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Morphology, physiology, and biochemistry responses of *Chenopodium Quinoa* Wild. to aqueous extract stress of *Anthurium andraeanum* L. and *Strelitzia reginae* Ait

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In recent years, Quinoa has become one of the important plants in arid and semi-arid regions of many countries due to its nutritional properties and high compatibility with the environment. This plant is derived originally from the same type of weed with a high genetic diversity. The Quinoa has entered Iran as an immigrant plant since 1995, so that its botanical changes have not been done as a native plant. Also, if the Quinoa is planted once as a garden crop will grow for many years due to high seed loss. Field research showed that *Strelitzia reginae* and *Anthurium andreanum* species grow in some gardens of northern Iran. Therefore, an experiment was conducted to assess the allelopathic stress of different parts (i.e., root, stem, and leaf) of two ornamental plants (i.e., *A. andraeanum* and *S. reginae*) on the characteristics of morphology, physiology, and biochemistry of the Quinoa under hydroponic culture conditicon editions experiment was carried out as a factorial based on the completely randomized design with three replications in 2022. According to the results, the response of the morphological, physiological and b,iochemical characteristics of the Quinoa to the allelopathic stress of the two tested plants depended on the species and organ type. Findings also showed that the plant dry weight of the Quinoa was significantly stimulated in response to allelopathic stress of both root and stem of *S. reginae* aqueous extracts by 11.11% and 15.43%, respectively. The results also showed that stem extract of *S. reginae* significantly increased total chlorophyll content of the Quinoa with value of 13.33% only. In contrast, the plant growth of Quinoa significantly decreased with application of root, stem and leaf extracts of *A. andraeanum* by 16.67%, 42.59%, and 50%, respectively. Generally, both root and stem dry weight of the Quinoa with application of *A. andraeanum* leaf extract showed more significant reduction than other organs and leaves of *S. reginae* aqueous extract. According to the results, accumulation of osmolytes (proline and soluble sugars), ion leakage, total phenolic compounds, total flavonoids, and the enzyme's activity (catalase) in the Quinoa under oxidative stress of all plant part extracts tested of *A. andraeanum* and *S. reginae* leaf extract increased. It indicates the intensity of allelopathic stress in some treatments was more than the tolerance of plants, which caused an increase in oxidation of cell membrane. Therefore, due to the lack of full utilization of all the organs of ornamental plants, including the tested plants, it may be possible to introduce the obtained bioactive compounds of these plants as an environmentally friendly herbicide and even production of reinforcing fertilizers, medicines, and edible plants. This requires phytochemical analysis of compounds in these plants and screening their effect on a broad spectrum of plant species.

Keywords: *Hydroponic culture, Leaf extract, Oxidative stress, Osmolytes, Total phenolic compounds, Catalase activity*

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INTRODUCTION

Strelitzia reginae Ait. is a toxic plant that belongs to the Strelitziaceae family. It is a monocotyledonous plant and one of the typical ornamental plants in South Africa, and its flowers are known as the bird of paradise. The genus of *Strelitzia* has only five permanent evergreen species in the whole world (i.e., *S. reginae*, *S. juncea*, *S. nicolai*, *S. caudate*, and *S. alba*) (Londonkar and Rajani, 2021), of which only one species, *Strelitzia reginae*, is used in gardening. The other four species are wild types. *Anthurium andraeanum* is an economically important cut flower species in tropical and subtropical countries. *Anthurium* is one of the important genera in the Araceae family. *Anthurium* includes different species such as *A. andreanum*, *A. scherzerianum*, *A. crystallinum* and *A. faustinomirandae*. *A.* andreanum species is the most important and common species in some gardens in northern Iran. Cut flowers of Orchids and *Anthuriums*, produced in tropical regions, are ranked first and second in world markets, respectively (Dufour and Guerin, 2003; Chen et al., 2003). *A. andraeanum* flowers are tiny and arranged on a stalk-like structure called the spadix. Preliminary studies indicate the presence of several primary and secondary metabolites in *S. reginae* leaf extract. Unlike primary metabolites, secondary metabolites do not play a role in the growth and development of plants but have a defensive role against biotic and abiotic factors (Londonkar and Rajani, 2021). Allelopathy is one of the primary forms of competition in plants. A plant, by producing certain chemical compounds, affects the growth and development of the adjacent plant (Scavo and Mauromicale, 2021). Generally, these chemical compounds are of secondary type, which include phenols, long chain fatty acids, alkaloids and flavonoid derivatives, organic acids and quinines. These compounds inhibit the seed germination, and plant mechanisms (i.e., photosynthesis, respiration, water and ions absorption, and their transferring) (Ullah et al., 2015). Zhang et al. (2021) reported that disturbances in cell divisions, photosynthesis mechanisms, amino acids, and enzyme activity are known actions of allelochemicals. These allelopathic compounds can reduce 25% of the adjacent plant performance. Rob et al. (2021) and Krumsri et al. (2022) showed the dependence of the plants studied

on doses of plant extracts *Garcinia xanthochymus* and *Senna garrettiana.*

In addition, many studies have shown that plants have diverse biochemical compounds (i.e., alkaloids, glycosides, phenolics, flavonoids, proteins, tannins, and steroids) with different functions and biological activities (Saboon et al., 2019; Bachheti et al., 2020). In a study it was reported that chemical compounds with different structures have different effects on the target plants (Dayan et al., 2000). Several researchers reported the toxic effects of 3,4-dihydroxyphenyl-ethanol compound on cress and timothy. These researchers stated that this is due to the allelopathic stress caused by reactive oxygen species and the production of H_2O_2 (Zhang et al., 2021; Huang et al., 2020). The most crucial stage in the growth and development of plants is germination. In general, some immigrant plants are a serious threat to native plants due to their diverse genetic structure, adaptability, and lack of biological agent (Ma et al., 2020). Invasive plants in the ecosystem prevent the germination and growth of plants at the species level by releasing several allelopathic compounds (Chen et al., 2017; Kato-Noguchi, 2020). The Quinoa (*Chenopodium Quinoa* Wild.) belongs to the family Amaranthaceae. It is mainly grows in the Altiplano and arid areas of Andean from South America, although is currently cultivated on all continents (Del Castillo et al., 2008; Bazile et al., 2014). The genus *Chenopodium* consists of various species of herbaceous perennial or annual flowering plants known as goosefoot and found almost everywhere in the world (Gelin et al., 2003). The goosefoot family contains many of the species that we consider to be weeds (Robson, 2008). Charles et al. (1974) reported that both species of the *C. Quinoa* of the Andes and *C. nuttalliae* may have been derived from the same original wild type, a weedy Quinoa , *C. Quinoa* var. melanospermum from South America. As we know, the Quinoa has diverse genotypes with weed characteristics, which eas directly introduced from South America to other countries, including Iran, in 1995 and 1996. So, its botanical changes due to high genetic diversity have not been done as a native plant in most countries. Our field studies also showed that the Quinoa grows for many years in some gardens in the north of Iran if planted once. It was also observed that *S. reginae* and *A. andreanum* grow in some gardens in northern Iran. Therefore, due to the widespread use of plants with ornamental and medicinal flowers, especially *S. reginae* and *A. andraeanum* in cut form and the lack of proper use of their biomass in domestic and industrial uses for extracting and exploiting their effective substances, an experiment was conducted to evaluate the allelopathic potential of different plant parts of *S. reginae* and *A. andraeanum* aqueous extract on the morphology, physiology, biochemistry, and antioxidant activity characteristics of the Quinoa .

MATERIALS AND METHODS

Sampling of the plant and preparation

In this research, ornamental plant of *S. reginae* samples were collected from Dr. Beski's greenhouse located in Gonbad Kavous City, Golestan province, Iran (37° 15′ 00″ N, 55°10′01″ E) during the flowering stage in 2022. Samples of *A. andraeanum* also was collected from Romak Company located in Amol city, Mazndaran province, Iran (36° 28′ 01″ N, 52°21′25″ E). A herbarium specimen of *S. reginae* and *A. andraeanum* was authenticated by color flora of Iran (Ghareman, 1996). At first, the root, stem and leaf parts of both tested plants were separated from each other. Then, each part was washed in distilled water for a short time (to prevent the leaching of allelochemicals) to remove dust particles. Then, the studied parts were air-dried in the shade for three days. In the following, plant sample parts were dried by oven at a temperature of 60° C until reaching a constant weight (10% of the base weight) [Caceres, 2000]. The obtained plant sample parts were crushed by a grinder and kept in plastic bags.

Aqueous extracts preparation of *S. reginae* and *A. andraeanum* for quantification of total phenolic compounds and a hydroponic culture experiment

To quantify total phenolic compounds, five g of powdered plant parts of *A. andraeanum* and *S. reginae* were extracted with 100 ml of distilled water solvent (1:20 W/V), separately. A similar study was done but it was different in terms of plant species (*Solanu nigrum*) and extract ratio (1:10 W/V) (Abeed et al., 2022). Then the fresh extract of each plant part was centrifuged (model NF 200) at 1000 rpm. In the following, total phenolic compounds were measured using the Folin-Ciocalteu method (Mallick and Singh, 1980). Then, total phenolic compounds in different part extracts were calculated based on the gallic acid standard curve (Table 1). For the preparation of the extract in hydroponic culture, studied parts of tested plants powder were mixed with distilled water in a ratio of 50 g: 1000 ml (W/V), separately. So that after straining and washing several times, the final volume was brought to 1000 ml 600 ml of resultant filtrate aqueous extract was used in 4-liters of Yoshida's

nutrient solution (Yoshida et al., 1976), separately (Table 2).

Preparation of Quinoa seedlings under the influence of different part extracts of *A. andraeanum* and *S. reginae* in hydroponic culture

Certified Quinoa seed was purchased from Pakan Bazr Company (Iran). Seeds disinfection was done by treating seeds with 1% sodium hypochlorite and then washed three times with distilled water. To do this experiment, sterilized seeds of the Quinoa were germinated in a petri dish. Then 10-day-old Quinoa seedlings were transferred to containers containing Yoshida solution (pH=5.5). After applying the plant part extracts of each plant (600 ml of extract in four liters of nutrient solution, 15:100 V/V), the seedlings were kept at an ambient temperature (25 ± 3° C), with a photoperiod of 16 hours of light and 8 hours of darkness, a growth chamber with 1400 lux illumination and 75% relative humidity for four weeks (Cadho and Rajender, 1995). Yoshida's solution was changed every seven days, and the treatments were replaced again with the previous solution. The pH was adjusted every two days. Finally, some characteristics such as morphology, physiology, and biochemistry in the Quinoa were measured as follows:

Measurement of morphological characteristics: The length of root and stem as well as dry weight of root, stem, and leaf of the seedlings of Quinoa were determined by selecting 27 samples from each container.

Measurement of physiological characteristics: All the samples were tested in triplicates to measure physiological traits.

Relative water content of the Quinoa leaf: The relative water content of the leaf of the Quinoa was measured using the following equation (Slavick, 1979):

LRWC = [(FW-DW)/(TW-DW)] x 100 equation (1)

which, FW: fresh weight, TW: turgid weight (measured after 24 hours of saturation on deionized water at 4º C in the dark condition), and DW: the dry weight of the sample (after 48 hours at 80º C).

Photosynthetic pigments: Photosynthetic pigments content in the Quinoa leaf extract was measured using chilled acetone method (80%) (Arnon, 1949). The absorption points were measured at wavelengths of 663, 645, and 470 nm for chlorophyll a, b and carotenoid pigments, respectively. Based on current relationships, the content of chlorophyll a, b and total (a+b) as well as carotenoids were reported as mg per g fresh weight.

The osmolytes: The content of proline in the Quinoa leaf was calculated using the Bates et al. (1973) method. Using calibration curve of pure proline, the content of this osmolyte was determined based on the µmol per g fresh weight. The leaf soluble sugars content also was determined by phenol- sulphuric acid method (Kochert, 1978). With the help of the calibration curve of pure glucose, the soluble sugars content was determined as mg per g sample dry weight.

Ion leakage: Using the following equation, ion leakage in the Quinoa leaf was calculated as follows:

$$\text{Ion leakage } (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100 \text{ equation (2)}$$

which, EC1 was the rate of leaf ion leakage in distilled water after 24 hours; EC2 was the ion leakage rate in a boiling water bath at 95° C temperature for 90 minutes.

Enzyme activity: Catalase activity was determined by the Cakmak and Horst (1991) method at A240 nm. This enzyme was calculated based on the µmol of H_2O_2 decomposed per mg of protein per minute.

Measurement of biochemical characteristics

Total phenolic compounds content: The total phenolic compounds were estimated using the Folin-Ciocalteu method (Mallick and Singh, 1980). It was calculated based on the following equation according to the calibration curve of Gallic acid and expressed based on the mg GAE per 100 g sample dry weight.

$$y = 0.0027x - 0.0055 \quad R^2 = 0.999 \text{ equation (3)}$$

which, x, Y, and R were the absorbance, Gallic acid equivalent, and determination coefficient, respectively.

Total flavonoid content: The flavonoid content was estimated using aluminum chloride colorimetric method (Nabavi et al., 2008). Quercetin was used for preparation of the standard calibration curve, and the mixture absorbance was recorded at wavelength of 415 nm. Then using the following equation, the total flavonoid content was calculated as mg quercetin per g of sample dry weight.

$y = 0.0291x-0.0397$, $R^2 = 0.9904$ equation (4)

which, x: the absorbance, Y: mg quercetin per g of sample dry weight, and R: determination coefficient.

Activity of antioxidant

The antioxidant activity was estimated by the DPPH method at 517 nm wavelength. DPPH free radical inhibition percentage was estimated with the following equation (Brand-Williams et al., 1995).

Table 1. Total phenolic compounds content in different plant extracts of *Anthurium andraeanum* and *Strelitzia reginae*

Plant species	Organ extracts	Total phenolic compounds content (mg GAE per 100 g sample dry weight)
Anthurium andraeanum	root	11.22
	stem	13.98
	leaf	16.22
Strelitzia reginae	root	6.54
	stem	5.45
	leaf	12.78

Table 2. Ingredients of Yoshida nutrient solution

	Element		Chemical	Amount (mg/L)
Macro-Nutrient	Nitrogen	N	NH4NO3	91.4
	Phosphor	P	NaH2PO4.H2O	35.6
	Potassium	K	K2SO4	71.4
	Calcium	Ca	CaCl2.2H2O	117.35
	Magnesium	Mg	MgSO4.7H2O	324
	Manganese	Mn	MnCl2.4H2O	1.5
	Molybdenum	Mo	(NH4)6Mo7O24.4H2O	0.074
	Zinc	Zn	ZnSO4.7H2O	0.035
Micro-Nutrient	Boron	B	H3BO3	0.934
	Copper	Cu	CuSO4.5H2O	0.031
	Iron (ferric chloride)	Fe	FeCl3.6H2O	7.7
	Citric acid	-	C6H8O7.H2O	11.9

The DPPH radical inhibition (%)= ([(A_0 – A_1/ (A_0)] × 100% equation (5)

which, A_0 and A_1 were the absorbance values of the blank sample and the test sample, respectively. The percentage of stimulation and inhibition effect of morpho-physiological and biochemical traits in the Quinoa was calculated using the following methods:

$I = [(R_2 – R_1) / R_1 \times 100$ equation (6)

in which, I: the value of inhibition or stimulation percentage, R_1, and R_2: the control and treated plant response.

Statistical analysis

This experiment was done as a factorial in a completely randomized design in three replications (aqueous extract of different parts of both plants, respectively, n=24). The data obtained from the hydroponic culture was normalized using the Minitab software (version 14). Then, the data were analyzed using SAS software (version 9.3). Using the least significant difference test, mean data comparison was done at a 5% probability level.

RESULTS AND DISCUSSION

The interaction effect of different part extracts of *A. andraeanum* and *S. reginae* on the morphological characteristics of the Quinoa

As the findings showed, the effect of species and plant part extracts on the leaf number, the length of root and stem, the dry weight of plant parts such as root, stem, and leaf were significant at a 1% confidence level. However, the interaction of species and plant parts on all characteristics except the leaf number was also significant (Table 3). The Quinoa leaf number under allelopathic stress of different extracts of *A. andraeanum* parts significantly decreased. The highest reduction effect was obtained in the stem extract (45.88%). Root and leaf organ extracts were ranked second (Figure 1). The Quinoa leaf number increased using aqueous extracts of different parts of *S. reginae*. Most leaf number of Quinoa was obtained with the application of leaf extract, although its difference with the stem extract was insignificant (Figure 2). Studying the changes in the Quinoa root length under the root and stem aqueous extracts of *S. reginae*, unlike *A. andraeanum* showed that this characteristic was significantly increased by 88.23% and 102.93% compared to the control, respectively. On the contrary, the root length of the Quinoa was significantly reduced by applying leaf extract of *A. andraeanum* and *S. reginae* with values of 39.42% and 23.54%, respectively (Table 4).

According to the findings, the root and stem extracts of *S. reginae* also showed a significant increase effect on the stem length of the Quinoa by 40.24% and 55.47%, respectively. In contrast, the stem length of Quinoa was significantly reduced by applying all aqueous extracts of rese *A. andraeanum* parts. The greatest reduction effect was obtained in the leaf extract with the value of 17.69%. The root and stem

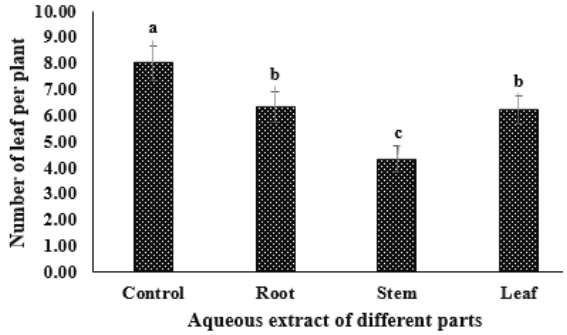

Figure 1. The effect of different organ extracts of the *Anthurium andraeanum* on the leaf number per plant of the quinoa (mean ± standard error).

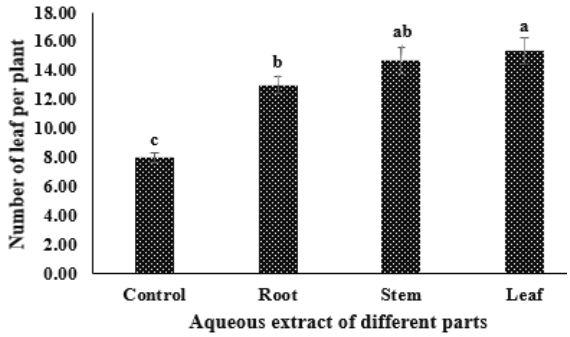

Figure 2. The effect of different organ extracts of the *Strelitzia reginae* on the leaf number of the quinoa (mean ± standard error).

parts were ranked second (Table 4). In the present study, the Quinoa root length was more influenced than the stem length under the allelopathic stress caused by the different part extracts of both treated plants. Considering that the root is the first organ exposed to allelopathic stress, this is not far from expected. Several secondary metabolites such as alkaloid, flavonoid, phenol, steroid, and tannin in *Anthurium andraeanum* were identified (Shazhni et al., 2016). Fuxiang et al. (2002) also expressed that polyphenols are an important secondary metabolite in *S. reginae*. In this case, Hossen and Hisashi (2020) reported that the radicle growth of plants such as cress, lettuce, barnyard grass, Italian ryegrass, timothy, and alfalfa was more sensitive to the *Acacia catechu* extracts as compared to the shoot.

The findings also indicated that the dry weight of root, stem, and leaf of the Quinoa decreased by application of different plant part extracts of *A. andraeanum* and S*. reginae* leaf extract. The most decreased effect in the Quinoa root dry weight was obtained by leaf extract of *A. andraeanum*, although its difference with some treatments was not significant statistically. In contrast the dry weight of root, stem and leaf of Quinoa were significantly increased by both root, and stem extracts of *S. reginae*. The highest dry weight of the root, stem, and leaf of the Quinoa was observed in the stem, stem, and root extracts of *S. reginae* with values of 18.35%, 16.64%, and 13.35%, respectively. However, the effects of both plant parts extract on the growth of the Quinoa seedlings were like the dry weight of the different parts (Table 4). Based on the obtained results, responses of morphological characteristics of the Quinoa depended on the plant species and parts type. These results agreed with results of other researchers (Rice, 1984; Saha et al., 2018; Bachheti et al., 2020), who reported that composition and concentration of allelochemicals can be different in plant parts. In most cases, the morphological characteristics of the Quinoa under allelopathic stress of *A. andraeanum* leaf extract showed a more significant decrease than the root and stem parts. Considering that the leaves are the most vital organs for the synthesis of secondary metabolites, this is not far from expected. However, the lower reducing effect of *S. reginae* leaf extract on the morphological characteristics of the Quinoa compared to the effect of the same part in *A. andraeanum* may be attributed to the sufficient and higher concentration of secondary compounds in the species under study.

The interaction effect of different part extracts of *A. andraeanum* and *S. reginae* on the characteristics of physiology and biochemistry of the Quinoa

Results indicated a significant difference in the effect of plant parts extracts and the interaction effect of species and plant part extracts on the physiological characteristics such as content of leaf relative water, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, proline, soluble sugars, ion leakage, total phenols, total flavonoid, and activity of catalase and antioxidant. However, the effect of species on all the measured characteristics was significant except for the content of chlorophyll a and carotenoids, as well as catalase and antioxidant activity (Table 5). As the findings showed, the extract of root, stem, and leaf in *A. andraeanum* significantly decreased the relative water content of the Quinoa with a value of 16.49%, 13.00%, and 22.06%, respectively. The effects of the plant parts extract of *S. reginae* on the relative water content of the Quinoa were like the effect of leaf relative water content of *A. andraeanum,* with the difference that *S. reginae* leaf extract had the most inhibitory effect on this trait compared to other plant parts (Table 6).

Table 3. Variance analysis (mean square) for the effect of various organ extracts of *Anthurium andraeanum* and *Strelitzia reginae* on the morphological characteristics of the quinoa

S.O.V	df	Number of leaves	Root length	Stem length	Root dry weight	Stem dry weight	Leaf dry weight	Seedling dry weight
a	1	96.00**	114.84**	5922.042**	2128.17**	301.042**	38.76**	160.17**
b	3	10.00**	37.88**	2643.71**	394.78**	53.82**	78.54**	46.78**
a× b	3	0.00000001^{ns}	24.45**	1480.37**	372.72**	46.93**	32.37**	19.61*
Error	16	0.0000001	1.18	56.42	25.042	1.79	1.14	2.67
Coefficient of variance (%)	-	1.50	14.35	11.61	8.51	7.78	9.31	9.07

**: indicates significance at 1 and 5% confidence level, and ^{ns}: non-significant

Table 4. Mean comparison (mean± standard error) of the interaction effect of various organ extracts of *Anthurium andraeanum* and *Strelitzia reginae* on the morphological characteristics of the quinoa

Plant species/ characteristics	Organ extracts	Root length (cm)	Stem length (cm)	Root dry weight (mg per plant)	Stem dry weight (mg per plant)	Leaf dry weight (mg per plant)	Plant dry weight (mg per plant)
Anthurium andraeanum	Control	56.67±3.33^{b}	54.67±2.67^{b}	20.0±0.00^{b}	14.0±0.58^{b}	20.0±1.15^{ab}	54.0±1.53^{b}
Sterlitzia reginae	Root	51.67±4.40^{bc}	44.00±2.08^{c}	15.0±0.58^{c}	13.0±0.58^{b}	17.0±1.15^{c}	45.0±1.53^{c}
	Stem	53.33±3.33^{bc}	54.00±3.05^{b}	10.0±1.15^{d}	7.0±0.58^{c}	14.0±0.58^{d}	31.0±1.73^{e}
	Leaf	34.33±0.67^{d}	45.00±2.89^{c}	9.67±0.67^{d}	6.67±0.88^{c}	10.67±0.67^{e}	27.0±0.58^{e}
	Control	56.67±3.33^{b}	54.67±2.67^{b}	20.0±0.00^{b}	14.0±0.58^{b}	20.0±1.15^{ab}	54.0±1.53^{b}
	Root	106.67±3.33^{a}	76.67±3.33^{a}	22.67±0.88^{a}	14.67±0.67^{ab}	22.67±0.33^{a}	60.00±1.00^{a}
	Stem	115.00±8.66^{a}	85.00±2.89^{a}	23.67±1.20^{a}	16.33±0.33^{a}	22.33±1.33^{a}	62.33±2.40^{a}
	Leaf	43.33±3.33^{cd}	56.7±3.33^{b}	16.67±0.67^{c}	5.83±0.60^{c}	17.33±0.67^{bc}	39.83±1.17^{d}
	LSD 5%	13.00	8.66	2.32	1.84	2.83	4.55

Similar letters in each column indicate significance at a 5% confidence level (mean ± SE).

Table 5. Variance analysis (mean square) for the effect of various organ extracts of *Anthurium andraeanum* and *Strelitzia reginae* on the characteristics of physiology, biochemistry, and antioxidant activity in the quinoa

S.O.V	df	Leaf relative water content	Chlorophyll a content	Chlorophyll b content	Total chlorophyll content	Ratio of chlorophyll a to b	Carotenoids content	Proline content
a	1	1312.76**	36.38 ^{ns}	0.313**	0.02**	0.48**	0.11 ^{ns}	0.11**
b	3	526.73**	1430.56**	0.041**	0.03**	0.103**	16.90**	0.022**
a× b	3	248.15**	551.58**	0.042**	0.004**	0.06**	6.13**	0.021**
Error	16	6.93	23.53	0.003	0.0001	0.003	0.17	0.001
Coefficient of variance (%)	-	5.64	6.16	11.76	7.91	9.08	12.58	9.74

S.O.V	df	Soluble sugars content	Ion leakage	Total Phenolic compounds content	Total flavonoids content	Catalase activity	Antioxidant activity
a	1	0.0038**	28.25*	150.10**	635.61**	0.13 ^{ns}	11.76 ^{ns}
b	3	0.0025**	307.62**	189.50**	452.12**	71.66**	29.82*
a× b	3	0.0005*	142.52**	68.46**	274.88**	21.45**	17.26*
Error	16	0.0001	4.48	2.331	3.95	0.77	5.023
Coefficient variance (%)	-	16.77	11.88	3.41	8.98	12.95	24.40

**, *: indicates significance at the 1 and 5% probability level, respectively, and ^{ns}: non-significant

According to the results, extracts of various plant organs of *A. andraeanum*, in addition to *S. reginae* leaf extract, caused a significant decrease in chlorophyll a content in the Quinoa . The greatest decrease in chlorophyll a content of Quinoa was observed under the application of *A. andraeanum* leaf extract by 58.82%. It was also observed that chlorophyll a content in the Quinoa significantly increased when the stem aqueous extract of *S. reginae* was used (37.25%). In this study, chlorophyll b content of the Quinoa significantly decreased by the aqueous extract of different parts of both plants. The phytotoxicity effect of the leaf extract of *A. andraeanum* was significantly higher than the other parts and even plant parts of the *S. reginae*. However, the total chlorophyll content (a+b) increased only under the application stem extract of *S. reginae* by 13.33%.

Table 6. Mean comparison (mean± standard error) of the interaction effect of various organ extracts of *Anthurium andraeanum* and *Strelitzia reginae* on the characteristics of physiology, biochemistry, and antioxidant activity in the quinoa

Plant species/ characteristics	Organ extracts	Leaf relative water content (%)	Chlorophyll a content (mg per g sample fresh weight)	Chlorophyll b content (mg per g sample fresh weight)	Total chlorophyll content (mg per g sample fresh weight)	Ratio of Chlorophyll a to b	Carotenoids content (mg per g sample fresh weight)	Proline content (µmol per g fresh weight of the sample)
Anthurium andraeanum	Control	91.82±3.71^{a}	0.51±0.04^{bc}	0.25±0.003^{a}	0.753±0.04^{b}	2.05±0.15^{c}	0.39±0.03^{b}	3.81±0.23^{d}
	Root	76.68±2.32^{c}	0.30±0.01^{d}	0.12±0.003^{c}	0.413±0.01^{d}	2.48±0.16^{c}	0.30±0.01^{c}	6.97±0.32^{c}
	Stem	79.88±0.46^{bc}	0.30±0.01^{d}	0.073±0.003^{d}	0.375±0.01^{d}	4.04±0.18^{ab}	0.22±0.01^{d}	7.41±0.82^{c}
	Leaf	71.56±2.51^{c}	0.21±0.02^{e}	0.05±0.00^{e}	0.26±0.015^{e}	4.16±0.31^{ab}	0.18±0.01^{d}	9.20±0.25^{b}
Sterlitzia reginae	Control	91.82±3.71^{a}	0.51±0.04^{bc}	0.25±0.003^{a}	0.753±0.04^{b}	2.05±0.15^{c}	0.39±0.03^{b}	3.81±0.23^{d}
	Root	87.79±4.26^{ab}	0.57±0.04^{b}	0.16±0.01^{b}	0.723±0.03^{b}	3.74±0.39^{b}	0.41±0.01^{b}	4.40±0.28^{d}
	Stem	89.21±1.60^{a}	0.70±0.03^{a}	0.15±0.01^{b}	0.85±0.035^{a}	4.75±0.13^{a}	0.51±0.02^{a}	4.26±0.29^{d}
	Leaf	41.27±1.61^{d}	0.45±0.03^{c}	0.17±0.01^{b}	0.61±0.04^{c}	2.73±0.28^{c}	0.32±0.01^{c}	14.34±1.97^{a}
	LSD 5%	8.40	0.08	0.02	0.09	0.71	0.06	1.52

Plant/ characteristics	Concentrations (%)	Soluble sugars content (mg per g sample dry weight)	Ion leakage (%)	Total phenolic compounds content (mg GAE per 100 g sample dry weight)	Total flavonoids content (mg quercetin per g sample dry weight)	Catalase activity (µmol H_2O_2 decomposed per mg protein per minute)	Antioxidant activity (%)
Anthurium andraeanum	Control	12.98±1.10^{d}	14.46±1.14^{d}	12.49±0.14^{d}	0.06±0.003^{bc}	8.88±0.39^{b-d}	38.63±0.81^{e}
	Root	18.76±1.40^{c}	34.82±3.52^{c}	25.63±0.88^{c}	0.06±0.01^{bc}	10.31±0.67^{ab}	42.04±1.45^{d}
	Stem	20.75±1.50^{bc}	65.47±2.23^{b}	37.91±0.33^{a}	0.08±0.01^{b}	9.34±0.48^{bc}	43.51±1.29^{cd}
	Leaf	23.17±0.53^{b}	92.59±2.50^{a}	33.13±0.19^{b}	0.11±0.01^{a}	11.02±3.23^{ab}	44.88±0.85^{bc}
Sterlitzia reginae	Control	12.98±1.10^{d}	14.46±1.14^{d}	12.49±0.14^{d}	0.06±0.003^{bc}	8.88±0.39^{b-d}	38.63±0.81^{e}
	Root	10.64±1.25^{d}	16.16±0.23^{d}	12.79±0.43^{d}	0.03±0.003^{e}	6.02±0.42^{dc}	46.30±0.20^{b}
	Stem	9.56±0.96^{d}	14.61±1.00^{d}	9.48±0.42^{d}	0.04±0.01^{de}	5.28±0.87^{d}	44.50±0.53^{b-d}
	Leaf	33.79±1.61^{a}	19.17±2.72^{d}	33.24±3.04^{b}	0.08±0.01^{bc}	13.76±1.04^{a}	59.64±0.32^{a}
	LSD 5%	3.67	6.24	3.44	0.02	3.88	2.64

Similar letters in each column indicate significance at a 5% confidence level (mean ± SE).

In contrast, all plant part extracts of *A. andraeanum* as well as *S. reginae* root extract showed an inhibitory effect on the total chlorophyll content. The highest decrease in total chlorophyll content of the Quinoa was also obtained using the leaf extract of *A. andraeanum* by 65.33%. In this study, the ratio of chlorophyll a to b in the Quinoa depended on the type of plant part and extracts of the treated species. The highest increase in the chlorophyll a to b ratio was achieved by stem treatment of *S. reginae* with a value of 4.75. However, statistically, its difference with some treatments was insignificant. The response of carotenoid pigments in the Quinoa under allelopathic stress of both studied plant parts varies from 0.18 to 0.51 mg per g sample fresh weight. The highest carotenoid content was found with the application of *S. reginae* stem extract, while the *A. andraeanum* leaf extract showed the lowest carotenoid content. However, the carotenoid content of the Quinoa was significantly decreased when the seedlings were exposed to all plant part extracts of *A. andraeanum* as well as *S. reginae* leaf extract (Table 6). In this study, the decrease in chlorophyll b and carotenoid pigments is probably due to the reduction of their light protection role, the excited chlorophyll formation and reactive oxygen species in chloroplast, which causes peroxidation of the cell membrane. Carotenoids are auxiliary pigments in photosynthesis that protect cellular organelles by receiving additional energy from sunlight (Lu et al., 2022). In a study reported that photosynthetic pigments such as chlorophyll and carotenoids in *Cyperus rotundus* weed decreased using sesame leachate (Hussain et al., 2017).

The Quinoa proline accumulation increased when exposed to the plant extracts of *A. andraeanum* and *S. reginae*. The highest proline content in Quinoa was obtained in response to allelopathic stress of *S. reginae* leaf extract, with a value of 14.34 µmol per g sample fresh weight (Table 6). Similarly, soluble sugars accumulate in the Quinoa seedlings increased when the different plant parts extracts of *A.*

andraeanum and *S. reginae* leaf extract were applied. Findings also showed that both root and stem extracts of *S. reginae* had no significant effect on the Quinoa soluble sugar content compared to control (Table 6). The reduction in the seedlings dry weight of Quinoa under allelopathic stress of different parts of *A. andraeanum* and *S. reginae* leaf, despite an increase in proline and soluble sugars content, indicates the insufficiency of these compounds in the tissues of Quinoa seedlings and oxidative stress, which caused by the toxicity of harmful substances such as phenolic compounds which were present in different parts of species under study.

Compatible osmolytes such as proline and soluble sugars can act as electron acceptors and prevent damage to the photosystem in plants against photoxidation. It was reported that proline plays an essential role in reducing oxidative stress in plants (Zali and Ehsanzadeh, 2018; Ghaffari et al., 2019). Bohnert and Jensen (1996) also stated that soluble carbohydrates also stabilize the function of cell membranes by inhibiting reactive oxygen species (ROS) and maintaining cell turgorescence (Jouve et al. 2004). In addition to the role of cellular osmolyte, these compounds stabilize the structure and action of macromolecules (Radi, 2013).

The finding also showed that different extracts from the *A. andraeanum* parts, unlike *S. reginae* part extracts significantly increased the percentage of ion leakage in Quinoa. The highest percentage of ion leakage was obtained in leaf extract, with a value of 92.59% (Table 6). The cell membrane is one of the first organs to be damaged by allelopathic stress. Generally, the ion leakage of the cell increases with an increase in the cell membrane permeability. Therefore, cell membrane permeability can be considered as a criterion for stress. Yan et al. (2015) reported that cellular lipid peroxidation causes changes in lipid bilayer membrane fluidity and permeability, which changes cell integrity.

According to the results, different part extracts of *A. andraeanum* as well as *S. reginae* leaf extract had an increase effect on the content of the Quinoa total phenolic compounds compared to the control. The most significant total phenolic compounds in Quinoa seedlings were obtained under stem extract of *A. andraeanum* with a value of 37.91 mg GAE per 100 g dry weight of the sample (Table 6). According to the results, the total flavonoids content in Quinoa was the maximum in the leaf extract of *A. andraeanum* (0.11 mg quercetin per g sample dry weight). In contrast, both root and stem extracts of *S. reginae* resulted in the lowest values of total flavonoids content by 0.03 and 0.04 mg quercetin per g dry weight of the sample, respectively (Table 6). These results agreed with the results of John and Sarada (2012), who reported that phenolic compounds are major plant allelochemicals in the ecosystem. Generally, these compounds play a fundamental role against allelopathic stress (Ozyigit et al., 2007) reported that stress and pathogen often cause the accumulation of phenolic compounds in plant sub-epidermal tissues. The quantity and quality of phenolic compounds depend on many biotic and abiotic factors such as plant physiology, phenological stages, climate conditions, etc. Generally, phenolic compounds are synthesized from phenylalanine through the shikimate acid pathway. These compounds have an aromatic ring and nucleophilic properties (Michalak, 2006), and by transferring electrons to peroxidase enzymes, they provide the basis for the hydrogen peroxide detoxification in the cell and act as antioxidants in the cell (Sakihama et al., 2002).

In a study, the difference in the effect of *Lactuca serriola* extract on the germination and physiological traits of *Lepidium sativum* was reported to allelochemical concentration threshold (Heivachi et al., 2023). Generally, α-amylase and α- glucosidase are essential enzymes for the decomposition of carbohydrates into glucose, so some non-enzymatic antioxidants, including polyphenols, especially flavonoids, phenolic acids, and tannins, have the essential efficacy of inhibiting these enzymes (Lin et al., 2016). Balasundram et al. (2006) stated that the interference in cellular oxidation-reduction mechanisms is one of the most prominent functions of phenolic compounds. Phenolic compounds affect the biosynthesis of indolyl-3-acetic acid hormone, which is responsible for regulating the growth and development of plants (Zaprometov and Nikolaeva, 2003; Kovaleva, 2007). A study reported that flavonoids also inhibit free radicals such as superoxide, hydroxyl, and hydrogen peroxide have an antioxidant role in cytoplasm and the enzymes responsible for the free radicals production (Agati et al., 2012).

Catalase activity in Quinoa varied from 5.29 and 13.76 mmol of H_2O_2 decomposed per mg of protein per minute. The activity of this enzyme in Quinoa was the highest and lowest using *S. reginae* leaf and stem extracts. The results also showed that the catalase activity in the treated plant increased with the

application of all plant part extracts of *A. andraeanum.* However, statistically, its difference with some treatments was insignificant (Table 6). In general, the enzymes such as catalase, guaiacol peroxidases, and ascorbate peroxidase play an essential role in preventing oxidation and cell damage. It has been reported that catalase is a common enzyme in almost all cells that can convert hydrogen peroxide into oxygen and water (Shim et al., 2003; Baker et al., 2023). Inhibition of catalase probably increases the sensitivity to cell damage caused by ROS. Therefore, the reduction of catalase activity leads to harmful effects on the cell due to the absorption of superoxide and hydrogen peroxide (Khan et al., 2023). In the present study, the antioxidant activity in Quinoa seedlings also significantly increased in response to allelopathic stress of all studied part extracts of both treated plants. The lowest and highest increase in treated plant part extracts was found in *S. reginae* leaf and *A. andraeanum* root extracts by 42.04 and 59.64%, respectively (Table 6).

In general, this study showed that the plant growth, relative water content, and photosynthetic pigments content in all plant part extracts of *A. andraeanum* and *S. reginae* leaf decreased despite the relative increase in protective pigments content (i.e., chlorophyll b and carotenoids), the osmolytes, ion leakage, phenolic compounds, and the catalase activity. Therefore, this is probably due to the inadequacy of these protectors in cells against oxidative stress caused by the different part extracts. This study showed that the intensity of allelopathic stress in some treatments was higher than plant tolerance. Therefore, the balance of ROS and antioxidant agents was disrupted, which led to an increase in the oxidation of the cell membrane. Further, it caused a decrease in photosynthesis and dry weight of the Quinoa seedlings. Goraya and Asthir (2016) reported that the ROS causes oxidative stress and increase lipid peroxidation in the cell membrane. Anjum et al. (2017) and Liu et al. (2019) reported that when the production rate of ROS is higher than its removal by enzymatic or non-enzymatic antioxidants, it will cause oxidative stress and cell cytotoxicity. Based on the findings of this study, it could be argued that the phenolic compounds, adaptive osmolytes and the photo-protector's content were essential molecules to change the response of plants. Therefore, it can be concluded that the content of adaptive osmolytes, ion leakage, phenolic compounds as a non-enzymatic antioxidant can be criteria for evaluating allelopathic stress in the Quinoa plant.

CONCLUSION

Based on the results, the morphological, physiological, and biochemical response of the Quinoa was dependent on the type of plant species and the concentration of organ aqueous extracts. In most cases, the leaf extract of both species had the highest inhibitory effect on the tested plant of the Quinoa . The results also showed that the root and stem extracts of *S. reginae* and *A. andraeanum* had significant stimulatory and inhibitory effects on the different characteristics of the Quinoa seedlings, respectively. This was probably due to the difference in the concentration of allelochemicals present in the organs, which caused different physiological responses of the Quinoa seedlings. However, this study showed that the seedlings growth of the Quinoa under oxidative stress of all plant part extracts of *A. andraeanum* and *S. reginae* leaves extract despite increased content of auxiliary pigments, adaptive osmolytes, phenolic compounds and enzymes activity decreased. According to the findings, stress intensity in some treatments was more than the tolerance of this plant. In general, due to the widespread use of ornamental medicinal flowers, especially *A. andraeanum* and *S. reginae* in cut form and the lack of exploitation of their production biomass, they can be considered suitable candidates to produce similar substances with hormonal effects, drugs, and edible plants, and even herbicides of biological origin. This requires phytochemical analysis of compounds in these plants, additional experiments, and screening their effect on the morpho-physiological and biochemical responses of adjacent plant species.

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