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EFFICIENCY OF VARIOUS CHEMICAL TREATMENTS AGAINST FOLIAR DISEASE OF ENDIVE

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ABSTRACT

Isolates of *Botrytis cinerea, Alternaria cichorii and Penicillum sp.*were isolated from diseased endive leaves collected from two governorates, *i.e.* Giza and Behera. Results indicated that *Botrytis cinerea* was the most dominant in the two governorates. All the isolated fungi were pathogenic to endive plants except *Penicillum sp.* Some salts, antioxidants, Sulphar(natural substances and the fungicide Switch 62.5%(Cyprodil+Fludioxonil)were used to control endive diseases under greenhouse conditions. Results indicated that all tested materials reduced infection of endive by both fungal pathogen and increased the survived plants, plant yield and improved its quality. However, these materials varied in their efficiency. The fungicide Switch 62.5% was the best in controlling the disease followed by Sulphar (natural substances) and salicylic acid. Also, oxalic acid (as antioxidant) was moderately effective, while calcium chloride showed the lowest effect.

Keywords: Endive, foliage disease, control, fungicide alternatives, antioxidants, and mineral salts.

INTRODUCTION

Endive **(***Cichorium endivia* L**.)** is an edible annual leafy plant of the family Asteraceae. Endive was introduced to England, Germany, Holland, and France in the 13th century. The French used it primarily for medicinal purposes to "comfort the weake and feeble stomack and to help gouty limbs and sore eyes". Today, the main growing countries are Belgium, France, Holland, and Germany. The earliest mention of

it in North America was in 1803, and ever since, has created confusion in the culinary world. The ancient Egyptians ate, and probably cultivated, endive. Endive is foods high in vitamin K such as vegetables typically are associated with low cancer risk. Endive is a good source of beta-carotene, which is inverted in the body into vitamin A, and effective antioxidant and immune system booster. 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, an elevated homocysteine level is a risk factor for heart diseases (Omar, 2010).

In Egypt, endive has been newly introduced to agriculture. Endive diseases include Anthracnose (*Microdochium panattonianum)*, Botrytis gray mold (*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 (Jegyvig 2010). In Europe most of the studies included primarily *Rhizoctonia solani* (Camporota et al . 1989) and *Septoria intybi* Pass. (Cappelli and Stravato 1996); reports on biology, suppression, and other activities concerning pathogenic fungi are rather few *Alternaria cichorii* Nattrass (ascomycota, Dothideomycetes, Pleosporales) is also in Solvania a relatively in sufficiently known pathogenic fungus, although it can be and usually is especially in moist periods- economically the most damaging pathogen on endive plants. One of the few studies of *A. cichorii* deals with the fact that its organic extract contains at least six substances which are phytotoxic to various degrees (Stierle et al. 1993). *Alternaria cichorii* is infectious also for other species of the Asteraceae family, such as Lettuce, Chicory, Catalonha, and annual Sowthistle. Disease symptoms vary for different hosts, but it is known that they start sooner and develop much more rapidly on endive plants **(**Koike and Butler 1998**,** and Lima et al. 2003). In Egypt, *F. oxysporum* and *F. solani* were the most wilt and rot root pathogenic (El- Far et al., 2010). Trdan et al., 2008 reported that during 2004 and 2005 the activity of sulphur, soya lecithin, salicylic acid on endive and on the fungus *Alternaria cichorii* were investigated in a field block experiment were significantly less infected than untreated plants. (Trdan et al. 2004) reported the same substances previously showed activity in the growing of chicory under conditions where the fungus

Erysiphe cichoracearum DC. 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.

Recently, there are several attempts to use fungicide alternatives for controlling plant disease. In this concern, some mineral salts as potassium chloride, mono- potassium sulphate and potassium sulphate are reported to have antifungal activities against several plant pathogenic fungi (Napiro and Oosthuyse, 1999**)**

The present study aimed to evaluate the efficiency of some slats, antioxidants, sulphar and fungicide Switch 62.5% as spraying of endive leaves to control endive foliar diseases under greenhouse cultivation.

MATERIALS AND METHODS

Disease symptoms

1) Leaf spots grow very quickly and the exteriorl leaves turn yellow and disintegrate, black spots to large zonate lesions with brown centres.

2) Invasion normally begins on lower leaf margins and progresses throughout the head until it is a rotted slimy mess.

The disease may occur over a wide range of temperatures but is strongly favored by high humidity periods, and moderate temperatures

1. Isolation and identification of causal pathogen

Naturally infected endive plants (var. Vintor) showing leaf symptoms were collected from Giza and Behera governorates for isolation the causal organisms. Infected leaves were cut into small pieces, washed thoroughly with tap water, surface sterilized with sodium hypochlorite solution (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 technique. The growing fungi were then transferred onto Petri-dishes containing water 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 and morphological and microscopical characteristics described by Singh (1982). Identification was confirmed at the Dept. of Fungal Taxonomy, Plant Pathology Research Institute, ARC, Giza , Egypt.

2. Pathogenicity tests

Fungi isolated from the infected leaved were tested for their pathogenicity on endive leaved through infestation technique. The fungi isolated from Giza and Behera governorates were individually grown on PDA medium for 7 days and incubated $28^{o}C$. The result fungi growth was blended (in a blinder medium speed) with sutible amounts of distilled water (20ml) for 2 minutes. Suspensions were filtered through two layers of sterile clothcheet and mycelial fragments concentration was ($1x10^{5}$ fragments / ml), by using Hamocytometer slide technique according to Shahin and Shepared (1979). Inoculia,s suspensions were sprayed on endive leaves 15 days after planting using an atomizer at the same time, one group was sprayed with water to serve as control. Pots (20cm in diam.) were sterilized by immersing in a 5% formalin solution for 15 minutes and then left for 7 days before use. Each pot was filled by Nile silt soil. Sixteen pots were used for each particular treatment (one root for each pot).They were divided into four replicates, each consisted of three pots. Pots were completely randomized in the greenhouse. Seven leaves were chosen randomly from each pots, and were classified according to scale of 5 point rating representing a percent of surface infected area. The disease severity was estimated after 7 and 15 days of infected leaves, using the following scal:

Class	Infected surface area %
1	1 to 10 %
2	10.1 to 25 %
3	25.1 to 50 %
4	50.1 to 75 %
5	75.1 to 100 %

The following disease severity formula proposed by Kesevan and Chounhury (1977) was used to assess:

$$\sum (f \times v) / n \times X \times 100$$

F= frequency of a numerical rating.
V=numerical rating of the scal (1-5).
N= total number of tested plants.
X= maximal value (5) of the evaluation scale.

3- Control agents

a. Natural subsatances

Sulphar at the rate of 2.5 g/ l

b. Chemical salts

Calcium chloride, each at the rate of 4.0 g/l water.

c. Antioxidants

Salicylic acid (SA) and oxalic acid, were used at the rate of 1.0g/l.

d. Standard fungicide

Switch 62.5 % (Cyprodil+ Fludioxonil) at the rate 0.75 g/l..

3.1. Effect of different chemical on the linear growth of *Botrytis cinearia* and *Alternaria cichorii*

Different concentrations of various tested chamical treatment were study their inhibitory effect on linear growth of *Botrytis cinearia and Alternaria cichorii* in vitro.Three concentration of calcium chloride, salicylic acid and oxlalic acide (0.5,1.0,2.0), Sulphar (0.5,1.0,2.5), and Switch (0.1,0.5,1.0) were added individually to conical flasks containing sterilized PDA medium to obtin the proposed concentrations then mixed gently and dispensed in sterilized Petri plates (10-cm-dimeter). Plates were individually inoculated at the center with equal disks (6-mm).The average linear growth of fungus was estimated after 10 days.

3.2. Effect of different chemical on diseases severity under greenhouse experiment

The tested materials were used as spraying treatment, leaves were artificially, inculated, 7 days before treatment.The leaves were

two infested with the inoculum of tested fungi (*Botrytis cinereai* and *Alternaria cichorii*).

Infested plants were put in pots (20 cm in diam.) and sown with endive roots previously washed in tap water. Percentages of disease severity were estimated 15 days after inoculation with fungi. As well as the efficiency of the fungicides was calculated according to the following formula:

Efficiency % = Control - Treatment / Control x100

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 leaves plants showing leaf spots and gray mold symptoms yielded several fungi. The isolated fungi were identified as *Botrytis cinerea and Alternaria cichorii* .Data in Table (1) demonstrate that several fungi were isolated from endive leaves plants collected from two governorates; however, they showed different frequencies. *Botrytis cinerea* showed the highest frequency followed by *Alternaria cichorii* and *Penicillum sp.* In most cases, the higher frequency was obtained from Behera governorate. These variations among the isolated fungi may be due to the prevailing meteorological conditions, suitable requirements for each fungus and / or the susceptibility of the cultivated variety.

Table (1): Frequency of fungi isolated from endive plants, naturally grown in two governorates in Egypt during two successive seasons2009 and 2010.

Isolated fungi	Frequency of the isolated fungi, %					
	season 2009			season 2010		
	Giza	Behera	Mean	Giza	Behera	Mean
Alternaria ichorii .	30.8	32.0	31.4	36.7	42.7	39.7
Botrytis cinerea	45.8	46.7	46.3	48.3	40.0	44.2
Penicillum sp.	23.3	21.3	22.3	15.0	17.3	16.2

Pathogenicity test

Pathogenic capabilities of the fungi isolated from endive leaves were tested. Data in Table (2) show that only three fungi associated with endive leaves were pathogenic; however, the percentage of infection was differed from one pathogen to another. *Botrytis cinerea* showed the highest infection followed by *Alternaria ichorii*.and *Penicillum sp.* Generally, the pathogenic fungi were more active during the15 days obtained date are in agreement with those found by Trdan *et al.* (2008) and Jegyvig (2010) who found that *Botrytis cinerea* and *Alternaria ichorii* were able to infect endive plants.

Table (2): Pathogenicity of fungi on endive plants (var.Vintor) under greenhouse conditions.

Fungus	Disease severity , %		Mean
	After 7 days	After 15 days	
Botrytis cinerea	67.2	76.4	71.8
Alternaria cichorii	49.8	54.0	51.9
Penicillum sp.	0.0	0.0	0.0
Control	0.0	0.0	0.0
Mean	29.3	32.6	-

L.S.D at 0.05 for
Fungi (F) = 1.95 Period(p) = 3.21 F x P = 2.31

Effect of different chemical on the linear growth of *Botrytis cinearia* and *Alternaria cichorii*

Three concentrations of various tested chamical treatment (calcium chloride, salicylic acid and oxlalic acide 0.5,1.0,2.0, Sulphar 0.5,1.0,2.5, and Switch 0.1,0.5,1.0) were study their inhibitory effect on linear growth of *Botrytis cinearia and Alternaria cichorii* in vitro.

Data in Table (3) indicated that the reduction growth of *Botrytis cinearia and A. cichorii* in response of different chemical treatment. All tested concentrations reduced the mycelial growth of the pathogens compared with control. Also, it was observed that increase the tested concentrations of different chemical decreased the mycelial growth and increased the reduction. Result also, indicated that the highest reductions were the fungicide at 0.5 %, sulphar at 1 % and salicylic acid at 1 %, while the lowest one was the calcium chloride. Meanwhile, sulphar showed higher reduction on *A. cichorii* than on *Botrytis cinearia* .

Disease Control under greenhouse conditions.

Different materials (salts, biocides and antioxidants) compared with the standard fungicide Switch 62.5% were evaluated for controlling endive leaf spot and gray mold. Data in Table (4) indicate that all treatments significantly reduced the percentages of disease incidence. Results also, indicate that the most effective treatment were the fungicide Switch 62.5% ,Sulphar and Salicylic acid (SA) which gave the best control followed by oxalic acid , while the lowest one was the calcium chloride. Meanwhile sulphar treatment showed better effect on *A. cichorii* than on *B. cinerea* while calcium chloride show high effect on *Alternaria cichorii*.

Many investigators evaluated the tested materials, as antimicrobial inhibitors, for plant disease suppression. Nagaich et al. (2003), reported that foliar application of sulphur has a positive effect on the yield of vegetables. In can be concluded that sulphur applied on endive plants, even more than for chicory, acts more as a foliar fertilizer and less as an antifungal agent. This makes sense, since sulphur is not a fungicide registered against leafspots. The results of this study show that sulphur is suitable for the spraying of endive plants,since plants treated with sulphur had – compared to untreated plants. But it is not easy to determine the role of sulphur as a plant strengthener in the context of less pronounced infection of endive plants with *A. cichorii* .

On the other hand, salicylic acid is known to promote cell elongation and increase cell wall extensibility (Hossain et al. 2002). Moreover, the results another study also proved the considerable efficacy of salicylic acid against Alternaria leafspots (Colson-Hanks and Deverall 2000) and other pathogens (Cao et al. 2006).

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.

The results of the present study indicate that the tested materials such as mineral salts, and antioxidants gave good control directly after Switch 62.5 % as a standard fungicide against endive leaf spots and gray mold; 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 air borne plant pathogens in addition to, the avoidance of environmental pollution due to the decrease in the usage of chemical fungicides.

Table (3): Reduction of growth of *Botrytis cinearia* and *Alternaria cichorii* in response of different chemical treatment.

Treatment (T)	Concentration % ©	*Botrytis cinearia*		*Alternaria cichorii*	
		Linear growth (mm)	Reduction %	Linear growth (mm)	Reduction %
CaCl2	0.5	59.0	34.4	54.6	39.3
	1.0	48.2	46.4	46.4	48.4
	2.0	0.0	100	0.0	100
SA	0.5	34.2	62.0	32.2	64.4
	1.0	20.0	77.7	22.8	74.7
	2.0	0.0	100	0.0	100
Sulphar	0.5	22.8	74.7	20.0	77.7
	1.0	18.0	80.0	17.8	80.2
	2.5	0.0	100	0.0	100
Oxalic acid	0.5	47.6	47.1	48.0	46.7
	1.0	32.2	64.2	34.2	62.0
	2.0	0.0	100	0.0	100
Switch 62.5%	0.1	18.0	80.0	17.6	80.4
	0.5	10.0	88.8	11.0	87.8
	1.0	0.0	100	0.0	100
Control	0.0	90.0	0.0	90.0	0.0
LSD at 0.01 %		T = 2.09 C = 2.15 TxC = 4.82		T = 1.81 C = 2.13 TxC = 4.78	

Table (4): Effect of treatment endive leaves by some salts, biocides, antioxidants and the fungicide (Switch 62.5%) on the percentage of disease severity under greenhouse conditions during 2009/2010.

Treatments	Rate of application %	Disease severity, %			
		Botrytis cinerea	Efficiency	*Alternaria cichorii*	Efficiency
Calcium chloride	4.0 g	58.8	16.0	44.3	19.9
Salicylic acid	0.1 g	39.5	43.6	32.4	41.4
Oxalic acid	0.1 g	45.7	34.7	36.2	39.5
Sulphar	2.5g	24.3	65.3	17.1	69.1
Swith 62.5%	2.0 ml	7.2	89.7	8.3	85.0
Control	---	70.0	00.0	55.3	00.0

L.S.D at 0.05 for
Fungi(B) =0.21 Treatment (A)= 0.12 AxB = 0.34

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فاعليه المعاملات الكيماويه ضد امراض المجموع الخضري لنباتات الشيكوريا

إيهاب محمود الفار– نجلاء عبد الباقي سلام مهنا– رزق محمد عبد الغني شطا
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- تعتبر نباتات الشيكوريا احد النباتات حديثه الزراعه في مصر وتزرع لغرض التصدير والتسويق المحلي.
- لوحظ وجود تبقعات علي اوراق نباتات الشيكوريا صنف فينتور خاصه الاوراق الخارجيه في اماكن زراعتها الرئسيه (محافظة الجيزة والبحيرة) و تبين من العزل لهذه التبقعات أن فطر بوتريتس سيناريا اكثر الفطريات تكرارا ثم الترناريا شيكوريا ثم البنسليوم.
- تم اختبار القدرة المرضية للفطريات بمفردها علي الصنف فينتور حيث تبين أن كلا الفطريين أحدثا إصابة بدرجات متفاوته.
- تم اختباربعض المواد الكيماويه الامنه من املاح المعادن في المعمل بتركيزات مختلفه وهي كلوريد الكالسيوم ،سالسيلك اسد، اوكساليك اسد،الكبريت مقارنه بالمبيد الفطري سويتش (62.5 %) وتاثيرها علي النمو الميسليومي للفطريات المختبره وكان افضل المواد في تاثيرها لمقاومة نمو الفطريات هي المبيد سويتش، الكبريت،وسالسلك اسد،اوكساليك اسد علي التوالي واقلها تاثيرا كلوريد البوتاسيوم .
- تم استخدام التركيز الافضل للمواد المختبره لمقاومة أمراض الشيكوريا صنف فينتور تحت ظروف الصوبة حيث أظهرت المواد المستخدمة 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 القياس سويتش أعلاها فعالية.
- من هذه الدراسة فانه يمكن مقاومة إمراض المجموع الخضري علي الشيكوريا باستخدام الكبريت وسالسيلك اسد وذلك لتفادي استخدام المبيدات الفطرية خاصة لما لها من اثار ضاره وغير امنه علي محاصيل الخضر الورقيه .