Alleviating the Negative Impact of Brackish Water and Reclaimed Wastewater on *Vicia faba* Plants through Treatment with Jasmonic Acid.

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DEDICATION

To my husband Khaled for great love, patience, and good understanding for being busy during the study.

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Abstract

Water scarcity is the major constrain to the agricultural sector in the Palestinian Territories. Consequently, the search for alternative water resources for irrigation is vital for the sustainable development of agriculture in this area. Both reclaimed wastewater and brackish water represent highly attractive water sources for irrigation of crops. However, both sources cause various strains stresses on cultivated plants, where salinity stress is the most damaging one. In this respect, the use of natural growth regulators (e.g. Jasmonic acid -JA-) to alleviate stress may be a possible mean to alleviate stress imposed by a 'Mix' of both reclaimed wastewater and brackish water. In this research, Seeds of Broad Bean *Vicia faba* (cv. Primarenca*)* which are classified as sensitive plant to salinity, were planted on December, 2004 in 42 L pots, filled with soil mixture composed of, peat moss, sand, and clay in 2:1:1 ratio (by volume). Plants were randomly distributed in the green house and divided into three blocks. Experimental design was completely randomized block design, with two plants per replicate and three replicates per treatment. Plants were divided into two groups: plants in the first group were irrigated with reclaimed water, whereas plants in the second group were irrigated with a composed of reclaimed wastewater and salt (NaCl) and designated as 'Mix' in the following sections; EC of the 'Mix' was started with 1.5 dS m^{-1} increased gradually and ended with 7.0 dS m^{-1}. Within each group, there were several treatments as follows: JA treatments with different rates (0.0 mM, 0.5 mM, 1.0 mM, and 1.5 mM). Exogenous applications of Jasmonic acid tend to

improve the tolerance of plants irrigated with ‘Mix’-water that has an EC-value of 5 to 7 dS m^{-1}. That was evident in lower salt injuries and less wilting, but with higher average fruit weight, higher Ca and K level in leaves for plants treated with higher level of JA. Most other parameters did not differ significantly between treatments, and no obvious trends can register.

Table of Contents

Acknowledgment
Abstract
Table of contents
List of figures
List of tables
List of appendices
Symbols and abbreviations

CHAPTER 1: INTRODUCTION 1

1.1. Background: 1

1.2. Objectives: 3

CHAPTER 2: LITERATURE REVIEW 4

2.1. Water Impact on Plant Growth and Development 4

2.2. Stress and Plant Growth and Development 5

2.3. Reclaimed Wastewater and Reuse in Agriculture 8

2.4. Plant Growth Regulators to Alleviate Stress 11

CHAPTER 3: MATERIALS AND METHODS 13

3.1. Experimental Site Characterization 13
    3.1.1. Plant material, growing conditions, and soil media 13
    3.1.2. Treatments 14
    3.1.3 Irrigation system: 15
    3.1.4. Irrigation and fertilization schedule 15
    3.1.5. Preparation of Jasmonic acid 15

3.2. Investigated Parameters 16

3.2.1. Field Measurements 16

3.2.2. Plant Tissue Analysis 16
    3.2.2.1. Total Nitrogen 17
    3.2.2.2. Phosphorus, Potassium and Calcium 18

3.2.3. Visual Inspection 19

3.2.4. Fruit Quality 19

3.2.5. Statistical analysis 20

CHAPTER 4: RESULTS 21

4.1. Field measurements 21
4.1.1. Influence on number of leaves 21
4.1.2. Influence on number of branches 22
4.1.3. Influence on plant height 23
4.1.4. Influence on leaf color (yellowing) 24
4.1.5. Influence on plant wilting 25
4.1.6 Influence on salt injury 26
4.1.7 Influence on fruit weight: 27

4.2. Plant tissue analysis 28
4.2.1 Influence on calcium concentration of new Leaves 28
4.2.2. Influence on calcium concentration of old leaves 29
4.2.3. Influence on total phosphorus concentration of new leaves 30
4.2.4. Influence on total phosphorus concentration of old leaves 31
4.2.5. Influence on potassium concentration on new leaves 32
4.2.6. Influence on potassium concentration on old leaves 33
4.2.7. Influence on total nitrogen and protein concentrations in new leaves 34
4.2.8. Influence on total nitrogen and protein concentrations on old leaves 35

4.3. Fruit Quality: 37
4.3.1. Contamination of fruits 37

CHAPTER 5: DISCUSSION 38

5.1. Field measurements 38
5. 1. 1. Influence of reclaimed wastewater treatments on vegetative growth 38
5. 1. 2. Influence of JA on vegetative growth, yellowing, wilting and salt injury 39

5.2. Plant tissue analysis 41
5. 2. 1. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on mineral composition of leaves (Ca and K) 41
5. 2. 2. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on total phosphorus level in plant tissue 43

5. 2. 3. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on nitrogen uptake 43

5.3. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on fruit weight: 44

5.4. Fruit crop quality: 45

CHAPTER 6: CONCLUSION AND RECOMMENDATION 46

6.1 Conclusions: 46

6.2 Recommendations: 46

REFERENCES: 47

LIST OF FIGURES

FIGURE 4.1: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON NUMBER OF LEAVES OF *VICIA FABA* PLANTS. 21
FIGURE 4.2: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON NUMBER OF BRANCHES OF *VICIA FABA* PLANTS. 22
FIGURE 4.3: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON PLANT HEIGHT (CM) OF *VICIA FABA* PLANTS.................................... 23
FIGURE 4.4: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON YELLOWING OF *VICIA FABA* PLANTS AT DIFFERENT DATE................ 24
FIGURE 4.5: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON WILTING OF *VICIA FABA* PLANTS AT DIFFERENT DATE. 25
FIGURE 4.6: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON SALT INJURIES OF *VICIA FABA* PLANTS ON DIFFERENT DATE. 26
FIGURE 4.7: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON FRUIT WEIGHT (G) OF *VICIA FABA* PLANTS....................................... 27
FIGURE 4.8: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON CA-LEVEL OF NEW LEAVES OF *VICIA FABA* PLANTS......................... 28
FIGURE 4.9: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON CA-LEVEL OF OLD LEAVES OF *VICIA FABA* PLANTS. 29
FIGURE 4.10: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON P-LEVEL OF NEW LEAVES OF *VICIA FABA* PLANTS. 30
FIGURE 4.11: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON P-LEVEL OF OLD LEAVES OF *VICIA FABA* PLANTS. 31
FIGURE 4.12: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON K-LEVEL OF NEW LEAVES OF *VICIA FABA* PLANTS........................... 32
FIGURE 4.13: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON K-LEVEL OF OLD LEAVES OF *VICIA FABA* PLANTS............................ 33
FIGURE 4.14: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON N-LEVEL OF NEW LEAVES OF *VICIA FABA* PLANTS........................... 34
FIGURE 4.15: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON PROTEIN LEVEL OF NEW LEAVES OF *VICIA FABA* PLANTS................. 35
FIGURE 4.16: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON N-LEVEL OF OLD LEAVES OF *VICIA FABA* PLANTS............................ 36
FIGURE 4.17: INFLUENCE OF RECLAIMED WASTEWATER TREATMENTS (RWW+JA AND MIX+JA) ON PROTEIN LEVEL OF OLD LEAVES OF *VICIA FABA* PLANTS.................. 36

LIST OF TABLES

Table 3:1 Treatments through the study……………………………………………14

List of Appendices

Appendix 1: Detailed mean length (cm) of plant with time for each treatment during ithe experiment. i
Appendix 2: Detailed mean leaves number of plant with time for each treatment during the experiment. ii
Appendix 3: Detailed mean branches number of plant with time for each treatment during the experiment. ii
Appendix 4: Detailed mean Fruit number of plant with time for each treatment during the experiment. iii
Appendix 5: Detailed mean Fruit weight (gm) of plant with time for each treatment during the experiment iii
Appendix 6: Detailed mean score of plant wilting with time for each treatment during the experiment iv
Appendix 7: Detailed mean score of plant yellowing with time for each treatment during the experiment iv
Appendix 8: Detailed mean score of plant salt injury with time for each treatment during the experiment v
Appendix 9: Detailed mean Ca -level in plant leaves. v
Appendix 10: Detailed mean total P -level in plant leaves. vi
Appendix 11: Detailed mean K -level in plant leaves 7
Appendix 12: Detailed mean N -level and protein in plant leaves 7

List of Abbreviations

ABA	Abscisic acid
BOD5	Biological Oxygen demand
DI-H_2O	Deionized water
dS m^{-1}	decisiemens per miter
EC	Electrical Conductivity
FW	Fresh water
g	Gram
JA	Jasmonic acid
JIPs	Jasmonates induced proteins
Km	Kilometer
L	Liter
M	Molarity
m	meter
M m^3	million cubic meters
M $m^3.year^{-1}$	million cubic meters per year
MeJA	methyl jasmonate
mg L^{-1}	milligram per Liter
ml	milliliter
mm	millimeter
mmho cm^{-1}	millimohs per Centimeter
NaCl	Sodium Choride
ppm	Part Per Million
Pq	Paraquat
ROS	Reactive oxygen species

RWW	reclaimed wastewater
TDS	Total Dissolved Solid
UNDP	United Nations Development Programme
WHO	World Health Organization
wt	Weight
VSP	Vegetative storage protein
Mix	reclaimed wastewater + salt

CHAPTER 1: Introduction

1.1. Background:

Water is a vital resource, necessary for all aspects of human and ecosystem survival and health. In recent years, new alarms have been sounded about growing water scarcity and contamination, and the likely inability to meet the water requirements of rapidly growing populations (Postel, 1992; Gleick, 1993; Engleman and LeRoy, 1993). As the world's supply of freshwater becomes ever scarcer, it is necessary to find alternative resources for agricultural purposes; because water deficit is one of the main environmental factors limiting plant growth and development. Drought causes visible injuries to leaves, induces stomata closure, leaf rolling, a faster decline in the chlorophyll and protein content, and altering both the structure and function of membranes (Hare and Cress, 1998).

In Palestine, shortage in water supply for domestic, industrial and agricultural purposes is a chronic problem (ARIJ, 2006). Scarcity of water resources in Palestine imposed the search for alternative resources. Both brackish water and reclaimed wastewater represent major resources that can be used to alleviate the increasing demand on the continuously decreasing freshwater supplies, since desalinization of seawater is extremely expensive and this resource could not be considered as an alternative, even in the far future. However, both resources are problematic, since they impose stress to growing plants. Under salinity stress, key metabolic systems such as photosynthesis are among the most affected (Mass and Grieve, 1987); the capacity of the electron transport chain in such conditions exceeds the consumption of reduction equivalents delivered to the stoma side of the thylakoid membranes (Niyogi et al., 1997), Duration of this constraint is harmful to plants, because it triggers the production of reactive oxygen species (ROS), such as hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide (Lamb and Dixon, 1997; Bolwell, 1999; Bartels, 2001). Consequently, means are needed to alleviate stresses, in particular salt stress, imposed by the use of brackish water and/or reclaimed wastewater. Jasmonic acid (and methyl jasmonate), is one of the newly discovered plant growth regulator, and proved to be efficient in alleviating various types of stresses, such as chilling and drought stress (Creelman and Mullet, 1995).

This research was initiated in response to the fact that the semi-arid climate and water deficit are the major constraints for agricultural development and sustainability in Palestinian Territories (PT's). Consequently, the reuse of treated wastewater (also known as reclaimed wastewater) 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 M $m^3.year^{-1}$ (million cubic meters per year) for the main Palestinian cities by the year 2010 (Meerbach, 2004). On comparison, the total agricultural water demand is projected to increase by 50M m^3 over the years 2005–2010 (Meerbach, 2004). In the current political climate, this increased demand is unlikely to be met by an increase in freshwater supply, and therefore water reuse is the key to the development of the agricultural sector. However, several factors and conditions restrict the use of treated wastewater in agriculture. In this respect, health risks could be considered as the main factors that restrict the reuse of treated wastewater. Furthermore, salinity effect, crop type and irrigation system are additional factors.

1.2. Objectives:

The hypothesis of this research is, treating growing plants with Jasmonic Acid (JA), may alleviate the salt stress imposed through the use of brackish water and/or reclaimed water. The present work focuses on using a mixture of brackish water with treated wastewater, to dilute the brackish water in order to use it as an irrigation source. The objectives of this study can be consequently listed as the following:

- To introduce and enhance the reuse of wastewater and brackish water as a non-conventional source of water for irrigation, and consequently to evaluate the suitability of reclaimed wastewater for Broad Bean grown in semi-arid conditions (Palestinian territories).
- To evaluate the impact of reclaimed wastewater and brackish water on plant growth, development.
- Examine the physiological and biochemical changes in broad bean plants treated by JA during salinity stress.

CHAPTER 2: Literature Review

2.1. Water Impact on Plant Growth and Development

Plant growth and development are result of three processes: cell division, cell enlargement, and cell differentiation (Salisbury et al., 1985). Commonly, growth is the increase in the amount of living material, which leads to an increase in cell size and ultimately cell division (Janick, 1979). Plant growth and development are greatly affected by the environment, and if any environmental factor is less than ideal, it may limit the growth of plants (Smirnoff, 1998). In most cases, environmental stress weakens a plant and makes it more susceptible to disease or insect attack (Bressan et al., 1998). The most important environmental factor, however, is water. Most growing plants contain about 90 % water, and water plays many vital roles in plants, mainly in photosynthesis, and all metabolic reactions (Danity, 1976). Furthermore, water is needed to transport the essential elements from the soil to upper parts (e.g. leaves) of plants; plants need 17 elements for normal growth (Marshner.1986). Most of the essential nutrients are absorbed from the soil-water solution, and only about 2 % are actually extracted from soil particles (Kramer, 1983).

Water movement through the soil, plant and air can be understood through the concept of water potential (Boyer, 1985) This is measured in pressure units (MP) and water always moves from high to low water potential, whatever the reason for the difference in potential (Kramer, 1983). Pure water at ground level and air at 100% relative humidity, have zero water potential.

In order to keep growing, cells need to keep accumulating sugars and salts in their vacuoles to maintain a low water potential and offset the dilution as they take up water (Boyer, 1985), water uptake occurs through the semi-permeable plasma membrane and continues as long as the water potential of the vacuole is lower than the water potential outside the cell. Each cell in the plant "sits" on a water potential gradient from the soil to the atmosphere. As long as the water potential is lower in the cells than outside, the plant can take up water. So cells in the leaves at the top of a tall tree will be at lower water potential than those down below.As the soil dries out, water potential falls towards that in the cell, which continues to lose water to the air, This leads to shrinkage of the cell contents so that turgor pressure is lost and the plant wilts (Xiong and Zhu, 2002), An equivalent situation occurs when salts accumulate in the soil; one effect of salinity is that cells cannot take up water against the water potential gradient. They cannot grow and may even lose water to their surroundings (Hare and Cress, 1998).

2.2. Stress and Plant Growth and Development

Plants are exposed to a range of biotic stress (e.g. pathogens, animals and other plants), and abiotic stress (e.g. excess light, deep shade, high/low temperatures, drought and nutrient toxicity) (Smirnoff, 1998). Biological stress is defined as ‘any change in environmental conditions that might reduce or adversely change a plant’s growth and development (Salisbury and Ross, 1992). One of the most common stress factors is the salinity stress (Yeo, 1998), in which salinity stress leads consequently to a water deficit stress. As water transpire from the plants, salts in the protoplasm may reach dangerous level leading to substantial damage to cells, particularly enzymes (Hare and Cress, 1998). Plant species differ in their response to salinity stress; some species are highly sensitive, such as citrus fruit trees and apples, while others are tolerant, such as date palm, lettuce and alfalfa. The common link among different stresses is that they all produce an oxidative burst. Chloroplasts, which are the major source of activated O_2 in plants (Foyer et al., 1994), and antioxidants, which play an essential role in preventing oxidative damage, are greatly affected by environmental stress (Bowler et al., 1994). Concerning salinity stress, it is well documented that the quality of irrigation water has been determined by the quantity and kind of salts present (Pettygrove and Asano, 1984). As salinity in the reclaimed wastewater increases above a certain level, the probability of soil, water and cropping problems also increases. Potential problems are related to the total salt content, to the types of salt, or to excessive concentration of one or more elements; these problems are not different from those caused by salinity or specific ion in fresh water (Hoffman, et al., 1980). Usually, no salinity problems are expected for water having an EC< 0.7 mmhos cm^{-1}, and no special management practices are required. But water in the 0.7- to 3.0-mmhos cm^{-1} range (Slight to moderate salinity) may require special practices. Water with EC> 3.0 mmho cm^{-1} requires very intensive and careful management to control salinity, including such drastic steps such as changing to a more salt tolerant crop, or greatly increasing the leaching fraction. Salt sensitive crops show drastic yield reduction at EC> 3.0 mmho cm^{-1}, even under the best management (Pettygrove and Asano, 1984).

In arid regions, the quality of irrigation water became even more important, where extremes of temperatures and low relative humidity result in higher rates of evaporation, with consequent deposition of salt that tends to accumulate in the soil profile. Another aspect of agricultural concern is the effect of dissolved solids (TDS) on the growth of plants. Dissolved salts increase the osmotic potential of soil water, and consequently water potential become more negative; that increases the osmotic pressure of the soil solution, which increases the amount of energy the plants must expend to take up water from the soil (Hasegawa et al., 2000), which results in an increased respiration , and the growth and yield of most plants decline progressively. Although most plants respond to salinity as a function of the total osmotic potential of soil water, some plants are susceptible, however, to specific ion toxicity (Zhu et al., 1997). Many of the ions, which are harmless or even beneficial at relatively low concentrations, become toxic to plants at high concentration, either through, direct interference with metabolic processes, or through indirect effects on other nutrients, which might be rendered inaccessible (Krista et al., 2003). Morishita (1985) has reported that irrigation with nitrogen-enriched polluted water can supply a considerable excess of nutrient nitrogen to growing rice plants, and resulted in a significant yield loss of rice through lodging, failure to ripen and increased susceptibility to pests and diseases.

2.3. Reclaimed Wastewater and Reuse in Agriculture

Wastewater reuse has been growing over the previous three decades and it is now considered an essential management strategy in areas of the world where water is in short supply (Mara and Cairncross, 1989). Many countries now consider wastewater reuse as a method to secure water resources (Asano and Levine, 1996). The benefits of reclaimed wastewater for irrigation include: increasing crop yields, decreasing the use of fertilizers while providing increases in nutrients and organic matter for soil conditioning, soil conservation and potential reduction of desertification, improving of the environment by enabling zero-discharge to receiving bodies, and enabling the reallocation of freshwater supplies for urban use. However, the hygienic problem represent the major constrain on the reuse of reclaimed wastewater. Wastewater-related diseases can be divided into those caused by chemical substances, such as heavy metals and other toxins in mismanaged industrial effluent, and those caused by biological agents or pathogens (Giles and Brown, 1997). Both chemical substances and biological pathogens are a threat to public health as they can be transferred up the food chain when contaminated wastewater is used to irrigate crops or used in aquaculture (Furedy et al., 1994; Asano and Levine, 1996). The main issues regarding reclaimed wastewater are the protection of public health. Unlike fresh water irrigation, reclaimed wastewater is restricted to certain uses due to public health or water quality concerns (Mills and Asano, 1996). Reuse of wastewater and protection of public health are achieved through following a control algorithm that includes:

(1) Wastewater treatment to reduce pathogen concentrations to meet the WHO guidelines (1989); (2) crop restrictions to prevent direct exposure to those consuming uncooked crops; (3) application methods (irrigation) reducing the contact of wastewater with edible crops; and, (4) human exposure control for workers, crop-handlers and final consumers (WHO, 1989; Mara and Cairncross, 1989; Strauss and Blumenthal, 1990). Restricted irrigation refers to the irrigation of crops not directly consumed by humans (*e.g.*, trees, fodder crops). For restricted irrigation, wastewater effluent must contain ≤ 1 viable intestinal nematode egg per liter. Unrestricted irrigation refers to the irrigation of vegetable crops eaten directly by humans, including those eaten raw, and also to the irrigation of sports fields, public parks, hotel lawns, and tourist areas (Mara and Cairncross, 1989). The criteria for unrestricted irrigation, contains the same helminthes criteria as restricted irrigation, in addition to a restriction of no more than a geometric mean concentration of ≤1000 faecal coliforms per 100 ml/treated effluent. These guidelines have been introduced to directly protect the health of consumers who may eat uncooked crops such as vegetables and salads (Mara and Cairncross, 1989).

In the period 1981 to 1987, the Ministries of Agriculture and Public Health, with assistance from the United Nations Development Programme (UNDP), carried out studies designed to assess the effects of using treated wastewater on crop productivity, and on the hygienic quality of crops and soil (Shuval, 1999). Treated wastewaters from the La Cherguia (Tunis) and Nabeul activated sludge plants were used in the studies, and irrigation with groundwater was used as a control. At La

Soukra, tests were conducted on sorghum *(Sorghum vulgare)* and pepper *(Capsicum annuum),* using flood irrigation and furrow irrigation (FAO, 1992). Respectively Clementine and Orange trees were also irrigated in Nabeul with treated wastewater. In order to assess the long-term effects of irrigation with treated wastewater, investigations were carried out on the perimeter area of La Soukra, where irrigation with treated effluent had been practiced for more than 20 years (Bahri, 1988). The use of treated wastewater resulted in annual and perennial crop yields higher than yields produced by freshwater irrigation (FAO, 1992). Bacterial contamination of citrus fruit picked from the ground irrigated with treated wastewater, or fertilized with sewage sludge, was significantly higher than the level of contamination of fruit picked from the trees. Irrigation with treated wastewaters was not found to have an adverse effect on the chemical and bacteriological quality of shallow groundwater (Bahri, 1988). Another studies show the long-term impact of irrigation with reclaimed water effluent on soil, crops and ground water. In India they used the reclaimed water from domestic and industrial effluent (FAO, 1992), and sewage effluent is appreciated as a potential source of plant nutrients, although it may build up heavy metals. In China, national policy has been developed that promotes the development of water-efficient technologies, and encourages the reuse of reclaimed municipal wastewater in agriculture first, and then for industrial and municipal uses (Zhongxiang and Yi, 1991). However, with California experiences, Adin and Asano (1986) show that using reclaimed water increased the problems with soil, water, and plants, as salinity in the reclaimed water may increase above a certain level.

2.4. Plant Growth Regulators to Alleviate Stress

Stress may induce common responses, which may include changes in hormonal balance. The effects of plant growth regulators and hormones depend largely on the target tissue and the biochemical environment (Stewart, 1985). Although textbooks and literature listed five groups of plant growth regulators, recent findings indicate the existence of another classes of hormone namely Jasmonic acid (or its methyl ester) believed to be one of these new classes (Rojo et al., 1988). The jasmonates, consisting of cis-jasmone (CJ), methyl jasmonate (MJ), and jasmonic acid (JA) are a family of plant stress hormones, naturally occurring growth regulators found in higher plants (Anderson, 1989; Creelman, et al., 1992). Jasmonic acid is a plant signaling compound necessary for the regulation of growth and development, as well as for the response of plants to environmental stress factors (Stuhlfelder et al., 2004). The intensive research on plant response to various environmental stresses during the last few years has revealed a role for jasmonates as stress modulating compounds that have been involved in plant response to wounding (Farmer and Ryan, 1992), UV irradiation (Conconi et al., 1996), and pathogen infection (Creelman and Mullet, 1997). Increased endogenous levels of jasmonates have been also found in plants suffering from drought and osmotic stress (Creelman and Mullet, 1995), wounding (Creelman et al., 1992; Baldwin et al., 1997), after treatment with fungal elicitors (Gundlach et al., 1992), or upon pathogen attack (Farmer and Rayan, 1992). Exogenous treatment with Jasmonic acid (or Methyl Jasmonate) proved to be an effective way to alleviate various biotic and abiotic stresses (Mulpuri et al., 2000). In recent years jasmonates have been the focus of much attention because of their ability to provide protection to salinity stress (Tsonev et al., 1998), or UV irradiation (Mackerness et al., 1999), leading to the suggestion that jasmonates could mediate the defense response to salinity stress. Concerning the precise mechanism of Jasmonates, it is not clear to date, whether methyl jasmonate itself acts as a signal or if its conversion to jasmonic acid is mandatory prior to the induction of a defense response (Stuhlfelder et al., 2004). In plants, these compounds activate cellular responses to diverse conditions, including cell death, to protect plants that come under biotic stress such as insect attack or wounding (Robert et al., 1992). JA markedly induced a pathogenesis-related protein, and the salt stress-responsive salt protein in roots; most JA-responsive proteins (JIPs) from roots also accumulated when plants were subjected to salt stress (Moons et al., 1997). Anderson et al. (2005) concluded that JA-ethylene signaling pathways modulate defense and stress responsive gene expression in response to biotic and a biotic stress. Jasmonic acid appears to affect plant development and physiology, which could in turn affect plant growth and development; exogenous jasmonic acid stimulates the expression of defensive chemistry (Reinbothe et al., 1994). The latter fact indicates that it is possible to apply jasmonic acid to stimulate plant resistance on large scale in the field with out damaging individual plants.

CHAPTER 3: Materials and Methods

3.1. Experimental Site Characterization

Al-Bireh city is located in the middle of the West Bank, 16.5 km to the north of Jerusalem. The elevation of Al-Bireh city is 800 m above sea level, with an average annual rainfall about 600 mm, and the annual average temperature ranges between l5-20° C. The experimental site of this study was the Wastewater Water Treatment Plant (WWTP) in Al Bireh inside a plastic house. The Al-Bireh WWTP is located east of Ramallah and treats approximately $1.25x10^6 m^3$ $year^{-1}$ of raw municipal wastewater. The treatment at the WWTP consists of oxidation ditches, secondary clarifiers and a UV-disinfection system for pathogen removal. The reclaimed water has a tested quality of 10/10 mg l^{-1} BOD/TSS (Biochemical Oxygen Demand < 10 mg l^{-1} and Total Suspended Solids < 10 mg l^{-1}), Total Nitrogen 30–40 mg l^{-1} and a faecal coliform level lower than 100 CFU.100 ml^{-1}.

3.1.1. Plant material, growing conditions, and soil media

Seeds of Broad Bean *Vicia faba* (cv. Primarenca*)* were planted on (15, December, 2004) in 42 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 grams of 14: 7: 28 (N: P: K) starter fertilizer. Plants were randomly distributed in the green house and divided into three blocks. Experimental design was completely randomized block design, with two plants per replicate and three replicates per treatment.

3.1.2. Treatments

Plants were divided into two groups: plants in the first group were irrigated with reclaimed water, whereas plants in the second group were irrigated with a mixture of reclaimed wastewater and salt (NaCl) and designated as ‘Mix’ in the following sections; EC of the ‘Mix’ was started with 1.5 mmho cm^{-1} increased gradually and ended with 7.0 mmho cm^{-1} (see below for details). Within each group, there were several treatments as follows: JA treatments with different rates (0.0 mM, 0.5 mM, 1.0 mM, and 1.5 mM). Consequently there were the nine treatments as listed in the following table.

Table 3:1 Treatments through the study.

Treatments	**Water Source**
JA 0.0 mM Mix	RWW+NaCl
JA 0.5 mM Mix	RWW+NaCl
JA 1.0 mM Mix	RWW+NaCl
JA 1.5 mM Mix	RWW+NaCl
JA 0.0 mM RWW	RWW
JA 0.5 mM RWW	RWW
JA 1.0 mM RWW	RWW
JA 1.5 mM RWW	RWW
Control	FW

3.1.3 Irrigation system:

Three tanks were used to supply water for drip Irrigation systems. The first tank was used to supply freshwater, the second tank to supply reclaimed wastewater, and the third tank to supply the ‘Mix’. In the third tank, NaCl salt was added to the reclaimed wastewater to raise the salinity level; in the first 45 days EC was increased from 1.5mmho/cm to 3mmho/cm, after 45 days from planting the salinity level was increased to 5-mmho/cm, and finally the salinity level was increased after 100 days from planting to reach 7-mmho/cm until the end of growing season.

3.1.4. Irrigation and fertilization schedule

At the beginning, all plants were irrigated with freshwater three times per week, until plants reach a height of 15 cm. After that, each pot received the designated treatment. Each plant was fertilized with 2 grams of 14: 7: 28 (N: P: K) at weekly intervals, and the quantity of irrigated water was increased proportionally in relation to plant growth and climatic conditions to reach 2.5 liters of water daily by the end of experiment.

3.1.5. Preparation of Jasmonic acid

Jasmonic acids with different concentrations (0.0, 0.5, 1.0, and 1.5 mM) were applied exogenously to plants using hand held sprayer three times during plants growing season: the first time was on 18.1.2005 (42 days from planting). Second application was at the beginning of flowering (70 Days from planting), and the final application was after 120 days from planting. In brief, methyl jasmonate was prepared according to Farmer et al. (1992). 1.5 ml of methyl jasmonate was mixed with 15 mL MeOH, 450 µL H_2O, and 1.5 g K_2CO_3. The mixture was incubated at 60º C for 45 minutes in sealed vial, then added to 90 mL water. This aqueous mixture was extracted four times with 45 mL pentane to remove methyl jasmonate; 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 jasmonic acid. JA was mixed after that with 3 ml acetone and the volume was increased to 2 liter with distilled water, and that give a stock solution with a concentration of 1.5 mM JA. The designated solutions were prepared through dilution with distilled water.

3.2. Investigated Parameters

3.2.1. Field Measurements

To assess the impact of the treatments on the vegetative and reproductive growth, the following parameters were recorded biweekly, except fruit number which was recorded more than once per week: plant height, number of leaves, number of branches, fruit number and weight.

3.2.2. Plant Tissue Analysis

110 days after planting, 20 leaves were taken randomly from each plant, ten leaves from upper part of the plant and other ten leaves from lower part, and shock-frozen in liquid nitrogen. Tissues were stored after that at -10 ˚C until required. The following measurements were conducted using these tissues:

3.2.2.1. Total Nitrogen

Nitrogen determinations were conducted using Kjeldahl method based on ICARDA Manual (Ryan, et al. 1999). One gram of finely ground plant material was placed in a plastic vial, and dried at 60 °C in an oven (over night), and then cooled in a desicator. 0.25 g of dry plant material was transferred to 100-ml digestion tube, and few pumice-boiling granules were added. Three grams of the catalyst mixture were added using a calibration spoon, and further 10 ml concentrated sulfuric acid were added, and stirred using the vortex tube stirrer until mixed well. Tubes were placed in block–digester set at 100 °C for 20 minutes. After that, tubes were thoroughly agitated, and then placed on the block –digester set at 380°C for 2 hours after clearing. Following digestion, tubes were removed, cooled, and the volume was completed to 100-ml volume with DI-H_2O. Each batch of samples for digestion contained at least one reagent blank (on plant), and one chemical standard (weigh 0.1 g EDTA standard digest), and one standard plant sample (internal reference). After that, distillation apparatus was steamed out for 10 minutes. Tubes were thoroughly shaked to mix its contents. Then 10 ml aliquots were placed into a 100-mL distillation flask and further 10 mL 10 N sodium hydroxide solution were added. Immediately after that flask was connected to distillation unit and distillated. After that 35 ml distillate was collected in the collecting dish, and water was drained from the condenser jacket. Finally the distillate was titrated to pH 5.0 with standardized 0.01 N H_2SO_4 using the Auto-Titrator. Titration volume of acid was recorded to complete calculation.

Calculation:

%N = (T - B) x N x 14.01

Where: T = mL of sample titrated; B = ml of blank titrated; N = acid normality; 14.01= Atomic weight of N.

%N in sample= % N/sample weight (mg)*100.

%Crude protein in sample = % N in sample*6.25

3.2.2.2. Phosphorus, Potassium and Calcium

Total P, K and Ca, determinations were conducted using dry –ashing procedure based on ICARDA Manual (Ryan, et al.1999). One gram of ground material was placed on porcelain crucibles, and ashed in a cool muffle furnace at 550 °C for 5 hours, and then cooled in a desiccator. After that, the cooled ash was dissolved in 5 ml 2 N HCl and mixed with a plastic rod; aliquot's volumes were brought to 50 ml using DI-H_2O. To measure P in sample, 10 ml of aliquots were placed into a 100-mL volumetric flask, and further 10 ml ammonium- vanadomolybdate reagent were added, and the solution was diluted to volume with DI-H_2O. By Spectrophotometer, absorbance of standards, blank and samples were read to measure P at 410 nm wavelength. Then, the standard curve was prepared, and P concentrations in the unknown samples were read from the calibration curve. To measure K and Ca, the aliquots were read using Flame photometer; absorbance of standards, blank and samples were read to measure K at 768 nm wavelength. Absorbance of standards, blank and samples were read to measure Ca at 620 nm wavelength also calibration curves were prepared for both nutrients.

Calculations:

% Total Phosphorus in plant:

%P= ppm P (from calibration curve)*R/Wt*100/1000

Where: R= ratio between total volume of the digest /aliquot and the digest /aliquot volume used for measurement; Wt=Weight of dry plant (g).

% Potassium in plant

%K= ppm K (from calibration curve)*R/Wt*100/1000

Where: R= ratio between total volume of the digest /aliquot and the digest /aliquot volume used for measurement; Wt=Weight of dry plant (g).

% Calcium in plant

%Ca= ppm Ca (from calibration curve)*R/Wt*100/1000

Where: R= ratio between total volume of the digest /aliquot and the digest /aliquot volume used for measurement; Wt=Weight of dry plant (g).

3.2.3. Visual Inspection

80, 120, and 135 days after planting, plants were inspected visually for yellowing, wilting, and salt injuries. These parameters were evaluated visually on a scale of 1 to 5, where 1 means plant with no salt injury, no wilting symptoms and greener, and 5 means extremely wilting and yellowing plants.

3.2.4. Fruit Quality

Fruits were tested for Fecal Coliform, Total Coliform, *Salmonella*, and *E.coli*.

Fecal coliform and total coliform were detected according to FDA Bacteriological Analytical Manual (2001). 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, it was diluted in Eosin methylene blue (EMB) medium by spread plate technique, and incubated for 18-24 hours at 44.5 °C .To test Total coliform, samples were diluted on Violet red bile lactose (VRBL) medium, and incubated for 24 hours at 35 ° C. *Salmonella* were detected according to FDA Bacteriological analytical Manual (2001). Samples were prepared by adding 25 g of product to 225 ml peptone water. Then it was blended in a stomacher for one minute at medium speed, and incubated for 16 hours at 37 °C. After that *Salmonella* was isolated by adding 10 ml of culture (peptone water) to 100 ml of Selenite cystine medium, and incubated for 24 hours at 37 °C.

3.2.5. Statistical analysis

All obtained data were subjected to analysis of variance (ANOVA) at $p \leqq 0.05$, and mean separation was conducted using Duncan’s Multiple Range Test (DMRT) using (SPSS) software.

CHAPTER 4: Results

The impact of treatments was monitored over the entire growing season, and results presented here reflect the main trends.

4.1. Field measurements

4.1.1. Influence on number of leaves

The influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) is shown in Figure 4.1. It is clear that plants irrigated with FW (control) had more leaves than other treatments. Plants treated with 1.5 mM JA, either irrigated with Mix or RWW, had significantly lower number of leaves when compared to control plants. Number of leaves for other treatments ranged between these two values, and no obvious trend can be found.

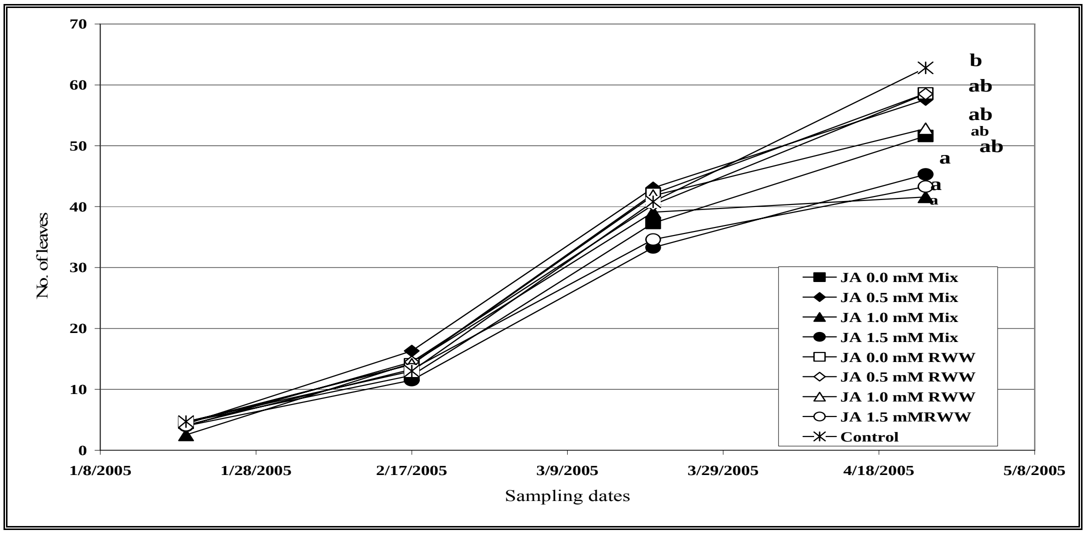

Figure 4.1: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on number of leaves of *Vicia faba* plants.

*p $\leq$ 0.05, n= 3 samples/ treatment.

4.1.2. Influence on number of branches

Figure 4.2 illustrates the effects of reclaimed wastewater treatments on the number of branches. All treatments followed the same trend, except with control plants where number of branches continues to increase significantly through all the growth period. However, treating plants with lower concentration of JA (0.5 mM) tend to increase significantly number of branches in the period from 20th March to the end of April; Plants irrigated with 'Mix' form more branches than plants irrigated with RWW.

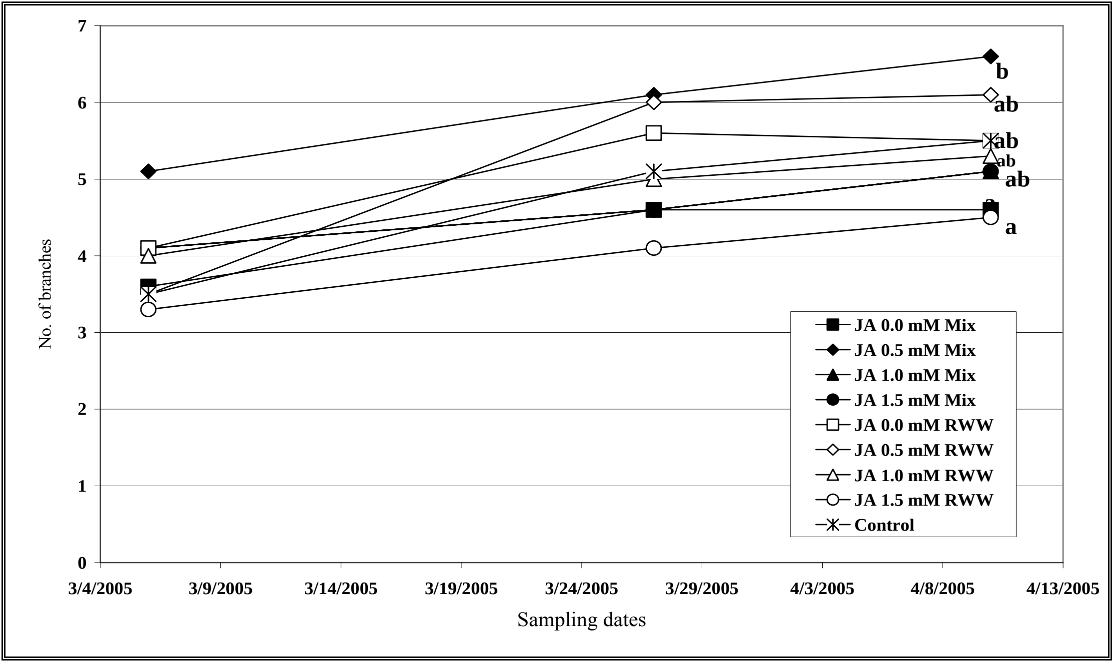

Figure 4.2: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on number of branches of *Vicia faba* plants.

*$p \leq 0.05$, n = 3 samples/ treatment.

4.1.3. Influence on plant height

The influence of JA and reclaimed wastewater treatments is shown in Figure 4.3. It is clear that plants irrigated with FW (control) reached longer length than plants in other treatments. Plants treated with 1.5 mM JA, either irrigated with Mix or RWW water, reached significantly less heights than the control plants. Plant lengths for other treatments ranged between these two values, and no obvious trend can be found.

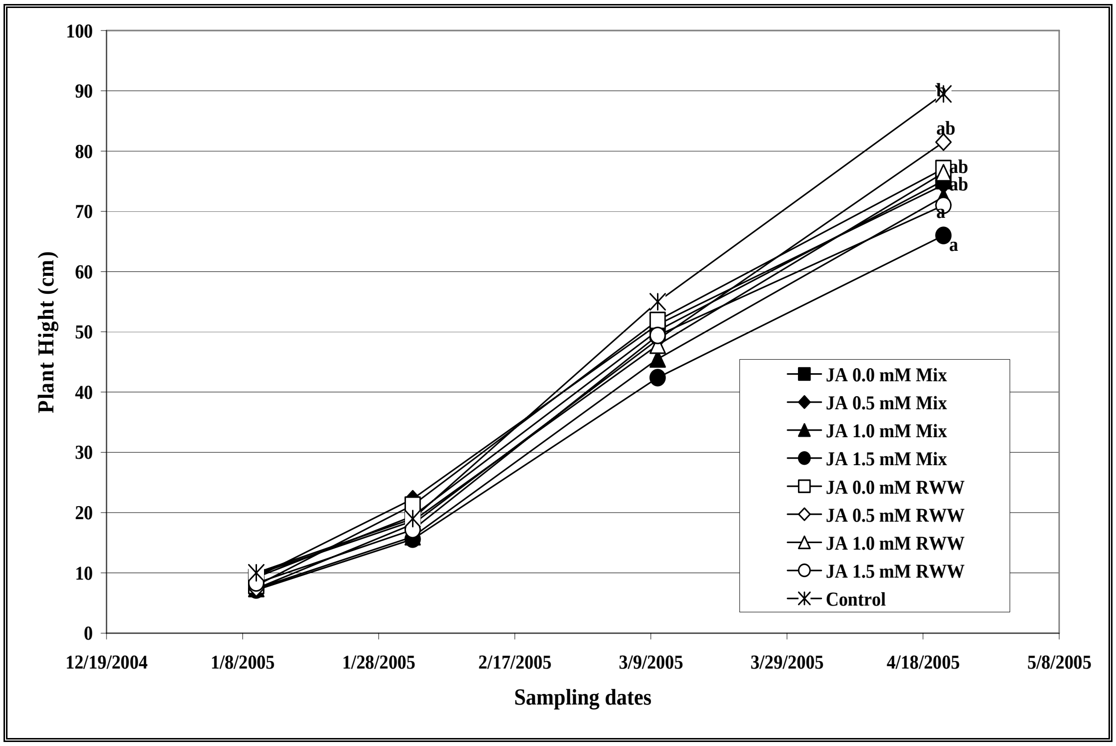

Figure 4.3: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on plant height (cm) of *Vicia faba* plants.

*$p \leq 0.05$, $n = 3$ samples/ treatment.

4.1.4. Influence on leaf color (yellowing)

Plants irrigated with RWW, and treated with 1.5mM JA, were the only plants, which were greener than other treatments, ever more than control (Figure 4.4). It is obvious that decreasing JA concentrations led to more yellowing, with no significant differences between plants irrigated either with Mix or RWW water; the comparisons are between the equivalents JA treatments.

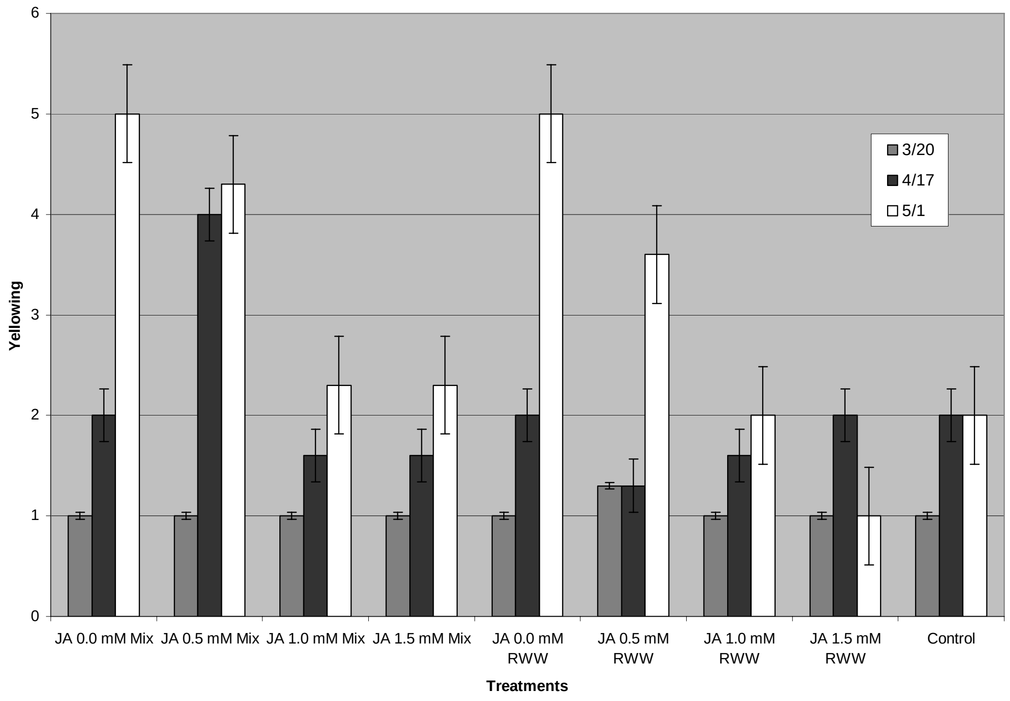

Figure 4.4: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on yellowing of *Vicia faba* plants at different date.

*$p \leq 0.05$, vertical pars represent ± 1 SE, n = 3 samples/ treatment.

*Yellowing score: 1 = green, 5 = complete yellow.

4.1.5. Influence on plant wilting

Plants treated with 1.5mM JA and RWW were the only treatment where plants show no symptoms for wilting (Figure 4.5). Treating plants with JA tend to prevent wilting of plants, with no significant differences between mix and reclaimed wastewater.

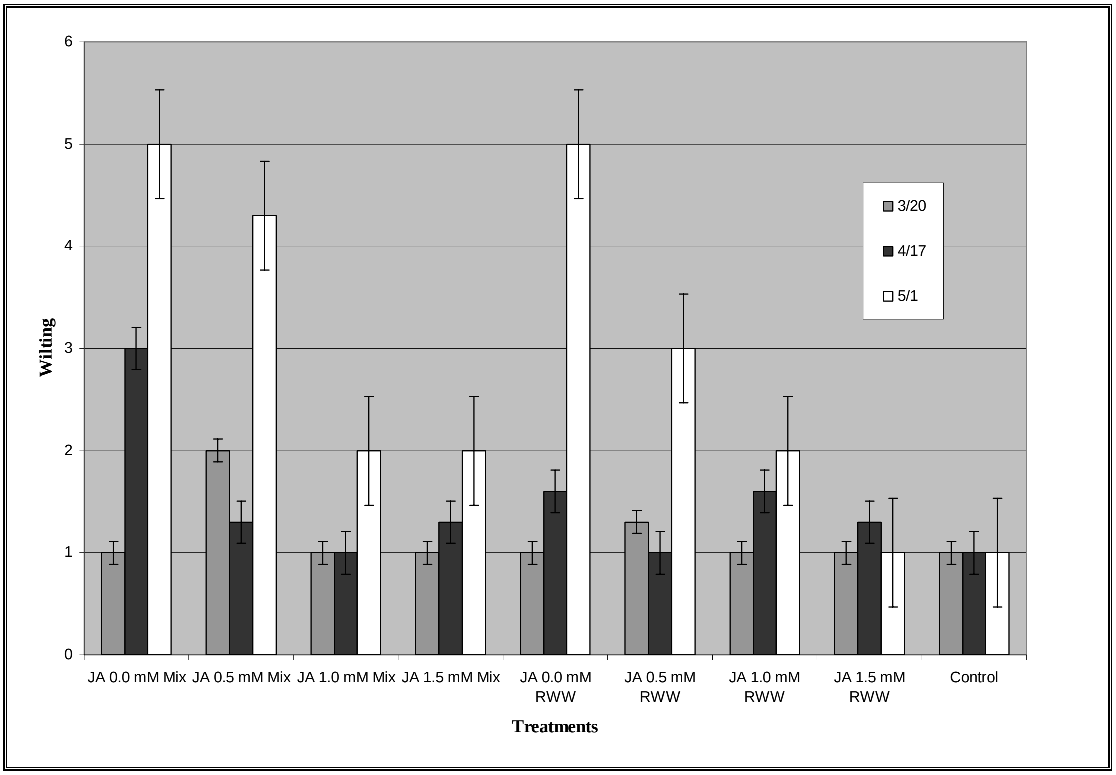

Figure 4.5: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on wilting of *Vicia faba* plants at different date.

*p ≤ 0.05, vertical pars represent ± 1 SE, n = 3 samples/ treatment.

*Wilting score: 1= no wilt, 5 = complete wilt.

4.1.6 Influence on salt injury

The influence of JA and reclaimed wastewater treatments is shown in Figure 4.6. It is clear that plants irrigated with FW (control) had less injury than other treatments. Plants treated with 1.5 mM JA, either irrigated with Mix or RWW, had significantly lower salt injuries compared to control plants. Plants that received no JA treatment exhibited significantly severe injuries (the highest score for salt injury), irrespective of the irrigation source, the 'Mix' or RWW alone.

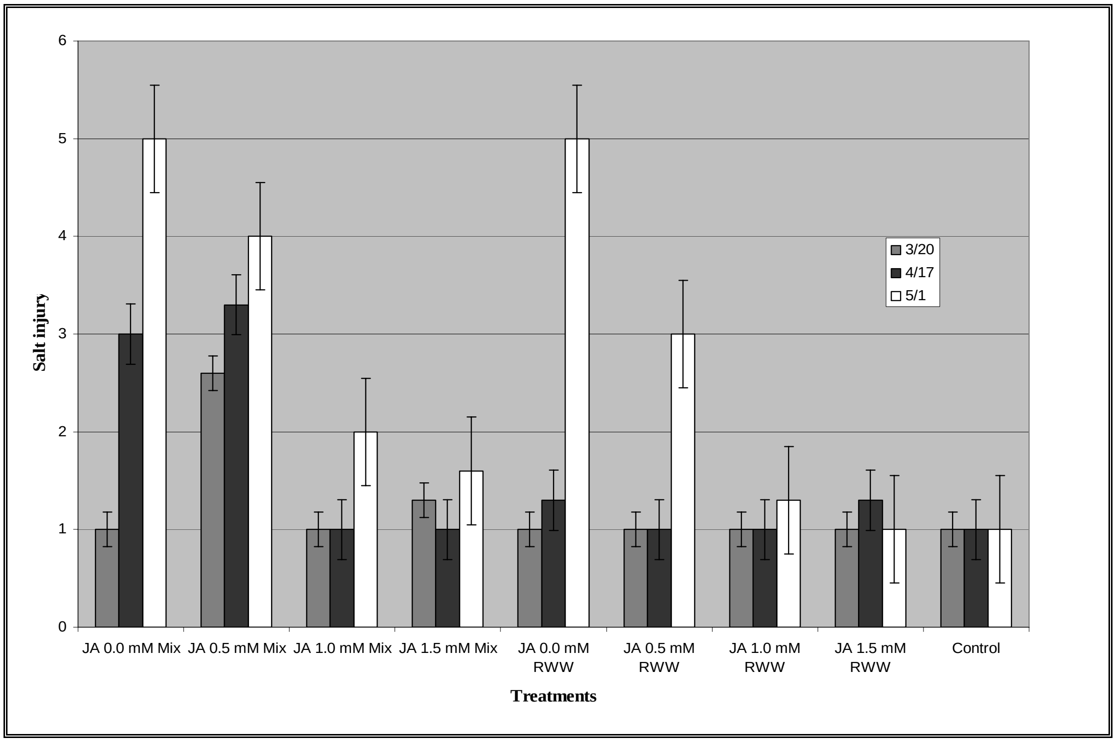

Figure 4.6: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on salt injuries of *Vicia faba* plants on different date.

*$p \leq 0.05$, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

*Salt injury score: 1= no salt injury, 5 = complete injury.

4.1.7 Influence on fruit weight:

The influence of JA and reclaimed wastewater treatments is shown in Figure 4.7. Fruit weight decreased drastically upon the irrigation with ‘Mix’ water. However, treating plants with JA, in particular at high levels (1.5 mM) led to fruit weight almost equivalent to fruits produced on plants irrigated with fresh water. Results show that plants irrigated with reclaimed wastewater alone gave, in most cases, heavier fruits compared to plants irrigated ‘Mix’; comparisons at each JA level.

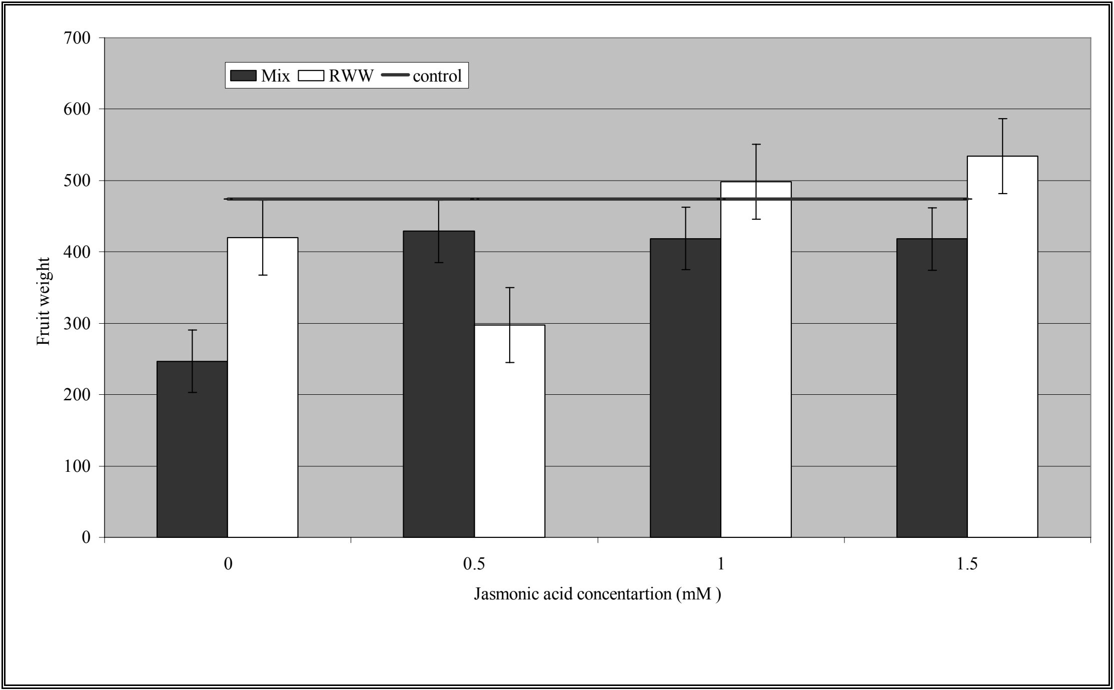

Figure 4.7: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on fruit weight (g) of *Vicia faba* plants.

*$p \le 0.05$, vertical pars represent ±1 SE, n= 3 samples/ treatment.

4.2. Plant tissue analysis

4.2.1 Influence on calcium concentration of new Leaves

The effects of reclaimed wastewater and JA treatments on Ca-level of new (upper) leaves are illustrated in Figure 4.8. Ca -level in plant irrigated with reclaimed wastewater and received no JA treatment was significantly higher. As JA concentration increased, Ca -level increased significantly in plant irrigated with reclaimed wastewater.

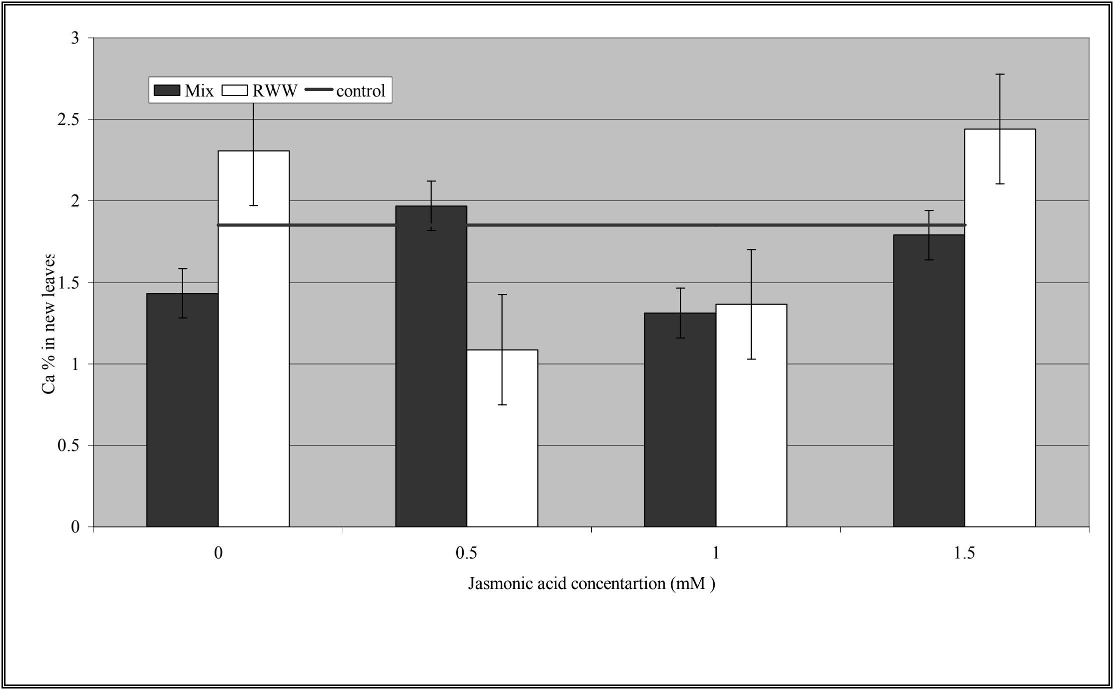

Figure 4.8: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on Ca-level of new leaves of *Vicia faba* plants.

*p ≤ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.2.2. Influence on calcium concentration of old leaves

Figure 4. 9 illustrates the effects of salinity and JA on Ca-level in down (old) leaves. It is obvious that increasing concentration of JA did not alter Ca-level in old leaves of plants, either those irrigated with mix water or those irrigated with reclaimed wastewater, and no significant differences exist between all treatments.

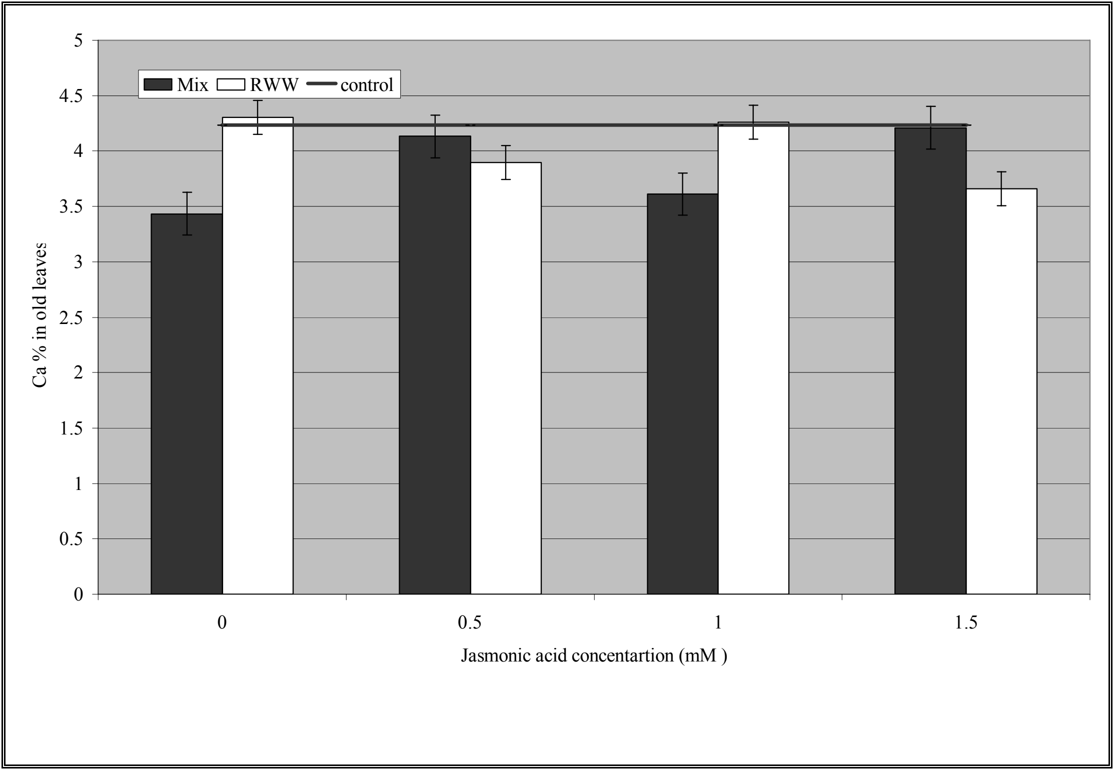

Figure 4.9: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on Ca-level of old leaves of *Vicia faba* plants.

*p ≤ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.2.3. Influence on total phosphorus concentration of new leaves

Phosphorus levels in leaves tend to increase upon the irrigation with ‘Mix’ water more than with RWW (Figure 4.10). Moreover, differences between JA treatments are not significant, although a slight decrease in P-level can be observed by increasing JA-level.

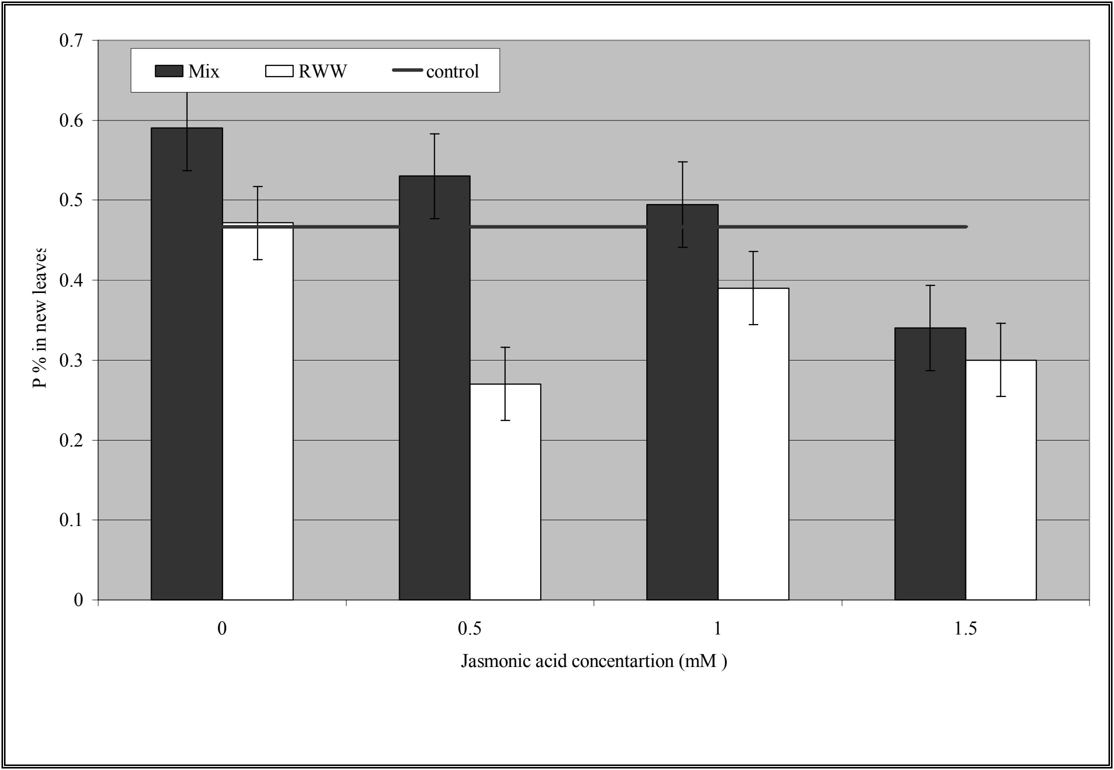

Figure 4.10: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on P-level of new leaves of *Vicia faba* plants.

*p $\leq$ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.2.4. Influence on total phosphorus concentration of old leaves

All plants irrigated with 'Mix' water had higher P –level than plants irrigated with reclaimed wastewater at all JA concentrations (Figure 4.11). Although the increases in JA-level led to a decrease in P-level, differences between JA treatments are not significant.

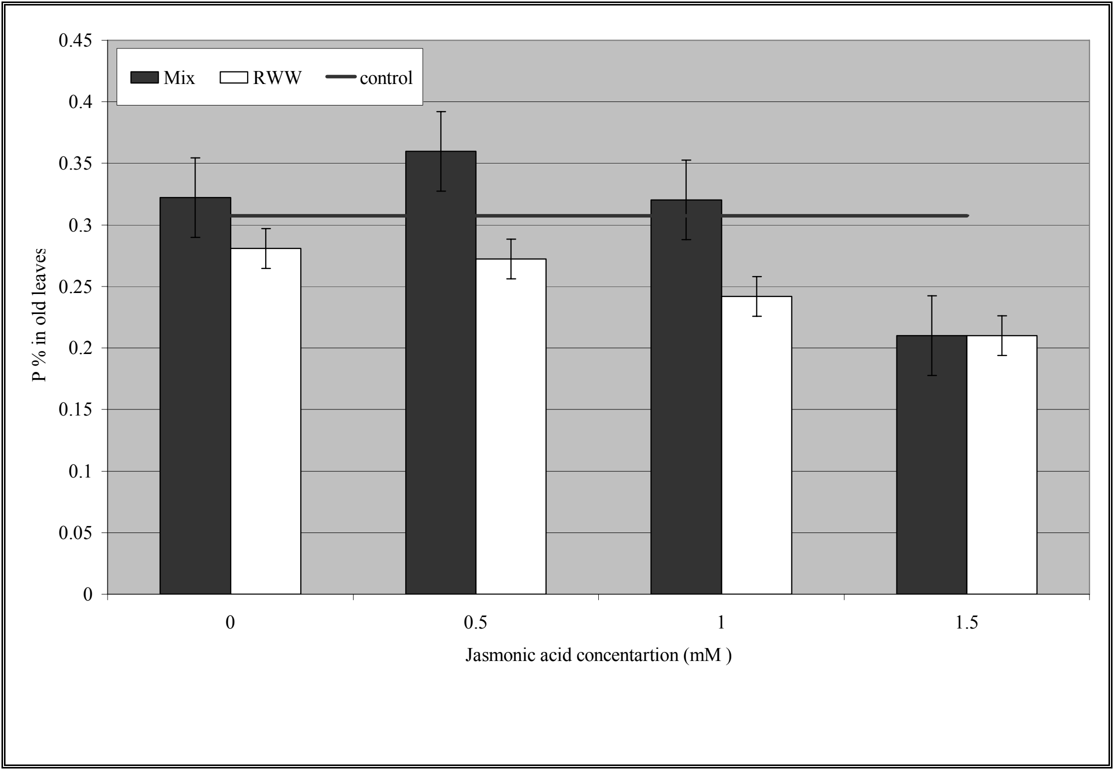

Figure 4.11: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on P-level of old leaves of *Vicia faba* plants.

*p ≦ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.2.5. Influence on potassium concentration on new leaves

Figure 4.12 illustrates the effect of reclaimed wastewater and JA treatments on K - level of new leaves. Higher K values were recorded in plants irrigated with RWW, compared with plants irrigated with Mix water, although without significant differences. Differences between JA treatments are not significant too.

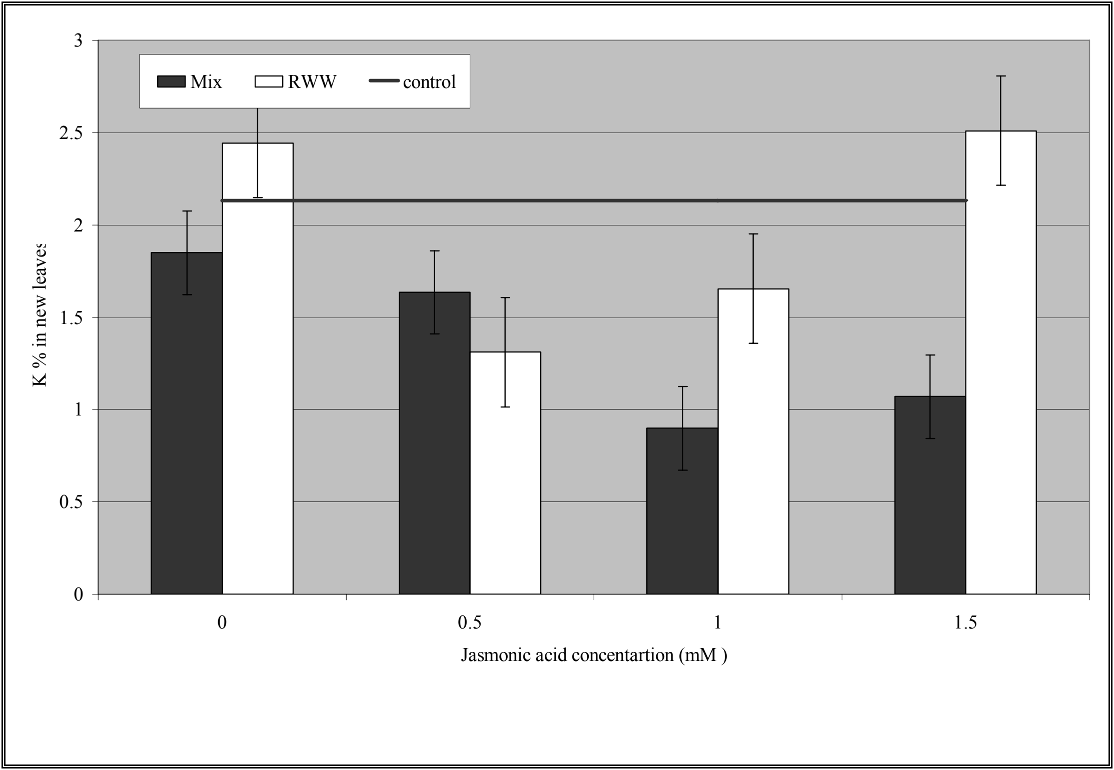

Figure 4.12: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on K-level of new leaves of *Vicia faba* plants.

*p ≦ 0.05, vertical pars represent ± 1SE, n= 3 samples/ treatment.

4.2.6. Influence on potassium concentration on old leaves

The effect of reclaimed wastewater and JA on K -level of old leaves is illustrated in Figure 4.13. The K -level in plants irrigated with RWW was significantly higher than with plants irrigated with 'Mix' water. Moreover, it is obvious that K -level increases as JA concentration increase for plants irrigated with RWW, which may explain the lower salt injuries on these plants.

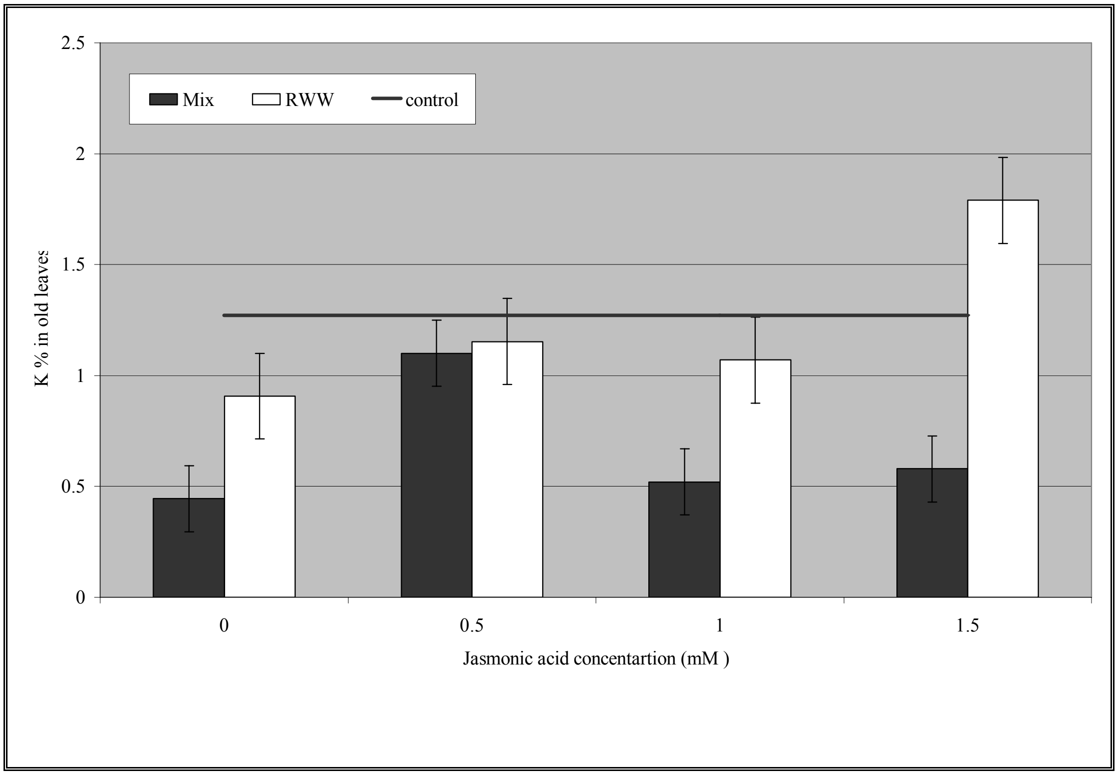

Figure 4.13: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on K-level of old leaves of *Vicia faba* plants.

*$p \le 0.05$, vertical pars represent ± 1SE, n= 3 samples/ treatment.

4.2.7. Influence on total nitrogen and protein concentrations in new leaves

N- level of leaves tend to increase in plants irrigated with ‘Mix’ water (Figure 4.14) Moreover, differences between JA treatments are not significant, although a slight increase in N-level can be observed by increasing JA-level. The same results were observed on protein-level (Figure 4. 15).

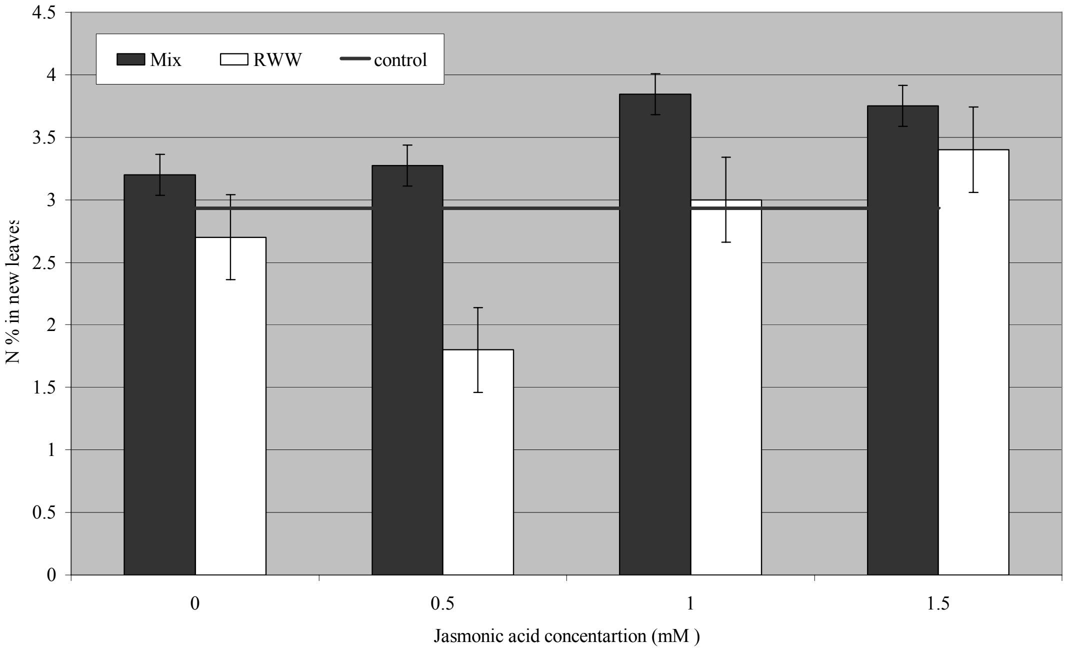

Figure 4.14: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on N-level of new leaves of *Vicia faba* plants.

*p ≦ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

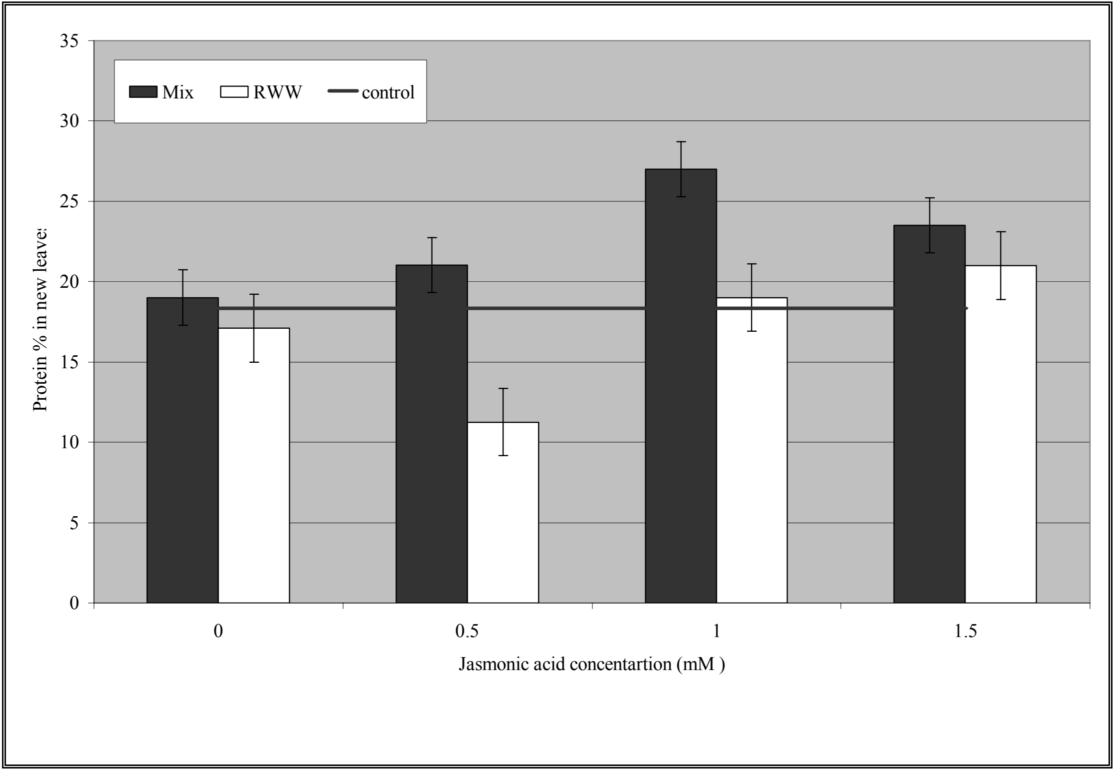

Figure 4.15: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on protein level of new leaves of *Vicia faba* plants.

*p ≤ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.2.8. Influence on total nitrogen and protein concentrations on old leaves

Figure 4. 16 show the effects of reclaimed wastewater and JA treatments on N -level of old leaves. Higher N values were recorded in plants irrigated with ‘Mix’ water compared to those irrigated with RWW. Moreover, differences between JA treatments are not significant, and similar results are observed on protein levels (Figure 4.17).

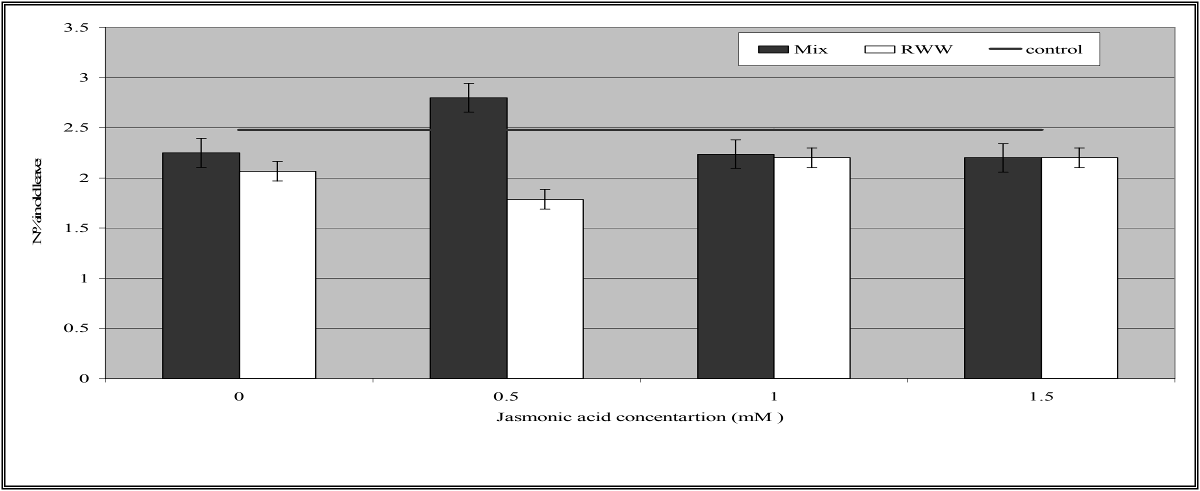

Figure 4.16: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on N-level of old leaves of *Vicia faba* plants.

*p $\leq$ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

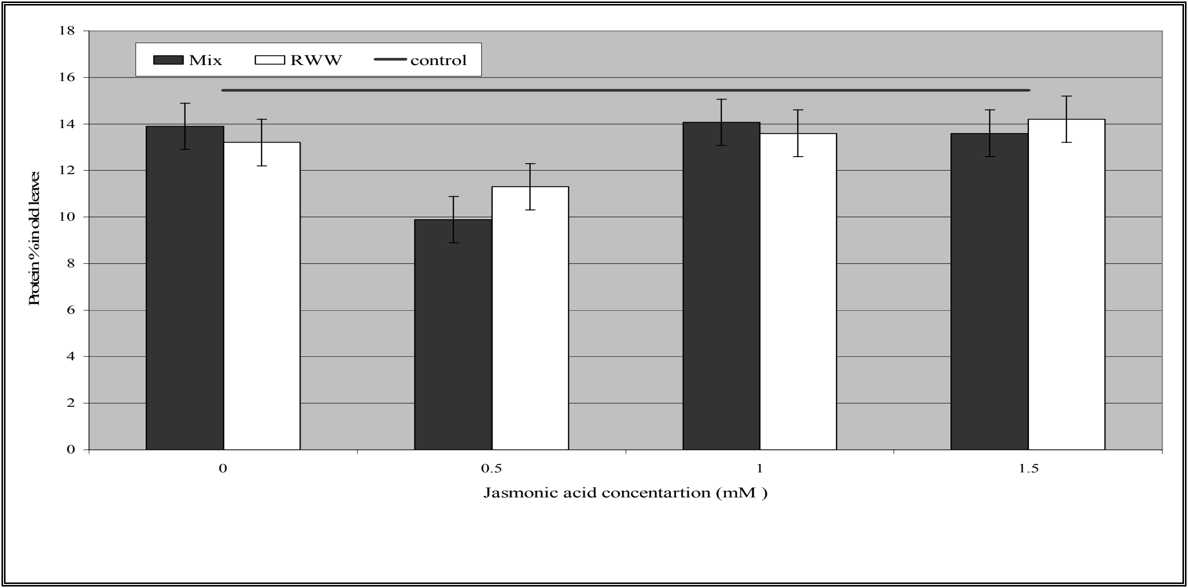

Figure 4.17: Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on protein level of old leaves of *Vicia faba* plants.

*p $\leq$ 0.05, vertical pars represent ± 1 SE, n= 3 samples/ treatment.

4.3. Fruit Quality:

4.3.1. Contamination of fruits

The contamination of fruits with various bacteria was investigated, and no sign of contamination was found. Parameters examined were: Total Coliform, fecal Coliform, *Salmonella,* and *E.coli.*

CHAPTER 5: Discussion

5.1. Field measurements

5. 1. 1. Influence of reclaimed wastewater treatments on vegetative growth

Broad bean has been considered as a salt sensitive crop; the threshold of electrical conductivity (EC) is 0.7 dS m^{-1}. Results show that increasing salinity level of reclaimed wastewater (Mix) led to reductions in plant height, number of leaves, and number of branches (Figure 4.1, 4.2, 4.3). This agreed with Jeschke et al. (1986). Salinity has a dual effect on plant growth, firstly via its osmotic effect on water uptake, and secondly on specific ion toxicities (Sheldoni et al., 2004; Bolarin et al., 2001). It is clear that water potential of the soil solution became more negative, and subsequently the plant ability to take soil water decreases. In order to maintain water uptake from saline soil, plants must adjust it osmotically, and this is achieved either by taking up salt or compartmentalizing it within plant tissue, or by synthesizing organic solutes (Sheldoni et al., 2004). Harmer and Benne (1995) stated that decreasing water movement from soil to plant decreased mineral uptake by plants, which may reduce growth. Moreover, a highly negative water potential in the soil may lead to a decreased transpiration rate, and stomatal opening, which directly reduced the amount of CO_2 taken by plants; reduced CO_2 uptake means lower photosynthesis rate and consequently lower growth, yellowing, and plant salt injury, and subsequently lower yield (Rawson and Munns, 1984; Mass and Grieve, 1987; Chaves, 1991; Cornic and Massacci, 1996).

5. 1. 2. Influence of JA on vegetative growth, yellowing, wilting and salt injury

Results show that exogenously applied JA reduced plant growth (Figure 4.1, 4.2, 4.3) and this is in agreement with Horton (1991), and with Liu et al. (2002) who found that exogenously applied JA induced stomatal closure in broad bean, and also with barley (Tsonev et al., 1998). Lee et al. (1996) and Pospilisova (2003) confirmed that JA inhibits carbon dioxide fixation, independently of ABA or stomatal closure, although stomatal closure generally occurs when plants are exposed to drought. In some cases and under severe stress, photosynthesis may be more controlled by the chloroplast's capacity to fix CO_2 than by the increased diffusive resistance (Faver et al., 1996; Herppich and Peckmann, 1997), and as stomatal closure and CO_2 fixation decrease, the growth was also decreased. The present investigation showed that plant growth decreased due to the effect of JA application, but JA application altered and alleviate, although partially, the symptoms of salt injury (Figure 4.6), such as leaf burning, chlorosis, wilting and yellowing (Figure 4.5) These effects may be related to inhibitory effect of JA on NaCl absorption by plant, and these findings cope with the results reported by Tsonev et al. (1998) and Fedina and Tsonev, (1997). Swiatek et al. (2003) reported also that root growth inhibition is a typical plant reaction to exogenous application of jasmonate, which indicates that systemic signals that are elevated in stress responses, like JA and ABA, can directly influence vegetative growth. The decrease in plant growth, due to exogenous application of JA, could be explained by the decreased rate of photosynthetic CO_2 fixation (Popova et al., 1988; Popova et al., 2003). In the other hand, exogenous application of JA on plant may induce a reduced rate in the biosynthesis of ribulose bisphosphate carboxylase/oxygenase (Rubisco) enzyme (Weidhase et al., 1987; Popova and Vaklinova, 1988; Rakwal and Komatsu, 2001). The hormonal balance plays also important role. Under Environmental stresses, several plant hormones, such as salicylic acid, abscisic acid, jasmonic acid and ethylene play a crucial role in altering plant morphology in response to stress. Jasmonic acid (JA) and abscisic acid (ABA) were found to prevent DNA replication in tobacco plants grown under salinity stress (Swiatek et al., 2003). Furthermore, jasmonates induced accumulation of a number of Jasmonate-induced proteins (JIPs) in many plant species (Muller-Uri et al., 1988). Maslenkova et al. (1992) adduced experimental data showing an induction of JIPs mainly belonging to the thylakoid-bounded polypeptides. Most of the JA-induced polypeptides were identical to ABA- and NaCl-induced proteins, leading to the assumption that exogenously applied jasmonates act as stress agents. Creelman and Mullet (1995) reported that the level of endogenous of jasmonates increased in plants suffering from drought and osmotic stress. However, Popova et al., (2003) reported that Salicity acid (SA) and Methyl Jasmonate (MeJA) could be responsible for protection of photosynthesis against paraquat oxidative stress, Moreover they hypothesized that both regulators could improve the rate of the carboxylating, protection of chlorophyll and protein breakdown, and the activation of the antioxidative enzymes in chloroplasts, which in turn would increase tolerance against Pq, or trigger various defense-related genes. In our experiment, it is possible to hypothesize that exogenously applied JA alleviate salinity stress by protecting chlorophyll, which expressed itself as leaves appear greener in JA-treated plants (Figure 4.4). These finding are, however, in contrast to Ueda and Kato (1980), Weidhase et al., (1987), and Chou and Kao (1992) who described the JA as a senescing hormone that accelerate leaf senescence.

5.2. Plant tissue analysis

5. 2. 1. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on mineral composition of leaves (Ca and K)

The present study showed that increasing salinity of reclaimed wastewater (Mix) led to decrease in Ca and K levels in leaves (Figure 4.8, 4.9, 4.12, and 4.13) which was reported also with Raid and Smith, (2000). Ca-level in old leaves (Figure 4.9), for all treatments, was higher than in young leaves (Figure 4.8), which is related to that fact that Ca-is considered as immotile plant nutrient (Mccauly, 2003). The lower Ca and K-level levels may be attributed to the competition between Na and K or Ca (Taban et al, 1999), since salt stress (excess Na) caused rapid efflux of cations, particularly K, which led usually to cation deficiency under saline conditions (Marschner 1995; Maathnis and Amtmann, 1999). In addition to that, salinity is known to reduce Ca 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). Moreover, 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. Cell membrane function may be compromised as a result of Na^+ replacing Ca^{2+}, resulting in increased cell leakiness (Orcutt and Nilsen 2000). The changes in the concentrations of cations, particularly K^+, control pressure potential and water uptake by the plant (Marschner 1995). It was obvious in the present study (Figure 4.8) that Ca level in plant new leaves increased upon JA application, regardless of JA concentrations. Increasing K uptake may be related to the increase in Ca level in plant tissue (Figure 4.12), which decreases the salinity effect on K uptake. The addition of Ca in plant tissue tends to decreases the uptake of Na ion and enhances K uptake, which help to maintain the permeability of leaf cell membranes (Hansen and Munns, 1988; Zekri and Pearson, 1990). Cationic content, especially Ca, may help in alleviating the inhibitory effects of Na toxicity and restored growth when compared to control plants. It well documented that Ca, in addition to several plant growth regulators such as kinetin, ethephon, gibberellin, and some amino acids, such as proline, are known to alleviate salinity stress of plants (Shaddad, 1990; Zekri and Pearson, 1990). In this respect, Jyothsna and Sudhakar (2003) found that exogenous JA application increased in free proline content. The Jasmonic acid may also affect, although indirectly, the plant salinity resistance. The possible mechanism of JA and MeJA action on stomatal opening is probably similar to that of ABA in suppression of H^+ efflux and K^+ influx (Raghavendra and Reddy 1987). Since K^+ and Ca^{+2} have important osmotic roles in plants (Tesidale et al., 1985; Fagaria et al., 1991; Ammar et al., 2004), an increase in their content in leaves will improves drought resistance, and maintains the selectivity and integrity of the cell membranes. That will lead to a reduced water loss and wilting.

5. 2. 2. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on total phosphorus level in plant tissue

Results showed that as salinity level of reclaimed wastewater (Mix) increased, total phosphorus increased in plant tissue (Figure 4.10, 4.11). These results are in agreement with Grattan et al. (1988a); Grattan et al. (1988b); Aiden et al. (1999). The increase of plant P upon salinity may be due to the increased availability of P in the soil, or synergetic effect of Na, which is involved in P uptake. Aiden et al. (1999) reported leaf P concentrations tended to increase with increasing P levels, to a greater extent under saline conditions.

In the present study JA appeared to reduce P uptake, and that could be explained in two ways, 1) by closing the stomata and consequently reduce both photosynthesis, and phosphate synthesis. 2) JA may, as it is mentioned previously, inhibit NaCl effect, which reduced the availability of P up take by plant (Tsonev et al., 1998).

5. 2. 3. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on nitrogen uptake

The present investigations indicate that as salinity level of reclaimed wastewater (Mix) increased, nitrogen content and protein were increased (Figure 4.14), which may be related to higher nitrogen fixation and greater uptake of N from the soil under the salt-stress conditions (Rao et al., 2002). Other studies, however, suggest that some proteins produced under salinity stress, such as glycinebetaine, and proline, which may play a role in osmotic adjustment subjected to salinity stress (Braze, 2004).

Jasmonic acid application tend to increase nitrogen and protein content in plant tissue (Figure 4.14 4.15), and that could be related to known effect of Jasmonate, in many plant species, in the induction and accumulation of certain proteins known as JIPs protein (Maslenkova et al., 1992; Moons et al., 1997; Muller-Uri et al., 1988). Rossato et al. (2001b) and Beardmore et al. (2002) showed that MeJA increases N cycling within the plant at the expense of leaves, leading to a transient accumulation of N within roots, and that led to increase vegetative storage protein. VSP is thought to play an important role in the temporary storage of N because it accumulates abundantly in several immature organs (Paul et al., 1991).

5.3. Influence of reclaimed wastewater treatments (RWW+JA and Mix+JA) on fruit weight:

Results show that increasing salinity level of reclaimed wastewater (Mix) led to decreased fruit weight (Figure 4.7), and that agreed with Rao et al. (2002) and Singleton and Bahlool (1984), who found that production of grain legumes is severely reduced in salt-affected soils. As a result, salinity led to lower fresh and dry-weights, which means lower yields (Osowa, 1960; Rush & Esptein, 1975; Levitt, 1980; Sharma, 1980; Robinson et.al., 1983; Sharma et.all Garg, 1983). In contrast to fruit number, the fruit weight increased with JA treatments, and that could be attributed to inhibiting effect of JA on the yellowing of leaves (Figure 4.4) Under such condition, leaves live longer, and the filling period of pods will be subsequently longer, which may be the reason for bigger fruits (pods).

5.4. Fruit crop quality:

Present results show that fruits were free from pathogenic bacteria, which may be related to high quality of the effluent, which was cleared by using sand filter (Meerbach, 2004). In addition to that, drip irrigation system was used, which prevent water from reaching fruits, and significantly reduce contamination. This is in agreement with Sadavoski et al. (1975) and Goldberg et al. (1976), who found that drip irrigation is the better choice to use the reclaimed wastewater.

CHAPTER 6: Conclusion and Recommendation

.In this experiment, results show that exogenous application of Jasmonic acid tend to improve the tolerance of plants irrigated with ‘Mix’-water that has an EC-value of 5 to 7 dS m^{-1}. That was evident in lower salt injuries and less wilting, but with higher average fruit weight and higher Ca and K level in leaves for plants treated with higher level of JA.

6.1 Conclusions:

- Increased salinity level of reclaimed wastewater inhibited plant growth, and reduced fruit weight.
- Increased salinity of reclaimed wastewater changed the nutrient uptake of plants.
- Exogenous applications of JA alleviate partially the salinity stress ,probably by protecting chlorophyll, which expressed itself, as leaves appear greener in JA-treated plants.

6.2 Recommendations:

- Further research studies are needed to explain JA efficiency in alleviating the plant resistance to salinity stress.
- Reuse of Al-Bireh WWTP effluent in agriculture, is recommended.
- Using drip irrigation system in restricted irrigation is highly recommended
- Further research studies are needed to assess the impact of reclaimed wastewater and brackish water on the soil flora and fauna.

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APPENDICES

Appendix 1: Detailed mean length (cm) of plant with time for each treatment during the experiment.

Date	Mix				RWW				Control
	Jasmonic Acid (mM)				Jasmonic Acid (mM)				
	0.0	**0.5**	**1.0**	**1.5**	**0.0**	**0.5**	**1.0**	**1.5**	
10/01/05	9.3	9.3	16.8	23.9	7.9	15.3	25.1	33.4	18.0
19/01/05	13.1	13.8	24.4	35.4	11.7	22.2	33.5	43.8	24.4
27/01/05	15.9	17.5	31.0	43.8	16.4	30.8	46.2	59.8	33.4
02/02/05	19.4	22.3	38.3	54.0	21.3	39.4	58.0	75.2	40.3
09/02/05	19.4	21.9	38.8	54.8	19.8	38.8	57.8	75.8	39.0
17/02/05	22.1	25.5	45.8	66.3	25.3	47.9	70.8	89.8	48.8
24/02/05	31.0	34.3	61.9	87.5	34.3	65.8	98.5	126.5	67.3
03/03/05	40.0	41.7	75.4	108.9	40.9	78.4	117.8	156.2	82.5
10/03/05	50.3	51.3	96.8	139.2	51.9	100.7	148.5	197.9	107.0
17/03/05	50.3	55.4	103.1	150.0	53.8	107.8	164.3	215.9	115.8
24/03/05	58.3	59.6	117.3	167.5	60.6	120.1	177.0	236.1	128.6
03/04/05	69.2	69.5	137.8	199.8	64.3	135.0	205.3	270.5	149.3
10/04/05	71.8	71.6	141.5	205.0	67.5	141.3	214.0	280.2	151.3
21/04/05	75.2	74.4	146.8	212.8	77.2	158.7	235.1	306.1	166.7

Appendix 2: Detailed mean leaves number of plant with time for each treatment during the experiment.

Date	Mix				RWW				Control
	Jasmonic Acid (mM)				Jasmonic Acid (mM)				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
19/01/05	4.7	4.3	2.5	4.0	4.7	4.0	4.5	4.2	4.8
27/01/05	6.8	7.5	6.5	5.2	6.8	5.8	7.0	8.3	7.0
02/02/05	11.2	11.5	10.0	9.3	11.3	10.0	11.7	10.5	11.5
09/02/05	12.0	12.0	10.5	10.3	11.8	11.4	13.0	11.0	12.2
17/02/05	12.3	16.3	14.3	11.5	14.2	14.5	14.2	13.3	13.0
27/02/05	19.2	23.7	18.8	16.2	21.3	20.5	22.7	20.2	19.2
06/03/05	24.3	29.8	24.7	21.5	28.2	25.8	26.8	23.7	27.7
13/03/05	29.2	37.3	31.2	26.8	36.7	33.5	34.3	31.0	34.2
20/03/05	37.3	43.2	39.2	33.3	42.2	40.3	41.8	34.7	40.8
27/03/05	38.5	47.3	42.5	36.8	46.5	44.7	44.7	35.3	44.8
03/04/05	39.2	48.3	46.2	36.7	46.3	43.7	44.0	37.8	44.2
10/04/05	40.7	52.5	47.7	42.0	49.5	45.2	48.0	43.0	46.8
24/04/05	51.7	57.7	41.7	45.3	58.7	58.5	52.8	43.3	62.8

Appendix 3: Detailed mean branches number of plant with time for each treatment during the experiment.

Date	Mix				RWW				Control
	Jasmonic Acid (mM)				Jasmonic Acid (mM)				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
06/03/05	3.7	5.2	4.2	4.2	4.2	3.5	4.0	3.3	3.5
13/03/05	3.8	5.5	4.5	4.5	4.7	5.2	4.5	3.5	4.0
20/03/05	4.2	5.8	5.0	5.0	5.2	6.0	4.8	3.8	4.8
27/03/05	4.7	6.2	4.7	4.7	5.7	6.0	5.0	4.2	5.2
03/04/05	4.7	6.7	5.2	5.2	5.5	6.2	5.3	4.5	5.5
10/04/05	4.8	6.7	5.3	5.3	6.0	6.2	5.8	4.8	6.0
24/04/05	4.8	5.8	4.5	4.5	5.8	5.7	5.2	4.5	5.8

Appendix 4: Detailed mean Fruit number of plant with time for each treatment during the experiment.

Date	Mix				RWW				Control
	Jasmonic Acid (mM)				Jasmonic Acid (mM)				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
7/4/2005	3.0	10.3	13.7	9.3	12.0	6.7	7.7	10.0	9.0
14/4/2005	18.3	27.3	17.3	19.0	17.3	15.0	23.7	24.0	20.7
1/5/2005	12.0	9.7	11.3	13.0	15.3	11.3	14.0	9.3	14.7
Total no.	33.3 a	47.8a	43.3a	42.8a	44.7a	33.5a	46.3a	44.8a	44.3a

Appendix 5: Detailed mean Fruit weight (gm) of plant with time for each treatment during the experiment

Time	Mix				RWW				Control
	Jasmonic Acid (mg/l)				Jasmonic Acid (mg/l)				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
7/4/2005	13.5	81.2	159.7	101.3	104.5	53.7	107.3	142.0	79.0
14/4/2005	152.8	295.6	152.9	221.6	167.7	160.8	221.4	286.9	205.8
1/5/2005	89.5	52.2	106.0	95.2	147.7	83.0	169.7	105.3	189.3
Total (wt)	255.8a	429.4abc	419.6abc	419.6abc	419.9abc	298.0ab	499.4c	535.7c	474.1bc

Appendix 6: Detailed mean score of plant wilting with time for each treatment during the experiment

Treatment	Jasmonic Acid	20.3.2005	17.04.2005	1.5.2005
	(mM)	mean	mean	mean
Mix	**0**	1.0a	3b	5d
	0.5	2a	1.3ab	4.3cd
	1	1.0a	1.0a	2abc
	1.5	1.0 a	1.3ab	2abc
RWW	**0**	1.0a	1.6ab	5d
	0.5	1.3a	1.6ab	3bcd
	1	1.0a	1.6ab	2abc
	1.5	1.0a	1.3ab	1.0a
Control	**0**	1.0a	1ab	1ab

Appendix 7: Detailed mean score of plant yellowing with time for each treatment during the experiment

Treatment	Jasmonic Acid	20.3.2005	17.04.2005	1.5.2005
	(mM)	mean	mean	mean
Mix	**0**	1a	2a	5c
	0.5	1a	4b	4.3bc
	1	1a	1.6a	2.3ab
	1.5	1a	1.6a	2.3ab
RWW	**0**	1a	2a	5c
	0.5	1.3a	1.3a	3.6bc
	1	1a	1.6a	2ab
	1.5	1a	2a	1a
Control	**0**	1a	2a	2ab

Appendix 8: Detailed mean score of plant salt injury with time for each treatment during the experiment

Treatment	Jasmonic Acid (mM)	20.3.2005 mean	17.04.2005 mean	1.5.2005 mean
Mix	**0**	1.0a	3bc	5c
	0.5	2.6b	3.3c	4bc
	1	1.0a	1.0a	2ab
	1.5	1.3ab	1.0a	1.6ab
RWW	**0**	1.0a	1.3ab	5c
	0.5	1.0a	1.0a	3abc
	1	1.0a	1.0a	1.3ab
	1.5	1.0a	1.3ab	1.0a
Control	**0**	1.0a	1.0a	1.0a

Appendix 9: Detailed mean Ca -level in plant leaves.

Treatment	Jasmonic Acid (mM)	Ca % Upper leaves mean	Ca% Down leaves mean
Mix	**0**	1.43ab	3.43a
	0.5	1.97abc	4.13a
	1	1.31a	3.61a
	1.5	1.79abc	4.21a
RWW	**0**	2.31bc	4.3a
	0.5	1.09a	3.9a
	1	1.38ab	4.14a
	1.5	2.44c	3.66a
Control	**0**	1.85abc	4.24a

Appendix 10: Detailed mean total P -level in plant leaves.

Treatment	Jasmonic Acid (mM)	P% Upper leaves mean	P% Down leaves mean
Mix	**0**	0.59a	0.32bc
	0.5	0.53a	0.35c
	1	0.49a	0.32bc
	1.5	0.34a	0.21a
RWW	**0**	0.47a	0.28abc
	0.5	0.27a	0.27ab
	1	0.39a	0.24ab
	1.5	0.30a	0.21a
Control	**0**	0.46a	0.30bc

Appendix 11: Detailed mean K -level in plant leaves

Treatment	Jasmonic Acid (mM)	K % Upper leaves Mean	K % Down leaves mean
Mix	**0**	1.8abc	0.44a
	0.5	1.64abc	1.1c
	1	0.9a	0.52a
	1.5	1.07a	0.58ab
RWW	**0**	2.44c	0.91bc
	0.5	1.31ab	1.15c
	1	1.64abc	1.07c
	1.5	2.51c	1.79d
Control	**0**	2.13bc	1.27c

Appendix 12: Detailed mean N -level and protein in plant leaves

Treatment	Jasmonic Acid (mM)	Upper	Leaves	Down	Leaves
		%N mean	Protein mean	%N mean	Protein mean
Mix	**0**	3.2ab	19ab	2.2a	14.0a
	0.5	3.2ab	21.0ab	2.8a	9.8a
	1	3.84b	27b	2.2a	14.0a
	1.5	3.75b	23.5b	2.2a	13.6a
RWW	**0**	2.7ab	17.1ab	2.0a	13.2a
	0.5	1.8a	11.26a	1.7a	11.3a
	1	3.0ab	19ab	2.2a	13.6a
	1.5	3.4ab	21ab	2.2a	14.2a
Control	**0**	2.9ab	18.3ab	2.4a	15.4a