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Allelopathy activity of wild Atriplex species on germination and seedling growth of Portulaca oleracea L.

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Abstract: Genus Atriplex (family Chenopodiaceae) is global distribution and annuals, herbaceous perennials or shrubs, with mealy crust. The present study aimed to estimate the phytochemical constituent and to evaluate the antioxidant as well as allelopathic potential of four Atriplex species growing in the Deltaic Mediterranean coast (Atriplex halimus L., A. lindleyi Moq., A. portulacoides L. and A. semibaccata R. Br). The antioxidant activity was measured using DPPH assay while the allelopathic potential of the Atriplex species was studied on the germination of Portulaca oloraceae. The obtained results revealed that A. lindleyi was the richest in its phytochemical constituents among the other studied Atriplex species followed by A. semibaccata, A. portulacoides and A. halimus, respectively. A. lindleyi also expressed the highest antioxidant activity among the studied species (IC50 = 0.59 mg ml-1) followed by A. semibaccata (IC50 = 0.82 mg ml-1), A. portulacoides (IC50 = 1.06 mg ml-1) and A. halimus (IC50 = 1.18 mg ml-1), respectively. The phytotoxicity of the extracts of the studied Atriplex species increased significantly with increasing their concentration. At concentration of 40 mg ml-1 the germination of P. oloraceae reached maximum inhibition of 82.93 and 80.54 %, plumules inhibited by 80 and 76.80% and radicles were inhibited by 93.58 and 92.33% for A. lindleyi and A. semibaccata, respectively. In conclusion Atriplex species could be used as natural antioxidant and biocontrol of weeds.

keywords: Allelopathy, *Atriplex*, antioxidants, biocontrol of weeds, secondary metabolites

1. Introduction

Salt tolerant plants could use instead of conventional crops as food, fuel, fodder, resins and essential oils [1]. Such these plant could be produced using the large areas of coastal salt land in the developing countries [2]. The genus Atriplex belongs to family chenopodiaceae represent in Egypt by 15 species [3]. This genus comprises annuals, herbaceous perennials or shrubs with mealy crust and alternate leaves. The plant of this genus is globally distributed in Europe, North and South Africa, Western Asia and Australia. In Egypt it is distributed in Nile region in the delta and Mediterranean coastal strip, Egyptian desert and Sinai Peninsula [4.[

Allelopathy simply refers to any effect: direct or indirect, stimulatory or inhibitory, in which the bio-chemicals released into the environment from one plant impair germination, growth, survival, reproduction, and behaviour of other plants [5]. These chemicals, termed as secondary metabolites, allelochemicals, natural products or growth-inhibitors, are a major factor in regulating the structure of plant communities [6]. Previous chemical investigations on the species from the genus Atriplex revealed the presence of saponins, alkaloids, betains, proteins, amino acids, mineral salts [7], and phytoecdysteroids [8]. In particular, the flavonoids quercetin, kaempferol [9], isorhamnetin, spinacetin (6-methoxy-isorhamnetin), patuletin (6-methoxy-quercetin) and other 6-methoxyflavonols were isolated from Atriplex species [10.[

Weed control could be achieved using a wide range of, physical or chemical and biological methods. Nontraditional plants could be used as herbicides through allelopathic research to get of unwanted plants [5, 11-15]. This research aimed to determine the

phytochemical constituents, allelopathic and antioxidant potential of four Artiplex species (Atriplex halimus L., A. lindleyi Moq., A. protulacoides L. and A. semibaccata R. Br.) growing in the Deltaic Mediterranean coast, Egypt.

2. Materials and methods

1.Preparation of plant material

Shoots of four Atriplex species (Atriplex halimus L., A. lindleyi Moq., A. protulacoides L. and A. semibaccata R. Br.) were collected at vegetative stage from different sites of the Deltaic Mediterranean coast, Egypt during March 2019. The identification of the species was done according to Boulos [4]. The aerial plant parts were washed with distilled water several times and dried at room temperature. The dried samples were ground into a powder using a blender and preserved in a polyethylene bag in a refrigerator until use .

2.Phytochemical analysis

Total phenolics, flavonoids, and alkaloids were estimated using spectrophotometric techniques adapted by Harborne [16], Sadasivam and Manickam [17] and Boham and Kocipai-Abyazan [18], respectively. Tannins were determined according to Van-Buren and Robinson [19], while saponin content was estimated by the method adopted by Obadoni and Ochuko [20.[

3.Evaluation of DPPH free radical scavenging activity

Antioxidant activity was determined using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH [21]. Two ml of 0.15 mM DPPH was added to 2 ml of plant extracts in different concentrations (1000, 800, 600, 400, 200 and 100 ppm). A control was prepared by adding 2 ml of DPPH to 2 ml solvent. The mixture was kept in the dark at 37 ºC for 30 min. The absorbance was recorded at 517 nm, and the IC50 was calculated graphically. The antioxidant activity was expressed as:

%Radical scavenging activity = [1– (A sample / A control)] × 100

4.Allelopathic bioassay

4.1Weed seed source

The seeds of Portulaca oleracea were collected from the different localities of study area, sterilized by 0.3% calcium hypochlorite, rinsed by distilled water and shade dried again on the filter paper in the laboratory at room temperature for 7 days [22.[

4.2 Preparation of plant material

The plant was harvested at a vegetative stage. The plant tissues were clipped by hand 1 cm above the soil, washed with distilled water and left to dry in room temperature in shaded place for several day till complete dryness. The dried samples were ground to pass a 1 mm screen and then stored in a refrigerator.

4.3.Preparation of methanol extract

For bioassay tests, stock extract (10 % w/v) were diluted with distilled water to obtain concentrations of 5, 10, 20 and 40 mg ml-1 test extracts. All osmotic concentrations of bioassay solutions were less than 0.1 Mpa and hence not considered a factor affecting germination [23]. The solutions were filtered thought a double layers of muslin cloth followed by a Whatman No.1 filter paper, the pH values were adjusted to 7 with 1M HCl, these were kept in refrigerator at 4 ºC until further use [24.[

4.4 Germination bioassays

Two layers of Whatman No. 1 filter paper were placed in 90 mm diameter glass petri dishes. In each petri 25 seeds were placed and 10 mm of each plant extract added in a concentration of 5, 10, 20 and 40 mg.ml-1. A check treatment was assigned with distilled water and let at room temperature. Starting from the first day after experiment set on, germinated seeds were counted and removed daily. A seed with 0.5 cm of radical was considered germinated. Experiment designed was RCB (Randomized Complete Block) with three replicate and experiment repeated twice. Rate of germination was calculated by dividing the number of germinated seed each day by the number of days and summing the values. The inhibition percentage was calculating using the following equation :

Inhibition percentage = [(CG – TG /CG) ×100[

where CG; germination rate in check treatment, TG; germination rate in extract treatment .

4.5 Growth bioassay

The seeds of Portulaca oleracea were germinated on filter paper in the dark at room temperature for 2 days. Fifteen germinated seeds were transferred to petri dishes which were filled with 25 g quartz sand and 10 ml of shoot powder extract added in concentrations of 5, 10, 20 and 40 mg.ml-1. In addition, a check added to experiment without any powder treatment. Experiment designed was RCB with three replicate and experiment peated twice. The shoot and root lengths of seedlings were measured on 14 day after treatment (DAT) and growth inhibition at shoot and root lengths were calculated using the following equation:

Growth inhibition = [(LC – LT)/LC) ×100[

where, growth inhibition in %; LT, shoot or root length of powder treated weed; LC, shoot or root length of untreated check weed .

3. Results and Discussion

1. Phytochemical constituents

Dry and saline habitat of the studied Atriplex species are considered as precursors for synthesis of various classes of secondary metabolites such as phenolics, flavonoids, alkaloids, saponins and many other compounds that possess pharmaceutical and medicinal properties [25]. The concentrations of the phytochemicals present in the studied Atriplex species are presented in Table 1.

The total phenolic compounds were 19.96, 17.28, 13.08 and 11.38 mg GAE/g DW for Atriplex lindleyi, Atriplex semibaccata, Atriplex halimus and Atriplex portulacoides methanolic extracts in descending order, respectively. While, the total flavonoids compounds were 9.34, 7.66, 6.06 and 5.48 mg quercetin equivalent/g DW for Atriplex lindleyi, Atriplex semibaccata, Atriplex portulacoides and Atriplex halimus methanolic extracts in descending order, respectively (Table 1)

The total alkaloids contents were 5.84, 4.16, 3.86 and 3.22 mg /g DW for Atriplex lindleyi, Atriplex semibaccata, Atriplex halimus and Atriplex portulacoides methanolic extracts in descending order, respectively. The total saponins content were 25.60, 25.54, 22.92 and 20.09 mg/g DW for Atriplex lindleyi, Atriplex portulacoides, Atriplex semibaccata and Atriplex halimus methanolic extracts in descending order, respectively. Atriplex lindleyi was found to be the richest among the other studied Atriplex species in the phytochemical constituents (Table 1.(

This result is supported by the study of El-Amier and Abdullah [26] on the same wild plant. These results are higher than Rumex vesicarius (Polygonaceae) belong to the same family as mentioned by Hariprasad and Ramakrishna [27]. By comparing these results to that for other plant species, the secondary metabolites of Atriplex species found to be lower than those reported in the plants growing in dry habitats like Anthemis arvensis and Artemisia campestris [28] and Senecio glaucus and Cakile maritima [26]

Table 1. The concentration of the bioactive secondary compounds (mg/g dry weight) in the selected *Atriplex* species.

Bioactive constituent	Atriplex halimus	Atriplex lindleyi	Atriplex portulacoides	Atriplex semibaccata
Saponins	20.09±1.83	25.60±2.33	25.54±2.32	22.92±2.08
Tannins	14.08±1.19	25.58±1.81	26.16±1.03	22.89±1.57
Total phenol	13.08±1.28	19.96±2.33	11.38±2.38	17.28±1.98
Alkaloids	3.86±0.35	5.84±0.53	3.22±0.29	4.16±0.38
Flavonoids	5.48±0.50	9.34±0.85	6.06±0.55	7.66±0.70

2. Antioxidant activity

The free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used for evaluation of the antioxidant scavenging activity of the methanolic extracts of the studied *Atriplex* species through measuring the concentration of the antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) as a parameter used to measure the antioxidant activity. The IC_{50} is inversely

proportional to the antioxidant power where the lower the IC_{50}, the higher the antioxidant activity [29]. Catechol was employed as standard compound in this assay. The evaluation of the antioxidant activity of the four plant extracts is presented in Table 2. By

increasing the plant extract concentration there was a corresponding continuous increase in scavenging activity. In case of *A.* lindleyi, A. semibaccata, A. portulacoides and A. halimus extracts the increase was up to 1000 µg/mL where the scavenging activity was 61.18%, 53.15%, 47.39% and 39.09, respectively.

The data represented in Table 2 indicated that the methanolic extract of *A. lindleyi* (IC_{50}=0.59 mg/mL) was higher in its free radical scavenging activity followed by *Atriplex semibaccata* (IC_{50}=0.82 mg/mL), *A. portulacoides* (IC_{50}=1.06 mg/mL) and *A. halimus* (IC_{50}=1.18 mg/mL) in descending order, respectively. All the tested extracts have considerable antioxidant scavenging activities but with values lower than that of catechol

Table 2. Percentage of DPPH radical scavenging activity and IC_{50} values of methanolic extracts of the studied *Atriplex* species.

Plant species	Concentration (µg/mL)	Scavenging activity (%)	IC_{50} (mg/m)
Atriplex halimus	1000	39.09	1.18
	800	36.21	
	600	28.25	
	400	19.95	
	200	7.16	
	100	4.08	
Atriplex lindleyi	1000	61.18	0.59
	800	53.61	
	600	50.33	
	400	42.03	
	200	27.91	
	100	13.65	
Atriplex portulacoides	1000	47.39	1.06
	800	39.76	
	600	29.72	
	400	24.03	
	200	16.00	
	100	9.57	
Atriplex semibaccata	1000	53.15	0.82
	800	51.61	
	600	43.64	
	400	32.20	
	200	16.53	
	100	8.10	
Catechol	400	71.67	0.15
	300	65.00	
	200	56.33	
	100	32.47	

3. Allelopathic activity

The allelopathic effect of the methanolic extracts of the four studied *Atriplex* species (*Atriplex halimus*, *A. lindleyi*, *A. protulacoides* and *A. semibaccata*) on the germination of *Portulaca oleracea* four days after treatment is showed in Figure (1). The obtained data revealed that, the degree of inhibition was concentration-dependent. At lowest concentration 5 mg ml^{-1}, *A. portulacoides* showed the highest inhibition percentage (37.19%), while the methanolic extract of *A. halimus*, *A. lindleyi* and *A. semibaccata* inhibited the germination of *P. oleracea* by about 26.12%, 32.16% and 32.16%, respectively. On the other hand, at concentration 40 mg ml^{-1}, *A. lindleyi* showed the highest inhibition percentage (82.93%), while the extracts of *A. halimus*, *A. semibaccata* and *A. portulacoides* inhibited the germination of *P. oleracea* by about 60.24%, 80.54% and 74.34%, respectively.

The phytotoxic effects of methanolic extracts of *Atriplex* extracts on the shoot growth inhibition percentage of *Portulaca oleracea* after fourteen–days treatment is showed in Figure (2). At concentration 5, 10, 20, and 40 mg ml^{-1}, the inhibition percentages in shoot growth in case of *A. halimus* the values were 28.67, 50.22, 57.98, and 70.05% respectively. In *A. lindleyi*, the values were 20.92, 29.54, 64.88, and 80.39% respectively, *A. portulacoides* were 36.43, 43.33, 66.60 and 69.19%, respectively and *A. semibaccata* were 35.57, 55.40, 68.33 and 76.08%, respectively. On the other hand, the effects of different methanolic extracts from *Atriplex* species on the root growth inhibition percentage of *P. oleracea* after fourteen days treatment (DAT) are shown in Figure (3). At concentration 5, 10, 20, and 40 mg ml^{-1}, the inhibition percentages in root growth in case of *A. halimus* the values were 16.41, 23.81, 53.44, and 62.70% respectively. In *A. lindleyi*, the values were 77.20, 88.41, 93.58, and 95.30% respectively; *A. portulacoides* were 51.59, 73.81, 81.22 and 90.48%, respectively and *A. semibaccata* were 40.48, 84.92, 86.78 and 92.33%, respectively.

The present results showed the potent allelopathic effect of *Atriplex* species on the nuisance weed *P. oleracea*, which could be ascribed to the high content of of saponins, alkaloids, betains, proteins, amino acids, mineral salts [7] and phytoecdysteroids [8]. In particular, the flavonoids quercetin, kaempferol [9], isorhamnetin, spinacetin (6-

methoxyisorhamnetin), patuletin (6-methoxyquercetin) and other 6-

methoxyflavonols [10] were isolated from *Atriplex* species

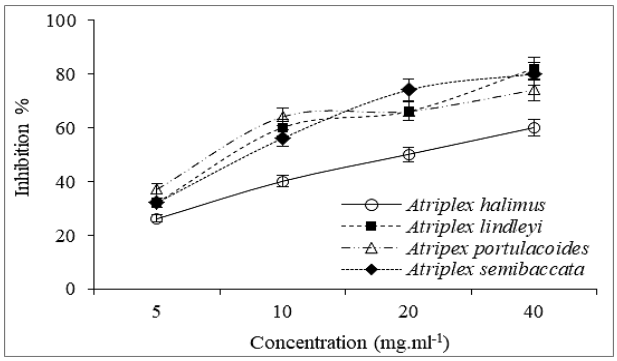

Figure 1: Effect of different methanolic *Atriplex* spp. extracts on the germination inhibition percentage of *Portulaca oleracea* 4 DAT.

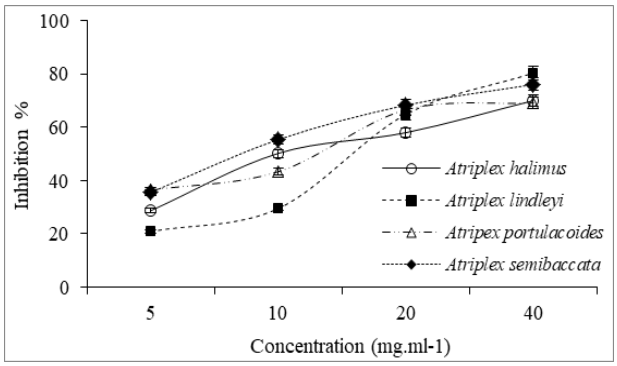

Figure 2. Effect of different methanol *Atriplex* spp. extracts on the shoot growth inhibition percentage of *Portulaca oleracea* 14 DAT.

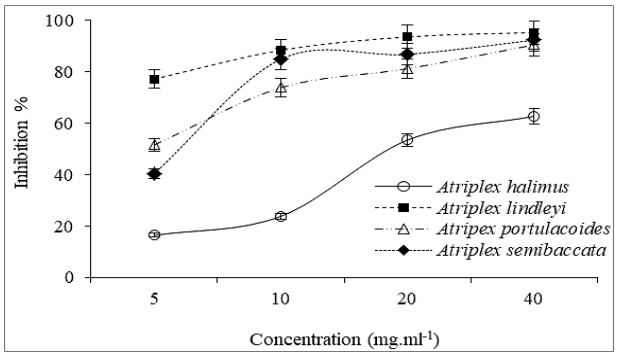

Figure 3: Effect of different methanolic *Atriplex* extracts on the root growth inhibition percentage of *Portulaca oleracea* 14 DAT.

Conclusion

From the results of this study, it is concluded that seed germination and seedling growth of *Portulaca oleracea* varied under different concentration of methanolic extracts of four *Atriplex* species (*Atriplex halimus, A. lindleyi, A. portulacoides* and *A. semibaccata*) collected from Deltaic Mediterranean coast, Egypt. From the above findings of the current experiment it could be suggested that *A. lindleyi,* and *A. semibaccata* had strong and moderate detrimental effect on *P. oleracea*. Moreover, *A. lindleyi* shows a potent antioxidant capacity which may be ascribed to the high content of phenolics, tannins and saponins. Furthermore, the allelochemicals responsible for germination and growth reduction of *P. oleracea* should be isolated and identified.

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