

Biological activity of selected plant ethanolic extracts against phytopathogenic fungi, *Fusarium oxysporum* f.sp. *cicer*

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ABSTRACT

In this present study six different plants used in traditional Sudanese medicine; *Allium sativum* L. (Liliaceae), *Azadirachta indica* (Meliaceae), *Capsicum frutescence* L. (Solanaceae), *Momordica balsamina* (Cucurbitaceae), *Petroselinum crispum* (Apiaceae) and *Pulicaria undulata* L.(Asteraceae), were examined against *Fusarium oxysporum* f.sp. *cicer*., the causal agent of vascular wilt in chickpea. We used disc agar method to determine the in vitro antifungal activity of these plants. The ethanolic extracts of studied plants exhibited varying degrees of inhibition activity against the tested fungi. Among the 6 plants studied only two plants (*A. sativum*, *P. crispum*) showed high activity, other plants (*M. Balsamina*, *A. indica*, *C. frutescence*, *P. undulata*) showed moderate activity. These results suggest that the plants may have individual components that can be useful for managing *Fusarium oxysporum* f.sp. *cicer*.

Keywords: *Fusarium oxysporum* f.sp. *cicer*., inhibition activity, medicinal plants, inhibition zone.

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INTRODUCTION

Many of compounds extracted from plant have pharmacological and biological activities such as anticancer, antiviral, antibacterial, antifungal insecticidal and nematicidal effects (Pandey et al., 2000; Srinivasan et al., 2001; Soliman and Badeaa, 2002; Dissanayake and Jayasinghe, 2013; Abd-Ulgadir et al., 2015).

The interest on use of plant material has been increased as alternative of synthetic fungicides to control plant diseases. However, that is needed because of her eco-friendly and the negative public perceptions about the use of synthetic chemicals, high cost of new chemicals and resistance to fungal pathogens (Dissanayake and Jayasinghe, 2013). Therefore, researchers are doing scientific studies on traditionally used plants for the control and management of different diseases and these could be valuable sources for new natural products (Lee et al., 2007; Dwivedi and Sangeeta,

2015).

Antifungal activity of plant extracts has been studied and showed a wide range of activity against fungi, in previous studies, tested plants showed antifungal activity against several fungi such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Penicillium* sp., *Alternaria solani*, *Fusarium solani*, *Fusarium oxysporum* f.sp. *melonis*, *Macrophomina phaseolina*, *Candida albicans* and *Trichophyton* sp. (Burger et al., 2010; Soumya and Bindu, 2012; Shrivastava and Kshma, 2014; Ajaib et al., 2015; Ikegbunam et al., 2016; Linde et al., 2016).

F. oxysporum f. sp. *ciceri*, associated with seeds of chickpea, and adversely affects quality and health of seeds in most of the chickpea growing areas of the world (Jalali and Chand, 1992). In addition, chickpea is the major source of food for human as well as animal food, it is a highly nutritious value (proteins, carbohydrates), also

very good source of fibre, vitamins and minerals (Chibbar et al., 2010).

Mandhare and Suryawanshi (2009) reported that the extracts of *Azadirachta indica* and *Allium sativum* showed antifungal activity against *F. oxysporum* f.sp. *ciceri*, and it was the same result recorded by Singh and Chand (2004). In addition, the antifungal potential of *A. indica* was determinate on tested fungi by Mukhtar (2007) and Jibhkate et al. (2010).

But, there have been no reports of extracts obtained from *Capsicum frutescence* L., *Momordica balsamina*, *Petroselinum crispum* and *Pulicaria undulata* L. used to control *Fusarium oxysporum* f.sp. *cicer*, hence, the principle aim of the present work is to assess the biological activity of Selected Plant Ethanolic Extracts namely; garlic (*Allium sativum* L.), neem (*Azadirachta indica*), hot paper (*C. frutescence* L.), bitter melon (*Momordica balsamina*), parsley (*Petroselinum crispum*) and rabble (*Pulicaria undulata* L.), against Phytopathogenic Fungi, *Fusarium oxysporum* f.sp. *cicer*.

MATERIALS AND METHODS

Plant materials

Studied Plants were collected from different local area in Sudan, where these plants are available such as shambat- Khartoum, and Kordufan. Identified and authenticated by Herbarium unit – department of phytochemistry. Herbarium material was deposited at Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), NCR. Khartoum. Sudan.

Preparation of plants extracts

Extracts prepared in ethanol at room temperature by simple extraction method (Sukhdev et al., 2008). Parts of plants (corms of garlic, leaves of neem, fruits of hot paper, and aerial parts of rabble, aerial parts of parsley and stems of bitter melon), were dried under room temperature, samples (100 g) of dried parts from each plant were aseptically weighed and placed in separate sterile glass flasks. One litre of 70% ethanol was added to each flask, then left to macerate for three days at room temperature, with occasional stirring. After this extraction period, the 70% ethanol extracts were filtered through Whatman No. 1 filter paper and these filtered extracts concentrated under reduced pressure using a rotary evaporator. Solvents (ethanol) were removed from the concentrates by incubated them into room temperature at 37°C for 24 h. after full dried extracts from each plant, only 2 g from each plant extract were added to 2 ml of distilled water and diluted into different concentrations (0.1, 0.05, 0.025, 0.012, and 0.006 ppm).

Preparation of fungal suspension

F. oxysporum f.sp. *ciceris* obtained from Microbial Type Culture Collection, Central lab of Plant Pathology, Plant Protection Directorate. This fungus was grown on Sabouraud dextrose agar plate at 28°C and maintained with periodic sub-culturing at 4°C. The fungal cultures of *F.oxysporum* f. sp. *ciceris*, were maintained on Sabouraud dextrose agar (SDA), incubated at 25°C for 7 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, to

produce a suspension containing about 10⁸ to 10⁹ CFU/ml. The suspension was stored in the refrigerator at 4°C till used.

Biological assay of antifungal activities

We used disc agar method (NCCLS, 1999; Duru et al., 2003), to determine a biological activity of ethanol crude extract of six medicinal plants used in traditional Sudanese medicine, namely: *Allium sativum* L. (Liliaceae), *Azadirachta indica* (Miliaceae), *Capsicum frutescence* L. (Solanaceae), *Momordica balsamina* (Cucurbitaceae), *Petroselinum crispum* (Apiaceae) and *Pulicaria undulata* L.(Asteraceae), against phytopathogenic fungi, *Fusarium oxospoeum* f.sp. *cicer*. Fungal growth was swabbed uniformly on surface of Sabouraud dextrose agar, sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the Sabouraud dextrose agar and soaked with 20 µl of a solution of each plant extracts. The inoculated plates incubated into room temperature in the inverted position to allow growth of tested fungi, and for each treatment were used in triplicate, then after 24 h, the diameters of clear zones were measured (the diameter around the filter paper disc with plant extract).

RESULTS AND DISCUSSION

Biological activity of six plants was assayed and data on effect of plant extracts on the growth of *F. oxysporum* f.sp. *cicer*., presented in Table 1. The extracts of studied plants exhibited varying degrees of inhibition activity against the tested fungi; the results were expressed in terms of the diameter of the growth-inhibition zone (clear zones) according to Mukhtar and Ghori (2012). Among the 6 plants studied only two plants (*Allium sativum*, and *Petroselinum crispum*) showed high activity (18 to 22 mm), other plants (*Momordica balsamina*, *Azadirachta indica*, *Capsicum frutescens*, *Pulicaria undulata*) showed moderate activity (13 to 17 mm).

Extracts from *Capsicum frutescence* L., *Momordica balsamina*, *Petroselinum crispum* and *Pulicaria undulata* L. were used for the first time to inhibit growth of *F. oxysporum* f.sp. *cicer*. and showed promising antifungal potentiality against tested fungi.

The best result of inhibition activity of ethanolic extracts found in the corms of garlic (*A. sativum*), at all concentrations (0.006 to 0.1 ppm) ranging between 18 and 22 mm, the similar result with Mandhare and Suryawanshi (2009). In a previous study, the essential oil of *P. crispum* showed antifungal activity against several of fungi such as *Aspergillus* sp., *Penicillium* sp., and *Trichophyton* sp. (Linde et al., 2016). However, in the present study, the ethanolic extract of parsley, also was good antifungal activity in all concentrations.

M. balsamina showed antifungal potential against *F. oxysporum* f.sp. *cicer*., the same result was found against *Fusarium oxysporum* f.sp. *melonis* (Burger et al., 2010). *A. indica* showed moderate activity against tested phytopathogen, but the lower activity found in lower concentration (0.006 ppm), in 2010 Jibhkate et al. reported that, neem seed extract had antifungal activity against *F. oxysporum* f.sp. *cicer*.

Table 1. Biological activity of plant extracts of six Sudanese medicinal plants against *F. oxysporum* f.sp. *cicer*. (IZ; diameter of clear growth-Inhibition zone).

Plant	Part used	Concentration (ppm)	Mean of IZ (mm)
<i>M. balsamina</i> (Cucurbitaceae)	Stems	0.1	17
		0.05	17
		0.025	17
		0.012	16
		0.006	14
<i>A. indica</i> (Miliaceae)	Leaves	0.1	17
		0.05	17
		0.025	14
		0.012	14
		0.006	12
<i>P. crispum</i> (Apiaceae)	Aerial parts	0.1	18
		0.05	18
		0.025	18
		0.012	18
		0.006	17
<i>P. undulata</i> L. (Asteraceae)	Aerial parts	0.1	16
		0.05	15
		0.025	15
		0.012	-
		0.006	-
<i>C. frutescence</i> L. (Solanaceae)	Fruits	0.1	16
		0.05	15
		0.025	14
		0.012	14
		0.006	13
<i>A. sativum</i> L. (Liliaceae)	Corms	0.1	22
		0.05	22
		0.025	19
		0.012	19
		0.006	18

Soumya and Bindu, reported in 2012, that aqueous leaf and fruit extracts of *C. frutescens* had effectiveness against *Aspergillus flavus*, *A. niger*, *Penicillium* sp. and *Rhizopus* sp fruit. But the fruit extract were lower compared to the leaf extract. In the present work, *C. frutescens* showed moderate activity against tested fungi in all concentration ranging between 13 and 16 mm.

In this study work no inhibition zone recorded in concentration (0.012/0.006 ppm) of *P. undulate* extract, however, other concentration showed moderate activity. However although, it has been reported that *P. undulate* had the greatest activity on *A. niger*. (Muhammad Ajaib et al., 2015). So, it is necessary to do more *in vitro* experimental in *P. undulate* extract to assess their high effectiveness for genus of fungi.

CONCLUSION

The results indicated that the extracts of studied plants had a biological activity against *F. oxysporum* f.sp. *cicer*. So, additional studies on plants under greenhouse and field conditions are still necessary to verify their activity *in vivo* interaction, also more studies *in vitro* are needed to determine the active compounds, and to develop new an alternative of synthetic fungicides to be potentially useful for the integrated management of check pea wilt.

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