarized field naturally infested with *Stromatinia cepivora* the causal of white rot

Table 4: Effect of soil solarization for six weeks combined with bioagents on incidence of onion white rot disease in field naturally infested with *Stromatinia cepivora*.

Treatment	Onion disease incidence (%)			
	1 st season	2 nd season	1 st season	2 nd season
	Un-solarized soil		Solarized soil	
Control	75.3±1.4 a	72.7±1.4 a	31.7±0.8 e	33.3±1.6 f
<i>T. koningii</i>	67.3±0.3 c	62.3±0.8 cd	8.0±0.5 fg	7.7±0.8 g
<i>T. viride</i>	67.3±0.3 c	62.3±0.8 cd	8.0±0.5 fg	7.7±0.8 g
<i>T. virens</i>	66.7±0.8 c	64.0±0.5 bcd	7.0±0.5 g	8.3±1.2 g
<i>P. oxalicum</i>	61.0±0.5 d	55.7±2.3 e	2.3±0.8 h	2.0±0.5 h
<i>P. fluorescens</i>	67.0±0.0 c	66.0±1.1 bc	10.0±0.5 f	8.3±0.6 g
<i>B. subtilis</i>	65.3±0.3 c	61.3±0.3 d	7.3±0.3 g	6.0±0.5 g
<i>B. pumilus</i>	66.3±0.3c	63.7±1.6 bcd	6.7±0.3 g	5.7±0.8 g

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05

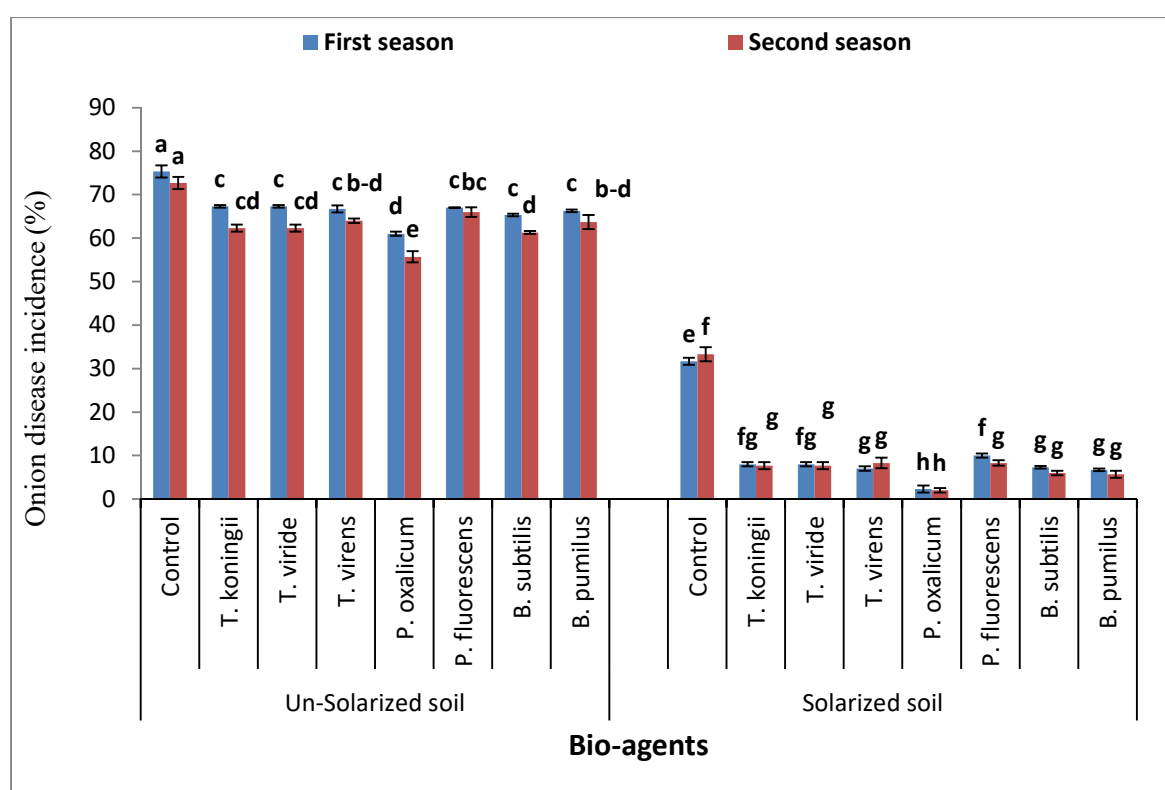


Fig. 3: Efficacy of integration between soil mulching and biocontrol agents on the percentage of white rot infection and yield of onion plants grown in field naturally infested with *Stromatinia cepivora*.

Effect on disease incidence

Regarding onion plants, there was a significant reduction in white rot infection in onion plants grown in bio-solarized or solarized soil treatments compared to plants grown in un-solarized soil whether treated or untreated with bioagents. Data in Table (4) and Fig. (3) revealed that treating onion transplants and the stem base with bio-agents to control white rot disease in a field naturally infested with *S. cepivora* whether cultivated in solarized or un-solarized soil, led to a significant reduction in disease incidence in all solarized treatments. In solarized soil, the bio treatment of *P. oxalicum* showed disease infection by 2.3%-2.0% at the first and second growing seasons, followed by treatments of *B. pumilus*, *T. virens*, *B. subtilis* and further, *T. koningii*, *T. viride* and *P. fluorescens* which they ranged between 5.7% and 10.0% compared 31.7%-33.3% in control treatment throughout the two successive growing seasons. In un-solarized soil, the recorded white rot infection was similar to those of un-solarized soil but to a lesser extent.

Effect on yield

An increase in onion bulb yield was observed in Table (5) and Fig. (4). In un-solarized soil, *P. oxalicum* achieved the highest yield among all tested bio-agents at 11.7 kg/plot followed by the other applied treatment which produced yields of approximately 10.0 kg/plot. However, when combined with soil solarization, *P. oxalicum* produced yield reached 22.6 kg/plot followed by *T. koningii*, *P. fluorescens*, *B. subtilis* and *B. pumilus* 21.4 kg/plot, 21.2 kg/plot, 21.7 kg/plot and 21.8 kg/plot, respectively compared with control treatment which produced a yield of 17.7 kg/plot.

Table 5: Effect of soil solarization for six weeks combined with bioagents on onion bulbs yield (kg/plot) grown in field naturally infested with *Stromatinia cepivora*.

Treatment	Onion bulbs yield (kg/plot)			
	1 st season	2 nd season	1 st season	2 nd season
	Un-solarized soil		Solarized soil	
Control	9.9±0.17 g	9.4±0.06 g	17.7±0.52 d	16.3±0.05 e
<i>T. koningii</i>	10.5±0.03 fg	9.9±0.20 g	21.4±0.1 bc	20.1±0.06 a
<i>T. viride</i>	10.6±0.05 f	9.7±0.11 g	20.9±0.26 c	18.8±0.41 b
<i>T. virens</i>	10.6±0.1f	9.8±0.03 g	20.9±0.03 c	18.2±0.14 c
<i>P. oxalicum</i>	11.7±0.14 e	10.8±0.12 f	22.6±0.08 a	19.6±0.03 a
<i>P. fluorescens</i>	10.3±0.06 fg	9.7±0.06 g	21.2±0.21 c	17.2±0.23 d
<i>B. subtilis</i>	10.4±0.06 fg	9.6±0.08 g	21.7±0.03 b	18.3±0.15 c
<i>B. pumilus</i>	10.5±0.05 fg	9.7±0.05 g	21.8±0.05 b	17.8±0.17 c

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at P < 0.05

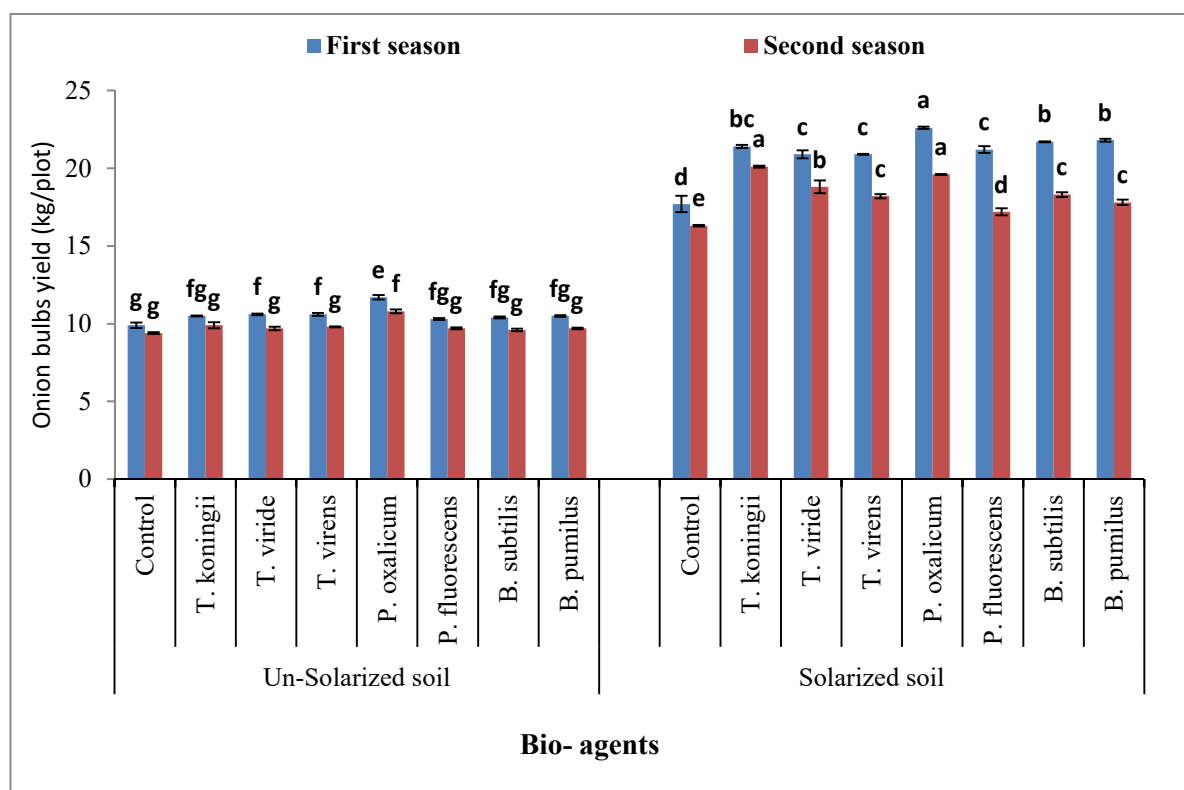


Fig. 4: Percentage of harvested onion bulbs yield in response to plants treated with biocontrol agents and grown in solarized soil

4. Discussion

The basic idea of soil solarization is to heat the soil using solar energy, such as by covering it with transparent polyethylene under the appropriate climatic conditions, this process helps in killing soilborne pests, improving plant health, and ultimately increasing yields. Soil solarization has proven to be effective for various cultivated crops by reducing the infection of soilborne pathogens cause plant diseases as well as increasing the yield, as shown in previous studies (Domínguez *et al.*, 2014; Chamorro *et al.*, 2015). In the current study, soil temperatures during the mulching period increased to 54°C compared to 49°C in un-mulched soil for the surface soil layer (0-10cm). Soil temperatures were measured at three different depths and were higher in mulched soil compared to un-mulched soil. The temperatures in the upper layers of solarized soils can reach 44-50°C, which is responsible for the significant reduction in pathogen populations observed in solarized soils (Katan 1981; Stapleton and DeVay 1986). Several investigations have reported similar findings regarding the use of solar heating to combat soil borne pathogenic microorganisms that cause diseases in various plants. Soil solarization using transparent polyethylene plastic on moist soil during the hot months before planting which typically increases soil temperature to levels lethal to many soil-borne plant pathogens, weeds, and nematodes (Pullman *et al.*, 1981; Stapleton *et al.*, 2000). Katan (1981) previously noted that increasing soil temperatures can reduce the population of soil-borne fungi by 62 to 100% at depths of 5 to 25-cm in solarized soil. Solar heating of soil is a method of soil disinfestation for disease control that aims to eradicate or reduce, prior to planting, using drastic means, the inoculum existing in the soil (Katan 1981). An increase in soil temperatures is the most apparent pronounced effect of solarization. Moreover, soil solarization alone or in combination with biocontrol agents reduced the incidence of white rot of onion and increase its bulbs yield at the same time. The use of biocontrol agent in combination with soil mulching has been found to enhance the efficacy of soil solarization methods (Gamliel and Stapleton, 1995; Mazzola, 2011; Martínez *et al.*, 2011; Domínguez *et al.*, 2014). Solar treatment was successfully reduced disease incidence, increased the percentage of plant stand and subsequently raised the harvested yield (Greco and Brandonisio, 1990). Our study found that the yield of garlic and onion crops grown in bio-solarized soil was significantly higher than those grown in un-biosolarized one. These findings are consistent with those reported by Satour *et al.*, (1989); Melero-Vara *et al.* (2002). Additionally, Domínguez *et al.* (2014), stated that using chicken manure in soil bio-solarization led to an increase in fruit yield of strawberries reaching 70,5 kg/ha. Soil mulching for more than four weeks has been shown to increase plant growth and garlic bulb yield (Katan, 1997).

5. Conclusion

Under the sunny weather conditions of Egypt an, increase in soil temperature was expected using solarization technique. This method is considered a safe and effective physical control method and an alternative to chemical soil pesticides for managing soil borne plant pathogens. The integration of biological and solarized soil resulted in a significant reduction in white rot disease incidence in garlic and onion plants, ultimately leading to an increase in yield. These findings suggest that utilizing soil solarization integrated with biocontrol agents is fully compatible with organic farming production systems, and should be broadly applicable to the highly intensive agro-ecosystems characteristic of several economic crops.

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