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Egypt. J. Phytopathol., Vol. **38**, No. 1-2, pp. 201-211 (2010)

Application of Fungicide Alternatives for Controlling Endive Wilt and Root Rot

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Isolates of *Fusarium solani, F. semitectum, F. oxysporum, Trichoderma harzianum* and *Chaetomium* sp. were isolated from diseased endive roots collected from two governorates, *i.e.* Giza and Behera. Results indicated that *F. solani* was the most dominant in the two governorates.

All the isolated fungi were pathogenic to endive plants except *Trichoderma harzianum* and *Chaetomium* sp., however, pathogenic fungi varied in their virulence. In general, *F. oxysporum* and *F. solani* were the most pathogenic.

The antagonistic effect of *T. harzianum* and *Chaetomium* sp. against mycelial growth of *F. solani, F. semitectum* and *F. oxysporum* was *in vitro* investigated. Generally, the bioagents tested significantly decreased the mycelial growth of the pathogenic fungi.

Some salts, bioagents, antioxidants and the fungicide Topsin M70 were used to control endive diseases under greenhouse conditions. Results indicated that all the tested materials reduced infection by endive root diseases and increased the survived plants as well as their yield and improved its quality. These materials varied in their efficiency. The fungicide Topsin M70 was the best treatment for controlling the disease followed by Plant guard (as commercial bioagent) and salicylic acid (as antioxidant). Also, oxalic acid (as antioxidant) was moderately effective, while salts, *i.e.* mono-potassium sulphate and calcium chloride showed the lowest effect.

Keywords: Antagonism, antioxidants, bioagents, endive, fungicide alternatives and salts.

Endive (*Cichorium endivia* L.) is an edible annual leafy plant of the family Asteraceae, variably believed to have originated in Egypt and Indonesia and cultivated in Europe since the 16th century (Omar, 2010). Endive varieties form two groups, the much curled or narrow leaved and the Batavian or broad leaved as a biennial. About 3 months after sowing, the plants outer leaves are tied together or covered to exclude light. This prevents the development of the natural bitter taste. This bleaching process takes 10 days to 4 weeks (Jegyvig, 2010).

Endive contains sufficient amount of vitamins A, B and C. Endive juice along with carrot and celery is very rich in vital minerals, *i.e.* iron, sodium and calcium. Endive juice also has many medicinal uses in cases of asthma, skin diseases, biliousness, poor blood, gall-stones and gall- bladder irritation, diseases of the urinary tract, stomach ulcers, inflammation of middle ear and for general body building. Endive is a good source of beta-carotene, which is inverted in the body into vitamin A. Beta-carotene, is widely regarded as an effective antioxidant and immune system booster. An elevated homocysteine level is a risk factor for heart diseases. Endive is a good source of heart healthy potassium, with one average-sized head of endive delivering over 50% of the potassium found in a banana and endive delivers significant levels of vitamins B and C, as well as folate and selenium (Dean *et al.*, 2007).

In Egypt, endive has been newly introduced to agriculture. Roots are usually imported from the Netherlands to be used as propagative materials. Endive diseases include Anthracnose (*Microdochium panattonianum*), Botrytis gray mould (*Botrytis cinerea*), Bottom rot (*Rhizoctonia solani* and *Fusarium* spp.), Damping-off (*Pythium* spp.), Downy mildew (*Bremia lactucae*), Drop (*Sclerotinia sclerotiorum* and *S. minor*) and many viral and bacterial diseases (Davis *et al.*, 1997 and Zitter and Provvidenti, 1984).

Biological control of plant pathogens using antagonistic microorganisms has great interest during the past decades. This interest is in part, due to the desire to enhance the sustainability of agriculture and horticulture and also because biocontrol may provide control of plant diseases that cannot or only partially, be managed by other methods (Cook and Baker, 1983 and Guarro *et al.*, 1995).

On the other hand, acquired resistance to pathogen infection has been observed in a number of angiosperms, including tobacco, cucumber, and different monocots (Kessmann *et al.*, 1994 and Hammerschmidt and Smith, 1997). When plants are pretreated with a necrotizing pathogen, long-lasting, broad-spectrum resistance may be induced to subsequent pathogen infections (Ryals *et al.*, 1994). Such acquired resistance can be expressed locally at or very near the pretreatment site (Ross, 1961a), or systemically (Ross, 1961b and Ryals *et al.*, 1996). Activation of acquired resistance seems to depend on prevent the development of pathogen due to plant cell death (Dangl *et al.,* 1996 and Ryals *et al.,* 1996). Ertan *et al.* (2008) found that salicylic acid (SA) as foliar applications resulted in greater shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight as well as higher cucumber plants under salt stress. Generally, the greatest values were obtained from 1.00 mM SA application. Based on these findings, the SA treatments may help alleviate the negative effect of salinity on the growth of cucumber.

Fungicides could successfully control root-rot diseases, however, it negatively affect human health and environment (Rauf, 2000). Therefore, recently there are several attempts to use fungicide alternatives for controlling plant diseases. In this concern, some salts as potassium chloride, mono-potassium sulphate and potassium sulphate are reported to have antifungal activities against several fungi (Napier and Oosthuyse, 1999; Abd-Alla, 2003; El-Mougy, 2002 and El-Mougy *et al.,* 2004).

Also, El-Mougy (2004) used salicylic acid (SA) or acetyl salicylic acid (ASA) as seed dressing or soil drenches which reduced root-rot infection of lupin plants under greenhouse conditions. Furthermore, resistance against plant pathogens can be also increased by salicylic acid or acetylsalicylic acid (Floryszek and Wieczorek, 1993). Moreover, the effect of Salicylic acid (SA) on induced resistance of cucumber seedlings against downy mildew was widely studied (Sun and Yang, 2004).

The present study aimed to evaluate the efficiency of some slats, antioxidants and bioagents as root soaking of endive to control endive root-rot and wilt diseases under greenhouse.

Materials and Methods

1. Isolation and identification:

Naturally infected endive plants (var. Vintor) showing root rot and wilt symptoms were collected from Giza and Behera governorates, during the two successive seasons 2007/08 and 2008/09, for isolation the causal organisms. Infected roots were cut into small pieces, washed thoroughly with tap water, surface sterilized with sodium hypochlorite (5% chlorine) for one minute, washed several times with sterilized water and then dried between folds of sterilized filter papers. Infected pieces were then placed on PDA and incubated at 28 °C for 5 days. The developed fungal colonies were purified by hyphal tip and single spore techniques. The growing fungi were then transferred onto Petri-dishes containing plain agar (Brown, 1924 and Keitt, 1915). Purified fungi were placed on slaps of PDA medium and kept for further studies.

Identification of the isolated fungi was carried out according to the cultural properties, morphological and microscopical characteristics described by Barnett (1960) and Singh (1982). Identification was confirmed in the Dept. of Fungal Taxonomy, Plant Pathol. Res. Inst., ARC, Giza, Egypt.

2. Pathogenicity tests:

Fungi isolated from the infected roots were tested for their pathogenicity on endive roots through soil infestation technique. Inocula were prepared by inoculating the isolates separately into autoclaved sand corn medium (25g clean sand, 75g corn and enough tap water to cover the prepared mixture in 500ml bottles) using agar discs obtained from the periphery of 7 days old colony of the desired fungus and incubated at 25±2°C for two weeks.

Pots (20cm in diam.) were sterilized by immersing in a 5% formalin solution for 15 minutes and left for 7 days before use. Each pot was filled by Nile silt soil, then infested with the desired inoculum at the rate of 5% of soil weight. Inocula were thoroughly mixed with the soil and watered regularly for one week before planting to insure the distribution and growth of the tested fungi (Whithchead, 1957). Pots used for control were filled with the same soil mixed with similar sterilized amount of autoclaved uninoculated sand corn medium. Sixteen pots were used for each particular treatment (one root for each pot). They were divided into four replicates, each consisted of four pots. Pots were completely randomized in the greenhouse. Root rot and wilt infection was assessed as percentage of dead plants 30 days after planting.

3. In vitro effect of the antagonistic fungi on the pathogenic fungi:

The antagonistic effects of *T. harzianum* and *Chaetomium* sp. on the linear growth of *F. oxysporum, F. solani* and *F. semitectum* were *in vitro* investigated using PDA plates. Each Petri dish was inoculated at one side with a disc, 5mm. of each antagonistic fungus taken from the margins of 7 days old cultures and the opposite side was inoculated with a 5 mm. disc taken from 7 days old cultures of each pathogenic fungus. Dishes were incubated at 28°C for 7 days.

Percentage of reduction in linear growth of the tested fungi was determined using the following formula:

$$R = C\text{-}T / C \times 100$$

Whereas: R = Percentage of growth reduction.
C = Diameter of pathogenic hyphal growth in the control.
T = Diameter of pathogenic hyphal growth in the antagonistic treatment.

4- Greenhouse Experiment:

Some salts, bioagents, antioxidants and the fungicide Topsin M70 were used to control endive diseases under greenhouse conditions as follows:

a. Biocides:

The commercial product Plant Guard *(Trichoderma harzianum* at the rate of 3.0 ml/l. water), *Trichoderma harzianum* and *Chaetomium* sp. at rate of ($1x10^5$ spores/ml.) were tested for their efficacy for controlling the disease.

b. Chemical salts:

Calcium chloride and mono potassium sulphate, each at the rate of 4.0 g/l water.

c. Antioxidants:

Salicylic acid (SA) and oxalic acid, each was used at the rate of 0.1 g/l.

d. Standard fungicide:

Topsin M70 (methyl thiophanate) at 2.0 g/l water.

The above mentioned materials were used as root soaking for 30 min and as soil drench 7 days before soil infestation. Soils were artificially, separately, infested with the inoculum of the tested fungi as previously stated (*F. oxysporum, F. solani* and *F. semitectum*).

Infested soil was filled in pots (20 cm in diam.) and planted with endive roots previously washed in tap water.

Root rot and wilt incidence was assessed as percentage of dead plants 30 days after planting.

Statistical analysis:

The obtained data were subjected to analysis of variance (Steel and Torrie, 1960) whereas the differences between treatments were tested by the calculated Least Significant Differences (LSD) at 5% level.

Results and Discussion

Isolation from diseased endive plants (showing wilt and root rot symptoms) yielded several fungi. The isolated fungi were identified as *Fusarium oxysporum, F. semitectum, F. solani, Chaetomium* sp. and *Trichoderma harzianum*.

Data in Table (1) demonstrate that several fungi were isolated from endive roots grown in the two governorates, however, they showed different frequencies. *F. oxysporum* showed the highest frequency followed by *F. solani*, *F. semitectum*, *T. harzianum* and *Chaetomium* sp. In most cases, the higher frequency percentages were obtained from Behera governorate. These variations among the isolated fungi may be due to the prevailing meteorological conditions, soil type and the suitable requirements for each fungus and/or the susceptibility of the cultivated variety. Also, endive infection with wilt and root rot was more severe during of 2008/2009 than during of 2007/2008.

Table 1. Frequency of fungi isolated from endive plants naturally grown in two governorates during the two successive seasons 2007/08 and 2008/09

Isolated fungus	Frequency (%) of the isolated fungi					
	season 2007 / 08			season 2008 / 09		
	Giza	Behera	Mean	Giza	Behera	Mean
Fusarium oxysporum Schlecht	28.0	29.3	28.7	29.6	30.0	29.8
F. solani (Mart.) Sacc.	37.3	40.0	38.7	39.2	40.0	39.6
F. semitectum Berk&Rav	17.3	18.7	18.0	19.2	20.0	19.6
Trichoderma harzianum Rifai	10.7	10.7	10.7	9.6	6.7	8.2
Chaetomium sp.	6.7	1.3	4.0	2.4	3.3	2.9

Pathogenicity test:

Pathogenic capabilities of the fungi isolated from endive roots were tested. Data in Table (2) show that only three fungi associated with endive roots were pathogenic, however, the percentage of infection was differed from one pathogen to another. The two fungi, *Chaetomium* sp. and *T. harzianum* were not pathogenic.

F. oxysporum showed the highest infection followed by *F. solani* and *F. semitectum*. Generally, the pathogenic fungi were more active during the second season. The obtained data are in agreement with those found by Davis *et al.* (1997) who found that *Fusarium* spp. was able to infect endive plants.

Table 2. Pathogenicity of fungi on endive plants (var. Vintor) under greenhouse conditions (2007 / 08 and 2008 / 09)

Tested fungus	Dead plants (%)		Mean
	2007/ 2008	2008/2009	
F. oxysporum	81.3	87.5	84.4
F. solani	56.3	62.5	59.4
F. semitectum	43.8	50.0	46.9
Trichoderma harzianum	00.0	00.0	00.0
Chaetomium sp.	00.0	00.0	00.0
Control	00.0	00.0	00.0
Mean	30.23	33.33	
L.S.D. at 0.05 for: Fungi (A)= 1.86	Growing season (B)= 0.93		A x B= 0.33

Antagonism in vitro:

The effect of antagonists on mycelial growth of the tested fungi (*F. oxysporum*, *F. solani* and *F. semitectum*) is shown Table (3). Data show that the bioagents tested decreased the mycelial growth of the pathogenic fungi on PDA medium incubated for 7 days at 28°C.

Side by side, several bioagents are usually found along with the pathogens, which show an antagonistic reaction for instance, most species of the genus *Trichoderma* are able to antagonize many plant pathogenic fungi and sometimes give equal control effects to those of certain fungicides (Elad *et al.*, 1980 and Bell *et al.*, 1982).

Table 3. ***In vitro*** **effect of some fungal bioagents on the pathogenic fungi growth**

Tested fungus	Antagonistic bioagent			
	T. harzianum		*Chaetomium* sp	
	Linear growth	Reduction (%)	Linear growth	Reduction (%)
F. oxysporum	1.5	83.3	3.0	57.1
F. semitectum	2.0	77.7	3.0	57.1
F. solani	2.0	77.7	2.5	64.3
T. harzianum	9.0	0.0	4.5	35.7
Chaetomium sp	2.5	72.2	7.0	00.0

Disease control under greenhouse conditions:

Different materials (salts, biocides and antioxidants) compared with the standard fungicide Topsin M70 were applied to evaluate their efficiency in controlling endive wilt and root rot. Data in Table (4) indicate that all treatments significantly reduced the percentages of disease incidence. Results also, indicate that the most effective treatment was Topsin M70 (standard fungicide). Salicylic acid (SA) gave the best control followed by Plant guard, while the lowest one was the mono-potassium sulphate alternative and calcium chloride. Also, Plant guard gave the higher efficacy effect than both *T. harzianum* and *Chaetomium* sp. each alone. Meanwhile the obtained results show that mono-potassium sulphate, calcium chloride and *Chaetomium* sp. showed moderate effect on *F. semitectum* infection.

Many investigators evaluated similar tested materials, as antimicrobial inhibitors, for plant disease suppression. In this concern, the food preservatives potassium sulphate or sodium benzoate were reported to have antifungal activities against several fungi as well as post harvest decaying fungi (Oliver *et al.*, 1999).

Also, El-Mougy (2002 and 2004) and El-Mougy *et al.* (2004) used potassium sulphate, sodium benzoate and acetyl salicylic acid as alternatives of fungicides to control cowpea root-rot under greenhouse and field conditions. They found that these materials proved to be active in reducing pre- and post-emergence damping-off and increasing the plant survival and gave high cowpea yield.

Table 4. Effect of treatment endive roots by some salts, biocides, antioxidants and the fungicide (Topsin M70) on the percentage of dead plants under greenhouse conditions during 2007/08

Treatment	Rate of application (%)	Dead plant (%)					
		F. oxysporum	Efficiency	*F. solani*	Efficiency	*F. semitectum*	Efficiency
a) salts:							
Mono potassium sulphate	4.0 g	75.0	11.1	62.5	00.0	37.5	00.0
Calcium chloride	4.0 g	68.8	18.5	56.3	9.[illegible]	37.5	00.0
b) antioxidants:							
Salicylic acid	0.1 g	31.5	62.7	25.0	60.0	18.8	49.9
Oxalic acid	0.1 g	37.5	55.6	25.0	60.0	25.0	33.3
c) bioagents:							
Plant Guard	3.0 ml	25.0	70.4	18.5	70.4	12.5	66.7
T. harzianum	1 x 10^5 spores/ml	50.0	40.8	37.5	49.6	31.5	16.0
Chaetomium sp	1 x 10^5 spores/ml	56.3	33.3	43.8	29.9	37.5	00.0
D)Fungicide:							
Topsin M70	2.0 ml	12.5	85.2	6.3	89.9	6.3	83.2
Control		84.4	00.0	62.5	00.0	37.5	00.0
L.S.D. at 0.05 for:	Fungi (A)= 0.38		Treatment (B)= 023			A x B= 045	

Salicylic acid and/or acetyl salicylic acid (ASA) were also reported to induce resistance in many host-pathogen systems (Okuno *et al.,* 1991 and Walters *et al.*, 1993). Also, Schneider and Ullrich (1994) found that ASA increased the activities of chitinase and *B*-l, 3-glucanase. Moreover, Oliver *et al.* (1999) reported that treating potato plants with acetyl salicylic acid induced resistance against late and early blight diseases and increased tuber yield under field conditions. Ibrahim *et al.* (2003) found that Promot and Rhizo-N as bioagents and Kaligreen (82% potassium bicarbonate and sodium bicarbonate) were effective in controlling powdery mildew on cantaloupe plants. Moreover, El-Shimy *et al.* (2009) found the most effective treatment was salicylic acid at 0.05 g/l which recorded the lowest cucumber downy mildew severity on the three tested hybrids.

Results in the present study indicate that the tested materials such as salts, bioagents and antioxidants gave good control directly after Topsin-M 70 as a standard fungicide against endive root-rot, however, the latter has a high risk due to environmental pollution and its residual effects. The alternative materials are considered non harmful and have many advantages. Therefore, such materials could be used as effective and safe method for controlling soil borne plant pathogens in addition to, the avoidance of environmental pollution due to the decrease in the usage of chemical fungicides.

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(Received 18/10/2010;
in revised form 21/12/2010)

استخدام بعض بدائل المبيدات في مكافحة ذبول وعفن جذور نبات الشيكوريا

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تم الحصول علي عزلات الفطريات فيوزاريوم سولاني ، فيوزاريوم أوكسيسبورم ، فيوزاريوم سيميتكتام ، ترايكوديرما هيرزيانم ، كيتوميم وتم عزلها من جذور نبات الشيكوريا صنف فينتور وذلك من كلا من محافظة الجيزة والبحيرة.

تبين من النتائج أن فطر فيوزاريوم سولاني و فيوزاريوم أوكسيسبورم كانا اكثر الفطريات تكرارا . تم اختبار القدرة المرضية للفطريات والعزلات التي تم الحصول عليها من الصنف فينتور واختبرت بمفردها حيث تبين أن جميع الفطريات أحدثت إصابة بدرجات متفاوته في ما عدا الفطريين ترايكودرما وكاتوميوم .وجد ان كلا من الفطر فيوزاريوم سولاني و الفطر فيوزاريوم أوكسيسبورم هما الأكثر قدرة علي إحداث إصابات مرضية .

تم إجراء اختبار تأثير التضاد لكلا الفطريين (ترايكودرما وكاتوميوم) علي النمو الميسليومى في أطباق بتري علي باقي الفطريات المتحصل عليها. وقد وجد ان الفطريين المضادين كان لهما تأثيرا فعالا علي نقص النمو الميسليومي لكل الفطريات المختبرةالناميه في الأطباق علي بيئة البطاطس وأجار الدكستروز علي درجة حرارة ٢٨°م لمدة ٧ أيام .

تم اختبار بعض المواد لمقاومة أمراض الشيكوريا صنف فينتور وذلك بنقع الجذور ومعاملة التربة والنباتات وكانت المواد المستعملة بعض الأملاح والمركبات الحيوية ومضادات الأكسدة وقورنت تلك المواد بالمبيد القياسي (توبسن ام ٧٠) تحت ظروف الصوبة ، أظهرت المواد المستخدمة تحت ظروف الصوبة انخفاضا واضحا في نسبة الإصابة بالأمراض وأدت إلي زيادة معنوية في الصفات المحصولية .

تفاوتت المواد السابقة سواء في قدرتها على مقاومة الأمراض حيث كان المبيد توبسن ام أعلاها فعالية.

إيماء إلى النتائج المتحصل عليها من هذه الدراسة فانه ينصح بمقاومة إمراض الشيكوريا باستخدام المركبات السابقه وذلك لتجنب تلوث البيئة باستخدام المبيدات الفطرية خاصة إن أوراق هذا النبات هي التي تستخدم في الطعام.