FUNGAL DISEASES OF ALFALFA IN ISMAILIA GOVERNORATE

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(Manuscript received 15 June 2014)

Abstract

A survey of fungal diseases on alfalfa (cv. Sewa) in Ismailia governorate was carried out during 2011 and 2012. Percentages of infection were determined in five districs. Ismailia district, however, showed the highest percentage of infection by root rot / wilt disease and foliage diseases (0.0-60.0 %). Data indicated that the major fungal diseases affecting alfalfa in Ismailia governorate are damping-off and root-rot caused by *Rhizoctonia solani* and vascular wilt caused by *Fusarium oxysporum* and *Vertcillium albo-atrum*. The most important fungal foliar diseases were black stem (*Phoma medicaginis*) anthracnose (*Colletotrichum trifolii*), leaf spot (*Stemphylium botryosum* and *Alternaria* sp.) , Downy mildew (*Peronospora* sp.) and rust (*Uromyces trifolii*). On the other hand, *R. solani* gave the highest percentage in isolation trials from alfalfa seeds followed by *F. oxysporum*, while *Macrophominia phsolina* was the least isolated . Also, *R. solani* was the most pathogenic fungus to alfalfa seeds in laboratory, followed by *S. sclerotiorum*, while *M. phaseolina* was the least.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) or Lucerne, the oldest and the most important forage crop in the world, is currently cultivated as a nitrogen source and soil-conserving perennial crop in low-imput agricultural systems (Stuteville and Erwin, 1990). In Egypt, it is one of the most promising forage crops in the new reclaimed areas (Mohamed *et al*., 1992). There is a gab between the demand and the consumption of green forages, especially in the summer season where the available forages are limited. Several soil borne fungi are reported as the causal fungal organisms of root-rot, wilt and crown rot diseases, *i.e*. *Rhizoctonia solani* **,** *Fusarium solani*, *Macrophomina phaseolina*, *Selerotium rolfsii*, *Sclerotinia* sp**.,** *Fusarium oxysporum* and *Vertcillium* sp**.** (El-Barougy, 1997and Couture *et al*., 2002)**.** Also, several airborne fungi were recorded as the causal pathogens of leaf spot, downy mildew, rust, black stem and anthracnose diseases, *i.e*. *Alternaria* sp., *Stemphylium botryosum*, *Peronospora trifrumolio, Uromyces striatus* , *Phoma medicaginis* and *Colletotrichum* sp. (Billar 1989, El-Barougy, 1997, Naseri & Marefat, 2008 and Abdel-Monaim *et al*., 2012). *Phoma medicaginis* var. *medicaginis*, *Pseudopeziza medicaginis*, *Peronospora trifoliorum*, *Stemphylium botryosum*, *Colletotrichum trifolii* and *Rhizoctonia solani* were the important diseases occurring in Ankara (Mishrace, 2006).

However, these various diseases usually cause economic losses in plants stand and yield.

In Egypt, mild to acute fluctuation in temperature and humidity started to occur during the last decade, therefore, prevalence, time and duration of the disease symptoms appearance began to change. The present work aimed to study the fungal diseases attacking roots and foliage of alfalfa in Ismailia governorate and their relative importance as will as pathogenic potential with special reference to the associated seed –borne fungi.

MATERIALS AND METHODS

Field survey:

A field survey was carried out in five locations: namely Ismailia, Fayed, El-Tal El-Kabeer, El-Quntra Gharb and El-Quntra Sharq, which represent the most producing areas of the crop. The survey was carried out during 2010/2011and 2011/2012 seasons. The percentages of roots and foliage fungal diseases were determined in twenty five random diseased plants picked diagonally from each field for laboratory isolation.

Isolation and identification of the associated fungi:

Roots, stems and leaves of the diseased plants showing typical symptoms of root rot, wilt, crown rot, black stem and leaf spot were washed several times with running tap water**.** Infected tissues of each sample were cut into small pieces and separately surface-sterilized in 2 % sodium hypochlorite for 3 min. then washed twice in sterilized distilled water. The sterilized plant pieces were dried and directly plated on PDA medium, (5 pieces / Petri-dish), then incubated at 25C° for 7 days. Emerging fungi were purified and identified microscopically according to their morphological characters according to Barnett and Hunter (1998). Also, the identification was confirmed by the staff of Mycology Research & Disease Survey Department, Plant Path. Res. Inst., Agric. Res**.** Center, Giza, Egypt. Stock cultures were maintained on PDA slant and kept in a refrigerator at 5 C° for further studies.

Pathogenicity test:

The pathogenic potential of each of the most important fungi of rot and wilt diseases, *i.e. R. solani, F. solani, F. oxysporum*, *V. albo-atrum*, *M. phaseolina*, *S. sclerotiorum* and *Sclerotium rolfsii*, was determined. The fungal inocula were prepared by growing each fungus on autoclaved sorghum medium in 500 ml glass bottles at 25C° for two weeks. Formalin- disinfested pots (25-cm-diam.) containing formalin-disinfested sandy soil (4 kg / pot) were infested separately with each fungal growth at the rate of 2% (w / w). Four pots were used for each treatment. Apparently healthy twenty seeds (cv. Sewa) were sown in each pot 7days after soil infestation. Disease severity index(DSI) of root rot was recorded using scale proposed by Hankock (1985).

The inocula of the most important foliar fungi, *i.e. Cereospora* sp., *Phoma medicaginis, Colletotrichum trifolli*, *Stemphylium botryosum* and *Alternaria* sp**.** were prepared from 14- days- old growth grown on PDA medium as spore suspensions (10^5 spores / ml). The inocula were sprayed on 45 days old plants. Inoculated plants were kept under high humidity for 3 days**.** Diseases Severity index (D.S.I) of crown necrosis, leaf spot and black stem were recorded using scales proposed by Turner and Van Alfan (1983) and Boland and Brochu (1989), respectively.

Isolation of alfalfa seeds associated fungi :

- Source of seed samples:

Twenty seed samples of Alfalfa (cv. Sewa) were collected from five districts during summer season 2012, *i.e.* Fayed, El-Tal El-Kabeer, El-Quantara Sharq, El-Quantara Gharb and Ismailia. Twenty five seeds were plated in a 12-cm diameter Petri dish (8 dishes) containing PDA medium and incubated at 20± 2 C° for 4 days under cool white fluorescent light with alternating cycles of 12-h light and 12-h darkness (Uzma and Nusrat, 2011). Seeds were examined under a stereoscopic microscope to determine the percentage of infected seeds. The occurrence of different fungal species was recorded according to the recommended methods of Mathur and Knogsdal (2003).

Identification of the isolated fungi:

The isolated fungi were identified based on their habit characters under stereomicroscope and light microscope according to Barnett and Hunter (1998).

Determination of aggressiveness for the seed fungal isolates:

The aggressiveness of five fungi namely: *R. solani*, *F. solani*, *F. oxysporum*, *M. phaseolina* and *S. sclerotirum* was tested after they were grown on water agar medium at 24 ± 2C°. Seeds (cv. Sewa) were surface sterilized with 2 % sodium hypochlorite solution for 3 min, washed several times with sterilized water and 10 seeds per plate were placed onto the prepared fungal cultures. Seeds also were placed on water agar plates as uninoculated control. The plates were incubated at 24± 2 C° for one week before the disease ratings were determined. Each fungal isolate was considered as a treatment and four replicates (4 plates) for each were used. The rating scale for aggressiveness on all seeds was measured as proposed by Zhang and Yang, (2000) as follow:

0 = seed germinated without visible infection, 1 = germinated with light discoloration on roots, 2 = germinated with short severely discolored roots, 3 = died after germination, 4 = died before germination. Measurement of aggressiveness of all isolates was calculated as a disease index from the following equation:

$$DI = \frac{\text{Sum of(Disease score x no. seeds with that score)}}{\text{No. of seeds in sample x highest rating category}}$$

Statistical analysis of the data:

The data on disease severity was subjected to single factor analysis of variance (CRD).

RESULTS AND DISCUSSION

1- Diseases survey:

Data presented in Table (1) indicate that plants with symptoms of root-rot / wilt, leaf spot and rust were found in all inspected fields, but the average percentage of infection varied from one district to another. In general, average percentages of infection were highest in Ismailia district followed by El Quantara Sharq. While, El-Quantra Gharb district showed the lowest of infection percentages. Rust infection reached 60 % in Ismailia district and 47.5, 42.5 and 40.0 % at El-Tal El-Kabeer, Fayed and El-Quantara Sharq, respectively. On the other hand, infection by black stem, anthracnose and downy mildew at El-Quantara Gharb was absent (0.0 %) and anthracnose was not noted in Fayed district. The present results are somewhat similar to those obtained by Billar (1989), Naseri and Marefat (2008) with respect to the multiplicity of diseases of alfalfa which varied in kind and frequency from one location to another.

Table 1. Average percentages of infection by fungal diseases on Alfalfa plants at five districts of Ismailia governorate.

District	NSF*	Average percentage of infection					
		Root-rot	Black stem	Anthracnose	Leaf spot	Downy mildew	Rust
Ismailia	9	8.7	37.6	18.2	41.2	15.4	60.0
Fayed	6	5.4	12.4	0.00	18.8	5.6	42.5
El-Tal El-Kabeer	7	6.3	12.5	9.3	23.7	8.1	47.5
El-Quantara Sharq	5	2.1	0.00	0.00	9.3	0.00	26.5
El-Quantara Gharb Gharb	6	8.3	12.5	13.0	21.0	8.9	40.0
Mean		6.2	15.0	8.1	22.8	7.6	43.3

NSF * = number of surveyed fields.

2-Isolation and identification of the causal organisms:

A- Plant roots:

Isolation from rotted roots yielded 8 fungi namely: *R. solani*, *F. solani*, *F. oxysporum* (Fig.1), *Fusarium* sp., *V. albo-atrum*, *M. phaseolina*, *S. rolfsii,* and *S.*

sclerotiorum (Table, 2, Fig.2). All fungi, were isolated from samples of Ismailia and Fayed, whereas *M. phaseolina* was not isolated from samples of El- Tal El-Kabeer and El-Quantara Sharq, Gharb) Also, *S. rolfsii* was not isolated from samples of El Tal El-Kabeer and El Quantra Gharb. Other fungi were found at all locations at different frequencies. The variation in the kind and frequency of different fungi isolated from plants growing at different locations could be attributed to the variation in environmental conditions and / or the cropping systems. *F. oxysporum* followed by *R. solani* and *Fusarium* sp. recorded the highest means (29.00, 19.70 and 19.00 %) of frequencies in isolation from all the locations tested, while *S. rolfsii* (2.6 %) and *M. phaseolina* (2.7%) recorded the least percentages. These results are in agreement with those of Salas and Stack (1987) who found that *F. oxysporum* was the most common fungus isolated from alfalfa infected roots. In this respect, Uddin *et al.* (1991) mentioned the fusaria isolated from crown rot of alfalfa in north Nevada survey were *F. solani* (43.0 %), *F. acuminatum* (36.0 %), *F. oxysporum* (17.0 %), *F. sambucinum* (3.0 %) and *F. avenaceum* (1.0%). Also, Several fungi including *R. solani*, *F. oxysporum*, *V. albo-atrum* and *C. trifolli* were isolated from diseased plants as reported locally by El-Barougy (1997).

Table 2. Frequency of root infecting fungi isolated from diseased alfalfa plants.

District	NSF*	Frequency %							
		A	B	C	D	E	F	G	H
Ismailia	8	28.3	6.3	21.9	15.6	9.3	9.3	3.1	6.2
Fayed	5	12.5	4.2	41.6	16.7	8.3	4.2	4.2	8.3
El-Tal El-Kabeer	5	16.7	8.3	16.7	33.3	16.7	0.00	0.00	8.3
El-Quantara Sharq	10	17.6	5.9	29.4	11.8	17.6	0.00	5.9	11.8
El-Quantara Gharb	5	23.5	5.9	35.4	17.6	11.7	0.00	0.00	5.9
Frequency(Total)		98.6	30.6	145	95	63.6	13.5	13.2	40.5
Mean		19.7	6.1	29.0	19.0	12.7	2.7	2.6	8.1

NSF * = number of surveyed fields.

A = *R. solani* B = *F. solani* C = *F. oxysporum* D = *Fusarium* sp.

E = *V. albo-atrum* F = *M. phaseolina* G = *S. rolfsii* H= *S. sclerotiorum*

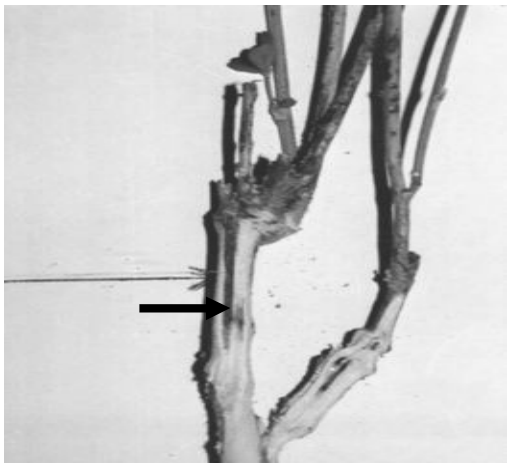

Fig. 1. Symptoms of stem infection by (*F. oxysporum*).

Fig. 2. Symptoms of foliage wilt caused by *S. sclerotiorum* infection.

B- Foliage diseases :

The fungi isolated from the infected foliage were: *Phoma medicaginis* (Fig.3), *Colletotrichum trifolli* (Fig.4), *Colletotrichum* sp., *Cercospora* sp., *Stemphylium botryosum, Alternaria* sp., *Helminthosporium* sp., and *Pseudopeziza* sp. (Table 3). *Stemphylium botryosum* (24.4%), *Alternaria* sp.(21.7%), *Colletotrichum* sp. (16.6%) and *Phoma medicaginis* (13.3 %) however, recorded the highest means of frequencies in isolation trials., while, *Pseudopeziza* sp. and *Cercospora* sp. recorded 0.9% and 3.87 respectively. On the other hand, *P. medicaginis*, *Colletotrichum* sp., *S. botryosum* and *Alternaria* sp. were present in samples of all districts. In this respect, Billar (1989) mentioned the presence of summer and spring black stem and leaf spot (*P. medicaginis*) Stemphylium leaf spot (*Stemphylium* sp.) and anthracnose (*Colletotrichum* sp.) among the fungal diseases affecting alfalfa plants.

Fig. 3. Symptoms of leaf spot caused by *Phoma medicaginis*.

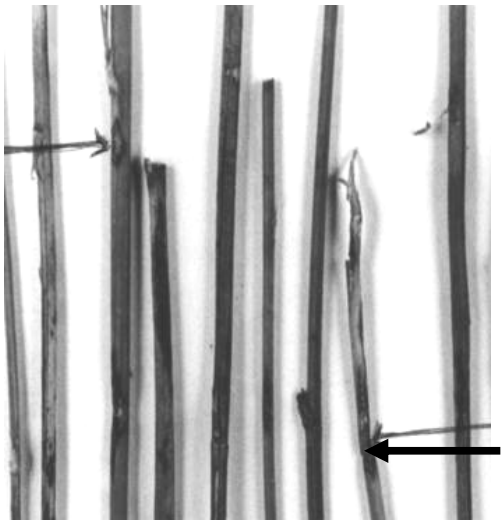

Fig. 4. Symptoms of stem rot caused by *Colletotrichum trifolii*

Table 3. Frequency of fungi isolated from diseased A foliage of alfalfa.

Frequency	NSF*	% Frequency of fungi isolated from foliar diseased of alfalfa plants.							
		A	B	C	D	E	F	G	H
Ismailia	9	7.4	14.8	14.8	10.4	22.2	18.5	7.4	4.5
Fayed	6	12.5	0.00	25.0	0.00	25.0	37.5	0.00	0.00
El-Tal El-Kabeer	7	9.1	18.2	9.1	0.00	36.4	18.2	9.1	0.00
El-Quantara Sarq	5	33.3	0.00	16.7	0.00	16.7	16.7	16.7	0.00
El-Quantara Gharb	6	4.3	13.0	17.4	8.7	21.7	17.4	17.4	0.00
Frequency (Total)		66.6	46.0	83.0	19.1	122	108.3	50.6	4.5
Mean		13.3	9.2	16.6	3.8	24.4	21.7	10.1	0.9

NSF * = number of surveyed fields.

A = *Phoma medicaginis,* B = *Colletotrichum trifolli*, C = *Colletotrichum* sp.,

D = *Cercospora* sp., E= *Stemphylium botryosum.*, F = *Alternaria* sp.,

G = *Helmenthosporium* sp. & H = *Pseudopeziza* sp.,

2- Pathogenicity tests:

A- Root- infecting fungi :

All fungi isolated from infected roots of alfalfa plants were found to be pathogenic, causing different degrees of pre- and post-emergence damping-off. The highest percentage of pre-emergence damping-off was caused by *R. solani* (70.0 %), followed by *S. rolfsii* (31.25 %) and *F. solani* (28.75 %), while the lowest percentages

was caused by *M. phaseolina* (17.5 %) and *F. oxysporum* (16.25 %). The lowest percentage of survival was recorded with *R. solani* (17.5 %), while the highest was with *M. phaseolina* (77.5 %). Zwatz (1991)found that *R. solani* and *F. solani* as the most destructive pathogens attacking alfalfa roots, while *V. albo-atrum* caused the highest degree of wilt. Beshir *et al.* (1997) mentioned that *R. solani* was the most destructive causing the highest percentage of pre-emergence damping-off, followed by *F. solani* and *P. debaryanum*.

Table 4. Effect of soil infestation with fungi on damping-off disease incidence 15 and 45 days, respectively.

Tested fungi c	Damping-off		Survivals (%)	DSI*
	Pre-emergence %	Post-emergence %		
Rhizoctonia solani	70.0	12.50	17.5	3.92
Fusarium solani	28.75	22.50	48.75	1.92
Fusarium oxysporum	16.25	46.25	37.50	3.17
Verticillium albo-atrum	21.25	37.50	41.25	2.25
Macrophomina phaseolina	17.5	5.00	77.5	1.25
Scelrotium rolfsii	31.25	15.00	53.75	1.84
Sclerotinia sclerotiorum	23.75	13.75	62.50	1.42
Control (Without fungus)	2.5	0.00	97.5	Healthy
L.S.D. at 5 %	8.36	4.64	9.31	

* DSI :Disease severity index.

B- Foliage – infecting fungi :

Data in Table (5) indicated that all the tested fungi were pathogenic. *P. medicaginis* was the most aggressive fungus with leaf spot severity of, 7.4% followed by *S. botryosum* (5.8%) and *Alternaria* sp. (4.7%) with limited numbers of small brown necrosis mainly on the leaves and stems. *Colletotrichum trifolii* was the only fungus causing typical anthracnose symptoms on stem, crown and leaf with disease severity of 1.5 , 12.3 and 6.5 %, respectively. These results are in agreement with Basu (1983), who reported that high incidences of *P. medicaginis,* followed by *C. trifolii* and *P. megasperma* were recorded on alfalfa plants. Lenssen *et al.* (1991) stated that *C. trifolii* was the fungus responsible for causing alfalfa anthracnose type symptoms, whereas, Boland and Brochu (1989) reported *C. desructivum* as the main causal of anthracnose in Canada.

Table 5. Pathogenicity of foliage infecting fungi on Alfalfa and the characteristics of the appeared symptoms.

Tested fungi	Disease severity of infection on :			Symptoms on alfalfa plants
	Leaf	Stem	Crown	
Phoma medicaginis	7.4	3.2	0.0	Spring black stem and leaf spot
Colletotrichum trifolii	1.5	12.3	6.5	Anthracnose and crown rot or spot.
Stemphylium botryosum	5.8	2.6	0.0	Leaf spot
Alternaria sp.	4.7	1.8	0.0	Leaf spot
Cercospora sp.	2.1	0.0	0.0	Summer black , stem and leaf spot
Control	0.0	0.0	0.0	____
L.S.D. at 5 %	0.21	0.15	0.04	

3 – Fungi associated with alfalfa seeds :

Fungi were usually isolated from seeds (Table 6) where *Rhizoctonia solani* gave the highest frequency (27.02 %), followed by *Fusarium oxysporum* (24.32%). *Fusarium solani* recorded moderate frequency (13.51 %) and *Macrophomina phaseolina* was the least (5.4 %). Data also showed that Ismailia district was higher in frequency of fungi (29.73 %) followed by El- Tal El-Kabeer (24.32 %), while El-Quantra Sharq was the least (8.10 %). Several fungi were isolated from ten varieties of legume seeds Quantara Sharq where *R. solani*, *Pythium aphanidermatum* and *S. sclerotiorum* recorded the highest frequency (Abdullah, 2010). Twenty four genera and thirty five species of fungi were isolated from alfalfa seeds (Al-Askar *et al.,* 2013). *Alternaria alternata*, *Cladosporium* sp., *Aspergillus* sp., *Stemphylium* sp., and *Penicillium* sp., were the genera most commonly found as saprophytic fungi, while *Stemphylium botryosum* and *Fusarium incarnatum* were common pathogenic fungi. Also, *C. trifolii* and *R. solani* gave the highest percentages of rotted alfalfa seeds (31.6 and 26.45 %, respectively) followed by *F. equiseti* (17.45 %) and *F. ncornatum* (15.7 %).

Table 6. Occurrence of fungi on alfalfa seeds, collected from five districts in Ismailia governorate, during (2012) season.

District	Disease index on seeds of Petri dishes							
	A	B	C	D	E	F	Total	Frequency
Ismailia	3	2	3	1	____	2	11	29.73
Fayed	2	1	2	___	1	2	8	21.62
El-Tal El-Kabeer	3	1	2	___	2	1	9	24.32
El-Quantara Sharq	1	1	___	1	___	___	3	8.10
El-Quantara Gharb	1	____	2	___	1	2	6	16.21
Total	10	5	9	2	4	7	37	_____
Frequency	27.02	13.51	24.30	5.40	10.81	18.91	___.	_____

A= *R. solani* , B= *F.solani* , C= *F. oxysporum* ,
D= *M. phaseolina* , E= *S. sclerotirum* & F= *Fusarium* sp.

Pathogenicity of the associated fungi to seeds:

Data in Table (7) expressed in terms of disease index showed that *R. solani* was more aggressive(0.95) than the other fungi, followed by *S. sclerotiorum* (0.91), *F. oxysporum* was moderate (0.85) and *M. phaseolina* was the least pathogenic (0.52). Some fungi can infect seeds before harvest but many of these fungi were not favored by storage conditions. Similar results were obtained by Pand *et al.*(2005). Pathogenic fungi viz. *Fusarium moniliforme*, *Fusarium oxysporum, Macophomina phaseolina* and *Rhizoctonia solani* were isolated from chickpea seeds. Several legume crops were tested under laboratory conditions for susceptibility to the pathogen isolated from seeds showed that *S. sclerotiorum* was more aggressive followed by *R. solani* (Abdullah, 2010).

Table 7. Aggressiveness of seed isolates using Petri dish technique:

Fungus	Disease index on seeds				Mean
	1	2	3	4	
Rhizoctonia solani	0.98	0.95	0.95	0.93	0.95
Fusarium solani	0.53	0.63	0.55	0.58	0.57
Fusarium oxysporum	0.75	0.88	0.93	0.83	0.85
Macrophomina phasolina	0.53	0.55	0.50	0.48	0.52
Sclerotinia sclertiorum	0.85	0.90	0.92	0.98	0.91
Control	0.0	0.0	0.0	0.0	0.0

L.S.D at 5 % = 0.11

Based on the obtained results, it is recommended that seed health testings should be done for the different seed lots and appropriate treatments be made before being planted. Also, disease surveys are recommended for different production areas to avoid those with high frequencies of destructive diseases.

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الأمراض الفطرية للبرسيم الحجازي في محافظة الإسماعيلية

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تم عمل حصر للأمراض الفطرية على البرسيم الحجازى فى محافظة الإسماعيلية وقد تم هذا خلال موسمي ٢٠١٠/ ٢٠١١& ٢٠١١ / ٢٠١٢ وقد تم تحديد النسبة المئوية لكل مرض حيث شوهد ان مركز الإسماعيلية كان أعلى في نسبة الإصابة بأعفان الجذور والذبول . وبالنسبة لأمراض المجموع الجذري أتضح أن الفطريات التى تفتك بالبرسيم الحجازى فى محافظة الإسماعيلية تتمثل في الفطر رايزوكتونيا سولانى المسبب لأمرض موت البادرات وأعفان الجذور وفطري فيوزاريوم اكسبسبورم و الفرتيسليم البواترم مسببا مرض الذبول الوعائي . بالنسبة لأمراض المجموع الخضري وجد ان أهم الأمراض الفطرية تتمثل فى مرض الساق الأسود ويسببه الفطر فوما ميديكاجينس الانثراكنوز ويسببه الفطر كلليتوتتركم ترايفولياى تبقعات الأوراق وتتسبب عن الفطرين استنفيليوم بوتريوسم والترناريا البياض الزغبى ويسببه الفطر برونوسبورا والصدأ ويسببه الفطر يوروميسس ترا يفولياى .

تم العزل من بذور البرسيم الحجازى التى تم جمعها حيث كان الفطر رايزوكتونيا سولانى الأكثر تكرارا يليه الفطر فيوزاريوم اكسيسبورم واقلهم الفطر ماكروفومنا فاصولينا٠

تم عمل اختبارالقدره المرضيه على الاطباق فى المعمل للفطريات المعزوله من بذور البرسيم الحجازي ووجد أن الفطر رايزوكتونيا سولانى الأعلى في القدرة المرضية يليه الفطر اسكروتينيا اسكروتيورم بينما كان الفطر ماكروفومنا فاصولينا الاقل فى هذا المجال.