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Chapter 8
Alleviation of Salinity Stress Imposed on Broad Bean (*Vicia faba*) Plants Irrigated With Reclaimed Wastewater Mixed With Brackish Water Through Exogenous Application of Jasmonic Acid

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Abstract Farmers in Palestine suffer from a continuous shortage of water due to its scarcity. It is important to note that both brackish water and reclaimed wastewater represent major sources, although both resources are problematic, as they impose stress to growing plants. Consequently, alleviation of these stresses is required, particularly salt stress, imposed by the use of brackish water or reclaimed water. The aim of this study is to search for the means to alleviate stress through irrigation with reclaimed wastewater mixed with brackish water (mix). Jasmonic acid (JA), a plant growth regulator, proved to be efficient in alleviating various types of stresses, such as chill and drought stress. JA was tested in this study to determine whether

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it would alleviate salt stress imposed through irrigation of broad bean (*Vicia faba*) plants by a mix of reclaimed wastewater and brackish water (Electrical conductivity [EC] = 7 dS/m). Broad beans plants are considered sensitive to salinity. Results showed that treating plants with JA lessened, although only slightly, the negative impact of mix. Moreover, applying treated wastewater using drip irrigation in addition to cultivating plants in pots prevented the contamination of fruits with the pathogens. Treating plants with JA enhanced the plant's tolerance to stress conditions imposed through irrigation of plants with alternative water resources.

8.1. Introduction

Palestine is located in southwest Asia on the eastern shore of the Mediterranean, in the center of the Middle East. There are two distinct climatic seasons in Palestine: a wet winter and a dry summer. The rainy season extends from mid-November to the end of April, with an average annual rainfall in the Palestinian Territories (PT) of approximately 450 mm. However, temperatures are relatively high: January is the coldest month with average temperatures between 8 and 12 °C, while August is the warmest month, with temperatures ranging between 22 and 34 °C. The Jordan River system is the only surface water resource in the PT, but there are two aquifers: the Mountain Aquifer underlying the West Bank, and the Coastal Aquifer underlying the Gaza Strip. The problems related to water that Palestine presently faces are abundant and varied. Palestine and Jordan, as well as most other Middle Eastern countries, are generally characterized as arid and lacking in water resources. The PT are expected to experience a serious water deficit in the year 2020, the shortage of which will be 271×10^6 m^3. Numerous studies and plans for expanding water resources exist, including desalination and water transfers from other basins. However, in most cases, these plans are expensive and face daunting logistical and political barriers (Mimi et al., 2003).

In recognition of the scarcity of water and inevitable population growth in the region, it has become vital to conserve existing water. The supply and management of water resources and wastewater remain a key priority for the PT. The wastewater-related problems in the environment have been documented as continuously increasing due to the increasing discharge of wastewater as a result of the increasing demand of fresh water. These environmental problems include the gradual increase in nitrates of both groundwater wells and some freshwater springs (Ministry of Planning and International Cooperation, 1998). The use of treated wastewater in the PT to meet increasing agricultural water demands has been identified as one of the main objectives of the Palestinian water sector. The total volume of treated urban wastewater suitable for reuse is projected to be 12.1×10^6 m^3/year for the main Palestinian cities by the year 2010. In comparison, total agricultural water demand is projected to increase by 50×10^6 m^3 over the years 2006 to 2010 (Meerbach, 2004). Due to the current political climate, an increase in the fresh water supply is not a viable option. Therefore, water reuse is the key to agricultural development. Various studies have been conducted to assess the feasibility of reuse at several locations in the West Bank, but implementation of a comprehensive water reuse project is still pending the approval and construction of wastewater treatment plants.

From the agronomic side, several factors and conditions restrict the use of treated wastewater in agriculture, the most important of which are crop type, irrigation system and socio-cultural factors. However, some of the potential hazards of using reclaimed wastewater for plants are salinity, specific ion or element toxicity, direct injury to leaves (Johnson and Parnell, 1998), nitrogen overdose (Feigin et al., 1991) and water stress. Consequently, using reclaimed wastewater forced horticulturists to adopt various techniques and means to deal with these hazards. Among these techniques are the leaching of accumulated salts by over-irrigation and mixing water of different sources to attain a lower irrigation water salinity level, application of Ca-source (e.g., $CaSO_4$) to counteract the sodicity and treating plants with growth regulators (e.g. Jasmonic acid [JA]) to lessen the negative impact of salinity.

The main goal of this research is to investigate the suitability of using reclaimed wastewater mixed with brackish water (mix) as alternative water resources in irrigating agricultural crops in the Palestinian highlands, combined with the use of the natural growth regulator (JA) to alleviate the negative impact of reclaimed wastewater and/or brackish water.

8.2. Materials and Methods

8.2.1. Location

The experiments were conducted at the wastewater treatment plant in Al-Bireh City. The treatment plant processes approximately 1.25×10^6 m^3/year of raw municipal wastewater. It consists of oxidation ditches and secondary clarifiers. The reclaimed water has a tested quality of 10/10 mg/L biochemical oxygen demand/total suspended solids (BOD/TSS; BOD <10 mg/L, TSS <10 mg/L), 30 to 40 mg/L total nitrogen and less than 100 CFU/100 mL fecal coliform level. Because there are no adjacent agricultural lands, the effluent is not normally reused and is discharged to the Wadis.

8.2.2. Planting Material

Broad bean seeds (*Vicia Faba*) cv. Primarence were planted in 12-L pots filled with soil mixture composed of peat moss, sand and clay in 2:1:1 ratio (by volume). Each pot was fertilized before planting with 10 g of 14:7:28 ($N:P_2O_5:K_2O$) starter fertilizer. Plants were distributed in the greenhouse and divided to three groups. Water was applied to all plants using drip irrigation. Plants in group one were irrigated with reclaimed water (RW), plants in the group two were irrigated with mix and plants in group three were irrigated with fresh water. The electrical conductivity (EC) of the mix was 1.5 dS/m at the start of treatment period, and increased gradually to 7.0 mmohs.cm^{-1} by the end of the growing season. During the first three weeks of the growing season, all plants were irrigated with freshwater three times per week until plants reached a height of 15 cm. After that, each pot received the designated treatment. Each plant was fertilized with 2 g of 14:7:28 (N:P_2O_5:K_2O) at weekly intervals, and the quantity of water applied was increased gradually in proportion to plant grown and climatic conditions, and reached 2.5 L by the end of experiment.

8.2.3. JA Treatments

Within each group, there were four JA treatments (0.0, 0.5, 1.0 and 1.5 mM). JA solutions, containing a few drops of Tween-20, were applied exogenously to plants three times during the plant growing season: the first time was 42 days from planting, the second application was at the beginning of flowering (after 70 days from planting) and the final application was 120 days from planting. Each plant was sprayed with 0.25 L at the first application time and with 0.5 L at the following application times. JA was synthesized from methyl Jasmonate (MJ), according to Farmer and Rayan (1992). In brief, JA was prepared by dehydration of MJ under mild alkaline conditions, by mixing 1.5 mL MJ with 15 mL MeOH, 450 µL H_2O, and 1.5 g K_2CO_3. The mixture was incubated at 60 °C for 45 minutes in a sealed vial, then added to 90 mL water. This aqueous mixture was extracted four times with 45 mL pentane to remove MJ, and the aqueous phase was then titrated to pH 4.5 with 2.0 M HCl and extracted four times with 60 mL diethyl ether. The diethyl ether was removed by rotary evaporation, leaving JA. After that, JA was mixed with 3 mL acetone and the volume was increased to 2 L with distilled water, which provided a stock solution with a concentration of 1.5 parts per million (ppm) JA. The designated solutions were prepared through dilution with distilled water. Accordingly, there were nine treatments with five replicates (total 50 pots), and the experimental design was randomized complete block design (RCBD).

8.2.4. Assessment of Vegetative and Reproductive Parameters and Mineral Composition of Plants

To assess the impact of the treatments on vegetative and reproductive growth, the plant height, number of leaves, and number of branches were recorded weekly, whereas fruit number and weight were recorded more than once per week. For mineral composition determination, 20 leaves were taken randomly from each plant after 110 days of planting. Ten leaves were taken from the upper plant and 10 leaves from the lower part and then shock-frozen in liquid nitrogen. Tissues were then stored at −10 °C until required. Total nitrogen was determined according to the Kjeldahl method based on the ICARDA Manual (Ryan et al., 2001), and total phosphorus, total potassium and total calcium (Ca) were determined by using dry–ash procedure bases (Ryan et al., 2001). Phosphorus readings of samples, standards and blanks were taken at 410 nm using the spectrophotometer, whereas readings for K and Ca were taken at 768 and 620 nm, respectively, using a flame photometer.

8.2.5. Visual Inspection

Plants were inspected visually for yellowing, wilting and salt injuries after 80, 120 and 135 days of planting. These parameters were visually evaluated on a scale of 1 to 5, in which 1 indicates no salt injuries, no wilting symptoms and greener plants, and 5 indicates extreme wilting and yellowing plants.

8.2.6. Assessment of Soil Properties

Soil samples were tested at the end of the growing season for the following properties: pH value, which was determined for soil paste extract using an HI 9017 microprocessor pH meter; EC, using an TH-2400 auto-ranging EC meter; and organic matter, which was estimated by digesting soil with concentrated sulfuric acid, further addition of potassium dichromate in the presence of concentrated phosphoric acid, and finally titration with ferrous sulfate.

8.2.7. Assessment of Fruit Contamination

Harvested fruits were tested for fecal coliform, total coliform, *Salmonella* and *Staphylococcus aureus*. Determination of fecal coliform and total coliform on products was conducted according to the Bacteriological Analytical Manual (Bennett and Lancette, 1998). Samples were prepared by adding 50 g of test sample to 200 mL peptone water, and then blended in a stomacher for one minute at medium speed. To test fecal coliform, samples were diluted in eosin methylene blue medium by the spread plate technique, and incubated for 18 to 24 hours at 44.5 °C. To test total coliform, samples were diluted on violet red bile lactose on medium speed and incubated for 24 hours at 35 °C. *Salmonella* were detected by adding 25 g of product to 225 mL peptone water, further blended in a stomacher for one minute at medium speed and incubated for 16 hours at 37 °C. *Salmonella* was then isolated by adding 10 mL of culture (peptone water) to 100 mL of selenite cystine medium and incubating for 24 hours at 37 °C.

8.2.8. Statistical Analysis

All results were subjected to analysis of variance using the CoStat-software (CoHort Software, Monterey, CA). The mean separations were calculated by Duncan's multiple range test at $P \leq 0.05$.

8.3. Results

8.3.1. Impact on Vegetative and Reproductive Growth

Treating broad bean plants, which were irrigated with either reclaimed wastewater or mix, with 0.5 mM JA resulted in significantly more branching compared to the plants treated with the highest level of JA (1.5 mM) and irrigated with reclaimed wastewater only (Table 8.1). Concerning plant height, it is evident that JA treatments have a retarding effect, in particular with plants irrigated with reclaimed wastewater or mix and having received the highest level of JA (1.5 mM). Plants that received the higher JA treatments formed fewer leaves compared to plants irrigated with fresh water and no JA treatment. Concerning fruit number, no significant differences were registered, though fruit weight differs significantly upon treatment with JA and the irrigation source. Fruits from plants irrigated with mix and treated with 0.5 mM JA were significantly lighter than control fruits.

Table 8.1 Effects of Jasmonic acid and reclaimed wastewater treatments on the branching, plant height, fruit number, and fruit weight of bread bean plants*

Treatments**	Number of branches 101 days after planting (DAP)	Plant height (cm) 107 DAP	Number of leaves 101 DAP	Number of fruit (whole season)	Total fruit weight (g; whole season)
JA 0.0 mM WWS	4.6 ab	75.1 ab	51.6 ab	31.3 a	246.8 a
JA 0.5 mM WWS	6.1 b	74.4 ab	57.6 ab	47.3 a	428.9 abc
JA 1.0 mM WWS	4.6 ab	72.4 a	41.6 a	42.3 a	418.6 abc
JA 1.5 mM WWS	4.6 ab	66.0 a	45.3 a	41.3 a	418.1 abc
JA 0.0 mM WW	5.6 ab	77.1 ab	58.6 ab	44.6 a	419.8 abc
JA 0.5 mM WW	6.0 b	81.5 ab	58.5 ab	33.0 a	297.4 ab
JA 1.0 mM WW	5.0 ab	76.4 ab	52.8 ab	45.3 a	498.4 c
JA 1.5 mM WW	4.1 a	71.0 a	43.3 a	43.3 a	534.2 c
Control	5.1 ab	89.5 b	62.8 b	44.3 a	474.1 bc

* Means within each column marked with different letters are significantly different ($p \leq 0.05$, Student-Newman-Keuls range test).

** JA, Jasmonic acid; WWS, reclaimed wastewater + brackish water "mix"; WW, reclaimed wastewater.

8.3.2. Impact on Salinity Stress Symptoms

Although plants were monitored visually three times (80, 120 and 135 days after planting), the results shown in Table 8.2 are for two times, due to similarities between the last inspection times. Three stress symptoms were monitored over the entire growing season, namely yellowing, salt injuries and wilting. It is obvious that treating plants with JA resulted in significant preservation of the green leaf color. Concerning salt injuries, only the higher JA levels were sufficient to alleviate salt injury imposed by both irrigation sources. Treating plants with JA resulted in reducing wilting symptoms on plants. It is important to note that the degree of stress symptoms was larger in mix-exposed plants, regardless of JA concentration, compared to controls.

8.3.3. Impact on Mineral Composition of Leaves and Soil Characteristics

The effect of various treatments on mineral composition of leaves, both old and young, is presented on Table 8.3. Concerning nitrogen (N) levels, no significant differences were registered between most treatments. However, the promotion effect of JA treatments on N-level can be seen, in particular with the higher JA levels (1 and 1.5 mM). With potassium (K), irrigation of plants with mix resulted in lower K-levels of old leaves, in most treatments, although no clear trend can be observed with young leaves. Furthermore, it is obvious that JA treatments did not have any influence. With both phosphorus (P) and Ca, no clear trends can be found.

Table 8.2 Effects of Jasmonic acid and reclaimed wastewater treatments on the yellowing, salt injuries, and wilting of bread bean leaves*

Treatments**	Yellowing		Salt injury		Wilting	
	94 days after planting (DAP)	134 DAP	94 DAP	134 DAP	94 DAP	134 DAP
JA 0.0 mM WWS	1.0 a	5.0 c	0.3 a	5.0 c	0.6 a	5.0 d
JA 0.5 mM WWS	1.0 a	4.3 bc	2.6 b	4.0 bc	2.0 a	4.3 cd
JA 1.0 mM WWS	1.0 a	2.3 ab	0.0 a	2.0 ab	0.0 a	2.0 abc
JA 1.5 mM WWS	1.0 a	2.3 ab	1.3 ab	1.6 ab	0.6 a	2.0 abc
JA 0.0 mM WW	1.0 a	5.0 c	0.0 a	5.0 c	0.0 a	5.0 d
JA 0.5 mM WW	1.3 a	3.6 bc	0.3 a	3.0 abc	1.3 a	3.0 bcd
JA 1.0 mM WW	1.0 a	2.0 ab	0.3 a	1.3 ab	0.6 a	2.0 abc
JA 1.5 mM WW	1.0 a	1.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Control	1.0 a	2.0 a	0.0 a	0.0 a	0.0 a	1.0 ab

* Means within each column marked with different letters are significantly different ($p \le 0.05$, Student-Newman-Keuls range test).
** JA, Jasmonic acid; WWS, reclaimed wastewater + brackish water "mix"; WW, reclaimed wastewater.

Table 8.3 Effects of Jasmonic acid and reclaimed wastewater treatments on the mineral composition of broad bean leaves, and properties of the growing media*

Treatments**	% N in young leaves	% N in old leaves	% K in young leaves	% K in old leaves	% P in young leaves	% P in old leaves	% Ca in young leaves	% Ca in old leaves	pH	EC	OM
JA 0.0 mM WWS	2.2 a	3.2 ab	1.8abc	0.44a	0.59a	0.32bc	1.43ab	3.43a	7.7ab	8.31de	2.93 b
JA 0.5 mM WWS	2.8 a	3.2 ab	1.64abc	1.1c	0.53a	0.35c	1.97abc	4.13a	7.67ab	10.05e	3.09 b
JA 1.0 mM WWS	2.2 a	3.84 b	0.9a	0.52a	0.49a	0.32bc	1.31a	3.61a	7.92b	6.74cd	2.90 b
JA 1.5 mM WWS	2.2 a	3.75 b	1.07a	0.58ab	0.34a	0.21a	1.79abc	4.21a	7.9b	6.35cd	3.64 ab
JA 0.0 mM WW	2.0 a	2.70 ab	2.44c	0.91bc	0.47a	0.28abc	2.31bc	4.3a	7.55a	5.18bc	4.52 ab
JA 0.5 mM WW	1.7 a	1.80 a	1.31ab	1.15c	0.27a	0.27ab	1.09a	3.9a	7.71ab	3.32ab	4.73 ab
JA 1.0 mM WW	2.2 a	3.00 ab	1.64abc	1.07c	0.39a	0.24ab	1.38ab	4.14a	7.68ab	3.6ab	5.11 a
JA 1.5 mM WW	2.2 a	3.40 ab	2.51c	1.79d	0.30a	0.21a	2.44c	3.66a	7.69ab	2.63a	5.30 ab
Control	2.4 a	2.90 ab	2.13bc	1.27c	0.46a	0.30bc	1.85abc	4.24a	7.73ab	2.27a	3.08 b

* Means within each column marked with different letters are significantly different ($p \leq 0.05$, Student-Newman-Keuls range test).
** JA, Jasmonic acid; WWS, reclaimed wastewater + brackish water “mix”; WW, reclaimed wastewater.

Irrigating plants with both irrigation sources resulted also in significant changes in soil properties. Soil pH did not differ significantly among the treatments as compared to the control. However, the soil paste EC increased dramatically and significantly upon irrigating plants with mix or with reclaimed wastewater alone. In both cases, it is clear that treating plants with JA (1.0 and 1.5 mM) resulted in reductions in the soil paste EC. The organic matter content of soil did not differ highly between treatments, although it is obvious that irrigating with reclaimed wastewater alone tends to increase the organic matter content of the growing media.

8.3.4. Impact of Treatments on the Fruit Contamination

Fruit contamination with various bacteria was investigated, with no sign of contamination was found. Differences between all treatments, including fresh water-irrigated plants were not significant, with all parameters studied, which include total coliform, fecal coliform, *Salmonella* and *Staphylococcus aureus*.

8.4. Discussion

The main findings of this study are the reduction in vegetative growth upon treating plants with JA, the partial alleviation of salinity stress upon treatment with JA and the altered mineral composition of leaves. Furthermore, the finding that irrigating plants with reclaimed wastewater through drip irrigation resulted in the production of fruits, which are not more contaminated than fruits from plants irrigated with fresh water, is the most significant finding.

The partial alleviation of salinity stress, although coupled with the a reduction of vegetative growth upon exogenous application of JA is in agreement with Horton (1991) and Liu et al. (2002), who found that exogenously applied JA induced stomatal closure in broad bean and barley. Further, Lee et al. (1996) and Pospilisova (2003) stated that JA inhibits carbon dioxide (CO_2) fixation. The preservation of leaf greenness may be explained by the findings of Popova et al. (2003), who reported that MJ could be responsible for protection of photosynthesis against paraquat oxidative stress. Researchers hypothesized that MJ may improve the rate of the carboxylating and protection of the chlorophylls. On the other hand, the growth reduction found on plants irrigated with mix could be directly related to the increasingly negative water potential in the soil, which generally leads to lower transpiration rates and stomatal closure. Lower CO_2 uptake rates subsequently lead to lower photosynthesis rate and consequently lower growth (Rawson and Munns, 1984; Chaves, 1991; Cornic and Massacci, 1996).

Concerning the leaf mineral composition, the present study demonstrated that increasing the salinity level led to a decrease in Ca^{2+} and K^{+} levels in leaves, which was reported also with Reid and Smith (2000). The lower Ca^{2+} and K^{+}-level levels may be attributed to the competition between Na^+ and K^+ or Ca^{2+}, since salt stress caused rapid efflux of cations, particularly K^+ (Marschner, 1995). In addition, salinity is known to reduce Ca^{2+} activity in aqueous solution (Grieve et al., 1999). Accumulation of excess Na^+ may cause also metabolic disturbances in processes where low Na^+ and high K^+ and/or Ca^{2+} are required for optimum function (Marschner, 1995). Furthermore, a decrease in nitrate reductase activity, inhibition of photosystem II (Orcutt and Nilsen, 2000) and chlorophyll breakdown (Krishnamurthy et al., 1987) are also associated with increased Na^+ concentrations. It was obvious in the present study that Ca^{2+} and K^+ levels in plant old leaves increased upon JA application, in combination with irrigation with mix. Increasing K^+ uptake may be related to the increase in Ca^{2+} level in plant tissue, which decreases the salinity effect on the K^+ uptake, as reported by Marschner (1995).

The increase in P in plant upon salinity may be due to the increased availability of P in the soil, or the synergetic effect of Na^+, which is involved in P uptake. Moreover, the present investigations indicated that as salinity increased, nitrogen content also increased, which may be related to higher N fixation and greater N uptake from the soil under the salt-stress conditions (Rao et al., 2000). Additionally, several studies suggest that some proteins produced under salinity stress, such as glycinebetaine, may play a role in osmotic adjustment subjected to salinity stress (Meloni et al., 2004). It is also documented that JA application tends to induce the synthesis certain proteins known as JIPs protein (Maslenkova et al., 1992; Muller-Uri et al., 1988).

Concerning the reproductive growth, results clearly show that increasing salinity led to a decreased fruit weight, which agreed with Rao et al. (2000) and Singleton and Bahlool (1984). However, the fruit weight increased with JA treatments, which could be attributed to inhibiting effect of JA on leaf yellowing. Under such conditions, leaves lived longer, and the filling period of pods was subsequently longer, which may be the reason for bigger fruits (pods).

8.5. Conclusion

The finding in this study that fruits irrigated with reclaimed wastewater were not more contaminated than fruits irrigated with fresh water is the major finding, which is attributed mainly to the use of drip irrigation, which prevents direct contact between water and fruits. This indicates that an irrigation source like this could be widely used in the PT, in combination with both soil-less culture and mulching. The reuse of reclaimed water is essential to meet the expanding water demands of the agricultural sector in the PT, and therefore should be part of the integrated management of the available water resources. The demonstration project showed that high-quality reclaimed water can be used efficiently for the irrigation of broad beans, which are eaten cooked. Concerning the impact of the JA, our results indicate that alleviation of salinity stress imposed on plants through using water resources of inferior quality is possible. However, additional study is required.

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