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DIFFERENT SOURCES OF SAPONIN AFFECTING WHITE ROT DISEASE IN ONION (*Allium cepae* L.)

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ABSTRACT

The effect of different sources of saponin, rhizosphere of alfalfa plant, the fungicide folicur and the biocontrol agent *Trichoderma harizianum* (plant-guard) were tested for efficacy on white rot disease of onion cv. Giza-6 *in vitro*, in the greenhouse and in the field. *In vitro*, saponin from seeds of oldman salt bush (*Atriplex nummularia)* was the most effective source to reduce the linear growth of *Sclerotium cepivorum* giving 58.57% efficacy as compared with the non-treated control.

In the greenhouse experiment, lucerne synthetic saponin was as effective as the folicur giving a 66.3% efficacy in reducing the infected plants compared to the non-treated control. *Atriplex nummularia* had a similar effect to that of the non-treated control though it was the most effective source on PDA medium *in vitro*.

In the field experiments, at Malawi Research Station, the synthetic lucerne saponin was the most effective saponin source in reducing the infected plant's by white rot giving a 59.16% efficacy of disease control. Also, the highest yield of onion bulbs (11325 kg/ feddan) was recorded in plots where transplants were treated with the same source of saponin while the lowest (4856.3 kg/ feddan) was from the non-treated control.

At Shandweel Research Station the fungicide folicur was the superior treatment giving an 87.61% efficacy in reducing the percentage of plants infected by white rot. Also, the folicur treatment gave the highest yield (12075 kg/ feddan) of onion bulbs while the lowest (2441.3 kg/ feddan) was from the non-treated control. Among sources of saponin, alfalfa roots was the most effective giving 46.02% efficacy in disease control and higher yield (6247 kg/ feddan). The number of sclerotia per gram soil and their viability were slightly affected.

Key words: *onion, saponin, S. cepivorum, white rot*

1. INTRODUCTION

Onion, *Allium cepae L* ., is an important crop in many countries of the world including Egypt. The total cultivated area of onion in Egypt is estimated by 90,000 feddan (feddan = 4200 m^2) in 1999.

Onion is subject to infection with many diseases that affect its quantity and quality. White rot disease caused by *Sclerotium cepivorum* is the most important and is considered a limiting factor, especially in upper Egypt, for onion cultivation, production and exportation.

The control of white rot disease is achieved by different control strategies using agricultural practices, solarization, chemical and non-chemical compounds (Hanafi 1989 and Ali *et al*., 1994). One of the control strategies is the use of organic compounds produced from specific plants (Omar *et al*., 1996). One of these compounds is saponin that can be obtained from several plant species such as *Medicago sativa* (alfalfa) (Amarasingham *et al*., 1964 and Omar *et al*., 1999). The chemical form of saponin can be steroidal or tri-terpenoid glycosides. The concentration of saponin differs in different plant species, cultivars, parts of the same plant, and the growing season (Horber *et al*., 1974, Oleszek *et al*., 1990 and Abdel-Halim *et al*., 1992). Saponin was proven to have an antagonistic effect to some fungal species including *S. cepivorum* (Omar *et al*., 1996), *Phytophtora cinnamomi* (Zentmyer and Thompson 1967), and *S. rolfsii* (Omar and Aly 1996). In a previous study by Omar *et al*., (1996), saponin was found effective on *S. cepivorum* of onion *in vitro* and greenhouse experiments. This result was an observation for a further study under field conditions. The objective of the present study is to determine the effect of saponin from different sources on the growth of *S. cepivorum in vitro* and the incidence of white rot and yield of onion under greenhouse and field conditions.

2. MATERIALS AND METHODS

The effect of saponin from seeds of oldman salt bush (*Atriplex nummalaria*) coded (AT), seeds of leucaena glauco benth (*Leucaena leucocephala*) coded (AC) and roots of alfalfa (*M. sativa*) collected from the north-western coast of Egypt and the synthetic saponin was tested *in vitro* on the linear growth of *S. cepivorum*. The plant samples containing saponin were dried at 70 °C using a forced air-drying oven. The dried samples were ground in the Thompson Wiely mill. The saponin contents in different samples were determined by the modified method of Shany *et al.*, (1970) and Khamis (1989). Fruit of loofa (*Luffa aegyptica*) coded (LG) was not used *in vitro* due to the difficulty of mixing it with the PDA, but was used in greenhouse and field experiments. Saponin contents were 2% in AT, 2.3% in LG, 2.1% in AC and 2% in roots of alfalfa. The synthetic lucerne saponin was provided by ICN company, USA. The specific amounts of saponin from different sources were added to the specific amounts of potato-dextrose-agar (PDA) medium in conical flasks to create a concentration of 2% of saponin in the medium and autoclaved. A saponin-free PDA was also autoclaved to be used as control treatment. The saponin-amended or -free PDA was cooled to 50 C then poured in sterilized 7-cm-dim. Petri dishes. Four dishes were used as replicates to each saponin source treatment and four saponin-free PDA dishes were used as control. Disks of 0.5 cm were cut from the mycelium of *S. cepivorum*, previously grown on PDA, and placed on the center of each dish and incubated at 20 C. The linear growth of *S. cepivorum* was measured for each treatment after four days when the saponin-free PDA dishes were completely covered by the mycelial growth of *S. cepivorum*.

In the greenhouse, sixty-day-old onion transplants (cv. Giza-6) were dipped in water suspensions of 2% saponin for 30 minutes, plant-guard, 2.5 ml/liter, for same period, or in folicur, 20 ml/liter, for 10 minutes ,as recommended, just before planting. Also, soil from the rhizosphere of alfalfa plants was mixed with soil at 10 gm/ pot. Onion transplants of the control pots were dipped in water in a similar way to that used with other treatments. The fungal propagules were added at 2% (w/w) of soil. Four replicates were used for each treatment and five transplants were inserted in each pot (20-cm-diam.). Percentage of diseased plants was recorded for each treatment after 140 days of planting.

Field experiments were carried out in artificially infested fields at Malawi (1998) and Shandweel (1999) Agricultural Research Stations. Repeating the experiment at Shandweel Research Station in 1999 was due to some difficulties in using the location of Malawi Research Station. Giza-6 onion transplants were dipped in suspensions of saponin from different sources at the concentration of 2% saponin. Also, the fungicide folicur at 20 ml/ liter water and plant-guard were used at the same concentrations and periods of dipping as mentioned for the greenhouse experiment. Soil from rhizosphere of alfalfa plants was drenched in plots at 1kg soil/ plot just before planting. For the check plots, transplants were dipped in water. Four plots, 2 X 3 m each, were used for each treatment by using randomized complete block design. Percentage of the diseased plants and, yield of onion bulbs were recorded at the end of season, after 140 days from planting. Also, soil samples were collected randomly from Shandweel experiment and the number of sclerotia per gram soil and their viability were determined (on PDA medium).

Data from various experiments were subjected to analysis of variance technique through SAS (SAS Institute, Cary, NC 27511) program to determine the least significant difference among means of different treatments.

3. RESULTS

All the used sources of saponin and the fungicide folicur reduced the linear growth of *S. cepivorum* compared to the non-treated control. Folicur was the superior treatment in reducing the linear growth of *S. cepivorum* giving 91.43% reduction of linear growthcompared to that of the non-treated control. Among sources of saponin, *A. nummularia* (AT) was the most effective giving 58.57% while *L. leucocephala* (AC) and the synthetic lucerne saponin were the least effective sources of saponin giving 28.86 and 29.29% reduction, respectively, compared to the linear growth of the non-treated control (Table 1).

Table (1): Effect of different sources of saponin on the linear growth of *S. cepivorum in vitro*.

Treatment	Linear growth(cm) After 4 days	Reduction in linear growth compared to Control (%)
Synthetic lucernes saponin	**4.95**	**29.29**
***Atriplex numalaria* (AT)**	**2.90**	**58.57**
***Leucaena leucocephala* (AC)**	**4.98**	**28.86**
Alfalafa (ground roots)	**4.08**	**41.71**
Folicur	**0.60**	**91.43**
Control (non-treated)	**7.00**	**0**
LSD 0.01	**0.52**	

In the greenhouse, all sources of saponin , folicur and plant-guard reduced the incidence of white rot disease compared to the non-treated control. The synthetic lucerne saponin was as effective as folicur giving 66.3% efficacy in reducing the incidence of white rot and more effective than plant-guard (25% efficacy). Soil from the rhizosphere of alfalfa plants and AC treatments showed reasonable effects giving 50% and 58.38% reduction of white rot, respectively, followed by alfalfa roots and LG treatments giving 16.63% efficacy for each while AT treatment had no effect (0% efficacy) (Table 2).

Data from field experiment at Malawi Research Station indicate that all treatments including the different sources of saponin were effective in reducing the incidence of white rot disease. The lowest percentage (33.6%) of infected plants by white rot and highest efficacy (59.17%) was observed with plants treated with synthetic lucerne saponin compared to 82.3% infected plants in the non-treated control (Table 3). No difference was found between the effect of the synthetic lucerne saponin and that of AT or AC treatment while LG treatment or the treatment of roots of alfalfa had a lower effect in suppressing white rot compared to the other sources of saponin used in this experiment (Table 3). Compared to other treatments, the effect of folicur was similar to those of sources of saponin while the effect plant-guard was lower than that of the synthetic lucerne saponin and similar to other treatments (Table 3).

Table (2):Effect of different sources of saponin on the incidence of white rot disease in greenhouse.

Treatment	Infected plants (%)	Efficacy (%)
Synthetic lucerne saponin	**26.7**	**66.3**
***Atriplex numalaria* (AT)**	**80**	**0**
***Leucaena leucocephala* (AC)**	**33.3**	**58.38**
Alfalfa (ground roots)	**66.7**	**16.63**
Soil (from rhizosphere of alfalfa plants)	**40**	**50**
***Luffa aegyptica* (LG)**	**66.7**	**16.63**
Plant-guard	**60**	**25**
Folicur	**26.7**	**66.3**
Control	**80**	**0**
LSD 0.01	**14.8**	

Regarding the yield of onion bulbs, all treatments gave a higher yield than the non-treated control. The highest yield (11325 kg/ feddan) of onion bulbs was from plants treated with synthetic lucerne saponin while the lowest (8362.5 kg/ feddan) was from plants treated with the ground roots of alfalfa (Table 3). No difference was found between yield of plants treated with folicur (10557.5 kg/ feddan) and that from plants treated with the synthetic leucerne saponin (11325 kg/ feddan). On the other hand, the yield of plants treated with plant-guard (6825 kg/ feddan) was less than those from all treatments except that from the non-treated control.

Table (3). Effect of different sources of saponin on the incidence of white rot and yield of onion bulbs in the field.

Source of saponin or other treatment	Malawi research station			Shandweel research station		
	Infected plants (%)	Efficacy (%)	Yield (kg/ feddan)	Infected plants(%)	Efficacy (%)	Yield (kg/ feddan)
Synthetic lucerne saponin	33.6	59.17	11325	55.5	36.93	5985
Atriplex numalaria (AT)	36.5	55.65	8793.8	78.2	11.14	3123.8
Leucaena leucocephala (AC)	38.9	52.73	9450	62.4	29.09	4215.4
Alfalfa (ground roots)	49.6	39.73	8362.5	47.5	46.02	6247.5
Soil (from rhizosphere of alfalfa plants)	43.3	47.39	9725	74.3	15.57	3071.3
Luffa aegyptica (LG)	46.1	43.99	8925	66.8	24.10	4042.5
Plant-guard	42.2	48.72	6825	57.6	34.55	6063.8
Folicur	41.3	49.82	10557.5	10.9	87.61	12075
Control	82.3	0	4856.3	88	0	2441.3
LSD 0.01	8.45		1426.7	9.8		1209.6

In Shandweel experiment, folicur was the superior treatment in suppressing white rot disease where the percentage of the infected plants was 10.9% compared to 88% in the non-treated control (Table 3). *Atriplex nummularia* (AT) treatment was the least effective saponin source giving 11.14% efficacy in suppressing white rot disease and 78.2% infected plants compared to 46.02% efficacy and 47.5 % infected plants in case of roots of alfalfa treatment (Table 3). The Plant-guard treatment was mostly effective as the synthetic lucerne saponin treatment, less effective than roots of alfalfa treatment, but more effective than *A. nummularia* and soil form the rhizosphere of alfalfa plants treatments (Table 3).

Regarding yield of onion bulbs, most treatments gave higher yield than that from the non-treated control. The highest yield (12075 kg/ feddan) was from transplants treated with folicur while the lowest was from the non-treated control (2441.3 kg/ feddan) or from the alfalfa rhizosphere soil treatment (3071.3 kg/ feddan).

Table (4): Effect of some sources of saponin and other treatments on the numbers and viability of sclerotia of *S. cepivorum* (from Shandweel experiment).

Source of saponin or other treatment	Sclerotia/ gm soil	Viable sclerotia (%)
Synthetic lucerne saponin	3.4	81.9
Atriplex numalaria (AT)	4.13	76.4
Leucaena leucocephala (AC)	3.65	75.9
Alfalfa (ground root)	3.28	79.6
Soil (from rhizosphere of alfalfa plants)	4.05	83.1
Luffa aegyptica (LG)	3.5	73.6
Plant-guard	3.48	79.4
Folicur	2.98	68.8
Control	5.42	81.9
LSD 0.01	0.472	6.36

The numbers of sclerotia were negatively affected by different treatments compared with the non-treated control. The lowest number of sclerotia per gram soil (2.9) was obtained from the folicur treatment while the untreated control gave the highest number (5.42).

Most sources of saponin were similar in their effect on the number of sclerotia (Table 4). Percentage of the viable sclerotia was not affected by most treatments compared with the non-treated control except in the case of folicur (68.8%) or LG (73.6%) (Table 4).

4. DISCUSSION

The antagonistic effect of different sources of saponin to *S. cepivorum* *in vitro* and greenhouse confirms previous reports by Sidhu and Oakenfull (1986), Aly *et al*., (1996) and Omar *et al*., (1996). This indicates the antifungal effect of saponin on *S. cepivorum* regardless to the source of saponin. This antifungal effect of saponin can be attributed to its membraneolytic action where saponin combines with the membrane sterols causing the formation of pores and hence the loss of membrane integrity (Osbum, 1996 and Hostettmann and Marston, 1995). The varied effects of some sources of saponin on *S. cepivorum* could be attributed to the type of saponin, steroid glycoside, steroid alkaloid or tri-terpene glycoside, (Amarasingham *et al*., 1964). Based on that, the source of saponin should be considered for selecting the most effective source of saponin to control the white rot on onion.

Most saponin sources and other treatments were effective on *S. cepivorum* in the *in vitro*, greenhouse and field experiments. These findings support the primary findings by Omar *et al*., (1996) on onion and Aly *et al*., (1996) on cotton that revealed the antifungal effect of saponin on *S. cepivorum* or some other soilborne fungi. The higher yield of onion bulbs obtained from plants treated with different sources of saponin, compared to the non-treated control was associated with suppression of white rot disease. Therefore, this variation in yield can be attributed to the use of saponin. The variation in the effect of the same source of saponin on *S. cepivorum* either *in vitro*, greenhouse or field may be attributed to the ambient conditions or the coexisted microflora in each case. Similarly, variation in the effect of some treatments in Malawi and Shandweel

Research Station may be due to the soil type, inoculum density and/or the ambient conditions at the two locations.

Moreover, the effect of some saponin sources also extended to reduce the numbers of sclerotia and slightly affected their viability. This is in agreement with Bora (1975) who found that the extract of roots of alfalfa entirely prevented the development of microsclerotia of *Verticillium dahliae* on cotton. This result can be useful in limiting the density of sclerotia in soil specially when using saponin as a long-term strategy for controlling white rot disease. The infection by white rot was higher in Shandweel than in Malawi and this may be due to the higher inoculum density, the suitable environmental conditions in the season of 1999 and the successive cultivation of onion in Shandweel more than in Malawi. Also, this could be due to the ability of the isolate of *S. cepivorum* from Shandweel to detoxify the effect of saponin (Wubben *et al.,* 1996).

Compared to other treatments, saponin was found more effective than the biological control formula plant-guard and similar to or less than the fungicide folicur. Based on the previous findings, saponin is effective in controlling white rot disease on onion under greenhouse or field conditions. Further studies are needed to determine the most effective source of saponin, the ideal conditions for its activity, and its formulation to be applicable on a commercial scale.

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تأثير بعض مصادرالسابونين على مرض العفن الأبيض في البصل

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ملخص

تناول هذا البحث دراسة تأثير بعض مصادر السابونين و فعاليتها في مقاومة مرض العفن الأبيض في البصل والذي يسببه فطر أسكليروشيم سيبفورم تحت الظروف المعمليه والصوبه والحقل.

وقد أظهرت النتائج المعمليه أن بيئة البطاطس ديكستروز أجار (PDA) والمحتويه على 2% سابونين من كل مصدر على حده قد قللت من النمو الميسليومى للفطر وأن أكثرهم تأثيرا كان *Atriplex nummularia* كمصدر للسابونين حيث قلل النمو الميسليومىللفطر بنسبة 58% مقارنة بالكنترول غير المعامل.

وقد استخدمت شتلات البصل جيزه 6 عمرها 60 يومــــا فـــي تجـــارب الصوبه و الحقل بعد غمرها في 2% معلق سابونين لكل مصدر على حـــده وتـــم أيضا استخدام التربة المأخوذه من ريزوسفير البرســـيم الحجـــازي كمعاملـــه مستقلة نثرا في الخطوط قبل الزراعة. وبالاضافه إلى ذلك تم غمر الشتلات فـــي مبيد الفوليكور 2% أو المركب الحيوى بلانت جارد 2,5% (يحتوى على معلـــق جراثيم الفطر *Trichoderma harzianum*) لمقارنة تأثير السابونين.

وقد أوضحت نتائج الصوبه أن تأثيرالسابونين المخلق كان مماثلا لتـــأثير مبيد الفولكيور فى تقليل نسبة الأصابه بمرض العفن الأبيض معطيا 67% فاعليه , بينما وجد أن سابونين *Atriplex nummularia* ليس له تـــــأثيرعلى المـــرض بالمقارنه بالكنترول.

وبالنسبه لنتائج الحقل فى محطة بحوث ملوى وجد أن السابونين المخلـــق له التأثير الأقوى فى تقليل الاصابه بمرض العفن الأبيض (33.6%) وينتج أعلـــى محصول (11325 كيلوجرام/فدان). بينما فى محطة بحوث شندويل كـــــان مبيـــد الفوليكور له التأثير الأقوى فى تقليل الاصابه بمرض العفـــن الأبيـــض (10%) ويعطى أعلى انتاجيه (12075 كيلوجرام/فدان). فى حين وجد أيضا أن ســـابونين جذور البرسيم الحجازى له تأثير قوى (نسبة الأصابه 47.5%) وكذلـــك يعطـــى انتاجية (6247 كيلوجرام/فدان).

بالاضافه الى ذلك وجد أن عدد الأجسام الحجريه/جرام تربه كان أقل فى عينات تربه مأخوذة من معاملة الفوليكور وكذلك بعض مصادر السابونين مقارنـــة بالكنترول غير المعامل. فى حين أن حيوية الأجسام الحجريـــه لا تتـــأثر بشـــكل واضح بأى من هذه المعاملات.