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Assessing Efficacy of Some Egyptian Medicinal Plants Extract against Postharvest Fungi cause Spoilage of Fruits.

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ABSTRACT

Considerable losses of fruits during handling, packaging, transportation, and storage. Five infected fruits were collected [apple, grapes, mandarin, peach, and plums] from the local market of 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 dominant fungal species were isolated from apples and peaches, respectively. Results indicated that the aqueous extracts of three medicinal plants [*Eucalyptus citriodora* ĽHér, *Inula crithmoides* L., *Launaea nudicaulis* [L.] collected from the area around were tested against the two dominant fungi at four concentrations [0.0, 0.25, 0.5, 1%; w/v]. The greater relative inhibition of fungal growth was recorded at the concentration of 1% of both plants, but the extract of *Eucalyptus citriodora* was more effective against tested fungi than that of other plants. So, the use of *Eucalyptus citriodora* extracts as potential antifungal preservatives for fruits against fungal spoilage instead of synthetic fungicides because they are available, safe, inexpensive, and ecofriendly.

Key words: Biocontrol, fruits, medicinal plants, plant extracts, spoilage of fruits

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INTRODUCTION

Fruits are important food commodities not only in Egypt but all over the world. Egypt is still struggling to achieve self-sufficiency to feed about 100 million people. For this purpose, fruits have got their specific importance to provide a balance and healthy diet [1]

Postharvest diseases considered as a major problem for the spoilage of many edible fruits. According to [2], of all losses of fruits caused by plant diseases, occur after harvest and are the most costly. Fruits are highly perishable products; their quality affected by postharvest handling, transportation, storage and marketing [3]. The improper handling, packaging, storage, and transportation may result in decay by microorganisms [4, 5]. Fruits, due to their low pH, high moisture content and rich nutrient composition are very susceptible to attack by pathogenic fungi; which in addition producing mycotoxins; thereby making the fruits unfit for consumption [Moss, 2002]. Several species of fungi and in some cases, bacteria participate in postharvest deterioration and rots of fruits. These include species of *Aspergillus, Fusarium, Colletotrichum, Macrophomina, Penicillium and Rhizopus* amongst several others [6, 7].

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 [8].

An international trend toward the use of natural substances present in plants, fruits, vegetables, oilseeds, and herbs as antimicrobial [9, 10]. Environment-friendly plant extract agents have been shown to be of great potential as an alternative to the synthetic fungicides [11].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 [12; 13]. 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 [14].

The aim of the present study is to evaluate some medicinal plant extracts for controlling the dominant fungal pathogens causing postharvest diseases of some fruits like apple, grapes, mandarine, peach and plum.

MATERIALS AND METHODS

Collection of spoiled fruits

Five infected fruit species of economic importance growing in Egypt were collected from the local markets in Damietta Governorate [Table 1]

Table 1: Scientific and common names of fruits under study

Scientific name	Common name	Family
Malus domestica Borkh.cv. Barkher	Apple	Rosaceae
Vitis vinifera L. cv. Thompson	Grapes	Vitaceae
Citrus reticulata Blanco. cv. Balady	Mandarin	Rutaceae
Prunus persica (L.) Batsch	Peach	Rosaceae
Prunus domestica L. cv. Santa Rosa	Plum	Rosaceae

Preparation of potato dextrose agar [PDA] medium

Two hundred grams of potato extract and 20 g glucose were added to one liter of distilled water. After stirring, 20 g agar was added and the mixture was autoclaved at 1.5 p.s.i. and 121°C for 20 min. and the cooled medium was tipped on sterile Petri dishes [about 20 ml / 9 cm dish].

Isolation and identification of fungal pathogens

Fungal pathogens were isolated from fruits according the method of [15] 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 twice 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°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 [16, 17, and 18].The isolated fungi were maintained on PDA slants at 5°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 five fruits were recorded and expressed as percentage of the total number of isolates, according to [19].

$$\text{Percentage of occurrence} = X \times 100/N$$

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 [19]. 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 [20].

Plant samples collection

Three medicinal plant species were collected from different habitats of Damietta, Egypt [Table 2 and Fig. 2]. The plant species were identified according to [21, 22] and deposited as herbarium sheets at Botany and Microbiology Department, Faculty of Science, Damietta University.

Table 2: List of medicinal plants tested for preparation of plant extracts

Scientific name	Common name	Family	Part Used
Eucalyptus citriodora ĽHér	Myrtle	Myrtaceaea	Leaves
Inula crithmoides L.	*Golden Samphire*	Asteraceae	Aerial parts
Launaea nudicaulis (L.) Hook.f.	Launaea	Asteraceae	Aerial parts

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 100 ml distilled water and left at room temperature for 24 hours. The mixture was filtered through sterile Whatman filter paper No.1 and centrifuged twice at 4000 rpm for 10 minutes. The supernatant was poured in conical flasks and covered with cotton plugs and left for 10 minutes in a digital water bath at 100°C to avoid contamination [23].Four concentrations [0.0, 0.25, 0.5, 1%; w/v] were used.

Antifungal activity

The agar-amended media was used according to [24]. Aqueous plant extracts at the concentrations of [0.0, 0.25, 0.5, 1%; w/v] were tested against the two predominant fungal species [*Alternaria alternata* and *Fusarium oxysporum*] isolated from apples and peaches, 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.

RESULTS

Seven fungal pathogens were isolated from five spoiled fruit species; viz: *Alternaria alternata, Aspergillus niger, A. nidulans, A. ochraceous, Fusarium oxysporum, Penicillium expansum,* and *Rhizopus stolonifer*. The most prevalent fungal species were *Alternaria alternata* and *Fusarium oxysporum* with the relative occurrence of 40.4 % and 54.0 %, respectively, of the total number of isolates [Table 3]. Apples and peaches exhibited 100% successful infection by these two pathogens after the pathogenicity test.

These two fungal species were subjected to biological control *in vitro* by using aqueous extracts of three plant species at five concentrations [0.0, 0.25, 0.5, 1%; w/v]. Figure Table 4 shows highly significant effect of plant species fungal growth as well as a highly significant difference in fungal susceptibility to treatments.

Fig. 2 shows the effect of extracts [at 1.0%] of *Inula crithmoides* and *Eucalyptus citriodora* on the growth of *Alternaria alternata* and *Fusarium oxysporum*. *Inula* water extract was more effective on *A. alternata* than that of *Eucalyptus.*

Effect of water extracts on the linear mycelial growth of tested fungi [*A. alternate and F. oxysporum*] is shown in Table 4. Results indicated that the treatments of *Inula* and *Eucalyptus* were positively effective in reducing the mycelial growth of the fungi tested, compared to the control. The reduction of mycelial growth showed an increase when the concentration of the extracts was increased from 0.25-1.0 %. On the other hand, *Launaea* exhibited no significant inhibition of fungi tested.

At the concentration of 0.25%, the growth inhibition of *A. alternata* was 22.5, 29.8 and 3.4% for Eucalyptus, Inula and *Launaea*, respectively and for *F. oxysporum* it was 52.5, 31.9 and 3.4%, respectively. Moreover, at 0.5%, the growth inhibition of *A. alternata* was 29.1, 22.2 and 5.6%, respectively and for *F. oxysporum* it was 48.9, 36.2 and 1.4%, respectively**.** On the other hand**,** at 1.0%, the growth inhibition of *A. alternata* was 32.5, 38.1 and 7.0%, respectively and for *F. oxysporum* it was 55.4, 52.8 and 3.4%, respectively**.**

Figure 1: Plants used for extraction; A. *Eucalyptus citriodora*; B. *Inula crithmoides*; C. *Launaea nudicaulis*

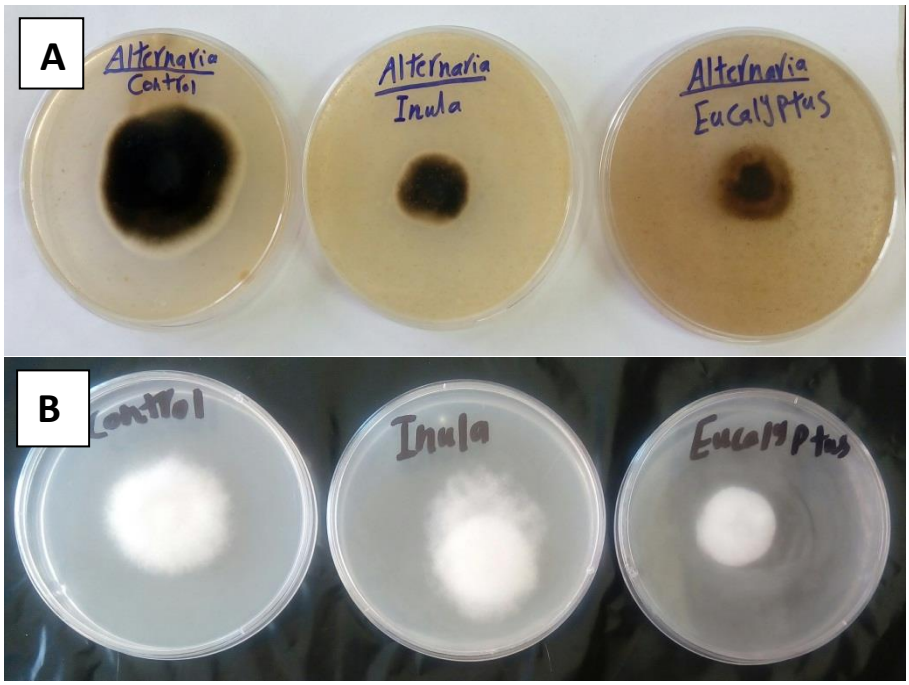

Figure 2. Effect of extracts (at 1.0%) of *Inula crithmoides* and *Eucalyptus citriodora* on the growth of *Alternaria alternata* (A) and *Fusarium oxysporum* (B).

Table 2: List of medicinal plants tested for preparation of plant extracts

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Table 4: Isolated fungal species from spoiling fruits. Each value is the mean of 5 replicates

Fruit	Disease	Isolated fungus	No. of colonies	Occurrence (%)
Apple	*Alternaria* rot Blue mold rot Black mold rot	*Alternaria alternata* (Fr.) Keissl *Penicillium expansum* Link *Aspergillus niger* van Tieghem	30 10 9	15.9 5.3 4.8
Grapes	*Dry rot* *Rhizopus rot*	*Fusarium oxysporum* Sch. Em. Syn. Hansen *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	15 6	7.9 3.2
Mandarin	*Alternaria rot* *Black mold rot* *Black mold rot*	*Alternaria alternata* (Fr.) Keissl *Aspergillus nidulans* G. Winter *Aspergillus ochraceus* Wilhelm	16 12 13	8.5 6.3 6.9
Peach	Dry rot Blue mold rot *Rhizopus* rot	*Fusarium oxysporum* Sch. Em. Syn. Hansen *Penicillium expansum* Link *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	25 10 8	13.2 5.3 4.2
Plums	*Alternaria* rot Dry rot *Rhizopus* rot	*Alternaria alternata* (Fr.) Keissl *Fusarium oxysporum* Sch. Em. Syn. Hansen *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill	16 14 5	8.5 7.4 2.6
Total colonies			189	

Table 4: Effect of different concentrations of aqueous extracts of plant species on the growth of *A. alternata* and *F. oxysporum.* Each value is the mean of 5 replicates ± SE.

Mean ± SE of inhibition of linear mycelial growth (mm)												
	0.25%				0.5%				1.0%			
Plant species	*Aa*	I %	*Fo*	I%	*Aa*	I%	*Fo*	I%	*Aa*	I%	*Fo*	I%
E. citriodora	23.4 ±0.4	22.5	16.8 ±0.3	52.5	21.4 ±0.2	29.1	18.1 ±0.4	48.9	20.4 ±0.4	32.5	15.8 ±0.2	55.4
I. crithmoides	21.2 ±0.3	29.8	24.1 ±0.5	31.9	23.5 ±0.6	22.2	22.6 ±0.3	36.2	18.7 ±0.2	38.1	16.7 ±0.4	52.8
L. nudicaulis	29.1 ±0.3	3.4	34.2± 0.4	3.4	28.5 ±0.2	5.6	34.9± 0.6	1.4	28.1± 0.3	7.0	34.2 ±0.5	3.4
The control	30.2		35.4		30.2		35.4		30.2		35.4	

Aa= Alternaria alternata; Fo = Fusarium oxysporum, I% = Inhibition percentage

DISCUSSION

Postharvest loss of fruits as a result of fungal infection is a severe problem facing the world particularly in the developing countries. The health, biological control of spoiled fruits by medicinal plant extracts is the update trend to solve traditional measure to limit this problem is the use of chemical fungicides. But, because of their dangerous consequences for human this problem [25].

The three plant species investigated in the present study exhibited diverse antifungal activities which varied according to the fungal species and plant species. In general, *Inula crithmoides* exhibited the strongest inhibition on *Alternaria* growth, while *Eucalyptus citriodora* showed the strongest effect, on *Fusarium* growth, whereas *Launaea nudicaulis* was the least effective. This is may be due to the variation in quality and quantity of the active constituents of different plant species.

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 [26 ; 27].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 [28].

The outstanding antifungal activity of *Eucalyptus citroidora* can be related to the unique secondary metabolites produced by the species. In this respect, [29] reported that the occurrence of several active antifungal compounds, including citronellal and isopulegol in *Eycalyptus citriodora* essential oil.

In agreement with this postulation, [30,31] reported that aqueous extracts of *Eucalyptus* spp. contain tannins, saponins, glycosides, steroids and anthraquinones but no alkaloids, flavonoids and terpenoids. The presence of these phytochemicals in *Eucalyptus* spp. justifies manipulation of the plant in the management and curing of various ailments.

The potency of the antifungal activity of plant extracts was estimated in terms of the relative inhibition of fungal growth below the control. 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 [29] 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*.

In conclusion further studies are required on, *Inula crithmoides* and *Eucalyptus citriodora* extracts which might use as preservatives for fruits.

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