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In vitro Antifungal Activity of Three Geophytic Plant Extracts against Three Post-harvest Pathogenic Fungi

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Abstract: Plant extracts appear to be one of the most effective alternative methods of plant diseases control which are less harmful to human beings and environment. *In vitro* antifungal activity of methanolic extracts of three promising wild geophytic plants against three post-harvest pathogenic fungi using radial growth technique was conducted. These extracts included the shoot system (S) and underground parts (R) of *Asparagus stipularis*, *Cyperus capitatus* and *Stipagrostis lanata*. The tested fungi were *Alternaria solani*, *Aspergillus niger* and *Rhizopus stolonifer*. The results exhibited that, all plant extracts had antifungal activity against the tested fungi. The antifungal activity greatly varied depending on plant parts and/or plant species. *R. stolonifer* was the most susceptible fungus to the tested plant extracts followed by *A. niger* and then *A. solani*. On the other hand, the most effective plant extracts against tested fungi were *S. lanata* (S) and *A. stipularis* (R). The most effective plant extracts against *R. stolonifer* were *S. lanata* (R) and *C. capitatus* (S). While, the extracts of *A. stipularis* (R) and *S. lanata* (S) were the most effective against *A. niger*. The extracts of *C. capitatus* (S) and *S. lanata* (S) exhibited the highest antifungal activity against *A. solani*. The results demonstrated that, the methanolic extracts of *A. stipularis*, *C. capitatus* and *S. lanata* had potential antifungal activity against *A. solani*, *A. niger* and *R. stolonifer*.

Key words: Antifungal activity, geophytic plants, post-harvest pathogenic fungi

INTRODUCTION

Post-harvest diseases are posing a major problem to the agriculture industry, where they account to about 50% losses in fruits stored in poor storage conditions (Agrios, 2005). Among of the most important fungi causing post-harvest diseases of plants are *Aspergillus* spp., *Alternaria* spp. and *Rhizopus stolonifer* (Ogawa *et al*., 1995). *Alternaria solani* is the main pathogen causing early blight disease and yield losses in numerous economically important crop plants of family Solanaceae such as potato, tomato and eggplant crops (Abd El-Khair and Haggag, 2007; Haggag and Farghaly, 2007). The early symptoms are in the form of small spots on the leaves which later on enlarge to form concentric rings. Also, this fungus can infects fruits and tubers in severe conditions (Sangvikar, 2012). Species of *Aspergillus* fungus are among the major reported fungi having the ability to produce mycotoxins during storage which reduce the quality of food products (Gautam and Bhadauria, 2008). *A. niger* is a saprophytic filamentous fungus found in soil, forage, organic debris and food product, causing black mould of onion and shallot; boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla and dried prune (Bobbarala *et al*., 2009). Rhizopus rot, caused by *Rhizopus stolonifer* is one of the most destructive post-harvest diseases. The spores of *R. stolonifer* are very common in the atmosphere and this infection of fruits occurs mainly at wound sites during harvest or packing (Ogawa *et al*., 1963). *R. stolonifer* is reported to cause food spoilage and decay in fruits, particularly peaches, strawberries, raspberries and grapes (Northover and Zhou, 2002).

The most important method of protecting the plants against the fungal attack is the use of synthetic fungicides, but their excessive use complemented with high costs, residues in plants and development of resistance, has left a negative effect on human health and the environment (Bull *et al*., 1997; Paster and Bullerman, 1988). Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides (Zhang *et al*., 2005). That plant extracts are cheap, locally available, non-toxic and easily biodegradable.

Wild plants may be contains a large source of effective secondary metabolites such as phenolic

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compounds which had antifungal activity. Among of these plants, *Asparagus stipularis* (Asparagaceae), *Cyperus capitatus* (Cyperaceae) and *Stipagrostis lanata* (Poaceae). These plants which are monocot geophytes (have subterranean organs) occur in harsh environments (saline and/or non-saline sand formations) in the Deltaic Mediterranean coast of Egypt (Eladly, 2009). The underground and aerial parts of *A. stipularis* and *C. capitatus* as well as the aerial parts of *S. lanata* contained high amounts of biologically active compounds such as total and simple phenolics, tannins, flavonoids, alkaloids, saponin and cyanogenic glycosides (Hassan and Maswada, 2012; Maswada and Elzaawely, 2013).

The main goal of this study was to investigate the potential of antifungal activity of three wild geophytic plants (*A. stipularis*, *C. capitatus* and *S. lanata*) against three post-harvest pathogenic fungi, *R. stolonifer, A. niger* and *A. solani*.

MATERIALS AND METHODS

Plant material: The studied plants, *Asparagus stipularis* Forssk., *Cyperus capitatus* Vend. and *Stipagrostis lanata* (Forssk.) De Winter, were collected during spring and summer seasons (April-July) of 2011 from their natural habitats in the Nile Delta coastal region of Kafr El-Sheikh Governorate, Egypt. These plants were identified by the first author. The voucher specimen was deposited at Laboratory of the Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt. The underground part (R) and shoot system (S) of each plant were separately cut and air dried. The dried materials were powdered and kept in the refrigerator till use.

Preparation of plant extracts: One hundred grams dried powdered form shoot (S) or underground (R) parts of each plant were extracted with one liter of 50% methanol for a week at room temperature. The extracts were separately collected, filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum and concentrated. The crude extract was dissolved in methanol (10 g/100 mL) to obtain 10% concentration.

The tested fungi: *Aspergillus niger*, *Alternaria solani* and *Rhizopus stolonifer* were isolated from their respective hosts, onion bulbs, tomato and strawberry fruits, respectively on PDA medium. These pathogenic fungi were identified by Prof. Dr. Hassan M. El-Zahaby, Professor of Plant Pathology and Head of Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt.

Antifungal assay: According to Torgeson (1969) and Nene (1971), the fungicidal activity of the methanolic plant extracts was conducted by using the radial growth technique. A define volume (9 mL) of the nutrient medium (PDA) was mixed well with 1 mL of each extract to obtain various concentrations (12.5, 25.0, 50.0, 125.0, 250.0 and 500.0 ppm). Then the mixture was poured in four sterilized Petri-dishes (7 cm diameter) and consider as one treatment. After solidification 0.6 cm disk inoculum made by cork borer of the tested fungi was taken from 7 day old culture and located in the center of the Petri-dish. The control was prepared as any treatment except the addition of the tested plant extract. The inoculated dishes were then incubated at 27-29°C for 7 days. Diameter of the developed growth was measured daily until the control growth covered the total area of the plate. Four replicated were made for each treatment. The fungi-toxicity of the extracts in terms of percentage inhibition of mycelial growth was calculated according to (Singh and Tripathi, 1999) by using the formula:

$$\text{Inhibition (\%)} = \frac{dc-dt}{dc} \times 100$$

Where:

dc = Average increase in mycelial growth in control
dt = Average increase in mycelial growth in treatment

The inhibitory percentages were plotted on probit graph paper against plant extract concentrations.

Statistical analysis: Data were statistically analyzed according to the method of Litchfield and Wilcoxon (1949). The obtained data including slopes of the regression lines and GR_{50} values with their 95% confidence limits had recorded.

RESULTS

Antifungal activity of the plant extracts against *Alternaria solani*: All plant extract had antifungal activity against *A. solani* (Table 1, Fig. 1). The antifungal activity greatly varied depending on plant parts and/or the tested plant species. The antifungal toxicities of certain plant extracts could be arranged descendingly as follows: *C. capitatus* (S) *S. lanata* (S) *A. stipularis* (R)≥*S. lanata* (R)≥*C. capitatus* (R)≥*A. stipularis* (S) (GR_{50}: 136.61, 364.78, 811.37, 908.60, 2207.81 and 3062.02 ppm, respectively). Based on the obtained results, the plant extracts could be classified into two groups according to their antifungal toxicity against *A. solani*. The first group includes the plant extracts that exhibited high antifungal

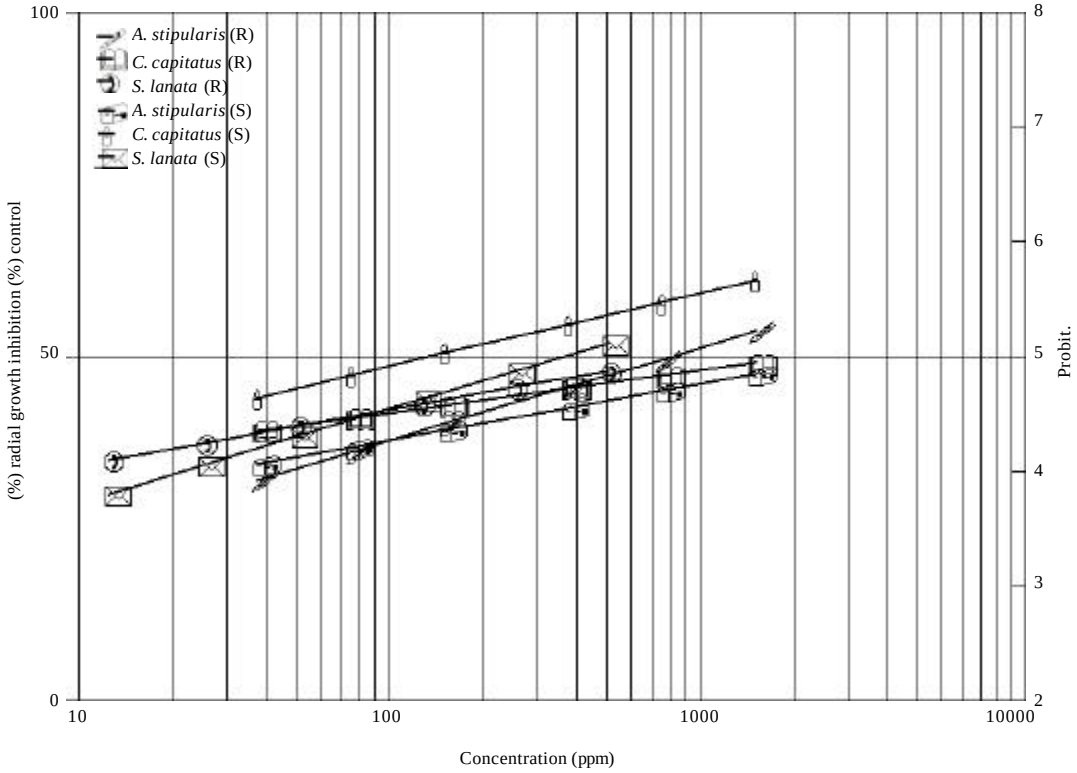

Fig. 1: Probit regression line for the inhibition effects of some methanolic plant extracts against *Alternaria solani* after 7 days from treatment using radial growth technique

Table 1: Toxicity of methanolic plant extracts against *Alternaria solani* after 7 days from treatment using radial growth technique

			Confidence limits (ppm)				
Plant species	Plant part	GR_{50} (ppm)	Lower	Upper	GR_{99} (ppm)	Slope value	R^2
A. stipularis	R	811.37	165.66	3974.03	604425.49	0.82	0.91
	S	3062.02	221.29	42370.31	$>10^6$	0.50	0.85
C. capitatus	R	2207.81	65.49	74428.35	$>10^6$	0.37	0.91
	S	136.61	17.83	1046.46	654749.53	0.64	0.93
S. lanata	R	908.60	63.39	13023.55	$>10^6$	0.49	1.00
	S	364.78	204.85	649.56	262189.77	0.82	0.98

S: Shoot system, R: Under ground part

toxicity, i.e., *C. capitatus* (S), *S. lanata* (S), *A. stipularis* (R) and *S. lanata* (R). The second group includes the plant extract that showed low toxicity, i.e., *C. capitatus* (R) and *A. stipularis* (S). According to GR_{99} values, all tested plant extracts can not be used as fungicides without mixed with synthetic or purification the effective compounds. Slop values is depressed which indicate that plant extracts had many effective antifungal compounds.

Antifungal activity of the plant extracts against *Aspergillus niger*: All plant extracts exhibited highly to moderate antifungal activity against *A. niger*, except *C. capitatus* (R) which had no relationship between plant extract concentrations and the inhibitory percentages (Table 2, Fig. 2). In addition, the relationship between plant extract concentration and the inhibitory effect are not statically significant in case of *A. stipularis* (S) (R^2 = 0.28). Among plant extracts, the extracts of *A. stipularis* (R) and *S. lanata* (S) exhibited the highest antifungal effect against *A. niger* (GR_{50}: 33.69 and 106.94 ppm, respectively). Other plant extracts had high antifungal activity. All plant extract had high GR_{99} values overcome 10^5 ppm. Accordingly, all tested plant extracts can not be used as fungicides without mixed with synthetic fungicide or purification the effective compounds. The slop value is very descent which means that plant extract had numerous active compounds.

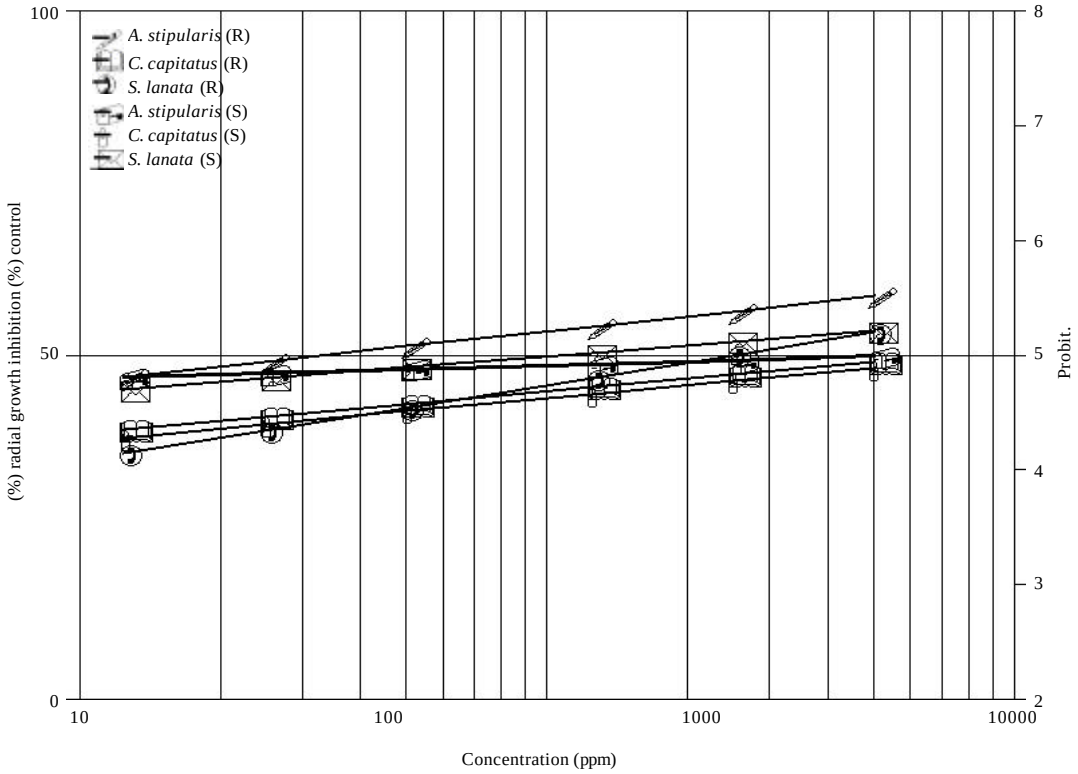

Fig. 2: Probit regression line for the inhibition effects of some methanolic plant extracts against *Aspergillus niger* after 7 days from treatment using radial growth technique

Table 2: Toxicity of methanolic plant extracts against *Aspergillus niger* after 7 days from treatment using radial growth technique

			Confidence limits (ppm)				
Plant species	Plant Part	GR_{50} (ppm)	Lower	Upper	GR_{99} (ppm)	Slope value	R^2
A. stipularis	R	33.69	11.59	97.96	$>10^6$	0.45	0.81
	S	630.72	9.70	41028.15	$>10^6$	0.11	0.28
C. capitatus	R	-	-	-	-	-	0.10
	S	951.06	280.72	3222.10	$>10^6$	0.39	0.88
S. lanata	R	251.90	123.33	514.53	$>10^6$	0.67	0.89
	S	106.94	24.29	470.79	$>10^6$	0.32	0.82

S: Shoot system, R: Under ground part

Antifungal activity of the plant extracts against *Rhizopus stolonifer*: All plant extracts exhibited very highly to moderate antifungal activity against *R. stolonifer* (Table 3, Fig. 3). The antifungal activity varied depending on plant parts and/or the plant species. The antifungal toxicities of investigated plant extracts could be arranged descendingly as follows: *S. lanata* (R)≥*C. capitatus* (S)≥*S. lanata* (S)≥*A. stipularis* (R) Plant extract≥*A. stipularis* (S)≥*C. capitatus* (R) where GR_{50} values were 20.84, 21.33, 105.37, 132.26, 137.17 and 586.31 ppm, respectively. According to the obtained results, the plant extracts could be classified into three groups according to their antifungal activity against *R. stolonifer*. The first group includes the plant extracts that exhibited very high antifungal activity, i.e., the extracts of *S. lanata* (R) and *C. capitatus* (S). The second group includes the extracts of *S. lanata* (S), *A. stipularis* (R) and *A. stipularis* (S) which had highly antifungal activity. The third group contains *C. capitatus* (R) extract which had moderate antifungal activity. According to GR_{99} values, all tested plant extracts had highly antifungal activity and can be used as bio-fungicides except, *A. stipularis* (R) and *C. capitatus* (S) extracts ($GR_{99}>10^5$). Based on the slope values of *S. lanata* (S) and *C. capitatus* (R) extracts which had ascent slop values, these extracts contain a small number of active compounds or affect on one or few sites of action on fungus mycelium. While, the slope values of other extracts is depressed which indicate that plant extracts had many of effective antifungal compounds.

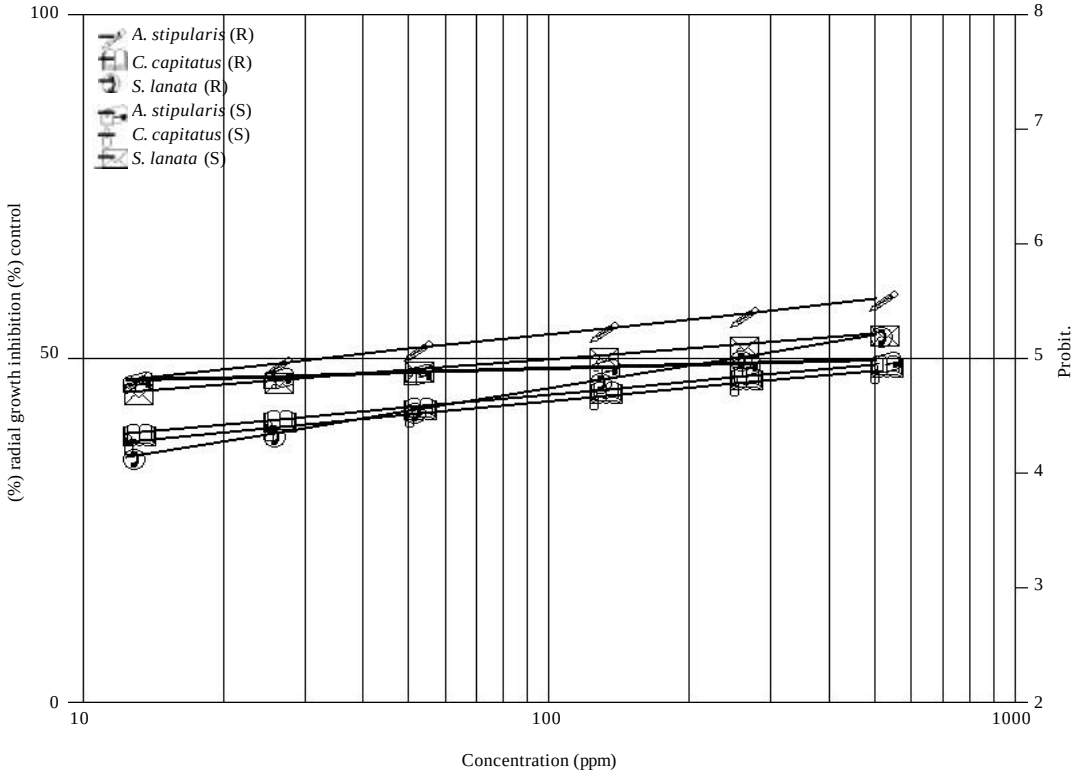

Fig. 3: Probit regression line for the inhibition effects of some methanolic plant extracts against *Rhizopus stolonifer* after 7 days from treatment using radial growth technique

Table 3: Toxicity of methanolic plant extracts against *Rhizopus stolonifer* after 7 days from treatment using radial growth technique

			Confidence limits (ppm)				
Plant species	Plant Part	GR_{50} (ppm)	Lower	Upper	GR_{99} (ppm)	Slope value	R^2
A. stipularis	R	132.26	13.88	1260.61	$>10^6$	0.58	0.95
	S	134.17	18.66	964.52	493678.75	0.66	0.94
C. capitatus	R	586.31	228.11	1506.98	29829.77	1.38	0.99
	S	21.23	0.14	3208.57	$>10^6$	0.26	0.99
S. lanata	R	20.84	11.11	39.09	27047.44	0.76	0.98
	S	105.37	76.71	144.74	3928.79	1.50	0.97

DISCUSSION

Plants are rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds, reported to have *in vitro* antifungal properties (Arif *et al*., 2009). The studied plants contained high amounts of bioactive compounds such as total and simple phenolics, tannins, flavonoids, alkaloids, saponin and cyanogenic glycosides (Hassan and Maswada, 2012; Maswada and Elzaawely, 2013). Therefore, the efficacy of different plant extracts of the underground parts (R) and shoot system (S) of these plants were tested against the mycelial growth of three pathogenic fungi, *A. solani*, *A. niger* and *R. stolonifer*.

There are some studies on the antifungal activity of plant extracts which are related to *A. stipularis*. From these plants, *Asparagus acutifolius*, *A. racemosus*, *A. officinalis*. Sautour *et al*. (2007) isolated six new steroidal saponins from the roots of *Asparagus acutifolius* which demonstrated antifungal activity against the human pathogenic yeasts, *Candida albicans*, *C. glabrata* and *C. tropicalis*. Mathur *et al*. (2011) stated that, the hydro-alcoholic extract (50 % v/v) of *Asparagus racemosus* had antifungal activity against *Aspergillus niger* (MIC = 0.5 mg mL^{-1}). Also, Sangvikar *et al*. (2012) found that, all the extracts (cold water, hot water, ethyl acetate and alcohol) of *A. racemosus* roots were found to be inhibitory for *Alternaria solani* and *Fusarium moniliforme*.

Wang and Ng (2001) isolated a Deoxy-ribonuclease from *Asparagus officinalis* seeds which had antifungal activity toward *Botrytis cinerea*. The crude saponin fractions of *A. officinalis* were ineffective against *Aspergillus niger* and *Rhizopus stolonifer* (Shimoyamada *et al*., 1990).

On the other hand, antifungal activity was assayed from plant extracts related to *Cyperus capitatus* such as *Cyperus kyllingia* and *Cyperus rotundus*. Khamsan *et al.* (2011) mentioned that, the essential oil from *Cyperus kyllingia* exhibited moderately active against *Aspergillus flavus* and *Candida albicans*. In addition, the ethanolic extracts of *Cyperus rotundus* had antifungal activity against the growth of *Candida albicans* (Al-Ebady and Al-Mohana, 2010). Ethyl acetate fractions of *C. rotundus* rhizomes have been found highly effective against some species of Alternaria including *A. solani* (Singh *et al*., 2011). Sharma and Singh (2011) reported that, although *Cyperus rotundus* rhizomes contained several metabolites such as polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil, their ethanolic extract were ineffective against *Aspergillus niger*. While, Parekh and Chanda (2008) found that, the methanolic extract of *C. rotundus* exhibited antifungal activities against *A. candidus*, *A. flavus* and *A. niger*.

The present study revealed that, all plant extracts had antifungal activity against the tested fungi. The pathogenic fungus, *R. stolonifer* was the most susceptible species to the tested plant extract while, *A. solani* was the most tolerant one. Agrios (2005) mentioned that, controlling plant diseases caused by *Alternaria* spp., can be difficult because it spreads so readily. In the present study, the most effective plant extracts against the three fungal species were *S. lanata* (S) and *A. stipularis* (R). The most effective plant extracts against *R. stolonifer* were *S. lanata* (R) and *C. capitatus* (S). While, *A. stipularis* (R) and *S. lanata* (S) extracts were the most effective plant extracts against *A. niger*. *C. capitatus* (S) and *S. lanata* (S) extracts exhibited the highest antifungal activity against *A. solani*.

The antifungal activity of *A. stipularis* (R) may be due to large amounts of saponins. Antifungal activities have been reported for several kinds of saponins (Shimoyamada *et al*., 1990). Saponins appear to act by disrupting the membrane integrity of fungal cells (Arif *et al*., 2009). On the other hand, the antifungal activities of *C. capitatus* may be as consequent to high amount of phenolic compounds. Alves *et al.* (1992) and Seabra *et al.* (1997, 1998) isolated numerous of 1-4 benzoquinon and methylaurone derivatives from *Cyperus capitatus* which had antifungal activity (Roussaki *et al*., 2012). Phenolic compounds have been divided into three major categories: phenolic acids, flavonoids and tannins (Chung *et al*., 1998). In addition, the increase in the production of phenolic compounds in plant extracts can be correlated with the induction of resistance in treated plant against phytopathogenic fungi (Hussin *et al*., 2009). Cushnie and Lamb (2005) reported that, many groups of flavonoids are possessing antifungal, antiviral and antibacterial activity. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Arif *et al*., 2009).

CONCLUSION

The results of this study signify the antifungal activity of the methanolic extracts of *A. stipularis, C. capitatus* and *S. lanata* plants against pathogenic fungi, *A. solani, A. niger* and *R. stolonifer*. However, further studies are needed to isolate and identify the most effective antifungal compounds of these promising plants.

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