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Antifungal activity of some medicinal plant extracts against the most prevalent fungal pathogens causing spoilage of some fruits

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Abstract

Fungi are the major disease-causing agents of fruits. They cause considerable losses to post harvest fruits during transportation and storage. Ten infected fruit species were collected from the local markets in Damietta Governorate, Egypt for surveying the fungal pathogens on these fruits. Ten fungal species were isolated and identified, among them, Alternaria alternata and Fusarium oxysporum exhibited the most prevalent fungal species isolated from mandarins and grapes, respectively. In this study, the aqueous and ethanolic extracts of eleven wild medicinal plants were tested against the two prevalent fungi at five concentrations (0, 1, 3, 5, 10%; w/v). The greater relative inhibition of fungal growth, along with the lower C50 values revealed that the ethanolic extract of the tested plants exhibited the stronger antifungal activity when compared to water extracts. Out of the eleven plant species investigated, Thymus capitatus and Eucalyptus citriodora were the most effective for fungal growth, while Nicotiana glauca was the least effective. Therefore, we recommend the using of Thymus capitatus and Eucalyptus citriodora extracts as potential antifungal preservatives for fruits against fungal spoilage.

Keywords: Alternaria alternata, Antifungal activity, Fusarium oxysporum, Postharvest diseases, Plant extracts.

Introduction

Postharvest diseases are posing a major problem for the agricultural industry, especially in the developing countries. It has been estimated that between 10% and 40% losses of agricultural production worldwide arise from the postharvest loss (Enyiukwu *et al*. 2014). According to Arya (2010), of all losses caused by plant diseases, those that occur after harvest are the most costly. Fruits are highly perishable products; their quality is affected by postharvest handling, transportation, storage and marketing (Wills *et al*., 1981; Liu and Ma, 1983). The improper handling, packaging, storage and transportation may result in decay and proliferation of microorganisms, which become activated because of the changing in physiological state of the fruits and vegetables (Wilson *et al*., 1991). Fruits, due to their low pH, high moisture content and rich nutrient composition are very susceptible to attack by pathogenic fungi; which

in addition to causing rots may also produce mycotoxins; thereby making the fruits unfit for consumption (Phillip, 1984; Stinson *et al*., 1981; Moss, 2002). Several species of fungi and in some cases, bacteria participate in postharvest deterioration and rots of tubers and agroproduce. These include species of *Aspergillus, Fusarium, Colletotrichum, Macrophomina, Penicillium* and *Rhizopus* amongst several others (Enyiukwu *et al*., 2014).

At a time of increased public awareness about chemical treatments and development of fungicide resistance by postharvest pathogen populations, the adoption of alternative control means seems to be essential. However, an effective way to reduce losses of agroproduce requires the knowledge of epidemiology and complex interactions between host, pathogen, and control agents (Rouissi *et al*., 2013).

The most common method of protecting plants against the fungal attack is the use of synthetic fungicides, but their excessive use, complemented with high costs, the presence of residues in plants, and development of resistance, has imposed a negative effect on human health and the environment (Paster and Bulllerman, 1988).

In recent years, there has been a global trend toward the use of natural substances present in plants, fruits, vegetables, oilseeds, and herbs as antioxidants and functional foods (Kitts *et al*., 2000). Environment-friendly plant extract agents have been shown to be of great potential as an alternative to the synthetic fungicides (Zhang *et al*., 2005). The plant extracts have the advantages of being cheap, locally available, non-toxic and easily biodegradable. The antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Wild plants may contain a 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)

The aim of the present study is to evaluate some wild medicinal plant extracts for controlling the predominant fungal pathogens causing postharvest diseases of some fruits collected Damietta Governorate markets.

Materials and Methods

Collection of spoiled fruits

Ten infected fruit species of economic importance growing in Egypt were collected from the local markets in Damietta Governorate (Table 1). Spoiled fruits with fungal infections were chosen and transferred to laboratory in sterile plastic polyethelene bags and isolation of fungal pathogens was done in the same day.

Table (1): Scientific and common names of fruits under study

Scientific name	Common name	Family
Malus domestica Borkh.cv. Barkher	Apple	Rosaceae
Musa sapientum L. cv. William	Banana	Musaceae
Vitis vinifera L. cv. Thompson	Grapes	Vitaceae
Pisidium guajava L. cv. Bassateen El Sabahia	Guajava	Myrtaceae
Citrus reticulata Blanco. cv. Balady	Mandarin	Rutaceae
Mangifera indica L. cv. Alfonso	Mango	Anacardiaceae
Citrus sinensis (L) Obseck cv. Balady	Orange	Rutaceae
Prunus domestica L. cv. Santa Rosa	Plum	Rosaceae
Punica granatum (L) cv. Manfalouty	Pomegranate	Punicaceae
Fragaria ananassa L.cv. Camarosa	Strawberry	Rosaceae

Isolation and identification of fungal pathogens

Fungal pathogens were isolated from fruits according the method of Chiejina (2008). Thin sections (2 mm diameter) were cut from the periphery of diseased fruits; surface sterilized in 0.1% mercuric chloride for 2–3 min, and then rinsed 3 times with sterile distilled water. The sections were plated in water agar and the mycelium was transferred into clean Potato Dextrose Agar (PDA) plates containing penicillin (100,000 Units/L). The plates were incubated at 27 ± 2^{o}C for 6–7 days. Subcultures made aseptically from the plates into similar clean PDA plates and incubated under similar conditions until pure cultures were obtained. The identification of

the isolated fungi was done macroscopically and microscopically. Macroscopic identification was based on culture growth patterns and mycelial color. Small portions of the fungal cultures were teased and mounted in lactophenol in cotton blue and examined microscopically. Fungal identification was confirmed with the aid of fungal identification manuals (Barnett and Hunter, 1999; Alexopoulos *et al*., 2002; Agrios, 2005; Ellis *et al*., 2007). The isolated fungi were maintained on PDA slants at 5^{o}C for further use.

Determination of percentage of fungal occurrence

This was done to determine the frequency of occurrence of the different fungal isolates. Isolates taken from the spoiled fruits were cultured and the number of fungal isolates from each of the ten fruits were recorded and expressed as percentage of the total number of isolates, according to Ukeh and Chiejina (2012).

Percentage of occurrence = X×100/N

X = number of isolates of each organism in each fruit.

N = Total number of isolates of all organisms in all fruits.

Pathogenicity test

Each of the fungal isolate obtained from the spoiled fruits were tested for their ability to cause the same disease condition previously observed in healthy fruits by the method of Franck *et al*. (2005). Healthy fruits were washed in sterile distilled water and surface sterilized by dipping into 0.1% $HgCl_2$ for 2 minutes and, with the aid of a sterile cork borer, cylindrical cores were removed from each fruit. Pure cultures of the isolated fungi were introduced into the open cores made within the fruits and the cores were sealed with sterile Vaseline. The fruits were kept at room temperature for 7–10 days. With the establishment of the disease, inocula were taken from the infected fruits and cultured. The organisms were re-isolated and identified as mentioned before. This was taken as evidence that the originally isolated organism from the spoiled fruit is the causative agent of the disease, thus confirming Koch's postulates (Nweke and Ibiam, 2012).

Plant samples collection

Eleven wild plant species were collected from natural habitats (Table 2). The plant species were identified according to Täckholm (1974); Boulos (1999-2005) and deposited as herbarium sheets at Botany Department, Faculty of Science, Damietta University.

Table (2): List of plant species used for preparation of plant extracts

Scientific name	Common name	Family	Part Used
Moringa oleifera Lam.	Moringa	Moringaceae	Fruits
Ziziphus spina-christi (L.)	Sidr	Rhamnaceae	Leaves
Melia azedarach (L.)	Chinaberry tree	Meliaceae	Leaves
Nicotiana glauca Graham	Tobacco tree	Solannceae	Fruits
Cyperus rotundus (L.)	Purple nutsedge	Cyperaceae	Rhizomes
Schinus terebinthifolius Raddi	Brazilian pepper	Anacardiaceae	seeds
Lantana camara (L.)	Tickberry wild sage	Verbenaceae	Leaves
Zygophyllum aegyptium (L.)	Rotrate	Zygophyllaceae	Shoot
Delonix regia (Boj. ex Hook.) Raf	Royal poincina	Fabaceae	Bark
Eucalyptus citriodora L'Hér	Myrtle	Myrtaceaea	Shoot
Thymus capitatus (L.) Hoffmgg. et Link	Thyme	Lamiaceae	Shoot

Preparation of plant powder

The plant samples were washed with tap water 3 times and then rinsed in distilled water and dried under shade at laboratory temperature (25-29 ºC) till they become crispy. Dried parts of the plants were ground using a blender and sieved to remove coarse particles.

Preparation of plant extracts

A known weight (1gm) of the used part of each plant was taken into 10 ml solvent, either distilled water or 95% ethanol and extracted on a shaker (Lab Line Company) at 150 rpm for 24 hours at 25°C. The mixture was filtered through sterile Whatman filter paper No.1 and centrifuged (Xiangshui Fada Medical Apparatus Factory, Centrifuge model 800 D) twice at 4000 rpm for 10

minutes. In case of the aqueous extract, it was concentrated into half of the original volume and in case of the ethanolic extract, ethanol was completely evaporated and the dry residue was resuspended in half of the original volume and DMSO (dimethyl sulfoxide). The supernatant was poured in conical flasks and covered with cotton plugs and left for 10 minutes in a digital water bath (Kottermann model 3042) at 100°C to avoid contamination (Madavi and Singh, 2005). Different volumes (0, 1, 3, 5 and 10 ml) were taken into 10 ml double strength PDA media to give 0, 1, 3, 5 and 10% (w/v) extract respectively.

Estimation of antifungal activity

The agar-amended media was used according to Kumar *et al*. (2009) with some modifications. Aqueous and ethanolic plant extracts at the concentrations of 0, 1, 3, 5 and 10% were tested against the two predominant fungal species (*Alternaria alternata* and *Fusarium oxysporum*) isolated from mandarins and grapes, respectively. The solidified extract-amended media in the Petri dishes were inoculated, each alone at the center with 7 mm inoculum-disc of each tested fungus and incubated at 25 ± 2°C for 7 days for *Alternaria alternata* and 4 days for *Fusarium oxysporum*. The diameter of fungal growth (cm) was measured and the percentage inhibition of fungal growth was estimated relative to the control. The experiment was factorial with four main factors and three replicates (Petri dishes) in a completely randomized design. The four factors were: fungus species with 2 levels (*Alternaria alternata* and *Fusarium oxysporum*), plant species with eleven levels, extract with two levels (aqueous and ethanolic) and concentration of extract with five levels (0, 1, 3, 5 and 10% w/v). The relative potency of plant extracts was estimated in terms of the concentration leading to 50% inhibition of fungal growth (C_{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 version 22 and the effect of the main factors (fungus, plant species, extract and concentration of the extract) and their interaction were assessed using four ways ANOVA. Main separation was performed using the Duncan's multiple range tests at p□0.05.

Results

Ten fungal pathogens were isolated from ten spoiled fruit species; viz: Alternaria alternata, Aspergillus niger, A. nidulans, A. flavus, A. ochraceous, Cladosporium herbarum, Fusarium oxysporum, Penicillium chrysogenum, P. expansum, and Rhizopus stolonifer. The most prevalent fungal species were Alternaria alternata and Fusarium oxysporum with the relative occurrence of 25% and 18%, respectively, of the total number of isolates (Table 3). Mandarins and grapes exhibited 100% successful infection by these two pathogens after the pathogenicity test (Fig. 1).

These two fungal species were subjected to biological control *in vitro* by using aqueous and ethanolic extracts of eleven plant species at five concentrations (0-10%). Table 4 shows highly significant effect of plant species and type of extract on fungal growth as well as a highly significant difference in fungal susceptibility to treatments.

Tables 5 and 6 shows that *F. oxysporum* was more affected by the water and ethanolic extracts of all plants than *A. alternata*, but ethanolic extract exhibited a more potent inhibitory effect on the growth of the two fungal species than that of aqueous extract. Out of the 11 plant species investigated, *Thymus capitatus* was the most potent with an average inhibition (for both aqueous and ethanolic extracts) of 41% and 53%, respectively. In addition, *Eucalyptus citriodora* came the second with an average inhibition of 28.4% and 42%, respectively when compared with the control. On the other hand, *Nicotiana glauca* exhibited the least effective plant with an average inhibition of 6% and 4.8%, respectively. The remaining eight plant species exhibited moderate inhibition with different magnitude and rank, depending on the type of extract and fungal species.

Increasing the concentration of the plant extract led to a progressive inhibition of fungal growth; yet the concentration-response relationship differed in the two fungal species according to plant species and type of extract. For example, a saturable trend, with variable magnitude depending on the fungus, plant species and the extract was exhibited in terms of both extracts of *Moringa oleifera*, *Ziziphus spina-christi* and *Delonix regia* on both fungal species and of *Melia azedarach* on *A. alternata*, and ethanolic extract only of *Thymus capitatus*, *Lantana camara*, *Cyperus rotundus*, *Schinus*

terebinthifolius, *Zygophyllum aegyptium* and *Eucalyptus citroidora* on both fungal species, and aqueous extract only of *Thymus capitatus*, *Schinus terebinthifolius*, *Zygophylleum aegyptium* and *Eucalyptus citriodora* on *F. oxysporum* (Figs. 2 and 3). This saturable trend was more frequent in case of ethanolic extract than aqueous extract in case of *F. oxysporum* than *A. alternata*. By contrast, a linear trend was appeared in case of both extracts of *Nicotiana glauca* and by the aqueous extracts only of *Cyperus rotundus* and *Lantana camara* on both fungal species and by the aqueous extracts of *Schinus terebinthifolius*, *Zygophyllum aegyptium*, *Thymus capitatus* and *Eucalyptus citriodora* on *A. alternata* and of *Melia azedarach* on *F. oxysporum* and by only the ethanolic extracts of *Melia azedarach* on *F. oxysporum*.

Table (3): The isolated fungal species from spoiling fruits. Each value is the mean of 5 replicates ± SE

Fruit	Disease	Isolated fungus	No. of colonies	Relative occurrence (% of total)
	Alternaria rot	*Alternaria alternata* (Fr.) Keissl	13	4.48
Apple	Blue mold rot	*Penicillium expansum* Link	10	3.44
	Black mold rot	*Aspergillus niger* van Tieghem	9	3.1
Banana	Alternaria rot	*Alternaria alternata* (Fr.) Keissl	15	5.17
	Rhizopus rot	*Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	9	3.1
	Black mold rot	*Aspergillus niger* van Tieghem	4	1.37
Grapes	Dry rot	*Fusarium oxysporum* Sch. Em. Syn. Hansen	20	6.89
	Rhizopus rot	*Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	6	2.06
Guajava	Black mold rot	*Aspergillus ochraceus* Wilhelm	10	3.45
	Black mold rot	*Aspergillus niger* van Tieghem	6	2.06
	Alternaria rot	*Alternaria alternata* (Fr.) Keissl	20	6.89
Mandarin	Black mold rot	*Aspergillus nidulans* G. Winter	12	4.14
	Black mold rot	*Aspergillus ochraceus* Wilhelm	13	4.48
Mango	Black mold rot	*Aspergillus niger* van Tieghem	3	1.03
	Black mold rot	*Aspergillus flavus* Link	5	1.72
	Sooty spot	*Cladosporium herbarium* (Pers.) Link	16	5.52
Orange	Black mold rot	*Aspergillus niger* van Tieghem	10	3.45
	Blue mold rot	*Penicillium chrysogenum* Thom	9	3.1
	Black mold rot	*Aspergillus flavus* Link	7	2.41
Plums	Alternaria rot	*Alternaria alternata* (Fr.) Keissl	25	8.62
	Dry rot	*Fusarium oxysporum* Sch. Em. Syn. Hansen	20	6.89
	Rhizopus rot	*Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	5	1.72
Pomegranate	Blue mold rot	*Penicillium expansum* Link	20	6.89
Strawberry	Rhizopus rot	*Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	10	3.45
	Dry rot	*Fusarium oxysporum* Sch. Em. Syn. Hansen	13	4.45
Total number of colonies			290	

Table (4): Four-way ANOVA of the effect of the main factors (plant species, type of extract and concentration of the extract) and their interactions on the percentage inhibition of *Alternaria alternata* and *Fusarium oxysporum.*

Source of variation	df	F	Sig.
Fungus	1	1055.266	.000
Plant species	10	893.187	.000
Extract	1	2101.675	.000
Conc.	4	4044.657	.000
Fungus * Plant species	10	163.753	.000
Fungus * Extract	1	38.524	.000
Fungus * Conc.	4	77.348	.000
Plant species * Extract	10	70.280	.000
Plant species * Conc.	40	114.209	.000
Extract * Conc.	4	158.929	.000
Fungus * Plant species * Extract	10	33.380	.000
Fungus * Plant species * Conc.	40	17.574	.000
Fungus * Extract * Conc.	4	6.930	.000
Plant species * Extract * Conc.	40	15.317	.000

Fungus * Plant species * Extract * Conc.	40	8.586	.000

Table (5): Effect of plant species and type of extract on the growth of *A. alternata.* Each value is the mean of 5 replicates ± SE. Means with common letters are not significantly different at p<0.05.

Plant species	**Inhibition of fungal growth (% of control)**	
	Aqueous extract	**Ethanolic extract**
Nicotina glauca	5.23 ± 1.31^{a}	6.89 ± 1.76^{a}
Schinus terebinthifolius	6.18 ± 1.44^{ab}	12.4 ± 2.25^{bcd}
Lantana camara	6.23 ± 2.22^{abc}	20.4 ± 3.78^{e}
Delonix regia	6.27 ± 1.21^{abcd}	10.7 ± 1.85^{b}
Ziziphus spina-christi	10.8 ± 1.85^{e}	11.8 ± 2.13^{bc}
Melia azedarach	12.1 ± 3.64^{ef}	34.5 ± 6.03^{i}
Eucalyptus citriodora	15.0 ± 3.86^{g}	41.8 ± 8.32^{j}
Zygophyllum aegyptium	18.2 ± 4.26^{h}	28.6 ± 5.04^{gh}
Cyperus rotundus	19.1 ± 3.65^{hi}	22.6 ± 4.45^{f}
Moringa oleifera	27.0 ± 4.37^{j}	27.5 ± 4.58^{g}
Thymus capitatus	35.6 ± 8.15^{k}	46.8 ± 9.37^{k}
Total of all species	14.7 ± 1.33^{a}	24.0 ± 1.79^{c}
Total of *A. alternata*	19.34 ± 1.14^{a}	

Table (6): Effect of plant species and type of extract on the growth of *F. oxysporum.* Each value is the mean of 5 replicates ± SE. Means with common letters are not significantly different at p<0.05.

Plant species	**Inhibition of fungal growth (% of control)**	
	Aqueous extract	**Ethanolic extract**
Nicotiana glauca	0.38 ± 0.66^{a}	9.24 ± 2.14^{a}
Melia azedarach	7.46 ± 1.67^{b}	20.2 ± 5.08^{c}
Schinus terebinthifolius	11.2 ± 3.46^{c}	31.7 ± 4.43^{g}
Delonix regia	11.7 ± 2.18^{cd}	17.7 ± 2.89^{b}
Ziziphus spina-christi	16.9 ± 2.39^{e}	28.4 ± 3.92^{def}
Cyperus rotundus	18.1 ± 3.85^{ef}	27.8 ± 4.75^{de}
Zygophyllum aegyptium	20.2 ± 2.83^{g}	46.6 ± 7.11^{hi}
Moringa oleifera	25.1 ± 4.07^{h}	27.4 ± 4.37^{d}
Eucalyptus citriodora	33.4 ± 5.27^{i}	50.6 ± 7.40^{j}
Lantana camara	37.7 ± 5.53^{j}	45.0 ± 6.61^{h}
Thymus capitatus	47.1 ± 9.92^{k}	59.0 ± 9.53^{k}
Total of all species	20.9 ± 1.67^{b}	33.0 ± 2.02^{d}
Total of *F. oxysporum*	26.95 ± 1.35^{b}	

Fig. 1. Pathogenicity tests on healthy fruits showing the appearance of fungal infection on (**a)** Grapes with *Fusarium oxysporum* and (**b)** Mandarin with *Alternaria alternata*.

Although the ethanolic extract of the studied plant species exerted, in general, a stronger inhibitory effect on fungal growth than the aqueous extract, the concentration at which the maximum difference between the two extracts exists differed in the two fungal species and depending on the plant species. This difference was progressive with the increasing of extract concentration in the following cases: the effect of *Melia azedarach, Schinus terebinthifolius, Eucalyptus citriodora* and *Delonix regia* on both fungi, and of *Ziziphus spina-christi, Nicotiana glauca* and *Zygophyllum aegyptium* on *F. oxysporum*. Another pattern of maximum difference in the moderate concentrations of the extract was pronounced by *Cyperus rotundus, Lantana camara* and *Thymus*

capitatus on both fungi and of *Nicotiana glauca* and *Zygophyllum aegyptium* on *A. alternata*. A third case of negligible difference between the two extracts encountered in the case of *Moringa oleifera* on both fungi and of *Ziziphus spina-christi* on *A. alternata*.

Table 7 shows the relative potency of the aqueous and ethanolic extracts of the studied plant species on fungal growth in terms of C_{50}; calculated from the concentration-response relationships of Figures 2 and 3. In general, the value of C_{50} of the ethanolic extract of the different species was much lower than that of the aqueous extract. In the majority of the studied species, the magnitude of inhibition of fungal growth by the aqueous extract was too low to allow the calculation of the $C_{50.}$ It was only possible to estimate C_{50} of the aqueous extract for the most potent species (*Thymus capitatus*) on the two fungal species (4.7% and 3% for *A. alternata* and *F. oxysporum*, respectively) and for *Eucalyptus citriodora* and *Schinus terebinthifolius* (6.9% and 6% respectively) on *F. oxysporum*.

Table (7): The concentration of plant extracts leading to 50% inhibition of fungal growth (C_{50})

	C_{50} (%) *Alternaria alternata*		C_{50} (%) *Fusarium oxysporum* ooxysporum	
Plant species	**Aqueous**	**Ethanolic**	**Aqueous**	**Ethanolic**
Nicotiana glauca	-	-	-	-
Schinus terebinthifolius	-	-	6	3
Lantana camara	-	-	-	-
Delonix regia	-	-	-	-
Zizyphus spina- christi	-	-	-	-
Melia azedarach	-	6.2	-	9.8
Eucalyptus citriodora	-	3.7	6.9	1.7
Zygophyllum aegyptium	-	8.3	-	2.5
Cyperus rotundus	-	-	-	10
Moringa oleifera	-	10	-	-
Thymus capitatus	4.7	2.7	3	1.6

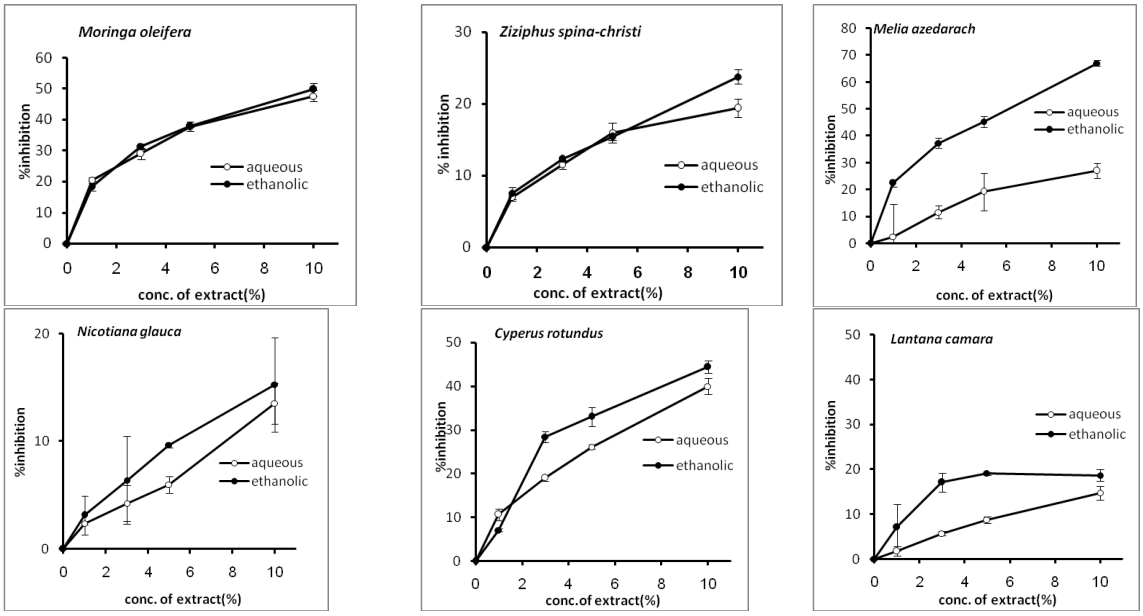


Fig. 2. Effect of aqueous and ethanolic plant extracts of *Moringa oleifera*, *Ziziphus spina-christi*, *Melia azedarach*, *Nicotiana glauca*, *Cyperus rotundus* and *Lantana camara* on growth of *A. alternata.* Each value is the mean of three replicates ± S.E.

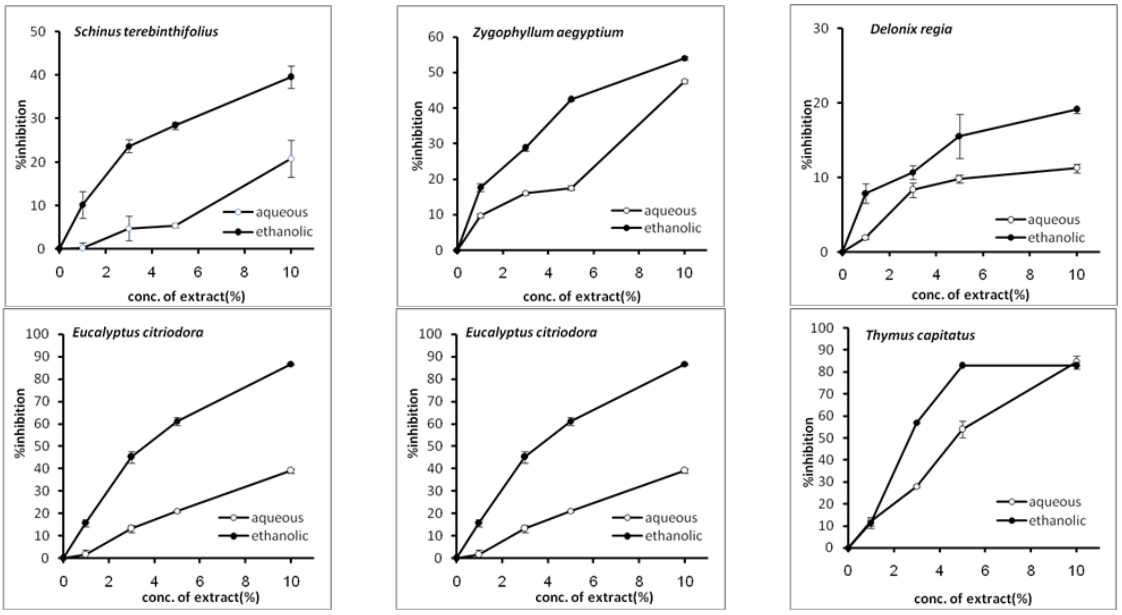


Fig. 2 cont. Effect of aqueous and ethanolic extracts of *Schinus terebinthifolius, Zygophyllum aegyptium, Delonix regia, Eucalyptus citriodora* and *Thymus capitatus* on growth of *A. alternata.* Each value is the mean of three replicates ± S.E.

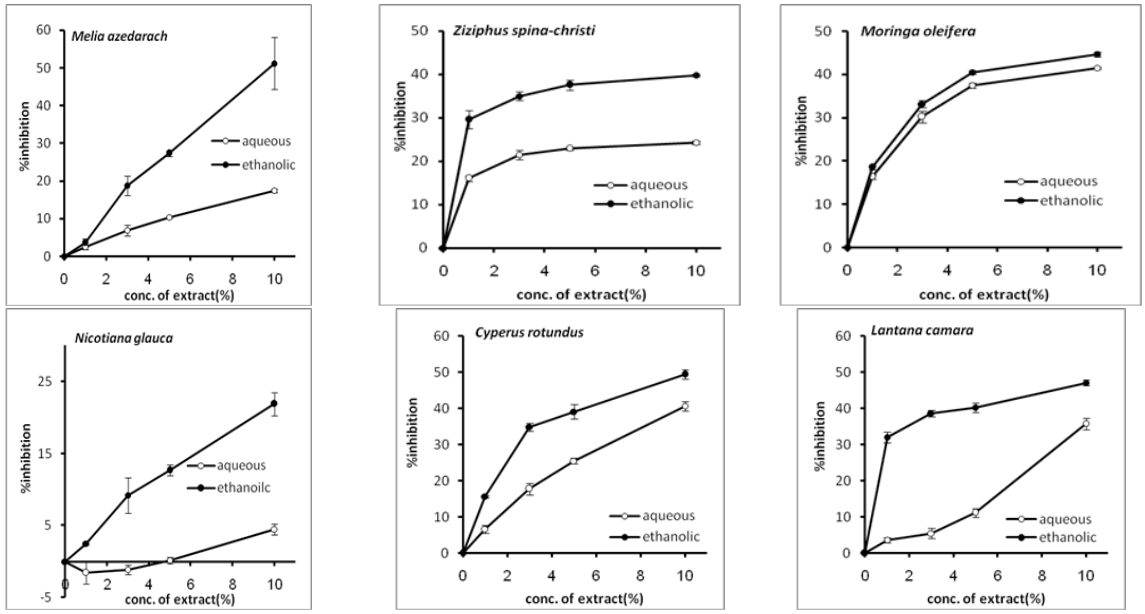
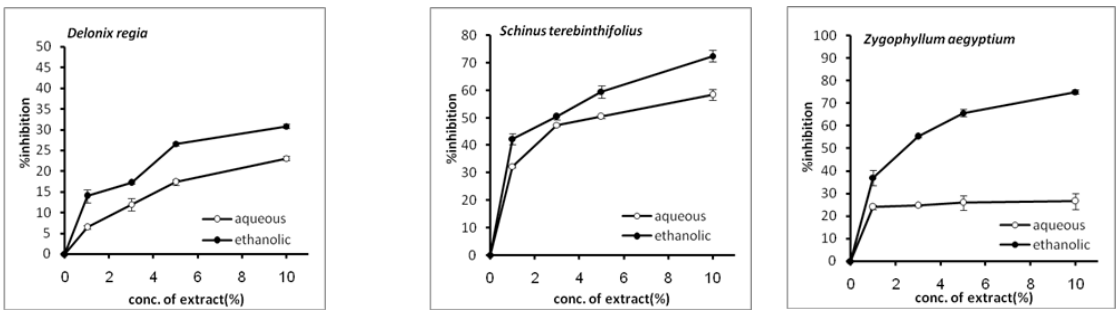


Fig. 3. Effect of aqueous and ethanolic extracts of *Moringa oleifera, Ziziphus spina-christi, Melia azedarach, Nicotiana glauca, Cyperus rotundus* and *Lantana camara* on growth of *F. oxysporum.* Each value is the mean of three replicates ± S.E.



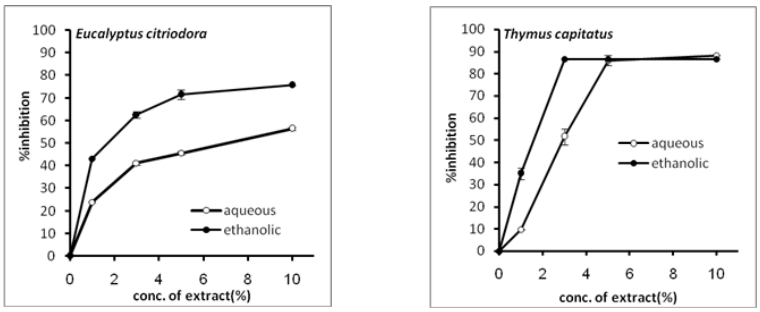


Fig. 3 cont. Effect of aqueous and ethanolic extracts of *Schinus terebinthifolius, Zygophyllum aegyptium, Delonix regia*, *Eucalyptus citriodora* and *Thymus capitatus* on growth of *F. oxysporum.* Each value is the mean of three replicates ± S.E.

Discussion

Postharvest loss of fruits as a result of fungal infection is a severe problem facing the world particularly in the developing countries. The traditional measure to limit this problem is the use of chemical fungicides. But, because of their dangerous consequences for human health, biological control of spoiled fruits by medicinal plant extracts is the update trend to solve this problem.

The eleven plant species investigated in the present study exhibited diverse antifungal activities which varied according to the fungal species, plant species and type of extract. In general, *Thymus capitatus* and *Eucalyptus citriodora* exhibited the strongest effect, whereas *Nicotiana glauca* was the least effective; and the ethanolic extract was more potent than the aqueous extract in the majority of species. This is may be due to the variation in quantity of the active constituents of different plant species, and also to the efficiency of the solvent to extract these substances. In this study, ethanol had a greater capability for the extraction of active substances from testing plants than water did which in agreement of the results obtained by Stephan *et al.* (2005).

The antifungal activity of plant extracts may be related to the presence of many bioactive compounds such as flavonoids, terpenoids, alkaloids, tannins, steroids, glycosides, 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 is 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).

The outstanding antifungal activity of *Thymus capitatus* and *Eucalyptus citroidora* can be related to the unique secondary metabolites produced by the two species. In this respect, Lee *et al*. (2007) reported that the occurrence of several active antifungal compounds, including citronellal and isopulegol in *Eycalyptus citriodora* essential oil and ρ-cymene, γ-terpinene and thymol in *Thymus capitatus*. These active substances, because of their considerable lipophilicity, are subjected to extraction by ethanol to a greater extent than by water, which can partially explain the stronger antifungal efficiency of the ethanolic extract.

In agreement with this postulation, Sani *et al*. (2014) and Shagel *et al*. (2012) reported that the ethanolic and aqueous extracts of *Eucalyptus* spp. share some components, but differ in others. Both the ethanolic and aqueous extracts contain large amounts of saponins, while the aqueous extract contains tannins, saponins, glycosides, steroids and anthraquinones but no alkaloids, flavonoids and terpenoids; and the ethanolic extract contain tannins and steroids but no glycosides and anthraquinone. The presence of these phytochemicals in *Eucalyptus* spp. justifies manipulation of the plant in the management and curing of various ailments. Likewise, it has been reported that *Thymus capitatus* has a powerful antifungal activity by virtue of its high content of a wide range of bioactive compounds like essential oils which can act as biogenetic precursors of phenolic compounds such as ρ -cimene, γ-terpinene, and β-cariophyllene; in addition to its high content of phenols such as carvacrol (Mariateresa et *al*., 2013). The mechanism of action of carvacrol and thymol as fungicides appears to be through the inhibition of ergosterol biosynthesis and disruption of membrane integrity of the fungus as reported by Bouchra *et al*. (2003) and Ahmed *et al*. (2011). In addition, preliminary phytochemical screening of *Thymus capitatus* revealed the presence of saponins, resins, flavonoids, essential and fixed

oils; compounds of profound inhibitory effect against several bacteria and fungi (Kandil *et al*., 1994). In addition, Tabti *et al*. (2015) identified fifteen fatty acids accounting for 95% of the lipid content responsible for the *in vitro* antifungal activity of this species.

The potency of the antifungal activity 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 C_{50}. The two measures lead to the same conclusion that the ethanolic extract is more powerful than the aqueous extract and that the most potent species are *Thymus capitatus* and *Eucalyptus citriodora*. From the data of Table 7, it is clear that the values of C_{50} of the ethanolic extracts are more frequent and of lower magnitude than those of the aqueous extracts and that *Thymus capitatus* and *Eucalyptus citriodora* yielded the lowest C_{50} among the studied species. Normally, the lower the C_{50} the more potent is the antifungal activity of the extract; and whenever an extract has no value for C_{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 (10% w/v).

The two fungal species examined exhibited different susceptibility towards the action of plant extract; and in general *F. oxysporum* was more affected than *A. alternata.* The differential susceptibility of fungal species to active plant ingredients is well documented and Lee *et al*. (2007) reported that out of the five fungal species examined, *F. oxysporum* proved to be the most susceptible fungus to the action of the essential oils of five plant species including *Eucalyptus citriodora* and *Thymus spp*.

The difference in the concentration-response relationship between the different plant species and according to the fungal species and type of extract demonstrated in Figures 2 and 3 suggests different modes of action of the different plant extracts on the two fungal species. The saturable relationship always refers to a stronger effect of the extract which approaches an asymptote at a lower dose, thus yielding a lower C_{50} compared to the extract exhibiting the linear trend. In support to this postulation, the saturable trend was more frequent, and more evident in the ethanolic extracts of the majority of the studied plant species than in the aqueous extracts, and the reverse was true for the linear trend. In this respect, Abdel-Mogib *et al.* (2001) found that the ethanolic extract of *Tamarix aphylla* was inhibitory to the growth of a diverse array of microorganisms of bacteria and fungi including *Fusarium* spp. than the aqueous extract.

Conclusion

In conclusion, we recommend using *Thymus capitatus* and *Eucalyptus citriodora* extracts as preservatives for fruits against fungal attack.

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الملخص العربي

عنوان البحث: النشاط الضد فطري لمستخلصات بعض النباتات الطبية ضد الفطريات الممرضة السائدة التي تسبب فساد بعض الفواكه

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تعتبر الفطريات من أهم العوامل الرئيسية التي تصيب معظم الفواكه بعد الحصاد إذ انها تسبب خسائر فادحه لهذه الفواكه وذلك أثناء النقل والتخزين والتداول وخاصة إذا كان ذلك فى ظروف غير صحية. في هذا البحث تم جمع عشرة أنواع من الفواكه المصابه بمعدل خمسة من كل نوع من أسواق مختلفة في محافظة دمياط، وقد تم عزل وتعريف عشرة أنواع من الفطريات من على هذه الفواكه والتي تسبب فسادها وكان أكثر الفطريات شيوعا فطري الترناريا الترناتا وفيوزاريم اوكسيسبورم وتم عزلهما من اليوسفى والعنب على الترتيب، وللحد من استخدام المبيدات الفطريه الكيميائيه والتى تسبب ضررا للإنسان والبيئة فكان الإتجاه لاستخدام مستخلصات نباتية طبيعية لمقاومة هذه الفطريات بيولوجيا، ولقد تم اختبار مستخلصات عشرة أنواع من النباتات ضد هذين الفطرين عن طريق الاستخلاص بمذيبين هما الماء والايثانول بمعدل خمسة تركيزات لكل مستخلص وهم ٠% و ١% و ٣% و ٥% و ١٠%، وقد وجد أن المستخلص الايثانولى للنباتات عموما أكثر تثبيطا للفطرين عن مثيله المائي وأن مستخلصات نبات الزعتر البري والكافور الليمونى هما الأفضل على الإطلاق كمثبطات لكل من الفطرين بينما كان نبات الدخان هو الأضعف لذلك يمكن استخدام مستخلصات الزعتر والكافور كمواد حافظه للفاكهة للحد من الفطريات المسببة لأمراض ما بعد الحصاد.