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FIRST RECORD OF ROOT ROT AND WILT DISEASES OF THE MEDICINAL PLANT *RUTA GRAVEOLENS* L. IN EGYPT AND THEIR CONTROL

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Abstract

Surveying plantations of rue (*Ruta graveolens*) exhibited a new record of root rot and wilt fungal diseases for the first time in Egypt or elsewhere. Isolation trials yielded several fungi belonging to 10 genera. *Fusarium moniliforme* (21.3%), *F. solani* (17.6) and *F. oxysporum* (17.2) showed the highest percentages of frequency, followed by *Rhizoctonia solani* (13.3%) and *F. semitectum* (11.1%), while *Pythium debaryanum* occurred at 10.9%. Pathogenicity studies using seeds or seedlings in planting indicated that *F. oxysporum*, *P. debaryanum* and *R. solani* were the most aggressive fungi.

In infested soil, the use of Topsin M and Vitavax/Thiram as seed dressing, significantly controlled damping-off diseases in case of *F. moniliforme, F. oxysporum and Sclerotinia sclerotiorum,* while Plant Guard (*Trichoderma harzianum,* $3x10^7$ cfu/ml) or Rhizo-N (*Bacillus subtilis,* $3x10^7$ cfu/gm) were the least effective. Also, these fungicides or bioagents successfully decreased infection significantly in comparison with the control when soils were infested with *P. debaryanum* or *R. solani.* On the other hand, dipping in Topsin M solution was generally the most effective treatment for the transplants against rue diseases, although it failed to give sufficient control in some cases. Efficacy of Plant Guard and Rhizo-N as transplants dipping treatments varied according to the pathogen. Rhizo-N was generally the best one. Positive effective values of the bioagents were approximately equal to that of Topsin M. Moreover, they were successful in some cases where fungicides were not.

INTRODUCTION

Rue (*Ruta graveolens* L.) is a perennial sub-shrub belonging to the family *Rutaceae* and originating from the Eastern part of the Mediterranean. As a medicinal plant, rue has been known in Europe for 1500 years (Hornok, 1992). The parts used are flowering tops known as rue or sazab. It is an important medicinal plant with constituents of volatile oil, rutis and rutinic acid, quercetin and resin (Mahran, 1967), usually planted in various locations of Egypt in small areas.

Unfortunately, wilt and root rot diseases began to appear, for the first time in Egypt, on growing mature plants at the Farm of Medicinal and Aromatic Plants Research Station, El-Kanater El-Khairia, Qalubyia governorate. Infection resulted in pronounced losses in plant stand and yield. The same diseases were also recorded on seedlings of nursery and mature plants in Giza and Beni-Suief during survey performed during 1999-2000. The recorded diseases were, however, considered among the most important and destructive diseases of other different medicinal and aromatic plants all over the world including Egypt (Abdel Sattar *et al.*, 1987b; Agnihorti, 1991 and Hilal *et al.*, 1998).

According to the available literature, root rot and wilt diseases of rue were not recorded in Egypt or elsewhere. Therefore, incidence of these diseases and their causal pathogens as well as chemical and biological control were studied.

MATERIALS AND METHODS

Isolation, purification and identification of the causal pathogens:

Wilted and rotted plants were collected from the Farm of Medicinal and Aromatic Plants Res. Sta. at El-Kanater El-Khairyia, Qalubyia governorate. The samples were thoroughly washed with tap water, cut into small pieces and surface sterilized with sodium hypochlorite (1%) for 3 minutes, washed several times with sterilized distilled water and dried between sterilized filter paper. The sterilized pieces were aseptically transferred to Petri dishes of PDA medium and incubated at 25°C for 7 days.

The growing fungi were purified using single spore or hyphal tip techniques and identified according to Booth, (1971) and Domsch *et al.*, (1980). Also, the identification was kindly confirmed by Mycol. Res. and Pl. Dis. Survey Dept., Pl. Path. Res. Inst., ARC, Giza.

Pathogenicity studies:

Fungi were grown on PD liquid media (200 gm potatoes and 20 gm dextrose per liter of medium) in 500 ml glass bottles . Inoculation was carried out with 5mm fungal discs taken from the margins of 10-days-old cultures of *Fusarium oxysporum, F. moniliforme* (No. 6 and 9), *F. semitectum, F. solani, Pythium debaryanum, Rhizoctonia solani* (No. 7 and 8) and *Sclerotinia sclerotiorum*. The inoculated bottles were incubated at 25±1°C for 20 days.

Formalin-sterilized pots (10 and 20 cm diam.) were filled with autoclaved soil.

The potted soil was infested with each fungus at the rate of 1% (v/w). Five pots were used for each treatment, and each pot was considered as one replicate. Apparently healthy seedling (45-days-old), or 5 seeds were used for planting in 20 and 10 cm diam. pots, respectively.

Pre- and post-emergence damping-off were recorded, 20 and 60 days after seed planting, respectively, while percentages of infection were recored 3, 6, 9 weeks after planting, when seedlings were used. However, occurrence of the diseases was recorded when symptoms of yellowing, stunting, wilting and/or rotting were observed.

Chemical and biological control:

Three commercial formulations namely Topsin M 70% WP, Vitavax/Thiram 75%WP and Humix were used. The first two fungicides were used either at the rate of 3g/L water (dipping treatment) or at the rate of 3g/kg seeds (seed treatment), and Humix (Humic acid 12% + manganese 0.5% + zinc 1% + ferric 1% + copper 200 ppm + boron 100 ppm + calcium 200 ppm + traces of sulphur, molybdenum and cobalt) at the rate 12 ml/L water were used for seedlings only. Also, Plant Guard (*Trichoderma harzianum*; $3x10^7$ cfu/ml) and Rhizo-N (*Bacillus subtilis*; $30x10^7$ cfu/gm) as commercial bioagents were used. Seeds were immersed in plant Guard (4 ml/L water) for 12 hours before planting, while Rhizo-N was used as seed dresser (4 g/ kg seeds) with gum Arabic as sticker. As for seedlings, apparently healthy ones (45-days-old) were, however, dipped in suspension of each tested compound (2 gm/L water) or biocide (4 ml or 4 gm/L water) for 20 minutes before planting in infested or uninfested soil.

Percentages of pre-and post-emergence damping-off, 20 and 60 days after seed planting, respectively, or percentages of infection 3, 6 and 9 weeks after planting seedlings were recorded.

RESULTS

I. Isolation, identification and frequency of the isolated fungi:

One hundred purified fungal isolates were obtained from rotted root and wilted seedlings and plants (Fig. 1). Seven species belonging to four genera of the 10 isolated were identified as: *Fusarium moniliforme, F. oxysporum, F. semitectum, F. solani, Pythium debaryanum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Table 1). *Fusarium moniliforme* (21.3%), *F. oxysporum* (17.2%) and *F. solani* (17.6%) showed the highest occurrence percentages, followed by *F. semitectum* (11.1%), *P. debaryanum*

Fig. 1. Rue plants (10-months-old) showing natural infection symptoms of root rot on foliar growth at the beginning of disease development. Symptoms began to appear at the flowering stage.

(10.9%) and *R. solani* (13.3%). On the other hand, *Alternaria, Aspergillus, Helminthosporium, Nigrospora, Rhizopus* and *Trichoderma* recorded the lowest percentages of occurrence being 1.7%, 0.4%, 0.2%, 0.9%, 0.6% and 0.5%, respectively.

Table 1. Occurrence percentages of the fungi isolated from infected roots and stems of rue.

Fungi	Frequency %	Source and symptoms
Fusarium moniliforme	21.3	root rots
F. oxysporum	17.2	root & stem* rot & wilt
F. semitectum	11.1	root rots
F. solani	17.6	root rots
Pythium debaryanum	10.9	root & stem rots
Rhizoctonia solani	13.3	root & stems
Scelrotinia scelrotiorum	4.3	stem rots
Alternaria sp.	1.7	root rots
Aspergillus sp.	0.4	stem rots
Helminthosporium sp.	0.2	stem rots
Nigrospora sp.	0.9	stem rots
Rhizopus sp.	0.6	root rots
Trichoderma sp.	0.5	root rots

* Stems = basal stems.

II. Pathogenicity studies:

1. Seeds planting:

Data in Table (2) show that most of the tested fungi recorded more than 50% of pre-emergence damping-off. *Pythium debaryanum* (80.0%) and *R. solani* (60.0-66.7%) were, however, the most aggressive fungi, whereas *F. oxysporum* (33.3%), *S. sclerotiorum* (33.3%) and *F. semitectum* (46.7%) showed the least incidence percentages. On the other hand, all the tested fungi caused the post-emergence phase of the disease (6.7%-46.7%). Both *F. oxysporum* and *S. sclerotiorum* resulted in 46.7% disease incidence. As for survivals after 60 days of planting, only 13.3% to 33.3% were recorded and the least percentages (13.3%) was recorded with *P. debaryanum.*

Table 2. Pathogenicity tests of nine fungi to *Ruta graveolens* planted with seeds, under greenhouse conditions.

Fungi	% infection		%
	Pre-emergence	Post-emergence	Healthy
	20 days after planting	60 days after planting	survivals
Fusarium moniliforme (No. 6)	53.3	20	26.7
Fusarium moniliforme (No. 9)	53.3	26.7	20
F. oxysporum	33.3	46.7	20
F. semitectum	46.7	20	33.3
F. solani	53.3	26.7	20
Pythium debaryanum	80	6.7	13.3
Rhizoctonia solani (No. 7)	66.7	13.3	20
R. solani (No. 8)	60	13.3	26.7
Scelrotinia scelrotiorum	33.3	46.7	20
Control (without fungus)	0	0	100
LSD at 5%	9.4	8.4	-

2. Seedlings planting:

All the tested fungi (Table, 3) were able to cause root rot and wilt diseases to rue seedlings (Figs. 2, 3). Percentages of infection were gradually increased by time elapse from 3 to 6 and 9 weeks. It reached (11.1% - 66.6%), (33.3%-88.9%) and (44.4% - 88.9%) at 3, 6 and 9 weeks after transplanting, respectively. *R. solani* No. 8 (66.6%) and *P. debaryanum* (88.9) after 3 and 6 weeks of transplanting gave significantly higher percentages of infection than the other tested fungi in most cases. On the other hand, *F. oxysporum* (11.1%) and *P. debaryanum* (11.1%) followed by *R. solani* No. 8 (22.3%) resulted in the least percentages of healthy survival plants, while *F. semitectum* (44.5%) and *S. sclerotiorum* (55.6%) gave the highest ones.

III. Control studies:

A. seed dressing:

Data (Table 4) indicate that the effectiveness of fungicides and bioagents in controlling the studied diseases varied according to the fungus and the type of pesticide used. However, the incidence of pre-and post-emergence-damping-off caused by *F. moniliforme, F. oxysporum* and *S. sclerotiorum* was significantly decreased by using either Vitavax/Thiram or Topsin M, while, the bioagents were not effective. *Pythium*

Table 3. Pathogenicity tests of nine fungi to *Ruta graveolens* seedlings, under greenhouse conditions.

Fungi	% infection after (weeks)			Mean	Healthy survival
	3	6	9		
Fusarium moniliforme (No. 6)	22.2	44.4	66.6	44.4	44.4
Fusarium moniliforme (No. 9)	22.2	55.5	66.6	48.1	44.4
F. oxysporum	44.4	66.6	88.9	66.6	11.1
F. semitectum	11.1	44.4	55.5	37	44.5
F. solani	22.2	55.5	66.6	48.1	44.4
Pythium debaryanum	44.4	88.9	88.9	74	11.1
Rhizoctonia solani (No. 7)	55.5	55.5	66.6	59.2	44.4
R. solani (No. 8)	66.6	66.6	77.7	70.3	22.3
Scelrotinia scelrotiorum	11.1	33.3	44.4	29.6	55.6
Control (without fungus)	0.00	0.00	0.00	0.00	0.00
Mean	33.3	56.7	69.1	-	

LSD at 5% for Fungi (F) = 14.0
Periods (P) = 7.7
F x P = 24.3

Table 4. Effect of chemical and biological treatments on the percentage of damping-off of *Ruta graveolens* as seed dressing, under greenhouse conditions.

Fungi		Vitavax/Thiram	Topsin M	Rhizo N	Plant Guard	Control (without treatment)
Fusarium moniliforme *	Pre-	13.3	20	20	26.7	60
	Post-	6.7	20	20	33.3	40
F. oxysporum	Pre-	6.7	6.7	13.3	13.3	20
	Post-	26.7	33.3	46.7	60	66.7
Pythium debaryanum	Pre-	26.7	33.3	33.3	33.3	60
	Post-	13.3	13.3	13.3	20	33.3
*R. solani***	Pre-	20	20	26.7	33.3	53.3
	Post-	6.7	13.3	13.3	20	26.7
S. scelrotiorum	Pre-	6.7	6.7	13.3	20	33.3
	Post-	20	26.7	33.3	33.3	40
Mean	Pre-	14.7	17.3	21.3	25.3	45.3
	Post-	14.7	21.3	25.3	33.3	41.3

* *F. moniliforme* (Nos, 6 & 9).
** *R. solani* (Nos. 7& 8).

LSD at 5% for:

	Pre-	Post-
Fungi (F) =	3.1	3.6
Fungicides (Fu) =	3.1	3.6
F x Fu =	7.0	8.0

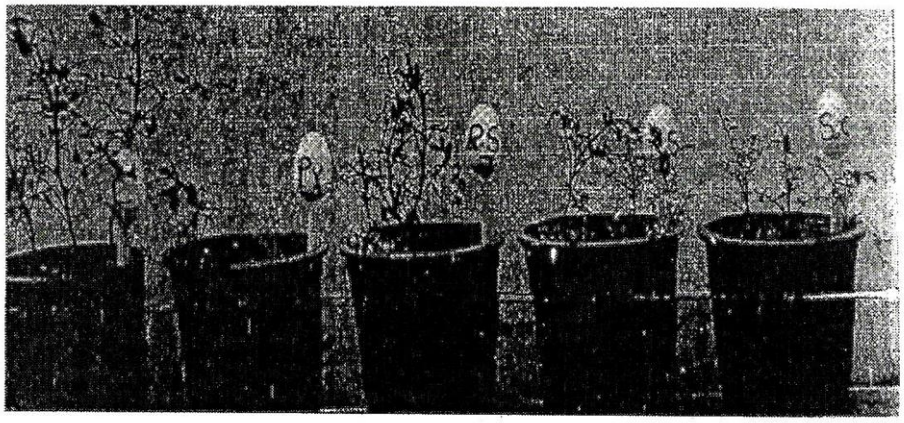

Fig. 2. Showing symptoms of stunting, yellowing, weathering and/or defoliation as a result of root rot incited by *P. debaryanum*, (Py), *R. solani* No. 7, (R.s7) *R. solani* No. 8 (R.s8) *S. sclerotiorum* (Sc) and control (c) 60 days after transplanting in infested soil.

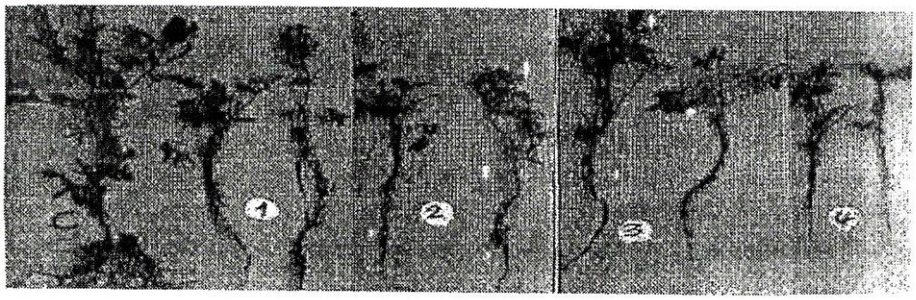

Fig. 3. Symptoms of roots rots caused by *F. solani* (No. 1), *R. solani* (No. 3) and *S. sclerotiorum* (No. 4), wilt incited by *F. oxysporum* (No. 2) and control (c).

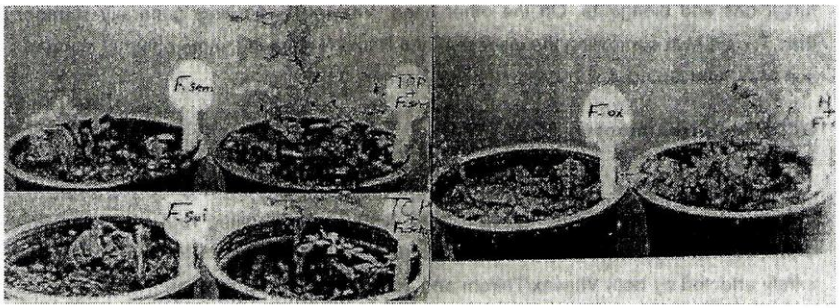

Fig. 4. Showing effectiveness of using Topsin M (dip treatment) against *F. semitectum* (*F.sem*) and *F. solani* (*F.so*) and Humix (H) against *F. oxysporum* (*F.ox*).

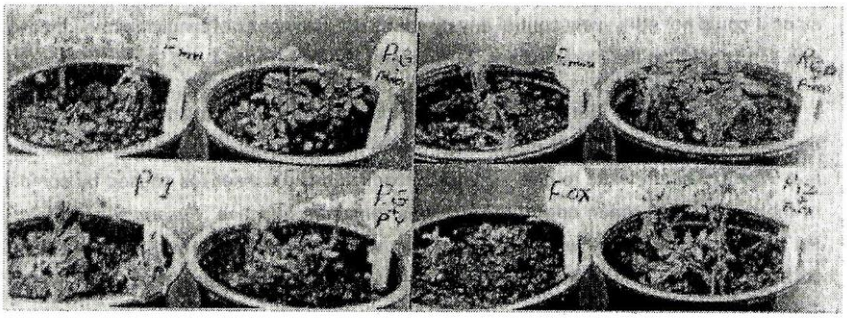

Fig. 5. Showing effectiveness of using Plant Guard (p.G), (dip treatment) against *P. debaryanum* (Py) and *F. moniliforme* (*F.mon*) and Rhizo-N (Rizo) against *F. moniliforme* (*F.mon*) and *F. oxysporum* (*F.ox*).

debaryanum and *R. solani* were, however, significantly controlled by each one of the fungicides and bioagents. On the other hand, Vitavax/Thiram was generally superior than Topsin M in controlling the diseases, and Rhizo-N gave the same effect in comparison with Plant Guard.

B. Dipping treatments:

Data presented in Table (5) show that Vitavax/Thiram was generally the most effective commercial product among those tested in controlling root rot and/or wilt diseases. Infection with *F. moniliforme* (No. 6 & 9) and *R. solani* (No. 7) were significantly affected by both Vitavax/Thiram and Topsin M., in certain cases. Also, *F. moniliforme;* No. 6 (Topsin M), *F. semitectum* (Vitavax/Thiram) and *S. sclerotiorum* (Topsin M) were significantly controlled after 9 weeks with the refered fungicides.

Both bioagents (Rhizo-N and Plant Guard) significantly decreased diseases incidence in comparison with the control in case of only *F. moniliforme* (No. 6) and *F. solani,* whereas, Rhizo-N alone gave significantly effective action against *F. oxysporum*, *F. semitectum* and *P. debaryanum* and Plant Guard against *F. moniliforme* (No. 9) and *R. solani* (No. 7 and 8). On the other hand, Humix product was the inferior compound, since it could not effectively control any diseases. Effectiveness of fungicides and bioagents in controlling the studied diseases is clearly shown in Figs (4 and 5).

Effectiveness of Plant Guard or Rhizo-N in controlling root rot and/or wilt diseases were approximately equal to those of Topsin M. In spite of the fact that Topsin M and Vitavax/Thiram failed to give significant reaction against diseases caused by some of the tested fungi, each one of the biocides remained effective. *Fusarium monilifore* (9) incidence was 44.4% with Vitavax Thiram, while it was (22.2%) with Rhizo N and 33.3% with Pland Guard. Also, *Fusarium solani* infection percentage was 33.3% with Topsin M, while it was 6% and 11.1% with Plant Guard. *Rhizoctonia solani* infection percentage was 33.3% with Topsin M, while it decreased to 22.2% with Plant Guard.

Table 5. Effect of chemical and biological control on percentages of root rot and wilt diseases of *Ruta graveolens*, under greenhouse conditions.

Fungi		Vitavax/ Thiram	Topsin M	Rhizo N	Plant Guard	Humix	Control (without treatment)
Fusarium moniliforme "6"	3 *	0.0	11.1	11.1	0.0	11.1	22.2
	6 *	11.1	22.2	11.1	22.2	33.3	44.4
	9 *	33.3	55.5	44.4	44.4	66.6	77.7
Fusarium moniliforme "9"	3 *	11.1	0.0	11.1	11.1	22.2	33.3
	6 *	33.3	22.2	22.2	22.2	33.3	44.4
	9 *	44.4	44.4	22.2	33.3	55.5	66.6
F. oxysporum	3 *	0.0	11.1	11.1	11.1	11.1	33.3
	6 *	11.1	22.2	22.2	33.3	44.4	55.5
	9 *	22.2	44.4	22.2	55.5	66.6	77.7
F. semitectum	3 *	0.0	0.0	0.0	11.1	0.0	22.2
	6 *	33.3	11.1	22.2	22.2	33.3	44.4
	9 *	33.3	22.2	22.2	44.4	44.4	55.5
F. solani	3 *	11.1	11.1	0.0	0.0	11.1	22.2
	6 *	11.1	33.3	33.3	11.1	44.4	66.6
	9 *	11.1	33.3	33.3	22.2	55.5	66.6
P. debaryanum	3 *	11.1	0.0	11.1	0.0	22.2	33.3
	6 *	33.3	0.0	44.4	33.3	55.5	66.6
	9 *	33.3	22.2	44.4	55.5	55.5	77.7
R. solani "No. 7"	3 *	0.0	0.0	11.1	11.1	11.1	33.3
	6 *	11.1	33.3	33.3	22.2	44.4	55.5
	9 *	33.3	33.3	44.4	22.2	44.4	55.5
R. solani "No. 8"	3 *	0.0	0.0	11.1	0.0	11.1	22.2
	6 *	0.0	33.3	33.3	11.1	33.3	44.4
	9 *	22.2	33.3	44.4	22.2	44.4	66.6
S. scelrotiorum	3 *	0.0	0.0	0.0	11.1	0.0	33.3
	6 *	0.0	11.1	0.0	22.2	22.2	33.3
	9 *	11.1	22.2	22.2	22.2	22.2	44.4
Mean	3 *	3.7	3.7	7.4	6.2	11.1	28.4
	6 *	16.0	21.0	24.7	22.2	38.2	50.6
	9 *	27.1	34.5	33.3	35.8	50.6	65.4

* weeks after planting.

LSD at 5% for:

Fungi (F) =	7.4	F x P =	12.9
Fungicides (Fu) =	6.1	Fu x P =	10.5
Period (P)	4.3	F x Fu x P =	31.4
F x Fu =	18.2		

DISCUSSION

According to the available literature, there are no previous studies concerning fungal diseases on rue (family *Rutaceae*) in Egypt or elsewhere; however, the same observed diseases were recorded on medicinal and aromatic plants belonging to the rue family. Root rots and wilt diseases were reported to cause considerable losses on several medicinal and aromatic plants in Egypt and all over the world. These plants were pelargonium and marjoram (Hilal *et al.*, 1998), chamomile (Abdel-Sattar *et al.*, 1987b and Hilal *et al.*, 1998) cumin (Hilal *et al.*, 1993 and 1998), coriander and fennel (Agnihotri, 1991) and basil (Garibaldi *et al.*, 1997 and Hilal *et al.*, 1998). However, *Fusarium* spp., *Pythium* spp., *R. solani* and *S. sclerotiorum* are among the causal pathogens of root rots and wilt of the afore-mentioned plants similar to those found on rue plants in the present investigation.

Using fungicides as an effective procedure of soilborne diseases management either as seed dressing or dipping treatment for cuttings or seedlings of medicinal and aromatic plants are recommended by several investigators (Abdel-Sattar *et al.*, 1987a; Mohamed *et al.*, 1987 Hilal *et al.*, 1993; Agnihotri, 1991; Garibaldi *et al.*, 1997). Topsin M and/or Vitavax/Thiram were, however, effective fungicides against root rots and/or wilt of basil, chamomile, coriander, cumin, fennel, marjoram and pelargonium similar to those found against rue diseases.

Plant Guard (*Trichoderma harzianum*) and Rhizo-N (*Bacillus subtilis*) were found to be promising alternatives to chemical fungicides, since their values as seed dressing were approximately equal to Topsin M. Moreover, they were sometimes more effective as dipping treatments than Topsin M and Vitavax/Thiram. Considerable values of these two bioagents against root rots and wilt diseases of rue were also found on a wide range of plant varieties including medicinal plants (Cook and Baker, 1983; Hilal and Helmy, 1998 and Hilal and Baiuomy, 2000). Biological control is, also, very useful in reducing undesirable environmental pollution and public exposure to pesticides.

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تسجيل امراض اعفان الجذور والذبول لنبات السذب الطبي لأول مرة في مصر ومقاومتها

عالية عبد الحميد حلمي ، محمد احمد محمد بيومي ، عرفه عبد الجليل هلال

قسم بحوث أمراض نباتات الزينة والطبية والعطرية - معهد بحوث امراض النباتات - مركز البحوث الزراعية

تم تسجيل بعض الأمراض الفطرية الجديدة من خلال الحصر الذي أجرى على زراعات السذب والتي لم تكن معروفة من قبل في مصر أو أي مكان آخر وهي امراض اعفان الجذور والذبول، ولقد تم عزل عشرة أجناس فطرية حيث كان الفطر فيوزاريوم مونيليفورم اكثرها تكراراً في العزل (٢١,٣٪) يليه الفطرين فيوزاريوم سولاني (١٧,٦٪) وفيوزاريوم أكسيسبورم (١٧,٢٪) بينما احتلت الفطريات ريزوكتونيا سولاني (١٣,٢٪) وفيوزاريوم سيتكتم (١١,١٪) وبيثيم ديباريانم (١٠,٩٪) المرتبة الثانية في هذا المجال. ولقد أثبتت تجارب العدوى الصناعية بزراعة البذور أو البادرات أن الفطريات فيوزاريوم أوكسيسبورم، بيثيم ديباريانم وريزوكتونيا سولاني كانت أكثر الفطريات المختبرة قدرة على إحداث المرض.

أدت زراعة البذور المعاملة بالمبيدين الفطريين توبسن م وفيتافاكس - ثيرام وزراعتها في تربة ملوثة بالفطر إلى مقاومة امراض ذبول البادرات المتسببة عن الفطريات فيوزاريوم مونيليفورم، فيوزاريوم اكسيسيورم وسكليروتنيا سكليروتيورم بدرجة معنوية في حين لم يؤد استخدام المبيدات الفطرية أو الحيوية (بلانت جارد وريزو - ن) إلى خفض الاصابة بدرجة معنوية في التربة الملوثة بأي من الفطرين بيثيم ديباريانم أو ريزوكتونيا سولاني، ومن جهة أخرى كان المبيد الفطري فيتافاكس ثيرام هو أكثر المبيدات الفطرية المستخدمة في معاملة الشتلات فعالية لمقاومة أمراض السذب، على الرغم من فشله في إظهار فعالية كافية لبعض هذه الأمراض في بعض الحالات، ولقد اختلفت فعالية المبيدين الحيويين بلانت جارد وريزو (ن) عند استخدامها لغمر الشتلات تبعاً للمسبب المرضي. ولقد كان الريزو (ن) هو أفضل المبيدات الحيوية المختبرة بصفة عامة، ولقد تساوت فعالية المبيدات الحيوية في مقاومة هذه الأمراض تقريباً مع المبيد الفطري توبسن م بل إنها نجحت في بعض الحالات التي فشلت فيها استخدام المبيدات الفطرية.