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In vitro, evaluation of some medicinal plants extracts against *Fusarium oxysporum f. sp. lycopersici*

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Abstract

This work evaluates the efficacy of aqueous and ethanolic extracts of fourteen medicinal plants as antifungal agents against *Fusarium oxysporum* f. sp. *lycopersici*. Extracts were used at five concentrations (0.0, 0.5, 1.5, 2.5, 5% w/v). *Allium sativum* was the most efficient when used in both aqueous and ethanolic extracts, followed by *Datura stramonium* whereas the least effective species were *Lantana camara* and *Ficus elastica*. In general, the ethanolic extract exhibited a more potent inhibitory effect on the growth of *F. oxysporum* f. sp. *lycopersici* than the aqueous extract, and this was particulary evident for *Citrus reticulata*, *Eucalyptus rostrata*, *Mentha piperita*, *Cupressus macrocarpa* and *Eugenia jambolana*. However, *Allium sativum* and *Datura stramonium* were the most potent species in addition to *Malva parviflora*, *Althaea rosea*, *Nigella sativa* and *Artemisia herba-alba*. The dose-response relationship differed according to plant species and the extract used.

Keywords: *Fusarium oxysporum f. sp. lycopersici*, Antifungal activity, *Fusarium* wilt, Plant extracts.

Introduction

Tomato (*Solanum lycopersicon* L.) is an important vegetable crop in Egypt. It is grown in winter, summer and autumn, representing 3% of Egypt's available planting land **(Glala *et al.*, 2005).** It has diversified use high nutritive value, particularly as a source of vitamins A and C; In addition, it has the protective effect by virtue of its content of valuable bioactive components with antioxidant properties (**Borguini and Ferraz Da Silva Torres, 2009**). *Fusarium oxysporum* f. sp. *lycopersici* W.C Snyder and H.N. Hans is the causal agent of vascular wilt disease of tomato. This pathogen is a soil- borne facultative parasite. The pathogen enters the host roots directly through penetration hyphae and colonizes the cortex by intracellular and intercellular growths (**Fuchs *et al.*, 1997; Pietro *et al.*, 2001**). As in other fusaria, its identification has generally been based on morphological criteria such as the shape of micro and macroconidia, the structure of microconidiophores, formation and disposition of chlamydospores (**Henni *et al.*, 1994; Pietro *et al.*, 2003**).

As Fusarium wilt disease of tomato is a soil-borne in nature, application of fungicides to control this disease is not practical and expensive. These chemicals have a serious effect on human health, contaminate the environment and kill various beneficial microorganisms (**Hayes and Laws, 1991**). Also, frequent application of fungicides leads to the developing of new resistant

strains of pathogens (**Smith and Littrell, 1980**). **Williams (1984)** reported that the resistance expressed by a set of cultivars in one geographical region may not be effective in another region due to the existence of pathogens and/ or the variability in environmental conditions**.**

The previous factors emphasize the need for new methods of plant disease control (**Wilson *et al.*, 1987**). So, there is an urgent need to find safer alternatives to fungicides for both human and the environment. The natural substances present in plants, fruits, vegetables, oil seeds, and herbs are considered as antioxidants and functional foods (**Kitts *et al.*, 2000**). Environment-friendly plant extracts have been shown to be of great potential as alternative to the synthetic fungicides (**Zhang *et al.*, 2005**). The plant extracts are considered as cheap, non-toxic, available and easily biodegradable.The antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (**Cowan, 1999**). Natural plant substances include large source of effective secondary metabolites such as phenolics, alkaloids, tannins, saponins, glycosides and flavonoids, which may exert an antifungal activity (**Hassan and Maswada, 2012; Maswada and Elzaawely, 2013**).

However, the aim of this study is to evaluate *in vitro*, some medicinal plant extracts for controlling *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of wilt disease of tomato.

Materials and Methods

Fungal strain used:

Fusarium oxysporum f. sp. *lycopersici* was kindly provided by the Department of Plant pathology, Faculty of Agriculture, Mansoura University. *Fusarium* was grown on potato dextrose agar (PDA) medium and incubated at the laboratory conditions (25^{o}C) for 7 days.

Preparation of potato dextrose agar (PDA) medium

Two hundred g of potato extract and 20 g of glucose that added to one liter of distilled water. After the dissolving of all glucose by stirring, then the total volume was distributed to desired volumes in conical flasks. After that the agar was added in a percent of 2% and autoclaved at 15 p.s.i. and 121°C for 15 min**.**

Plant samples collection:

Fourteen plant species were collected from the natural habitats (Table 1). The plant species were identified according to **Boulos (2005),** and deposited as herbarium sheets at Botany Department, Faculty of Science, Damietta University.

Table 1. **List of plant species used for preparing extracts**

Scientific name	Family name	Part used	Common name
Allium sativum L.	Liliaceae	Bulb	Garlic
Althaea rosea L.	Malvaceae	Leaves	Hollyhock
Artemisia herba-alba Asso.	Asteraceae	Shoots	White wormwood
Citrus reticulata Blanco.	Rutaceae	Peels	Mandarin peels
Cupressus sempervirens L.	Cupressaceae	Leaves	Mediterranean cypress
Datura stramonium L.	Solanaceae	Leaves	Datura
Eucalyptus rostrata L.	Myrtaceae	Leaves	Camphor
Eugenia jambolana Lam.	Myrtaceae	Leaves	Jambolin
Ficus elastica Var.	Moraceae	Leaves	rubber plant
Juniperus oxycedrus L.	Cupressaceae	Seeds	Prickly juniper
Lantana camara L.	Verbenaceae	Leaves	Tickberry wild sage
Malva parviflora L	Malvaceae	Leaves	Egyptian mallow
Mentha piperita L.	Lamiaceae	Leaves	Peppermint
Nigella sativa L.	Ranunculaceae	Seeds	Black seeds

Drying of plant samples

The plant materials were air-dried until being crispy. Dried plants parts were ground into a fine powder using a blender and sieved to remove coarse particles.

Preparation of plant extracts

A known weight (5gm) of powdered plant material was extracted with 50 ml of 95% ethanol, boiling distilled water. The conical flask was covered with aluminum foil to prevent the contamination and extracted on a shaker at 150 rpm for 24 hours at 25°C.The mixture was filtered through sterile Whatman filter paper No.1. In case of the ethanolic extract, ethanol was completely evaporated at 45°C and the residue was resuspended in 10 ml of distilled water**.** Different volumes (0, 1, 3, 5 and 10 ml) were taken into 20 PDA medium for 0.0, 0.5, 1.5, 2.5 and 5% (w/v) extract, respectively.

Antifungal assay

The antifungal assay was done according to the procedure of **Kumar *et al.* (2009)** with some modifications. About 1, 3, 5, 10 ml of aqueous and ethanolic plant extracts were taken into 20 ml liquid PDA medium to give the concentrations of 0.0, 0.5, 1.5, 2.5 and 5% w/v and tested against *Fusarium oxysporum* f. sp. *lycopersici*, then 7-mm inoculum-disc of the fungus was placed in the center on solid PDA medium and incubated at 25°C for 7 days. The diameter of fungal growth (cm) was measured and the inhibition of fungal growth was estimated as a percentage of the control. The relative potency of plant extract was estimated in terms of the concentration leading to 50% inhibition of fungal growth (LC_{50}).

Statistical analysis*:*

The relative inhibition of fungal growth estimated as a percentage of the control was arcsine transformed before performing statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS (**SPSS, 2004**) version 22 and mean separation was performed using the Duncan's multiple range test at $p < 0.05$ (**Duncan, 1955**).

Results

Treatments (plant species, extractant and concentration of the extract) had a highly significant effect ($P< 0.001$) on growth of *F. oxysporum* f. sp. *lycopersici* was shown in Table 2. Aqueous and ethanolic extracts of 14 plant species were tested against *F. oxysporum* f. sp. *lycopersici* in five concentrations (0, 0.5, 1.5, 2.5 and 5%). Table 3 shows that the most active plant species was *Allium sativum* with an average inhibition for aqueous and or ethanolic extracts of 61% and 54%, respectively, followed by *Datura stramonium* and *Citrus reticulata* with an average inhibition of 40% for both aqueous and ethanolic extracts; whereas the least effective species were *Lantana camara* and *Ficus elastica* with an average inhibition of 0.5% and 12% for aqueous and ethanolic extracts, respectively. Figure 1 shows that the most effective aqueous and ethanolic plant extracts on the growth of *Fusarium oxysporum* f. sp. *lycopersici* at the concentration of 5% in both of *Allium sativum* and *Datura stramonium*. The remaining plant species exhibited a moderate inhibition, depending on the type of used extract.

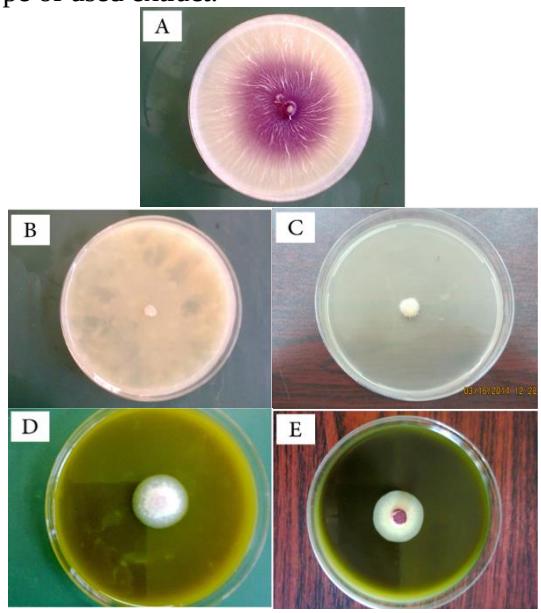

Fig.1. Effect of the most effective plant extracts on the growth of *Fusarium oxysporum* f. sp. *lycopersici* at the concentration of 5%. A. control; B. aqueous Garlic extract; C. ethanolic Garlic extract; D. aqueous Datura extract; E. ethanolic Datura extract.

In general, the ethanolic extract exhibited a potent inhibitory effect on the growth of *F. oxysporum* f. sp. *lycopersici* than the aqueous extract, and this was particularly evident for *Citrus reticulata*, *Eucalyptus rostrata*, *Mentha piperita*, *Cupressus sempervirens* and *Eugenia jambolana*. However, *Allium sativum* and *Datura stramonium* were the most potent species in addition to *Malva parviflora*, *Althaea rosea*, *Nigella sativa* and *Artemisia herba-alba*. Increasing concentration of the plant extract led to a progressive inhibition of fungal growth; yet the dose-response relationship differed according to plant species and the type of extract. For example, a saturable trend was found in both ethanolic and aqueous extracts of *Allium*

sativum, *Datura stramonium*, *Citrus reticulata* and ethanolic extract of *Ficus elastica*, *Eucalyptus rostrata* and *Cupressus sempervirens*, and aqueous extract of *Eugenia jambolana* and *Althaea rosea* were recorded. In addition, a linear trend was found in both extracts of *Juniperus oxycedrus*, *Nigella sativa*, *Mentha piperita*, *Artemisia herba-alba*, *Lantana camara* and *Malva parviflora* as well as the ethanolic extracts only of *Eugenia jambolana* and *Althaea rosea* and aqueous extracts only of *Eucalyptus rostrata*, *Ficus elastica* and *Cupressus sempervirens* were detected in Figures 2, 3 and 4.

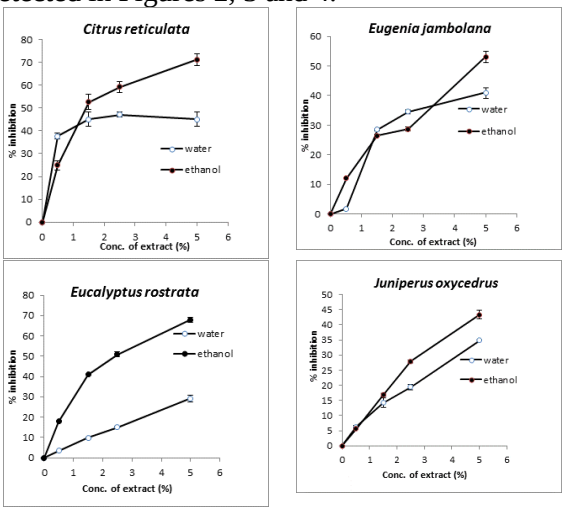

Fig.2. Effect of aqueous and ethanolic extracts of *Citrus reticulata*, *Eugenia jambolana*, *Eucalyptus rostrata* and *Juniperus oxycedrus* on growth of *F. oxysporum* f. sp. *lycopersici.* Each value is the mean of three replicates ± S.E.

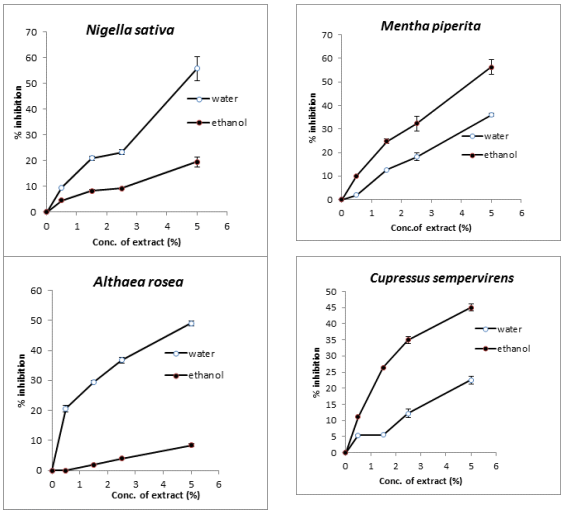

Fig.3. Effect of aqueous and ethanolic extracts of *Nigella sativa*, *Mentha piperita*, *Althaea rosea* and *Cupressus sempervirens* on growth of *F. oxysporum* f. sp. *lycopersici*. Each value is the mean of three replicates ± S.E.

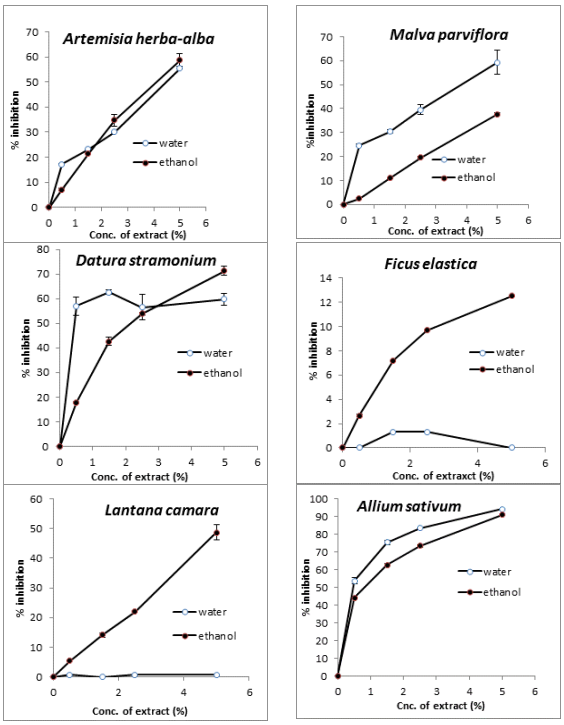

Fig.4. Effect of aqueous and ethanolic extracts of *Artemisia herba-alba*, *Malva parviflora*, *Datura stramonium*, *Ficus elastica*, *Lantana camara* and *Allium sativum* on growth of *F. oxysporum* f. sp. *lycopersici.* Each value is the mean of three replicates ± S.E.

Table 4 showed the relative potency of the aqueous and ethanolic extracts of the examined plant species against *F. oxysporum* f. sp. *lycopersici* in terms of LC_{50}, calculated from the dose-response relationships of Figures 2, 3 and 4. The effective concentration of the aqueous extract at which 50% pathogen inhibition occurred was 0.45% for both *Allium sativum*, *Datura stramonium*, 3.8% for *Malva parviflora*, 4.45% for *Artemisia herba-alba* and 4.55% for *Nigella sativa*. The value of LC_{50} of the ethanolic extract was 0.8% for *Allium sativum*, 1.4% for *Citrus reticulata*, 2.1% for *Datura stramonium* and 2.4% for *Eucalyptus rostrata*.

Table 2. ANOVA of the effect of the main factors (plant species, type of extract and concentration of the extract) and their interactions on the inhibition percentage of *F. oxysporum* f. sp. *lycopersici*

Source of variation	df	F	Sig.
Species	13	1181.542	.000
Extractant	1	19.400	.000
Conc.	4	4842.088	.000
Species * Extractant	13	284.078	.000
Species * Conc.	52	97.975	.000
Extractant * Conc.	4	87.894	.000
Species * Extractant * Conc.	52	40.459	.000

Table 3. Effect of plant extracts on the growth of *F. oxysporum* f. sp. *lycopersici.* Each value is the mean of 3 replicates ± SE. Means with common letters are highly significant different at $p < 0.05$

Plant species	Inhibition of fungal growth (% of control) Aqueous extract	Ethanolic extract
Lantana camara	0.45 ± 0.09^{a}	18.0 ± 4.56^{e}
Ficus elastica	0.52 ± 0.17^{ab}	6.41 ± 1.21^{b}
Cupressus sempervirens	9.10 ± 2.09^{c}	23.5 ± 4.33^{g}
	11.5 ± 2.76^{d}	35.5 ± 6.42^{k}
Eucalyptus rostrata	13.7 ± 3.48^{e}	24.7 ± 5.23^{ghij}
Mentha piperita	15.0 ± 3.21^{f}	18.7 ± 4.17^{ef}
Juniperus oxycedrus	21.1 ± 4.56^{g}	24.0 ± 4.78^{gh}
Eugenia jambolana	21.9 ± 5.10^{gh}	8.26 ± 1.75^{c}
Nigella sativa	25.1 ± 4.83^{i}	24.4 ± 5.62^{ghi}
Artemisia herba-alba	27.1 ± 4.40^{j}	2.83 ± 0.83^{a}
Althaea rosea	30.7 ± 5.26^{k}	14.1 ± 3.64^{d}
Malva parviflora	35.0 ± 4.81^{l}	41.6 ± 6.94^{m}
Citrus reticulata	47.1 ± 6.43^{m}	37.1 ± 6.80^{kl}
Datura stramonium	61.3 ± 8.92^{n}	54.1 ± 8.29^{n}
Allium sativum		
Total of all species	22.83 ± 1.64^{a}	23.8 ± 1.62^{b}

Table 4. The concentration of plant extracts leading to 50% inhibition of *F. oxysporum* f. sp. *lycopersici* growth (LC_{50})

Plant species	LC_{50} (% w/v)	
	Aqueous extract	Ethanolic extract
Lantana camara	-	-
Ficus elastica	-	-
Cupressus sempervirens	-	-
Eucalyptus rostrata	-	2.4
Mentha piperita	-	4.3
Juniperus oxycedrus	-	-
Eugenia jambolana	-	4.65
Nigella sativa	4.55	-
Artemisia herba-alba	4.45	4.05
Althaea rosea	-	-
Malva parviflora	3.80	-
Citrus reticulata	-	1.4
Datura stramonium	0.45	2.1
Allium sativum	0.45	0.8

Discussion

The extracts of fourteen plant species investigated in the present study exhibited diverse antifungal activities against *F. oxysporum* f. sp. *lycopersici* which varied according to the plant species and type of extract. In general, aqueous and ethanolic extracts of *Allium sativum* and *Datura stramonium* exhibited the potent effect on the pathogen. The aqueous extract of *Lantana camara* was the least effective on the pathogen. Moreover, the ethanolic extract of *Althaea rosea* was the least effective on the pathogen. The antifungal activity of plant extracts may be related to the presence of many bioactive compounds such as flavonoids, terpenoids, alkaloids, tannins, steroids, glycosides and phenolics (**Leicach *et al.*, 2010; Yaber Grass and Leicach, 2011**). These secondary metabolites, also known as allelochemicals, are normally produced by the medicinal plants to provide protection against stress conditions, invasion of pathogens and also involved in the plant-plant interaction; thus allowing the successful survival of the plant against other species and the invading microorganisms (**Matsuki *et al.*, 2011**).

Aqueous extract of garlic showed a better activity than that of ethanol. Previous studies supported these results (**Gull *et al.*, 2012**). Garlic was reported to have antifungal *properties* and inhibit formation of aflatoxins (**Graham and Graham, 1987**) and penicillic acid (**Ismaiel, 2009**). The antimicrobial activity of garlic is totally dependent on the allicin compound. Studying the antimicrobial properties of garlic indicated that garlic is full of anion compounds, including nitrates, chlorides, sulfides and organosulphur compounds that can be easily resolved in water and are responsible for antibacterial properties (**Shobana *et al.*, 2009**). **Srivastava and Yadav (2008)** evaluated extracts of 45 medicinal plants against *F. oxysporum* f. sp. *lycopersici* and proved the antifungal properties of these medicinal plants. These results were also obtained by **Ranawane *et al.* (2010)** who reported that *Datura stramonium* extract had the inhibitory effect against the growth of many fungal species. Also, **Sharma *et al.* (2014)** investigated that the aqueous leaf extracts of *Datura stramonium* had the antimicrobial activity against *Alternaria solani* and *Fusarium oxysporum*. *Datura stramonium* plants contain bioactive compounds such as hyoscyamine, scopolamine and atropine in the seeds and flowers, besides its hallucinogen activity (**Rajesh and Sharma, 2002; Kaushik and Goyal, 2008**).

The ethanolic extract of *Citrus reticulata* is better than its aqueous extract; this can be related to its content of flavonoids, tannins, phenolics, vitamin C, proteins and carbohydrates. High flavonoid content expressed as catechin equivalent was found in the ethanolic extract (92.4mg CT/gm). Vitamin C content was 115.5 mg AAE/gm, tannin content was 132 mg TAE/gm (**Justin *et al.*, 2014**). The aqueous extract of *Malva parviflora* showed better activity than the ethanolic extract. These results are in agreement with those of **Abdel-Ghani *et al.* (2013).** The antifungal activity of *Malva* might be due to the presence of alkaloids, essential oils and phenolic

quleoside as reported by **Abad *et al.* (2007).** The antifungal activity of *Artemisia herba-alba* might be due either to the flavonoids or to the combine effect of glycosides, saponins, alkaloids, tannins and flavonoids (**Juvatkar *et al.*, 2012**). The aqueous extract of *Nigella sativa* showed more antifungal activity than the ethanolic extract which coincides with the results of **Zahra *et al.* (2011**) and this may be due to the higher content of glycosides, tannins, saponins, flavonoids and alkaloids in the aqueous extract. Antifungal activity of thymoquinone, present in *Nigella sativa*, against *Aspergillus niger* was reported **(Al Jabre *et al.*, 2003).** The antifungal potency of plant extracts was estimated in terms of the relative inhibition of fungal growth below the control. Nevertheless, this efficiency can be estimated also in terms of the LC_{50}. The results of LC_{50} revealed that *Allium sativum* was the most efficient in both aqueous and ethanolic extracts and *Datura stramonium* came next with the lowest LC_{50} (0.45% as an average of the aqueous extract of the 2 species and 0.8% and 2.1% for ethanolic extract of *Allium sativum* and *Datura stramonium*, respectively). Normally, the lower the LC_{50} the more potent is the antifungal activity of the extract; and whenever an extract has no value for LC_{50}, this means that the antifungal activity of this extract is too weak to the extent that the relative inhibition of fungal growth never attained 50% even at the top concentration used (5% w/v). These results are in agreement with previous reports **(Alkhail, 2005; Shrestha and Tiwari, 2009; Taskeen-Un-Nisa and Mir, 2010).**

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الملخص العربي

عنوان البحث: تقييم مستخلصات بعض النباتات الطبية ضد فطر فيوزاريم اوكسيسبورم ليكوبرسيسي

زكريا عوض محمد بقا ومحمود متولى نور الدين ومحمد إسماعيل ابو دبارة و فاطمه الزهراء يوسف المنير

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في هذا البحث تم تقييم فعالية المستخلصات المائيه والايثانولية لأربعة عشر نباتاَ طبياَ تم جمعهم من البيئة المحيطة ضد فطر فيوزاريم اوكسيسبورم ليكوبرسيسي المسبب لمرض الذبول في الطماطم، واستخدمت هذه المستخلصات عند تركيزات 5. و1.5و2.5و5% وكان مستخلص الثوم بنوعيه هو الأكثر كفاءة لمقاومة الفطر، يليه الداتورا سترامونيوم أما نباتي اللانتانا كامارا والفيكس إلاستكا كانا هما الاقل تأثيرا. وبشكل عام كان المستخلص الإيثانولى لمعظم النباتات هو الأكثر تثبيطا لنموالفطر عن مثيله المائي وهذا واضح في قشور الحمضيات، الكافور البلدى، النعناع الفلفلى، السرو الليمونى و البامبوزيا، ومع ذلك كان الثوم والداتورا هما الأكثر فعاليه بالاضافه الى الخبيزه ، الخطميه، الحبه السوداء والشيح. وكانت علاقه جرعة الاستجابة تختلف طبقا لأنواع النباتات و نوع المستخلص.