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ANTIFUNGAL ACTIVITY OF SELECTED HALOPHYTES AGAINST ROOT PATHOGENIC FUNGI

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ABSTRACT

The fungicidal effectiveness of *Atriplex stocksii* Boiss, *Suaeda fruticosa* (L.), and *Salsola imbricata* Forssk. parts (leaves and stem) was observed against root pathogenic fungi *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (Kühn) and *Fusarium oxysporum* (Schlecht) using two methods namely agar well diffusion and paper disc methods. Results showed that leaves extracts of *S. fruticosa* and *S. imbricata* at 100 and 50% v/w concentrations gave pronounced inhibition in the growth of *M. phaseolina* and *F. oxysporum* as compared to stem extracts by both methods. However, *R. solani* showed no zone of inhibition against any plant extract by using any of above methods.

Key words: *Atriplex stocksii*, *Suaeda fruticosa* and *Salsola imbricata*, root rot fungi, concentrations, plant extracts.

INTRODUCTION

Various scientists have used biological screening of plant extract as a tool for determining antifungal activity worldwide. Using plant extract is better option because synthetic chemicals produce environmental pollution, harmful for terrestrial life and human health. Keeping the environmental losses and economic circumstances in mind many scientists prefer to use plant extract as cheaper and healthier way to control plant diseases (Gerresten and Haagsma, 1951). Medicinal plants have antimicrobial and antifungal activity on other plants and also on human beings, many researches have shown that medicinal plant product usually leaves contain alkaloids that protect plants from microbial infections, so it is mandatory to find out medicinal plant's activity against microbes and its existing in possibility to produce antimicrobial process (Mothana and Lindequist, 2005; Bajpai *et al.*, 2005).

Halophytes have medicinal properties due to presence of enzymatic and non-enzymatic antioxidant compounds. They have ability to cope with stress condition (Ksouri *et al.*, 2009). Many halophytes contain phenolic compounds that act as bioactive compounds. These phenolic compounds are used in many therapeutic purposes (Watt and Pretorius, 2001). The most abundant macromolecule, which is present in all living organism, is polysaccharides (Wang *et al.*, 2016). It has been reported that most of antioxidant and biological properties are mainly found in naturally occurring polysaccharides (carbohydrate) which is largely found in all living organism (Yan *et al.*, 2012).

Halophyte *Suaeda fruticosa* is widely distributed in salt marshes and salinized areas belonging to Chenopodiaceae. It is a medicinal plant and contains hypoglycaemic and hypolipidaemic activity in its leaf and stem making it an important plant in the field of medicine (Bennani-Kabchi *et al.*, 1999, Benwahhoud *et al.*, 2001). *Salsola imbricata* is profoundly distributed in sea belt, deserted and semi-deserted areas of Pakistan belonging to the family chenopodiaceae. *Salsola* spp. is important due to its medicinal value like *S. monoica* contains triterpenoid and other sterols which have ability to heal wound (Ravikumar *et al.*, 2010). Leaves extract of *Salsola imbricata* contains alkaloids which is used to cure ocular diseases, also used as a diuretic and against curing wounds (Rashid *et al.*, 2000; Al-Saleh *et al.*, 1993). Another halophytic plant *Atriplex* naturally tolerate high salinity soil condition, drought and dry climate, distributed throughout the world in arid and semi-arid area. Belonging to the chenopodiaceae family *Atriplex* contains important chemical compounds and high amount of protein which make it more important in the field of medicine. Another distinguishing property of *Atriplex* is that it absorbs salty water and store salts in salt exude glands at the surface of leaf which helps to improve vegetation in deserted soil.

Root rot fungi namely *Macrophomina. phaseolina* causing charcoal rot in arid region, widely distributed throughout the world (Hoes, 1985). *M. phaseolina* is soil inhabiting fungi cause rotting of root leads to dry root rot wilting with dark colored spots and stalk rot of many species of plant (Ullah *et al.*, 2019). Another destroying root rot fungi is *Rhizoctonia solanii* that damage cultivated and wild crop by producing early and late emerging infection on root and stem of a plant (Khouray and Alcorn, 1973). Sturrock *et al.* (2015) revealed that damping off of wheat and oil seed rape is caused by *R. solani*, which penetrates through root hair and damages taproot system causing maceration of tissues. *Fusarium oxysporum* is best defined as widely distributed among all types of soil considered as well correspondence in soil borne population (Burgess *et al.*, 1974). It is root rot fungi penetrate its hyphae into

root and intrude into vascular system where it causes blockage or sometimes produce root rot without penetrating into vascular system (Olivain and Alabouvette, 1997; Ortiz *et al*., 2014).

Present research would be carried out to study the fungicidal potential of halophytic plants in the management of root infecting pathogen and on growth of plant.

MATERIALS AND METHODS

Plant Collection

Healthy leaves and stems of *S. imbricata, A. stocksii* and *S. fruticosa* were collected from Karachi University campus. Leaves and stem were dried in shade and powdered with the help of electrical grinder and placed in glass jars in order to keep the material dried.

Preparation of extract

Ten g of leaves and stem of *S. imbricata, A. stocksii* and *S. fruticosa* were soaked in 90mL water separately and kept it for overnight. The prepared extracts were used as stock solution with 100 w/v concentration. Supernatant was filtered by using Whatman filter paper No.1 into 250mL glass beaker. The filtrate was diluted again with sterilized distilled water to prepare 75 and 50% w/v concentrations. Electrical conductivity (EC) of aqueous extract of each plant was recorded following the method of Sparks (1999).

Paper disc and agar well diffusion methods:

Paper disc and agar well diffusion methods were used to detect antimicrobial activity against *M. phaseolina, F. oxysporum* and *R. solanii* with aqueous extracts of *S. imbricata, A. stocksii* and *S. fruticosa* stems and leaves with 100, 75 and 50% w/v concentrations, respectively. In the paper disc method sterilized filter paper disc of 6mm in diameter were soaked in 100, 75 and 50% w/v concentrations of stems and leaves aqueous extracts, respectively. Disc of three different concentrations were placed at three different sides of petri plate while fourth disc were soaked in sterilized distilled water and placed in fourth side of petri plate served as control (Nair *et al*., 2005). The disc of each root rot fungi *M. phaseolina, F. oxysporum* and *R. solanii* with diameter of 5mm were placed in the center of each petri plate. Similarly in agar well diffusion method wells of 5mm in diameter and 2.5mm in depth were made with the help of sterilized borer on the surface of petri plates containing agar medium, wells were filled with 50µL of 100, 75 and 50% w/v concentrations of stems and leaves aqueous extracts of *S. imbricata, A. stocksii* and *S. fruticosa*. Fourth well were filled with sterilized distilled water served as control. Disc of root rot fungi were placed in the center of each petri plate separately. Three replicates of each root infecting fungi were prepared and incubated for 5 to 6 days at room temperature (27-33^{o}C). After incubation time period zone of inhibition were measured in millimeters (Ghazala *et al*., 2003).

RESULTS

Three halophytic plants were used for their antifungal activity using paper disc and agar well diffusion methods. Electrical conductivity (EC) of aqueous extract showed that maximum EC was recorded with leaves extract of *S. fruticosa* followed by *A. stocksii* leaves extract. Stem extract of all the three plants showed less EC compared to leaves. However less EC result was obtained from 50 % aqueous extract of *A. stocksii* (Table 1).

Table 1. Electrical conductivity (EC) in mS/cm of three halophytic plants.

Halophytic plants	**Parts**	**Concentrations (%)**		
		100	**75**	**50**
Suaeda fruticosa	Leaves	0.634±0.0027	0.522±0.0045	0.374±0.0088
	Stem	0.275±0.0006	0.247±0.0006	0.224±0.0026
Salsola imbricata	Leaves	0.357±0.000	0.307±0.0005	0.23±0.0005
	Stem	0.292±0.0023	0.157±0.0005	0.111±0.0008
Atriplex stocksii	Leaves	0.532±0.015	0.409±0.003	0.288±0.0015
	Stem	0.216±0.0048	0.216±0.0086	0.089±0.002
distilled water		0.02±0.00	0.02±0.00	0.02±0.00

Table 2. *In vitro* inhibition of root rot fungi by aqueous extracts of *Atriplex stocksii*, *Salsola imbricata* and *Suaeda fruticosa* parts with different concentrations by using paper disc methods.

Plants	Part	*Fusarium oxysporum*				*Macrophomina phaseolina*				*Rhizoctonia solani*			
Extract %		0 (Control)	50	75	100	0 (Control)	50	75	100	0 (Control)	50	75	100
Atriplex stocksii	Leaves	3.33±1.45	2.33 ± 0.88	0.33 ± 0.33	4.66 ± 2.33	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Stem	0.33 ± 0.33	4.0 ± 3.05	1.66 ± 1.66	8.66 ± 1.33	0 ± 0	0 ± 0	0 ± 0	0.33 ± 0.33	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Salsola imbricata	Leaves	0 ± 0	14 ± 3.05	11 ± 4.51	19 ± 2.64	3 ± 3.00	3.66 ± 1.85	5.33 ± 2.18	5.66 ± 3.48	0 ± 0	3.6 ± 0.88	4.00 ± 1.52	8.33 ± 0.66
	Stem	0.33 ± 0.33	1.33 ± 1.33	5.00 ± 8.66	4.66 ± 3.28	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Suada fructicosa	Leaves	0 ± 0	9.66 ± 1.66	7.66 ± 1.454	7.0 ± 2.08	0 ± 0	3.33 ± 0.88	2.66 ± 1.45	1.66 ± 1.20	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Stem	0 ± 0	8.66 ± 4.63	8.00 ± 4.04	1 ± 0.00	1.66 ± 1.66	4.66 ± 1.66	1.66 ± 0.66	1.0 ± 1.00	0 ± 0	0 ± 0	0 ± 0	0.33 ± 0.33

Table 3. *In vitro* inhibition of root rot fungi by aqueous extracts of *Atriplex stocksii*, *Salsola imbricata* and *Suaeda fruticosa* parts with different concentrations by using well diffusion methods

Plants	Part	*Fusarium oxysporum*				*Macrophomina phaseolina*				*Rhizoctonia solani*			
Extract %		0 (Control)	50	75	100	0 (Control)	50	75	100	0 (Control)	50	75	100
Atriplex stocksii	Leaves	7.33 ± 3.48	4.33 ± 1.66	1.66 ± 1.66	6.00 ± 1.73	0 ± 0	1.00±1.00	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Stem	0 ± 0	0 ± 0	3.0 ± 2.08	0.33 ± 0.33	0 ± 0	2 ± 2.00	2.66 ± 2.66	1 ± 1.00	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Salsola imbricata	Leaves	5 ± 5.00	6.33 ± 0.33	4.33 ± 1.66	13 ± 1.00	2.66 ± 2.66	2.66 ± 2.18	6.66 ± 0.88	6.0 ± 0.00	0 ± 0	2.00 ± 2.00	1.66 ± 1.66	2.0 ± 2.00
	Stem	0 ± 0	0 ± 0	1.66 ± 1.66	1.0 ± 1.00	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Suada fructicosa	Leaves	0 ± 0	4.33 ± 2.33	4.66 ± 1.20	10 ± 2.31	4.33 ± 1.76	5.0 ± 0.00	5.66 ± 0.33	8.33 ± 0.33	0.6 ± 0.66	3.6 ± 0.33	3.3 ± 1.33	5.00 ± 4.00
	Stem	9.33 ± 5.24	7.00 ± 3.46	5.0 ± 4.00	5.33 ± 2.188	4.33 ± 1.66	2.33 ± 0.66	2.33 ± 1.85	3.0 ± 1.00	0 ± 0	0 ± 0	0 ± 0	0.66 ± 066

±S.E = Standard Error

Paper disc method showed that *F. oxysporum* was inhibited by leaves extract of *S. imbricata* at 100% w/v concentration followed by leaves extract at 50% w/v. However, stem part of all halophytic plants at all concentrations gave restricted growth of *F. oxysporum* (Table 1). Similarly leaves extract of *S. imbricata* at 100 and 75% concentrations resulted in maximum zone of inhibition against *M. phaseolina* while stem part of *S. imbricata* did not produce any effect on *M. phaseolina.* It is interesting to note that all the three halophytic plants did not affect the growth of *R. solani* and showed full growth of fungi at all concentrations. However, *R. solani* was reduced by using leaves extract of *S. imbricata* at 100% followed by 75 % of leaves extract (Table 2).

In case of well diffusion method, *S. imbicata* leaves part at 100 % w/v exhibited maximum zone of inhibition against *F. oxysporum.* The second highest inhibition zone was recorded by *S. fructcosa* leaves part at 100 % w/v. However, *M. phaseolina* showed reduced growth by using aqueous extract of *S. fruticosa* leaves part at 100 % w/v and 50 % w/v *S. fruticosa* gave pronounced zone of inhibition against *R. solani*. On the other hand, no effect was recorded by stem part of all the three halophytic plants on *R. solani* infection (Table 3).

In comparison to stem aqueous extract, leaves extract showed greater activity against all three root pathogenic fungi by both methods. Maximum zone of inhibition were recorded by leaves extract of *S. imbricata* and *S. fruticosa* by using both methods. Excellent growth inhibition of *M. phaseolina* and *F. oxysporum* were observed by both halophytic plants whereas *R. solanii* produced no sufficient result in both plants extracts.

DISCUSSION

Recent study revealed that leaves extracts of *S.imbricata* and *S.fruticosa* showed pronounced inhibition in root rot infection of *M. phaseolina,* and *F.oxysporum* whereas excellent zone of inhibition were observed on 100 and 50% w/v concentrations. Bibi *et al.* (2018) reported that *S.imbricata* is an important medicinal plant due to the production of antifungal secondary metabolites that rupture fungal cell wall leads to cell lysis and death. *S.imbricata* is helpful in treatment of various disorders in human being (Aslam and Janbaz, 2017). Oueslati *et al.* (2012) reported that *S. fruticosa* shoot extract contains valuable phenolic and abundant antioxidant capacity. *S. frcticosa* is an interesting source of antioxidant and contains highest potency of phenols (Bechlaghem *et al.*, 2019).

Suaeda vermiculata contains high amount of alkaloids, flavonoids, phenolic compounds and its antioxidant capacity makes it antimicrobial against many pathogenic fungi (Al-Tohamy *et al*., 2018). Leaves extracts of *Salsola vermiculata* and *Suaeda vermiculata* contains compounds of fats that cause lysis of fungal cell wall which make them antifungal and medicinally important plants. Flavonoid, phenolic compound in addition to thick tannin were reported in photosynthetic organ of *Salsola kali*. Leaves extract of *S. kali* showed significant capacity of antioxidant and antimicrobial activities (Boulaab *et al.,* 2019). *S. fruticosa* showed effectiveness against low blood sugar and high cholesterol problems in human being, due to this activity it is also used in medical treatment. (Bennani-Kabchi *et al.*, 1999; Benwahhoud *et al.*, 2001). Species of *Salsola* contains high cadmium hyper-accumulation which is used for plant remedies and it is also used by patients of low blood pressure because of its medicinal property. (De La Rosa *et al.*, 2004). Root rot infection of many leguminous and non leguminous plants are inhibited by using *Acacia nilotica* and *Sapindus mukorossi* leaves which helps to improve growth of leguminous and non leguminous plants (Rafi and Dawar, 2015). Root rot infection caused by *M. phaseolina, R. solanii* and *F. oxysporum* can also be reduced by leaves, stem, root and fruit of *eucalyptus* spp. (Dawar *et al*. 2007). Leaf, stem and pneumatophore of *Avicennia marina* is also used against root rot fungi (Tariq *et al*., 2006).

Phenolic compounds, flavonoids, alkaloids, tannins, saponins, glycoalkaloids, sesquiterpenes, terpenes, lactones, phenol and terpenoids are present in different plant parts make them medically very important (Tiwari and Singh, 2004). Major focus of scientists is maximum use of natural products in comparison to synthetic chemicals against plant pathogens (Kiran *et al*. 2006).

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