Influence of exogenous polyamines on antioxidant defence and essential oil production in valerian (*Valeriana officinalis* L.) plants under drought stress

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ABSTRACT

The objective of this study was to determine the effects of foliar application of polyamines (PAs) on antioxidant defence and essential oil production of valerian (*Valeriana officinalis* L.) grown under different drought stress treatments (100, 70, 50 and 30% available water content). This study was carried out using pots in greenhouse condition. Drought–stressed valerian seedlings were sprayed with 1 mM concentration of each putrescine (Put), spermidine (Spd) and spermine (Spm). The results showed that drought stress significantly affected most biochemical characteristics of valerian plants. Characteristics including leaf relative water content, chlorophyll a and b contents were decreased, while carotenoids and electrolyte leakage were increased with the increase of water stress. In this research, defensive characteristics comprising proline content, soluble sugars, catalase, and ascorbate peroxidase were increased followed by drought stress to ameliorate the adverse effect of it. Results revealed that foliar application of Spd and Spm provoked the antioxidant enzymes activity as well as proline accumulation in valerian which alleviate the membrane damages. Consequently, Spd and Spm increased photosynthetic pigments which act as energy supply for plant growth and production. Here, putrescine had detrimental effects on CAT activity and Chl a content. Albeit, PAs presented remarkable effects under moderate drought stress condition but it showed reverse trends in severe drought stress condition. In terms of quantity and quality yield, drought stress reduced root growth but increased the concentration of essential oils. PAs are able to alleviate water deficit-induced diminish root growth. These results suggest that in moderate drought stress, growers can use PAs to increase productivity valerian.

Key words: biochemical characteristics, drought stress, essential oils, polyamines, *Valeriana officinalis*

IZVLEČEK

VPLIV DODAJANJA POLIAMINOV NA ANTIOKSIDATIVNO OBRAMBO IN PRODUKCIJO ETERIČNIH OLJ PRI ZDRAVILNI ŠPAJKI (*Valeriana officinalis* L.) V RAZMERAH SUŠNEGA STRESA

Namen raziskave je bil določiti učinke foliarnega dodajanja poliaminov (PAs) na antioksidacijsko obrambo in tvorbo eteričnih olj pri zdravilni špajki (*Valeriana officinalis* L.) gojeni pri različni oskrbi z vodo (100, 70, 50 in 30 % vsebnosti razpoložljive vode). Raziskava je bile izvedena kot lončni poskus v rastlinjaku. Sadike špajke v sušnem stresu so bile škropljene z 1 mM raztopino putrescina (Put), spermidina (Spd) in spermina (Spm). Rezultati so pokazali, da je sušni stres značilno vplival na večino analiziranih biokemičnih parametrov v zdravilni špajki. Parametri kot so relativna vsebnost vode, vsebnost klorofila a in b so se zmanjšali, medtem ko sta se vsebnost karotenoidov in iztok elektrolitov povečala z večanjem sušnega stresa. Obrambni mehanizmi rastline, ki obsegajo vsebnost prolina, topne sladkorje, aktivnost katalaze in askorbat peroksidaze so se z večanjem sušnega stresa povečali, da bi omilili škodljive učinke stresa. Rezultati so pokazali, da je foliarno dodajanje Spd in Spm sprožilo antioksidacijsko aktivnost encimov in akumulacijo prolina v zdravilni špajki, kar je zmanjšalo poškodbe membrane. Posledično sta Spd in Spm povečala vsebnost fotosinteznih barvil, ki rastlini omogočajo oskrbo z energijo za rast in produkcijo. Dodajanje putrescina je imelo negativen učinek na aktivnost katalaze (CAT) in vsebnost klorofila a. Pozitivni učinki dodajanja poliaminov so bili opazni le v razmerah zmernega sušnega stresa in so dobili nasproten trend ob njegovi zaostritvi. Z vidika kakovosti in količine pridelka je sušni stres zmanjšal rast korenin, a povečal vsebnost eteričnih olj. Poliamini lahko oblažijo zmanjšano rast korenin, ki jo povzroča pomanjkanje vode. Ti rezultati nakazujejo, da lahko pridelovalci v razmerah zmernega sušnega stresa z dodatkom poliaminov povečajo pridelek zdravilne špajke.

Ključne besede: biokemični parametri, eterična olja, poliamini, *Valeriana officinalis*, sušni stres

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1 INTRODUCTION

Valerian (*Valeriana officinalis* L.) which belongs to the family Valerianaceae is an important medicinal plant that is, due to its mild sedative and sleep aid, widely used in traditional medicine (Bos *et al.*, 1996). The subterranean organs of valerian contain several compounds including the essential oils and their sesquiterpenoid derivatives (valerenic acids), epoxy iridoid esters (valepotriates) and their decomposition products such as baldrinal and homobaldrinal, and alkaloids (Isetts, 2010). Valerian is the 8th top-selling herbal supplement in North America (Blumenthal, 2001), making it very attractive to improve agronomical practices and in general its production increases. Therefore, eliminating the unfavourable circumstance conditions for valerian is one of the primary and most important strategies in it`s production. The genus *Valeriana* contains about 200 species, which are mostly found in the cold and temperate regions and majority of species possessed high water requirement (Bernatb, 1999). It is reported that the lower water saturation levels had an adverse effect on valerian production (Berbec, 1965). Considering literature, plant growth and functionality suppression under drought stress condition result from morphological, physiological and molecular changes. In limiting water states, plants tend to close their stomata, resulting in reduction of CO_2 flow into the leaves for fixation. As result, far less amounts of $NADPH^+H^+$ and ATP are consumed within the Calvin cycle. Consequently, the concentration of $NADP^+$, and thus that of potential electron acceptors for electron transport chain decreases. Thereafter, electron flow on free oxygen and finally oxygen radicals are induced. The ROS are highly reactive and can seriously damage plants by protein degradation, DNA fragmentation, lipid peroxidation, membrane destruction, and ultimately cause cell death (Beligni and Lamattina 1999).

In plants, several defence systems contribute with together to alleviate effects of any stress factor. One of the important defensive systems is the involvement of phytohormons (Farooq *et al.*,

2009). Literatures have emerged that abiotic stress modulated the accumulation of polyamines in many plant species (Alcázar *et al.*, 2011; Shi and Chan, 2014), and these observations revealed the possible implication of polyamines to increase plant stress tolerance. Polyamines (PAs) including putrescine (Put), spermidine (Spd), and spermine (Spm), are mainly accumulated in plants exposed to environmental stresses, and play a key role in stress tolerance which depends on species and stress intensity and duration (Tavladoraki *et al.*, 2012). Limited water resources beside progressive demand for food production encouraged researchers to improve water use in agriculture. Recently, researches pursue to apply polyamines exogenously to ameliorate the adverse effect of environmental stresses such as drought stress (Mustafavi *et al.*, 2015). For instance, Nayyar *et al.*, (2005) found that exogenous application of Put and Spd substantially improved the drought tolerance in soybean. It was also shown that plants mainly change range of essential oils production as defense system when exposed to stress conditions (Ramakrishna and Ravishankar, 2011). Saeidnejad *et al.*, (2013) reported that drought stress increased essential oil content of *Bunium persicum* (Boiss.) B. Fedtsch. plants. Recently researches have deliberately applied drought stress during the cultivation of medicinal plants in order to stimulate the accumulation of pharmaceutical products such as essential oils. In this regard there is a fundamental question which exogenously applied substance can trigger in plant the accumulation of secondary metabolites with no impact on medical use. Therefore, in this study we followed the hypothesis that by providing polyamines exogenously, the synthesis of pharmaceutical metabolites would be increased.

This study was aimed to determine the effects of several drought stress states accompanied with exogenous application of PAs on biochemical characteristics and essential oil production of valerian plants.

2 MATERIALS AND METHODS

A pot experiment was conducted in greenhouse condition at the University of Maragheh, Maragheh, Iran. Pots with 8 L content were filled with 12 kg of soil (clay: clean sand; 2/1 v/v). The soil had been passed through a mesh number 10. Then, 20 days-old valerian seedlings were obtained from medicinal plants institute, Tehran, Iran. Uniform seedlings were collected and then transplanted into each pot. The pots were placed under a rain-shelter based on completely randomized design. During the next 30 days after transplantation, plants were irrigated with tap water, and then divided into four lots subjected to different water levels. Drought stress levels were measured using control plants with 100 % water availability (L1), 70 % (L2), 50 % (L3) and 30 % (L4) of available water. The pots were weighed daily and water was added to maintain soil moisture content. In this project, polyamines including putrescine, spermidine and spermine, (Sigma Chemical Co.) were sprayed 2 times in 1 mM concentration. In order to eliminate the effect of water from test results, untreated plants were sprayed with distilled water. The first spray was made 35 days after transplanting and the second repeated after 15-days. The plants were sprayed with a manual pressure pump at an average of 10 ml. All of the observations, except essential oil content, were taken three days after later foliar application of PAs.

Water status of leaves was determined by measuring relative water content (RWC). The leaves were subsequently rehydrated in distilled water for 4 h to obtain the turgid mass (TM), and dried for 48 h at 72 ◦C to obtain the dry mass (DM). RWC was calculated by the ratio: RWC = $[(FM − DM)/(TM − DM)] \times 100$. For measurement of electrolyte leakage, 20 leaf discs from the young fully expanded leaves were placed in 50 mL glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 mL of distilled water and allowed to stand in the dark for 24 h at room temperature. Electrical conductivity (EC_1) of the bathing solution was determined at the end of incubation period. Vials were heated in a temperature-controlled water bath at 95°C for 20 min, and then cooled to room temperature and the electrical conductivity (EC_2) was measured. Electrolyte leakage was calculated as percentage of EC_1/EC_2.

Chlorophyll-a, -b, and carotenoid were determined according to the method of Arnon (1949). Fresh leaves were taken from the plants and triturated in 80 % acetone. The absorbance of the extracts was measured at 663, 642 and 472 nm using a spectrophotometer (BioTek, PowerWave, USA). Proline content of leaf tissues was estimated spectrophotometrically following the ninhydrin method described by Bates *et al*., (1973). Total soluble sugars were extracted and determined by the anthrone method of Riazi *et al*., (1985).

The shoot tissues (0.5 g fresh mass) were homogenized in 2 mL of 100 mM potassium phosphate buffer, pH 7 containing 1 mM of EDTA and 1 % (w/v) polyvinylpyrrolidone (PVP). The extract was then centrifuged at 4°C for 15 min at 12.000 ×g in a cooled centrifuge. This supernatant was used to measure the activities of guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT). Activity of GPX was determined at 25°C with guaiacol (Bergmeyer, 1974). Activity of APX was measured by following the rate of hydrogen peroxide-dependent oxidation of ascorbic acid (Nakano and Asada 1981). Finally, the activity of CAT was assayed following H_2O_2 decomposition.

Finally, 50 g of dry root was taken for determination of essential oil percentage using Clevenger instrument (European Pharmacopoeia, 2005). Essential oil yield was determined using the following formula: Essential oil yield = Essential oil percentage × Root yield. Analysis of variance appropriate to the experimental design was conducted using SPSS software (version 16). Means of each trait were compared according to Duncan multiple range test at $P<0.05$ by means of MSTATC software.

3 RESULTS AND DISCUSSION

3.1 Relative water content (RWC) and electrolyte leakage

Regarding Table 1, relative water content (RWC) was significantly decreased with decreasing the irrigation from 100 % to 30 % AWC. Levels of 70 %, 50 % and 30 % of the available water content (AWC) lead to 14 %, 34 % and 35 % of RWC to respectively (Table 1). Leaf RWC reflected the metabolic activity in tissues and it significantly declined due to water stress (Table 1). It seems that decrease in leaf RWC could have been due to unavailability of water in the root zones, which is not able to compensate for water, lost by transpiration. In this project, based on RWC measurements, foliar application of polyamines especially putrescine had not any ameliorate effects against drought stress. Paralleled with our findings, Kubis *et al.* (2014) reported that application of spermidine did not improved water status of water-stressed cucumber plants. Similar results were reported by Bolat *et al.*, (2014) who found that leaf RWC decreased with intensifying stress.

Cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance. Electrolyte leakage (El) level increased from 20 to 42 %, when AWC dropped from 100 to 30 %. Except for Put, plants treated with PAs showed a significant reduction of stress-induced electrolyte leakage depending on its type and drought stress level (Table 1). Under moderate drought stress (70 % AWC), application of Spd and/or Spm vigorously decreased El but with increasing the water shortage their positive effect was markedly reduced (Figure 1a). Similar results were also reported by Shaddad *et al.*, (2011) who reported that at very severe drought stress condition, application of polyamines did not alleviate the adverse effect of drought stress. Results reported in the present paper showed that exogenous Spd and Spm could effectively alleviate membrane damage induced by water shortage up to 50 % AWC (Figure 1). The observation is in harmony with the results of Chattopadhayay *et al.*, (2002) on rice and Li *et al.*, (2014) on white clover plants. They proposed that exogenous spermidine and spermine effectively protected cells against damage by inhibition of protease and RNAse activities, which probably helped in maintaining membrane integrity. In addition, polyamines may mediate a decrease in ion fluxes across the vacuolar membrane by blocking fast-activating vacuolar channels under salt stress, as suggested by Pottosin *et al.* (2014). Besford *et al.*, (1993) reported that dicotyledons mainly accumulate Spd and Spm with a decline in Put. Therefore, according to their findings it seems that Put had detrimental effect on dicotyledons plant such as valerian.
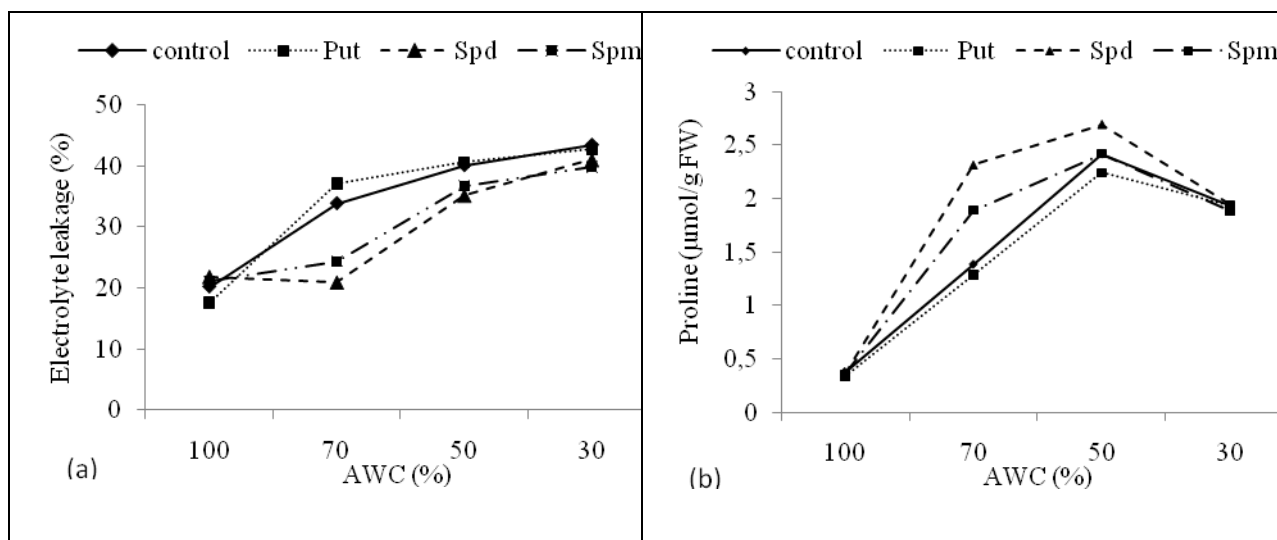

Figure1: Effect of foliar PAs treatments on valerian leaves (a) electrolyte leakage and (b) proline concentration under different available water content levels (AWC).

3.2 Osmotic adjustment

Proline is considered to act as a compatible osmoticum and is perhaps involved in drought tolerance (Bandurska, 2000). In the present study, in response to drought stress, proline concentration was gradually increased reaching a peak value at 50 % AWC and then commenced to reduce (Table 1). The lowest proline content was obtained with normal irrigation (control), while the highest was obtained at irrigation with 50 % AWC. Proline accumulation is in accordance with the fact that many higher plants accumulate free proline in response to drought stress (Farooq *et al.*, 2009). Several possible roles have been attributed to accumulation of proline: osmoregulation, detoxification of free radicals, conservation of nitrogen and energy for the post-stress period and regulating the stress protective proteins (Khedr *et al.*, 2003). Although stressed plants sprayed with Spd and Spm exhibited 19.7 and 7.2 % higher accumulation of proline compared to PA-untreated shoots, their effects mainly depend on soil AWC (Table 1). In this respect, Spd and/or Spm treatments stimulated proline accumulation whenever soil AWC lowered and reached to 50 % (Figure 1b). Our results are in line with the results of Kubis *et al.*, (2014). They concluded that cucumber seedlings treated with PAs exhibited a definitely higher stress-evoked proline accumulation. Shi and Chan (2014) reported that polyamine and proline biosynthesis pathways might share some common substrates, and exogenous application of polyamines might result in more substrates for proline biosynthesis, especially under stress conditions.

Total soluble sugars concentration increased with increasing the drought stress levels up to 50 % AWC, however, differences were not significant, and then they reduced markedly. Foliar application of PAs failed to revert changes in carbohydrates concentration caused by the water shortage treatments (Table 1). In general, the inability of PAs in reducing water loss (Table 1) suggests that osmotic adjustment in valerian leaves cannot be regulated by PAs under drought conditions.

3.3 Antioxidant enzymes activity

Results indicated that drought stress had a significant effect ($P < 0.01$) on antioxidant enzymes activity in leaves of valerian plants. The activities of catalase (CAT) and ascorbate peroxidase (APX) were increased with the increase of drought stress from the control (100 % AWC) to 50 % AWC. However, further increase in water shortage reduced CAT activity, but maximum value of APX activity was achieved at 30 % AWC. In contrast to CAT and APX, the activity of guaiacol peroxidase (GPX) tended to decrease by increased water deficit. Maximum GPX (10.7 units/min·mg.pro) contents were noted from well-watered valerian plants, which were decreased significantly upon exposure to drought stress (Table 1). Liu *et al.*, (2011) reported that catalase activity was higher under mild drought stress than under severe drought stress and well-watered treatments. The increase of CAT and APX activity in plants under drought stress was also reported in other studies (Anjum *et al.*, 2012; Huseynova, 2012).

In current study, except for Put, exogenous PAs significantly promoted activities of CAT relative to untreated plants. High and low CAT activities were recorded from 1 mM Spd and Put, respectively (Table 1). Efficacy of PAs application on APX and GPX activities depends on water stress levels. According to Figure 2a, under moderate drought stress, only Put induced marked changes in APX activity, but by further increase in drought stress levels, treatments had not statistically significant differences with control, and adverse effects of drought stress eclipsed PAs effects. In the case of GPX activity, at all drought stress levels, exogenously applied Put and/or Spd had no significant effect on GPX activity, while Spm had dual effects, under moderate and severe drought stress, positive and negative, respectively (Figure 2b). The same result was obtained in study of Li *et al.*, (2014) who showed that exogenous Spd significantly promoted activities of antioxidant enzymes under drought stress. These findings revealed that enzymatic antioxidant activities of valerian leaves were substantially induced by Spd and Spm application (Table 1). Enhanced antioxidant defense system in PAs treated plants resulted in improving cell membrane stability, as demonstrated by lower electrolyte leakage (Table 1). Pottosin and Shabala (2014) reported that polyamines may play a dual role in the ROS scavenging process. First, PA may play a critical

role in drought stress signalling to confer adaptive responses (Toumi *et al.*, 2010). On the other hand, PAs are known to significantly enhance activity of both enzymatic (Radhakrishnan and Lee, 2013) and non-enzymatic (Asthir *et al.*, 2012)

antioxidants. Therefore, the PA control over the balance between ROS production and scavenging may "shape" H_2O_2 signal, conferring differential stress responses between species and genotypes.
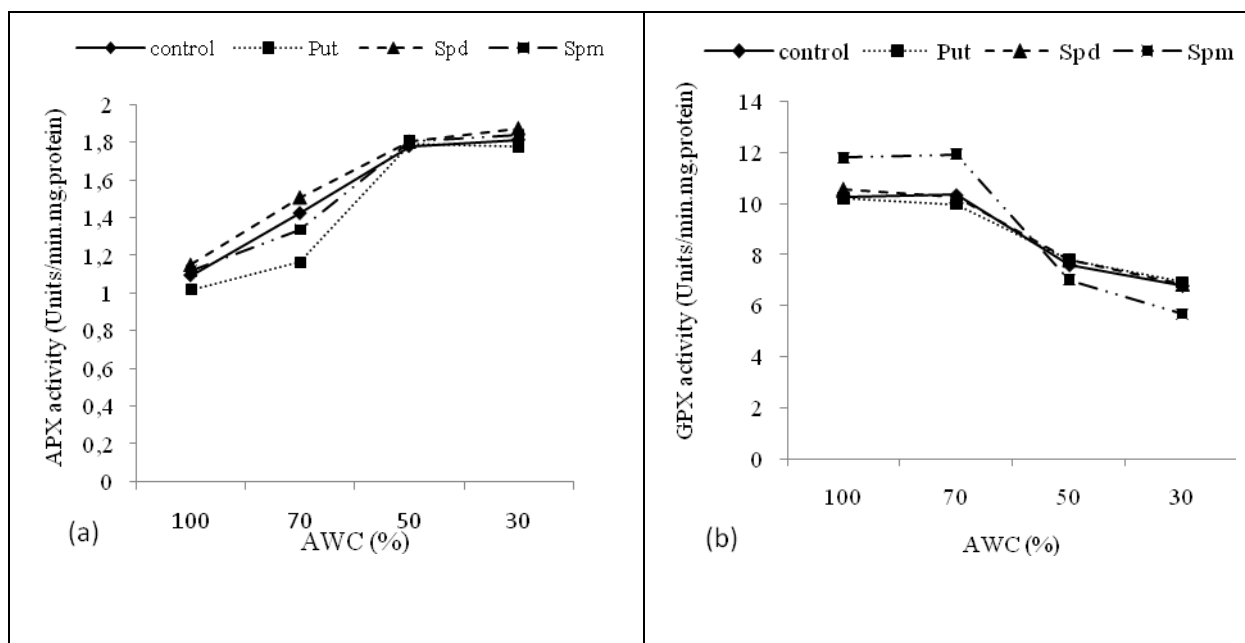

Figure 2: Effect of foliar PAs treatments on Ascorbate peroxidase (APX) activity (a) and Guaiacol peroxidase (GPX) activity (b) in leaves of valerian under different available water content levels (AWC)

3.4 Photosynthetic pigments

Drought stress significantly affected chlorophyll a, b, and carotenoids contents (Table 1). The results revealed that Chl. "a, b" contents decreased by decreasing the soil moisture content. Drought stress up to 70 % AWC induced a slight effect on the chl-a, chl-b of valerian leaf, then it reduced them markedly by the further increase in the level of drought stress. Contrary to these results, plants carotenoid values increased by drought stress increasing up to 50 % and then commenced to reduce (Table 1). Our results about chl-a and chl-b changes in response to drought stress are in agreement with reports of Liu *et al.*, (2011). Reduction in chlorophyll pigments concentration can be as a drought response mechanism in order to minimize the light absorption by chloroplasts (Pastenes *et al.*, 2005). Since carotenoid plays an important role in photo-protection (Munne-Bosch and Penuelas, 2003), the increased carotenoid content under drought conditions, indicate a higher need of photo-protection by carotenoid (Elsheery and Cao, 2008).

PAs foliar application had significant effect on chlorophyll a and b contents depending on available water content levels. According to Figure 3a, with increasing water shortage up to 50 % AWC, exogenously applied PAs had significant effects on chl.a content, but under severe conditions, differences among the treatments were not statistically significant. Under moderate drought stress, Spd and Spm application increased chl.a content, while Put had detrimental effect. In the case of chl.b content, application of Spd had positive effect when sprayed on stressed-plants. At all water shortage levels, Spm foliar application slightly increased chl. b content (Figure 3b). In normal plants, moderate drought stress lead to increasing in chla/b ratio while this ratio tends to decrease followed by severity of drought stress (Figure 3c). Results (Figure 3c) depicted that foliar application of PAs did not make any remarkable changes on chla/b ratio. Our findings manifested that Spd could positively influenced total chlorophyll (a + b) content comparing to other PAs

in all drought stress states (Figure 3d). All PAs treatments had not significant effect on carotenoid content (Table 1). In connection with these results, Chattopadhayay *et al*., (2002) found that the exogenous application of polyamines enhanced the total chlorophyll level of salt-stressed rice plants. This enhancement effect of Spd and Spm may be

attributed to increased stability of thylakoid membranes (Chattopadhayay *et al*., 2002). Thus polyamines could bind to the negatively charged phospholipid head groups on membranes, thereby influencing stability and permeability characteristics of these membranes.
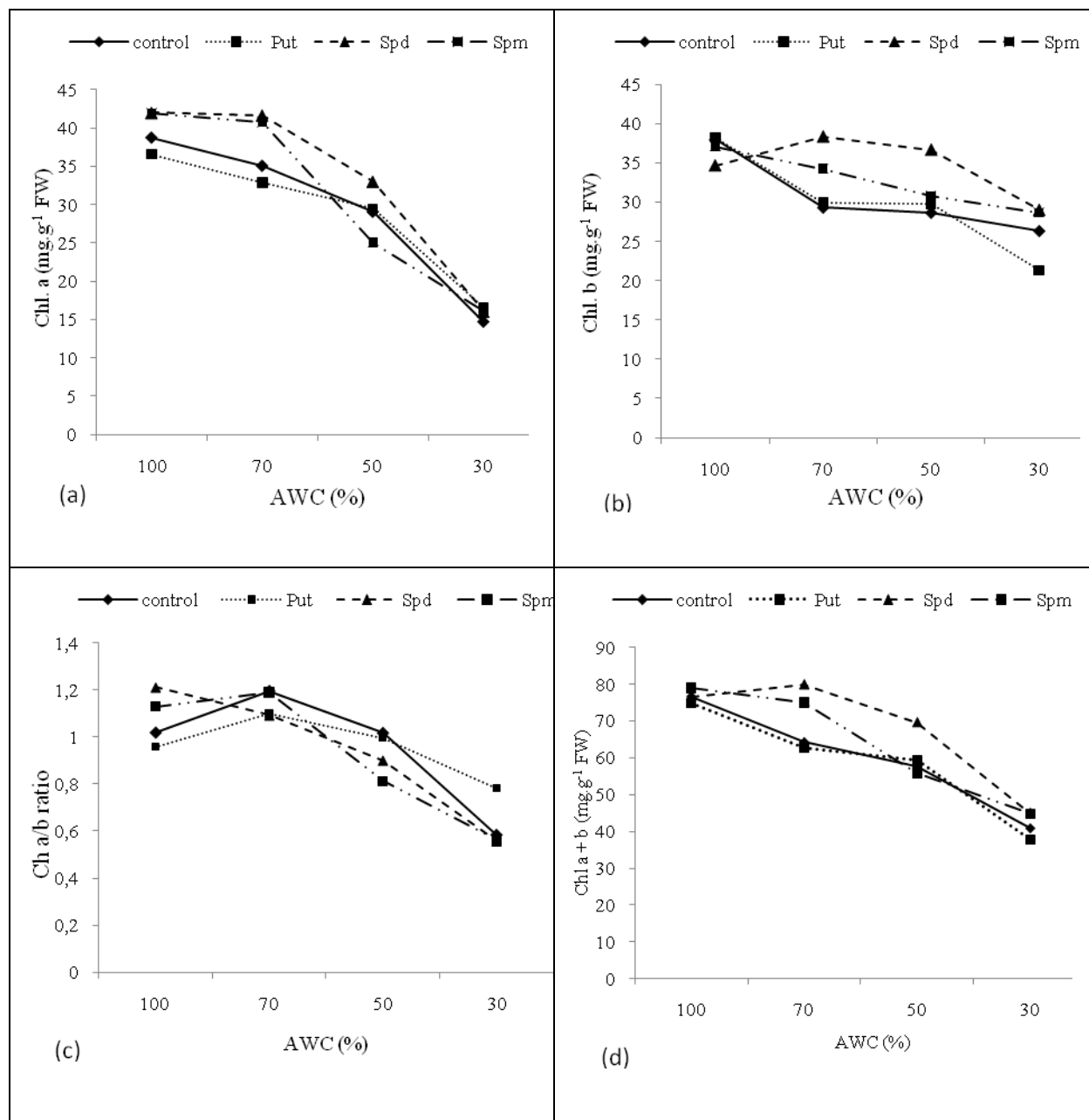
Figure 3: Effect of foliar PAs treatments on chlorophyll a content (a), chlorophyll b content (b), chlorophyll a/b ratio (c) and total chlorophyll (a + b) content in leaves of valerian under different available water content levels (AWC).

3.5 Growth and Essential oil production

Plant aerial biomass as well as root biomass was severely inhibited under drought stress, and both of them decreased with increasing the water shortage (Table 2). Aerial parts growth decreased by 28-50 % while subterranean organs growth decreased by 21-35 %. Therefore, roots were less affected by drought stress than aerial parts (Table 2). Root is the first organ to come into contact with water stress in the rhizosphere, thus supplying assimilate for this organ may be a best strategy for their resistance. Application of PAs significantly improved root and above ground organs growth under drought stress (Table 2). Maximum root biomass (economical part) was recorded from Spm foliar application, which was at par with Spd treatment (Table 2). PAs exogenously applied to plants grown under drought stress mitigated the adverse effect of stress on plant growth which may be attributed to stimulatory effects of PAs on defence systems and chlorophyll protection as as our data indicated (Table 1). Our results are in line with previous studies (Shaddad *et al.*, 2011; Amooaghaie, 2011) where exogenous PAs alleviate stress-induced growth inhibition possibly due to protection of membranes and minimization of oxidative damage.

Essential oil percentage increased when soil AWC dropped to 50 %, and then it declined with drought stress intensifying (Table 1). The highest essential oil content was achieved at 50 % AWC, which accumulated by 63 % when compared with control. As shown in Table 1, increasing of drought stress level from 50 % to 30 % had an adverse effect on essential oil accumulation in valerian. Bernatb (1999) reported that sufficient and continuous water supply did not only enhance essential oil content of valerian plant but also it could decrease essential oil content. Our results are in agreement with those previously documented by Rebey et al., (2012) on *Cuminum cyminum* L. and Hassan and Ali (2014) on *Coriandrum sativum* L. who revealed that the essential oil content was positively affected by drought stress treatments. Exogenously applied PAs remained generally ineffective on essential oil accumulation (Table 2). It seems that the products of polyamine catabolism, more involved in the synthesis of proline and did not contribute in terpen biosynthesis. Essential oil yield is a dependent variable determined by root yield and essential oil percentage. The highest essential oil yield (301 mg) was achieved in plants exposed to 50 % AWC. The lowest value for essential oil yield was achieved when plants exposed to very severe drought stress (30 % AWC). In PA-treated valerian plants, the essential oil yield increased by 18 %, 20 % and 19 % with Put, Spd and Spm, respectively. Essential oil improvement by PAs mainly attributed to positive effects of these growth regulators to root growth.

Table 1: Effect of foliar PAs treatments on some physiological and biochemical traits of valerian under drought stress

	RWC (%)	El (%)	Chl.a	Chl.b	Car	CAT	APX	GPX	Proline (μmol. g^{-1} FW)	Total soluble sugars ($mmol.g^{-1}$ DW)
			(mg.g^{-1} FW)			Units/min·mg.protein				
AWC (%)										
100	78.17a	20.15d	39.77a	37.0a	1.27c	12.08d	1.09c	10.7a	0.35d	99.8a
70	67.44b	29.02c	37.55b	32.9b	1.32b	18.56b	1.35b	10.6a	1.71c	108.2a
50	51.59c	38.12b	29.12c	31.4b	1.39a	21.80a	1.79a	7.55b	2.44a	110.0a
30	51.05c	41.74a	15.81d	26.3c	1.24c	14.30c	1.82a	6.55c	1.92b	80.58b
A	**	**	**	**	**	**	**	**	**	**
Control	63.05ab	34.36a	29.33bc	30.5bc	1.30a	14.96c	1.52b	8.74a	1.52c	93.58a
Putrescine	58.35b	34.49a	28.83c	29.8c	1.31	14.63c	1.43c	8.72a	1.45c	100.6a
Spermidine	65.61a	29.69b	33.16a	34.6a	1.32a	20.03a	1.58a	8.86a	1.82a	100.2a
Spermine	61.24ab	30.49b	30.93b	32.7ab	1.29a	17.13b	1.52b	9.10a	1.63ab	104.1a
B	*	**	**	**	ns	**	**	ns	**	ns
A*B	ns	**	**	**	ns	ns	**	**	**	ns

Different letters in each column indicating significant difference at $p \le 0.05$.

ns: non-significant   Significant at: $*P>0.05$, $**P>0.01$, $\# P>0.1$

Table 2: Effect of foliar PAs treatments on quantitative and qualitative yield of valerian under drought stress

	Aerial biomass (g/plant)	Root biomass (g/plant)	Essential oil content (%)	Essential oil yield (mg/pod)
AWC (%)				
100	116.9a	64.40a	0.44c	283a
70	83.66b	50.30b	0.54b	274a
50	64.16c	45.46c	0.72a	301a
30	58.25c	41.74c	0.42c	193b
A	**	**	**	**
Control	76.5b	44.38c	0.53a	230b
Putrescine	81.5ab	49.34b	0.56a	271a
Spermidine	80.1ab	53.45ab	0.52a	276a
Spermine	84.8a	54.74a	0.51a	273a
B	#	**	ns	#
A*B	ns	ns	ns	ns

Different letters in each column indicating significant difference at $p \leq 0.05$.
ns: non-significant   Significant at: $*P>0.05$, $**P>0.01$, $\# P>0.1$

4 CONCLUSION

To sum up, present study suggest that foliar application of Spd and Spm could ameliorate the deleterious effects of drought stress by stimulating the antioxidant enzymes activity and increasing the accumulation of proline, which protect cell membrane integrity and decrease chlorophyll loses. Albeit, PAs possessed less tangible effects on valerian plants in severe drought stress state but, it is more effective in moderate drought stress state through induction of biochemical changes. It is inferred that PAs which is economical and environment friendly alternative can be implicated to improve productivity of valerian in current scenario of drought and climate change.

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