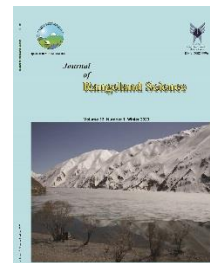




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Research and Full Length Article:

Effects of a Growth Season Rain Pulse on Physiological Parameters and Phytochemical Compounds of Caper (*Capparis spinosa* L.)

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Abstract. Growth season rain pulses affect biological and physiological activities of wild plants in arid and semi-arid regions. Despite of numerous reports on the effects of rain pulses on plant physiological indices, there is little information on how rain pulses may affect secondary metabolites of medicinal plants. Caper (*Capparis spinosa* L.) plays a critical role on soil conservation and economy of arid and sub-tropical inhabitants. Accordingly, present research was conducted to investigate the physiological and phytochemical responses of caper to a growth season rainfall in its natural habitat in Mazdavand, Sarakhs, Iran. Leaf samples (4 replicates) were taken 2, 4, 7 and 10 days after a natural rain event (10 mm) in 17 May 2018. Results showed increase of antioxidant enzymes (catalase and ascorbate peroxidase activity by 77 and 34 %, respectively) and decrease (23%) in proline content by the day after the rain pulse, whereas, photosynthetic pigments were not affected by soil moisture changes. Total alkaloid content was higher (16%) in vegetative than reproductive phase. From the secondary metabolites and 14 major phytochemical compounds detected in leaf extract, phenol content was reduced whereas alkaloid content increased by decreasing soil moisture during the days after rainfall. In conclusions, the phytochemical compounds showed contrasting (increase or decrease) responses to the soil moisture pulse and inter-pulse conditions. These results indicate the best time of leaf harvest to achieve highest concentrations of specific secondary metabolites after the growth season rain pulses.

Key words: Arid-land plants, Eco-physiology, Medicinal plants, Secondary metabolites

Introduction

Rainfall is one of the most important abiotic factors that affects physiological processes and ecological adaptability of plants in arid regions (Huang and Zhang, 2016). In such regions, rainfall is episodic or “pulsed” and highly variable in amount, time, and space. It is usually followed by long dry periods, and hence creates a dynamic soil moisture regime (Noy-Meir 1973; Yoda and Tsuji, 2015). Rainfall events produce biologically significant “pulses” of soil moisture recharged in soil depth associated with rainfall variability that is favorable for plant growth for only brief periods (Gebauer *et al.*, 2002).

In water-limited ecosystems, pulses of rainfall can trigger a cascade of plant physiological responses that have been studied in arid and semi-arid plants such as *Eucalyptus crebra* and *Callitris glaucophylla* (Zeppel *et al.*, 2008) and *Haloxylon ammodendron* (Qiyue *et al.*, 2014). Xiong *et al.* (2017) studied rainfall events in semiarid region, typically characterized as pulses, and showed that small rainfall pulses affected leaf photosynthesis because of a complex interplay between rainfall size, species and season. The physiological parameters of Mediterranean woody and annual species have shown great resistance to change under new rainfall pattern scenarios (Ladron de Guevara *et al.*, 2015). Rainfall pulses can significantly influence carbon cycling in water-limited ecosystems (Tang *et al.*, 2018). Woody plants of arid and semi-arid regions can respond to pulses of summer rain especially after prolonged periods of drought. Short term responses of individual plant to inter-pulse period has been considered as drought-resistance mechanism in arid and semi-arid plants (Golluscio *et al.*, 1998). Rainfall pattern-driven changes in soil respiration composition interact with physiological activity and abiotic factors (Wang, *et al.*, 2019). Liu *et al.* (2020) modeled the physiological responses of a

desert shrub to rainfall pulses in an arid environment and shown the existence of a clear non-linear relationship between the physiological responses of plant and both pulse magnitude and antecedent moisture, highlighting the importance of considering initial soil moisture conditions, previous pulse history, and changes in soil water content following rainfall events, in order to understand plant responses to precipitation pulses. Drought often causes oxidative stress (Akula and Ravishankar, 2014). The generation of reactive oxygen species (ROS) is characteristic of oxidative stress in drought conditions. Plants use several enzymatic (superoxide dismutase, catalase, glutathione peroxidase), and non-enzymatic (ascorbates, glutation, phenolic compounds, tocopherols, carotenoids, monitols and polyphenols) scavenging ROS (Blokhina *et al.*, 2003). Plants' responses to rain pulse may also differ in phenological stages. Juvenile plants can use effectively rain pulses more than an adult plant, which can be considered as an adaption for using early-season rain pulses (Jankju-Borzelabad and Griffiths, 2006).

Changes in phytochemical and antioxidant activity affected by environmental stress (especially drought) have been investigated in several lab or field studies (Daniels *et al.*, 2015; Zhang *et al.*, 2012; Wei *et al.*, 2013; Quan *et al.*, 2016 and Amirjani, 2013). Despite numerous reports on the effects of pulses on physiological indices, however, there is a research gap on phytochemical responses of wild plants to the season rain pulses in their natural habitats. The available data are on general responses to drought stress, e.g. changes in the production of different metabolite classes (Sampaio *et al.*, 2016) and increases in concentrations of secondary plant products (Selmar and Kleinwachter, 2013).

Caper (*Capparis spinosa* L., Capparaceae family) is a valuable plant for inhabitants of arid and semi-arid areas (Fici, 2014), which

is used as food, spice, medicine, sand dune fixation. In terms of phytochemical constituents, *Capparis spinosa* is by far one of the most studied medicinal plants to date (Kulusic-Bilusic *et al.*, 2012; Tlili *et al.*, 2015; Tlili *et al.*, 2009). The chemical compositions of the various parts include alkaloids, flavonoids, glucosinolates, phenolic acids, terpenoids and more which are important for their valuable medicinal potential (Lansky *et al.*, 2014). This study aimed to evaluate phytochemical and physiochemical responses of *Capparis spinosa* to soil moisture changes during the days after a natural rain pulse event and also compare the reaction of this plant to a growth season rain pulse at vegetative and reproductive phases. The results of this research can be useful for determining the best harvesting time of caper leaves, after a growth season water pulse, in terms of concentrations of secondary metabolites.

Materials and Methods

Study Area; Research was performed in Mazdavand, Sarakhs Khorasan Razavi Province, northeast of Iran; latitude N36°07', longitude E60°41' and 721 m above the sea level, with an area about 10 ha. The climate at site study is categorized as arid according to the climatic classification of De Martonne system and cold arid according to Emberger. The mean annual precipitation is 224 mm and the average temperature is 15°C according to long-term (2000-2018) meteorology data. Precipitation occurs from December to May (Fig 1), which indicates a long term drought period during June-September, with high temperature and low precipitation (<https://irimo.ir/>). Monthly rainfall and temperature variations are compared for the year of study (2018) and the long term data (2000-2018) in Fig 1.

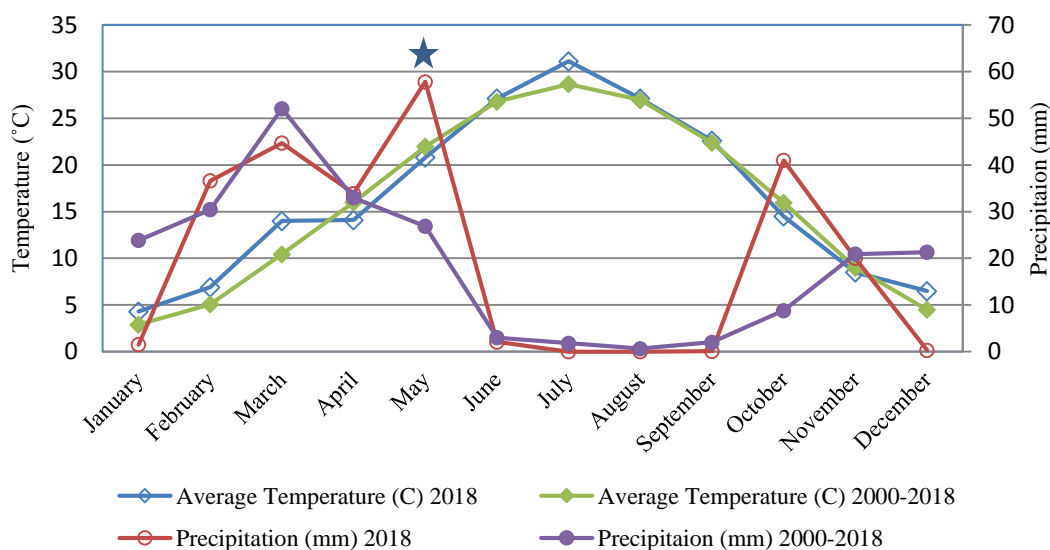


Fig. 1. Monthly changes in temperature and precipitation of Mashhad Weather station, long term data (2000-2018) is compared with the year of study (2018). The measured pulse event is indicated by ★

Plant material; this study was conducted on caper (*Capparis spinosa* L.) a spiny and aromatic semi shrub species from Capparaceae family. In the study area, vegetative growth of caper starts from April;

it flowers during May and June, fruit and seed ripening occurs from July to September (Agah *et al.*, 2020). However, the phenological stages can overlap with each other (Legua *et al.*, 2013). We could find

caper stands close to each other that were growing at two stages of vegetative and flowering in 17th of May, when the rain pulse event was studied in this research (Fig 1).

Sampling; Fresh leaf samples of caper were taken at 2, 4, 7 and 10 days after a rain event (10 mm) on 17 May 2018 from eight individual plants (4 replications of plants at vegetative phase and 4 replications at reproductive phase). Leaf samples were immediately frozen in liquid nitrogen and then stored at -20°C. In the laboratory, 0.1 g of fresh leaves were taken for measuring physiological assays. Canopy temperature was measured by a digital thermometer.

Soil samples (about 500 g) were taken from the root distribution depths (20-50 cm from the surface), for measuring soil moisture content. Samples dried in oven at 102°C for 24 h and weighed. Soil moisture content was measured using followed equation:

$$\text{Soil moisture content (\%)} = [(W_1 - W_2)/W_2] \times 100$$

where: W_1 = initial weight, W_2 = dry weight

Sampling carried out at 6 am, immediately after the leaf sampling and canopy measurements. Soil samples were preserved in a fridge (4°C), before being dried in an oven.

Physiological and Phytochemical Measurement; Chlorophylls (a, b and a+b) and carotenoids content were measured by UV-VIS spectroscopy according to Lichtenthaler (1987). Free proline concentration was determined using ninhydrin-based method (Bates *et al.*, 1973), secondary metabolites included total phenolic content by the Folin-Ciocalteu micro-method (Slinkard and Singleton, 1977), total flavonoid content by aluminium chloride colorimetric method (Chang *et al.*,

2002) and spectrophotometric determination of total alkaloid content (Mano *et al.*, 1986).

Catalase activity was assayed by measuring the initial rate of hydrogen peroxidase disappearance according to Velikova *et al.* (2000), peroxidase activity was estimated based on Srinivas *et al.* (1999) method and ascorbate peroxidase activity was measured by Yamaguchi *et al.* (1995) method. Ethanol leaf extract also used to identify chemicals using Gas Chromatography (7890A), Mass spectrometry (5975C), Palo Alto, Agilent Technology, USA.

Data Analysis A two way ANOVA was applied in which growth stage (vegetative and reproductive) and days (after the rain event) were considered at two independent factors with four replications, physiological and phytochemical compounds parameters were considered as dependent variables. Duncan test was used for comparison of means (at $P \leq 0.05$).

Results

An analysis of variation (Table 1) indicated significant effects of rain pulse and phenology stage. From the all physiological and phytochemical traits, only total alkaloid content was differed between vegetative and reproductive phases. Soil moisture was a more influencing factor. It significantly affected free proline concentration, catalase, and ascorbate peroxidase activity, total phenolic, and alkaloid content. However, photosynthesis pigments content, peroxidase activity, and total flavonoid content were not affected by the soil moisture variation during days after rain event. Interaction of phenology and soil moisture variation did not show any significant effect on all physiological and phytochemical traits (Table2).

Table 1. Analysis of variation for effects of rain pulse on physiological and phytochemical traits in *Capparis spinosa*; Means of Squares are shown

Sources	df	Physiological traits								Phytochemical traits		
		Chl a	Chl b	Chl _(a+b)	Car _(x+c)	Pro	CAT	POX	APX	TPC	TFC	TAC
Phenology(P)	1	86.70 _{ns}	11.15 _{ns}	160.06 _{ns}	1.32 _{ns}	1.31 _s	7.44 _{ns}	1146.5 _{ns}	557.3 _s	0.19 _s	0.00 _s	4.76*
Days (D)	3	7.54 _{ns}	2.16 _{ns}	16.60 _{ns}	2.83 _{ns}	9.77*	18.27*	2672.4 _{ns}	1166.1*	4.35*	1.98 _s	1.52*
P × M	3	27.06 _{ns}	4.11 _{ns}	43.65 _{ns}	2.18 _{ns}	3.86 _s	0.10 _{ns}	175.7 _{ns}	151.1 _s	0.84 _s	0.25 _s	0.11 _s
Error	2 4	23.40	3.37	38.86	1.67	2.97	6.12	3031.9	293.4	1.18	1.22	0.35

Abbreviations: Chl a: Chlorophyll a, Chl b: Chlorophyll b, Chl_(a+b): total chlorophylls, Car_(x+c): total carotenoids (xanthophylls and carotenes), Pro: free proline concentration, CAT: Catalase activity, POX: Peroxidase activity, APX: Ascorbate peroxidase, TPC: Total Phenolic Content, TFC: Total Flavonoid Content, TAC: Total Alkaloid Content. ns: non significance, * significance at P<0.05.

Table 2. Analysis of variation for effects of rain pulse on physiological and phytochemical traits in *Capparis spinosa*; Means of Squares are shown

Groups if traits	Name of traits	Abbrev.	MS			
			Phenology (P) (df=1)	Soil moisture (M) (df=3)	P × M (df=3)	Error (df=24)
Physiological	Chlorophyll a	Chl a	86.70 _{ns}	7.54 _{ns}	27.06 _{ns}	23.4
	Chlorophyll b	Chl b	11.15 _{ns}	2.16 _{ns}	4.11 _{ns}	3.37
	Total chlorophylls	Chl _(a+b)	160.06 _{ns}	16.60 _{ns}	43.65 _{ns}	38.86
	Total carotenoids	Car _(x+c)	1.32 _{ns}	2.83 _{ns}	2.18 _{ns}	1.67
	Proline content	Pro	1.31 _{ns}	9.77*	3.86 _{ns}	2.97
	Catalase activity	CAT	7.44 _{ns}	18.27**	0.10 _{ns}	6.12
	Peroxidase activity	POX	1146.5 _{ns}	2672.4 _{ns}	175.7 _{ns}	3031.9
	Ascorbate peroxidase	APX	557.3 _{ns}	1166.1*	151.1 _{ns}	293.4
Phytochemical	Total Phenolic Content	TPC	0.19 _{ns}	4.35*	0.84 _{ns}	1.18
	Total Flavonoid Content	TFC	0.00 _{ns}	1.98 _{ns}	0.25 _{ns}	1.22
	Total Alkaloid Content	TAC	4.76**	1.52*	0.11 _{ns}	0.35

ns: non significance, * significance at P<0.05

Microclimate; Gravimetric soil moisture under the canopy of caper was reduced from beginning until 10 days after the rain pulse event (Fig. 2). Maximum moisture was 16% at the first day after rain pulse, but it reduced to 2% at the day 10. The soil temperature variation showed the reverse trend. Soil

temperature was 18°C on the first day after the rain event, but it increased to 30°C on the last day. Since there was no significant difference between plant responses under vegetative or reproductive stages, so the results of pooled data are shown in Fig.2.

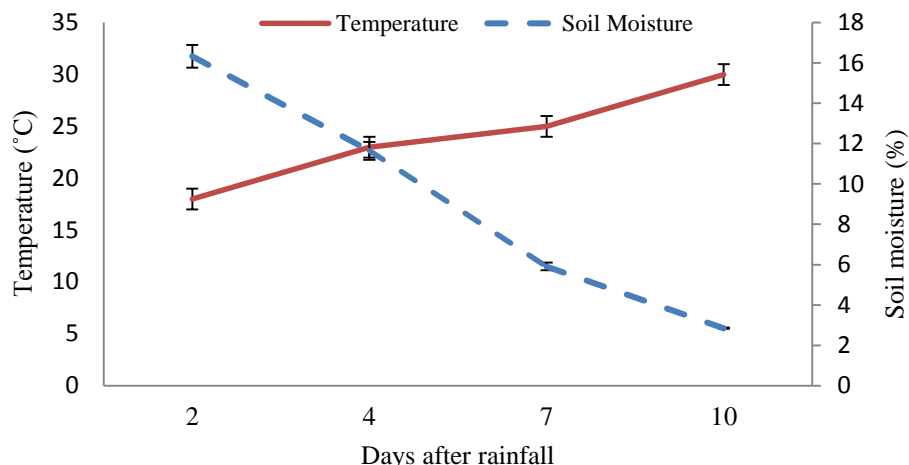


Fig. 2. Changes in soil temperature (°C) and soil moisture (%) under the canopy of caper days after the rain event

Caper physiological responses to rain pulse:

Enzymes activity: Rain pulse led to significant increases in catalase (CAT) and ascorbate peroxidase activity (APX) during days after the rain event (Table 3). CAT and APX activities were lowest on the first day after the rain event (1.02 and 54.36 unit. g⁻¹FW, respectively) and raised to their maximum amount (4.46 and 83.56 unit. g⁻¹FW) in the last day of sampling.

Photosynthesis pigments: Photosynthetic pigments were not significantly affected by rain pulses. However, we found a non-significant increasing trend of chlorophyll b but a decreasing trend for chlorophyll a, by reducing the soil moisture during the days after rain event. Caper could withstand drought by preserving carotenoids, i.e. there were no significant changes in total

carotenoids content during days after the rain event (Table 3).

Free proline concentration: Changes in free proline concentration are shown in Table 3. The concentration of free proline decreased significantly during the days after the rain event. Maximum (11.30 μmol.g⁻¹FW) and minimum (8.64 μmol.g⁻¹FW) proline concentrations were respectively observed at first and 10th day after rain event (Table 3).

A comparison of physiological traits of caper during the days after the rain pulse event showed no significant differences between individual plants that were growing at vegetative or reproductive stages, although data of reproductive were relatively smaller (Table 4).

Table 3. Changes in physiological traits during the days after the rain event

Physiological traits	Abbre.	Days after the rain event			
		Day 2	Day 4	Day 7	Day 10
Catalase activity (unit. g ⁻¹ FW),	CAT	1.02^b ± 0.27	3.04^{ab} ± 1.09	3.91^a ± 1.02	4.46^a ± 0.67
Peroxidase activity (unit. g ⁻¹ FW)	POX	88.69 ^a ± 15.15	106.90 ^a ± 15.79	109.45 ^a ± 25.54	133.21 ^a ± 14.05
Ascorbate peroxidase (unit. g ⁻¹ FW)	APX	54.36^b ± 4.55	64.95^b ± 7.81	68.05^{ab} ± 4.87	83.56^a ± 6.18
Chlorophyll a (mg.g ⁻¹ FW)	Chl a	26.28 ^a ± 1.20	26.21 ^a ± 1.46	26.91 ^a ± 2.40	24.63 ^a ± 1.90
Chlorophyll b (mg.g ⁻¹ FW)	Chl b	14.65 ^a ± 0.50	14.38 ^a ± 0.80	14.11 ^a ± 0.67	15.32 ^a ± 0.71
Total chlorophylls (mg.g ⁻¹ FW)	Chl (a+b)	40.93 ^a ± 1.61	40.59 ^a ± 2.14	42.24 ^a ± 2.85	38.75 ^a ± 2.54
Total carotenoids (mg.g ⁻¹ FW)	Car (x+c)	3.58 ^a ± 0.43	4.08 ^a ± 0.59	2.65 ^a ± 0.38	3.57 ^a ± 0.40
Proline content (μmol.g ⁻¹ FW)	Pro	11.30^a ± 0.80	9.54^{ab} ± 0.36	9.95^{ab} ± 0.35	8.64^b ± 0.77

Means of rows followed by the same letters are not significantly different by Duncan's test at 5% level of probability.

Table 4. Changes in physiological traits between vegetative and reproductive phases

Physiological traits	Abbre.	Phenology phases	
		Vegetative	Reproductive
Chlorophyll a (mg.g ⁻¹ FW)	Chl a	27.657 ^a ±0.32	24.365 ^a ±1.00
Chlorophyll b (mg.g ⁻¹ FW)	Chl b	15.21 ^a ±0.49	14.02 ^a ±0.41
Total chlorophylls (mg.g ⁻¹ FW)	Chl _(a+b)	42.86 ^a ±1.76	38.39 ^a ±1.23
Total carotenoids (mg.g ⁻¹ FW)	Car _(x+c)	3.68 ^a ±0.32	3.27 ^a ±0.35
Proline content (µmol.g ⁻¹ FW)	Pro	10.06 ^a ±0.50	9.66 ^a ±0.46
Catalase activity (unit. g ⁻¹ FW),	CAT	2.63 ^a ±0.47	3.59 ^a ±0.78
Peroxidase activity (unit. g ⁻¹ FW)	POX	115.55 ^a ±15.71	103.58 ^a ±9.58
Ascorbate peroxidase activity (unit. g ⁻¹ FW)	APX	71.90 ^a ±4.83	63.56 ^a ±4.73

Means of rows followed by the same letters are not significantly different by Duncan's test at 5% probability level.

Caper phytochemical responses to rain pulse:

Total phenolic content (TPC) decreased significantly during days after the rain event. The highest phenolic content (6.28 mg Gal.g-1FW) was found in the first day. There were no significant changes in TPC from day 4 to 10 (Table 5). Total flavonoid content (TFC) was not affected by the rain pulse, despite a gradual reduction during the days after the rain event. Total alkaloid content (TAC) increased significantly during

days after the rain event. Lowest TAC was observed on the first day after the rain event (3.75 mg.g-1FW) that reached to highest concentration in day 10 (4.72 mg.g-1FW).

A comparison of phytochemical changes during the days after the rain event indicated significantly higher total alkaloid content (4.59 mg.g-1FW) in the reproductive than the vegetative phase (3.82 mg.g-1FW) (Table 6). Other secondary metabolites (TPC and TFC) were not affected by phenology phases.

Table 5. Changes in phytochemical traits during the days after the rain event

Phytochemical traits	Abbrev.	days after the rain event			
		Day 2	Day 4	Day 7	Day 10
Total Phenolic Content (mg Gal.g ⁻¹ FW)	TPC	6.28 ^a ±0.52	4.67 ^b ±0.31	5.02 ^b ±0.33	4.80 ^b ±0.24
Total Flavonoid Content (mg QUE.g ⁻¹ FW)	TFC	5.33 ^a ±0.51	4.90 ^a ±0.43	4.45 ^a ±0.26	4.20 ^a ±0.13
Total Alkaloid Content (mg.g ⁻¹ FW)	TAC	3.75 ^b ±0.27	3.96 ^b ±0.24	4.38 ^{ab} ±0.23	4.72 ^a ±0.22

Means followed by the same letters are not significantly different by Duncan's test at 5% probability level.

Table 6. Changes in phytochemical traits between vegetative and reproductive phases

Phytochemical traits	Abbrev.	Phenology phases	
		Vegetative	Reproductive
Total Phenolic Content (mg Gal.g ⁻¹ FW)	TPC	5.27 ^a ±0.33	5.12 ^a ±0.26
Total Flavonoid Content (mg QUE.g ⁻¹ FW)	TFC	4.71 ^a ±0.24	4.72 ^a ±0.29
Total Alkaloid Content (mg.g ⁻¹ FW)	TAC	3.82 ^b ±0.10	4.59 ^a ±0.21

Means followed by the same letters are not significantly different by Duncan's test at 5% probability level.

Changes in some phytochemical indices in leaf extract of caper were compared between vegetative and reproductive phenological stages also during days after rain pulse (Table 7). In this research, more than 50 phytochemical components were detected in caper leaves, but 70% of the total essential oil composition only consisted of 14 major

components. Hexacosane, Eicosane, and β-sitosterol, that account for 50% of the total essential oil composition, were not affected by the phenological stages. n-Hexadecanoic acid (Palmitic acid) showed a decreasing trend during the vegetative phase but an increasing trend during the reproductive stage. Fatty acid 9,12-Octadecadienoic acid

(Z,Z)- (Linoleic acid) was observed only in the vegetative stage and in the 2 and 4 days after the rainfall.

Table 7. Changes in some phytochemicals in leaf extract during the days after the rain event

leaf phytochemicals traits	phenology phases							
	Vegetative stage (days)				Reproductive stage (days)			
	2	4	7	10	2	4	7	10
Hexacosane	0.72	23.25	26.65	29.02	29.79	25.72	13.53	17.03
Eicosane	0.53	17.48	5.38	21.47	15.2	17.13	30.1	20.5
Heneicosane	-	2.32	2.59	-	3.08	-	3.06	2.01
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	22.41	2.61	3.6	-	2.65	2.49	2.73	4.24
n-Hexadecanoic acid	17.69	7.44	7.48	1.91	5.61	6.87	7.64	10.8
beta.-Sitosterol	10.1	12.41	10.5	6.75	10.73	12.84	10.17	11.3
Neophytadiene	6.88	5.85	4.57	2.83	6.75	5.54	6.89	5.35
beta.-D-Glucopyranose, 1,6-anhydro	3.73	0.24	-	-	0.23	-	0.04	0.27
3,7,11,15-Tetramethyl-2-hexadecen-1-OL	2.99	2.12	-	-	-	0.86	1.06	-
Acetic acid, 3,7,11,15-tetrameth...	2.52	0.24	0.21	-	-	-	0.29	0.22
1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.29	0.42	0.68	0.65	0.45	0.56	0.52	0.59
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	0.57	0.66	0.67	0.28	0.48	0.34	0.33	0.29
9,12-Octadecadienoic acid (Z,Z)-	3.95	0.13	-	-	-	-	-	-
Vitamin E	0.49	0.37	0.44	0.59	-	0.53	0.29	0.65
Total (%)	72.87	75.54	62.77	63.5	74.97	72.88	76.65	73.25

Discussion

In water-limited ecosystems, an ecologically significant rainfall pulse is defined as a rainfall event that alters both soil water status and plant physiological activity (Hao *et al.*, 2012). The episodic rainfalls lead to pulses of soil moisture availability, when the plant nutrient uptake often occurs during such pulses (Gebauer and Ehleringer, 2000). In this research also a growth season rain pulse caused considerable increase in soil moisture, which was gradually decreased over the time after the event. The rain pulse in growth season led to physiological and phytochemical responses in caper. Synthesis and accumulation of some phytochemical components were critically dependent on the environmental (soil moisture and temperature) conditions.

We found the highest activity of antioxidant enzymes (catalase and ascorbat peroxidase) in caper when soil moisture was low and the temperature was high, at the final days of measurement (day 10). It can be considered as an index of caper adaptation to drought stress. Numerous studies have reported increase of antioxidant

enzymes in response to moisture changes (Siddiqui *et al.*, 2016; Xiao *et al.*, 2008). Similar to our results, an up-regulation in catalase and ascorbate peroxidase activity was also observed in drought-stressed *Capparis ovata* (Ozkur *et al.*, 2009), olive (Sofa *et al.*, 2008) and ornamental shrubs (Toscano *et al.*, 2016). In the present study, free proline concentration and photosynthetic pigments content were not affected by soil moisture changes. It could be due relatively short term (only 10 days) exposure to drought stress after the rain pulse event.

Changes in phytochemical and secondary metabolites also were observed in caper after the rain pulse event in growth season. A significant increase in total alkaloid content (an important secondary metabolite in caper) found by decreasing soil moisture during the days after the rainfall. Among the chemical compounds, alkaloids are extensively (at least 20 types of alkaloids) in all organs of the *Capparis spinosa* (Lansky *et al.*, 2014) and their content in the plant is an important index for estimation of medicinal quality; e. g. they are responsible for some pharmacological effects like anti-

inflammatory and immunological properties (Lansky *et al.*, 2014). Anticancer effects of *Capparis spinosa* also refers to its alkaloids and flavonoids (especially quercetin) content (Matsuyama *et al.*, 2009).

In the present research, the highest amount of total phenol content was observed in high soil moisture immediately after the rain event, which was reduced by increasing drought stress at the following days after the rain pulse. However, total flavonoid content was not influenced by drought at days after rain pulse. Some studies have shown that the mild drought improved the synthesis and accumulation of alkaloids, in the process of cultivation, a short time of mild drought treatment would activate alkaloids content (Wei *et al.*, 2013; Li *et al.*, 2007). Previous researchers have shown contrasting responses of flavonoids and total phenolic compounds to drought stress; some have found an increase (Trabelsi *et al.*, 2013) other have reported decrease in the rate of total flavonoid content or no significant changes (Daniels *et al.*, 2015; Chehrit-Hacid *et al.*, 2015). For phenolic and flavonoids compounds however, higher accumulation have been found in response to long and continuous drought stress (Boscaiu *et al.*, 2010; Quan *et al.*, 2016).

For most of the measured parameters, caper did not show significant differences in response to pulse, when it was compared between the vegetative or reproductive phases. We only found a significantly higher total alkaloid content increase for caper under vegetative than the reproductive stage. Some researchers (e.g. Jankju-Borzelabad and Griffiths, 2006) have shown higher responses of a juvenile than adult seedlings of *Panicum antidotale* to the water and nutrients pulses.

In the present study, moisture reduction after rain event increased palmitic acid in the reproductive phase. Effect of drought stress on fatty acids and yield have been reported in some researches e.g. significant decrease

in *Salvia officinalis* (Bettaieb *et al.*, 2008) and increase in *Capparis spinosa* l. (Liu *et al.*, 2011) under drought stress.

Soil moisture deficit highly reduced Linoleic acid by day 7 and 10 after rain event; hence it was not detectable in the reproductive phase. In Sampaio *et al.*, (2016) study on the effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*, a seasonal pattern was observed for the occurrence of metabolites that included sugars, sesquiterpenes lactones and phenolic in the leaf and stem parts, which can be referred to rainfall and in temperature changes. β -sitosterol was observed in all sampling days in both vegetative and reproductive phases and its concentration was not affected by moisture changes. Also, other researchers have shown in their studies that moisture changes did not affect the percentage of essential oil components (Misra and Sricastatva, 2000). The anti-rheumatic property of caper plant is attributed to of β -sitosterol.

Conclusions

A rain pulse event in growth season could significantly affect the physiological and phytochemical traits of caper. Caper showed adaptive responses to drought stress during the days after the rain pulse (inter-pulse), via changing some physiological properties such as antioxidant enzymes activities. Rain pulse also showed contrasting effects on pharmacological traits of caper; while some phytochemical traits such as total alkaloid content increased, total phenolic content decreased during the days after the rain event. These results indicate the best time leaf harvest to achieve highest concentrations of specific secondary metabolites after the growth season rain pulses. They can also be used for predicting caper plant response to climate change.

References

- Agah, F., Esmaeili, M. A., Farzam, M., Abasi, R., 2020. Modeling of *Capparis spinosa* L. growth in natural habitat for cultivation in low input farming systems. *Iranian Journal of Medicinal and Aromatic Plants*, 35(6), 1002-1016. <http://doi.org/10.22092/IJMAPR.2020.127872.2645>. (In Persian.).
- Akula, R., Ravishankar, G. A., 2014. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, 6(11), 1720-1731. <https://doi.org/10.4161/psb.6.11.17613>
- Amirjani, M. R., 2013. Effects of drought stress on the alkaloid contents and growth parameters of *Catharanthus roseus*. *ARNP Journal of Agricultural and Biological Science*, 8(11), 745-750.
- Bates, L. S., Waldren, R. P., Teare, I. D., 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39, 205-207. <https://doi.org/10.1007/BF00018060>
- Blokhina, O., Virolainen, E., Fagestedt, K. V., 2003. Antioxidants, oxidative damage and oxygen privation stress: A review. *Annals Botany*, 91(2), 179-194. <https://doi.org/10.1093/aob/mcf118>
- Bettaieb, I., Zakhama, N., Wannas, W. A., Kchouk, M. E., Marzouk, B., 2008. Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Science Journal of Horticulture*, 120 (2), 271-275. <https://doi.org/10.1016/j.scienta.2008.10.016>
- Boscaiu, M., Sanchez, M., Bautista, I., Donat, P., Lidon, A., Lliinares, J., Llul, C., Mayoral, O., Vicente, O., 2010. Phenolic compounds as stress markers in plants from gypsum habitats. *Bulletin UASVM Horticulture*, 67(1), 44-49.
- Chang, C., Yang, M., Wen, H., Chen, J., 2002. Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. *Journal of Food and Drugs Analysis*, 10 (3), 178-182. <https://doi.org/10.38212/2224-6614.2748>
- Chehrit-Hacid, F., Derridj, A., Moulti-Mati, F., Mati, A., 2015. Drought stress effect on some biochemical and physiological parameters; accumulation of total polyphenols and flavonoids in leaves of provenance seedlings *Pistacia lentiscus*. *International Journal of Research in Applied Natural and Social Sciences*, 3(9), 127-138.
- Daniels, C. W., Rautenbach, F., Marnewick, J. L., Valentine, A. J., Babajide, O. J., Mabusela, W. T., 2015. Environmental stress effect on the phytochemistry and antioxidant activity of a South African bulbous geophyte, *Gethyllis multifolia* L. Bolus. *South African Journal of Botany*, 96, 29-36. <https://doi.org/10.1016/j.sajb.2014.10.004>
- Fici S., 2014. A taxonomic revision of the *Capparis spinosa* group (Capparaceae) from the Mediterranean to Central Asia. *Phytotaxa*, 174 (1), 001-024. <https://doi.org/10.11646/phytotaxa.174.1.1>
- Gebauer, R. L. E., Schwinning, S., Ehleringer, J. R., 2002. Interspecific competition and resource pulse utilization in a cold desert community. *Ecology*, 83(9), 2602-2616. [https://doi.org/10.1890/0012-9658\(2002\)083\[2602:ICARPU\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2602:ICARPU]2.0.CO;2)
- Gebauer, R. L. E., Ehleringer, J. R., 2000. Water and nitrogen uptake patterns following moisture pulses in a cold desert community. *Ecology*, 81(5), 1415-1424. [https://doi.org/10.1890/0012-9658\(2000\)081\[1415:WANUPF\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1415:WANUPF]2.0.CO;2)
- Golluscio, R. A., Sala, O. E., Lauenroth, W. K., 1998. Differential use of large summer rainfall events by shrubs and grasses: a manipulative experiment in the Patagonian steppe. *Oecologia*, 115, 17-25. <https://doi.org/10.1007/s004420050486>
- Hao, Y. B., Kang, X. M., Cui, X. Y., Ding, K., Wang, Y. F., Zhou, X. Q., 2012. Verification of a threshold concept of ecologically effective precipitation pulse: From plant individuals to ecosystem. *Ecological Informatics*, 12, 23-30. <https://doi.org/10.1016/j.ecoinf.2012.07.006>
- Huang, L., Zhang, Zh., 2016. Effect of rainfall pulses on plant growth and transpiration of two xerophytic shrubs in a revegetated desert area: Tengger Desert, China. *Catena*, 137, 269-276. <https://doi.org/10.1016/j.catena.2015.09.020>
- Jankju-Borzelabab, M., Griffiths, H., 2006. Competition for pulsed resources: an experimental study of establishment and coexistence for an arid-land grass. *Oecologia*, 148, 555-563. <https://doi.org/10.1007/s00442-006-0408-1>
- Kulisic-Bilusic, T., Schmöller, I., Schnäbele, K., Siracusa, L., Ruberto, G., 2012. The anticarcinogenic potential of essential oil and aqueous infusion from caper (*Capparis spinosa* L.). *Food Chemistry*, 132(1), 261-267. <https://doi.org/10.1016/j.foodchem.2011.10.074>
- Lansky, E. P., Paavilainen, H. M., Lansky, Sh., 2014. *Caper The Genus Capparis*. CRC Press, Taylor & Francis Group, pp: 334.
- Ladron de Guevara, M., Lazaro, R., Amau-Rosalen, E., Domingo, F., Molina-Sanchis, I., Mora, J. L., 2015. Climate changes effects in a semiarid grassland: Physiological responses to shifts in rain patterns. *Acta Oecologica*, 69, 9-20. <https://doi.org/10.1016/j.actao.2015.08.001>
- Legua, P., Martínez, J. J., Melgarejo, P., Martínez, R., Hernández, F., 2013. Phenological growth stages of caper plant (*Capparis spinosa* L.) according to the Biologische Bundesanstalt, Bundessortenamt and Chemical scale. *Annals of Applied Biology*,

- 163(1), 135–141. <https://doi.org/10.1111/aab.12041>
- Li, X., Wang, Y., Yan, X. F., 2007. Effects of water stress on berberine, jatrorrhizine and palmatine contents in *amur corktree* seedlings. *Acta Ecologica Sinica*, 27(1), 58-63. [https://doi.org/10.1016/S1872-2032\(07\)60011-0](https://doi.org/10.1016/S1872-2032(07)60011-0)
- Lichtenthaler, H. K., 1987. Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. *Methods in Enzymology*, 148, 350-382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Liu, W., He, Y., Xiang, J., Fu, C., Yu, L., Zhang, J., Li, M., 2011. The physiological response of suspension cell of *Capparis spinosa* L. to drought stress. *Journal of Medicinal Plants Research*, 5(24), 5899-5906.
- Liu, H., Wu, C., Yu, Y., Zhao, W., Yang, Q., Wang, S., Liu, J., 2020. Modeling the physiological responses of a desert shrub to rainfall pulses in an arid environment in northwestern China. *Journal of Arid Environments*, 183, article 104277. <https://doi.org/10.1016/j.jaridenv.2020.104277>
- Mano, Y., Nabehima, S., Matsui, C., Ohkawa, H., 1986. Production of tropane alkaloids by hairy root cultures of *Scopolia japonica*. *Agricultural and Biological Chemistry*, 50(11), 2715-2722. <https://doi.org/10.1080/00021369.1986.10867820>
- Matsuyama, K., Villareal, M.O., El Omri, A., Han, J., Kchouk, M. E., Isoda, H., 2009. Effect of Tunisian *Capparis spinosa* L. extract on melanogenesis in B16 murine melanoma cells. *Journal of Natural Medicines*, 63(4), 468-472. <https://doi.org/10.1007/s11418-009-0355-3>
- Misra, A., Sricastatva, N. K., 2000. Influence of water stress on Japanese mint. *Journal of Herbs, Spices and Medicinal Plants*, 7(1), 51-58. https://doi.org/10.1300/J044v07n01_07
- Noy-Meir, I., 1973. Desert ecosystems: Environment and producers. *Annual Review of Ecology and Systematic*, 4, 25-51. <https://doi.org/10.1146/annurev.es.04.110173.000325>
- Quan, N.T., Anh, L.H., Khang, D.T., Tuyen, P.T., Toan, N.P., Minh, T.N., Minh, L.T., Bach, D.T., Ha, P.T.T., Elzaawely, A.A., Khanh, T.D., Trung, K.H., Xuan, T.D., 2016. Involvement of secondary metabolites in response to drought stress of Rice (*Oryza sativa* L.). *Agriculture*, 6(2), 23. <https://doi.org/10.3390/agriculture6020023>.
- Ozcur, O., F. Ozdemir, M. Bor, and I. Turkan., 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environmental and Experimental Botany*, 66(3), 487–492. <https://doi.org/10.1016/j.envexpbot.2009.04.003>
- Qiyue, Y., Wenzhi, Z., Bing, L., Hu, L., 2014. Physiological responses of *Haloxylon ammodendron* to rainfall pulses in temperate desert regions, Northwestern China. *Trees*, 28(3), 709-722. <https://doi.org/10.1007/s00468-014-0983-4>
- Sampaio, B. L., Edrada-Ebel, R., Da Costa, F. B., 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. *Scientific Reports*, 6, 29265. <https://doi.org/10.1038/srep29265>
- Selmar, D., Kleinwächter, M., 2013. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops and Products*, 42, 558-566. <https://doi.org/10.1016/j.indcrop.2012.06.020>
- Siddiqui, Z. S., Shahid, H., Cho, J., Park, S. H., Ryu, T. H., Park, S. C., 2016. Physiological responses of two halophytic grass species under drought stress environment. *Acta Botanica Croatica*, 75(1), 31-38. <https://doi.org/10.1515/botcro-2016-0018>
- Slinkard, K., Singleton, V. L., 1977. Total phenol analysis; automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.
- Sofa, A., Manfreda, S., Dichio, B., Florentino, M., Xiloyannis, C., 2008. The olive tree: a paradigm for drought tolerance in Mediterranean climates. *Hydrology and Earth System Science*, 12, 293-301. <https://doi.org/10.5194/hessd-4-2811-2007>
- Srinivas, N. D., Rashmi, K. R., Raghavarao, K. S. M. S., 1999. Extraction and purification of a plant peroxidase by aqueous two-phase extraction coupled with gel filtration. *Process Biochemistry*, 35(1-2), 43-48. [https://doi.org/10.1016/S0032-9592\(99\)00030-8](https://doi.org/10.1016/S0032-9592(99)00030-8)
- Tang, Y., Jiang, J., Chen, C., Chen, Y., Wu, X., 2018. Rainfall pulse response of carbon fluxes in a temperate grass ecosystem in the semiarid Loess Plateau. *Ecology and Evolution*, 8, 11179-11189. DOI: 10.1002/ece3.4587
- Tlili, N., Mejri, H., Anouer, F., Saadaoui, E., Khaldi, A., Nasri, N., 2015. Phenolic profile and antioxidant activity of *Capparis spinosa* seeds harvested from different wild habitats. *Industrial Crops and Products*, 76, 930-935. <https://doi.org/10.1016/j.indcrop.2015.07.040>
- Tlili, N., Munne-Bosch, S., Nasri, N., Saadaoui, E., Khaldi, A., Triki, S., 2009. Fatty acids, tocopherols and carotenoids from seeds of Tunisian caper "*Capparis spinosa*". *Journal of Food Lipids*, 16(4), 452-464. <https://doi.org/10.1111/j.1745-4522.2009.01158.x>
- Trabelsi, N., Waffo-Te'guo, P., Snoussi, M., Ksouri, R., Me'rillon, J. M., Smaoui, A., Abdelly, C.,

2013. Variability of phenolic composition and biological activities of two Tunisian halophyte species from contrasted regions. *Acta Physiologiae Plantarum*, 35, 749–761. <https://doi.org/10.1007/s11738-012-1115-7>
- Toscano, S., Farieri, E., Antonio, F., Romano, D., 2016. Physiological and biochemical responses in two ornamental shrubs to drought stress. *Frontiers in Plant Science*, 7, 645. <https://doi.org/10.3389/fpls.2016.00645>.
- Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Science*, 151(1), 59-66. [https://doi.org/10.1016/S0168-9452\(99\)00197-1](https://doi.org/10.1016/S0168-9452(99)00197-1)
- Wei, H., Li, L., Yan, X., Wang, Y., 2013. Effects of soil drought stress on the accumulation of alkaloids and flavonoids in Motherwort. *Advance in information sciences and service sciences*, 5(6), 795-803.
- Xiao, X., Xu, X., Yang, F., 2008. Adaptive responses to progressive drought stress in two *Populus cathayana* populations. *Silva Fennica*, 42(5), 705-719. <https://doi.org/10.14214/sf.224>
- Xiong, P., Shu, J., Zhang, H., Jia, Z., Song, J., Palta, J. A., Xu, B. 2017. Small rainfall pulses affected leaf photosynthesis rather than biomass production of dominant species in semiarid grassland community on Loess Plateau of China. *Functional Plant Biology*, 44, 1229-1242. <https://doi.org/10.1071/FP17040>
- Yamaguchi, K., Mori, H., Nishimura, M., 1995. A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. *Plant Cell Physiology*, 36(6), 1157-1162. <https://doi.org/10.1093/oxfordjournals.pcp.a078862>
- Yoda, K., Tsuji, W., 2015. Evaluation of the Effect of a Rain Pulse on the Initial Growth of *Prosopis* Seedlings. *Arid Land Research and Management*, 29(2), 210-221. doi.org/10.1080/15324982.2014.943376
- Wang, Y., Li, X., Zhang, C., Wu, X., Du, E., Wu, H., Yang, X., Wang, P., Bai, Y., Wu, Y., Huang, Y. 2019. Responses of soil respiration to rainfall addition in a desert ecosystem: Linking physiological activities and rainfall pattern. *Science of the Total Environment*, 650, 3007-3016. <https://doi.org/10.1016/j.scitotenv.2018.10.057>
- Zeppel, M., Macinnis-Ng, C.M.O., Ford, C. R., Eamus, D., 2008. The response of sap flow to pulses of rain in a temperate Australian woodland. *Plant and Soil*, 305, 121-130. <https://doi.org/10.1007/s11104-007-9349-7>
- Zhang, L., Wang, Q., Guo, Q., Chang, Q., Zhu, L., Liu, L., Xu, H., 2012. Growth, physiological characteristics and total flavonoid content of *Glechoma longituba* in response to water stress. *Journal of medicinal plants research*, 6(6), 1015-1024. <https://doi.org/10.5897/JMPR11.758>

اثرات پالس باران فصل رشد روی پارامترهای فیزیولوژیک و ترکیبات فیتوشیمی کور (*Capparis spinosa* L.)

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چکیده. پالس‌های باران فصل رشد روی فعالیت‌های بیولوژیک و فیزیولوژیک گیاهان خودرو در مناطق خشک و نیمه خشک تاثیر می‌گذارد. علیرغم گزارش‌های متعدد درباره اثرات پالس‌های باران روی شاخص‌های فیزیولوژیک گیاه، اطلاعات اندکی در مورد نحوه اثر پالس‌های باران بر متابولیت‌های ثانویه گیاهان دارویی موجود است. کور (*Capparis spinosa* L.) نقش اساسی در حفاظت خاک و اقتصاد رویشگاه‌های خشک و نیمه-گرمسیری دارد. پژوهش حاضر به منظور بررسی پاسخ‌های فیزیولوژیک و فیتوشیمیایی کور به بارش‌های فصل رشد، در رویشگاه طبیعی واقع در مزداوند سرخس در ایران انجام شد. در روز دوم، چهارم، هفتم و دهم پس از وقوع باران (۱۰ میلی‌متر) در ۲۷ اردیبهشت ۱۳۹۷ از برگ گیاه (در چهار تکرار) نمونه برداری شد. نتایج نشان دهنده افزایش آنزیم‌های آنتی‌اکسیدانت (کاتالاز و آسکوربات پراکسیداز به ترتیب ۷۷ و ۳۴ درصد) و کاهش محتوای پرولین (۲۳٪) در روزهای پس از پالس باران بود، در حالی که رنگدانه‌های فتوسنتزی تحت تاثیر تغییرات رطوبت خاک قرار نگرفتند. محتوای آلکالوئید کل در فاز رویشی بالاتر از فاز زایشی (۱۶٪) بود. از بین متابولیت‌های ثانویه و ۱۴ ترکیب اصلی فیتوشیمیایی شناسایی شده در عصاره برگ، با کاهش رطوبت خاک در طول روزهای پس از بارندگی محتوای فنول کاهش و محتوای آلکالوئید افزایش یافت. به عنوان نتیجه‌گیری کلی، ترکیبات فیتوشیمیایی پاسخ‌های ثابت (افزایشی یا کاهش) به پالس رطوبت خاک و شرایط بین پالسی نشان دادند. این نتایج، بهترین زمان برای برداشت برگ به منظور دستیابی به بالاترین غلظت متابولیت ثانویه خاص بعد از پالس‌های باران فصل رشد را نشان می‌دهند.

کلمات کلیدی: اکوفیزیولوژی، گیاهان دارویی، گیاهان مناطق خشک، متابولیت‌های ثانویه