

***In vitro* Antifungal Activity of Some Plant Extracts against Seed-borne Pathogens**

F. A. Abushaala¹, A. R. Ben Ramadan¹, M. A. S. Fahej²

¹Microbiology Department, Faculty of Science, Misurata University, Libya

²Biology Department, Faculty of Science, Elmergib University, El-khums, Libya.

Abstract: Chemical fungicides cause serious environmental problems. Plant metabolites and plant based pesticides considered to be better alternatives as they are known to have minimal environmental impact in contrast to synthetic pesticides. In this study some plant extracts in different concentrations were tested in vitro for their activity against some cucurbit seed-borne fungi. All tested plant extracts significantly reduced the growth of the tested damping-off pathogens at all tested concentrations. *F. semitectum* and *F. solani* were more sensitive to plant extracts treatment than the other tested pathogens, where *R. raetam* extracts with 15% and 10% concentrations as mean were more effective to tested damping-off pathogens, whereas *R. raetam* 5% concentration and *P. dactylifera* 15% concentration were similarly efficient in suppression (30.0 and 30.2 mm, respectively). *C. trifurcatum* gave lowest effect, whereas *P. dactylifera* 5% and *C. trifurcatum* 15% gave the equal values (27.2%). The highest inhibitory zone values were obtained by the plant extract *R. raetam* (32.53 mm as a mean values). *F. solani*, *F. semitectum* and *F. moniliforme* were more sensitive with the highest inhibitory zone values due to plant extract treatments (37.0, 36.0 and 36.0 mm, respectively).

Keywords: Damping-off pathogens, Plant extract, Cucurbit seed-borne fungi, Antifungal activity.

I. Introduction

Cucurbits are important vegetable crops that daily consumed world over. The family *cucurbitaceae*, includes cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), cantaloupe (*Cucumis melo* var. *cantaloupensis*), snak cucumber (*Cucumis melo* var. *flexuosus*), sweetmelon (*Cucumis melo*) and pumpkin (*Cucurbita* sp.). Serious soil-borne and seed-borne pathogens, i.e. *Pythium debaryanum*, *P. ultimum*, *P. dissotocum*, *P. oligandrum*, *P. violae*, *P. aphanedermatum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, *F. semitectum*, *Aphanomyces euteiches*, *Sclerotinia sclerotiorum* and many species of *Verticillium* and *Cladosporium*., can cause damping-off and root rot diseases, resulting in great economic losses in crop yield and quality.

The use of fungicides for control of these pathogens has met moderate success and their future use is in question due to increased regulatory restrictions. So, the modern approach in plant disease control is directed toward minimizing the fungicidal use to decrease environmental pollution and finding alternatives to chemical fungicides. Hence, in recent time application of plant extracts as well as plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly where botanicals place an important role (Sahayaraj *et al.*, 2009). Several investigation studies have been conducted in order to screening different plants for their antifungal properties (Stephan *et al.*, 2005 and Satish *et al.*, 2010) and biochemical compounds that these plants have. Studies revealed a highly significant antifungal activity of some water extracts or essential oils of plants. Since some plants are already known to possess several biological activities (Amin *et al.*, 2009 and Belabid *et al.*, 2010).

1. Plant extracts:

The antimicrobial activity of secondary metabolites and lectins, compounds usually associated to defense mechanisms of plants. Secondary metabolites are separated into nitrogen compounds (alkaloids, non-protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and nonnitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins). Lectins are carbohydrate-binding proteins and their biological properties include cell-cell interactions. This chapter reports solvent organic extracts (mixture of secondary metabolites), isolated secondary metabolites and lectins from plants with antimicrobial activity against Gram-negative and Gram-positive bacteria as well as antifungal activity towards human and plant pathogens. Effects of plant secondary metabolites and lectins on deleterious human and plant microorganisms indicate their perspectives of antimicrobial uses (Paiva *et al.*, 2010). Solvent organic extracts contain a mixture of secondary metabolites including alkaloids, flavonoids, terpenoids and other phenolic compounds; these molecules are associated to defense mechanisms of plants by their repellent or attractive properties, protection against biotic and abiotic stresses and maintenance of structural integrity of plants (Tepe *et al.*, 2005).

1.1. *Retama raetam*:

Retama raetam (Forssk.) Webb & Berthel. (Fabaceae alt. Leguminosae), commonly known as 'raetam' or 'broom bush', is a desert shrub native to several countries of North-Africa (e.g., Algeria, Egypt, Libya, Morocco and Tunisia), temperate Asia (e.g., Israel, Jordan, Lebanon, Palestine and Syria) and south-eastern Europe (e.g., Sicily in Italy) (GRIN Database, 2011). This plant has long been used as an abortifacient, a purgative and a vermifuge in the traditional medicine systems of the source countries (Dr. Duke's Phytochemical and Ethnobotanical Databases, 2011). Previous pharmacological studies on this plant have revealed its antibacterial, antifungal, antihypertensive, anti-oxidant, antiviral, diuretic and hypoglycemic properties and hepatoprotective, nephroprotective and cytotoxic effects (Hayet *et al.*, 2008; Koriem *et al.*, 2009; Algardaby *et al.*, 2010 and Edrizi *et al.*, 2010). In continuation of our phytochemical and pharmacological studies on the Libyan medicinal plants (Auzi *et al.*, 2008; Elouzi *et al.*, 2008 and Geroushi *et al.*, 2010).

1.1.1. Phytochemical Screening:

During the last decades, a huge number of chemical compounds have been isolated from the genus *Retama*, including flavonoids, alkaloids, saponins, lignans and others (Harborne, 1969 and Harborne *et al.*, 1971). Abdel Halim *et al.*, (1997) reported that, *Retama* species contain flavonoids and alkaloids. Phytochemical studies have shown that *Retama* species are very rich in flavonoids, triterpenoides, saponins, alkaloids, phytosterols, cellulose, hemicellulose and lignin (Moubasher, 1998; Kassem *et al.*, 2000 and Cheriti *et al.*, 2004). Djeddi *et al.*, (2013) found that the isoflavones genistein, 6-hydroxygenistein, 3'-O-methylrobo, pratensein, biochanin A, the flavones 6-hydroxyapigenin and luteolin, the flavonol kaempferol, as well as the phenolic compound p-coumaric acid reduce significantly the pain at a concentration dose of 1 mg/kg. Mobin *et al.*, (2015) reported that, the phytochemical screening of the *R. raetam* extracts revealed the presence of alkaloids, phenols, flavonoids, terpenoids, tannins and carbohydrates. However, saponins were absent.

1.1.2. Antifungal activity:

Previous pharmacological studies on the plant have revealed its various medicinal properties: antibacterial, antifungal, antihypertensive, antioxidant, antiviral, diuretic, hypoglycaemic, hepatoprotective, nephroprotective and cytotoxic effects (Edziri *et al.*, 2010). Hayet *et al.*, (2007) showed the activity of *Retama raetam* was low against the different *Candida* spp., i.e. *C. glabrata*, *C. albicans*, *C. parapsilosis* and *C. kreusei*. Edziri *et al.*, (2010) indicated that the antibacterial, antifungal and cytotoxic activities of two flavonoids isolated from *R. raetam* flowers. The compounds licoflavone C and derrone showed important antifungal activity. Strong antifungal activity against *Candida* species (7.81 µg/mL) was for example found with derrone. These two compounds may be interesting antimicrobial agents to be used against infectious diseases caused by many pathogens.

1.2. *Phoenix dactylifera*:

1.2.1. Phytochemical Screening:

Whole plant contains Carbohydrates, steroids, alkaloids, flavonoids, tannins and vitamins. Its phenolic profile shows that it contains cinnamic acids, falconoid glycosides and flavones. Dates contain nutrients as carbohydrates (44-88%), dietary fibers (6.4-11.5%), fats (0.2-0.5%) and proteins (2.3-5.6%). Dates contain fatty acids as palmitoleic acid, Oleic, linoleic and linolenic acid. In dates, 23 amino acids are found. Furthermore vitamin A, B1, B2 and nicotinic acid are also found in dates stated by Abdu, 2011. Several studies on antimicrobial activity of *P. dactylifera* have been reported (Vyawahare *et al.*, 2009); however antifungal potential of date palm extracts have not been explored.

Fungi are very large and diverse group of organisms, which are responsible for causing large number of diseases to human beings, animals and plants (Mehrotra, 1993). Delphin *et al.*, (2014) revealed the presence of seed ethanolic extracts of *Phoenix dactylifera* contains saponin, terpenoids, phenolic compounds and glycosides but none contains tannins, flavonoids, carotenoids, protein, carbohydrate and alkaloids. Chinelo *et al.*, (2012) studied the aqueous extract of the fruits of *Borassus aethiopum*, *Borassus flabellifer*, *Balanite aegyptiaca*, *Phoenix dactylifera* and *Tamarindus indica* were tannins, saponins, phlobotannins, terpenoids, flavonoids, cardiac glycosides, glycosides, phenol, anthracene, free-anthraquinone, carotenoids, steroid, reducing compounds and alkaloids. All plant specimens seem to potential as a source of useful drugs.

1.2.2. Antifungal activity:

Bokhari and Perveen (2012) tested water, methanol and acetone extract of leaves and pits of Dates on seven pathogenic fungi *F. oxysporum*, *Fusarium* sp., *F. solani*, *A. flavus*, *A. alternata*, *Alternaria* sp. and *Trichoderma* sp. by agar well diffusion and agar dilution method against fungi. Methanol pits extract was highly effective against most of the tested fungi followed by methanol leaves extract, acetone pits and acetone leaves extract. The most sensitive fungus was *A. alternata*, its growth inhibition was maximum (51.6%) by methanol

pits extract of var. Barhee. 50% inhibitory effect (IC₅₀) of methanol pits extract of var. Barhee against *F. oxysporum*, *F. solani*, *Fusarium* sp., *A. alternata* and *Alternaria* sp. was found to be 6.7, 4.9, 3.4, 2.8 and 3.7 mg/ml, respectively. Water extracts of both varieties showed no effect on growth of tested fungi. Strong antifungal activity was seen with methanol pits and leaves extract against *A. alternata*. Poor to negligible antifungal activity was seen with all extracts against *A. flavus*. Order of activity according to zone of inhibition against almost all fungi is as follows: Methanolic pits extract > Methanolic leaves extract > Acetone pits extract > Acetone leaves extract. Boulenouar *et al.*, (2011) stated that dichloromethane extract of date fruit has also antifungal activity.

1.3. *Chrysanthimum trifurcatum*:

1.3.1. Phytochemical Screening:

Ukiya *et al.* (2002) reported that the phytochemical profile of *Chrysanthemum* species has shown the presence of terpenoids. Ahlem *et al.* (2008) indicated that the most plentiful constituent of flowers' head, leaves and stems essential oils from Tunisian *C. trifurcatum* was limonene. But in the roots oil, α -Calacorene was the predominant compound. Although, the essential oils from all parts of *C. trifurcatum* were rich in terpenoid components. Lin and Harnyl (2010) reported that the phytochemical profile of *Chrysanthemum* species has shown the presence of flavonoids and phenolic compounds. Zhang *et al.* (2012) showed that the ethyl acetate extract of *C. indicum* contains terpenes and flavonoids mainly. Besides, the content of flavonoids in ethanol extract is the highest by comparison with different extracts. Umadevi and Sujatha (2013) reported that the methanolic and acetone crude extracts of *C. trifurcatum* leaves from India contained tannins, flavonoids, terpenoids, alkaloids, saponins, cardiac glycosides, reducing sugars and triterpenoids. Ahlem *et al.* (2014) reported that, tannins, alkaloids and flavonoids were detected in the methanolic extracts of all parts of *C. trifurcatum*. But, all petroleum ether extracts do not contain tannins, flavonoids and alkaloids. Among, all ethyl acetate extracts showed the presence of tannins. Based on this preliminary phytochemical analysis, the methanol extract of *C. trifurcatum* showed the presence of important bioactive compounds, namely tannins, flavonoids and alkaloids. Other researches, suggested that, flavonoids, triterpenes, vitamin, and polysaccharides were the main active ingredient of *C. morifolium* (Lin and Harnyl, 2010 and Xiaoyan *et al.* 2011). Recently, it has been signaled that *C. morifolium* was rich in flavonoids, glycosides and caffeoylquinic acids (Tao *et al.*, 2013). In addition, *C. coronarium* and *C. segetum* were rich in flavonoids (Valant-Vetschera *et al.*, 2003).

1.3.2. Antifungal activity:

Bahraminejad *et al.*, (2013) showed that methanolic crude extracts of 32 plant species included *Chrysanthemum* sp. belonging to 21 families collected from the west of Iran were screened for antifungal activity against three phytopathogenic fungi, *Phytophthora drechsleri*, *Pythium aphanidermatum* and *Rhizoctonia solani*. Bioassay of the extracts was conducted by agar diffusion method on agar plate cultures with five replications. Twenty-seven of the 32 (85%) plant species tested, showed inhibitory activity against at least one of the fungi. Seventeen (53%), thirteen (41%) and seventeen (53%) tested plant species showed inhibitory activity against mycelia growth of *P. drechsleri*, *P. aphanidermatum* and *R. solani*, respectively. Ahlem *et al.* (2014) reported that the most active *C. trifurcatum* extracts tested against bacterial strains, contained tannins, flavonoids and alkaloids. They can be toxic to bacteria, yeasts and filamentous fungi (Scalbert, 1991). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and they also complex with polysaccharide (Ya *et al.* 1988). Furthermore, flavonoids were known to be synthesized by plants in response to microbial infection (Dixon *et al.* 1983). Ahlem *et al.* (2014) showed that, essential oils and organic extracts were tested for antibacterial activities against eight Gram-positive and Gram-negative strains, using a microdilution method. Antifungal activity of *R. raetam* and *C. trifurcatum* has not been reported earlier. We carried out this study to investigate the antifungal, activities of the ethanolic extracts of tested plant parts. Therefore, the objective of this work is controlling the isolated damping-off and root rot pathogens, using some plant extracts *in vitro*.

II. Materials and Methods

2. Disease Control:

2.1. Plant Extract:

2.1.1. Preparation of Plant Crude Extracts:

The plants namely, *Retama raetam* (flowers, Stems & fruits) and *Phoenix dactylifera* (seeds) were collected from Misurata region in north Libya, while *Chrysanthemum trifurcatum* (mixed of leaves, stems and roots) was collected from Alojyate and Mizda in west and south Libya during spring season. They were cut into small pieces and dried at room temperature (25°C \pm 2°C) for 3 days. The air-dried plant (1000g) was extracted with ethanol by Soxhlet extractor. Extracts were concentrated by Rotary evaporation and dry at 45°C under reduced pressure for 15-30 min following solvent preparation of extracts and finally the plant with the maceration of water for 24h and then the extract was freeze dried.

2.1.2. Qualitative phytochemical screening:

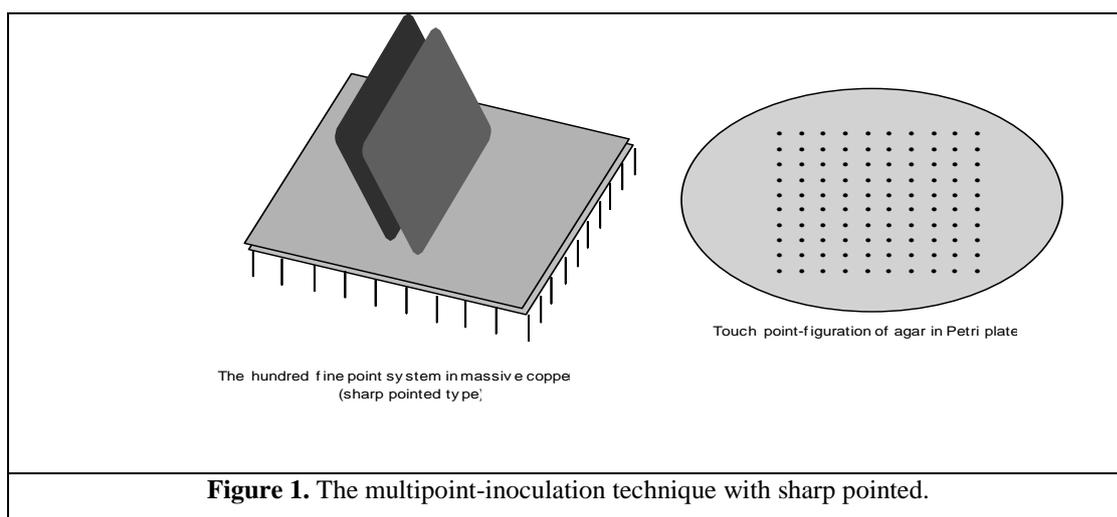
Detection of presence of phytoconstituent in plant extract was as described by Ciulei, (1994), Sofowora, (1993), Umesh *et al.*, (2010) and Harborne, (1998).

2.1.3. *In vitro* experiment:

A laboratory study was performed to examine the sensitivity of *F. solani*, *F. moniliforme*, *F. semitectum* and *M. phaseolina* to the plant extracts namely, *Retama raetam* (flowers, Stems and fruits), *Phoenix dactylifera* (seeds) and *Chrysanthemum trifurcatum* (mixer of leaves, stems and roots).

2.1.3.1. Fungal inhibition diameter method (FID):

The multipoint-inoculation technique with needles such as curved sharp pointed (Poppe, 1991) was used to inoculate Petri dishes with the tested fungi (Fig. 1). Drenched plant extract paper-disks at three concentrations, i.e (5%, 10% and 15%) in sterile distilled water, were placed on the inoculated PDA medium plate. Paper-disks soaked in sterile water were used as a control. Four replicates were used for each treatment. The fungal-inhibition-diameters (FID) were measured periodically as the growth of each fungus in control treatment reached to the dish edge (Amer, 1995).



III. Experiment Results

3. Plant Extracts:

This part planned to evaluate efficacy of some plant extracts in controlling diseases caused by the five tested seed-borne pathogens *in vitro*.

3.1. Preliminary Phytochemical screening:

The screening of these different plant species namely, *Retama raetam*, *Chrysanthemum trifurcatum* and *Phoenix dactylifera* for phytochemical constituents was performed using generally accepted laboratory technique for qualitative determinations. From Table (10), The study indicates the presence of Alkaloids, Flavonoids, Phenolic compounds, Carbohydrates, Glycosides, Acetate, Steroides, Saponins, Tanins and Terpenoids in all the ethanolic extract of the *R. raetam* (fruits, flowers and Stems), *C. trifurcatum* (mixed of leaves, stems and roots) and *P. dactylifera* (seeds). Alkaloids and carbohydrates were found in all plant extracts, while flavonoids was absent in all plant extracts except *C. trifurcatum*, phenolic compounds were found in all parts of *R. raetam*. Glycosides and acetate were found in fruits and Stems of *R. raetam* but not found in flowers, while steroides was found only in the *P. dactylifera* and tannin was found only in *C. trifurcatum*.

Table 1. Phytochemical screening of the plant extracts.

Phytochemical Compounds	<i>R. raetam</i> (fruits, flowers & Stems)			<i>C. trifurcatum</i> (all of plant)	<i>P. dactylifera</i> (seeds)
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	+	-
Phenolic compounds	+	+	+	*	*
Carbohydrates	+	+	+	+	+
Glycosides	+	-	+	-	*
Acetate	+	-	+	*	*
Steroides	*	*	*	-	+
Saponins	*	*	*	-	-
Tannins	*	*	*	+	-
Terpenoids	*	*	*	-	*

+ = Present ; - = Absent; * Not tested.

1.2. *In vitro* trials:

1.2.1. Fungal inhibition diameters (FID) technique:

Laboratory tests were carried out to check the effect of the tested plant extracts, i.e. *R. raetam* (fruits, flowers and leaves), *C. trifurcatum* (all of plant) and *P. dactylifera* (seeds) with different concentrations (5%, 10% and 15%) on growth and development of tested pathogens, i.e. *F. solani*, *F. moniliforme* , *F. semitictum*, *S. sclerotiorum* and *M. phaseolina*. Determination of plant extracts efficacy was carried out using fungal inhibition diameter (FID) methods. The obtained results were statistically analyzed, then illustrated in (Table 2 and Fig. 2). All concentrations application rates of the tested plant extracts were, generally, significantly effective in increasing inhibition zone diameters (IZD) of the tested pathogens, where IZD values ranged 14 mm to 37 mm. Moreover, concentration 15% was more effective in inhibition of the tested pathogens than other concentrations (Table 2).

All the plant extracts tested showed antifungal activity, results obtained in the present study show that plants extract possesses potential antifungal activity against the tested seed-borne pathogens; however, concentration 15% and concentration 10% were found to be more effective antifungal agents than the concentration 5%.

Table 2. Inhibition percent of mycelia growth of some seed-borne pathogens by different concentrations of some plant extracts.

Pathogens	Control	Inhibition zone (mm)								
		Plant Extracts								
		<i>R. raetam</i>			<i>P. dactylifera</i>			<i>C. trifurcatum</i>		
		Concentration (%)								
		5	10	15	5	10	15	5	10	15
<i>F. solani</i>	0.0	30	35	37	29	30	32	22	24	28
<i>F. moniliforme</i>	0.0	31	33	36	27	28	30	14	17	26
<i>F. semitictum</i>	0.0	31	34	36	28	29	31	24	26	28
<i>S. sclerotiorum</i>	0.0	31	32	34	28	29	31	25	27	28
<i>M. phaseolina</i>	0.0	27	30	31	24	26	27	15	22	26
Mean	0.0	30	32.8	34.8	27.2	28.4	30.2	20	23.2	27.2

L.S.D. at 5% 1.23

Significant inhibition zones were induced in each of the tested pathogens by the tested plant extracts at all concentrations. However, the highest value of IZD was detected in *F. solani*, followed by *F. moniliforme* and *F. semitictum* (37 mm, 36 mm and 36 mm, respectively). Each of the tested plant extracts induced significant IZD values in all the tested pathogens, compared with control. The highest IZD values were detected in *R. raetam*. i.e., 15% to *F. solani*, *F. moniliforme* and *F. semitictum*, 10% to *F. solani* and 15% to *S. sclerotiorum* (37 mm, 36 mm, 36 mm, 35 mm and 34 mm, respectively). The lowest values (5%) were recorded in *C. trifurcatum* to *F. moniliforme* and *C. trifurcatum* to *M. phaseolina* (Fig.2).

Generally, *R. raetam* extracts were most effective, then *P. dactylifera* , followed by *C. trifurcatum*. However, *F. solani* was more sensitive than all tested pathogens, followed by *S. sclerotiorum*, while, *M. phaseolina* was low sensitive.

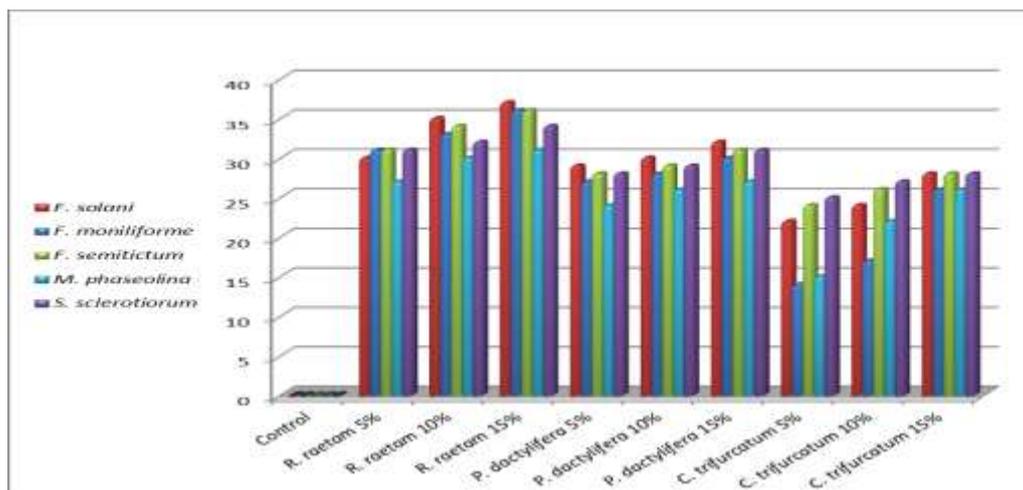


Fig.2. Inhibition percent of mycelia growth of some seed-borne pathogens by different concentrations of some plant extracts.

IV. Discussion

4.Plant extracts:

4.1.Phytochemical analysis:

In the present study, ethanol extracts of some medicinal plants: *Retama raetam* (stems, flowers and fruits), *P. dactylifera* (seeds) and *Chrysanthemum trifurcatum* (mixer of leaves, stems and roots). The study indicates the presence of Alkaloids and carbohydrates in the all tested ethanol extracts. Phenolic compounds were found only in all parts of *R. raetam*. Glycosides and acetate were found in the stems and fruits but absent in the flowers of *R. raetam*. Flavonoids were absent in all parts of *R. raetam*. These findings were similar to those reported by El-Shazly *et al.* (1996) and Abdel Halim *et al.*, (1997). Mobin *et al.* (2015) reported that, the phytochemical screening of the *Retama raetam* extracts revealed the presence of alkaloids, phenols, flavonoids, terpenoids, tannins and carbohydrates. However, saponins were absent.

Phytochemical analysis revealed the presence of alkaloids, steroides and carbohydrates in seed ethanol extracts of *P. dactylifera* but none contains flavonoids, saponin and tannins. These results were confirm by Al-daihan and Bhat (2012) and Mallhi *et al.*, (2014). They showed that, phytochemical analysis of leaf, fruit, seed and bark of *Phoenix dactylifera* revealed the presence of carbohydrates and alkaloids in all parts, and flavonoids, steroids, saponins and tannins were present in some parts. The phenolic profile of the plant revealed presence of mainly cinnamic acids, flavonoid glycosides, flavanols, four free phenolic acids and nine bound phenolic acids (Eong *et al.*, 2006 and Biglari *et al.*, 2008). Fresh dates contain Anthocyanins (Vembu *et al.*, 2012).

4.2. Antifungal activity:

In the present study, ethanol extracts of different plant species namely *R. raetam*, *P. dactylifera* and *C. trifurcatum* inhibited the growth of some cucurbits seed-borne pathogens (*F. solani*, *F. moniliforme*, *F. semitectum*, *S. sclerotiorum* and *M. phaseolina*). All the plant extracts tested showed antifungal activity, results obtained in the present study show that plants extract possesses potential antifungal activity against the tested seed-borne pathogens; however, concentrations 15% and 10% were found to be more effective antifungal agents than the concentration 5%. Alkaloids and carbohydrates were found in all plant extracts, while flavonoids was absent in all plant extracts except *C. trifurcatum*, phenolic compounds were found in all parts of *R. raetam*. Glycosides and acetate were found in fruits and Stems of *R. raetam* but not found in flowers, while steroides was found only in the *P. dactylifera*, tannin was found only in *C. trifurcatum*. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa *et al.*, 2007 and Mensah *et al.*, 2008.

In case of *Phoenix dactylifera* seeds, ethanolic extract contains saponin, terpenoids, phenolic compounds and glycosides. Ethanolic extract of *carica papaya* seeds contains saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds and carotenoids. Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested *in-vitro* (Delphin *et al.*, 2014). Their activity was probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Cowan, 2002). Tannins are also reported to have various physiological effects like antimicrobial. Earlier, it has been reported that methanol and acetone

extracts of the *P. dactylifera* pits moderately inhibited the growth of gram positive and gram negative bacteria (Sabah *et al.*, 2007; Ammar *et al.*, 2009). However reports on antifungal activity of *P. dactylifera* are unavailable. Different concentrations of *Spilanthes acmella* showed high inhibition zones in *F. oxysporum* and *F. moniliformis* followed by *Aspergillus niger* and *A. paraciticus* (Sabitha and Suryanarayana, 2006). Jabeen *et al.* (2008) found that seed extracts of *Moringa oleifera* inhibited the growth of *F. solani* and *Rhizoctonia solani*. Earlier various studies reported that plant extracts had a good potential to control various fungal diseases (Abd-El-Khair and Wafaa, 2007; Joseph *et al.*, 2008; Yasmin *et al.*, 2008; Mdee *et al.*, 2009; Yanar *et al.*, 2011; Salim, 2011).

The phytochemicals derived from root, stem, leaves, fruits, flowers and seeds of medicinal plants include phenolic compounds, essential oils, proteins and antioxidants, and together they work as biocontrol agents (Cragg *et al.*, 1996). The inhibition potential of plant extracts against the growth of microbes was attributed to the presence of antioxidants (Cutter, 2000; Puupponen *et al.*, 2001). It has been reported that the whole date plant (including pits and leaves) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed presence of mainly cinnamic acids, flavonoid glycosides, flavanols, four free phenolic acids and nine bound phenolic acids (Eong *et al.*, 2006 and Biglari *et al.*, 2008). The results of our study demonstrated positive antifungal activity by date palm extracts against tested pathogenic fungi, which might be due to the selective or synergistic action of various chemicals present in date palm. There were some differences in the antifungal activities of extracts which may be due to differences in phytochemicals found in two varieties of *P. dactylifera*. Previous pharmacological studies on the plant have revealed its various medicinal properties: antibacterial, antifungal, antihypertensive, antioxidant, antiviral, diuretic, hypoglycaemic, hepatoprotective, nephroprotective and cytotoxic effects (Maghrani *et al.*, 2005 and Edziri *et al.*, 2012). Hayet *et al.*, (2007) found that the best activity of *R. raetam* against Gram positive bacteria but low activity against the different *Candida* spp., i.e. *C. glabrata*, *C. albicans*, *C. parapsilosis* and *C. kreusei*. On the other hand Edziri *et al.*, (2012) indicated that *R. raetam* was active against *Pseudomonas aeruginosa* and *Escherichia coli* and showed important antifungal activity. Strong antifungal activity against *Candida* species as Ben Sassi *et al.*, (2008) reported that, all extracts of *Chrysanthemum* plants showed some degree of activity against one or more of the bacterial and yeasts strains.

C. parapsilosis was found to be the most sensitive yeast. The activity against both the types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins (Srinivasan *et al.*, 2001). The most active *Chrysanthemum* extracts tested against bacterial and yeasts strains, contained tannins. However, some anti-infective actions have been assigned to tannins (Haslam, 1996). They can be toxic to bacteria, yeasts and filamentous fungi (Scalbert, 1991). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and they also complex with polysaccharide (Ya *et al.*, 1988). Other extracts were active against bacteria and contained flavonoids, like methanolic extracts of *C. paludosum*, *C. grandiflorum*, *C. trifurcatum* leaves and flowers and *C. trifurcatum* leaves and stems. In fact, flavonoids were known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). It should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity was probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al.*, 1996). Methanolic extracts of *C. Myconis*, *C. trifurcatum* leaves and flowers and *C. trifurcatum* leaves and stems have antimicrobial activities and contained tannins, flavonoids and moreover alkaloids which are known to have antimicrobial activity (Omulokoli *et al.*, 1997). Previous studies reported that the other *Chrysanthemum* species such *C. indicum*, contained a phenolic compounds, terpenoids, flavonoids (Cheng *et al.*, 2005), oxygenated monoterpenes and sesquiterpenes which had antimicrobial activity against a large number of bacterial strains (Shunying *et al.*, 2005).

V. Conclusion

Retama raetam extracts have high antifungal activity. Fungus like *F. solani*, *F. semitictum* and *F. moniliforme* were more sensitive with the highest inhibitory zone values for the *R. raetam* plant extract treatments. Further studies are necessary to confirm the present results.

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