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Physiological and antimicrobial studies on some selected plants grown under stress conditions

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Abstract

In the current study, some of the physiological adaptive responses were evaluated in five true xerophytic plants, for their medicinal importance; *Ephedra alata* Dence., *Lycium shawii* Roem., *Nitraria retusa* (Forssk.), *Ochradinus baccatus* Del. and *Tamarix nilotica* (Ehrenb.) growing naturally (wild plants) at Wadi Hof represented by site 1(drought stress) and Red sea coast represented by site 2 (salinity stress). The selected plants were collected during spring and autumn seasons of the studied year (2019). The results revealed that the enzymatic activities of catalase, peroxidase, polyphenol oxidase and superoxide dismutase for the studied plants were higher in spring season compared with the autumn one and the highest activity of these enzymes was found in *O. baccatus* inhabiting site (1). Results showed that, the highest values of total phenols concentration of *E.alata* and *T.nilotica* were obtained during autumn season at site (1), while the lowest value of total phenols concentration of *O.baccatus* was achieved in the site (2) during spring season. The studied plants grown at site (2) possessed a highly significant increment in total flavonoids concentration during autumn season and the highest value was recorded in *T.nilotica* .On the contrary, the lowest value of total flavonoids was recorded in *O.baccatus* located at site (2) during spring season. DNA concentration in most studied plants was increased during spring season and the highest value was recorded in *L.Shawii* inhibiting site (1). The highest value of RNA was observed in *N.retusa* grown at site (2) during autumn season. Data showed that the methanol extracts of *T. nilotica,* grown at two studied sites during the selected seasons, had the highest antimicrobial activity against all tested bacterial and fungal strains compared with other tested plants.

Introduction

Drought is one of the most important abiotic stress factor which affects almost every aspects of plant growth. The drought tolerance of plants can be characterized by growth response, changes in water relations of tissue exposed to low water potential, accumulation of ions in tissues and stomatal conductance of leaves **(Krannich *et al.*,2015**).Desert plants generally follow two main strategies *i.e.*, they tolerate the drought through phenological and physiological adjustments referred to as tolerance or avoidance mechanisms contribute to the ability of a plant to survive under drought but it also depends on the frequency and sensitivity of the drought periods (**Youssef *et al.*, 2003).Vishnu *et al*.(2021)** showed that abiotic stress in plants is a crucial issue worldwide,especially,salinity and drought. These stresses may raise a lot of issues such as the generation of reactive oxygen species, membrane damage, loss of photosynthetic efficiency, *etc.* that could alter crop growth and developments by affecting biochemical, physiological and molecular processes causing a significant loss in productivity. Saline and salt affected lands occupy vast areas in the arid and semi-arid zones and the problems of salinity are aggravated by the inadequacy of rainfall to remove the salts. However, salinity is not entirely limited to such zones and halophytes are widely distributed in various climatic regions **(El-Khawaga, 2007).** Some plants are naturally adapted to afford harsh stress conditions as halophytes (Salt – tolerant plants), xerophytes (plants of arid lands), poor – nutrient plants, *etc.* **(Zhu, 2001)**. Such plants can survive and carry out their life stages under respective unfavorable environmental conditions **(Sanders, 2000)**. With respect to physiological and biochemical studies, stress responses in wild tolerant plants were mainly attracted to ion homeostasis and osmotic balance, synthesis of protective metabolites, antioxidant enzyme system as well as non-enzymatic antioxidants, *etc.* **(Zhu, 2000).** Drought or salinity causes oxidative stress that increase reactive oxygen species (ROS) leading to induced strong anti-oxidative defense systems and abundant natural resources include enzymatic or non-enzymatic antioxidants. Conservation and sustainable utilization of drought and/or salinity tolerant plants should be on a scientific basis **(Shawky, 2010)**. Catalase and Peroxidase enzymes have the ability to

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convert H_2O_2 directly into $H_2O + O_2$ which being important for ROS detoxification under stress conditions **(Jaleel *et al.*, 2007)**. Catalase and Peroxidase are one of the plants stress indicators because their level of synthesis increases after stress stimulation **(Jansen, 2001). Sudhaker *et al.* (2001)** verified the enhancement in the Pox activity as a result of soil salinity in *Morus alba*. Xerophytes and halophytes naturally growing in the Egyptian deserts may play an important role for the welfare of the Egyptians. Some of these plants are used for traditional medicine as they contain a wide range of substances that can treat chronic as well as communicable diseases. Additionally, medicinal plants represent a rich source of antimicrobial agents **(Harlev *et al.*, 2012).** Phenolic compounds recorded in some plants (e.g. phenolic acids and flavonoids) have the main antioxidant properties, many of these compounds show diverse of antimicrobial activities **(Gamal, 2015)** and bioactivities, such as antiageing, antiallergic, antimitotic, antimutagenic and antiviral effects **(Liyana and Shahidi, 2007).**

The aim of the present work was to study some physiological adaptive responses of five true xerophytic and halophytic plants which grow naturally at Wadi Hof and Red sea coast, to cope the adverse effects of their habitat conditions. Seasonal changes in some antioxidant enzymes, some secondary metabolites as well as nucleic acids were determined in plants grown in two studied sites during the tested seasons. Also, antimicrobial activity against some strains of bacteria and fungi was determined by using the extracts of the selected plants.

Materials and Methods

The present study was done to identify the adaptive responses of five true xerophytic and halophytic plants under a biotic stress conditions during spring and autumn periods (2019) along Wadi Hof represented by site (1) and Red sea coast represented by site (2). The selected plants comprised: *Ephedra alata* Dence., *Lycium shawii* Roem., *Nitraria retusa* Forssk., *Ochradinus baccatus* Del. and *Tamarix nilotica* Ehrenb. The samples of plants were taken during spring season (April) and autumn season (November) of the studied year. The collected fresh plant materials (leaves) were preserved and kept frozen for estimation of antioxidant enzymes and determination of nucleic acids (DNA, RNA). Oven dried plant materials (shoot system) were used for total phenols and flavonoids determinations. Air dried plant materials were put into practice for antimicrobial activity.

1-Estimation of antioxidant enzymes:

-Enzyme extraction and assay:

The fresh leaves were extracted and homogenized in 0.05 M cold phosphate buffer according to **Ma *et al.* (2012)** and centrifuged at 38000 g for 10 min. according to **Saroop *et al.* (2002).** The clear supernatant was taken as the enzyme source.

A- Assay of catalase (CAT) activity: The estimation of catalase activity was carried out by the method described by **Kar and Mishra (1967).**

B- Assay of polyphenol oxidase (PPO) activity: PPO activity was estimated according to **Beyer and Fridovich (1987).**

C- Assay of peroxidase (POX)activity: Pox was determined according to **Upadhyaya *et al.* (1985)**.

D-Assay of superoxide dismutase (SOD): SOD activity was measured by the nitro blue tetrazolium (NBT) reduction method **(Beyer and Fridovich, 1987).**

The enzymes activities were expressed as the changes in the optical density (µg/gFresh wt.).

2 **-Determination of total phenols:**

Total phenolic compounds of dry plant powder were determined colorimetrically according to the method reported by **Kil *et al.* (2009).**

3- Determination of total flavonoids:

Total concentration of flavonoids in dry plant powder was determined according to a colorimetric method described by **Adom and Liu (2002)**.

4- Nucleic acids:

Nucleic acids were estimated according to **Guinn (1966).** The results were calculated on fresh weight basis (mg/g) fresh wt.

5-Determination of Antimicrobial Activity:

Plants under investigation were air dried and ground to obtain coarse powder using a blender. The powdered plant materials were successively extracted with two solvents (ethyl acetate and methanol) according to the method of **Mishra *et al.* (2010)**. The effect of studied plant extracts on the growth of some microorganisms was investigated. Four microorganisms were used in this study as test organisms comprising clinical isolates of two bacteria, *Staphylococcus aureus* (as Gram-positive bacteria), *Escherichia coli* (as Gram negative bacteria) and two fungi (*Aspergillus niger* and *Candida albicans*).

Antibacterial activity was investigated according to **Abdel-Aziz *et al.* (2015).** Neomycin (100 UG/100Ul) was used as antibacterial standard drug. The antifungal activity was done by agar well diffusion method **(Cappuccino and Sherman, 2008).** Cyclohexamide (100 Ug/100 Ul) was used as antifungal standard drug. The inhibition zone (mm) was measured.

Data analysis:

The obtained data represented the mean values of three replications. Statistical design was made using SPSSV18 (SPSS Inc., Chicago, Il,USA).Two and three ways analysis of variance (ANOVA)was used to compare the sites, seasons and species at $P \leq 0.05$ according to **Gomez and Gomez(1984).**

Results and Discussion:

1-Changes in the activities *of* antioxidant enzymes:

Plant cells are protected by a complex antioxidant system comprised of non-enzymatic as well as enzymatic antioxidants, such as catalase (CAT), polyphenol oxidase (PPO), peroxidase (POX) and superoxide dismutase (SOD) **(Boguszewska *et al.*, 2010).** Our results in table (1) display seasonal changes in some antioxidant enzymes. The results showed that the highest significant increase in the activity of catalase, polyphenol oxidase, peroxidase and superoxide dismutase appeared during spring season compared with the autumn season in most studied plants at the two selected sites. The highest value of catalase, polyphenol oxidase, peroxidase and superoxide dismutase activity were recorded in *O. baccatus* in site 1 during spring season. On the other hand, the lowest value of catalase and peroxidase activity was found in *N. retusa* during autumn season at site 1. The minimum value of superoxide dismutase was recorded in *N. retusa* grown at site (2) during autumn season. On the contrary, **El-Khawaga (2011)** indicated that the enzymatic activities of catalase, peroxidase and polyphenol oxidase for *Launaea spinosa* (forssk.) and *Leptadenia pyrotechnica* (forssk.) were higher in dry than in wet season under xeric condition of Wadi Hagul in Egypt. Also, **Abd El-Kawy (2015)** found that superoxide dismutase has higher activity compared with other enzymes followed by poly phenol oxidase in leaves and roots of *Derverra tortuosa* and *Asphodelus aestivus* species (plants growing naturally under drought and salinity stresses in the North western and Eastern coats of Egypt during dry season.

In this concept, **Omolbanin *et al.* (2016)** have demonstrated a significant increase in total SOD and Pox activities in the leaves of *Hyssopus officinalis* under salinity stress. Similarly, **Yazici *et al.* (2007)** examined the SOD role in *Portulaca oleracea* and stated that under salinity and drought stress the synthesis of SOD was increased. Superoxide dismutase is the first step in reactive oxygen species scavenging system being responsible for scavenging toxic superoxide free radicals ($O_{2^{\cdot}}^{-}$) in different cell organelles generated under oxidative stress and achieved tolerance **(Chen *et al.*, 2010).**

Polyphenol oxidase is a copper containing enzyme which catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-dihydroxyphenols to o-quinones utilizing molecular oxygen. These quinines covalently modify and crosslink to a variety of cellular constituents **(Partigton and Bolwell, 1996).**

Table1. Seasonal changes in some antioxidant enzymes (enzymatic activity (µg/g fresh wt.) of different plants at the studied sites.

Selected plants	Enzymatic activity (µg/g f.wt.)							
	Seasons	Catalase		Polyphenol oxidase		Peroxidase		Superoxide dismutase	
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
E.alata	Spring	0.14±0.010	0.17±0.015	0.31±0.006	0.47±0.020	0.28±0.010	0.35±0.026	1.07±0.044	1.28±0.030
	Autumn	0.05±0.003	0.074±0.006	0.34±0.021	0.58±0.025	0.087±0.005	0.084±0.004	0.23±0.015	0.42±0.020
L.shawii	Spring	0.17±0.017	0.58±0.052	0.38±0.015	1.11±0.040	0.34±0.032	1.16±0.107	1.24±0.032	2.14±0.156
	Autumn	0.032±0.001	0.064±0.001	0.23±0.010	0.49±0.006	0.058±0.003	0.070±0.001	0.17±0.010	0.34±0.006
N.retusa	Spring	0.13±0.006	0.17±0.010	0.25±0.010	0.44±0.006	0.28±0.010	0.34±0.021	1.13±0.044	1.20±0.097
	Autumn	0.027±0.001	0.082±0.001	0.20±0.10	0.63±0.006	0.050±0.002	0.090±0.001	0.14±0.010	0.45±0.010
O.baccatus	Spring	1.05±0.041	0.96±0.032	1.83±0.17	0.32±0.015	2.12±0.275	0.19±0.015	2.28±0.064	0.92±0.047
	Autumn	0.062±0.001	0.077±0.004	0.44±0.006	0.59±0.029	0.11±0.001	0.085±0.004	0.31±0.006	0.42±0.021
T.nilotica	Spring	0.059±0.010	0.47±.015	0.17±0.021	0.19±0.015	0.11±0.021	0.95±0.030	0.99±0.036	1.37±0.036
	Autumn	0.037±0.002	0.095±0.013	0.26±0.015	0.73±0.098	0.067±0.004	0.014±0.10	0.18±0.010	0.52±0.072
Plants		**	**	**	**	**	**	**	**
Seasons		**	**	**	**	**	**	**	**
Plants×Seasons		**	**	**	**	**	**	**	**
Plants×Site		**	**	**	**	**	**	**	**
Plants×Seasons×Site		**	**	**	**	**	**	**	**

****=significant at P≤0.05**

Site1: Desert habitat of Wadi Hof. Site2: Coastal habitat of Red Sea.

2- Changes in Non-enzymatic antioxidants (Phenolic and flavonoid compounds):

a- Phenolic compounds

Phenolic compounds are a diverse group of plant secondary metabolites. They are produced in the shikimic acid of plants and pentose phosphate through phenyl propanoid metabolism **(Randhri *et al.*, 2004).** The concentration and rate of metabolism of phenyl propanoids is enhanced in plants under stress conditions **(Velderrain Rodriguez *et al.*, 2014).** Data in table (2) indicated that there is a considerable change in total phenols of the studied plants. The highest values of total phenols concentration (3.98, 3.42 mg galic acid /g dry wt.) of *E. alata* and *T. nilotica* were obtained during autumn season, respectively at site 1. Moreover, the best values of total phenols concentration of *L. shawii* and *T.nilotica* (2.26, 2.25 mg galic acid /g dry wt.) grown at site 2 were recorded during the same season. On the other

hand, the lowest value of total phenols concentration (0.12 mg galic acid /g dry wt.) of *O. baccatus* was recorded in the second site during spring season. Also, the lowest value of total phenols concentration (0.72 mg galic acid /g dry wt.) of *N. retusa* was observed in the first site during the autumn season compared with the other season **(Table 2).** The results agreed with those obtained by **Abass (2019)** who stated that total phenols increased significantly during autumn season in *Echinops spinosus* and *Fagonia mollis* grown at Wadi Hagual. The results were also in consistency with the study of **Julkunen *et al.* (2015)** who reported that phenols accumulation is stimulated to cope with the increase in ROS under environmental stresses.

In this connection the accumulation of total phenols could be attributed to the lowest polyphenol oxidase activity and/or to the increase in phenol biosynthesis (**Parr and Bolwell, 2000).** Additionally, **Korkina (2007)** stated that the accumulation of phenolic compounds varies strongly with the growth state development and responses to environmental stresses and is a result of balance between biosynthesis and further catabolism. Phenols act as a substrate for many antioxidant enzymes, so it mitigates the stress injuries **(Lewis and Yamamoto, 1990**). In this regard, phenols protect cells from potential oxidative damage and increase the stability of cell membranes **(Burguieres *et al.*, 2006).**

b- Total flavonoids concentration:

Flavonoids play an important role in the interaction between the plant and its environments as well as its biological functions in plant protection against diverse stresses **(Pourcel *et al.*, 2007).**Flavonoid biosynthesis was predominantly activated under severe stress conditions **(Fini *et al.*, 2011).**In the present study, the selected plants grown at site (2) revealed a significant increase in total flavonoids concentration during autumn season and the highest value of flavonoids concentration was recorded in *T. nilotica* (1.22 mg QE /g dry wt.) .The results also showed that value of total flavonoids concentration was high in *L. shawii* and *N. retusa* located in the second site during the spring and autumn seasons. While the lowest value of total flavonoids (0.13 mg QE /g dry wt.) was detected in *O. baccatus* located in site (2) during spring season **(Table 3).** In this regard, **Nagy (2018**) found that the total flavonoids concentration was markedly increased in the studied *Zygophyllum* species during winter and spring seasons. Similar results were obtained by **Abass (2019**) who reported that a significant increase in total flavonoids concentration was recorded in *Echinops spinosus* and *Fagonia mollis* during autumn season. **Agate *et al.* (2009)** reported that different flavonoid compounds were multiplied in the leaves of *Ligustrum vulgare* to protect the plant tissues from oxidative damage that was stimulated by high intensity of sunlight. Additionally**, Hernandez *et al.* (2004**) evaluated the effect of drought on flavonoids in *Cistus clusii* leaves, they found that the flavonoids concentration was increased continuously during drought and reach its maximum values after several days (30) of stress. Flavonoids have a role in the plant cell since it is considered as anon-enzymatic antioxidant compound to protect the cells from the harmful effect of reactive oxygen species. It has been reported that the interaction between flavonoids and phenols with free radicals gives the free radicals chemical stability than the original radicals **(El-shora *et al.*, 2016).**

Table 2. Seasonal changes in total phenols (mg galic acid/g dry wt.) and flavonoids concentration (mg / quercetin g dry wt.) of different plants at the studied sites.

Contents		Phenols concentration (mg /g dry wt.)		Flavonoids concentration (mg /g dry wt.)	
Selected plants	Seasons	Site1	Site2	Site1	Site2
E. alata	Spring	0.96±0.005	0.13±0.007	0.48±0.015	0.71±0.010
	Autumn	3.98±0.007	0.83±0.006	0.20±0.030	0.73±0.026
L. shawii	Spring	1.92±0.004	1.94±0.020	0.83±0.015	0.96±0.025
	Autumn	3.33±0.006	2.26±0.059	0.12±0.021	0.15±0.003
N. retusa	Spring	1.34±0.008	1.42±0.032	0.18±0.015	0.37±0.015
	Autumn	0.72±0.004	1.37±0.007	0.35±0.026	0.94±0.025
O. baccatus	Spring	0.42±0.003	0.12±0.002	0.26±0.31	0.13±0.015
	Autumn	1.89±0.016	1.46±0.015	0.42±0.021	0.62±0.025
T. nilotica	Spring	1.61±0.003	1.93±0.011	0.21±0.040	0.86±0.021
	Autumn	3.42±0.006	2.25±0.060	0.61±0.021	1.22±0.031`
Plants		**	**	**	**
Plants × Seasons		**	**	**	**
Plants × Site		**	**	**	**
Plants× Seasons × Site		**	**	**	**

****=significant at P≤0.05 NS = Non significant at P≤0.05**

Site1: Desert habitat of Wadi Hof. Site2: Coastal habitat of Red Sea.

2- Changes in nucleic acid concentration (DNA and RNA)

There was a significant increase in DNA concentration in most studied plants during the spring season compared with autumn one. The highest levels of DNA concentration were detected in each *L. Shawii* inhabiting in site (1) and *E. alata* inhabiting site (2) during spring season. On the other hand, the lowest concentration of DNA was observed in *N. retusa* grown at site (1) and site (2) during autumn and spring seasons, respectively. The highest concentration of RNA was detected in *N. retusa* inhabiting site (2) and *E. alata* inhabiting site (1) during autumn season. On the contrary, the lowest concentration of RNA was observed in *L. shawii* and *N. retusa* during spring season at site (1) and site (2), respectively **(Table 3).** In this respect, **Elabsy (2006)** in her study on *N. retusa* and *Arthrocenumun macrostachym*, found that, DNA, RNA and their ratio increased during wet season. The higher levels of DNA during the wet season can be due to active cell division **(El-Shourbagy *et al.*, 1980). Jaleel *et al.* (2008**) found that drought stress lowered the DNA and RNA concentration to a large extent in the *Catharanthus roseus* plants. This reduction may be due to increased activities of DNAse and RNase **(Tewari and Singh, 1991).** Also, **El-Khawaga (2011)** reported that, there was a significant increase in each of DNA and RNA concentration during the wet season compared with the dry one in *Launaea spinosa* and *Leptadenia pyrotechnica*.

Table 3. Seasonal changes in nucleic acid concentration (DNA and RNA) (mg/g fresh wt.) of different plants at the studied sites.

Selected plants	Seasons	DNA (mg/g f. wt.)		RNA (mg/g f. wt.)	
		Site1	Site2	Site1	Site2
E. alata	Spring	0.770±0.138	0.809±0.184	4.11±1.024	2.80±0.622
	Autumn	0.734±0.081	0.508±0.111	5.53±1.428	2.75±0.508
L. shawii	Spring	1.063±0.040	0.300±0.053	2.39±0.511	4.20±1.077
	Autumn	0.276±0.033	0.381±0.051	4.33±1.008	3.84±0.729
N. retusa	Spring	0.318±0.074	0.209±0.037	4.55±0.871	2.65±0.567
	Autumn	0.230±0.019	0.367±0.026	4.26±0.938	8.55±1.664
O. baccatus	Spring	0.266±0.045	0.432±0.079	3.50±0.817	3.61±0.855
	Autumn	0.485±0.075	0.440±0.072	5.44±1.294	3.84±0.729
T. nilotica	Spring	0.737±0.116	0.701±0.012	5.20±1.378	6.67±1.180
	Autumn	0.249±0.002	0.323±0.012	3.66±0.728	7.51±1.293
Plants		**	**	**	**
Plants× Seasons		**	**	NS	NS
Plants × Site		**	**	**	**
Plants× Seasons × Site		**	**	**	**

****=significant at P≤0.05 NS = Non significant at P ≤0.05**

Site1: Desert habitat of Wadi Hof. Site2: Coastal habitat of Red Sea

4- Changes in Antimicrobial Activity

Antimicrobial substances are substances that inhibit the growth and existence of microorganisms. These microorganisms could be pathogenic or non-pathogenic; hence, antimicrobial substances are used in the treatment of various ailments. A number of antimicrobial substances exist, and they are gotten from diverse sources, such as microbial, plant, animal and chemical sources **(Silva *et al.*, 2012).** Antimicrobial activity of methanolic and ethyl acetate extracts of the studied plants showed variable results against four microbial stains, *Staphylococcus aureus* (as Gram positive bacteria), *Escherichia coli* (as Gram negative bacteria) and the fungal strains include *Aspergillus niger* and *Candida albicans*. The results of antimicrobial activity measured in term of inhibition zone diameter (mm).

a- Antibacterial effect:

Data in tables 4 and figures 1, 2, 3 and 4 indicated that there was a considerable seasonal change in antibacterial activity of the studied plants. It is clear that the methanol extract of *T. nilotica* showed the highest antibacterial activity against *S. aureus* during autumn season at site (2). Moreover, the ethyl acetate extract of the same plant exhibit antibacterial activity against *S. aureus* during spring and autumn seasons at site (1). Meanwhile, no antibacterial activity was detected by the methanol extract of *N. retusa* during spring season in both sites. Also, no antibacterial activity was detected by the ethyl acetate extract of *O. baccatus* during autumn season in both selected sites.

Additionally, the results showed that, *E. coli* was the most sensitive organism to the methanol extract of *T. nilotica* during spring and autumn seasons at two sites, as well as to the ethyl acetate extract of the same plant during the studied seasons at two sites. Similar results are in consistent with that obtained by **El-Demerdash (2016)** on *T. nilotica*, she concluded that the methanolic extract of *T. nilotica* showed the highest antimicrobial activity against all tested

microorganisms. This might be related to the high amount of the secondary metabolites especially phenols and flavonoids accumulated in *T. nilotica.* Also, **Abass (2019)** found that, crude extract and also different fractions of some wild plants in Wadi Hagul inhibit the growth of *S. aureus bacteria* with different rates.

Table 4. Zone of inhibition (mm) of ethyl acetate and methanol extract of the aerial parts of the studied plants at the two studied sites during spring and autumn seasons against bacterial activity.

Tested microorganisms		Gram + and Gram – Bacteria							
Selected solvents		Ethyl acetate extract				Methanol extract			
Selected plants	Seasons	*Staphylococcus aureus*		*Escherichia coli*		*Staphylococcus aureus*		*Escherichia coli*	
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
E. alata	Spring	8	9	NA	7	8	NA	NA	NA
	Autumn	8	NA	7	NA	7	8	7	6
L. shawii	Spring	6	9	7	8	NA	7	NA	7
	Autumn	6	7	8	NA	8	8	6	7
N. retusa	Spring	7	8	NA	8	NA	NA	6	NA
	Autumn	9	NA	8	NA	7	7	7	7
O. baccatus	Spring	7	8	6	NA	7	NA	NA	6
	Autumn	NA	NA	NA	NA	10	9	8	7
T. nilotica	Spring	11	7	16	11	7	7	14	6
	Autumn	10	10	13	9	9	17	NA	16

Site1: Desert habitat of Wadi Hof. Site2: Coastal habitat of Red sea NA:No Activity.

B-Antifungal effect:

Results in Tables (5) and figures 1, 2, 3 and 4 revealed that the ethyl acetat extracts of *E. alata* and *T. nilotica* plants had the most potent antifungal effect on the *A. niger* organism during autumn and spring seasons, respectively at the selected sites. On the other hand, the results showed that, the methanolic extracts of *E. alata* had no antifungal effect for both studied fungi at both selected sites during spring season. Additionally, the results showed that, during autumn season, the ethyl acetate and methanol extracts of *T. nilotica* exhibited high antifungal activities against *C. albicans*, this may be as a result of highly concentration of phenols and flavonoids in this plant. In this respect, **Nagy (2018)** concluded that *Z. album* extract has no antifungal activity against *A. fumigatus* during all seasons of the tested year and against C*andida albicans* during summer and autumn seasons. Meanwhile *Z. album* extract showed antifungal activity against C*andida albicans* during winter and spring seasons at Wadi Hagul site. Also, she observed that the extract of *Z. coccineum* was the most potent antifungal effect on fungal test organism.

Table 5. Zone of inhibition (mm) of ethyl acetate and methanol extract of the aerial parts of the studied plants at the two studied sites during spring and autumn seasons against fungal activity.

Tested microorganisms		Fungi							
Selected solvents		Ethyl acetate extract				Methanol extract			
Selected plants	Seasons	*Aspergillus niger*		*Candida albicans*		*Aspergillus niger*		*Candida albicans*	
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
E. alata	Spring	NA	NA	8	8	NA	NA	NA	NA
	Autumn	16	11	8	NA	7	NA	7	7
L. shawii	Spring	NA	7	7	7	7	NA	7	6
	Autumn	NA	15	7	NA	NA	NA	6	6
N. retusa	Spring	8	NA	7	7	NA	8	6	7
	Autumn	12	8	10	NA	NA	NA	7	NA
O. baccatus	Spring	NA	NA	7	7	NA	NA	7	NA
	Autumn	14	9	9	8	7	NA	10	8
T. nilotica	Spring	15	9	12	8	15	7	10	6
	Autumn	NA	NA	15	10	NA	NA	NA	13

Site1: Desert habitat of Wadi Hof. Site2: Coastal habitat of Red sea. NA:No Activity.

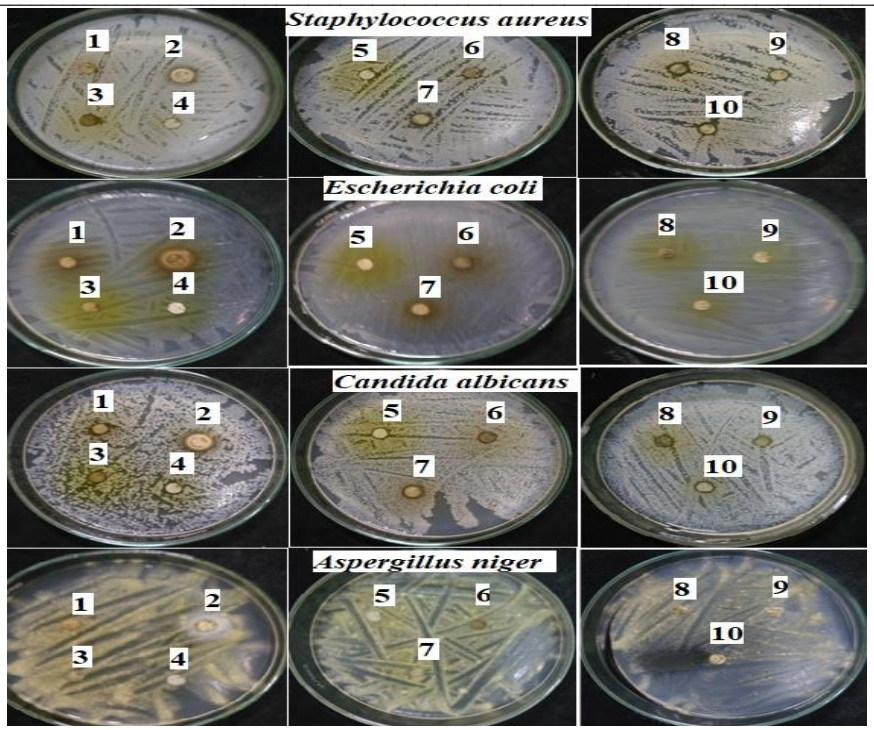

Figure (1) clear zone of ethyl acetate fractions during autumn season.
1,2,3,4,5= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 1.
6,7,8,9,10= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 2.

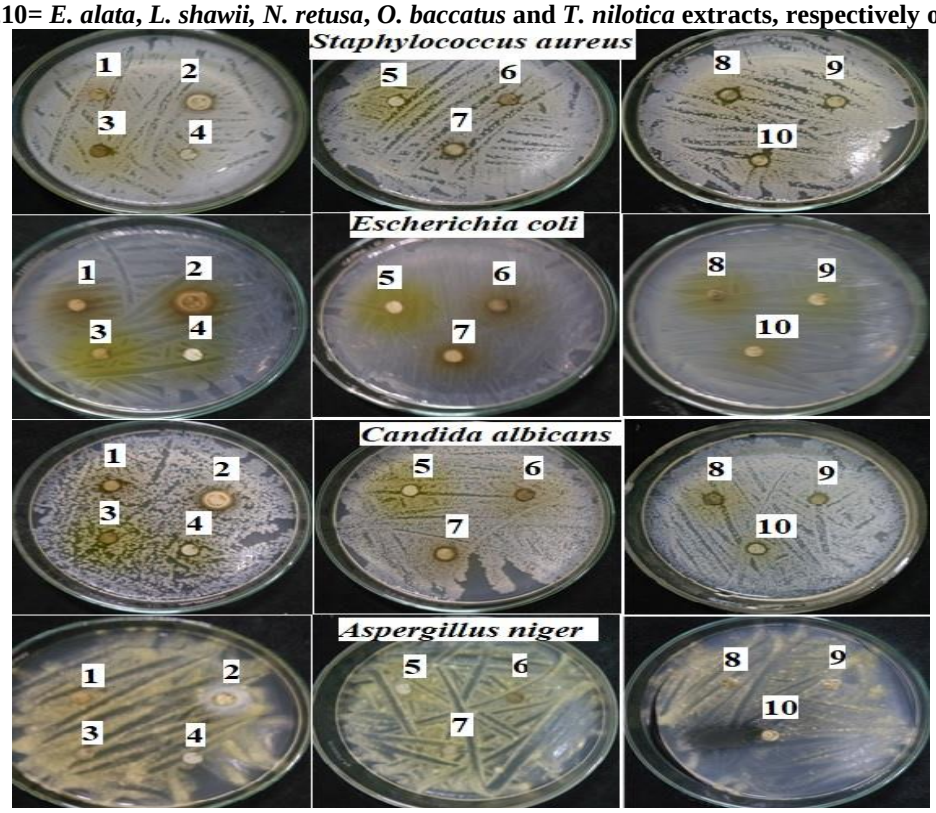

Figure (2) clear zone of methanol fractions during autumn season.
1,2,3,4,5= *E. alata, L. shawii, N. retusa, O. baccatus.* and *T. nilotica* extracts, respectively of site 1.
6,7,8,9,10= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 2.

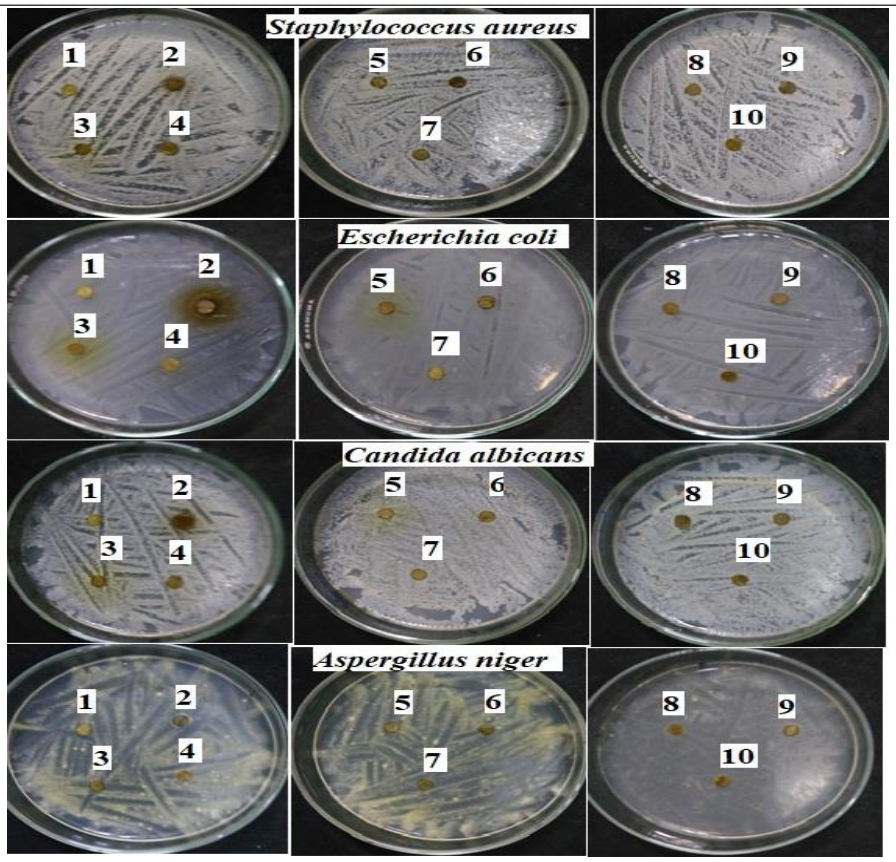

Figure (3) clear zone of ethyl acetate fractions during spring season.
1,2,3,4,5= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 1.
6,7,8,9,10= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 2.

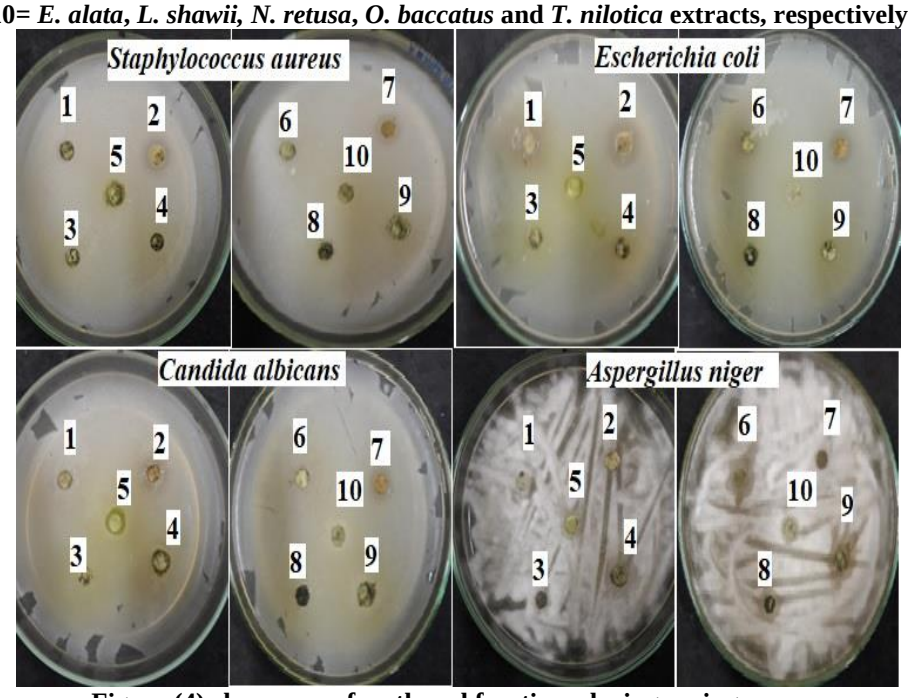

Figure (4) clear zone of methanol fractions during spring season.
1,2,3,4,5= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 1.
6,7,8,9,10= *E. alata, L. shawii, N. retusa, O. baccatus* and *T. nilotica* extracts, respectively of site 2.

Conclusion

From the outcome of the obtained results, it seems likely to conclude that the physiological responses of *Ephedra alata* Dence., *Lycium shawii* Roem., *Nitraria retusa* (Forssk.), *Ochradinus baccatus* Del. and *Tamarix nilotica* (Ehrenb.) to stress conditions (drought and salinity) were achieved through the activity of antioxidant enzymes in most studied species. The highest levels of DNA concentration were detected in each *L. Shawii* and *E. alata*. There is correlation between the increase of total phenols and flavonoids concentration with respect to antimicrobial activity of plant extracts. The highest values of total phenols concentration were observed in *E. alata* and *T. nilotica.* As well as, the highest value of flavonoids concentration was recorded in *T. nilotica.* Therefore, Methanol extracts of *T. nilotica* plant grown at two studied sites exhibited the highest antimicrobial activity against all tested bacterial and fungal strains; this may be due to the high concentration of phenols and flavonoids.

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