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Assess Effect of Abscisic/Salicylic Acid Mixing Ratio Application at Different Growth Stages on Quantitative and Qualitative Traits of Cowpea *(Vigna unguiculata* L.) under Ahvaz Climatic Conditions

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ABSTRACT

BACKGROUND: Salicylic acid (SA) is a small molecule phenolic substance and secondary metabolite, which is commonly found in different plants. Abscisic acid (ABA), one of the five recognized plant hormones.

OBJECTIVES: The purpose of this research was to investigate the most effective abscisic acid to salicylic acid ratio at different growth stages and its influence on crop production and qualitative trait of Cowpea.

METHODS: Current study was conducted in the summer of 2016 in Shahid Salemi farm according factorial experiment based on randomized complete block design with 3 replications. This experiment included different ratios of abscisic acid to salicylic acid at 3 levels [including no use (control), 1.5:2 and 3:4 Mm/(mg/g)] and also included three of foliar application in different periods of growth [1-Before 30 days (slow growth period) 2- Flowering time 3- Pod appearance].

RESULT: The difference between the different ratios of abscisic acid to salicylic acid in terms of pod length, number of pods per plant, number of seeds per pod, 1000 seed weight, seed yield, biological yield, harvest index and protein percentage were statistically significant at the 1% of probability level. The difference between the levels of different periods of foliar application in all measured traits was significant at the 1% of probability level. The interaction effects of treatments on seed yield and biological yield was significant at the 1% of probability level and pod length and the number of pods per plant at the 5% of probability level. The highest seed yield, with an average of 2859 $kg.ha^{-1}$, was related to the ratio of 1.5:2 abscisic acid to salicylic acid during vegetative growth, and the lowest (with an average of 1035 $kg.ha^{-1}$) was related to the ratio of 3:4 hormones and foliar application at the pod appearance stage.

CONCLUSION: According to the obtained results, it is recommended to apply the ratio of 3:4 abscisic acids to salicylic acid during vegetative growth to increase both quantitative and qualitative yield.

KEYWORDS: *Growth regulator, Phenology, Protein, Pulse, Seed yield.*

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1. BACKGROUND

Cowpea is an annual summer legume with short, dormant, and semi-dormant cultivars, reaching a height of 50-60cm. It exhibits unlimited growth in some cultivars, while others have more limited growth (Eradatmand Asli and Mehrpanah, 2009). Hormones are one of the factors that regulate the growth of plants and the hormonal regulation of plant growth and metabolism is very complex, resulting from mutual effects between hormones (Lenobel *et al*., 2004). Abscisic acid is considered as the main factor in regulating aging processes in plants (Pospisilova *et al*., 2005). Abscisic acid and salicylic acid participate in a complex network of signal induction, regulating plant growth and development in response to the environment (Szalai *et al*., 2011). These hormones play a key role in enabling plants to adapt to environmental stress, either through synergistic or antagonistic interactions (Jaillais and Chory, 2010). By regulating different responses, hormones aid plants in coping with stress conditions (Moradi, 2016; Wang *et al*., 2009). Abscisic acid hormone is produced when stress occurs in plants, and previous studies have highlighted its important role in enhancing plants' tolerance to such stresses (Shinozaki and Shinozaki, 2000). The main role of abscisic acid is probably related to stomatal closure, which occurs when the plant is exposed to water stress or an increase in the concentration of carbon dioxide in the guard cells, such as at night when photosynthesis stops and respiration takes place. Abscisic acid's inhibitory effect is crucial, as it acts as an anti-gibberellin substance, preventing the elongation of internodes and thus delaying the growth of the stem (Fahimi, 1997). Salicylic acid is a plant growth regulator found in trace amounts in plants, existing in both free and glycosylated forms (Lee *et al*., 1995). Salicylic acid plays an important role in creating resistance to environmental stresses, including cold stress in some plants (Raskin, 1992; Faroog *et al*., 2008). The use of salicylic acid decreased the activity of catalase and increased the level of hydrogen peroxide (Janda *et al*., 2003), indicating the role of hydrogen peroxide as a marker molecule that activates and increases the activity of antioxidant enzymes (Klessig *et al*., 2000). In recent years, extensive research has been conducted on the role of salicylic acid as an important messenger molecule in plant response to pathogens (Vicente and Plasencia, 2011). Moreover, salicylic acid, being soluble in water and an antioxidant compound, is among the plant hormones that play a crucial role in plant response to various abiotic stresses, such as drought, cold, toxic heavy metals, heat and osmotic stress (Zaki and Radwan, 2011). During times of stress, particularly water shortage stress, plants synthesize the abscisic acid hormone. This leads to decreased plant growth, leaf shedding, stomatal closure to maintain plant moisture, organ aging, and chlorophyll breakdown, making it one of the growth-inhibiting hormones (Ruggiero *et al*., 2004). In contrast, salicylic acid acts as an internal growth regulator, overseeing physiological processes in plants such as growth, photosynthesis, nitrate metabolism, ethylene production, heat production, and flowering. Additionally, it confers resistance against both biotic and abiotic stresses and promotes plant growth (Hayat *et al*., 2010).

2. OBJECTIVES

The purpose of this study was to investigate the most effective abscisic acid to salicylic acid ratio at different growth stages and its influence on crop production and qualitative trait of Cowpea.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out during the summer of 2016 at Shahid Salemi's farm, located in the northern part of Ahvaz. The experimental farm is situated at latitude 31 degrees 20 minutes north and longitude 48 degrees 40 minutes east, with an elevation of 12m above sea level. Considering the crucial role of soil condition during different stages of plant growth, soil samples were collected from the field at a depth of 0-30 cm for this experiment. The obtained results are presented in table 1.

Table 1. Physiochemical characteristics of the soil field

Soil depth (cm)	Soil texture	Sand (%)	Silt (%)	Clay (%)	EC ($ds.m^{-1}$)	pH	OC (%)	N (%)	P (ppm)	K (ppm)
0-30	clay loam	27	35	38	5.9	5.9	0.45	0.04	5.2	123

To investigate the impact of abscisic/salicylic acid mixing ratio at different growth stages on the growth process, quantitative and qualitative yield of Cowpea in Ahvaz, an experiment was conducted during the summer of 2016 at Shahid Salemi farm. The experiment followed a factorial design with a randomized complete block design (RCBD) and included three replications. This experiment includes different ratios of abscisic acid to salicylic acid at 3 levels (including no consumption (control) (P_0), 1.5:2 (P_1) and 3:4 (P_2)) and also three levels of foliar application at different growth periods (before 30 days (slow growth period (V_1), flowering time (V_2), appearance of pods (V3)). Foliar application was performed using a manual sprayer (Solo model 461) at the respective growth stages.

3.2. Farm Management

The land preparation process involved plowing the soil using a rotary plow followed by desiccation. Prior to planting, chemical fertilizers, including pure nitrogen and phosphorus fertilizers, were applied at the recommended rates of 30 and 80 $kg.ha^{-1}$, respectively, based on soil test results and fertilizer recommendations. Planting was carried out as one-sided cultivation adjacent to the stacks, with a distance of 20 cm between plants on the row. The experiment consisted of 3 repetitions, each with 27 plots. Each experimental plot contained 7 lines, each 6m long.

The distance between stacks was 65cm, and each plot had a width of 5m. The distance between each block (experimental repetition) was 1.5m, and there were 3 unplanted lines between two plots (experimental units). Planting was done manually by placing a few seeds in each hole. At the end, some soil was poured on each hole to cover the seeds and irrigation was done immediately. Operations including thinning, irrigation and weed control started immediately after planting. Then, the final harvest was done at the physiological stage and it was done manually from each plot with an area of 2 square meters after removing the margins to measure the yield and yield components.

3.3. Measured Traits

To determine the pod length, 10 pods were randomly separated from each treatment and their length was measured with a ruler. In order to determine the number of pods per plant, 5 plants were randomly separated from all harvested plants and their pods were counted, and their average was considered as the number of pods per plant. To determine the number of seeds per pod, 10 pods were randomly separated from all harvested pods and their seeds were counted, and their average was considered as the number of seeds per pod. To determine the 1000 seed weight of each treatment, two random samples of 500 seeds were selected from each plot and counted. The seeds in each sample were accurately weighed. If the weight difference between the two samples was less than 5%, the combined weight of both samples was considered as the 1000 seed weight for that treatment. The final harvest was done when all the leaves turned yellow from an area equal to 2 square meters, and after separating the components and separating the seeds, the seed yield of each plot was weighed separately. In order to determine the biological yield of each plot, the entire crop harvested from an area equal to 2 square meters was dried and weighed separately. The harvest index was calculated by dividing the seed yield by the biological yield of the same treatment in terms of percentage. To determine the seed protein percentage, the nitrogen content in the harvested samples (seeds) was first measured separately using the Kjeldahl apparatus. This method includes stages of digestion, distillation, and titration. After obtaining the percentage of seed nitrogen through the Kjeldahl method, it was multiplied by a fixed factor of 6.25, as per the formula described by Linn and Martin (1999), to calculate the percentage of crude protein.

3.4. Statistical Analysis

Analysis of the results was done by Minitab statistical software and comparison of treatment averages by using Duncan's test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. *Number of pods per plant*

The number of pods per plant was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and spraying time, and the interaction effect of treatments on the number of pods per plant was statistically significant ($P \leq 0.05$) (Table 2). The highest and lowest number of pods per plant was related to the ratio of 2:1.5 (with an average of 11.59 pods) and 3:4 ratio (with an average of 7.53), respectively (Table 3). The highest and lowest number of pods per plant belonged to the use of hormones during the growing season with an average of 11.07 pods per plant and the use of hormones at the time of pod appearance with an average of 8.47 pods per plant (Table 3). The highest number of pods per plant with an average of 13.62 pods was related to the ratio of 1.5:2 abscisic acid to salicylic acid and during vegetative growth, the lowest amount of this trait (with an average of 6.85) was also related to the treatment of 3:4 hormones and foliar application at the time of pods appearance (Table 4). The number of pods is one of the important components of the yield, which can determine the number of seeds and finally the seed yield. The findings of this experiment were consistent with the results of Azarnia (2011) regarding the increase in the number of pods in the chickpea plant with the application of abscisic acid compared to the control treatment. It seems that by sprinkling salicylic acid, the water balance in the plant is maintained to some extent and conditions are provided for the inoculation of more flowers, and as a result, the number of reproductive organs per plant increases (Sajedi and Norouzi, 2020). Khan *et al.* (2010) stated that salicylic acid increases the number of pods in broad bean. Exogenous application of salicylic acid affects a wide range of processes, including seed germination (Sumart *et al.*, 2010), ion uptake and transport (Joseph *et al.*, 2010), and membrane permeability. Salicylic acid treatment has a positive effect on the yield components by affecting photosynthesis and plant growth indicators, which is especially beneficial for the plant under drought stress conditions (Bideshki and Arvin, 2010).

4.2. *Number of seeds per pod*

The number of seeds per pod was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and the spraying time, but the interaction effect of the treatments on the number of seeds per pod was not significant (Table 2). The highest number of seeds per pod was related to the ratio of 1.5:2 hormones with an average of 11.09 seeds per pod, and the lowest number of seeds per pod was obtained in the treatment of 3:4 ratio of abscisic to salicylic acid with an average of 7.73 seeds per pod (Table 3). The seed per pod was related to the use of hormones during the growing season with 10.34 seeds per pod and the lowest was related to the use of hormones at the time of pod appearance with 8.51 seeds per pod (Table 3). The findings of this experiment were consistent with the results of Azarnia's research (2011) regarding the increase in the number of seeds per pod in chickpeas with abscisic acid application compared to the control treatment. Foliar application at the time of vegetative growth with ratio of 1.5:2 of hormones also increased the number of seeds per pod due to its effect on increasing the pod length.

Table 2. Results of analysis of variance of the data in which the mean square is shown

S.O.V	df	Number of pods per plant	Number of seed per pod	1000 seed weight
Replication(R)	2	$0.06^{n.s}$	$1.0839^{n.s}$	$2.64^{n.s}$
Hormones ratio(P)	2	38.941^{**}	25.410^{**}	991.65^{**}
Spraying time(V)	2	15.224^{**}	7.5918^{**}	129.50^{**}
P*V	4	2.073^{*}	$0.1501^{n.s}$	$10.01^{n.s}$
Error	16	0.478	0.3466	5.49
CV (%)		22.26	17.87	4.39

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	Seed yield	Biologic yield	Harvest index	Seed Protein content
Replication (R)	2	$665.6^{n.s}$	$1511^{n.s}$	$3.25^{n.s}$	1.589^{**}
Hormones Ratio (P)	2	22860.8^{**}	78149^{**}	493.03^{**}	132.85^{**}
Spraying time (V)	2	13509.4^{**}	45135^{**}	92.26^{**}	2.518^{**}
P×V	4	2060.3^{**}	10947^{**}	$0.47^{n.s}$	$0.021^{n.s}$
Error	16	332.7	997	4.78	0.244
CV (%)		34.29	20.49	24.17	13.81

^{ns}, * and **: no significant, significant at 5% and 1% of probability level, respectively.

The application of salicylic acid at the beginning of vegetative growth increased the number of seeds per spike in wheat (Shoaa and Miri, 2012). Salicylic acid causes an increase in some plant hormones including auxins and cytokinins, thereby improving growth and increasing photosynthesis, and as a result, affects the yield of the plant (Sakhabutidnova *et al*., 2003). It seems that its application in the vegetative growth period has increased the leaf area index, which has increased the absorption of solar radiation and assimilation and has a greater ability to produce seeds per pods. Hashemi *et al*. (2015) investigated the barley plant and stated that the application of salicylic acid improved the number of seeds per spike, so that the number of seeds per spike in foliar treatments at the beginning of tillering is significantly different from other application treatments. It seems that foliar application with ratio of 1.5:2 abscisic acids to salicylic acid has increased the number of seeds per pod by improving the physiological indicators of growth, absorption of radiation and more assimilation. With the usage of growth regulators, the distribution of growing materials to vegetative parts is reduced and the share of seeds from these materials increases. Therefore, the increase in the number of seeds can be seen as the result of reducing the proportion of sterile florets before pollination (Sedaghat and Emam, 2016). Abscisic acid improved the physiological indices of growth by affecting stomatal conductance and reducing evaporation from the leaf surface (Davies and Zhang, 1991), and with ratio of 1.5:2 of hormones, the number of seeds per pods increased.

Table 3. Mean comparison effect of treatment on studied traits

Treatment	Number of pods per plant	Number of seed per pod	1000 seed weight (g)	Seed yield ($kg.ha^{-1}$)
Hormones ratio				
P_0	10.34 ^{ab}	9.55 ^{b}	221.85 ^{ab}	1656.6 ^{b}
P_1	11.59 ^{a}	11.09 ^{a}	225.36 ^{a}	2214.6 ^{a}
P_2	7.53 ^{b}	7.73 ^{c}	205.68 ^{b}	1208.7 ^{c}
Spraying time				
V_1	11.07 ^{a}	10.34 ^{a}	221.36 ^{a}	2074.7 ^{a}
V_2	9.92 ^{b}	9.52 ^{b}	217.76 ^{b}	1705 ^{b}
V_3	8.47 ^{c}	8.51 ^{c}	213.78 ^{c}	1300.1 ^{c}

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

P_0: control, P_1: The ratio of abscisic acid to salicylic acid (2:1.5), P_2: The ratio of abscisic acid to salicylic acid (4:3).

V_1: Before the plant is 30 days old (slow growth period), V_2: Flowering time, V_3: Pod appearance time.

Continue table 3.

Treatment	Biological yield ($kg.ha^{-1}$)	Harvest index (%)	Seed protein (%)
Hormones ratio			
P_0	5328.5 ^{b}	30.87 ^{b}	19.6 ^{c}
P_1	6253.6 ^{a}	34.87 ^{a}	24.21 ^{b}
P_2	4389.9 ^{c}	20.53 ^{c}	27.33 ^{a}
Spraying time			
V_1	6039.2 ^{a}	31.67 ^{a}	24.14 ^{a}
V_2	5309.7 ^{b}	29.28 ^{b}	23.53 ^{b}
V_3	4623.1 ^{c}	25.32 ^{c}	23.22 ^{c}

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

P_0: control, P_1: The ratio of abscisic acid to salicylic acid (2:1.5), P_2: The ratio of abscisic acid to salicylic acid (4:3)

V_1: Before the plant is 30 days old (slow growth period), V_2: Flowering time, V_3: Pod appearance time.

4.3. *1000 seed weight*

The 1000 seed weight was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and the time of application, but the interaction effect of the treatments on this trait was not significant (Table 2). The highest 1000 seed weight was related to the ratio of 1.5:2 hormones with an average of 225.36 g. The lowest 1000 seed weight was obtained from the ratio of 4:3 with an average of 205.68g (Table 3). The highest and lowest 1000 seed weight (respectively with average of 221.36 and 213.78 g) were related to the use of hormones during vegetative growth and the time of pod appearance (Table 3). The 1000 seed weight is one of the main elements in increasing the yield of plants, and the increase in this component can be attributed to the role of salicylic acid in improving and increasing the absorption of nutrients, the process of photosynthesis and transferring more assimilates from the source to the sink, which is consistent with the findings of other researchers (Zhou *et al*., 1999; Grieve *et al*., 1992; Arfan *et al*., 2007). It can be stated that foliar application during the growing season gives the plant enough opportunity for maximum growth and making more assimilates, which led to an increase in the 1000 seed weight. The results of this experiment were consistent with the findings of Hashemi *et al*. (2015), who stated that late application of salicylic acid (at the flowering stage) caused a significant decrease in the 1000 seed weight. Abscisic acid is also known as one of the important and effective factors in regulating the transfer of photosynthetic nutrients to growing seeds or fruits (Bremer and Cheikh, 1995). However, conflicting information has been reported about the role of abscisic acid in regulating the phenomenon of aging and the retransmission of assimilate (Yang *et al*., 2002). The external use of abscisic acid caused an increase in the 1000 seed weight by inducing senescence and increasing the rate of redistribution of stored compounds from secondary sources, especially stems, to growing seeds (Saiedi *et al*., 2006).

4.4. *Seed yield*

Seed yield was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and spraying time and the interaction effect treatments (Table 2). The highest seed yield is related to ratio 2: 1.5 abscisic to salicylic acid with an average of 2214 $kg.ha^{-1}$, and the lowest belonged to the ratio of 4:3 hormones(with an average of 1208 $kg.ha^{-1}$) (Table 3). The highest seed yield was related to the application of hormones during the vegetative stage with an average of 2074 $kg.ha^{-1}$, and the lowest (with an average of 1300 $kg.ha^{-1}$) was related to the application of hormones at the time of pod appearance (Table 3). The highest seed yield with an average of 2859 $kg.ha^{-1}$ belonged to the treatment with ratio of 2:1.5 hormones at the time of vegetative growth stage (Table 4). Foliar application with a ratio of 2:1.5 abscisic acid to salicylic acid during vegetative growth increased the seed yield due to its effect on increasing physiological indices and yield components (number of pods per plant, number of seeds per pod and 1000 seed weight) (Table 4). According to the results, the treatment of plants with salicylic acid during vegetative growth had a greater effect on the number of pods per unit area, and considering that salicylic acid is known as a quasi-hormonal substance, it seems that this substance has an effect on vegetative and reproductive meristems and increases the number of pods. The exact mechanism of action of salicylic acid is not yet known, but it is possible that salicylic acid, like auxin, is involved in the regulation of cell elongation and division (Singh, 1980). These results were consistent with the findings of Krishna *et al*. (2004). They reported that spraying black mung beans with salicylic acid increased the number of pods per plant and ultimately increased the yield. It was also reported that the highest yield of corn was obtained when the corn plant was sprayed with 100 $mg.kg^{-1}$

salicylic acid solution (Shuting *et al*., 1997). Kothule *et al*. (2003) reported that the use of salicylic acid increased soybean yield. Saeidi *et al*. (2006) reported that foliar application of other regulators after the flowering stage had not effect on increasing seed yield. The use of abscisic acid in wheat led to an increase in seed yield. This effect was attributed to the maintenance of large amounts of chlorophyll and relative water content, which, in turn, supported the continuous process of photosynthesis (Travaglia *et al*., 2007).

Table 4. Mean comparison of interaction effect of treatment on studied traits

Hormones ratio	Spraying time	Number of pods per plant	Seed yield ($kg.ha^{-1}$)	Biological yield ($kg.ha^{-1}$)
P_0	V_1	11.38 abc	1985 bc	5881.6 b
	V_2	10.26 b	1629.3 c	5100.8 bc
	V_3	9.37 bc	1355.5cd	5003 bcd
P_1	V_1	13.62 a	2859.3 a	7518.9 a
	V_2	11.97 ab	2275.3 b	6457.9 ab
	V_3	9.2 bcd	1509.3 c	4783.9 c
P_2	V_1	8.22 c	1379.9 cde	4717 c
	V_2	7.53 cd	1210.5 de	4370.4 cd
	V_3	6.85 d	1035.6e	4082.4 d

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

P_0: control, P_1: The ratio of abscisic acid to salicylic acid (2:1.5), P_2: The ratio of abscisic acid to salicylic acid (4:3)

V_1: Before the plant is 30 days old (slow growth period), V_2: Flowering Time, V_3: Pod appearance time.

4.5. *Biological yield*

The results of table (2) showed that the biological yield was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and spraying time and the interaction effect of the ratio of hormones and spraying time. The highest biological yield was related to 2:1.5 ratio of hormones (with an average of 6253 $kg.ha^{-1}$) and the lowest was related to the ratio of 4:3 abscisic acid to salicylic acid (with an average of 4389 $kg.ha^{-1}$) (Table 3). The highest biological yield was related to the use of hormones during at vegetative stage with an average of 6039 $kg.ha^{-1}$, and the lowest was related to the use of hormones at the time of pod appearance (with an average of 4623 $kg.ha^{-1}$) (Table 3). The highest biological yield with an average of 7518 $kg.ha^{-1}$ was related to the application of 2:1.5 abscisic to salicylic acid treatment during vegetative growth stage. On the other hand, the lowest biological yield belonged to the treatment of abscisic acid to salicylic acid ratio of 4:3 and foliar application at the time of pod appearance with an average of 4082 $kg.ha^{-1}$ (Table 4). The results of this experiment were consistent with the findings of Keikhah *et al*. (2017), who stated that by increasing the amount of salicylic acid up to 50 ppm, the total dry weight increased compared to the control. By investigating the effect of salicylic acid on Canola plant, it was found that salicylic acid increases leaf fresh weight, leaf dry weight, leaf specific weight and total dry weight (Sadeghi *et al*., 2010). By investigating the effect of salicylic acid in corn and soybean leaves, it was found that salicylic acid increased the leaf area and dry biomass of these plants (Khan *et al*., 2003). By investigating the effect of salicylic acid on wheat, it was determined that the use of salicylic acid increased the dry weight of wheat seedlings (Singh and Usha, 2003). Biological yield is the result of the accumulation of photosynthetic materials in different parts of the plant. In agricultural plants, factors such as soil nutrients, variety and climate affect the final dry weight of the plants, and when the photosynthesis of the plant is limited due to adverse environmental factors or lack of nutrients, its effect is determined on the total dry weight (Salehi *et al*., 2003). Biological yield depends to a large extent on the amount of growth and production of dry matter before flowering. The research results of Pirasteh Anooshe and Emam (2012) on wheat showed that spraying salicylic acid on the leaves at the time of tillering increases the biological yield. Bayatian *et al*. (2015) reported that the concentration of one micro molar abscisic acid caused a significant increase in the dry weight of bean seedlings compared to other treatments, but with increasing its concentration, the dry weight of beans decreased.

4.6. *Harvest index (HI)*

The harvest index was significantly ($P \leq 0.01$) affected by the ratio of abscisic acid to salicylic acid and spraying time, but the interaction effect of treatments on the harvest index was not statistically significant (Table 2). The highest and lowest amount of harvest index was related to the ratio of 2:1.5 hormones (with an average of 34.87%) and the ratio of 4:3 (with an average of 20.53%), respectively (Table 3). The highest rate of HI was related to the use of hormones during at vegetative period with an average of 31.67% (Table 3). Hashemi *et al*. (2015) stated that the highest harvest index in the foliar treatment was at the beginning of tillering, which was consistent with the results of this experiment. It seems that the ratio of 2:1.5 abscisic to salicylic acid had more effect on seed yield than biological yield. As a result of this treatment, in addition to the effect on plant weight-increasing factors such as photosynthesis, it probably had a role in the transfer of more assimilates to reproductive destinations (seeds) and increased the harvest index. The results of this experiment were consistent with the findings of Keikhah *et al*. (2017) who stated that with the use of salicylic acid, the harvest index in mung bean increased by 30% compared to the control. In the study of the effect of salicylic acid on chickpea plants, it was observed that the treatment of seeds with salicylic acid increases the harvest index (Kumar *et al*., 1999). The use of abscisic acid, by stimulating the senescence process and reducing the photosynthetic capacity, caused a decrease in the production of dry matter. On the other hand, it accelerated the re-transfer of the stored compounds in the stem to the growing seeds, resulting in the maintenance of seed yield in exchange for a reduction in biomass yield compared to the conditions of no moisture stress (Yang *et al*., 2002). The significant decrease in biomass observed in the abscisic acid treatment, and the increase in biomass and seed yield by the use of growth regulators in the first stage of seed growth, clearly indicate the effects of these growth regulators on the growth processes (Xie *et al*., 2004 and Yang *et al*., 2002). The external use of abscisic acid has multiple effects on the plant. For example, by closing plant stomata, it reduces current photosynthesis, or by increasing the amount of ethylene or enhancing the plant's internal sensitivity to this growth regulator, it causes plant senescence (Yang *et al*., 2003). Despite the reduction in plant growth, abscisic acid probably increases the harvest index compared to other treatments by accelerating the transfer of stored materials from secondary sources, especially stems, to growing seeds. Yang *et al*. (2002) also reported that despite accelerating senescence, abscisic acid can enhance the re-transfer of reserve materials from secondary sources to growing seeds. This acceleration of senescence during the seed-filling phase is likely a prerequisite for accelerating retranslocation from secondary sources such as stems and leaves to growing seeds (Zhang *et al*., 2005).

4.7. *Seed protein percentage*

Seed protein percentage was significantly ($P \leq 0.01$) affected by abscisic acid to salicylic acid ratio and spraying time, but the interaction effect of abscisic acid to salicylic acid ratio and spraying time on protein percentage was not statistically significant (Table 2). The highest and lowest percentage of seed protein was related to abscisic acid to salicylic acid ratio of 4:3 with 27.23% and the treatment of no use of hormones (control) with 19.6%, respectively (Table 3). The highest percentage of protein related to the use of hormones during the vegetative growth with 24.14% and the lowest was related to foliar application of hormones at the time of pod appearance with 22.23% (Table 3). The increase in the amount of proteins under the effect of salicylic acid, especially at high concentration, can be due to the stress effect of salicylic acid and the increase of anti-stress proteins, or as a result of metabolic activities and the increase of storage proteins. The final opinion in this field needs additional detailed research. The effect of salicylic acid in stimulating the synthesis of proteins has been reported by researchers (El-Tayeb, 2005). Additionally, the increase in abscisic acid and the induction of stressful conditions have been associated with protein hydrolysis and an increase in free amino acids (Singh and Gautam, 2013).

5. CONCLUSION

The overall results of this experiment showed that the treatment of 2:1.5 abscisic acid to salicylic acid and foliar application during vegetative growth significantly improved the yield and yield components of cowpea compared to the control treatment. However, increasing the ratio of hormones to 4:3 abscisic acid to salicylic acid not only had no effect on the yield and yield components but also caused a decrease in these traits. The 4:3 ratio of hormones only affected the seed protein percentage, increasing this component. Foliar application during vegetative growth resulted in a better yield as the plants had more opportunity to grow and develop vegetative organs, leading to increased photosynthesis and plant growth rate, thereby enhancing the quantity and quality of seed yield. To maximize the efficiency in improving the vegetative and functional characteristics of cowpea in terms of quantity and quality, we recommend using 2:1.5 abscisic acid to salicylic acid during vegetative growth in the region.

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FOOTNOTES

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