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Full Length Research Paper

In vitro assessment of *Inula* spp. organic extracts for their antifungal activity against some pathogenic and antagonistic fungi

Faten Omezzine[1]*, Mejda Daami-Remadi[3], Asma Rinez[1], Afef Ladhari[1] and Rabiaa Haouala[2]

[1]Department of Biology, Faculty of Sciences of Bizerte, University of Carthage, Amilcar 1054, Tunisia.
[2]Department of Biological Sciences and Plant Protection, Higher Agronomic Institute of Chott Meriem, University of Sousse, Chott Meriem 4042, Tunisia.
[3]Laboratory of Plant Pathology, Regional Center of Research in Horticulture and Organic Agriculture, University of Sousse, Chott Meriem 4042, Tunisia.

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***Inula viscosa, Inula graveolens* and *Inula crithmoïdes* (Asteraceae) leaf and flower organic extracts (hexane, chloroform and methanol) were assessed for their antifungal activity against two *Trichoderma* species (*Trichoderma harzianum* and *Trichoderma viride*) and three *formae speciales* of *Fusarium oxysporum*. *I. viscosa* organic extracts showed an important inhibitory activity against all target fungal isolates. Growth inhibition percentage ranged between 17-61, 77-100, and 55-100% in presence of hexane, chloroform and methanol *I. viscosa* leaf extracts, respectively. Flower organic extracts reduced mycelial growth of all fungi by 32-66, 30-75, and 8-70%, respectively. For *I. graveolens,* Stem + leaf organic extracts showed more or less important inhibition depending on solvent nature, though, flower organic extracts were found to be the most effective against tested fungi: a total inhibition of growth was recorded with methanol fraction against all target fungi and with hexane fraction against *F. oxysporum* f. sp. *melonis* (FOM) and *T. viride*. In presence of *I. crithmoïdes* leaf organic extracts, a total growth inhibition was noted with the three extracts against *T. harzianum,* with hexane and methanol fraction against *T. viride* and with chloroform fraction against FOM. *I. crithmoïdes* flower extracts caused a highly significant growth inhibition of about 57-100, 66-100, and 100-100% with hexane, chloroform and methanol extracts, respectively. Therefore, *I. viscosa, I. graveolens* and *I. crithmoïdes* could be an important source of biologically active compounds useful for developing environmentally safe antifungal products.**

Key words: Antifungal activity, organic extracts, *Inula*, radial growth.

INTRODUCTION

The extensive use of agrochemicals especially fungicides, with more carcinogenic risk than other pesticides (Anonymous, 1987), may give rise to undesirable effects on animals and human (Pandey et al., 1992). Therefore, alternative control methods are needed. Plant extracts seem to be an alternative to currently used fungicides to control phytopathogenic fungi, as they are (i) rich source of bioactive chemicals (Kagale et al., 2005), (ii) biodegradable in nature, (iii) non pollutant and (iv) have no residual or phytotoxic properties.

These natural products have the potential to replace the present fungicides (Sharma and Tripathi, 2006; Varma and Dubey, 2001). Allelochemicals have been investigated as a possible alternative weed management strategy (Macias, 1995). Biologically active natural products (allelochemicals) are now being employed as herbicides and fungicides (Einhellig, 1984; Rice, 1985).

The genus *Inula* includes more than 100 species, widespread in the Mediterranean countries and four species are present in Tunisia: *Inula viscosa* (L.) Ait, *Inula graveolens* (L.) Desf, *Inula crithmoïdes* (L.) and *Inula montana* (L.) var. *calycina* (Pres l) Batt (Pottier-Alapetite, 1981). Several studies have shown that some *Inula* species contain some interesting compounds with

*Corresponding author. E-mail: faten.omez@yahoo.fr.

antifungal (Cohen et al., 2002), antibacterial (Stamatis et al., 2003), antiviral (Abad et al., 2000), cytotoxic (Golaw et al., 1993), and nematicidal activities (Yuji et al., 2001) and a protective effect against oxidative stress and genotoxicity (Mosaad et al., 2008).

Several studies have shown that *I. viscosa* possesses antifungal activity against some fungal species of medical or agronomic importance. In fact, *I. viscosa* ethanol extract was tested against *Candida albicans* (Ali Shtayeh et al., 1998; Cafarchia et al., 1999) found that flower and leaf extracts of *I. viscosa* obtained with different solvents showed an antifungal activity against dermatophytes and *Candida* species. The aqueous extract of *I. viscosa* leaves was also used against *Trichophyton mentagrophytes* at 15 µg/ml, where the inhibition recorded was more than 90%, as well as against *T. violaceum* and *Microsporum canis* (Ali Shtayeh and Abu Ghedeib, 1999). However, Ziv et al. (1996) noted a growth inhibition of *Botrytis cinerea* on tomato fruits and grapes with the aqueous extract of the leaf dry matter. Moreover, the foliar spray with acetone and hexane dry leaves extracts of *I. viscosa* showed antifungal activity against cucumber downy mildew (*Pseudoperonospora cubensis*) and powdery mildew on wheat (*Blumeria graminis* f. sp. *tritici*), late blight of potato and tomato (*Phytophthora infestans*) and sunflower rust (*Puccinia heliathi*) when applied at 2.1, 0.5 and 0.25% (w/v), respectively (Wang et al., 2004). Cohen et al. (2002) provided evidence for the antifungal activity in *I. viscosa* extracts prepared with organic solvents, including methanol, ethanol, ethylacetate, acetone, chloroform, and n-hexane. Using thin-layer chromatography overlay assays, seven inhibitory zones against *Cladosporium cucumerinum* were observed in the extracts (Cohen et al., 2002). Maoz et al. (1999) have isolated the tayunine from the petroleum ether leaf extract of *I. viscosa*. This compound, which is a sesquiterpene lactone. Showed inhibitory activity against *Microsporum canis* and *Trichophyton rubrum* when tested at 10 and 50 µg/ml, respectively.

Moreover, another sesquiterpene lactone, the tomentosine, was isolated by Cafarchia et al. (2001) from fresh *I. viscosa* flowers. This compound also exhibited an antifungal activity against *M. canis*, *M. gypseum* and *T. mentagrophytes* when applied at 1 mg/ml. These results indicate that *I. viscosa* is an important source of bioactive compounds against different fungal species. Nevertheless, to our knowledge, no studies were done on the antifungal activity of *I. graveolens* and *I. crithmoïdes.*

The present research was conducted to assess the antifungal activity of three organic extracts of *I. viscosa, I. graveolens* and *I. crithmoïdes* leaves and flowers against some pathogenic and antagonistic fungi.

MATERIALS AND METHODS

Plant

I. viscosa, I. graveolens and *I. crithmoïdes* were identified according to the flora of Tunisia (Pottier-Alapetite, 1981). A voucher specimen was collected, dried and deposited at the herbarium (Asteraceae 24) of the Higher Agronomic Institute of Chott-Meriem, University of Sousse, Tunisia. Plant material was collected at the flowering stage in October 2010 in the area of Monastir (latitude 35°46'0''N, longitude 10°59'0'' E, coastal region, East of Tunisia, with a sub-humid climate).

Fungi tested

Three phytopathogenic isolates of *Fusarium oxysporum* that is, *F.oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *tuberosi* (FOT) and two antagonistic fungi (*Trichoderma harzianum* and *Trichoderma viride*) were selected for the present screening. The host plants from which the different fungi were isolated were: melon for FOM, tomato for FOL and potato for FOT. *T. harzianum* and *T. viride* were isolated from soil. They were cultured during 7 days at 25°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate before use.

Extraction

Sequential extraction was carried out with organic solvents having increasing polarity: hexane, chloroform and methanol. Forty grams of dried powder of leaves and flower were immersed in the organic solvent for 7 days at room temperature. Organic extracts were evaporated to dryness under reduced pressure at 45-50°C, using Rotavapor R-114 (Buchi, France). Dry fractions were stored at 4°C until use.

Antifungal activity assay

Hexane, chloroform and methanol residues were dissolved in methanol (6 g/l). Fungal isolates were grown in 40 ml PDA amended with 5 ml of each leaf and flower organic extract of *I. viscosa, I. graveolens* and *I. crithmoïdes* (Satish et al., 2007). Agar plugs (6 mm diameter) removed from a fast growing fungal colony was plated in 9 cm Petri dishes amended or not with the extracts tested (three plugs per plate). The colony diameter was noted after 6 days of incubation at 25°C. Control received the same volume of sterilized distilled water.

Fungal growth was measured by averaging the three diameters taken at right angles for each colony. Percentage growth inhibition (%) of fungal colonies was calculated according to the following formula (Jabeen and Javaid, 2008):

Growth inhibition (%) = [(Growth in control - Growth in treatment) / Growth in control] × 100

Statistical analysis

The laboratory bioassays were conducted according to a completely randomized design with three replications. S.N.K tests were performed with PASW Statistics 18, for Windows program, to analyze treatment differences.

RESULTS AND DISCUSSION

Organic leaf and flower extracts of *Inula* spp. exhibited a marked variability in their effect on growth of

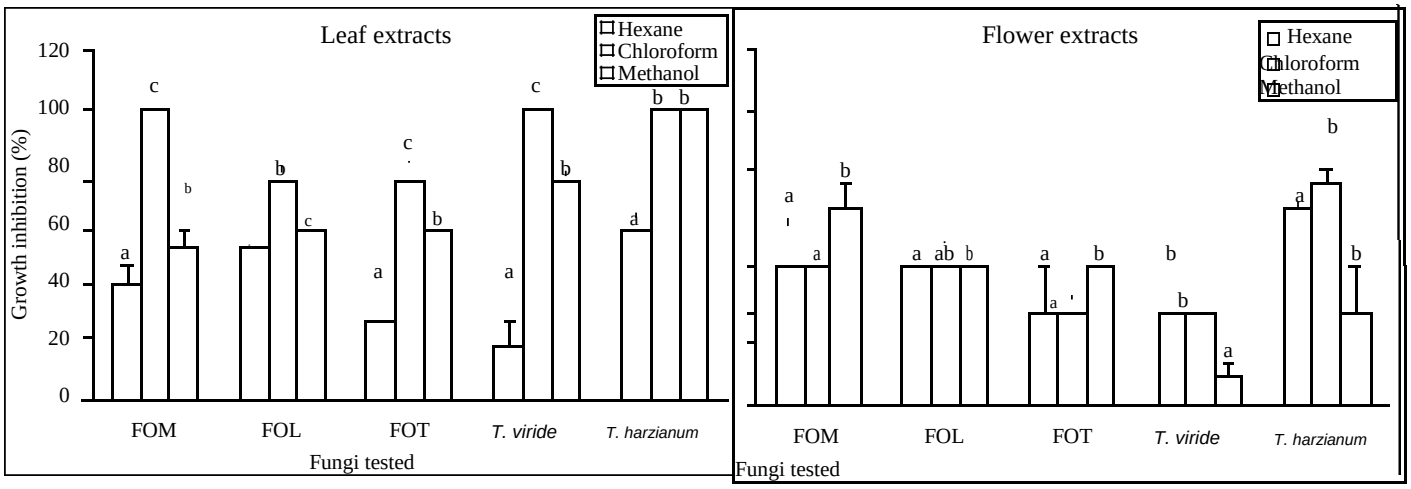

Figure 1. Growth inhibition of *Fusarium oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *tuberosi* (FOT), *Trichoderma harzianum* and *T. viride*) induced by *Inula viscosa* leaf and flower organic extracts (at 6000 ppm) and recorded after 6 days of incubation at 25°C. The bars on each column show standard error. For each organic solvent, bars affected by the same letter are significantly similar at $P \leq 0.05$.

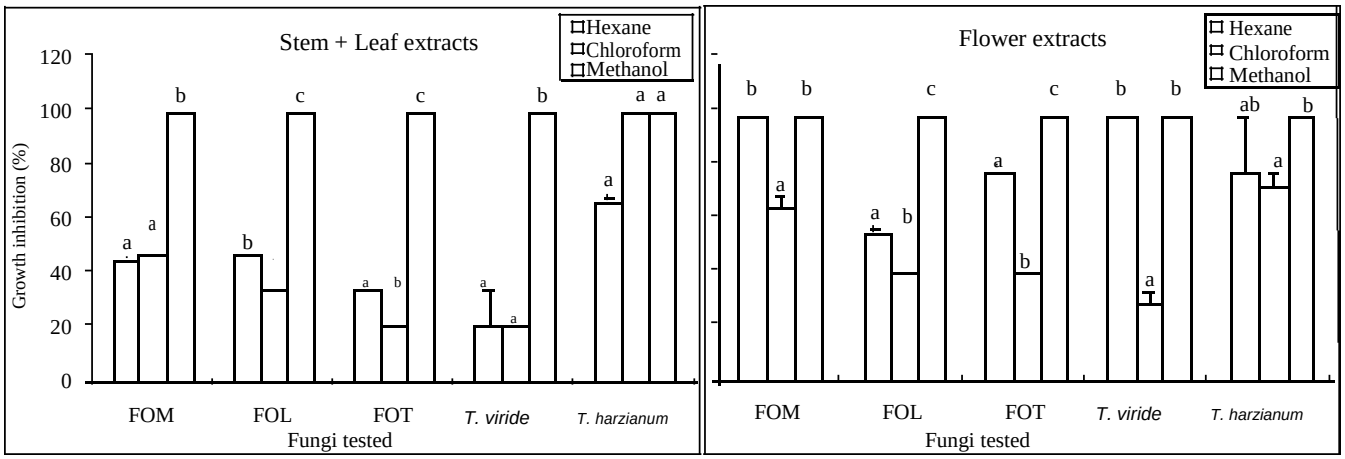

Figure 2. Growth inhibition of *Fusarium oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *tuberosi* (FOT), *Trichoderma harzianum* and *T. viride*) induced by *Inula graveolens* stem + leaf and flower organic extracts (at 6000 ppm) and recorded after 6 days of incubation at 25°C. The bars on each column show standard error. For each organic solvent, bars affected by the same letter are significantly similar at $P \leq 0.05$.

phytopathogenic and antagonistic fungal isolates tested (Figures 1, 2 and 3). Present findings showed that chloroform and methanol extracts were quite more effective than hexane extracts. Methanol was found to be a good solvent for extraction of plant antifungal compounds (De Rosa and Di Vincenzo, 1992; Gulden et al., 1990). Indeed, all *I. viscosa* extracts showed an important inhibitory activity against all target fungal isolates (Figure 1). A total inhibition (100%) was observed with chloroform *I. viscosa* leaf extract against FOM, *T. viride* and *T. harzianum* and with methanol leaf extract against *T. harzianum*. Among fungal isolates tested in the present study, *Trichoderma* spp. and FOM were found to be more sensitive to chloroform leaf extracts than FOL and FOT. In fact, the radial growth inhibition of target fungi ranged between 17-61, 77-100, and 55-100% in presence of hexane, chloroform and methanol extracts, respectively. Cohen et al. (2002) reported that acetone extracts from shoots of *I. viscosa* were effective in controlling late blight in potato and tomato, and downy mildew in grape in growth chambers. Wang et al. (2004) showing that *I. viscosa* organic extracts were effective in controlling late blight in potato and tomato, downy mildew in cucumber, powdery mildew in wheat, and rust in sunflower. Though, hexane and chloroform flower extracts of *I. viscosa* were found to be more active against FOM, FOL and *T. harzianum* than against FOT and *T. viride*. Indeed, mycelial growth was

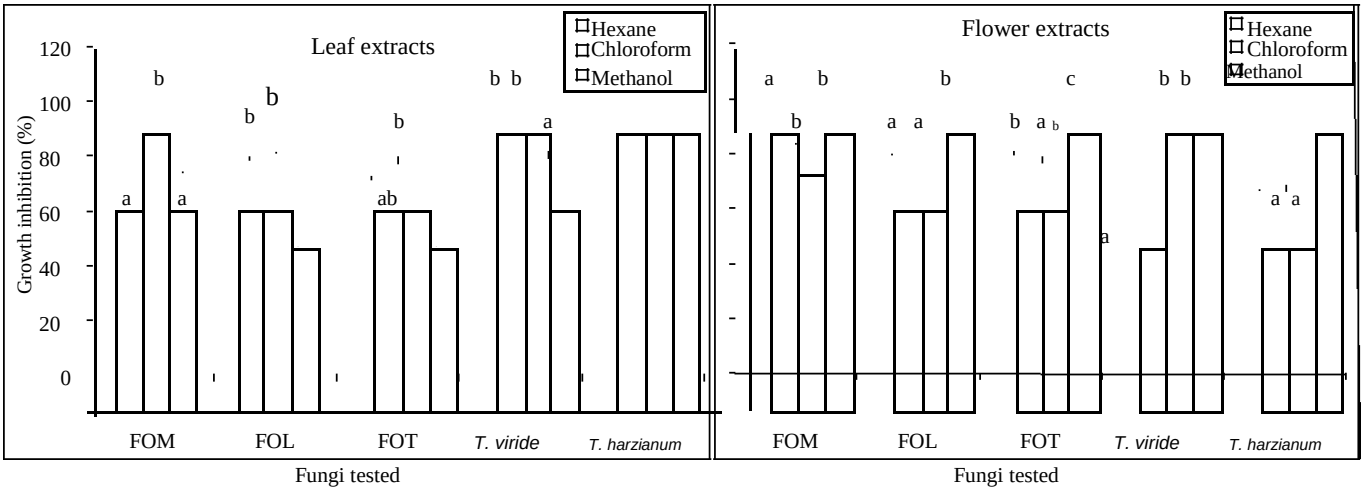

Figure 3. Growth inhibition of *Fusarium oxysporum* f. sp. *melonis* (FOM), *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *tuberosi* (FOT), *Trichoderma harzianum* and *T. viride*) induced by *Inula crithmoïdes* leaf and flower organic extracts (at 6000 ppm) and recorded after 6 days of incubation at 25°C. The bars on each column show standard error. For each organic solvent, bars affected by the same letter are significantly similar at $P \leq 0.05$.

reduced by 32-66, 30-75, and 8-70% with hexane, chloroform and methanol extracts, respectively, compared to the untreated control (Figure 1). Abu Jawdah et al. (2002) tested the antifungal activity of this plant against spore germination of plant pathogens. In fact, they found that extract with light oil *I. viscosa* applied at a concentration of 2 ml/98 ml PDA, had inhibited by 88% the development of *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis* and *Verticillium dahliae.*

Stem + leaf and flower organic extracts of *I. graveolens*, showed important antifungal activity with all fractions in comparison to the untreated control (Figure 2), and the colony diameter noted, after 6 days of incubation at 25°C, was found to vary depending on target fungi and tested organic extracts.

Flower organic extracts were found to be most effective against the tested fungi; a total inhibition of growth was recorded with methanol fraction against target fungi and with hexane fraction against FOM and *T. viride*. However, with chloroform flower extracts, the radial growth of FOM, FOL, FOT, *T. viride,* and *T. harzianum* was reduced by 63, 39, 43, 27 and 71%, respectively. Several works reported the inhibition of the mycelial growth of various species of *Fusarium* by extracts of several plants such as *Agave americana* (Pandey et al., 1992), *Allium sativum* and *Sapindus trifoliata* (Gohil et al., 1996), neem (Gour and Sharmaik, 1998), *Azadirachta indica, Atropha belladona, Calotropis procera, Ocimum basilicum, Eucalyptus amygdalina, Ailanthus excelsa* and *Lantana camera* (Bansal and Rajesh, 2000). However, stem + leaf organic extracts showed more or less important inhibition depending on extract type. A total growth inhibition of target fungi was obtained with methanol and chloroform fractions against *T. harzianum.* The methanolic, ethanolic and boiling water extracts of *Barringtonia racemosa* leaves, sticks and barks tested Hussin et al. (2009) at 50 mg/ml exhibited an inhibitory effect against *Fusarium* sp., *Trichoderma koningii*, *Penicillium* sp., *Ganoderma tropicum*, *G. lucidum*, *Aspergillus* sp. and *Rhizopus* sp. The highest antifungal activity was recorded with the methanolic extracts of *B. racemosa* aerial parts.

Leaf and flower organic extracts of *I. crithmoïdes* were the most effective in reducing the radial growth of the fungi tested as compared to extracts of the two other *Inula* species (Figure 3). In fact, the three leaf extracts totally had inhibited *T. harzianum* whereas hexane and methanol fractions had completely suppressed *T. viride* development. Similar absolute inhibition was obtained with chloroform fraction against FOM. Angelini et al. (2009) recorded fungistatic and fungicidal properties of the methanol extract of *Asafoetida oleogum* against *T. harzianum* strains and *Pleurotus* spp. at higher concentrations (30 and 40 µg/mL). In our study, the three organic fractions of *I. crithmoïdes* flowers were the most toxic and they caused a highly significant inhibition of colony growth. Indeed, the colony diameter was reduced by 57-100, 66-100, and 100% with hexane, chloroform and methanol extracts, respectively, compared to the control. Siva et al. (2008) noted a total inhibition of *Fusarium oxysporum* f. sp. *melongenae* by aqueous, ethanol and acetone leaf extracts of *Adhatoda vasica, Jatropha curcas* and *Sapindus emarginatus* and mycelial growth was inhibited by 60 to 98% with plant extracts of 17 different species of angiosperms.

Conclusion

Organic extracts of different plants or plant parts may be used as biofungicides against a variety of phytopathogenic fungi. However, the efficiency of such

extracts depends greatly upon the resistance offered by the different fungal species. Among the three selected plant species, organic extracts of *I. crithmoïdes* were proved to be the most effective. Further studies are required to investigate the *in vivo* efficacy of these extracts.

REFERENCES

Abad MJ, Geurra JA, Bermejo P, Iruruzum A, Carrasco L (2000). Search for antiviral activity in higher plant extracts. Phytother. Res., 14: 604-607.

Abu-Jawdah Y, Sobh H, Salameh A (2002). Antimycotic activities of selected plant flora, growing with in Lebanon, against phytopathogenic fungi. J. Agric. Food Chem., 50: 3208-3213.

Ali Shtayeh MS, Abu Ghedeib SL (1999). Antifungal activity of plant extracts against dermatophytes Mycoses, 42: 665-672.

Ali Shtayeh MS, Reem M, Yaghmour R, Faidi YR, Salam K, AL-Nuri MA (1998). Antimicrobial activity of 20 plants used in folkoric medicine in the Palestinian area. J. Ethnopharmacologie, 60: 265-271.

Angelini P, Pagiotti R, Venanzoni R, Granetti B (2009). Antifungal and allelopathic effects of *Asafoetida* against *Trichoderma harzianum* and *Pleurotus* spp. Allelopathy J., 23: 357-368.

Anonymous (1987). Regulating pesticides in food. The Delancy Paradox. National Academy Press, Washington, DC.

Bansal KR, Rajesh KG (2000). Evaluation of plant extracts against *Fusarium oxysporum*, wilt pathogen of fenugreek. Indian Phytopathol., 53: 107-108.

Cafarchia C, De Laurentis N, Milillo MA, Losacco V (2001). Fungistatic activity of a sesquiterpene lactone (Tomentosin) isolated from fresh *Inula viscosa* (Asteraceae) flowers from the Puglia region. Parasitologia, 43: 117-121.

Cafarchia C, De Laurentis N, Milillo MA, Losacco V, Puccini V (1999). Research on antifungal activity of flowers and leaves of *Inula viscosa* (Asteraceae). Parasitologia, 41: 579-582.

Cohen Y, Baider A, Ben-Daniel BH, Ben-Daniel Y (2002). Fungicidal preparations from *Inula viscosa*. Plant Protection. Sciences, 38: 629-630.

De Rosa S, Di Vincenzo G (1992). Isochelidonine, a benzophenanthridine alkaloid from *Chelidonium majus.* Phytochem., 31: 1085–1086.

Einhellig FA (1984). Allelopathy-natural protection, allelochemicals, in H. B. Mandaven (ed.). Handbook of Natural Pesticides. I. Theory, Practice, and Detection, CRC Press, Boca Raton, Florida, pp. 161-200.

Gohil VP, Vala GD (1996). Effect of extracts of some medicinal plants on the growth of *Fusarium moniliforme*. J. Mycol. Plant Pathol., 26: 110-111.

Golaw T, Sevil O, Hui-Ling S, Geoffrey AC, John MP, Candan BJ (1993). Cytotoxic and antibacterial sesquiterpenes from *Inula graveolens*. Phytochem., 33: 407.

Gour HN, Sharmaik C (1998). Inhibition of growth, Sporulation and Phytotoxicity of *Fusarium oxysporum* fungal species Cumini, a wilt pathogen of cumin by plant extracts. J. Mycol. Plant Pathol., 2: 76-77.

Gulden K., Gozler T, Shamma M (1990). Turkiyenine, a new alkaloid from *Chelidonium majus*. J. Nat. Prod., 53: 531-532.

Hussin NM, Muse R, Ahmad S, Ramli J, Mahmood M, Sulaiman MR, Shukor MYA, Rahman FA, Aziz KNK (2009). Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae). Afr. J. Biotechnol., 8: 2835-2842.

Jabeen K, Javaid A (2008). Antifungal activity of aqueous and organic solvent extracts of allelopathic trees against *Ascochyta rabiei*. Allelopathy J., 22: 231-238.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2005). Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas* pv *oryzae*. Physiological and Mole. Plant Pathol., 65: 91-100.

Macias FA (1995). Allelopathy in the search for natural herbicide models. pp. 310-329, in Inderjit, K. MM Dakshini, and FA Einhellig (eds.). Allelopathy: Organisms, Processes and Applications, ACS Symposium Series 582. American Chemical Society, Washington, D.C.

Maoz M, Kashman Y, Neeman I (1999). Isolation and identification of a new antifungal sesquiterpene lactone from *Inula viscosa*. Planta Med., 65: 281-282.

Mosaad A, Abdel-Wahhab S, Abdel-Azim H, Aziza A El-N (2008*). Inula crithmoides* extract protects against ochratoxin A-induced oxidative stress, clastogenic and mutagenic alterations in male rats. Toxicon, 52: 566-573.

Pandey JC, Kumar R, Gupta RC (1992). Possibility of biological control of rhizome rot of ginger by different antagonists. Progressive Hortic., 24: 227-232.

Pottier-Alapetite G (1981). Flore de la Tunisie (Tome 2) : Angiospermes-Dicotylédones, Gamopétales. Publication scientifique Tunisienne, pp. 970-972.

Rice EL (1985). Chemically mediated interactions between plants and other organisms. pp. 81-105, in G.A Cooper-Driver, T Swain and EE Conn (eds.). Allelopathy—An Overview. Plenum Press, New York.

Satish S, Mohana DC, Ranhavendra MP, Raveesha KA (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. J. Agric. Technol., 3: 109-119.

Sharma N, Tripathi A (2006). Fungitoxicity of the essential oil of *Citrus sinensis* on post-harvest pathogens. World J. Microbiol. Biotechnol., 22: 587-593.

Siva N, Ganesan S, Banumathy N, Muthuchelian (2008). Antifungal effect of leaf extract of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. Ethnobotanical Leaflets, 12: 156-163.

Stamatis G, Kyriazopoulos P, Golegou S, Basayiannis A, Skalts S, Skaltsa H (2003). *In vitro* anti-*Helicobacter pylori* activity of Greek herbal medicines. J. Ethnopharmacol., 88: 175-179.

Varma J, Dubey NK (2001). Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. Int. J. Food Microbiol., 68: 207-210.

Wang WQ, Ben-Daniel BH, Cohen Y (2004). Extracts of *Inula viscosa* control downy mildew caused by *Plasmopara viticola* in grapevines. (Abstr.) Phytoparasitica, 32: 208.

Yuji O, Bat-Hen B, Yigal C (2001). Nematicidal activity of powder and extracts of *Inula viscosa*. Nematol., 3: 735-742.

Ziv O (1996). Using extracts of *Inula viscosa* for controlling plant diseases on fresh and dry post-harvest products. Phytoparasitica, 24: 154-155.