

Effect of *Schinus molle* and *Schinus terebinthifolius* Extracts on Sweet Pea Damping-off

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Abstract

The potential efficiency of leaf and stem extracts of *Schinus molle* and *Schinus terebinthifolius* against *Fusarium solani* in addition to the phytochemical screening of both species was studied. Extracts were prepared using different solvents (water, ethanol, methanol and acetone). *Fusarium solani* was isolated and identified from naturally diseased sweet pea (*Lathyrus odoratus*) plants. According to the pathogenicity tests, the highest pathogenic isolate was used to test the antifungal activity of each extracts with different concentrations on mycelial growth of *F. solani in vitro*. The effect differed according to the extract type and concentration. The potential antifungal activity of ethanol extracts appeared highly significant when used with leaves, regardless of plant species, whereas acetone was effective when used with stems. The highest reduction of *F. solani* growth was obtained by ethanol and aqueous extracts of *S. terebinthifolius* leaves, ethanol extract of *S. molle* leaves, acetone extract of both *S. molle* and *S. terebinthifolius* stems, respectively. Effect of extracts at 64 mg/ml on damping-off incidence of artificially infected *L. odoratus* under greenhouse conditions was studied as well. The highest reduction of the disease was achieved by leaf extracts of *S. terebinthifolius* followed by *S. molle*. These results were attributed to the presence of alkaloid, phenolics, flavonoids, and tannins in leaves and stems of both plant species as revealed by the phytochemical screening. Significant negative correlation was detected between *in vitro* inhibition percentage of *F. solani* and the field incidence of damping-off in *L. odoratus* plants. These results suggest that extracts from *S. molle* and *S. terebinthifolius*, especially ethanol leaf extracts and acetone stem extracts, are promising naturally derived antifungal agents for *F. solani* and other plant pathogenic fungi.

Keywords: *Lathyrus odoratus*, *Fusarium solani*, pepper tree, solvent extracts

Introduction

The sweet pea (*Lathyrus odoratus* L.) belongs to Leguminosae family and it is popular for cultivation in many places all over the world including Egypt. It is available with wide range of flower colors and consequently high commercial demand as there are reports of commercial

sweet pea cut flower production however without accurate statistics for the production worldwide (Nakamura *et al.*, 2008 and references therein). Production of sweet pea and other Leguminosae family is highly affected by damping-off and wilt disease caused by several soil borne

fungi including *Rhizoctonia solani* (Kühn) and *Fusarium solani*, respectively (Burnchara and Camacho, 2000; El-Mougy, 2001). Taubenhaus (1914) claimed that Fusarium wilt or root rot of sweet pea was caused by a new species to which they tentatively gave the name *Fusarium lathyri*.

Controlling plant diseases depends mainly on using synthetic fungicides as excessive use of fungicides in agriculture system led to environmental pollution and hazardous of human health (Tepe *et al.*, 2004). Recently, considerable attention has been focused on the potential use of plant extracts as natural and safe alternatives instead of synthetic fungicides. Many potent and powerful antimicrobial agents have been derived from *Cassia fistula* showing high antifungal activity against *Fusarium solani*, *F. oxysporum* and *Rhizoctonia solani* (Ibrahim *et al.*, 2015).

Schinus (pepper tree) belongs to family Anacardiaceae, with the main species are *Schinus terebinthifolius* Roddi and *S. molle*. L. Both species have been introduced from South America to most of the tropical and subtropical areas of the world and to the Mediterranean (Taylor, 2005). Several previous studies indicated that extracts of different parts of these plants have high medicinal value and have been used in folk medicine for a long time to treat many illnesses. A few reports are available on effect of plant extracts against plant pathogens as stated by Rhouma *et al.* (2009), they compared leaf extracts from *S. terebinthifolius* and *S. molle* against some plant pathogens; *Agrobacterium tumefaciens*, *Pseudomonas savastanoi* pv. *savastanoi*, *Fusarium solani*

and *Rhizoctonia solani*. They found that aqueous and methanolic extracts showed a high level of antifungal and antibacterial activity compared to hexane and ethanolic ones, which positively correlated with total phenol contents of the extracts.

Several strategies were used for controlling such diseases including chemical control, cultural practices, biological control and plant extracts (Mezeal, 2014 and references therein). Therefore our current work to determine the potential efficiency of leaf and stem extracts of both *S. molle* and *S. terebinthifolius* using different solvents against *Fusarium* growth *in vitro* and *in vivo* as seed pre-sowing treatment against damping-off disease of sweet pea plants under greenhouse conditions.

Materials and Methods

Isolation of the pathogen

Diseased *Lathyrus odoratus* plants showing symptoms of damping off were collected from the Floriculture Experimental Farm, Faculty of Agriculture, Assiut University, Egypt, during 2012 season. Small pieces of roots with lesion were disinfested in 0.5% NaOCl for 1 min. Rinsed several times with sterilized water and dried with sterilized filter papers. The surface-sterilized plant pieces were placed on Petri dishes containing Potato Dextrose Agar medium (PDA) and incubated 7 days at 27°C. After 7 days of incubation, the developed fungal colonies were purified by hyphal tip technique. The obtained fungal isolates were identified according to their cultural and microscopical characteristics as described by Barnett and Hunter (1986). Subcultures of the obtained isolates were

kept on PDA slants and stored at 5°C for further studies.

Pathogenicity tests

Pathogenic capabilities of 3 isolates were tested on *L. odoratus* seedlings under greenhouse conditions during 2012 season. Inocula of the tested isolates were prepared by growing in sterilized glass bottles (500 ml) containing barley medium (150 g sorghum seeds, 50 g clean sand, 4 g glucose, and 200 ml water), autoclaved in two consecutive days and incubated at 25°C for 15 days. Sterilized pots (20 cm in diameter) were filled with sterilized clay soil and then infested with each isolate at the rate of 1%. Each pot was planted with 10 sterilized seeds. Five pots were considered for each treatment as replicates, whereas untreated pots with pathogens were used as control (Abo-Elyousr *et al.*, 2009). Emergence of *L. odoratus* seedlings at 20 days from planting was estimated for damping-off, pre-emergence damping-off (%) was calculated based on the number of non-emerged seeds in relation to the number of sown seeds.

Plant materials

Leaf and stem samples were collected from the two tested species; *Schinus molle* and *S. terebinthifolius* during 2013 season. The trees were locally grown at the Floriculture Experimental Farm, Faculty of Agriculture, Assiut University, Egypt. The plants were authenticated by senior colleagues of Department of Ornamental Plants and Landscape Gardening.

Extract preparation

Dry powder of leaves and stems (200 g) from each of *S. molle* and *S.*

terebinthifolius was macerated each alone in 2 liter of four solvents; distilled water and 40% of ethanol, methanol and acetone for 48 h at room temperature under constant shaking. The macerates were homogenized for 15 min. and then filtered with watmann filter paper under vacuum filtration. The residues were farther macerated twice in the same manner. The filtrates obtained were mixed and the solvents were evaporated using rotary evaporator (Hidolph VV2000). The residues were left in porcelain dishes to dryness. Dried extracts were weighed and calculated relative to plant sample fresh weight. Total dried extracts were kept in tightly stoppered bottles in refrigerator 2-4°C.

Phytochemical screening

Dry powder of leaves and stems of both *S. molle* and *S. terebinthifolius* was subjected to qualitative phytochemical screening for identification of various classes of active constituents according to the methods described by Evans (2002) and Khandelwal (2001).

Test for the presence of saponins:

1 mg of each extract was dissolved in distilled water (1 ml) and vortexed. The presence of saponins was verified by the formation of abundant and persistent foam.

Test for the presence of phenols and tannins:

three drops of ferric chloride (10%, in ethanol) were added to 2 ml of each extract (1 mg/ml, in ethanol). The presence of phenols was confirmed when color of the extracts varied between blue and red was. The formation of a dark blue precipitate indicates the presence of hydrolysable tannins and the forma-

tion of a green precipitate the presence of condensed tannins.

Test for the presence of flavonoids: alkaline reagent test was used where extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color becoming colorless upon addition of dilute acid indicates the presence of flavonoids.

Test for the presence of steroids and free tetracyclic triterpenes: 1 mg of each extract was dissolved in 1 ml of chloroform. Then, 1 ml of acetic anhydride was added and 3 drops of concentrated sulfuric acid were slowly added. The emergence of evanescent blue color then permanent green was indicative of the presence of free steroids and a brownish red for free pentacyclic triterpenes.

Test for the presence of alkaloids: For estimation of alkaloids, 0.5 to 0.6 g of each extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents of Maeyer's and Dragendorff's. Then, it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Antifungal activity assay *in vitro*

Laboratory works were directed to study the antifungal activities of sixteen plant extracts on linear growth of *F. solani* according to the method described by Mecteau *et al.* (2002) with some modifications. Each crude Extract solution was filtered through a 0.22 µm sterile filter (PES Syringe filter). Each plant extract was added singly to conical flasks (250 ml) contenting Potato dextrose agar (PDA) medium to get concentrations of 64, 32 and 16

mg/ml. Non treated PDA medium with extract was used as control. Each flask purred in Petri dishes (9 cm in diameter) and inoculated in the center with disks (5 mm) of *F. solani*, 7 days old culture. Four replicates were used for each treatment. Cycloheximide with concentration of 0.5 mg/ml was used as a positive control. Plates were incubated at 25±2°C. Linear growth was measured using a ruler, when *F. solani* growth filled up control plates. The percentage of inhibition in fungus growth was calculated as follow:

$$\text{Inhibition\%} = \frac{A - B}{A} \times 100$$

Where: A= colony diameter of control; B= colony diameter of treatment.

Testing of extracts against the pathogen under greenhouse conditions

Two experiments were carried out under greenhouse conditions in seasons 2013/2014 and 2014/2015 seasons. Pots (20 cm. in diam.) were sterilized by immersing in 5% formalin solution for 10 min. and left to dry in open air. Extracts treatments at 64mg/ml was applied as seed treatment. Greenhouse experiments were repeated twice, and pathogenic isolate no 2 was prepared as mentioned before in pathogenicity tests and mixed with sterilized soil in rate of 2% of the soil weight in the sterilized pots. The pots were left for one week, watered and mixed thoroughly to ensure even distribution of infested fungi. Each pot was planted with 10 sterilized seeds. Sterilized seeds were immersed in each extract at 64 mg/ml and left to dry. Untreated seeds with extracts were used as control. Each

treatment was replicated four times. Percentage of disease was recorded after 20 days; results were calculated as previously mentioned in pathogenicity tests.

Statistical analysis

The results were analyzed using ANOVA test and the means differences were regarded as significant using LSD test at 5% level of probability. The obtained antibacterial results were stated in as mean \pm SD for three replicates according to Gomez and Gomez (1984).

Results

Identification of the causal pathogen

Three fungal isolates were obtained from naturally diseased *Lathyrus odoratus* showing damping off symptoms and identified as *F. solani*, based on the morphological characteristics (Barnett and Hunter, 1986).

Pathogenicity tests

Results in Table 1 indicate that all the tested isolates of *F. solani* were able to infect *L. odoratus* plants causing typical symptoms of damping-off with different degrees of disease percentage of damping-off. Data indicate that isolate no 2 caused the highest disease percentage of damping-off followed by other isolates. On the basis of these results, isolate 2 was used in the following experiments.

Table 1. Pathogenicity tests of three isolates of *Fusarium solani* (Fs) on *Lathyrus odoratus* plants.

Isolates No.	% damping off
Rs1	20 b
Rs 2	40 a
Rs 3	20 b

The values in the column followed by same letter are not significantly different according to ANOVA (L.S.D. $p=0.05$)

Phytochemical screening

The results of the phytochemical analysis presented in Table 2 show the presence of alkaloids, phenolics, flavonoids, and tannins in leaves and stems of both *S. molle* and

S. terebentifolius. On the other hand, all extracts were tested negative for saponins, steroids and triterpenoids except stems of *S. terebentifolius* which were found positive for steroids and triterpenoids.

Table 2. Preliminary phytochemical screening of leaves and stems of both *S. molle* and *S. terebinthifolius*

Phytochemical constituents	<i>S. molle</i>		<i>S. terebinthifolius</i>	
	Leaves	Stems	Leaves	Stems
Alkaloids	+	+	+	+
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	-	-	-
Steroids and Triterpenoids	-	-	-	+
Tannins	+	+	+	+

Antifungal activity assay

The activities of different concentrations of sixteen plant extracts on mycelial growth of *F. solani* were tested *in vitro*. Table 3 illustrated that the addition of different concentrations of each plant extracts separately to the media affected significantly the mycelial growth of *F. solani*. The effect differed according to the tested extract and the concentration. The highest reduction of *Fusarium* growth was noticed at concentration of 64 mg/ml when the media were amended with ethanol and aqueous extracts of *S. terebinthifolius* leaves, ethanol extract of *S. molle* leaves, acetone extract of both *S. molle* *S. terebinthifolius* stems, respectively. Methanol gave moderate to high results with both leaves and stems of both species. Moreover, the plant extracts inhibited significantly the growth of *F. solani* at all tested concentrations compared to the control. Mycelial growth reduction was increased depending on the concentration reaching its maximum effect at concentration of 64 mg/ml. On the other hand, aqueous extract of *S. molle* stems and acetone extract of *S. molle* leaves gave the least reduction on *F. solani* with the three tested concentrations, respectively. The other plant extracts showed intermediate effects.

Effect of extracts on damping-off incidence under artificial infection under greenhouse conditions

Based on *in vitro* screening, the best concentration of each extract was selected to follow up their effects under greenhouse conditions against the pathogen. Seed treatments with each extract at 64mg/ml significantly reduced the damping-off incidence of *L. odoratus* compared with control plants under artificial infection with *F. solani* under greenhouse conditions in both tested seasons. The highest reduction of the disease was achieved by leaf extracts of *S. terebinthifolius* followed by *S. molle* then stems of *S. terebinthifolius* followed by *S. molle* as shown in Table 4. Seeds treated with leaf ethanol extract of *S. terebinthifolius* gave the best reduction of the disease in both seasons, while seeds treated with stems acetone extracts of *S. terebinthifolius* and *S. molle* showed the lowest percentage of damping-off compared with other solvents. Seeds treated with leaf methanol or aqueous extracts of *S. molle* and *S. terebinthifolius* showed moderate effect of damping-off percentage. Whereas, the highest percentage of the disease incidence was observed when seeds were treated with aqueous stem extract of *S. molle* followed by *S. terebinthifolius*. No significance appeared on the disease between ethanol and methanol stem extracts of *S. terebinthifolius*, but ethanol stems extracts of *S. molle* showed lower percentage of damping-off than methanol stems extracts. Results in Fig 1 show a sig-

nificant negative correlation at $p < 0.05$ between *in vitro* inhibition % of *F. solani* and damping-off % incidence of *L. odoratus* plants under artificial infection in greenhouse conditions during both tested seasons as

affected by different extracts of both *S. molle* and *S. terebinthifolius* leaves and stems. The regression (R^2) reached 0.765 and 0.645 in the first and second seasons, respectively.

Table 3. Antifungal activities of sixteen plant extracts on radial growth of *F. solani* *in vitro*

Plant material	Solvent	Concentration mg/ml	<i>F. solani</i> colony diameter in cm	Inhibition %
<i>S. molle</i> leaves	Water	64	2.32 fg	74.16
		32	3.12 jkl	65.27
		16	3.57 no	60.27
	Ethanol	64	1.57 bc	82.50
		32	1.82 d	79.72
		16	2.62 h	70.83
	Methanol	64	2.12 ef	76.38
		32	3.55 no	60.55
		16	4.12 pq	54.16
	Acetone	64	4.30 qrs	52.22
		32	4.60 tu	48.88
		16	5.27 v	41.38
<i>S. molle</i> stems	Water	64	4.47 stu	50.27
		32	4.60 tu	48.88
		16	5.12 v	43.05
	Ethanol	64	2.87 i	68.05
		32	3.02 ijk	66.38
		16	4.25 qr	52.77
	Methanol	64	3.42 mn	61.94
		32	4.17 q	53.61
		16	4.65u	48.33
	Acetone	64	1.85 d	79.44
		32	2.47 gh	72.50
		16	3.42 mn	61.94
<i>S. terebinthifolius</i> Leaves	Water	64	1.50 ab	83.33
		32	1.75 cd	80.55
		16	2.30 efg	74.44
	Ethanol	64	1.32 a	85.27
		32	1.57 bc	82.50
		16	2.30 efg	74.44
	Methanol	64	2.10 e	76.66
		32	2.97 ij	66.94
		16	4.17 q	53.61
	Acetone	64	3.17 jkl	64.72
		32	3.65 o	59.44
		16	4.40 rst	51.11
<i>S. terebinthifolius</i> Stems	Water	64	2.55 h	71.66
		32	3.27 lm	63.61
		16	4.40 rst	51.11
	Ethanol	64	3.22 klm	64.16
		32	3.50 no	61.11
		16	3.95 p	56.11
	Methanol	64	2.85 i	68.33
		32	3.17 jkl	64.72
		16	4.10 pq	54.44
	Acetone	64	1.87 d	79.16
		32	2.17 ef	75.83
		16	3.00 ij	66.66
Positive control (Cycloheximide)		0.5	1.40 VW	84.4%
Control		-	9.00 w	0.0
LSD 0.05			0.20	

* Means within the same column followed by different letters are significantly different ($P < 0.05$) based on LSD.

Table 4. Effect of *Schinus* plant extracts with different solvents at 64mg/ml on *F. solani* damping-off (%) of *L. odoratus* under greenhouse conditions during 2014 and 2015 seasons

Plant material	Solvent	Damping off (%)	
		2014	2015
<i>S. molle</i> leaves	Water	41.6 d	43.6 d
	Ethanol	24.4 b	28.0 b
	Methanol	41.6 d	40.0 c
	Acetone	64.0 i	60.0 i
	Mean	45.9 B	42.9 B
<i>S. molle</i> stems	Water	64.8 i	64.0 j
	Ethanol	52.4 g	52.0 g
	Methanol	55.6 h	56.0 h
	Acetone	42.0 de	49.2 f
	Mean	53.7 D	55.3 D
<i>S. terebinthifolius</i> Leaves	Water	42.2de	49.2 f
	Ethanol	22.0 a	20.0 a
	Methanol	40.0 cd	44.4 e
	Acetone	52.0 g	56.0 h
	Mean	40.8 A	36.1 A
<i>S. terebinthifolius</i> Stems	Water	56.0 h	48.4 f
	Ethanol	47.0 f	55.2 h
	Methanol	47.6 f	54.0 gh
	Acetone	44.0 e	48.8 f
	Mean	48.8 C	51.6 C
Control		80.0 jE	76.0 kE

* Means within the same column followed by the small letters are not significantly different ($P \leq 0.05$) based on LSD; Means within the same column followed by the capital letters are not significantly different ($P \leq 0.05$) based on LSD

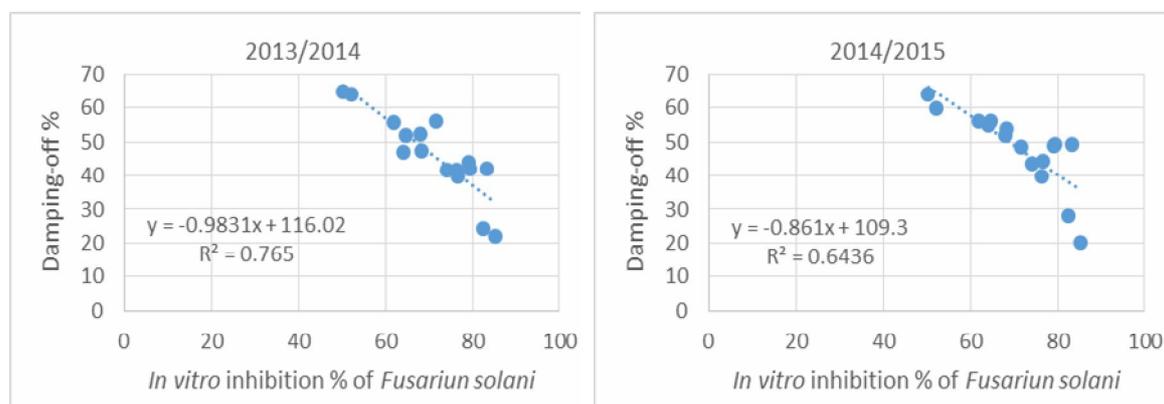


Fig. 1. Correlation between *in vitro* inhibition % of *F. solani* and damping-off % incidence of *L. odoratus* plants under artificial infection in greenhouse conditions during 2013/2013 and 2014/2015 seasons as affected by different extracts of both *S. molle* and *S. terebinthifolius* leaves and stems

Discussion

In the present study, three fungal isolates obtained from naturally diseased *Lathyrus odoratus* showing damping off symptoms were identi-

fied as *F. solani*, which is one of main causal pathogen of damping-off in sweet pea and other Leguminosae family (Burnchara and Camacho, 2000; El-Mougy, 2001). *In vitro* test for antifungal activity of the plant ex-

tracts on radial growth of *F. solani* revealed that inhibition percentage of *F. solani* growth compared with control significantly varied according to the extract source (leaves or stems) and solvent type. The potential antifungal activity of ethanol leaf extracts appeared highly significant, regardless of the plant species, where inhibition percentage reached more than 80%. On the other hand, acetone seemed effective when used with stems recording 79% growth inhibition. When the same extracts were assayed for their effect on damping-off incidence under artificial infection of *L. odoratus* in greenhouse conditions, almost the same general trend was obtained where ethanol leaf extracts and acetone stem extracts resulted in the least incidence of damping-off. This fact is further supported by the significant negative correlation between *in vitro* inhibition percentage of *F. solani* and the field incidence of damping-off in *L. odoratus* plants. Antifungal activity of aqueous and methanolic extracts of *S. molle* and *S. terebenthesfolius* trees was previously reported against *F. solani* (Rhouma *et al.*, 2009). Unlike the results of the current experiment, they found that aqueous and methanolic extracts showed a high level of antifungal and antibacterial activity compared to ethanolic extracts.

Most of the published work on both *S. molle* and *S. terebenthesfolius* plants focus on the identification of compounds found in the essential oils and their biological activity. It is necessary to explore phytochemical and biological properties of extracts from different parts of both plants species, which initiated the idea of this ex-

periment. According to the results of the current experiment, presence of alkaloid, phenolics, flavonoids, and tannins was confirmed in leaves and stems of both plant species. These results are in accordance with the findings of Rhouma *et al.* (2009) and Carvalho *et al.* (2013). In addition, presence of such compounds was a good indication for their potential antifungal activity as shown by several researchers such as Johann *et al.* (2010). In the current study, the detected antifungal effect of the tested extracts could be attributed to their major phytochemical components. Among them, tannins have proved biologically active as antibacterial and antifungal due to their complication with metal ions and macromolecules in addition to their antioxidant effect (Scalbert, 1991). Besides, Taguri *et al.* (2004) attributed the antimicrobial action to the astringent nature of the phenolic constituents including tannins and other polyphenols present in the extracts.

According to our results, ethanol and acetone, followed by methanol gave appreciable results which could be explained upon the ability of them to enhance the extraction of a large quantity of secondary metabolites due to their polarity, concentration or nature (Tekwu *et al.*, 2012). The plant methanol extract was found to be a potent antibacterial and antifungal agent by many authors such as Rhouma *et al.*, (2009) and Malla *et al.* (2014). Other groups such as alkaloids, flavonoids and phenols are important in the defense mechanisms of the plant against microbial pathogens (Calixto *et al.*, 2001). These results suggest that extracts from *S. molle*

and *S. terebinthifolius*, especially ethanol leaf extracts and acetone stem extracts, are promising naturally derived antifungal compounds against phytopathogenic fungi.

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تأثير مستخلصات من نباتي الفلفل الرفيع والفلفل العريض على مرض موت البادرات في بسلة الزهور

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الملخص

تهدف الدراسة الحالية إلى تقييم التأثير المحتمل لمستخلصات الأوراق والسيقان من نباتي الفلفل الرفيع والفلفل العريض ضد فطر الفيوزاريوم *Fusarium solani* في المعمل وتحت ظروف الصوبة وكذلك إجراء الاختبارات المبدئية للمحتوى الفيتوكيميائي لكلا النوعين النباتيين. تم تجهيز المستخلصات باستخدام مذيبات مختلفة وهي الماء والايثانول والميثانول والأسيتون. كما تم عزل سلالة الفيوزاريوم وتعريفها من نباتات بسلة الزهور المصابة طبيعياً بممثل نباتات الزينة بجامعة أسيوط. وبناءً على نتائج اختبار القدرة المرضية، تم تحديد العزلة ذات أعلى قدرة مرضية والتي استخدمت في الاختبارات التالية مثل النشاط المضاد لنمو فطر الفيوزاريوم معملياً. وأظهرت نتائج اختبارات التأثير المضاد للمستخلصات النباتية تبايناً في التأثير تبعاً لنوع وتركيز المستخلص حيث كان هناك تأثيراً معنوياً لمستخلصات الايثانول مع الأوراق في حين أظهر الأسيتون فاعلية واضحة عند استخدامه مع السيقان، وذلك بغض النظر عن النوع النباتي. كما تم تسجيل أعلى فاعلية مثبتة لنمو الفطر معملياً عند المعاملة بمستخلصات الايثانول والماء لأوراق الفلفل العريض، ومستخلص الايثانول لأوراق الفلفل الرفيع، ومستخلص الأسيتون من سيقان كل من الفلفل الرفيع والعريض، على التوالي. وعند دراسة تأثير المستخلصات على موت البادرات في نباتات بسلة الزهور تحت ظروف العدوى الصناعية في الصوبة، أظهرت النتائج تفوقاً معنوياً لمستخلصات الأوراق من نبات الفلفل العريض يليها أوراق الفلفل الرفيع. وأعزيت تلك النتائج لمحتوى الأوراق والسيقان لكلا النباتين من القلويدات والفينولات والفلافونيدات والتانينات بناءً على اختبار المحتوى الفيتوكيميائي. كما لوحظ ارتباطاً معنوياً سلباً بين التأثير المثبط لنمو الفطر معملياً والتأثير على حدوث المرض في الصوبة. وبناءً على تلك النتائج، يمكن التوصية بفاعلية مستخلصات نباتي الفلفل الرفيع والعريض، وخاصة مستخلصات الايثانول من الأوراق والأسيتون من السيقان، كمواد طبيعية مضادة لنمو فطر *F. solani*.

