Journal of Plant Sciences
2013; 1(3): 43-50
Published online November 20, 2013 (http://www.sciencepublishinggroup.com/j/jps)
doi: 10.11648/j.jps.20130103.15

Effect of combination between bioagents and solarization on management of crown-and stem-rot of Egyptian clover

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To cite this article:
Effat Abdel-Mageed Zaher, KhairyAbdel-Maksoud Abada , Marwa Abedl-Lateef Zyton. Effect of Combination between Bioagents and Solarization on Management of Crown-and Stem-Rot of Egyptian Clover. *Journal of Plant Sciences*. Vol. 1, No. 3, 2013, pp. 43-50.
doi: 10.11648/j.jps.20130103.15

Abstract: Fourteen isolates of *Trichoderma* sp. and eight isolates of *Bacillus* sp., isolated from a field has severe infection by stem and crown-rot of Egyptian clover plants were screened for their efficacy against the fungus *Sclerotinia sclerotiorum* Lib. de Bary, the causal of crown and stem-rot of Egyptian clover, *in vitro* and *in vivo*. In general, *Bacillus* spp. were more efficient in reducing the radial growth of *S.sclerotiorum* than *Trichoderma* spp and the opposite was found in case of sclerotial germination. In addition, the isolates of *B.thuringiensis* and *T.harzianum* showed maximum percentages of radial growth inhibition and sclerotial viability. Meanwhile, isolates of *B. pumilus* and *T.viride* resulted in the lowest percentages of radial growth inhibition and sclerotial viability . The tested bioagents, *i.e. B.thuringiensis*-1 and *T.harzianum*-3 as well as soil solarization resulted in significant reduction to the severity of clover crown and stem-rot with significant increase to the green forage yield compared with control treatment. In addition, *T.harzianum*-3. was more efficient than *B.thuringiensis*-1 and solarization ,when each of them was applied alone. Moreover, the combination among *B.thuringiensis*-1 + *T.harzianum*-3 + solarization was the most efficient in this regard, which no apparent infection by crown and stem-rot was detected and the highest green forage yield was obtained. However, the combination between solarization and any of the tested bioagents was of intermediate effect in this regard.

Keywords: Egyptian clover, *Bacillus* sp., Biological control, *Sclerotinia Sclerotiorum*, *Trichoderma* sp. and Viability

1. Introduction

Egyptian clover (*Trifolium alexandarenium* L.) is the main legume crop grown in winter for green and dry forage of high nutrient values for live stocks in Egypt. It is valued for its nitrogen fixation capacity, benefits in organic farming, high quality forage, and beneficial effects on the soil structure. It is liable to infection by many fungal diseases. However, crown and stem-rots are the most constrain diseases, especially that caused by *Sclerotnia* spp. Clover rot (clover cancer or Sclerotinia crown and stem-rot), caused by the ascomycete fungi *Sclerotinia sclerotiorum* Lib. de Bary and / or *Sclerotinia trifoliorum* Eriks., is an important disease all over the world including Egypt (Carroll, 1964 and Dixon and Doodson, 1974 and Gilbert, 1987 and Markovich and Kononova, 2003).

In late autumn, brown leaf spots appear then diseased leaves fall. The disease spreads to the crown and stems causing crown and stem-rot, where cold weather with rainfall are prevail and favor to aggressiveness the disease and the new growth wilts, dies, and may be covered with fungus growth. Hard, black, fungus bodies (sclerotia) are produced on diseased tissue during end of winter. Diseased plants initially may be in patches within the field (Gilbert, 1987). In addition, animals did not eat or recovery the rotted clover plants, due to the acidity of the infected plants due to secretion of oxalic acid by the causal fungus and / or the odor of pectin and lactic materials (Dixon and Doodson, 1974 and Markovich and Kononova, 2003)).

Due to clover is consume mainly as green forage, therefore, all diseases management rather than chemical control must be done. In this regard, biological control has emerged as an alternative and most promising means of the management of plant pathogens. Biological control of *S. sclerotioum* can be achieved by either promoting the native antagonists to reach a density sufficient to suppress pathogen(s) or by introducing alien antagonists. Among the several antagonists tested by various scientists, species of

Bacillus and *Trichoderma*….etc., have been found effective in inhibiting the causal fungus (Baker,1987 and Jacobsen, *et al*., 2004). Though introduction of several antagonists against this pathogen seems to hold great promise to suppress the disease and have been found effective in inhibiting the growth of the fungus and sclerotial germination under *in vitro* conditions.

The present investigation aimed to investigate the role of soil solarization in combination with *B. thuringiensis*. and *T.harzianum* as bioagents in management of crown and stem-rot of Egyptian clover.

2. Materials and Methods

2.1. Isolation, Purification and Identification of the Associated Fungi to Crown and Stem-Rot

Egyptian clover plants (Meskawy cv.) showing characteristic symptoms of crown and stem-rot were collected from five governorates, *i.e.* Dakahlia, Sharkia, Menofia , Kalubia and Giza. The infected stem and crown samples were thoroughly washed in running tap water and cut into small pieces with lesion having half healthy and half diseased tissue. The pieces were surface sterilized with 2 % sodium hypochlorite for two minutes. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then the pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at 18 ± 2°C and observed periodically for growth of the fungi. The pure cultures of the isolated fungi were obtained by hyphal tip method and/ or single spore technique and maintained on PDA slants throughout the investigation. The emerged fungi were identified on the basis of cultural, morphological characteristics and the description of Gilman (1957) ; Kohn (1979) and Jellis, *et al.*(1990).

2.2. Pathogenicity Test of S. Sclerotiorum and S.Trifoliorum

Thirty seeds (Meskawy cv.) were sown in plastic pots (25 cm. in diameter) contained disinfested clay soil. The plants were left to grow for 40 days under green house conditions (Fac. Agric., Cairo Univ.), which plastic containers were filled with water to arise the relative humidity (responsible for sever infection), during December of 2009 and January of 2010. Disks taken from the periphery of 5 days old of any of *S. sclerotiorum* and *S.trifoliorum* were stick by glue on the basal part of the stems and covered with transparent plastic bags for 48 hours. The plants were examined for the severity of infection by the two fungi 10 days after inoculation as mentioned under disease assessment. Plants inocutated with disks of PDA medium (free from the pathogen) were served as control. The plots were irrigated when it was necessary and fertilized with recommended doses as recommended by Min. of Agric. and Land Reclamation.

2.3. Isolation, Purification and Identification of the Antagonists

Soil samples collected from a field has severe infection by stem and crown-rot of clover plants, were used to isolate the antagonists. Serial dilution plate technique (Johnson *et al.*, 1959) was used to isolate native antagonistic *Trichoderma* spp. (Table,2) on PDA medium and *Bacillus* spp. (Table,1) on nutrient agar medium (Oedjijono and Dragar, 1993).

2.4. Effect of the Culture Filtrate on the Radial Growth and Sclerotial Viability

The effect of culture filtrate of the fourteen isolates of *Trichoderma* sp. and on the growth of *S. sclerotiorum* was studied as a method given by Dennis and Webster (1971). One hundred ml. of potato dextrose broth were put in each 250 ml flask and sterilized by steamer for three successive days. The medium was inoculated with a 5 mm. mycelial disc of any of the *Trichoderma* spp. bioagent(s) cut from the edge of four day old culture. Inoculated flasks were incubated at 28 ± 2°C on a rotary shaker at 200 rpm for 5 days. The culture filtrate was filtered through Whatman No.1 filter paper and the filtrate was collected in a flask. The culture filtrate of the bioagents was mixed with the component of PDA medium in different proportion (25, 50, 75 and 100%). The medium was then sterilized and poured into the Petri-dishes (20 ml /plate).

The effect of culture filtrate of the eight isolates of *Bacillus* spp. on the growth of *S. sclerotiorum* was also studied as mentioned before. One hundred ml. of nutrient medium were put in each 250 ml flask and sterilized by steamer for three successive days. The medium was inoculated with a loop of the bioagent(s) taken from two day-old culture. Inoculated flasks were incubated on a rotary shaker at 200 rpm for 2 days at 28 ± 2°C for 48 hr. The culture filtrate was filtered through Whatman No.1 filter paper and the filtrate was collected in a flask. The culture filtrate of the bioagents was mixed with the component of PDA medium in different proportion (25, 50 and 75%). The medium was then sterilized and poured into the Petri-dishes (20 ml/plate).

After solidification the Petri plates were carefully inoculated with 5 mm. discs of the test pathogen cut from the five days old culture. PDA plates inoculated with the test pathogen, but not amended with culture filtrate were maintained as control. Plates were then incubated in an incubator at 18±2°C. Four replications were maintained for each treatment. Periodic observations on radial growth of mycelium were recorded. Inhibition percentage of mycelial growth of the test pathogen was calculated by the formula:

$$I = (C- T)/C \text{ X}100$$

Where; I = Percent of inhibition in growth of test pathogen, C = Radial growth of pathogen (mm) in control, T = Radial growth of pathogen (mm) in treatment.

To assess the efficacy on sclerotial viability, sclerotia of *S.sclerotiorum* were placed on the surface of PDA plate,

which was put on the mycelium and spores of a 4 day-old culture of *Trichoderma* spp. as well as the 2 day-old slime growth of *Bacillus* spp.. Twenty sclerotia were kept in each Petri-dish and shack well then sealed with parafilm . Five dishs were carried out for each treatment. The dishes were incubated at 28±2° C in the darkness for up to 30 days. Untreated sclerotia (put on PDA medium) were served as the control. The viability of the sclerotia was estimated by placing them on water- agar medium for 48 hrs at 18±°C and the germination was recorded.

2.5. Effect of Combination between Solarization and some Bioagents on Management of Crown and Stem-Rot

In the present investigation, seeds of the Egyptian clover (Meskawy cv.) were taken from Agronomy Det., Fac. Agric., Cairo Univ. The pathogen was isolated from the stems and crowns by tissue segment method on PDA medium. The antagonistic *Bacillus* sp. and *Trichoderma* sp. were screened against the test pathogen, *S.sclerotiorum in vitro*. The most antagonistic isolated were selected to test their efficiency in management of crown and stem-rot in combination with soil solarization.

The upper 10 cm, layer of each plots (1 m^2), located in the experiment unit of Plant Pathol. Dept., Fac., Cairo Univ., was infested with 5 % inoculum level (grown on sterilized corn-sand medium in 500 ml. glass bottles) of the tested pathogen. The plots, were divided into the following treatments:

1 Three infested plots with *S.sclrotiorum* were solarized only.
2 Three infested plots with *S.sclrotiorum* were infested with the bioagent *B.thuringiensis*-1 at the rate of 5 L. /plot (1x0^6 cfu/ ml.water) after solirization .
3 Three infested plots with *S.sclrotiorum* were infested with the bioagent *T .harzianum*-3 at the rate of 5 % inoculum level
4 Three infested plots with *S.sclrotiorum* were solarized and infested with the bioagent *B.thuringiensis*-1 at the previous rate after solirization .
5 Three infested plots with *S.sclerotiorum* were solarized and infested with the bioagent *T .harzianum*-3 at the previous rate after solarization.
6 Three infested plots with *S.sclrotiorum* were solarized and infested with both bioagents at the previous rates after solirization.
7 Three infested plots with *S.sclrotiorum* were infested with both bioagents at the previous rates without solarization .
8 Three infested plots with *S.sclrotiorum* without another treatment.

The plots were then irrigated and left for three days and hoeing then covered with proliferated plastic sheets (50 µ thick) during first of August, 2011 for 40 days. The solarized plots were re-hoeing, irrigated and sown with Egyptian clover Meskawy cv. at the rate of 10 g./ plot . The plots were irrigated when it was necessary and fertilized with recommended disease as recommended by Min. of Agric. and Land Reclamation.

2.6. Disease Assessment

A visual assessment key (0-3) developed by Dixon and Doodson (1974) to test the resistance of red clover to Sclerotinia rot was used to assess disease severity (%) as follows:

$$\text{Disease severity \%} = \frac{\Sigma (nxv)}{3\,N} \times 100$$

Where: n = number of infected leaves in each category.
v = numerical values of each category.
N = total number of the infected leaves.

2.7. Statistical Analysis

Data were statistically analyzed using the standard procedures for complete randomize block and split designs as mentioned by Snedecor and Cochran (1967). The averages were compared at 5% level using least significant differences (L.S.D) according to Fisher (1948).

3. Results

3.1. Isolation, Purification and Identification of the Associated Fungi to Crown and Stem-Rot

Isolation trial from the rotted crown ant stems of Egyptian clover plants yielded many fungal isolates. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *Rhizoctonia solani, Sclerotinia sclerotiorum, S.trifoliorum* , *Stemphylium botryosum, Trichoderma* album, *T. harzianum* and *T. veredi*. Both *S. sclerotiorum* and *S.trifoliorum* were chosen, to select the most aggressive one under Egyptian conditions.

3.2. Pathogenicity Test of S. Sclerotiorum and S. Trifoliorum

Pathogenicty test of both *S. sclerotiorum* and *S. trifoliorum* reveal that *S. sclerotiorum* was more aggressive than *S. trifoliorum*, when the plants were inoculated with mycelial disks of the two pathogens on their stems, being 29.4 and 24.5% stem-rot severity and 17.3 and 14.1 % crown-rot severity, respectively. Therefore, *S. sclerotiorum* was chosen to carry out this work.

a *In vitro* screening and comparative effect of the antagonistic compounds of eight *Bacillus* sp. isolates on the radial growth of the tested pathogen:

Data presented in Table (1) reveal that culture filtrate of *Bacillus* spp. resulted in different degrees of reduction to the radial growth of *S.sclerotiorum,* 5 days after incubation at 18±2°C compared with control treatment. In this respect, the fungus failed to grow on the concentration of 75 % culture filtrate. The average linear growth reduction was 46.0 mm. at the concentration of 25 % then decreased to 33.0 mm. at the concentration of 50 %.

Both isolates of *B.thuringiensis* were the most efficient in reducing the radial growth , being 28.4- 29.5 mm. followed by the two isolates of *B. subtilis* ,being 31.4- 32.4 mm. then the two isolates of *B.chitinosporus*, being 36.0 - 35.0 mm. and two the isolates of *B. pumilus* , being 36.3- 36.6 mm., respectively.

a *In vitro* screening and comparative effect of fourteen *Trichoderma* spp. isolates on the radial growth of the tested pathogen :

Results (Table, 2) show that all the tested isolates of *Trichoderma* spp. resulted in different reduction to the radial growth of *S.sclerotiorum,* 5 days after incubation at 18±2°C compared with control treatment. The tested fungus failed to grow on the concentration of 100 % of all the tested *Trichoderma* spp. bioagents. However, isolates of *T. harzianum* were the most efficient in this regard, especially *T.harzianum*-3, being 23.7 mm.. Meanwhile, all isolates of *T.viride* were the lowest efficient in this regard (33.2-33.3 mm.). The other isolates of *T.album* and *T.hamatum* recorded intermediate values of inhibition.

Table 1. *In vitro screening and comparative effect of the antagonistic culture filtrate of eight Bacillus spp. isolates on the radial growth of S. sclerotiorum, 5 days after incubation at 18±2°C .*

Bacillus	**Radial growth (mm) at concentration of (%)**			
isolates	**25**	**50**	**75**	**Mean**
B.chitinosporus-1	43.6	28.4	0.0	36.0
B.chitinosporus-2	42.6	27.4	0.0	35.0
B. pumilus-1	44.0	29.2	0.0	36.6
B. pumilus-I	43.8	28.8	0.0	36.3
B.subtilis-1	39.6	25.2	0.0	32.4
B.subtilis-2	38.8	24.0	0.0	31.4
B.thuringiensis-1	35.2	21.6	0.0	28.4
B.thuringiensis-2	36.4	22.6	0.0	29.5
Control	90.0	90.0	90.0	90.0
Mean	46.0	33.0	10.0	------

L.S.D. at 5 % for:
Bacillus isolates(B)= 2.8, Concentration (C)= 3.2 and B x C= 4.5 .

Table 2. *In vitro screening and comparative effect of fourteen Trichoderma spp. .culture filtrate on the radial growth of S.sclerotiorum, 5 days after incubation at 18±2°C.*

Trichoderma	**Radial growth (mm) at concentration of (%)**				
isolates	**25**	**50**	**75**	**100**	**Mean**
T.album-1	61.4	38.4	23.0	0.0	30.7
T.album-2	60.8	37.2	22.2	0.0	30.1
T.album -3	59.2	38.8	22.0	0.0	30.0
T.hamatum-1	53.4	33.4	17.0	0.0	26.0
T.hamatum-2	52.4	33.8	16.0	0.0	25.6
T.hamatum-3	53.6	34.6	18.0	0.0	26.6
T.harzianum-1	51.8	31.2	14 .6	0.0	24.4
T.harzianum-2	53.4	32.0	16.0	0.0	25.4
T.harzianum-3	50.8	30.8	13.0	0.0	23.7
T.harzianum-4	53.6	32.2	14.0	0.0	25.5
T.viride-1	68.0	40.8	24.0	0.0	33.2
T.viride-2	69.2	39.6	24.0	0.0	33.2
T.virid-3	69.0	39.0	25.0	0.0	33.3
T.viridi-4	69.4	39.2	24.2	0.0	33.2
Control	90.0	90.0	90.0	90.0	90.0
Mean	61.1	39.4	25.9	6.0	----

L.S.D. at 5 % for:
Trichoderma isolates (T) = 3.1, Concentration (C) = 3.7 and T x C = 4.7 .

3.3. In Vitro Screening and Comparative Effect of the Antagonistic Compounds of the Tested Isolates on the Sclerotial Viability

Results shown in Table (3) indicate that all the tested isolates of *Bacillus* spp. and *Trichoderma* spp. resulted in significant reduction to the germinated sclerotia of the pathogenic fungus compared with control treatment. In general, the tested 14 *Trichoderma* spp. isolates were more efficient (more three times) in reducing the viability of *S.sclerotiorum* than the 8 isolates of *Bacillus* spp. The efficacy of the *Bacillus* spp. isolates in reducing the germinated sclerotia ranged between 16.0 % (*B. pumilus*-1) to 25.0 % (*B.thuringiensis*-1). Meanwhile, the efficacy of the *Trichoderma* spp. isolates in reducing the germinated sclerotia ranged between 75.0 % (*T.album*-2 and *T.viride*-3) to 83.0 % (*T.harzianum*-3).

Table 3. *In vitro screening and comparative effect of the antagonistic culture filtrate of some isolates of Bacillus spp. and Trichoderma spp. on the sclerotial viability of S.sclerotiorm, 5 days after incubation at 18±2°C.*

Bioagents	**Sclerotial viability (%)**	**Efficiency (%)**
isolates		
Bacillus spp.		
B.chitinosporus-1	80	20
B.chitinosporus-2	82	18
B. pumilus-1	84	16
B. pumilus-2	83	17
B.subtilis-1	79	21
B.subtilis-2	78	22
B.thuringiensis-1	75	25
B.thuringiensis-2	76	24
Trichoderma spp.		
T.album-1	23	77
T.album-2	25	75
T.album -3	24	76
T.hamatum-1	21	79
T.hamatum-2	20	80
T.hamatum-3	22	78
T.harzianum-1	21	79
T.harzianum-2	19	81
T.harzianum-3	17	83
T.harzianum-4	20	80
T.viride-1	24	76
T.viride-2	23	77
T.virid-3	25	75
T.viridi-4	24	76
Control	100	---
L.S.D. at 5 %.	3.4	----

Table 4. *Effect of combination between soil solarization , B.thuringiensis-1 and T.harzianum-3 on management of crown and root-rot of Egyptian clover (Meskawy cv.), plot experiment.*

Treatments	%, Severity of Crown-rot	Stem-rot	Weight of green forage (kg)/ plot ($1m^2$)
B.thuringiensis-1 (BT)	5.4	9.0	2.8
T.harzianum-3 (TH)	4.1	8.2	3.1
Solarization (S)	5.8	9.3	2.7
BT + TH	2.0	3.8	3.2
BT+S	3.2	5.1	3.1
TH+S	1.2	2.0	3.3
BT+TH+S	0.0	0.0	3.5
Control	16.8	28.3	1.4
L.S.D. at 5 %	2.9	3.2	0.9

3.4. Effect of Combination between Solarization, B. thuringiensis-1 and T.harzianum-3on Management of Crown an d Stem-Rot

Table (4) indicates that the tested bioagents, *i.e. B.thuringiensis*-1 and *T.harzianum*-3 as well as soil solarization resulted in significant reduction to the severity of clover crown and stem-rot with significant increase to the green forage yield compared with control treatment. In addition, *T.harzianum*-3. was more efficient than *B.thuringiensis*-1 and solarization ,when each of them was applied alone , being 4.1 , 5.4 and 5.8 % crown-rot, 8.2, 9.0 and 9.3 % stem-rot and 3.1, 2.8, and 2.7 kg. / plot green forage yield, respectively. However, the combination between solarization and any of the tested bioagents was more efficient in reducing both crown and stem-rot severity and increasing the green forage yield than when each of them was used alone. Moreover, the combination among *B.thuringiensis*-1 + *T.harzianum*-3 + solarization was the most efficient in this regard, which no apparent infection by crown and stem-rot were detected and the highest green forage yield was obtained (3.5 kg. / plot). Control treatment recorded 16.8 and 28.3 % crown and root-rot severity, respectively and 1.4 kg. green forage yield / plot.

4. Discussion

Nowadays, farmers are interested in reducing dependence on chemical inputs, so biological control can be expected to play an important role in Integrated Pest Management (IPM) systems. A model describing the several steps required for a successful IPM has been developed (Mc Spadden and Fravel, 2002).

Isolation trial from the rotted crown ant stems of Egyptian clover plants yielded many fungal isolates. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *Rhizoctonia solani, Sclerotinia sclerotiorum, S.trifoliorum*, *Stemphylium botryosum, Trichoderma* album, *T.harzianum* and *T.viride*. The isolated fungi were previously isolated by Ostazeski (1957) and Carroll (1964) as associated fungi and / or pathogenic ones..

Pathogenicty test of both *S. sclerotiorum* and *S.trifoliorum* revealed that *S. sclerotiorum* was more aggressive than *S.trifoliorum* , when the plants were inoculated with mycelial disks of the two pathogens on their stems. This may be due to the fungus *S.trifoliorum* needs low temperature and high relative humidity than *S.sclerotiorum* to the sever infection.

Culture filtrate of *Bacillus* spp. and *Trichoderma* spp. isolates resulted in different degrees of reduction to the radial growth of *S.scleotiorum,* 5 days after incubation at 18±2°C compared with control treatment. In this respect, the fungus failed to grow on the concentration of 75.0 % culture filtrate of *Bacillus* spp. and 100% of *Trichoderma* spp. Both the two isolates of *B.thuringiensis* were the most efficient in reducing the radial growth of the tested fungus followed by the two isolates of *B. subtilis* then the two isolates of *B.chitinosporus* and the two isolates of *B. pumilus*. Isolates of *T.harzianum* were the most efficient in this regard, especially *T.harzianum*-3. Meanwhile, all isolates of *T.viride* were the lowest efficient in this regard. The other isolates of *T.album* and *T.hamatum* recorded intermediate values of inhibition .The obtained results are accordance with those obtained by Zeng *et.al.*(2011) ; Barakat *et al.*(3013).

The tested isolates of *Bacillus* spp. and *Trichoderma* spp. resulted in significant reduction to the germinated sclerotia of *S.sclerotiorum* compared with control treatment. In general, the tested 14 *Trichoderma* spp. isolates were more efficient, more three times, in reducing the viability of the tested fungus than the 8 isolates of *Bacillus* spp. This may be attributed to the ability of *Trichoderma* spp. to secrete lytic enzymes and penetrate the tissues of the sclerotia with capacity than the isolates of *Bacillus* spp. as well as *Trichoderma* spp. can tolerate the dryness of the exposing time of sclerotia to the *Trichoderma* spp. growth than the growth of *Bacillus* spp.

The lines among competition, hyperparasitism, and antibiosis are generally blurred. Furthermore, some products of lytic enzyme activity may contribute to indirect disease suppression (Raaijmakers, *et al.,*2002).

The two tested bioagents, *i.e. B.thuringiensis*-1 and *T.harzianum*-3 as well as soil solarization resulted in

significant reduction to the severity of clover crown and stem-rot with significant increase to the green forage yield compared with control treatment. In addition, the combination between solarization and any of the tested bioagents was more efficient in reducing both crown and stem-rot severity and increasing the green forage yield than when each of them was used alone. Moreover, the combination among *B.thuringiensis*-1 + *T.harzianum*-3 + solarization was the most efficient in this regard, which no apparent infection by crown and stem-rot were detected and the highest green forage yield was obtained. The high efficiency of the combination between soil solarization and any of *B.thuringiensis*-1. and *T.harzianum*-3 may be greatly due to the drastic effect of solarization on the sclerotia, which make them unable to resist the invasion by the tested bioagents. Similar results were obtained by Vannacci and Materazzi (1988) and Stevens, *et al.* (2003).

The harmful effect of *S. sclerotiorom* comes from acidifies its ambient environment by producing oxalic acid (Rowe, 1993). This production of oxalic acid during plant infection has been implicated as a primary determinant of pathogenicity as in other phytopathogenic fungi (Marciano, *et al*., 1983 and Jeffrey and Dickman,2003). Since *S. sclerotiorum* acidifies the extracellular environment and yet cytosolic pH is assumed to remain relatively stable, any effects of external pH change on intracellular enzyme activities should be transient. These findings suggest that a signaling pathway responsive to external pH conditions regulates the expression of a gene(s) for oxalic acid biosynthesis. Also, oxalic acid synthesis and degradation may be tightly regulated to provide an optimal pH environment for lytic enzyme activities. Numerous pectinolytic, proteolytic, cellulytic, and other hydrolytic enzymes from *S. sclerotiorum* with acidic pH optima have been described (Hancock, 1966; Lumisden, 1976 and Jeffrey and Dickman,2003).

Phillips (1990) mentioned that solarization reduces the population of sclerotia of *S. sclerotiorum* in soil and reduces the ability of the surviving sclerotia to form apothecia. The greatest reductions occur in the top 5 cm layer of soil but significant effects are seen at 10 and 15 cm. depths. These reductions are mainly due to microbial colonization and degradation of sclerotia weakened by the sub-lethal temperatures produced by solarization. A beneficial side effect is significant reduction in the population of weeds in solarized plots. He added that solarization for 30 and 15 days affects recovery and viability of sclerotia in relation to depth of burial in the soil. Cartia and Asero,1994) found that during soil solarization, *S. sclerotiorum* sclerotia were completely killed at 45°C temperature after 3–4 h and at 35–40°C after 10–14 h. High temperature increases the exudate from sclerotia due to the high bacterial population on the cell surface.

Markovich and Kononova (2003) reported that Lytic enzymes of mycoparasitic fungi of the genus *Trichoderma*, capable of suppressing a number of fungal phytopathogens that originate in air or soil. The topics analyzed include (1) regulation of production of chitinases, β-1,3-glucanases, and proteases; (2) molecular and catalytic properties of purified enzymes; and (3) their *in vitro* ability to degrade cell walls and inhibit sclerotial germination in phytopathogenic fungi.

It has been mentioned that phytopathologists have begun to characterize the determinants and pathways of induced resistance stimulated by bioagents and other non-pathogenic microbes(Park,1995 and Bargabus, *et.al*.,2004) . The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes some of which may act directly to lyze invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death. A second phenotype, first referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria. Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.

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