Effect of Acetyl Salicylic Acid (Aspirin) on Growth and Yield of Faba Bean (*vicia faba* l.) under Salinity Stress

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الملخص.

يعتبر الإجهاد الملحي أحد اهم الاجهادات اللاحيوية التي تؤثر على نمو المحاصيل، مما يقلل بشدة من إنتاجيتها. يمكن استخدام حمض أسيتيل الساليسيليك (الأسبرين)، لما له من دور مهم كهرمون نباتي، لمنع أو تقليل خسائر المحاصيل تحت ظروف الإجهاد الملحي. هدفت الدراسة الحالية إلى تقييم تأثير الأسبرين في التخفيف من الآثار السلبية للإجهاد الملحي لنبات الفول .(*Vicia faba* L.) لهذا الغرض، أجريت تجربة شبه حقلية باستخدام اصص على نبات الفول حسي التصميم العشوائي الكامل وبأربعة مكررات. تم معاملة نباتات الفول باستخدام محلول كلوريد الصوديوم (0و100 ملي مولار من كلوريد الصوديوم)، ومعاملتها ايضا بمحلول الاسبرين وذلك برش اوراق نباتات الفول بمحلول الأسبرين (0 و75 مجم / لتر) بعد شهرين من الزرع. نميت النباتات في بيئة شبه حقلية وتحت المراقبة. أظهرت النتائج أن الري بالمياه المالحة أثر سلباً على كلا من سمات النمو وانتاجية نيات الفول ($P < 0.0001$) ومع ذلك، فإن رش اوراق النبات بالأسبرين، أظهر تعزيزًا لنمو وإنتاجية نبات الفول ($P < 0.0001$) ، والتي يستدل عليها من خلال الزيادة في ارتفاع النبات، وفي عدد فروع النبات لكل نبات، وعدد القرون لكل نبات، وانتاجية البذور لكل نبات. بينت نتائج هذه الدراسة ان المعاملة بالأسبرين كان له دور في تخفيف الآثار السلبية لسمية كلوريد الصوديوم على نباتات الفول. وتوصي الدراسة باجراء المزيد من الابحاث للتحقق من تركيز الأسبرين المستخدم.

الكلمات الدلة: الفول *Vicia faba* L؛ الأسبرين؛ الإجهاد الملحي؛ الانتاجية.

Abstract

Salinity stress is considered one of the major abiotic stress factors, which strongly reduces crop productivity. Acetyl salicylic acid (common aspirin) which plays an important role as plant hormones, may be used to prevent or lessen crop losses under stress condition. The present study aimed to evaluate the effect of Aspirin in alleviating the negative effects of salinity stress for faba beans (***vicia faba* l.**). For this purpose, pots experiment was conducted involving faba bean plants. The plants were subjected to 0, and 100 mM NaCl and also treated with and without aspirin solution (75mg/L). plants were grown under semi-controlled environment in randomized complete design (RCD) with four replicates. After two months of sowing, faba bean plants were treated with salinity (100mM NaCl) and foliar sprayed with aspirin solution (75mg/L), growth and yield responses were measure at harvest. The obtained results showed that irrigation with saline water negatively affected the growth and yield parameters of faba bean ($P < 0.0001$). However, the application of aspirin to NaCl stressed plants displayed enhancement of growth and yield traits of faba beans ($P < 0.0001$), which indicated by increasing plant height and number of branches plant^{-1}. Number of pods plant $^{-1}$ and seed yield plant^{-1}. In conclusion, aspirin applicattion alleviated the negative effects of NaCl toxicity in faba bean plants. However, further research is needed to validate the aspirin doses at user level.

Keywords: Faba bean (*vicia faba* l.); Aspirin; Salinity stress; Seed yield

Introduction

Faba beans (*vicia faba* l.) is one of the oldest domesticated plants that used worldwide for human and animal nutrition as of their seeds with higher protein content (about 25%) content as well as vitamins and minerals recourses (Musallam et al, 2004; Erdogan, 2012). Besides some faba bean varieties may significant livestock feed and used for animal feeding (Boghdady et al., 2017). Also, growing of this legume is beneficial to other crops as it intensifications soil fertility by biological nitrogen fixation and increase nitrogen content of the soil (El-Dabaa et al., 2019). In 2010 the area under legumes covered almost 76 M ha worldwide, of which broad bean and faba bean occupied over 2.5 M ha (Kulig et al., 2014). In Libya faba bean is a main legume crop due to its high nutritional value of the seeds.

Worldwide the drought and soil salinity are predicted to affect up to 50% of all arable lands by the year 2050 (Aliakbari et al., 2021). In North Africa and particularly in Libya the abiotic stresses such as drought, high temperature, and

salinity have become the main challenges facing crop production per unit. Increased tolerance to salinity stress in crop plants is necessary in order to increase productivity with limited water supplies and high salinity. The genetic development of salt tolerance in different crops is having more attention by the breeders to overcome salinity problem and to maintain increases in crop production (Munns 2005). In fact, many breeders achieved noteworthy result in improving salinity tolerance in a number of agriculturally potential crops throughout synthetic selection and conventional breeding techniques. The development of new salt tolerance genotypes is very important to get use of salt affected lands. However, different efforts have been made to improve plant growth and yield under saline condition using multidimensional approaches. lately different approaches are being employed to enhance the salinity tolerance in diverse plant species. One of these approaches is supplementation of some substances as a method to overcome negative effects induced by salinity. Many substances include abscisic acid, salicylic acid, jasmonic acid, calcium and some plant extractions have been recommended as transducers and can be used as plant growth enhancers which increase plant tolerance (Klessig and Malamy, 1994). A study on aqueous extract of (*Eruca sativa* Mill) as growth enhancer for growth and yield of faba bean suggested that the plant can be used efficiently by crop producers as growth enhancer for faba bean (Ehtaiwesh and Qarimidah, 2021). Recent studies reported that Moringa leaves extract can be effectively used as plant growth enhancers for wheat seedling growth (Ehtaiwesh and Yarboa, 2020, Khan et al, 2022). Also, growth regulators are one of the most important factors for yield improvement in various field crops.

Acetyl salicylic acid (Aspirin) and salicylic acid is considered as a hormone-like substance, that play an essential role in regulating a number of physiological processes and offer protection against biotic and abiotic stresses in plant (Azooz, 2009). Al-Hilfy et al., (2017) recommended spraying plants at different growth stages with different concentrations of acetyl salicylic acid (Aspirin) and salicylic acid to improve growth and yield of faba beans. In addition, many studies addressed the potential of Aspirin supplementation to improve faba bean under water stress condition (Abdelaal, 2015). Senaratna et al., (2000) reported that acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants by signaling role of these molecules, leading to the expression of tolerance. Another study concluded that aspirin can increase the tolerance of high temperature and synchronize germination of radish seed (Takaki

and Rosim, 2000). A new study showed that foliar-applied aspirin is an effective strategy that can be used to improve the tolerance of chickpea plants to drought stress (Hussain et al., 2020). One recent study suggested that priming of wheat seeds with aspirin solution is an effective method to improve seeds germination and seedling performance (Ghafoor etal., 2021; Ehtaiwesh and Almajdob, 2021). However, few work has been done to address the effect of ASP supplementation on and growth and yields of faba beans grown in Libya. Therefor the objective of this study was to evaluate the potential of aspirin (ASP) supplementation to improve growth and yield of faba beans under salinity stress.

Materials and methods

The experiment was conducted in fall of 2020 to determine the impact of aspirin application on growth and yield of faba bean plants grown under saline condition. This study was conducted in semi-controlled environment facilities at Jodam farm in Zawia City and at the plant Science Department, University of Az Zawia.

Plant Materials Seeds of faba bean were obtained from the local market and were used in this study.

Experimental details and growth conditions

Pots experiment based on randomized complete design (RCD) with four replications was employed. The experiment consisted of faba bean plants and two salinity treatments (0, and 100mM NaCl) with or without aspirin application (75mg/l). Loamy soil was collected from the soil surface (0-10 cm). The soil was passed through a 2-mm mesh screen, and thoroughly mixed and filed in 10L pots, 10 kg of soil in each pot. five healthy seeds of faba bean were sown in each pot and irrigated with fresh water until emergence, after 30 days from sowing, the seedlings were thinned to have three seedlings per pot. Two months of sowing, pots were divided into two groups, each group represented one salinity treatment (0 and 100mM NaCl). Salinity stress was induced three times 60, 80, and 100 days of sowing by irrigated plant with saline water (100 mM NaCl). Pots in each salinity treatment were divided into two groups, each group represented aspirin treatment (with or without aspirin treatment). Aspirin application (foliar sprayed) was applied four times (55, 75. 90 and 110 days of sowing) by spraying the plants until complete covering of the plant foliage. Other plants which were not treated with aspirin were treated with water until complete covering of the plant foliage. The N, P. K fertilizer was applied as 75 mg/kg per pot in three stages of plant growth (emergence, vegetative, and

flowering). During the experiment, the pots were kept under semi-controlled condition and water applied as needed until plants displayed physiological maturity and plants got harvested.

Data Collection:

At harvest, one plant was randomly selected from each replicate to measure plant height, number of branches, number of leave, number of pods plant^{-1}, number of seeds pod^{-1}. Then, plants were dried in the oven maintained at 65 °C for 73 hours to record the dry weight. Plants were dissected into pods, leave, stems and roots to record pods, leaves, stems and roots dry weight (g). Weights were estimated in g per plant using a balance. Then number of seeds plant^{-1}, 10 seeds weight (g) and seed yield (g/plant) were calculated. The 10 seed weight was recorded in g of the weight of 10 randomly selected seeds from each replication after threshing. and seeds yield was estimated in g per plant with total weight of seeds after threshing.

Statistical analysis:

The experimental design was a randomized complete design with a split-plot treatment structure in four replications. salinity stress was the main plot factor; aspirin application was assigned to sub-plots. For the treatments, salinity had two levels (0 and 100 mM NaCl), aspirin application had two levels (with and without aspirin foliar application). Data were analyzed using GLM procedure in statistical software SAS 9.4 for mean and standard error estimation. Separation of means was carried out using the LSD test ($P < 0.05$).

Results

The P-values for growth and yield traits are presented in table 1. The independent effect of salinity was extremely significant ($P < 0.0001$) for growth and yield traits that included in this study (Table 1). The independent effect of aspiring was extremely significant ($P < 0.0001$) for some growth and yield traits such as plant height, number of branches plant^{-1}, number of pods plant^{-1}, number of seeds plant^{-1}, weight of 10 seeds (g) and seeds yield plant^{-1}. (g). Aspirin application was highly significant ($P < 0.01$) for some growth and yield traits such as number of leaves plant^{-1}, number of seeds pod^{-1}, (g), leaves dry weight plant^{-1} (g) stem dry weight plant^{-1} (g), root dry weight plant^{-1} (g) and pods dry weight plant^{-1} (g). The interaction effect of salinity x aspirin was significant ($P < 0.05$) for all growth and yield traits included in this study (Table1).

Table:1. Probability values of the effect of salinity and aspirin on growth and yield of faba bean (*Vicia faba* L) Plants.

Traits	Salinity (SAL)	Aspirin (ASP)	ASP x SAL
Plant height (cm)	<.0001	<.0001	0.0485
Number of branches plant^{-1}	<.0001	0.0001	0.0489
Number of leaves plant^{-1}	<.0001	0.0012	0.0453
Number of pods plant^{-1}	<.0001	<.0001	0.0468
Number of seeds pod^{-1}	<.0001	0.0074	0.0459
Number of seeds plant^{-1}	<.0001	<.0001	0.0408
Leave dry weight plant^{-1} (g)	<.0001	0.0095	0.0482
Stem dry weight plant^{-1} (g)	<.0001	0.0018	0.0424
Root dry weight plant^{-1} (g)	<.0001	0.0014	0.0494
Pods dry weight plant^{-1} (g)	<.0001	0.0003	0.0490
Weight of 10 seeds (g)	<.0001	<.0001	0.0449
Yield of seeds plant^{-1}. (g)	<.0001	<.0001	<.0001

Results of the independent effect of salinity on growth and yield traits of faba bean are presented in (Tables 2). All growth and yield traits of faba bean dramatically decreased under salinity stress (100 mM NaCl). Data on table 2 showed the negative impact of salinity stress on all traits investigated in this study, which clearly indicated by the decreasing of all studied traits.

Table:2. The main effect of salinity on growth and yield of faba bean (*Vicia faba* L) Plants. Data are averaged across two aspirin treatment and four replications. Means was estimated using the GLM procedure in SAS.

Traits	Salinity level	
	0mM Nacl	100mM NaCl
Plant height (cm)	63.75^{a}	43.87^{b}
Number of branches plant^{-1}	5.4^{a}	3.4^{b}
Number of leaves plant^{-1}	105^{a}	60^{b}
Number of pods plant^{-1}	12^{a}	8^{b}
Number of seeds pod^{-1}	4.25^{A}	2.84^{b}
Number of seeds plant^{-1}	50.5^{a}	21.9^{b}
Leave dry weight plant^{-1} (g)	8.25^{a}	4.75^{b}
Stem dry weight plant^{-1} (g)	14.24^{a}	7.75^{b}
Root dry weight plant^{-1} (g)	3.36^{a}	2.94^{b}
Pods dry weight plant^{-1} (g)	73.37^{a}	15^{b}

Weight of 10 seeds (g)	15.02[a]	8.35[b]
Yield of seeds plant^{-1}. (g)	77.3[a]	18.8[b]

* Individual value is the mean of 4 plants. Values followed by different letters are significantly different according to LSD test ($P < 0.05$).

The results of the independent effect of aspirin application on growth and yield traits of faba bean are presented in (Tables 3). The application of aspirin significantly increased all growth and yield traits of faba bean. Faba bean plants treated with aspirin depicted better growth and produced high yields as compared to without aspirin treatment plants.

Table:3. The main effect of aspirin on growth and yield of faba bean (*Vicia faba* L) Plants.
Data are averaged across two aspirin treatment and four replications. Means was estimated using the GLM procedure in SAS.

Traits	Treatments	
	+ASP	-ASP
Plant height (cm)	58.75[a]	48.87[b]
Number of branches plant^{-1}	5[a]	3.75[b]
Number of leaves plant^{-1}	89.87[a]	74.5[b]
Number of pods plant^{-1}	11[b]	8.5[b]
Number of seeds pod^{-1}	3.75[a]	3.34[b]
Number of seeds plant^{-1}	42.56[a]	29.84[b]
Leave dry weight plant^{-1} (g)	6.9[a]	6[b]
Stem dry weight plant^{-1} (g)	11.86[a]	10.13[b]
Root dry weight plant^{-1} (g)	5[a]	4[b]
Pods dry weight plant^{-1} (g)	29.75[a]	22.62[b]
Weight of 10 seeds (g)	13[a]	10[b]
Yield of seeds plant^{-1}. (g)	61.68[a]	34.4[b]

* Individual value is the mean of 4 plants. Values followed by different letters are significantly different according to LSD test ($P < 0.05$).

The result showed that salinity stress (100mM NaCl) reduced plant height, number of branches and number of leaves plant^{-1} of bean plants as compared non-saline condition (0 mM NaCl). However, aspirin supplementation improved these growth traits of faba beans. This result indicted that ASP application was effective and increased the attributes of growth traits compared with control (Figure 1 A, B and C).

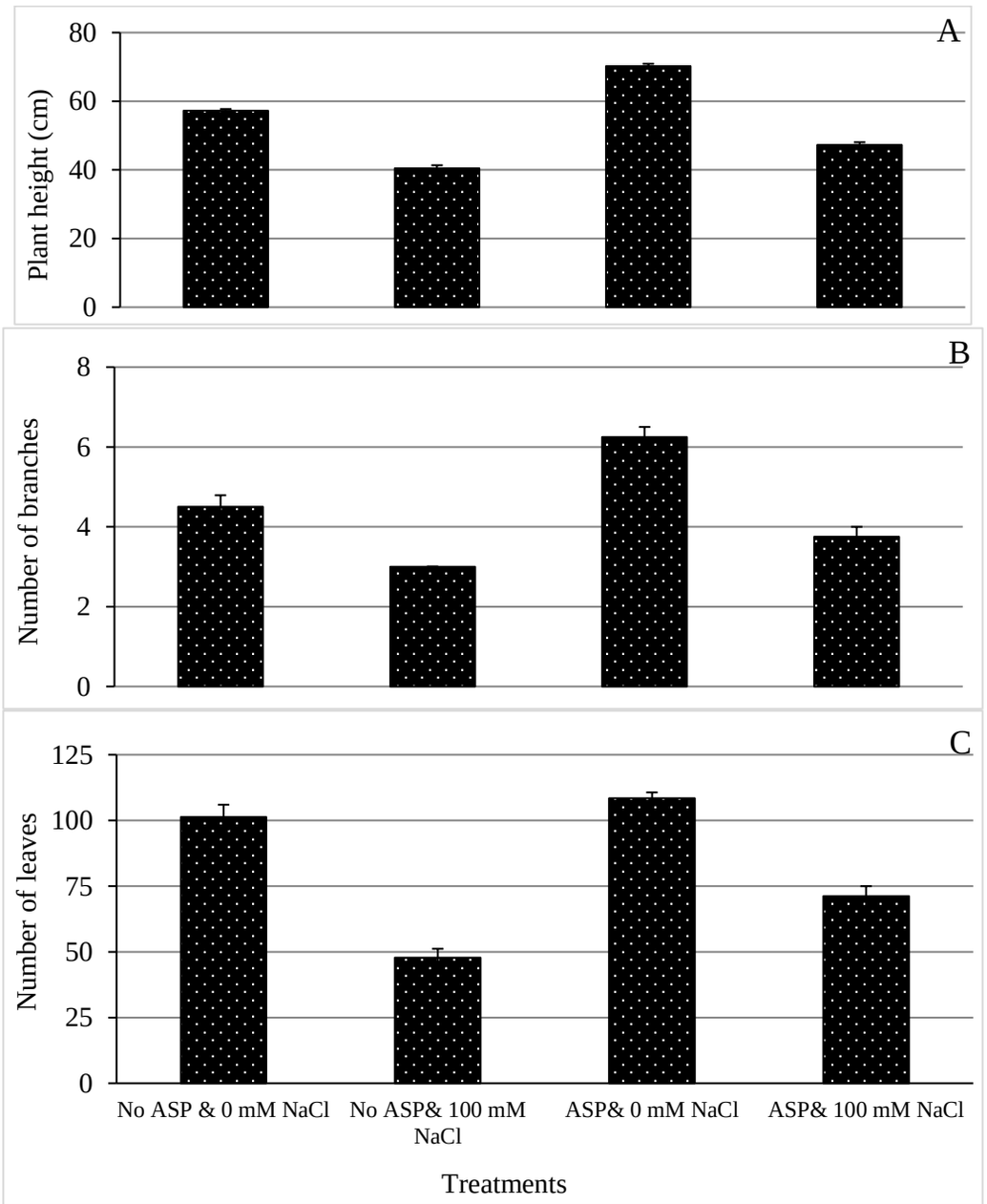

Figure 1. The effect of salinity and aspirin treatments on (A) plant height (cm), (B) number of branches and (C) number of leaves plant-1 of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

In addition, salinity stress effected plant growth and yield performance, which caused by reducing in the number of pods plant^{-1}, number of seeds pod^{-1} and number of seeds plant^{1-}. the result evidently showed a reduction in number of pods plant^{-1}, number of seeds pod^{-1} and number of seed plant^{1} as compared to the control. In fact, aspirin treatments used in this study improved number of pods plant^{-1}, number of seeds pod^{-1} and number of seed plant^{1} traits of faba plants under salinity stress as compared with untreated plants (Figure 2 A, B and C).

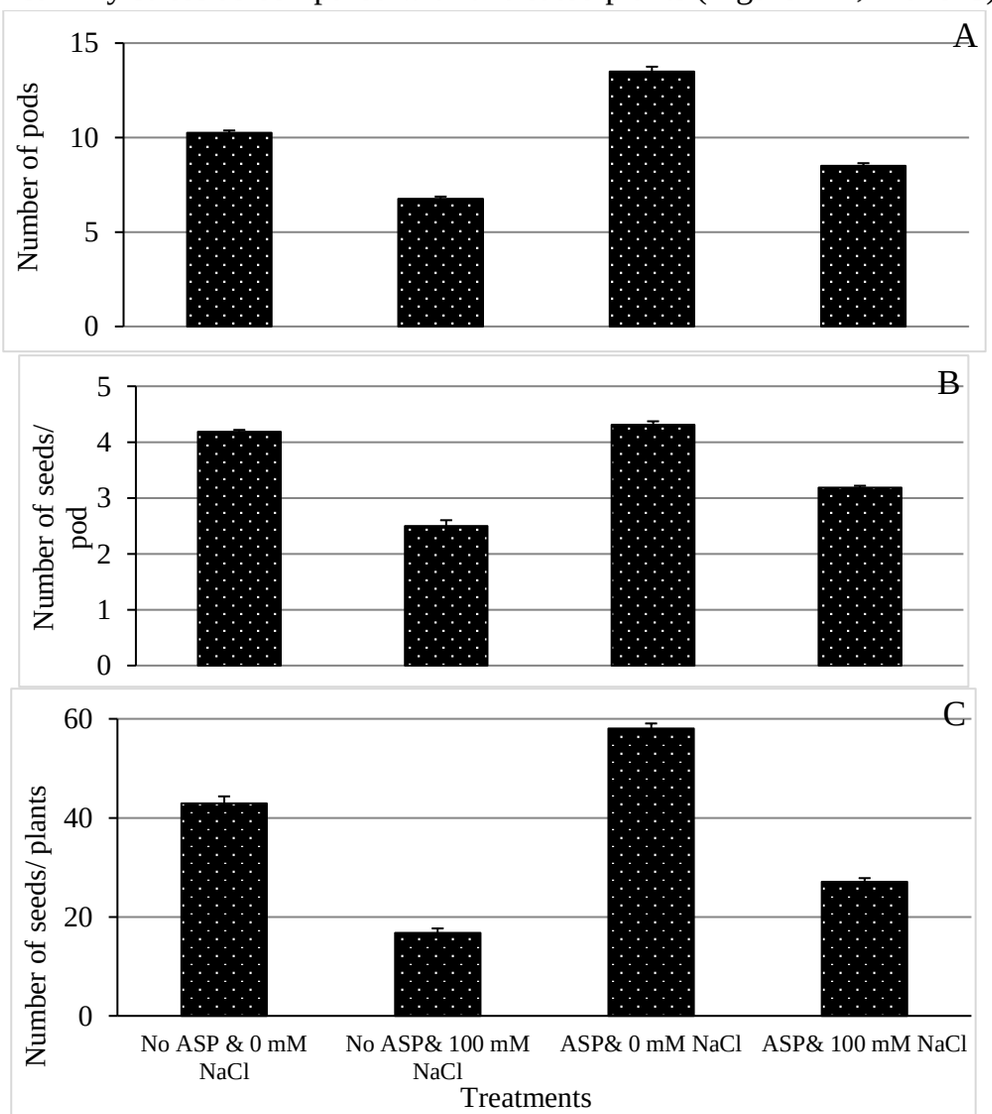

Figure 2. The effect of salinity and aspirin treatments on (A) Number of pods^{-1} plant, (B) number of seeds pod^{-1} (C) number of seeds plant^{-1} of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

Furthermore, due to aspirin treatment; leave, stem and root growth was also increased with the application of aspirin as compared with untreated seedlings in both saline and non-saline conditions. Remarkably; the result indicated that under salinity stress, leave, stem and root dry weight were reduced and the application of aspirin significantly improved those growth traits (Figure 3 A, B and C)

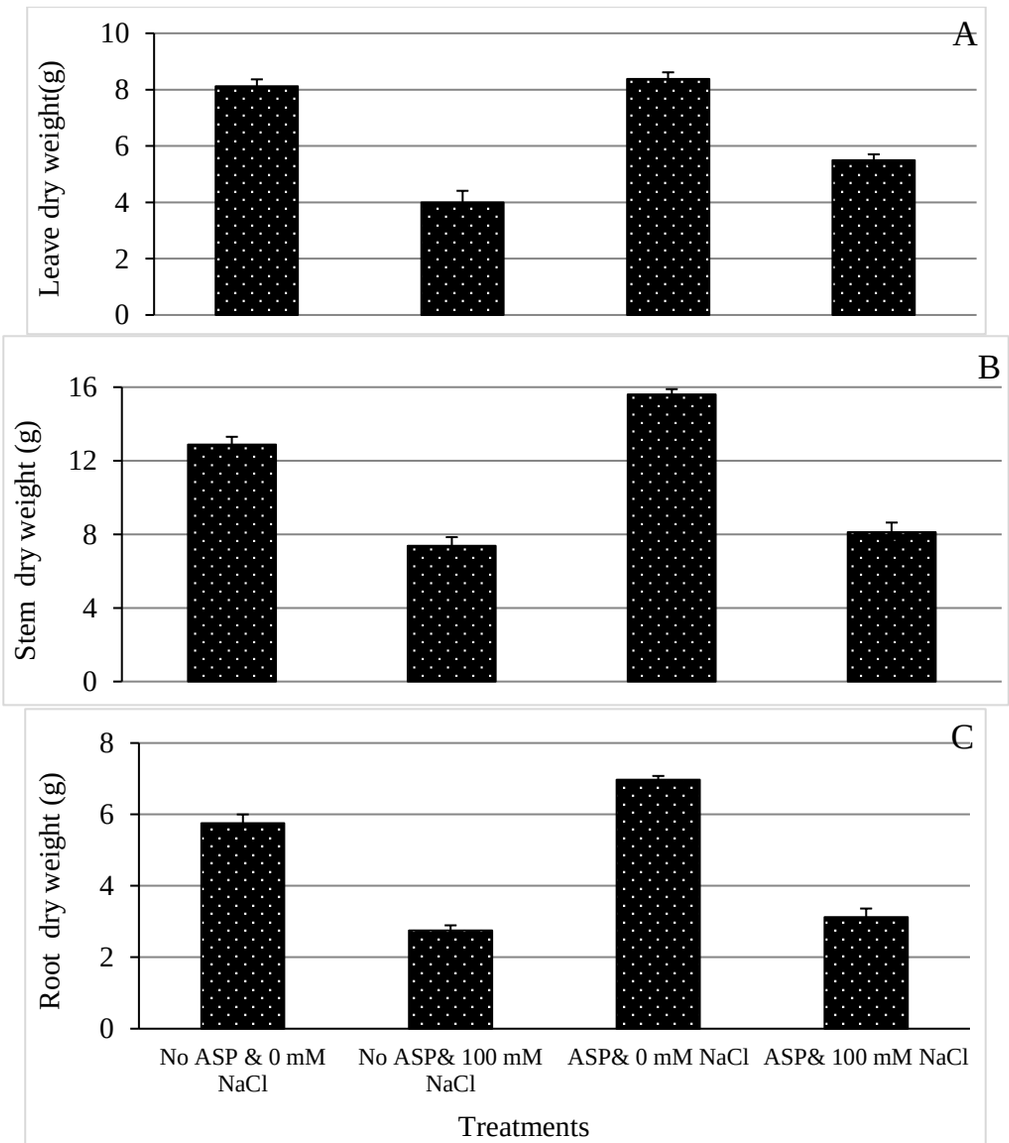

Figure 3. The effect of salinity and aspirin treatments on (A) leave dry weight (g), (B) stem dry weight (g) (C) roots dry weight (g) of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

Moreover, the result indicated that pods dry weight, seeds weight and seed yield affected by salinity stress. Nonetheless, aspirin treatment improved the above mentioned yield traits as compared with untreated plants (Figure 4 A, B and C).

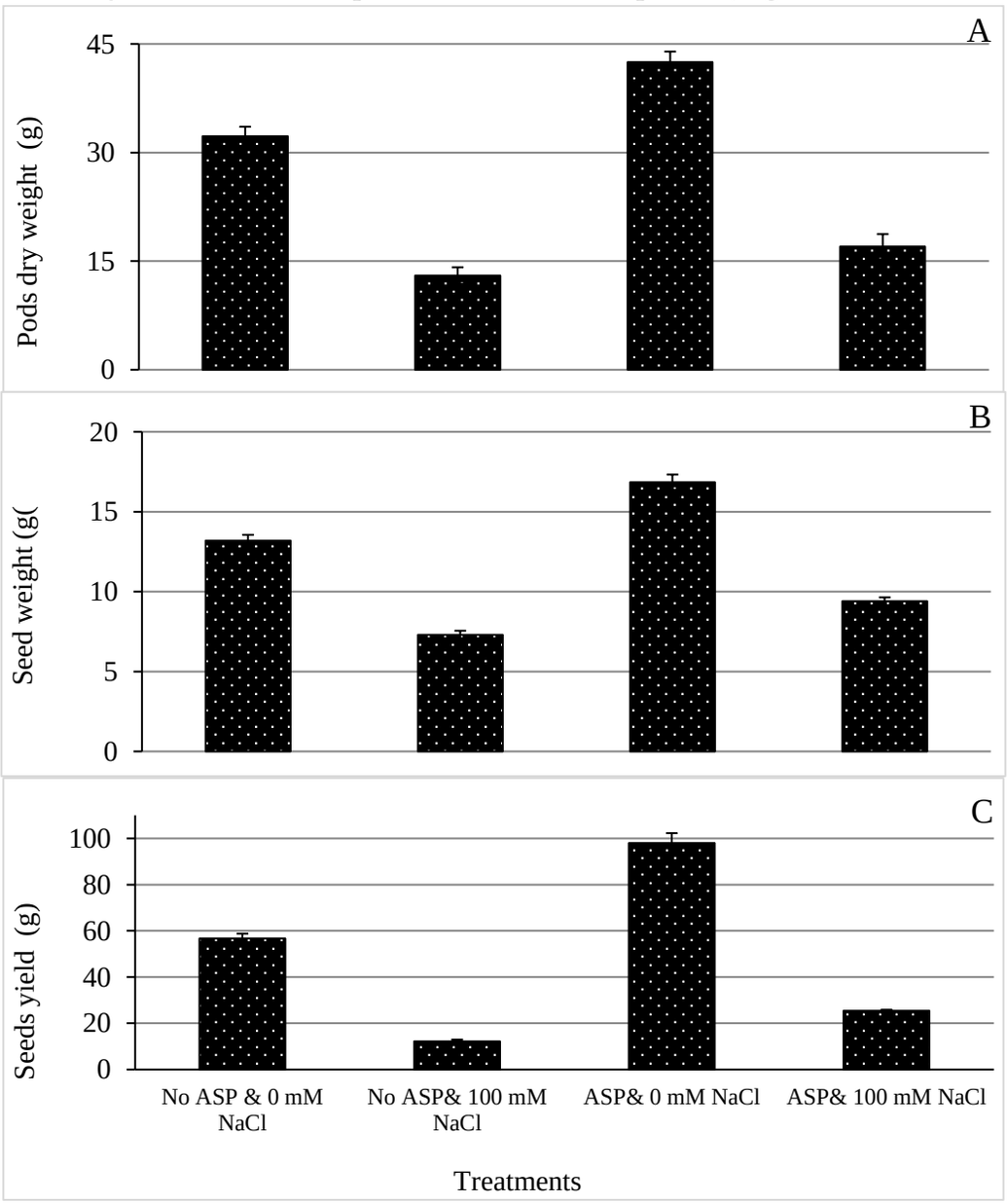

Figure 4. The effect of salinity and aspirin treatments on (A) pods dry weight plant^{-1} (g), (B) weight of 10 seeds (g) and (C) seeds yield plant^{-1} (g) of faba bean

plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

Discussion

The effects of aspirin treatment on growth and yield of faba bean (*vicia faba* l.) under salinity stress was investigated. The study indicated that salinity stress negatively affected plant growth and productivity. The effect of salinity on plant germination, growth and yield maybe due to changing in the osmotic balance between plant roots and soil solution and interfere with physiological and metabolic processes of plant (Ehtaiwesh and Rashed, 2019; Srivastava et al., 2019). Lately, different strategies are being applied to enhance the salinity tolerance in different plant species. Aspirin application have previously found to enhance plant growth in many plants include wheat, (Khan et al., 2021), Tomato (Bablee et al., 2019;), maize (Hussein et al., 2007), *Phaseolus vulgaris* L (Soliman et al., 2018) and broad leave bens (Al-Jubory and Jerry, 2020). Aspirin (acetylsalicylic acid) is a synthetic analogue of salicylic acid, which is a normally produced phenolic compound by plants and plays an important role in the controlling of some physiological processes of plants during abiotic stress (Fahad et al. 2014; Hussain et al., 2018; Djebar et al., 2020). The study revealed that the overall response of faba bean to salt stress resulted in a significant reduction in growth and yield. However, the foliar spray of aspirin revealed significant improvement in the growth and yield attributes as compared to untreated plants, this outcome agreed with the previous study that addressed the effect of aspirin on sugarcane under moisture stress condition (Sing et al., 2018). Also, salinity stress caused significant decreases in plant height, number of branches and leaves plant^{-1}. This reduction in growth parameters may be due to the reduction of water flow from the xylem to the different cells, which regulates cell division, stem elongation and leaves development as well as the decline in chlorophyll, which may lead to the reduction in photosynthetic process and influences gas exchange (Ehtaiwesh, 2016). Also the result indicated that salinity stress reduced number of pods plant^{-1}, number of seeds pod^{-1}, as well as number of seed plant^{-1}, which ultimately affected the final yield of faba bean. However; the application of aspirin had a supportive effect on growth and yield parameters such as pods and seed numbers, this was due to the fact that salicylic acid is a plant hormone and has important physiological roles in endorsing plant growth and increasing the efficiency of photosynthesis. Similar result was reported in a study of the effect of aspirin on growth and yield of Tomato plants (Sassine et

al., 2020). This study demonstrated that salinity caused a reduction in plant height, leave, stems, pod and root dry weight as compared with non-saline condition. This reduction was due to its effect on dry matter production. Yet, the application of aspirin alleviated the adverse effect caused by salinity on plant height, and leave, stem root dry weight due to the its positive role on growth and yield of plant (Rasheed, 2018). These results were similar with previous studies (Alzandi, 2018). For the weight of 10 seeds and seeds yield plant^{-1} of faba bean, the results showed that the stress decreased the two above stated traits. But the application of aspiring improved these traits. These findings were in similarity with Rasheed (2018), who found that salicylic acid's application improves seed yield of broad bean plant. These results may be as a result of salicylic acid role and its encourage of cell division which increases the level of auxin and cytokinins in plant tissues (Shakirova et al., 2003). The study illustrated that treatment of faba bean plants with aspirin under salt stress stopped the effect previously observed in the presence of salinity stress, and this result suggest that aspirin treatment could induce some mechanisms involved in the resistance and / or adaptive responses of salinity stress.

Conclusion

The purpose of the present study was to study the character of changes in growth and yield traits induced by aspirin foliar application on faba bean grown under salt stress conditions. The result conclude that the injuries caused by salinity stress to growth and yield traits were alleviated and the salt-tolerance of faba bean was elevated by aspirin application. The study concluded that the beneficial effect of aspirin application on faba bean increased salinity tolerance which may provide some practical basis for beans cultivation under salinity conditions. further studies still needed to test the efficacy of aspirin under different environmental conditions that may improve crop yield.

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