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Antifungal activity of *Citharexylum quadrangulare* Jacq. extracts against phytopathogenic fungi

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Antifungal activity of *Citharexylum quadrangulare* Jacq., an exotic tree introduced in Tunisia many years ago, was evaluated. Organic extracts using hexane, ethyl acetate and methanol solvents together with aqueous extracts tested at different concentrations were prepared from different organs (roots, stems, leaves and flowers). The fungitoxic activity for all extracts was evaluated against five phypathogenic fungi (*Fusarium culmorum, F. graminearum, Aspergillus flavus*, *Aspergillus niger* and *A. fumigatus*). All parameters were used in a principal components analysis (PCA) and a hierarchical clusters analysis (HCA). This study concludes that some aqueous and organic extracts of *C. quadrangulare* could be potential sources of natural fungicides to protect crops from fungal diseases.

Key words: Antifungal, biofungicides, *Citharexylum quadrangulare* Jacq., extracts, safe environment.

INTRODUCTION

A great diversity of fungi cause plant diseases; nearly all major groups are involved. Fungal infections are one of the major problems associated with our daily life. Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Dellavalle et al., 2011). Hence, there is a great demand for novel antifungals belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects, particularly from plant origin. The increased development of resistance against frequently used antimicrobial compounds by the microorganisms necessitates the discovery of new antimicrobial compounds in plant extracts.

Many plants synthesize substances that are useful for controlling the growth of microorganisms and plants are a possible source of antimicrobial agents non phytotoxic, systemic and biodegradable (Adesina et al., 2000; Ouedraogo et al, 2013; Sumathy et al., 2014; Kumari et al., 2015; Gond et al., 2015; Martins et al., 2015).

Recent studies showed that crude extracts of various plant species such as *Coronopus didymus* (L.) Sm., *Datura metel* L., *Solanum indicum* L., *Azadirachta indica* A. Juss., *Oxalis latifolia* Kunth and *Jatropha curcas* L. were very effective in the management of

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Table 1. Aqueous and organic extracts of *Citharexylum quadrangulare* used for antifungal tests and abbreviation.

Collection date	Collection locality	Plant part sampled	Plant source	Fungi
02/04/2010	MenzelBouzelfa	Fruits	Citrus	*Aspergillus flavus*
02/04/2010	Jendouba	Leaves	Wheat	*A. niger*
02/04/2010	Nefza	Leaves	Wheat	*A. fumigatus*
23/05/2007	Beja	Roots and stems	Wheat	*Fusarium culmorum*
23/05/2007	Beja	Roots and stems	Wheat	*F. graminearum*

phytopathogens namely *Sclerotium rolfsii* Sacc., *Fusarium oxysporum* f. sp. *lycopersici*, *Aspergillus flavus* and *Alternaria alternata* (Iqbal and Javaid, 2012; Jabeen et al., 2014; Anil and Raj, 2015; Abd El-Ghany et al., 2015). Researchers have also isolated many potential antifungal compounds from plants such as *β*-amyrin from *Melia azedarach* L. (Jabeen et al., 2011), tow flavonoids 7-O-glucoside and (-)-epi catechin from *Azadirachta indica* (Kanwal et al., 2011), and methyl gallate from *Melaphis chinensis* (Kuo et al., 2015). Gond et al. (2015) reported that lipopeptides secreted by bacterial endophytes naturally occurring in many maize varieties inhibit pathogens and for Martins et al. (2015) fungicidal effects proved to be dependent on the occurrence of phenolic compounds in tested extracts.

Citharexylum quadrangulare Jacq. (syn. *Citharexylum spinosum* L. and *Citharexylum fruticosum*) (Verbenaceae) (Wagner et al., 1999) is native to Caribbean (Turner and Wasson, 1997), was introduced in Tunisia many years ago and is cultivated along the roadsides and in gardens. This tree possesses medicinal properties and is useful for the treatment of various ailments. A decoction of young twigs was used for children thrush and bark decoction for treating colds (Lachman-White et al., 1992). The leaves are used as a source of an anti-allergic substance and as an alternative medicine in hepatic disorders (Balazs et al., 2006). *C. quadrangulare* was used in combination with other plants as anthelmintic (Lans, 2007). Studies concerning the antimicrobial activity of *C. quadrangulare* are not yet available except the work recently published by Ae and Patcharee (2014) where authors reported that flowers extracts and flower oil have an interesting antibacterial activity against eight microorganisms

So, the aim of this present study was to determine the antifungal potentialities of *C. quadrangulare* aqueous and organic extracts against five phytopathogenic fungi (*Fusarium culmorum*, *F. graminearum*, *Aspergillus flavus*, *A. niger* and *A. fumigatus*) causing serious damage in agriculture, resulting in losses of crop yield and quality.

MATERIALS AND METHODS

Plant material

C. quadrangulare different organs (roots, stems, leaves and flowers) were collected in the garden of the High Institute of Biotechnology of Monastir (latitude 35º46'0''N, longitude 10º59'0''E, coastal region, East of Tunisia, with a sub humid climate). A voucher specimen (CQV 12) was deposited at the Herbarium of the Laboratory of Botanic in the Institute. Roots were cleaned with tap water, and all the plant parts were air-dried in a shaded area at ambient temperature. Dried material was grounded into a powder using a Wiley mill and stored at 4ºC until use.

Preparation of aqueous and organic extracts

Two hundred grams of powder from each dried plant part were separately extracted by soaking in 100 mL distilled water at ambient temperature for 24 h to give a concentration of 2 g dry tissue per mL. The 16 crude aqueous extracts (Table 1) were filtered through a double layered muslin cloth followed by Whatman no. 1 filter paper and then passed through 0.22 µm micro-filter pore size to remove bacteria. Filtrates were preserved at 4°C. Aqueous extracts were used freshly within a week.

Sequential extraction was carried out in organic solvents with rising polarity: hexane, ethyl acetate and methanol. One hundred grams of powder were immersed in the appropriate solvent for 7 days at room temperature. The 12 organic extracts (Table 1) were evaporated to dryness under reduced pressure in a rotary evaporator at 45°C, to remove the solvent. After determination of the yield the extracts were stored at 4°C until use. Control was used with distilled water and methanol without organic extract.

Fungal isolates

Fungi used in this study were collected from various localities in Tunisia. Each fungus, the host and the plant part from which it was isolated, the locality and the date of collection are listed in Table 2. All fungal isolates were identified (Boughalleb et al., 2006; 2008) and samples of each fungus were deposited in the collection bank at the Plant Pathology Laboratory (High Institute of Agronomy of Chott-Meriem, Tunisia). Fungal isolates were maintained on potato dextrose agar (PDA, Difco Laboratories, Inc., Detroit, MI, USA), stored at room temperature and sub-cultured once a month (Deans and Svoboda, 1990) when needed.

Antifungal bioassays

The antifungal activity was determined according to the poisoned food technique of Grover and Moore (1962). PDA media with 0.25, 0.5, 1 and 2 g/mL of each aqueous extract were prepared. On the other hand each organic extract was dissolved in methanol and added in PDA medium at the concentration of 1 mg/mL before solidification. PDA medium was without aqueous extract and this amended only with methanol served as controls. Fifteen ml of the each medium was poured into Petri plate (9 cm diameter) and

Table 2. Fungal isolates used to test the antifungal activity of *Citharexylum quadrangulare*

Organic extracts	Abbreviation
Root, shoot, leaves, flowers hexane extracts	Rhex, Shex, Lhex, Flhex
Root, shoot, leaves flowers ethyl acetate extracts	Reac, Seac,Leac,Fleac
Root, shoot, leaves flowers methanol extract	Rmet, Smet, Lmet, Flmet
Aqueous extracts	Abbreviation
Aqueous root extracts at 0.25, 0.5, 1, 2 g/ml	AR0.25, AR0.5, AR1, AR2
Aqueous shoot extracts at 0.25, 0.5, 1, 2 g/ml	AS0.25, AS0.5, AS1, AS2
Aqueous leaves extracts at 0.25, 0.5, 1, 2 g/ml	AL0.25, AL0.5, AL1, AL2
Aqueous flowers extracts at 0.25, 0.5, 1, 2 g/ml	AFl0.25, AFl0.5, AFl1, AFl2

allowed to solidify. Then a disc (5 mm) of 7-days-old culture of each tested fungus was taken with a pre-sterilized cork borer and placed upside down on the centre of the dish. The Petri plates were incubated at 25±2°C in the dark for 7 days. The extension diameter (mm) of mycelia from the centre to the sides of the dishes was measured at 24 h intervals for seven days. The fungi toxicity in the aqueous and organic extracts in terms of percentage of mycelia growth inhibition (% MGI) of each fungus was calculated by using the formula of Jabeen and Javaid (2008):

% MGI = dc - dt/dc x 100

Where, dc = Average increase in mycelia growth in control, dt = Average increase in mycelia growth in treatment (aqueous and organic extract).

Statistical analysis

Percentages of mycelia growth inhibition (%MGI) of each fungus were transformed using arcsin-square root (arcsine √x) and subjected to conformity with assumptions of normality for analysis of variance (ANOVA) using SPSS 12.0, for Windows program. The significance of the differences between means was determined at $p < 0.05$ using *Duncans's* multiple range test. We evaluated whether the type of extract (Table 1) (or group of extracts) was useful in reflecting its fungitoxic effect. The %MGI reported with all extracts tested were subjected to Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) using SPSS 12.0 software (SPSS Inc. Chacago, IL, USA).

RESULTS

The percentages of the mycelia growth inhibition of each fungus were used for the PCA and the HCA (data not given) based on the *Euclidean* distance between groups. Two groups of extracts (1 and 2), identified by the fungus or group of fungus with which they correlate were identified, and data are reported in Table 3. Groups 1 and 2 clearly stand out forming separate groups in the PCA.

Group 1 (Reac, Fleac, Seac)

This group of extracts correlates with the percentages of mycelia growth inhibition for four out of the five fungi tested. In fact, the three extracts inhibited the growth of *A. flavus*, *A. niger* and *A. fumigatus* from 40.38 to 68.6% and to a lesser degree of *F. culmorum* mycelia (19.55-37.18%).

Group 2

The group 2 includes all the 25 other extracts, divided in 2 subgroups 2A and 2B. The subgroup 2A is divided into subgroups 2Aa and 2Ab and 2B into subgroups 2Ba and 2Bb. The later (2Bb) still divided into subgroups 2Bb1 and 2Bb2, and the 2Bb2 yet subdivided in 2 subgroups 2Bb21 and 2Bb22. Extracts from subgroup 2Aa (Smet, AS1, AS2) inhibited *A. niger* at percentages varying from 43.3 to 50.9%. Aqueous extract from stems at 1 mg/ml (AS1) inhibited *A. flavus* at 22.8% and AS1 and AS2 inhibited *F. graminearum* at 24.04%. *A. fumigatus* and *F. culmorum* were not susceptible to those extracts (0 - 10.3%). Those from the subgroup 2Ab (Shex, Lhex, Flhex) were highly positively correlated with the %MGI of *A. niger* (43.6 - 53.8%), and of *F. fumigatus* (39.1 - 50.6%), contrariwise, *A. flavus* and *F. graminearum* were not inhibited. In contact with the two extracts, AR1 and Leac, from the subgroup 2Ba, the %MGI of *A. flavus* were from 63.5 to 65.4%, however, %MGI of *F. culmorum* weakly correlated with those extracts (14.4 to 12.8%). Leaves ethyl acetate extract (Leac) inhibited also *A. fumigatus* (38.5%). *A. niger* and *F. graminearum* were no or weakly inhibited (4.2% for *F. graminearum* with AR1). Subgroup 2Bb1 with 7 aqueous extracts (AR0.25, AR0.5, AL0.5, AL2, AS0.25, AFl, AFl2) correlated with the %MGI of *A. flavus*, *F. graminearum* and *F. culmorum*, but were opposed to the inhibition of *A. niger* (0%) and *A. fumigatus* (0%). Percentages of MGI of *A. flavus* and of *F. graminearum* varied from 14.1 to 49.0% and from 25.9 to 48.4%, respectively. For *A. flavus* the best inhibition was obtained with AL0.5 and AR0.25 and for *F. gramineaum* with AR0.5. *A. niger* was inhibited only with AR0.25 (28.8%). *F. culmorum* was less affected by the 7 extracts (6.4 - 19.9%); extract AS0.25 have no effect on the mycelia growth of this fungus. Subgroup 2Bb2 was

Table 3. Percentages of mycelia growth inhibition in the aqueous and organic extracts (%MGI). The table was established according the Principal Component Analysis and the Hierarchical Clusters Analysis. Organic extracts were tested at 1 mg/ml and aqueous extracts at 0.25, 0.5, 1, 2 g/ml.

Groups/subgroups					*Aq/Org extracts	Mean % of mycelia growth inhibition				
						A. flavus	*A. niger*	*A. fumigatus*	*F. culmorum*	*F. graminearum*
Group 1					Seac	45.8±4.9f	42.6±9.3e	50.3±5.4g	19.6±1.9e-g	1.6±3.9a
					Fleac	68.6±3.6h	51.9±8.1gh	55.8±6.9h	37.2±6.0i	0.0±0.0a
					Reac	57.1±4.5g	49.4±8.2fgh	40.4±7.6f	29.8±7.1h	0.0±0.0a
	2A	2Aa			Smet	0.0±0.0a	46.8±9.2efg	0.0±0.0a	10.3±8.3bc	0.0±0.0a
					AS2	0.0±0.0a	50.9±1.1gh	0.0±0.0a	0.0±0.0a	24.0±9.6c
					AS1	22.8±5.9c-e	43.3±5.0ef	0.0±0.0a	0.0±0.0a	12.2±5.5c
		2Ab			Flhex	0.0±0.0a	53.8±7.1h	47.8±7.8g	6.4±7.3ab	0.0±0.0a
					Lhex	0.0±0.0a	43.6±9.9ef	39.1±4.2f	14.1±4.5c-f	0.0±0.0a
					Shex	0.0±0.0a	53.5±7.8h	50.6±4.2g	13.8±2.6c-f	0.0±0.0a
		2Ba			AR1	65,4±4.2h	00.0±0.0a	0.0±0.0a	14.4±1.1c-f	4.2±7.1a
					Leac	63.5±7.6h	00.0±0.0a	38.5±9.1f	12.8±5.9b-e	0.0±0.0a
			2Bb1		AR0.25	47,5±5.3f	28.8±6.3d	0.0±0.0a	11.5±6.2bc	25.9±9.9c
					AR0.5	17.0±9.3bc	0.0±0.0a	0.0±0.0a	11.2±8.9bc	48.4±9.5f
					AL0.5	49.0±2.0f	0.0±0.0a	0.0±0.0a	14.7±3.8c-f	36.9±8.3e
					AFl1	21.5±9.0cd	0.0±0.0a	0.0±0.0a	15.4±3.8c-f	35.9±9.7e
Group 2					AL2	21.1±7.9cd	0.0±0.0a	0.0±0.0a	19.9±2.3fg	28.2±7.7cd
					AFl2	14.1±8.1b	9.9±9.6b	0.0±0.0a	6.4±8.2ab	33.9±9.2de
					AS0.25	28.2±9.0e	0.0±0.0a	0.0±0.0a	0.0±0.0a	26.3±9.4c
	2B				AFl0.25	0.0±0.0a	22.8±4.3c	13.8±5.1b	13.1±5.4b-f	24.0±6.9c
		2Bb			AFl0.5	0.0±0.0a	0.0±0.0a	21.8±6.2c	22.4±9.2g	20.5±8.1c
					Flmet	0.0±0.0a	0.0±0.0a	20.8±4.6c	16.0±2.3c-g	11.5±4.7b
				2Bb22	Lmet	0.0±0.0a	0.0±0.0a	29.5±8.5e	12.5±5.7b-d	0.0±0.0a
					Rmet	0.0±0.0a	0.0±0.0a	49.7±7.0g	12.5±3.6b-d	0.0±0.0a
			2Bb2		AL1	0.0±0.0a	0.0±0.0a	0.0±0.0a	19.2±3.6d-g	0.0±0.0a
					AL0.25	24.7±9.9de	0.0±0.0a	0.0±0.0a	13.5±4.7c-f	0.0±0.0a
					AR2	27.6±8.6de	0.0±0.0a	0.0±0.0a	16.3±3.2c-g	0.0±0.0a
					AS0.5	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
				2Bb21	Rhex	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a

*Aq. Aqueous; org. organic; for abbreviation of extracts see Table 1. Means ± SE followed by different letters differ significantly at $p < 5\%$, as established by *Duncan's* test.

distributed in 2Bb21 and 2Bb22. Extracts from the former group (Rhex, AS0.5) did not inhibit all tested fungi. The subgroup 2Bb22 was formed by AFl0.25, AFl0.5, Flmet, Lmet, Rmet, AL0.25, AL1, and AR2 extracts. The first five extracts showed no effect on the development of *A. flavus* and *A. niger* mycelia, except AFl0.25 extract (22.8% against *A. niger*). *A. fumigatus, F. culmorum* and *F. graminearum* were inhibited (11.5 - 49.7%) by those extracts except Lmet and Rmet extracts (against *F. graminearum*). The highest percent of inhibition was exhibited with Rmet extract against *F. fumigatus* (49.7%). AL1, AL0.25, AR2 were ineffective against *A. fumigatus, A. niger* and *F. graminearum* but inhibited *F. culmorum* (13.5 - 19.2%). The extracts AL0.25 and AR2 were revealed to be able to reduce the mycelia growth of *A. flavus* (24.7, 27.6%, respectively). However, the extract AL1, did not inhibit this fungus.

DISCUSSION

Biocontrol is the safest and economical method of controlling plant pathogens by using extracts of different plant parts (Shafique et al., 2011; Kumari et al., 2015; Gond et al., 2015; Martins et al., 2015). The results of this conceptual study clearly reflect that *C. quadrangulare* has inherent ability to induce fungitoxic effects on the *in vitro* growth of the tested fungi species. According to the previously mentioned results, almost organic and aqueous

extracts (AR0.25, 0.5, 1; AL0.5; AS1,2; AFL1,2; Reac, Seac, Leac, Fleac, Shex, Lhex, Flhex, Smet and Rmet) inhibited at least one fungus growth (%MGI >40%). The percentage of *A. flavus* mycelia growth inhibition was higher than 60 with Fleac, Leac and AR1. However, the relative intensity of the antifungal effect varies with the target fungus, as well as the origin, type and concentration of the extract. Fungicidal effects proved to be dependent on the extracts concentration (Martins et al., 2015). The differences recorded between extracts in this fungitoxic test is likely due to the solubility of the active compound(s) in water or to the presence of inhibitors to the fungitoxic principle as noted by Amadioha (2001) and Okigbo and Ogbonnaya (2006).

Data of this study shows that *Aspergillus* species were the most susceptible to tested extracts and *A. niger* was the most affected, depending on the extract and its concentration. Susceptibility of *A. flavus*, *A. niger* and *A. fumigatus* to plant extracts has been previously also examined (Bansal and Gupta, 2000; Shafique et al., 2005; William, 2008; Abd El-Ghany et al., 2015). In literature, the mycelial growth of various species of *Fusarium* was inhibited by various plant extracts such as those from *Allium sativum* and *Sapindus trifoliata* (Gohil and Vala, 1996), neem seed (Gour and Sharmaik, 1998), *Eucalyptus amygdalina* (Bansal and Gupta, 2000) or *Azadirachta indica* and *Jatropha curcas* (Abd El-Ghany et al., 2015). The inhibition of the mycelial growth may be attributed to the presence of allelochemicals with detrimental effects on cell division, cell elongation and nutrient uptake (Blake, 1985). The variation in antifungal activity of the extracts in different solvents may be attributed to the chemical nature of the three solvents (Jabeen and Javaid, 2008). Moreover, results reveal that antifungal activity of the crude extracts was enhanced by increasing the concentration of the extracts. This finding is in agreement with the report of Banso et al. (1999), and Martins et al. (2015), who also observed that the highest concentration of antimicrobial substances showed more growth inhibition. In addition, the antimicrobial activity of plant extracts might not be due only to the action of a single active compound, but also to the synergistic effect of several compounds that are in minor proportion in a plant (Davicino et al., 2007).

The considerable importance of *C. quadrangulare* in folk medicine and the paucity of reports on the phytochemical constituents of the genus prompted us to test the biologically activity of extracts. Three flavone glycosides (Shalaby and Bahgat, 2003), one iridoid and two iridoid glucosides were isolated and identified from aerial parts of *C. quadrangulare* (Ayers and Sneden, 2002). Seven iridoid glucosides, were isolated from the fruits of *Citharexylum caudatum* (Ayers and Sneden 2002). From the aerial parts of *Citharexylum spinosum* L. (Syn. of *C. quadrangulare*) five iridoid glucoside, a lignan glucoside were identified (Balázs et al., 2006); these compounds may be responsible for the antifungal activity. Indeed, flavonoids have the ability to inhibit spore germination and growth of plant pathogens (Hussin et al., 2009; Kanwal et al., 2010; Bernini et al., 2015; Wang et al., 2015). Iridoids and iridoid glucosides have been proven also for their antifungal activity (Bolzani et al., 1996; Kawamura and Ohara, 2005). Gond et al. (2015) reported that the bacterial endophytes that naturally occur in many maize varieties may function to protect hosts by secreting antifungal lipopeptides that inhibit pathogens. The most pronounced antifungal properties reported for the decoction of sage (*Salvia officinalis* L.), was positively related with its highest concentration in phenolic compounds. Fungicidal and/or fungistatic effects proved to be dependent on the extracts of concentration (Martins et al., 2015).

Alkhail (2005) studied the antifungal activity of five plant extracts against *Fusarium oxysporum* f.sp. *lycopersici, Botrytis cinerea* and *Rhizotonua solani.* Results reveal that plant extracts obtained with cold distilled water had strong antifungal activity with significant inhibition on the growth of the three tested fungi. Similar antifungal properties of ethanolic leaf extracts of *Mangifera indica* against *A. niger, Alternaria alternata, Fusarium chlamydosporum, Rhizoctonia bataticola* and *Trichoderma viride* were reported by Aqil and Ahmad (2003). Shafique et al. (2005) have reported significant inhibition of seed-borne fungi of maize *viz*., *A. fumigatus*, *A. niger*, *Rhizopus arrhizus* A. Fischer and *Penicillium* sp. by aqueous extracts of *Melia azedarach.* More recently, Khan et al. (2013) reported that crude extracts and some fractionated samples of *Tamarix dioica* Roxb. leaves showed significant antifungal properties.

Conclusion

The present study demonstrates that aqueous and organic extracts of *C. quadrangulare* may possess antifungal potential and contain inhibitory substances. This result suggest that *C. quadrangulare* could be one of the useful natural resources for developing bio fungicides, besides crude extracts of this tree could be a cost effective way for crops protection against fungal pathogens. Further research in order to know the inhibitory substances from *C. quadrangulare* organs are underway.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Abd El-Ghany TM, Roushdy MM, Mohamed AA (2015). Efficacy of certain plant extracts as safe fungicides against phytopathogenic and mycotoxigenic fungi. Agric. Biol. Sci. J. 1(3):71-75.

Ae M, Patcharee P (2014). Chemical composition and antibacterial

activity of essential oil and extracts of *Citharexylum spinosum* flowers from Thailand. Nat. Prod. Commun. 9(5):707-710.
Adesina SK, Idowu O, Ogundaini AO, Oladimejil H, Olugbade TA, Onawunmi, GO, Pais M (2000). Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytother. Res. 14:371-374.
Alkhail AAA (2005). Antifungal activity of some extracts against some plant pathogenic fungi. Pak. J. Biol. Sci. 8: 413-417.
Amadioha AC (2001). Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch. Phytopath. Plant Prot. 33:499-507.
Anil KR, Raj KHG (2015). *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *Lycopersici*. Asian J. Plant Sci. Res. 5(1):22-27.
Aqil F, Ahmad I (2003). Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J. Microbiol. Biotechnol. 19:653-657.
Ayers S, Sneden AT (2002). Caudatosides A−F: New Iridoid Glucosides from *Citharexylum caudatum.* J. Nat. Prod. 65(11):1621-1626.
Balazs B, Toth G, Duddeck H, Soliman HSM (2006). Iridoid and lignan glycosides from *Citharexylum spinosum* L. Nat. Prod. Res. 20:201-205.
Bansal RK, Gupta RK (2000). Evaluation of plant extracts against Fusarium oxysporum, wilt pathogen of fenugreek, Indian. J. Phytopath. 53:107-108.
Banso A, Adeyemo SO, Jeremiah P (1999). Antimicrobial properties of *Vernonia amygdalina* extract. J. Appl. Sci. Manage. 3: 9-11.
Bernini R, Pasqualetti M, Provenzano G, Tempesta S (2015). Ecofriendly synthesis of halogenated flavonoids and evaluation of their antifungal activity. New J. Chem. 39: 2980-2987.
Blake NE (1985). Effect of allelochemicals on mineral uptake and associated physiological processes. In: Thompson AC (eds) The Chemistry of Allelopathy: Biochemical Interactions among the plants, American Chemical Washington, pp. 161.
Bolzani VS, Trevisan LMV, Izumisawa CM, Young MCM (1996). Antifungal Iridoids from the stems of *Tocoyena formosa*. J. Braz. Chem. Soc. 7(3):157-160.
Boughalleb N, Souli M, Karbous B, El Mahjoub M (2006). Identification and Distribution of *Fusarium* diseases affecting the ear and the foot of wheat in some regions of northern Tunisia. EPPO Bulletin. 36:512-516.
Boughalleb N, Souli M, Ouled Sghaier H (2008). Occurrence and geographic distribution of wheat *Fusarium* head blight and *Fusarium* root rot in Jendouba's areas of Tunisia. African J. Plant Sci. Biotech. 2(1):23-26.
Davicino R, Mattar MA, Casali YA, Graciela S, Margarita E, Micalizzi B (2007). Antifungal activity of plant extracts used in folk medicine in Argentina. Rev. Peru. Biol. 14: 247-251.
Deans SG, Svoboda KP (1990). Biotechnology and bioactivity of culinary and medicinal plants. Agbiotech. News Info. 2(2): 211-216.
Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Dalla Rizza M (2011). Antigungal activity of medicinal plant extracts against phytot pathogenic fungus *Alternaria* SPP. Chilean J.A.R. 71(2): 231-239.
Gohil VP, Vala GD (1996). Effect of extracts of some medicinal plants on the growth of *Fusarium moniliforme*. J. Mycol. Pl. Pathol. 26 (1): 110-111.
Gond SK, Bergen MS, Torres MS, White JF Jr (2015). Endophytic Bacillus spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiol. Res. 172:79-87.
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.
Grover RK, Moore JD (1962). Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. Phytopathology 52: 876-880.
Hussin NMR, Muse S Ahmad, J Ramli, Mahmood M, Sulaiman MR, Shukor MYA, Rahman MFA, Aziz KNK (2009). Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae). Afr. J. Biotechnol. 8 (12): 2835-2842.
Iqbal D, Javaid A (2012). Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii.* Nat. Prod. Res. 26:1638-1644.
Jabeen K, Javaid A (2008). Antifungal activity of aqueous and organic solvent extracts of allelopathic trees against *Ascochyta rabiei*. Allelopathy J. 22:231-238.
Jabeen K1, Javaid A, Ahmad E, Athar M (2011). Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Nat. Prod. Res. 25(3): 264-76.
Jabeen N, Javaid A, Ahmed E, Sharif A (2014). Management of causal organism of collar rot of bell pepper (*Sclerotium rolfsii*) by organic solvents extracts of *Datura metel* fruit. Pak. J. Phytopathol. 26: 13-18.
Kawamura F, Ohara S (2005). Antifungal activity of iridoid glycosides from the heartwood of *Gmelina arborea*. 59(2):153-155.
Kanwal Q, Hussain I, Latif Siddiqui H, Javaid A (2010). Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. Nat. Prod. Res. 24(20):1907-1914.
Kanwal Q, Hussain I, Latif Siddiqui H, Javaid A (2011). Antimicrobial activity screening of isolated flavonoids from *Azadirachta indica* leaves. J. Serb. Chem. Soc. 76(3):375-384.
Khan S, Ullah F, Mahmoud T (2013). *In vitro* antimicrobial and cytotoxic activity of *Tamarix dioica* Roxb. leaves. Turk. J. Biol. 37: 329-335.
Kumari S, Jain P, Sharma B, Kadyan P, Dabur R (2015). In Vitro Antifungal Activity and Probable Fungicidal Mechanism of Aqueous Extract of *Barleria Grandiflora*. Appl. Biochem. Biotechnol.
Kuo PC, Hsieh TF, Lin MC, Huang BS, Huang JW, Huang HC (2015). Analysis of antifungal components in the galls of *Melaphis chinensis* and their effects on control of anthracnose disease of chinese cabbage caused by *Colletotrichum higginsianum*. J. Chem. 2015:12-23.
Lachman-White DA, Adams CD, Trotz Ulric OD (1992). A guide to the medicinal plants of coastal Guyana. London: Commonwealth Science Council, London.
Lans C (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. J. Ethnobiol. Ethnomed. 3(3): 1-12.
Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira IC (2015). Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L. Food Chem. 170: 378-85.
Okigbo RN, Ogbonnaya UO (2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. Afr. J. Biotech. 5: 727-731.
Ouedraogo M, Konate K, Zerbo P, Barro N, SawadogoLaya L (2013). Phytochemical Analysis and in vitro antifungal profile of bioactive fractions from *Sterculia setigera* (Sterculiaceae). Curr. Res. J. Biol. Sci. 5(2):75-80.
Shafique S, Shafique S, Javaid A (2005). Fungitoxicity of allelopathic plant extracts on seed borne mycoflora of maize. Mycopath. 3(1-2): 23-26.
Shafique S, Bajwa R, Shafique S, Akhtar N, Hanif S (2011). Fungitoxic activity of aqueous and organic solvent extracts of Tagetes erectus on phytopathogenic fungus *Ascochyta rabiei*. Pak. J. Bot. 43: 59-64.
Sumathy V, Zakaria Z, Jothy SL, Gothai S, Vijayarathna S, Yoga Latha L, Chen Y, Sasidharan S (2014). In vitro and in vivo antifungal activity of *Cassia surattensis* flower against *Aspergillus niger*. Microb. Pathog. 77:7-12.
Shalaby NMN, Bahgat M (2003). Phytochemical and some biological studies of *Citharexylum quadrangulare* Jacq. Chem. Nat. Micro. Prod. 4:219-228.
Turner RJJr, Wasson E (1997). Botanica. NSW Australia, Mynah Publishing.
Waghner WL, Herbst DR, Sohmer SH (1999). Manual of the flowering plants of Hawai'i. Bishop Museum Special Publication 83, Honolulu, HI, University of Hawai'i and Bishop Museum Press, vol. 2.
Wang Q, Wu R, Wu X, Tai W, Dai N, Wu J, Han N (2015). Three flavonoids from the leaves of *Astragalus membranaceus* and their antifungal activity. Monatsh. Chem - Chemical Monthly
William Q (2008). Least toxic controls of plant diseases. Brooklyn Botanic garden. Natural Disease Control 11:225.