

FACTORS AFFECTING EFFICIENCY OF SAPONIN IN CONTROLLING DAMPING-OFF

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ABSTRACT

A greenhouse experiment was conducted to study the effect of different saponin sources on incidence of cotton seedling damping-off caused by *Rhizoctonia solani*, *sclerotium rolfsii*, *Macrophomina phaseolina*, *Fusarium moniliforme*, two isolates of *Fusarium oxysporum* and the mixture of these fungi on cotton cultivar Giza 86. The experiment conducted also on flax cultivar Giza 8 by using the mixture of the tested fungi. The sources of saponin were seeds of *Artiplex nummularia*, seeds of *Leucaena leucocephala*, roots of *Medicago sativa*, and seeds of *Luffa aegyptiaca*. Three concentrations (3, 6, and 9g or ml/kg seeds) of each saponin source were tested. The fungicide Monceren at rate 3g/kg seeds was used to compare the efficiency of different sources of saponin. The saponin sources at different concentrations and Monceren were used as seed dressing. Efficiency of saponin in controlling damping-off disease differed depending on source of saponin, its concentration, the tested fungi, the tested isolate, and the host plant. Cluster analysis was used to study the antifungal patterns of the saponin sources and to compare between the tested fungi in their response patterns to the different sources of saponin.

INTRODUCTION

Cotton seedling damping-off is caused by a complex of seed-borne and soil-inhabiting organisms. The pathogens most commonly involved in the disease complex are *Rhizostoma solani*, *Fusarium* spp, *Macrophomina phaseolina*, and *Sclerotium rolfsii* (El-Samawaty, 1999; Omar, 1999; Aly *et al.*, 1996; and Khashaba, 1972).

The widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian conditions. However, it is becoming increasingly evident that the widespread use is associated with some problems, such as the potential harmful effect on non-target organisms, the development of resistant races of the pathogens, and the possible carcinogenicity.

As a part of the environmentally safe system for plant disease control, natural plant products have been widely used for controlling plant pathogens (Aly *et al.*, 2000; Singh *et al.*, 1990; and Abdel-Rahman *et al.*, 1989).

Saponins, as a natural plant materials, are complex compounds that are composed of a saccharide attached to a steroid or triterpene that occur in a wide array of plants especially the plants belong to leguminosae (Wang, 1970 and Oleszczak *et al.*, 1990)

Saponin possesses antifungal activity against microorganisms. This toxic effect of saponin is attributed to saponogenin medicagenic acid which is considered the biologically active fraction of saponin (Gestelner *et al.*, 1971). The major mechanism of toxicity of saponins to fungi is believed to be due to their membraneolytic action. Saponins complex with membrane sterols

causing the formation of pores and, hence, the loss of membrane integrity (Osborn, 1996; and Hostettman and Marston, 1995).

The antifungal activity of different sources of saponin against a variety of soilborne fungal pathogens has been demonstrated in previous studies (Osman *et al.*, 2003; Abdel-Momen *et al.*, 2000; El-Sayed *et al.*, 2000; Aly *et al.*, 1996; and Omar and Aly, 1996).

The effect of saponins on fungal pathogens is variable depending upon saponin type and derivatives, concentration, plant part, plant age, degree of fungal sensitivity, or other factors (Osman *et al.*, 2003; El-Sayed *et al.*, 2000; Abdel-Ghani *et al.*, 1998; Omar and Aly, 1996; Abdel-Halim *et al.*, 1992; Oleszck *et al.*, 1990; and Leath *et al.*, 1972).

The objective of this study was to determine the effect of different sources of saponin on incidence of cotton seedling damping-off and the effect of host plant and pathogen isolate on the response to different sources of saponin.

MATERIALS AND METHODS

Experiments of the present study were conducted at greenhouse of cotton pathology section, Plant Path. Res. Inst., Agric. Res. Center, Giza

Fungal isolates:

Isolates of *Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tassi) Goid., *Sclerotium rolfsii* Sacc., *Fusarium oxysporum* Schlecht. (two isolates), and *Fusarium moniliforme* Sheld. were isolated from roots of cotton seedlings infected with damping-off disease. Isolation, purification, and identification of these fungi were carried out at Cotton Pathology Lab., Plant Path. Res. Inst., Agric. Res. Center, Giza.

Saponin sources:

Plants containing saponin are shown in Table 1. Saponin-containing seeds of AT, AC, and LG and roots of ALFA were dried at 70°C using a forced air-drying oven. The dried samples were ground in Thompson Wiley mill. Saponin contents in different samples were determined by the modified method of Shany *et al.* (1970) and Khamis (1989). Saponin contents were 2% in AT, 2.1% in AC, 2.3% in LG and 2% in ALFA.

Table 1. Saponin sources used in the present study

Code	Scientific name	English name	Family	Saponin-containing tissue
AT	<i>Atriplex nummularia</i>	Oldman salt bush	Chenopodiaceae	Seeds
AC	<i>Leucaena leucocephala</i>	Horse-tamarind	Fabaceae	Seeds
ALFA	<i>Medicago sativa</i>	Alfalfa	Fabaceae	Roots
LG	<i>Luffa aegyptiaca</i>	Smooth luffa Egyptian	Cucurbitaceae	Seeds

Effect of different sources and concentration of saponin and fungicide Monceren on incidence of cotton seedling damping-off under greenhouse conditions:

Cotton seeds (cultivar Giza 86) were dressed with different sources of saponin at three concentrations (3, 6, and 9g or ml/kg seeds). Cotton

seeds of the same cultivar were dressed with the fungicide Monceren at a rate of 3g/kg seed. Batches of autoclaved soil were placed on greenhouse benches and separately infested with inoculum of each fungus at rates of 0.05, 25, 50, 50 and 50g/kg soil for *R. solani*, *S. rolfsii*, *M. phaseolina*, *F. oxysporum* isolates, and *F. moniliforme*.

In case of fungal mixture, autoclaved soil was infested with mixture of the tested fungi at a concentration of 1%. The mixture consisted of the previously mentioned rates for the tested fungi. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots and these were planted with 10 treated seeds of cotton per pot. The greenhouse temperature during the experiment ranged from 23±4°C to 38.5±3.5°C. Percentage of infected seedlings was recorded 45 days after planting. Untreated seeds planted in infested soil served as autoclaved control.

Effect of different sources and concentrations of saponin and fungicide Monceren on flax-seed rot:

Flax seeds (cultivar Giza 8) were dressed with different sources and concentrations of saponin and also treated with fungicide Monceren as previously mentioned. Flax treated seeds planted in 15-cm-diameter pots containing infested soil with the mixture of tested fungi (20 seeds per pot). The greenhouse temperature during the experiment was ranged from 16±3°C to 21±2.5°C. Percentage of infected or rotted seeds was recorded 35 day after planting.

Statistical analysis of the data:

A randomized complete block design with five replicates was used in all experiments. Least significant difference (LSD) was applied for comparing means. Percentage data were transformed into arc sine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. ANOVA was performed with the MSTAT-C statistical package. Cluster analysis was performed with the software package SPSS 6.0.

RESULTS AND DISCUSSION

Effect of different sources of saponin on pathogenicity of fungi involved in cotton seedling damping-off under greenhouse conditions were shown in tables 2, 3 and 4.

Seedling damping-off of cotton caused by *S. rolfsii* reduced as a result of most saponins application. It is noteworthy that AC saponin was effective in reducing disease only at concentration 6gm, while low or high concentrations were ineffective in reducing disease incidence. Application of LG saponin was effective only on low concentration (3ml). Omar and Aly (1996) found that the use of saponin as seed-dressing at rate as low as 3g/kg seeds significantly decreased cotton damping-off, while none of the higher rates was able to induce significant reduction in damping-off. This finding may explained by the fact that saponin stimulates germination at low concentrations while it reduces it at higher concentration (Nord and Van Atta, 1960). Efficiency of some saponin sources applications were highest than efficiency of Monceren treatment.

Table 2. Effect of different sources of saponin on pathogenicity of *Sclerotium rolfsii* and *Macrophomina phaseolina* involved in cotton (cultivar Giza 86) seedling damping-off under greenhouse conditions.

Source	Rate ^a	Infection					
		<i>Sclerotium rolfsii</i>			<i>Macrophomina phaseolina</i>		
		(%)	Transformed ^b	Efficiency ^c	(%)	Transformed ^b	Efficiency ^c
AT	3g	64	(54.73)	34.15	66	(54.73)	31.71
	6g	72	(58.89)	24.39	70	(57.69)	26.83
	9g	70	(57.69)	26.83	70	(57.04)	26.83
AC	3g	84	(72.47)	----- ^d	54	(47.53)	46.34
	6g	58	(49.67)	41.46	68	(56.06)	29.27
	9g	94	(83.36)	-----	66	(55.03)	31.71
ALFA	3g	68	(56.48)	29.27	62	(52.37)	36.59
	6g	72	(58.24)	24.39	70	(57.69)	26.83
	9g	60	(51.04)	39.02	58	(50.73)	41.46
LG	3ml	54	(47.36)	46.34	48	(43.20)	53.66
	6ml	78	(62.82)	-----	62	(52.25)	36.59
	9ml	82	(73.15)	-----	58	(49.84)	41.46
Monceren	3g	70	(57.51)	26.83	30	(32.66)	75.61
Infested control		92	(75.25)	-----	92	(79.67)	-----
Autoclaved control		10	(14.31)	-----	10	(14.31)	-----
LSD (P < 0.05) =		16.11			16.83		
(P < 0.01) =		20.54			21.46		

^a per kg of seeds.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

^c Efficiency based on the corrected percentage data was calculated according to the following formula: [(Infested control - treatment)/infested control] x 100. { corrected percentage data = treatment - autoclaved control }

^d Efficiency was not calculated due to the lack of significant difference between treatment and the infested control.

All saponin sources application were effective in reduction of damping-off caused by *M. phaseolina*, but the efficiency of all saponin sources were less than efficiency of Monceren. This result was in agreement with results of El-Deeb *et al.* (2001) who mentioned that the fungicidal treatments were more effective than those of the alternative compounds (included synthetic saponin) in reducing root rots in peanut caused by most of tested fungi (*S. rolfsii*, *M. phaseolina*, and *R. solani*).

All saponin sources applications reduced damping-off caused by *F. moniliforme*. Efficiency of AT decreased at high concentration, while the efficiency of the other two concentrations were higher than efficiency of fungicide treatment. In contrast efficiency of Monceren in reducing damping-off caused by *F. oxysporum* was more effective than efficiency of application of all saponin sources.

Table 3. Effect of different sources of saponin on pathogenicity of *Fusarium moniliforme* and *Fusarium oxysporum* involved in cotton (cultivar Giza 86) seedling damping-off under greenhouse conditions.

Source	Rate ^a	Infection					
		<i>F. moniliforme</i>			<i>F. oxysporum</i>		
		(%)	Transformed ^b	Efficiency ^c	(%)	Transformed ^b	Efficiency ^c
AT	3g	20	(26.27)	82.76	22	(27.18)	73.08
	6g	18	(24.64)	86.21	28	(31.32)	61.54
	9g	38	(37.80)	51.72	30	(32.91)	57.69
AC	3g	36	(36.77)	55.17	22	(24.69)	73.08
	6g	40	(38.96)	48.28	24	(28.93)	69.23
	9g	36	(36.42)	55.17	36	(36.60)	46.15
ALFA	3g	38	(37.63)	51.72	36	(36.60)	46.15
	6g	36	(36.60)	55.17	32	(36.64)	53.85
	9g	36	(36.72)	55.17	32	(36.64)	53.85
LG	3ml	32	(34.24)	62.07	40	(39.13)	----- ^d
	6ml	42	(39.64)	44.83	28	(30.91)	61.54
	9ml	30	(32.96)	65.52	28	(28.75)	61.54
Monceren	3g	34	(34.62)	58.62	16	(23.31)	84.62
Infested control		68	(56.13)	-----	60	(50.82)	-----
Autoclaved control		10	(16.37)	-----	8	(12.69)	-----
LSD (P < 0.05) =			11.61			11.91	
(P < 0.01) =			14.8			15.19	

^a per kg of seeds.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

^c Efficiency based on the corrected percentage data was calculated according to the following formula: [(Infested control - treatment)/infested control] x 100. { corrected percentage data = treatment - autoclaved control }

^d Efficiency was not calculated due to the lack of significant difference between treatment and the infested control.

All sources of saponin significantly decreased seedling damping-off caused by *R. solani* compared with infested control except ALFA at concentration of 3gm/kg seeds. Increasing the concentration of ALFA to 6gm or 9gm/kg seeds caused highly significant reduction in disease incidence. This result is not in agreement with the results of Stureville and Skinner (1987) who found that alfalfa plants with low saponin content were more resistant to downy mildew than those with high saponin content. Efficiency of AC saponin at concentration 3gm, AT at concentration 6gm, and ALFA at concentration 6gm were somewhat similar to efficiency of fungicide Monceren. However, saponin may be superior as being natural plant material safe to the plants, animals and human beings, and don't cause pollution to environment.

Table 4. Effect of different sources of saponin on pathogenicity of *Rhizoctonia solani* and a mixture of fungi involved in cotton (cultivar Giza 86) seedling damping-off under greenhouse conditions.

Source	Rate ^a	Infection					
		<i>R. solani</i>			ixture		
		(%)	Transformed ^b	Efficiency ^c	(%)	Transformed ^b	Efficiency ^c
AT	3g	28	(28.33)	76.67	38	(37.68)	78.95
	6g	22	(26.70)	86.67	78	(67.67)	26.32
	9g	32	(33.17)	70.00	76	(66.69)	28.95
AC	3g	20	(26.27)	90.00	82	(65.36)	21.01
	6g	40	(38.36)	56.67	66	(54.93)	42.11
	9g	42	(40.28)	53.33	92	(79.67)	----- ^d
ALFA	3g	50	(48.01)	-----	72	(50.24)	34.21
	6g	22	(25.24)	86.67	82	(68.14)	21.05
	9g	34	(35.39)	66.67	(47.436)	57.89	
LG	3ml	34	(34.13)	66.67	46	(42.59)	68.42
	6ml	50	(39.13)	58.67	62	(52.20)	47.37
	9ml	38	(37.75)	60.00	52	(46.20)	60.53
Monceren	3g	24	(26.77)	83.33	38	(37.33)	70.95
Infested control		74	(59.57)	-----	98	(86.31)	-----
Autoclaved control		14	21.69)	-----	22	(27.60)	-----
HSD (P < 0.05) =		17.42			16.6		
(P < 0.01) =		22.22			21.17		

^a per kg of seeds.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

^c Efficiency based on the corrected percentage data was calculated according to the following formula: [(Infested control - treatment)/infested control] x 100. { corrected percentage data = treatment - autoclaved control m

^d Efficiency was not calculated due to the lack of significant difference between

Our results demonstrate that soilborne fungi were highly specific in their responses to saponin. Application of AC at rate 3g was highly effective against *R. solani*, while it was ineffective against *S. rolfsii*. LG application at rate 3ml was effective against *F. moniliforme* but it was ineffective against *F. oxysporum*.

Cotton seedling damping-off caused by mixture of the tested fungi significantly decreased as a result of application of all saponin treatments except AC at high concentration (9gm). Efficiency of all saponin sources in reducing disease were less than efficiency of fungicide except AT at the

lowest rate (3g) which was similar to efficiency of Monceren. It is noteworthy that application of AC at rate of 9g was effective in reducing damping-off caused by *R. solani* or caused by *F. moniliforme* while it failed to reduce the disease caused by the mixture of the tested fungi. Although the results of the present study demonstrate that saponin could be used as an environmentally safe substitute for fungicides in controlling cotton damping-off, however, this will require more experimentation under field conditions where seedlings are subjected to infection by mixture of fungi.

Effect of fungal isolate on response to different sources of saponin is shown in Table 5. Two isolates of *F. oxysporum* were evaluated as to their response to different sources of saponin. All sources of saponin were effective in reducing damping-off caused by *F. oxysporum* isolate no. 1 except LG at the lowest concentration (3ml).

Table 5. Effect of pathogen isolate on the efficiency of different sources of saponin in controlling damping-off of cotton (cultivar Giza 86) caused by *Fusarium oxysporum*.

Source	Rate ^a	Infection					
		<i>F. oxysporum</i> isolate 1			<i>F. oxysporum</i> isolate 2		
		(%)	Transformed ^b	Efficiency ^c	(%)	Transformed ^b	Efficiency ^c
AT	3g	22	(27.18)	73.08	36	(36.42)	56.25
	6g	28	(31.33)	61.54	54	(47.36)	----- ^d
	9g	30	(32.91)	57.69	40	(38.96)	50.00
AC	3g	22	(24.69)	73.08	52	(46.10)	31.25
	6g	24	(28.93)	69.23	38	(37.80)	53.13
	9g	36	(36.60)	46.15	60	(50.87)	-----
ALFA	3g	36	(36.60)	46.15	46	(42.52)	40.63
	6g	32	(36.64)	53.85	48	(43.85)	37.50
	9g	32	(34.11)	53.85	44	43.75	
LG	3ml	40	(39.13)	-----	34	(34.85)	59/38
	6ml	28	(30.91)	61.54	40	(39.01)	50.00
	9ml	28	(28.75)	61.54	64	6 (53.14)	8
Monceren	3g	16	(23.31)	84.72	46	(42.64)	40.63
Infested control		60	(50.82)	-----	72	(58.24)	-----
Autoclaved control		8	(12.69)	-----	8	-----	

LSD (P < 0.05) =
 11.91 11.91
 (P < 0.01) =
 11.91

11.9
 1

(P < 0.01) = q5.19 15.19

^a per kg of seeds.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

^c Efficiency based on the corrected percentage data was calculated according to the following formula: $\frac{(\text{Infested control} - \text{treatment})}{\text{infested control}} \times 100$.

{ corrected percentage data = treatment - autoclaved control }

^d efficiency (was not calculated due to the lack of significant difference between treatment and the infested control).

Efficiency of the fungicide in reducing the disease was higher than efficiency of all saponin sources. In contrast efficiency of Monceren in reducing disease caused by *F. oxysporum* isolate no. 2 was less than efficiency of most of saponin sources. It is noteworthy that LG saponin at the rate of 3ml was ineffective on isolate no. 1 while it was highly effective on isolate no. 2. When LG saponin used at high rate (9ml) it was effective in reducing damping-off caused by isolate no. 1 while it became ineffective on isolate no.2. These results suggested that isolate no. 1 was sensitive to high concentration of LG saponin, while isolate no. 2 was sensitive to low concentration of LG saponin. Application of AT saponin at the rate of 6g and AC saponin at the rate of 9g were effective on isolate no. 1 while they were ineffective on isolate no. 2. Leath *et al.* (1972) mentioned that microorganisms show highly specific response to saponin. Aly *et al.* (3000) reported that source of saponin varied in the level of its antifungal activity even against the same fungus.

Effect of host on the efficiency of different sources of saponin in controlling damping-off was shown in table 6.

Table 6. Effect of host on the efficiency of different sources of saponin in controlling damping-off caused by a mixture of fungi on cotton (cultivar Giza 86) and flax (cultivar Giza 8)

Source	Rate ^a	Infection					
		Cotton			Flax		
		(%)	Transformed ^b	Efficiency ^c	(%)	Transformed ^b	Efficiency ^c
AT	3g	38	(37.68)	78.95	51	(47.00)	----- ^d
	6g	78	(67.67)	26.32	33	(31.72)	-----
	9g	76	(66.69)	28.95	18	(23.81)	71.15
AC	3g	82	(65.36)	21.05	57	(48.28)	-----
	6g	66	(54.93)	42.11	34	(33.85)	-----
	9g	92	(79.67)	-----	38	(37.38)	-----
LG	3ml	46	(42.59)	68.42	56	(48.98)	-----
	6ml	62	(52.20)	47.37	47	(42.64)	-----
	9ml	52	(46.20)	60.53	49	(44.61)	-----
Monceren	3g	38	(37.33)	78.95	10	(17.86)	86.54
Infested control		98	(86.31)	-----	55	(48.27)	-----
Autoclaved control		22	(27.60)	-----	3	(4.56)	-----

LSD (P < 0.05) =	17.42	23.55
(P < 0.01) =	22.22	30.02

^a per kg of seeds.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

^c Efficiency based on the corrected percentage data was calculated according to the following formula: $[(\text{Infested control} - \text{treatment}) / \text{infested control}] \times 100$.

{ corrected percentage data = treatment - autoclaved control }

^d Efficiency was not calculated due to the lack of significant difference between Treatment and the infested control.

Application of different sources of saponin significantly reduced seedling disease of cotton except AC at rate 9g, while all saponin sources failed to reduce seedling disease of flax except AT saponin at rate of 9g.

Our results indicated that the taxonomy of the host was an important factor in determining the kind of response to saponin sources. El-Sayed *et al.* (2000) reported that the influence of saponins on plant growth depend entirely on saponin source and/or their concentration. The mechanism by which saponin affect plant growth is probably due to the nutritional value of plant extracts containing saponin and/or the antifungal activity of saponin against fungi which, in the absence of saponins may weaken plants. The efficiency of the fungicide was higher than both the efficiency of AT (9g) on flax and the efficiency of all saponin sources on cotton except AT (3g) which showed the same efficiency of the fungicide.

Our results demonstrated that the effect of saponin on plant pathogens was a complex phenomenon affected by the genus of the pathogen, the isolate of the pathogen, the genus of the host, source of saponin, and its concentration.

Cluster analysis (Table 7 and Fig. 1) showed that different sources of saponin differed in their antifungal patterns. High concentrations of AT saponin and LG saponin had different antifungal patterns from the low concentrations. Regarding AC saponin, the medium concentration (6g) had differed antifungal patterns from the high and low concentrations. Different concentrations of ALFA saponin exhibited different behavior regarding their antifungal patterns. Behavior of LG (at rates of 6ml and 9ml) regarding its antifungal patterns was similar to the fungicide Monceren.

Fig1

Table 8. Similarity matrix of fungi involved in cotton seedling damping-off based on their response patterns to different saponin sources

	Fungi	Fungi				
		1	2	3	4	5
1	<i>Rhizoctonia solani</i>					
2	Mixture of fungi	0.642				
3	<i>Sclerotium rolfsii</i>	0.234	0.127			
4	<i>Machrophomina phaseolina</i>	0.524	0.136	1.000		
5	<i>Fusarium moniliforme</i>	0.917	0.238	0.307	0.193	
6	<i>Fusarium oxysporum</i>	0.565	0.000	0.769	0.304	0.564

Fig2

The response patterns of fungi to different sources of saponin and Monceren are shown in table 8 and fig. 2. Both *S. rolfsii* and *M. phaseolina* were similar in their response patterns to different sources of saponin. Response of *F. moniliforme* to different sources of saponin was almost similar to response of *R. solani*. It is not worthy that *F. moniliforme* was more similar to *R. solani* than *F. oxysporum* regarding its response to saponin sources. The mixture of the tested fungi exhibited different response to saponin sources from the individual fungi.

The results of the present study demonstrate that saponin could be used as an environmentally safe substitute for fungicide in controlling cotton seedling damping-off; however, the confirmation of the greenhouse results requires more experimentation under field conditions.

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العوامل المؤثرة على فعالية السابونين في مقاومة موت البادرات

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أجريت تجربة تحت ظروف الصوبه لاختبار تأثير مادة السابونين المستخلصه من مصادر نباتيه مختلفه على اصابة القطن بمرض موت البادرات المتسبب عن كل من ريزوكتونيا سولاني، وسكليروشيوم رولفسياي، ماكروفيومينا فاسيولينا، فيوزاريوم مونيليفورمي، عزلتين من فيوزاريوم أوكسيسبورم، وخليط من هذه الفطريات. أجريت التجربه على صنف القطن جيزه ٨٦. كما أجريت التجربه أيضا على الكتان صنف جيزه ٨ باستخدام خليط الفطريات. تم اختبار السابونين المستخلص من كل من بذور نبات الأتريلكس نيومبولاريا ، وبذور نبات ليوكينا ليوكوسيفالا، وجذور نبات الميديكافو ساتيفا، وبذور نبات اللوفه اجينتيكا. أختبرت ثلاثة تركيزات من كل مصدر من مصادر السابونين هي ٣، ٦، ٩ جم أو مليلتر/كيلو جرام بذره. وقورنت فعالية هذه التركيزات بفعالية المبيد الفطري مونسرين المستخدم بمعدل ٣ جرام/كجم بذره. عوملت البذور بمصادر السابونين المختلفه وبالتركيزات المختلفه وكذلك بالمبيد الفطري. كفاءة السابونين في مقاومة مرض موت البادرات اختلفت باختلاف المصدر النباتي للسابونين والتركيز المستعمل في المعامله والفطر المختبر وعزلة الفطر والعائل النباتي. أظهر التحليل العنقودي أن مصادر السابونين المختلفه اختلفت فيما بينها من حيث نمط التأثير المضاد للفطريات. كما اختلفت الفطريات المختبره فيما بينها من حيث نمط استجابتها لمصادر السابونين المختلفه.

Table 7. Similarity matrix of different sources and concentrations of saponin based on their antifungal patterns

Source and rate of saponin	Source and rate of saponin												
	1	2	3	4	5	6	7	8	9	10	11	12	
1- AT 3g													
2- AT 6g	0.829												
3- AT 9g	0.811	0.971											
4- AC 3g	0.702	0.906	0.963										
5- AC 6g	0.571	0.545	0.751	0.709									
6- AC 9g	0.728	0.953	0.919	0.965	0.521								
7- ALFA 3g	0.244	0.057	0.095	0.168	0.448	0.199							
8- ALFA 6g	0.746	0.968	1.000	0.960	0.685	0.913	0.000						
9- ALFA 9g	0.955	0.925	0.832	0.741	0.391	0.829	0.097	0.815					
10- LG 3g	0.691	0.556	0.471	0.446	0.167	0.471	0.029	0.532	0.766				
11- LG 6g	0.863	0.770	0.826	0.883	0.670	0.848	0.367	0.769	0.792	0.560			
12- LG 9g	0.915	0.785	0.771	0.806	0.506	0.840	0.343	0.718	0.883	0.670	0.985		
13- Monceren	0.719	0.602	0.695	0.817	0.655	0.735	0.420	0.657	0.630	0.566	0.973	0.914	