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PLANT PROTECTION

Efficacy of *Lupinus termis* and *Trigonella foenum - graecum* Seed Extracts on *Pythium* and *Rhizoctonia* Diseases of Cucumber, Tomato and Radish Seedlings

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Abstract. An aqueous formulation of seed extracts of lupin (*Lupinus termis*) and fenugreek (*Trigonella foenum - graecum*) was tested against *Pythium aphanidermatum* and *Rhizoctonia solani* both in vitro and in vivo. In a food poison test, the seed extracts at the highest concentration tested (9 mg/ml) resulted in complete inhibition of *P. aphanidermatum* mycelial growth and a 77% suppression in the linear growth of *R. solani*. In the greenhouse, applying lupin and fenugreek seed extracts at the planting time and two weeks later, significantly suppressed Pythium damping-off of cucumber and tomato seedlings as well as radish damping - off caused by *R. solani*. Moreover, the seed extracts application had a significant promotional effect on the seedlings growth of the tested vegetables. The improved effect was obtained in both inoculated and non-inoculated plants.

Introduction

Damping off diseases caused by *Pythium* spp. and *Rhizoctonia solani* Kuhn are among most common soil-borne diseases causing serious destruction to vegetable crops grown in Saudi Arabia [1] as well as many other parts of the world [2;3]. Control of these diseases depends mostly on fungicides. However, many constraints are now being imposed on the use of synthetic fungicides due to their phytotoxicity, carcinogenicity and harmful residual effects. Several natural plant products have been reported to possess antifungal activity against plant pathogenic fungi [4]. These products have little or no phytotoxicity, high biological activity and are easily degraded by soil microbes [5]. Extracts of several legume crops especially alfalfa have proved its efficacy against various phytopathogenic fungi [6;7]. In the Middle East, other legumes such as lupin (*Lupinus termis* Forsk.) and fenugreek (*Trigonella foenum - graecum* L.) are also known for their antimicrobial effects. Their seeds are commonly ground and used as

poultice to treat abscesses and burns [8]. Recently, the antimicrobial activity of the aqueous extract of fenugreek seeds has been demonstrated [9].

The objectives of the present study were to: (1) detect the antifungal activity of lupin-fenugreek seed extracts [LFSE] against *Pythium aphanidermatum* (Edson) Fitzp. and *R. solani* as compared with the fungicides: Benomyl, Ridomyl, and Rizolex; (2) determine the efficacy of LFSE in protecting vegetable seedlings against damping - off diseases caused by *P. aphanidermatum* and *R. solani* in the greenhouse; (3) evaluate the impact of LFSE treatment on the growth of the tested vegetable seedlings as compared with that of the fungicides Ridomyl and Rizolex treatments.

Materials and Methods

Preparation of seeds aqueous extracts

Dried seeds of Balady cultivars of lupin and fenugreek were ground finely. One kg of seed powder of each species was soaked in two liters of distilled water at room temperature (20 ± 3°C). After one week the plant material was filtered through whatman No. 1 filter paper, the solid part was then removed. Our previous work proved that the effective concentration of LFSE was obtained when equal volumes of the two seed extracts were combined (El-Gizawy, unpublished). Therefore, equal volumes of each aqueous seed extract were mixed thoroughly, filtered through a 0.22 µm Metericel sterile membrane to form the final product of LFSE.

In vitro toxicity test

The appropriate volumes of the above mentioned solution of LFSE was added to 100 ml of potato-dextrose agar (PDA) to obtain the following concentrations: 0, 1, 3, 6, 9 mg/ml. For comparison, the following fungicides were also tested: Metalaxyl + Mancozeb (formulated as Ridomyl MZ 72 WP, Ciba - Geigy, Switzerland) at the concentration of 1.5 g a.i./liter. Benomyl (formulated as Benlate 50% WP, Du Pont, France) at the concentration of 0.5 g a.i/liter, and Tolcofos - methyl + Thiram (Formulated as Rizolex - T 60 WP, Sumitomo, Japan) used at 1 g a.i./liter. Five replicate plates were used for each treatment. A 5-mm plug from actively growing culture of either *P. aphanidermatum* or *R. solani* was placed in the center of each dish, and the dishes were incubated at 25 °C. Linear growth was measured after 72 hr of incubation.

Bioassays for the suppression of *Pythium* and *Rhizoctonia* damping-off with LFSE

The cucumber bioassay described by Chen *et al.* [10] was utilized to detect the suppression of *Pythium* damping - off with LFSE. A similar set of experiment was also conducted with tomato seedlings as the bioassay plant. Suppression of *R. solani* damping - off by LFSE was determined using the radish bioassay [11]. A mixture of Canadian sphagnum peat, and perlite (3:1, v/v) was autoclaved for 1 hr and placed in plastic pots (10 cm in diameter, 7.5 cm in depth). Cucumber seeds (*Cucumis sativus* L.,

Beit Alpha, 98% germination) were planted at the rate of five seeds per pot. Tomato seeds (*Lycopersicon esculantum* Mill., (Cv. Castle Rock), 99% germination) were sawn at the rate of 10 seeds per pot. The radish seeds (*Raphanus sativus* L., (Cv. Balady (common cultivar), 95% germination), were planted at the rate of five seeds per pot. *Pythium aphanidermatum* isolate was originally recovered from a diseased cucumber plant. *Pythium* inoculum was grown for 15 days on a sterilized mixture of peat moss and wheat bran (1:1. v/v), (Peat - bran) which was suggested by Roiger *et al.*[12] for the growth of *Phytophthora* sp. Soil inoculum of *R. Solani* was prepared by growing the fungus on moistened sterile wheat grain for 15 days at room temperature (20 ± 3 °C). Inoculum of each pathogen was air-dried for twenty-four hours in a laminar flow hood, ground in a mortar and pestle, then sieved through a 20 mm mesh sieve. Soil inoculum of either pathogen was then added to the pots to be infested at the rate of 0.5% of the soil mix dry weight. Plants were grown in the greenhouse at a temperature of 25 ± 3 °C under 16-hr illumination (200 μ Em^2 sec^1) per day and were watered daily. The treatments consisted of a control treatment (sprayed with water), a fungicide treatment with Ridomyl (1.5g a.i./liter) in case of the *Pythium* bioassays and with Rizolex (1 g a.i. / liter) in case of the *Rhizoctonia* bioassay, and LFSE treatments of 0.3%, 0.6%, and 0.9% of the crude extract diluted in water. All treatments were applied twice, at the date of planting and two weeks later.

The study was organized as a randomized complete block design and each treatment was replicated five times. Disease severity ratings were recorded two weeks after planting according to the following scale [13]: 1 =symptomless; 2 = diseased but not damped off; 3 = post - emergence damping - off; 4 = preemergence damping - off. After four weeks, fresh weight of the shoot system or sprout weight [3] was determined for 10 seedlings in each treatment. Diseased seedlings were surface sterilized in 0.5% sodium hypochlorite for 1 min, rinsed thoroughly in sterile distilled water, and placed on corn meal agar (CMA) for *Pythium* sp. or on acidified pototo dextrose agar (APDA) for *R. solani*, in order to reisolate the pathogens. All experiments were repeated twice and results combined. Analysis of variance was performed using PC SAS version 6.12 (SAS Institute, Carry, NC). Separation of means were based on Duncan's multiple range test ($P < 0.05$).

Results

In vitro assays

Lupin - fenugreek seed extract (LFSE) was effective in controlling the mycelial growth of *Pythium aphanidermatum* and *Rhizoctonia solani in vitro*. *P. aphanidermatum* was more senstive to LFSE as compared with *R. solani* (Table 1). The mycelial growth of *P. aphanidermatum* was substantially inhibited (68.2% inhibition) at the lowest concentration of LFSE (1 mg/ml). A complete inhibition of *P. aphanidermatum* mycelial growth was obtained at the highest concentration tested (9 mg/ml) of LFSE. Also Ridomyl MZ (1.5 mg/ml a.i.), and Rizolex T (1 mg/ml a.i.) completely inhibited *P.*

aphanidermatum mycelial growth. Benlate (0.5 mg/ml a.i.); however, did not demonstrate any efficacy against the tested isolate of *P. aphanidermatum*. The fungitoxic effect of LFSE against *R. solani* was less_pronounced (Table 1). *R. solani* mycelial growth was not completely inhibited even at the highest tested concentration (9 mg/ml) of LFSE. Also the fungicides Ridomyl MZ (1.5 mg/ml a.i.) and Benlate (0.5 mg/ml a.i.) inhibited the mycelial growth of *R. solani* by 85.9% and 84.7%, respectively. Only Rizolex T at the concentration of (1 mg/ml a.i.) resulted in 100% inhibition of *R. solani* (Table 1).

Table 1. Effect of lupin-fenugreek seed extract (LFSE) and fungicides on linear growth of *Pythium aphanidermatum* and *Rhizoctonia solani*, after 72 hr of incubation

Treatment	% Inhibition of fungal linear growth	
	P. aphanidermatum	*R. solani*
LFSE (1 mg/ml)	68.2	23.5
LFSE (3 mg/ml)	84.9	57.7
LFSE (6 mg/ml)	89.5	64.7
LFSE (9 mg/ml)	100.0	76.5
Ridomyl MZ (1.5 mg/ml a.i.)	100.0	85.9
Rizolex T (1 mg/ml a.i.)	100.0	100.0
Benlate (0.5 mg/ml a.i.)	0.0	84.7

Effect of LFSE application on suppression of *Pythium* and *Rhizoctonia* damping-off

The LFSE treatments resulted in a significant decrease in damping - off caused by either *P. aphanidermatum* or *R. solani* in the greenhouse (Tables 2, 3, 4). The three tested concentrations of LFSE were suppressive to *P. aphanidermatum* damping - off (Tables 2, 3). Mean disease severity values in the nontreated control of both cucumber and tomato seedlings were 3.9 and 3.2, respectively. Applying LFSE at 6 mg/ml lowered the disease severity incidence to 1.8 and 1.4 in cucumber and tomato, respectively [Tables 2,3]. Ridomyl MZ (1.5 mg/ml a.i.) represented the most effective treatment against *Pythium* damping-off in both cucumber and tomato bioassays. LFSE treatment, however, resulted in a significant increase in fresh weight of cucumber and tomato seedlings as compared with control (sprayed with water only) as well as the fungicide treatments (Tables 2, 3). Also increasing the dose of LFSE, lead to a significant increase in seedling weight. For example, while the cucumber sprout weight of the check (sprayed with water) was 0.92 g in the noninfested treatment, applying LFSE increased the sprout weight to 1.68 g, 2.20 g, 2.37 g at 3, 6, 9 mg/ml

of LFSE, respectively. A similar result was also obtained in case of the infested treatment (Table 2).

Table 2. Effect of lupin-fenugreek seed extract (LFSE) and Ridomyl MZ on damping - off caused by *Pythium aphanidermatum* and on sprout weight of cucumber seedlings

	Disease severity		Sprout weight[a] (g.)	
Treatment	Check	Infested	Check	Infested
LFSE (3 mg/ml)	1.1* b	2.3 b	1.68 c	1.52 c
LFSE (6 mg/ml)	1.3 a	1.8 d	2.20 b	1.83 b
LFSE (9 mg/ml)	1.3 a	2.0 c	2.37 a	2.04 a
Ridomyl MZ (1.5 mg/ml a.i.)	1.1 b	1.1 e	1.26 d	0.90 d
Check (sprayed with water only).	1.2 ab	3.9 a	0.92 e	0.57 e

* Means in a column followed by common letters are not significantly different according to Waller-Duncan K-ratio t test, K = 100 (approximates P=0.05).

[a] Based on the fresh weight of the shoot system of 10 seedlings 4 weeks after planting.

Table 3. Effect of lupin-fenugreek seed extract (LFSE) and Ridomyl MZ on damping-off caused by *Pythium aphanidermatum* and on sprout weight of tomato seedlings

	Disease severity		Sprout weight[a] (g.)	
Treatment	Check	Infested	Check	Infested
LFSE (3 mg/ml)	1.3* a	1.8 b	0.34 c	0.29 b
LFSE (6 mg/ml)	1.1 a	1.4 c	0.46 b	0.29 b
LFSE (9 mg/ml)	1.2 a	1.3 c	0.52 a	0.34 a
Ridomyl MZ (1.5 mg/ml a.i.)	1.1 a	1.1 d	0.25 d	0.18 c
Check (sprayed with water only).	1.3 a	3.2 a	0.25 d	0.19 c

* Means in a column followed by common letters are not significantly different according to Waller-Duncan K-ratio t test, K = 100 (approximates P=0.05).

[a] Based on the fresh weight of the shoot system of 10 seedlings 4 weeks after planting.

In the *R. solani* bioassay, the LFSE treatment has also resulted in a significant decrease in damping - off of radish seedlings (Table 4). No significant difference in disease severity was observed between the infested treatment sprayed with 6 mg/ml LFSE and that sprayed with 9 mg/ml LFSE. Evidently, Rizolex T (1 mg/ml a.i.) was the most effective treatment in controlling *R. solani* damping - off lowering disease severity to 1.3 as compared with 3.4 in case of the infested check. Again, applying LFSE at various concentrations had a significant increasing effect on the sprout weight of radish seedlings as compared with the control plants (sprayed only with water), as well as seedlings sprayed with Rizolex T. *R. solani* was easily recovered from damped - off radish seedlings on APDA. *P. aphanidermatum* was reisolated from diseased seedlings of both cucumber and tomato on CMA.

Table 4. Effect of lupin-fenugreek seed extract (LFSE) and Rizolex T on damping-off caused by *Rhizoctonia solani* and on sprout weight of radish seedlings

Treatment	Disease severity		Sprout Weight[a] (g.)	
	Check	Infested	Check	Infested
LFSE (3 mg/ml)	1.2* a	2.8 b	1.54 c	1.46 b
LFSE (6 mg/ml)	1.3 a	2.4 c	1.60 b	1.32 c
LFSE (9 mg/ml)	1.1 a	2.2 c	2.62 a	1.53 a
Rizolex T (1 mg/ml a.i.)	1.2 a	1.3 d	0.65 d	0.47 d
Check (sprayed with water only).	1.2 a	3.4 a	0.65 d	0.27 e

* Means in a column followed by common letters are not significantly different according to Waller-Duncan K-ratio t test, K = 100 (approximates P=0.05).

[a] Based on the fresh weight of the shoot system of 10 seedlings 4 weeks after planting.

Discussion

The results presented in this study are the first in Saudi Arabia to describe the effects of applying lupin-fenugreek seed extracts on suppression of some vegetable diseases and on enhancement of plants growth under greenhouse conditions. In the biocidal assays, LFSE was effective against *P. aphanidermatum* an Oomycete as well as on *R. solani* a member of the Deuteromycetes. This may indicate that the toxic substances in LFSE are active on a variety of fungi. *P. aphanidermatum* was more susceptible to LFSE than *R. solani*. While *P. aphanidermatum* mycelial growth was completely inhibited by LFSE at 9 mg/ml, approximately 77% of the linear growth of *R. solani* was inhibited by the same concentration of LFSE. On the other hand, Benomyl failed to suppress the mycelial growth of the tested *P. aphanidermatum* isolate at the used concentration of 0.5 mg/ml a.i.. The more specialized fungicides, Ridomyl MZ (1.5 mg/ml a.i.) and Rizolex T (1 mg/ml a.i.), completely inhibited their tested target organisms i.e. *P. aphanidermatum* and *R. solani*, respectively.

The obtained results demonstrated that the application of LFSE, as mentioned previously, significantly (P=0.05) reduced damping - off caused by *P. aphanidermatum* or *R. solani* under greenhouse conditions . Although this is the first report on the prophylactic treatment of plants with LFSE, similar treatments have been recently applied as an alternative to synthetic chemical pesticides [14; 15]. The mechanism by which LFSE affects plant pathogens is not yet known. Seed infusion of both lupin (*Lupinus termis*) and fenugreek (*Trigonella foenum - graecum*) have been traditionally used as anthelmintic against most common nematodes [16, pp.47,71;9]. The antifungal activity of lupin seed extract may be due to the presence of several flavonoids. Lupins are known to contain various flavonoids with antimicrobial activity [17;18,p.613;19]. Saxena and Vyas, [20] and more recently, Bhatti *et al.* [9], have demonstrated the antimicrobial activity of fenugreek aqueous seed extract, and attributed it to its flavonoids contents.

LFSE treatments had an improved effect on increasing the seedlings sprout weight of both infested and noninfested plants. For instance, sprout weight of cucumber seedlings infested with *P. aphanidermatum* increased by 166.7%, 221.1%, 257.9% when LFSE was applied at the concentrations of 3, 6, 9 mg/ml, respectively, as compared with the control (sprayed with water only) (Table 2). Sprout - weight was recommended by Wolk and Sarkar [3], as an easy to measure parameter for assessing damage caused by *P. aphanidermatum* on cucumber. The dramatic increase in seedlings fresh weight may be attributed to several factors. Lupins are known for their high nitrogenous content and have been successfully used in green manuring . Seed decoction of fenugreek is commonly used as a galactagogue [8]. Therefore, the nutritional properties of both lupin and fenugreek seeds may provide an explanation for the increased sprout weight of plants treated with LFSE.

In order for sustainable agriculture to be a reality worldwide, we will require new technologies to be encouraged including new crops, new biological control agents and new agrochemicals. The results presented in this work may provide farmers with a new and safe choice that may not only control vegetable damping-off, but also markedly increase seedlings growth. To complete the picture, a further study on the purification and identification of the chemical substances present in LFSE is currently under investigation.

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كفاءة مستخلص بذور الترمس والحلبة في مكافحة أمراض الذبول التي يسببها فطرا البيثيوم والريزوكتونيا لبادرات الخيار، الطماطم والفجل

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ملخص البحث. تمت دراسة تأثير مستخلص بذور الترمس والحلبة على نشاط فطرى البيثيوم والريزوكتونيا سواء في داخل المعمل أو تحت ظروف البيوت المحمية.

أظهرت نتائج الاختبار المعملي أن التركيز المرتفع لمستخلص البذور (٩ ملجم/مل) أدى إلى تثبيط كامل لنمو الميسليوم في فطر البيثيوم، كما أدى إلى تثبيط ما نسبته ٧٧٪ من نمو الميسليوم لفطر الريزوكتونيا.

أما في البيوت المحمية فكان لإضافة مستخلص بذور الترمس والحلبة مرتين (عند الزراعة وبعد ذلك بأسبوعين) إلى بيئة الزرع تأثير معنوي مثبط على مرض ذبول البيثيوم في بادرات الخيار والطماطم، وأيضا على مرض ذبول بادرات الفجل والذي يسببه فطر الريزوكتونيا سولانى. هذا بالإضافة إلى أن مستخلص البذور كان له تأثير معنوي منشط على نمو بادرات محاصيل الخضر تحت الدراسة وهذا التأثير المنشط كان ملحوظا سواء على البادرات النامية في تربة معداة صناعيا بأي من الفطرين تحت الدراسة أو تلك البادرات النامية في تربة معقمة.