An-Najah National University
Faculty of Graduate Studies

Determination of Nitrate and Nitrite Content in Several Vegetables in Tulkarm District.

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Dedication

My grateful thanks and appreciation are for my precious parents, for their encouragement, effort, and patience. My grateful thanks are also for my sisters, and for my husband who was very supportive and guided me all the way, and for everything they all did for me until I finished this work. My thanks are also for all my friends for their support.

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Abstract

A study of the content of nitrate and nitrite in few different vegetables in Tulkarm district was conducted. This district is part of the West Bank land (Palestine). A total of 75 samples of five different types of vegetables (tomato, cucumber, onion, potato, and cabbage) were collected from randomly selected farms in three different areas in Tulkarm district (Thenabe, Al-Sha'raweya, and Al-Kafriyat). The nitrate content in the samples was analyzed using HANNA spectrophotometer (HI93728-0), while the nitrite content was analyzed using the AOAC Official Method 973.31.

The results showed that the type of the vegetable has a great effect on the nitrate content, the highest nitrate content was found in potato with an average of 253.13mg/Kg, while the lowest nitrate content was found in tomato with an average of 16.95mg/Kg. The nitrate content was affected by fruit size; the small cucumber size shows a higher nitrate content than

the large size fruits; It was found that the peels of the cucumber fruits contain higher nitrate content than the essence. Cabbage as a leafy vegetable shows a high nitrate content. The nitrite concentrations were low (less than 1mg/Kg) in all test crops. These levels of nitrite are considered acceptable. The different locations show no significant effect on the nitrate and nitrite contents in vegetables tested in this study. This study high lights an important issue which was not investigated before. Under the conditions of the study, the results indicated acceptable levels of both nitrate and nitrite content. However more work is needed to elaborate these results with more samples and different crops.

Chapter One

Introduction

Introduction

Pollution issue is now among the most important issues, that the world countries are interested in, since this issue has a great effect on all life aspects and causes great threaten to animals, plants, environment, and human health. The pollution problems rose up after the industrial evolution.

Nitrates and nitrites seemed to be among the chemicals that may cause pollution; many studies have expected the effect of these compounds on the environment and on the living health. These studies focused on the nitrate and nitrite contents in water sources and in vegetables consumed by humans. In order to control the nitrate and nitrite intake by consumers in general and on babies in particular who are the most vulnerable to the adverse effects of these two compounds, a maximum acceptable limit of these compounds were suggested.

The Acceptable Daily Intake (ADI) of nitrate and nitrite set by European Commission's Scientific Committee for Food (ECSCF), is 3.7mg/Kg body weight, and 0.06 mg/Kg body weight, respectively [WHO,1995] [18, 42].

For methamoglobinaemia in infants, it was confirmed that the existing guideline value for nitrate ion in drinking water of 50 mg/L is protective. For nitrite, human data reviewed by (JECFA) support the current provisional guideline value of 3 mg/L [15].

Agriculture is considered the major source of nitrate and nitrite in the environment [16]. Fertilizers use is rapidly increased, causing excessive nitrogen loading to the environment [16, 47]. Globally, the use of fertilizers was increased from less than 14 million tones in 1950 to 135 million tones in 1996 [67, 69]. In 1998, the world used 137 million metric tons of chemical fertilizers. [24]

In our areas (in Palestine), farmers use higher amounts of fertilizers than they should, either due to their ignorance, or because they want to increase their production quickly when the prices of these products in markets are high. Plants can not utilize all the added fertilizers, so the excessive amounts will be leached out by irrigation water through the soil to reach ground water, or it may dissolve in run off water and flows into streams or lakes and rivers [10]. Nitrogen compounds are also accumulates in some plant tissues [10].

More than three quarter of our average nitrate intake comes from vegetables [54], which provide about 80% of the average daily dietary intake [15]. Vegetables that may accumulate nitrate in their tissues are leafy vegetables such as spinach, lettuce, and cabbage, or root crops like carrot, beetroots, potatoes, radish, and others like cauliflower, beans, and peas. The amount of nitrate in vegetables like lettuce is usually ranges between 90 and 3520 ppm [54].

The intakes of nitrate and nitrite from food were calculated as a global level on the basis of mean food consumption in the GEMS/Food regional diets [WHO, 1998], and the mean concentrations in food in Europe from the submitted data. Intake from drinking water was added, assuming a water consumption of 2 L/day. The mean concentration in water that was used in the intake calculations was 4 mg/L for nitrate and 0.3 mg/L for nitrite (Table 1&2), which are representative of the usual concentrations found in water [WHO, 1998]. An average body weight of 60 Kg was used for the global intake assessment [27].

Table (1): Nitrate intakes from sources other than food additives in the middle and far eastern [26].

Food	**Nitrate mg/Kg**	**Diet**					
		Middle Eastern			**Far Eastern**		
		Consumption (g/day)	**Intake (mg/day)**	**%total intake**	**Consumption (g/day)**	**Intake (mg/day)**	**%total intake**
lettuce	1700	2.3	4.0	10	0	0	0
spinach	1900	.05	.093	2	0	0	0
Chinese cabbage	3500	0.1	0.35	1	0.1	0.35	1
onion	110	23	2.5	6	12	1.3	5
potato	180	5.9	1.1	3	19	3.4	13
cabbage	340	5	1.7	4	9.7	3.3	12
tomato	15	82	1.2	3	7	0.1	0
Fruit*	25	19	0.48	1	78	1.9	7
water	4	2000	8	20	2000	8	29
ADI%*			20			10	

* Fruit: total fruit, minus apple and pear.

* ADI: Acceptable Daily Intake

Table (2): Nitrite intakes from sources other than food additives in the middle and far eastern [26].

Food	Nitrite (mg/Kg)	Diet					
		Middle Eastern			Far Eastern		
		Consumption (g/day)	Intake (mg/day)	%total intake	Consumption (g/day)	Intake (mg/day)	%total intake
Potato	0.7	5.9	0.0041	0	19	0.013	1
vegetable	0.6	230	0.14	7	180	0.11	5
Cereals	2.6	430	1.1	54	450	1.2	59
Fruit*	0.5	200	0.1	5	85	0.043	2
ADI%*			50			50	

* Fruit: total fruit.

* ADI: Acceptable Daily Intake.

1.1. Nitrate and nitrite effect:

The high concentration of nitrate has adverse effects on environment, animals and humans:

a. Environment: the high concentrations of nitrate in water causes a phenomenon known as "Eutrophication", which means an excessive growth of the algae in water which consumes the oxygen gas dissolved in water causing the death of fishes in that water [36].

b. Animals: especially ruminant animals such as cows, sheep and goats [5]. When ruminants consume feed with high nitrate levels, the nitrate can be converted to nitrite, which causes both nitrate and nitrite accumulation in the rumen [5].

As a result of the accumulation of both nitrate and nitrite in animal rumen, it causes acute and chronic symptoms which run as reduction in weigh gain, reduction in milk production, low appetite, aborted breathing, blue coloring of mucus membrane, rapid heartbeat, abdominal pain, vomiting, reproductive problems, abortions, and premature death of calves [5, 63].

The acute nitrate poisoning causes death, because the nitrate is reduced to nitrite in the rumen by bacteria. Nitrite is highly toxic because it combines with hemoglobin and form methemoglobin which is enabling to carry oxygen [3, 5, 33, 63].

A death occurs within few hours after the ingestion of a high nitrate feed [5].

c. Human: Nitrate itself is not toxic; however, the conversion of nitrate to nitrite in human and animal bodies is very dangerous if it accumulates in high concentrations [5].

On the other hand, the following could be occurring as a result of the accumulation (or uptake) of large dose of nitrate for human health:

1. Methemoglobenemia [62] [15, 68]: drinking water and vegetables are the major sources of nitrate consumed by human stomach [67]. Nitrate is reduced to nitrites which combine with hemoglobin to form methemoglobin (metHP). Methemoglobin is a compound that can not combine with oxygen, and that decreases the capacity of the blood to transport oxygen from lungs to body tissues causing a condition known as "Blue Baby Syndromes" or "methemoglobenemia" [47].

NO_2 + oxyHb (Fe^{2+})----→ metHb (Fe^{3+}) + NO_3 [15]

The normal met HP level in humans is less than 3% in infants under three months of age. However hypotoxic signs may develop at about 20%, while death occurs at 50% metHP or higher [15, 66].

Because of their high stomach acidity, infants less than one year old (3-6 months old), are highly infected by the methemoglobenemia, this acidity increases the conversion of nitrate to nitrite by providing an appropriate environment for the nitrate reducing bacteria [WHO,1996] [16].

Other groups that may be risk to form met HP are pregnant women [15, 22, 63], which may cause birth defects [62, 63] and miscarriages [63]. Also people who have deficiency in glucose-6- phosphate dehydrogenase or metHb reductase may be at risk to form metHP [15, 22].

Adults with lower gastric acidity can be infected with metHP. Fatalities have been reported after single intake of 4-50 g of nitrate (equivalent to 67-833 mg of nitrate per Kg of body weight) [WHO.1996].

The symptoms related to high levels of metHP in blood includes bluish discolorate of skin, headache, dizziness difficulty in breathing, in sever cases damage to brain and death may occur.

2. Carcinogenicity: nitrite reacts in stomach with nitrosatable compounds to form N-nitraso compounds [36] [15, 16]. These compounds have been found to be carcinogenic [10] [15, 22, 33].

The US National Research Council found an association between high nitrate intake and gastric and esophageal cancer [63].

High levels of nitrate intake were also linked with the Non-Hodgkin's lymphoma [63], bolder cancer [63], pancreatic cancer [14] and stomach cancer [1] [25, 54].

High levels of nitrate were also linked with the infection with the diabetes, the occurring of some birth defects, and miscarriages [63].

1.2. Study area:

The study was conducted in Tulkarm district as an agricultural area.

Tulkarm district lies on the western north part of West Bank in Palestine. It is bounded by Nablus and Jenin districts in the North, West, and South, and by the 1948 cease-fire line in the East.

Tulkarm district covers approximately 334,530 dunum.

Table (3) shows the classification of the land use in Tulkarm district, which shows inefficiency in the use of land, where both Palestinian built-up and Israeli settlements are built on lands of extremely rich soil for cultivation. This has led to the spread of built-up areas and industries at the cost of agricultural land.

Table (3): The land use classification in Tulkarm district [74].

Land use	Area/ dunum	% of land
Palestinian Build up Areas	18020.5	5.39
Israeli Settlements	3170	0.95
Israeli Military Bases	70.5	0.02
Industrial Park	550	0.16
Nature Reserves	1730.5	0.52
Forests	4140	1.24
Cultivated Areas	128100.5	38.3
Others*	178720.4	53.42
Total	334530	100

* Unused land or land used for grazing.

- The climate: Climate of the study area is a Mediterranean type with hot summers and warm winters.

- Precipitation: the rainy season starts in October and continues through May. Almost 70% of the annual rainfall occurs between December and February, while 20% of the annual rainfall occurs in October and November. The mean annual rainfall in Tulkarm city is 614.7 mm for the period from 1952 to 1995 (Tulkarm Agricultural Department).

- Humidity: humidity in Tulkarm district reaches high values with an annual average of 69.6%, which rises in winter to 75.9%in February, while it reaches its lowest value of 62.4% in May.

- Temperature: in Tulkarm district, the mean annual maximum temperature is 22.3C^{o}, and the mean annual minimum temperature is 15.6C^{o}.

- Winds: the wind direction in Tulkarm district mainly lies between the southwest and northwest with mean annual wind speed of 3.4Km/hr. Khamaseen winds blow over the area in the spring full with sand and dust, causing rising temperature and drop in humidity. The main daily wind speed from April to June is 3.2Km/hr.

Table (4): The average climatic parameters for Tulkarm district for the year 2005.

Month	Max. Temp(C^o)	Min. Temp(C^o)	Average Temp(C^o)	Humidity (%)	Wind speed (Km/hr)	Rainfall (mm) (1961-1995)
Jan.	19.0	9.2	15.5	64	4.0	218.8
Feb.	18	8.0	15.0	71	4.0	139.9
Mar.	22.5	10.5	18.0	63	3.0	17.1
Apr.	26.0	13.1	22.5	52	4.0	4.8
May	28.0	16.0	24.0	58	4.0	_
June	31.0	21.0	28.0	58	4.0	_
July	33.0	24.0	30.0	60	4.0	_
Aug	33.0	25.0	30.0	60	3.0	_
Sep.	34.3	23.1	30.0	59	4.0	_
Oct.	29.0	20.0	29.0	53	2.0	7.7
Nov.	23.7	11.7	20.6	59	5.0	56.6
Dec.	21.0	11.9	17.5	69	2.0	140.9
Average	22.3	15.6	18.95	69.6	3.4	
Total						663.3

*Tulkarm Weather Station.

Tulkarm is a very fertile area which explains its relative high population in comparison to other districts 583 person/km^2, while the population in Ramallah, Hebron, and Beithlahem are 200, 245, and 230 person/km^2, respectively.

The total area of Tulkarm is about 253,950 dunum. The arable area is 209,064 dunum, but the actually cultivated area is 138,368 dunum (ministry of agriculture 2004/2005). These areas are mainly cultivated with fruit trees, vegetables, and field crops.

Table (5) shows that fruit trees –mainly un-irrigated olive and irrigated citrus- forms the greatest area and it worth 27,655,000 U.S.A$, next comes the field crops and then the vegetables.

Table (5): The area, production, and value of different crops in Tulkarm district.

Crop	Area/dunum	Production/ton	Worth (1000 dollar)
Fruit trees	134,264	39,542	27,655
Vegetables	10,302	53,519	22,025
Field crops	482,848	6170	2,765

*Ministry of agriculture in Tulkarm.

In general, planting the open, protected and irrigated vegetables is considered the most widely spread type of agriculture; that is the number of green houses is 6642 and the number of high tunnels is 1375.

For the protected crops, the cucumber forms the most planted vegetables, with an area of about 1500 dunum with an average production of 6-8 tons/dunum.

Next to cucumber comes the tomato and other vegetable crops like beans and pepper. The faba bean, chick-pea and Egyptian cucumber are the most un-irrigated vegetable crops in Tulkarm district. For the irrigated field crops; the most planted crops are wheat and barely which occupies 6894 and 1339 dunum, respectively.

For the fruitful trees, citrus trees –mostly orange trees- are the most planted irrigated trees which occupy 3700 dunum.

Finally, the olive trees are the mostly planted un-irrigated trees, and it occupies 114,785 dunum according to Tulkarm Agricultural Department for the year 2003/2004. **[**9, 44**]**

1.3. Objectives:

Pollution issue has become one of the most important public awareness issue. The excessive use of the pesticides and fertilizers in agriculture with the threat of these chemicals in crops and water. Therefore great wonders have been raised about the cleanness and the quality of the vegetable crops grown in West Bank areas.

This work is a trial to find out the status of some chemical residues in vegetables. Therefore the aim of this work is to examine some of the most common vegetables grown in Tulkarm area for its nitrate and nitrite contents.

The objectives of the present work:

1- Determine the level of nitrate and nitrite in tomato, cucumber, potato, onion, and cabbage crops planted in Tulkarm district.

2- Determine the effect of size of fruits on the level of nitrate and nitrite

3- Comparison of nitrate and nitrite level in peel and essence of crops.

4- Comparing the content of nitrate and nitrite between underground crops (potato and onion) and aboveground crops (tomato and cucumber).

5- Comparing the nitrate and nitrite content between the onion crop and potato crop.

6- Comparing the content of nitrate and nitrite between the leafy crops (cabbage), and other edible crops.

CHAPTER TWO

LITERATURE REVIEW

Chapter Two

Literature Review:

2.1. Nitrate and nitrite in drinking water.

In a study surveyed by [32], the association between nitrate and atrazine from drinking water, and stomach cancer was studied. Existing data on the incidence on spectic types of cancers, contamination of drinking water with atrazine and nitrate, and related agriculture practices for the 40 ecodestricts in the province of Ontario, were obtained. It was found that the atrazine contamination levels range 50-649 ng/L, Maximum Acceptable Concentration (MAC) was 60 mg/L, were positively associated with stomach cancer incidence and negatively with colon cancer incidence. Nitrate levels (range 0-91 mg/L, MAC = 10 mg/L) were negatively associated with stomach cancer incidence.

2.2. Nitrate and nitrite in animals.

Hungry cattle and sheep introduced to stockyards containing a dominant growth of Dactylodenium radulans (button grass), suffered from acute nitrate-nitrite toxicity in four incidents in Island Queensland between 1993 and 2001. Deaths ranged from 16 to 44%. In all accidents, methemoglobenemia was noted at necropsies. An aqueous humor sample from one dead steer contained 75 mg nitrate/L, and from one dead sheep contained 100 mg nitrate/L, and 50 mg nitrite/L (normal = 5 mg nitrate/L). The dry button grass was toxic. The nitrate content of button grass from within the stockyards ranged from 4% to 12.9% as potassium nitrate equivalent in dry matter **[**40].

2.3. Nitrate and nitrite in vegetables.

Nitrate content was determined in several edible vegetables in Italy in 1996-2002. It was found that the highest content of nitrate was detected in chicory (6250 mg/kg) and rocket (6120 mg/kg), which are consumed in large quantities in some regions of Italy. Green salad and lettuce contained less nitrate (highest values were 4200 mg/Kg and 3300 mg/kg, respectively), but because they are consumed more generally, they provided 60% of the total intake of nitrates. Only a few samples were above the legal limits, with seasonal variation. Significantly higher nitrate content was found in organically grown green salad and rocket than in those conventionally produced. These data indicates that the average intake of nitrate from leafy vegetables is below the acceptable daily intake, i.e. 3.7 mg nitrate ion/Kg body weight per day, but the total intake should be monitored to protect groups at risk, such as children and vegetarians [39].

Nitrate (NO_3) and nitrite (NO_2) contents of various vegetables (Chinese cabbage, radish, lettuce, spinach, soybean sprouts, onion, pumpkin, green onion, cucumber, potato, carrot, garlic, green pepper, cabbage and Allium tuberosum Roth known as Crown daisy) were reported by **(**Chung et al., 2003**).** Six hundred samples of 15 vegetables cultivated during different seasons were analyzed for nitrate and nitrite by ion chromatography and ultraviolet spectrophotometry, respectively. No significant variance in nitrate levels was found for most vegetables cultivated during the summer and winter harvests. The mean nitrates level was higher in A.tuberosum Roth (5150 mg/kg) and spinach (4259 mg/kg), intermediate in radish (1878 mg/kg) and Chinese cabbage (1740 mg/kg), and lower in onion (23 mg/kg), soybean sprouts (56 mg/kg) and green pepper (76 mg/kg) compared with those in other vegetables. The average nitrite contents in various vegetables were about 0.6 mg/kg, and the values were not significantly different among most vegetables. It was observed that nitrate contents in vegetables varied depending on the type of vegetable and were similar to those in vegetables grown in other countries.

In Nigeria the contents of nitrate, nitrite, and ascorbic acid were determined in four samples of commercial and fifteen samples of home – prepared complementary infant foods. For the commercial food samples, the nitrate and nitrite values ranged from 3.1-3.9 mg NO_3-N/100 g and 5.0-

16.0 ug NO_2-N/100 g, respectively, while the ascorbic acid content ranged from 6.0-13 mg/100 g. For the home prepared complementary infant foods, the nitrate and nitrite content varied according to recipes with maximum values found in foods containing vegetables and legumes. The highest nitrate levels were found in yam and vegetables pottage (25.1 mg NO_3-N/100 g), and that of nitrite in pureed spinach vegetable (72.0 mg NO_2-N/100 g). The ascorbic acid content was very low. However, the nitrate and nitrite levels of these complementary foods were below tolerance levels, and these foods do not pose any health problem for infants [62].

In a study comparing the nitrate reductase activity (NRA) in different tissues of poplar plant, it was found that NRA was greater in leaves, and the highest leaf NRA was found in young leaves. Leaf and root NRA increased with increasing nitrate supply, whereas stem NRA remained constant. Leaf NRA was at least 10-fold greater than root NRA at all external nitrate concentrations. Nitrate reductase abundance increased in all tissues with increasing nitrate availability, and nitrate reductase abundance was at least 10-fold greater in leaves than in stems or roots at all nitrate availabilities. Tissue nitrate concentration increased with increasing nitrate supply and was greater in roots than in stems and leaves. Photoperiod influenced NRA, with leaf NRA declining in nitrate-fertilized plants with short daily photoperiods (8-h). It was concluded that different tissues of poplar vary in nitrate assimilation with little nitrate assimilation occurring in roots and the most nitrate assimilation taking place in leaves [12].

The influence of nitrogen concentration and the ratio of NO_3 to NH_4 on the distribution of inorganic elements within the tissues of cucumber fruit grown on rockwool were studied. Nitrogen was supplied at three nitrogen concentrations and four ratios of NO_3 to NH_4. Increases in the total nitrogen concentration within the nutrient medium significantly increased the NO_3 content of all the fruit tissues, but this effect was most pronounced in the skin, neck and apical region. The concentration of K, Ca, Mg and NO_3 in all regions of the fruit was higher when NO_3 constituted 75% or more of the total nitrogen in the nutrient medium, but was reduced by increasing concentrations of NH_4. The Mn content of the tissue in the central region of the fruit was reduced by NH_4 ions, whereas the Cu content of this tissue increased. No significant effect of nitrogen form on the B, Fe, Zn content of fruits was detected. In contrast, the B content of the apical tissue and the Zn content of the skin declined significantly with increasing nitrogen level. The highest concentrations of K and NO_3 were found in the neck and skin tissues, whereas the highest concentrations of Mn and Cu were observed only in the fruit skin. Mg concentrations were lowest in the tissues of the fruit neck, while B concentrations were highest in tissues near to the site of style abscission [31]

The contents of nitrate and nitrite in potato, cabbage, Chinese cabbage, scallion (shallot), celery, cucumber, tomato, eggplant and wax gourd taken from the north China market from 1998 to 1999 were determined. The highest content of nitrate was found in celery followed by Chinese cabbage, cabbage, scallion, wax gourd and eggplant. For all the products, a great variation in the content of nitrate was found. Generally, the nitrite content was low. The average intake of nitrate and nitrite from these vegetables was estimated as approximately 422.8 and 0.68 mg/day, respectively [73].

Nitrate accumulation in 48 varieties of vegetables was carried out at different seasons in China. The results showed that nitrate-nitrogen concentrations in 20 vegetables reached Pollution Level 4 (NO_3-N > 325 mg/kg), which accounted for 41.7% of the total number of the sampled vegetables and included all of the leafy, and most of the melon, root, onion and garlic vegetables. Among them, 5 leafy vegetables even exceeded level 4 (NO_3-N > 700 mg/kg). Although leafy vegetables were usually apt to heavily accumulate nitrate, most of them were with nitrate-nitrogen concentrations lower than Level 3 (NO_3-N < 325 mg/kg) in leave blades.

Further investigation showed that vegetable soils accumulated more nitrates in each layer from 0 cm to 200 cm than did cereal crop soil. The total amount of residual nitrate-nitrogen was 1358.8 kg/hm^2 in the 200 cm soil profile of usual vegetable fields, and 1411.8 kg/hm^2 and 1520.9 kg/hm^2 in the 2-years and the 5-years long plastic greenhouse fields, respectively. However that in the cereal crop fields was only 245.4 kg/hm^2. Nitrate residual in vegetable soils formed serious threats to underground water in vegetable growing areas [65].

Many researches have been conducted on this important issue, in the region; however, no such work has been conducted in our area (Gaza and West Bank, in Palestine). The following is a summary of the related work.

In a study conducted in Jordan, the nitrate content of several vegetable produced in the Jordan valley was measured. Results of the survey indicated that there was a general increase in the nitrate and nitrite levels in the sampled fruits. Tomatoes had lower levels of nitrate and nitrites than squash and cucumber. The same vegetables growing in plastic houses had higher levels of nitrate than those grown in open fields or tunnels. In addition, large size cucumber and squash fruits and their pulp had significantly ($p<0.01$) lower nitrate levels than small size fruits (88 - 98%), and their peels (52 - 79%).

The nitrate level ranged between 20.0-160.5 mg/kg in cucumbers, 63.9-248.7 mg/kg in squash, and below detection level in tomatoes. The levels of nitrate and nitrite found in vegetables surveyed in this study showed that the results are below the levels reported in similar products grown elsewhere in the world, especially in European countries [7].

In another study, the effect of different sources of nitrogen, time, and methods of application on growth and nitrogen content in potato plants was studied in Kenya. It was found that the late application of nitrogen fertilizers resulted in higher accumulation in both leaves and potato tuber [20].

In another study, cucumber plants (Cucumis sativus L. cv.Brunex) were grown under controlled conditions in an experimental greenhouse and treated with four rates of nitrogen in the form of KNO_3 (N1, 5 g/m^2; N2, 10 g/m^2; N3, 20 g/m^2; and N4, 40 g/m^2). The intermediate nitrogen rates (N2 and N3) gave higher utilization of NO_3^- in the leaves (highest NR activities) than treatment N1 (inadequate) and N4 (excessive). This latter rate (N4) appears to result in excessive foliar assimilation of NO_3^-, thereby increasing amino acids and proteins and inhibiting or reducing NR activity. N2 and, especially, N3 treatments strengthened the translocation of organic nitrogenous compounds (amino acids) towards the fruit, which enhanced the commercial yield [57].

The effects of cultivar and harvest time on the nitrate and nitrite content of the edible parts of several vegetables were studied. Harvest date was found to have a significant effect (*P*=0.05) on the nitrate content of the open-field-grown spinach, cabbage, and squash, and the nitrite content of the open-field-grown spinach, lettuce, and cabbage. Late-harvested vegetables had the lowest nitrate levels, while the pattern of their nitrite content was irregular with respect to the dates that gave highest nitrite levels in each vegetable. Harvest date had no significant effect on either nitrate or nitrite content of the greenhouse-grown vegetables.

Cultivar had a significant effect (*P*=0.05) only on the nitrate content of the greenhouse-grown tomatoes and squash, while it had no effect on either the nitrate or the nitrite content of all other vegetables irrespective of their cultivation method, although their levels in the greenhouse-grown vegetables were higher than those grown in open fields. Nitrate levels in these vegetables were generally low (lowest average of 0.13 mg/100 g in open-field-grown cauliflower, and highest of 4.77 mg/100 g in greenhouse-grown squash). Nitrite levels, on the other hand, were similar to those reported elsewhere in the world, ranging from non-detectable levels in open-field-grown cauliflower, to a maximum level of 0.43 mg/100 g in greenhouse-grown squash.

A highly significant, although low, positive correlation ($r = 0.55$, $P = 0.01$, $n = 108$) was found between nitrate and nitrite contents of the greenhouse-grown vegetables, compared to a non-significant, and much lower correlation between the two variables in the open-field-grown vegetables [8].

A research in Lowa community studied the relation between the nitrate and nitrite levels in drinking water and dietary sources with pancreatic cancer. The study suggests that long term exposure to drinking water with low levels of nitrate (about 10 mg/L) is not associated with pancreatic cancer, while the consumption of dietary nitrate from animal products may increase the risk [14].

The correlation between consuming food with high nitrate levels and the formation of carcinogenic nitrosoamines compounds was studied. This study concluded that the consumption of risk nitrate diet increased the risk of formation of carcinogenic nitrosoamines [36].

In another study, the nitrate and nitrite levels were determined in some common vegetables, which eaten row. In many samples the nitrate concentration was higher than the concentration in spinach (well known as rich source of nitrates), also commercial lettuce had a very high nitrate amount, while lettuce cultivated without fertilizing contained low amounts of nitrates [19].

In Poland, a study applied to determine the contents of nitrate and nitrite in samples of beetroot, white cabbage, parsley root, carrot, and potato. Mean nitrate concentration in products from conventional alloments, ranged from 203 in potatoes to 843 in beetroot (mg/Kg wet wt) vs 145 to 350 (mg/Kg wet wt) in the corresponding ‘ecological’ products. Mean nitrite concentration (mg/Kg wet wt) in the conventional products ranged from 0.53 in carrots to 1.54 in beetroot, vs 0.34 to 0.42 in the corresponding ‘ecological’ products [34].

In another study, the content of nitrate and nitrite in 28 commercial samples of (a) black tea, and 24 of (b) herbal and medical teas were determined. Nitrate (KNO_3) concentration in (a) ranged from 43.9 to 139.2, and in (b) ranged from 70 to 6207 (mg/Kg).

The effect of shading, harvesting time, culture system, and concentration of N, P, and Ca on accumulation of nitrate in leaves of Chinese cabbage were studied. Increasing shading rate increased the nitrate contents; this phenomenon was greater in autumn than in winter, while leaf nitrate content was lower when the plant was harvested in the late afternoon. Nitrate contents increased in the nutrient solution under a non circulating system, whereas no significant differences were found under a circulating system. Increased potassium concentration decreased the nitrate levels in the leaves, while increased concentration of calcium significantly decreased nitrate levels in summer, but not in winter [72].

The effect of time of harvesting and fruit size on the nitrate content in greenhouse cucumber was studied. The effect of fruit size was negligible. Harvesting time had a significant influence, the content of nitrate declined by 51% and 71% for the Sandra and Boloria var. respectively between the first and last harvesting. Sandra var. had a 62% higher nitrate content than the Boloria var [52].

In the Province of Lodz, a study was applied to determine the contents of nitrates and nitrites in 28-35 samples of 10 different vegetables (anion, cabbage, carrot, cucumber, parsley, tomatoes, leaks, lettuce, celery, and potatoes). Mean contents of $NaNO_3$ mg/Kg ranged from 41.2 mg/Kg in tomatoes to 1389 mg/Kg in lettuce, and mean nitrite contents of $NaNO_3$ mg/Kg ranged from 0.11 mg/Kg in tomatoes to 1.47 mg/Kg in lettuce [38].

Another study determined the contents of nitrate and nitrite in 33 samples of (fresh and boiled carrot, parsley, leek, celery and potato) was determined in another study. The nitrate content were reduced with boiling by approximately 50% and the nitrite content by 37-71% while the contents of nitrates and nitrites in carrot juice and in the raw carrot were the same [37].

In a study in Queensland, vegetables were collected and analyzed for nitrate and nitrite residues. Vegetables were collected near peak harvest. The survey also included a small sample of hydroponics produce levels of nitrate in potatoes, cabbages and beets were higher than those reported in other surveys. The median NO_3-N concentration measured in hydroponics lettuce (465 mg/kg NO_3 -N) was twice more than the median cancer far field grown lettuce [35].

The content of KNO_3 and $NaNO_2$ in samples of fresh vegetables, frozen vegetables, apple, soft fruits, frozen fruits and James was determined. Some vegetables contained >1000 mg KNO_3/kg (max 3506 mg/kg in leek). Low nitrate levels found in apple and higher levels in the soft fruits. Levels in the frozen products were slightly below the fresh products. Nitrite contents were very low in the fruit and vegetables (<1 mg/kg) [45].

The results of analyses of 1994 Total Diet Study (TDS) samples or nitrate and nitrite were reported. There was a comparison between the dietary intake of nitrate and nitrite with Acceptable Daily Intakes (ADI). Nitrate concentrations were highest in green vegetables (444 mg/Kg), potatoes (136 mg/Kg), and other vegetables (182 mg/Kg), were these food groups made the greatest contributions to dietary intake of nitrate with 39%, 37%, and 30%, respectively. Nitrite concentrations were low [42].

A study on Spanish and sorrel grown under nitrogen fertilization determined their nitrate contents which were (1131-1459 and 62-835 mg/Kg for Spanish and sorrel, respectively). It was also demonstrated that nitrate contents were not distributed in vegetative parts. Stalks, leaf petioles, and veins contained higher nitrate content [53].

Another study on spinach and beet determined their nitrate-nitrite levels in commercially and in home processed beets and spinach. Home processed beet were found to have higher nitrate contents than commercially processed beets, while home frozen spinach showed greater nitrate levels than commercially frozen spinach, although the difference was not significant. Length of storage of home processed beets and spinach did not affect the nitrate or nitrite content [11].

The nitrate contents in some species of wild edible herbs and vegetables common in Jordan were determined using the cadmium reduction method. The nitrate level varied widely ranging from 29 mg/kg the leaves of tetragonolbus (Tetragonolbus palaestinus) to 6743 mg/kg in fenugreek (Trigonella stellata). In general, the nitrate content in the stems was higher than in the leaves which in turn were higher than that in the roots and bulbs. The same species collected from irrigated farms were generally of higher nitrate content than those collected from pasture, forest, or non-irrigated farms [60].

Chapter Three

Materials and methods

Materials and Methods

3.1. Chemicals and reagents:

All chemicals used were of analytical grade.

Activated aluminum (Aluminum oxides): Riedel-de Haen (Aluminum oxides, neutral), for column chromatography: 30 cm length, and 2 cm in diameter.

Nitrate reagent: HI93728-0, for nitrate determination.

NAD reagent: N-(1-naphthyl) ethylenediamine.2HCl: SIGMA.

Sulfanilamide reagent: Sulfanilamide (P-Aminobenzenesulfonamide): SIGMA.

Other reagents used were: $NaNO_2$, and CH_3COOH.

3.2. Instruments:

Nitrate meter: HANNA Instrument, HI 93728-0.

UVPC spectrophotometer device: Schimadzu, UV-1601PC, UV-Visible spectrophotometer.

3.3. Sample collection: Five vegetable crops were collected for this study: cucumber, tomato, cabbage, potato, and onion. These crops are the most common cultivated crops in Tulkarm district at this season. In addition both cucumber and tomato fruits are usually consumed fresh, and grown above the ground, however, potato and onion represent the crops that grown under ground and the edible portion is the stem. On the other hand cabbage represents the leafy vegetables.

Seventy five samples of all these crops were collected, each sample represent a farm.

All samples were collected from two basic regions: As-Sha'raweya area (location no.1), which is a flat land, known as an agricultural land usually cultivated with almost all vegetable crops, especially Ateel- Al Deir area.

The second region is Thenabe (location no.2), which is a high area cultivated with different crops.

In addition to these two regions, cucumber samples were also collected from a third region known as Al-Kafryat (location no.3). Cucumber fruits were divided into two groups, large samples (>100 mm), and small samples (<100 mm).

Samples were collected between January and July 2004.

From each farm one fruit sample was collected. Farms and samples were chosen randomly (Table 6).

Table (6): Number of samples collected from the different locations for each vegetable type.

Number of samples collected from each location			
Type	**As.Sha'raweya No.1**	**Thenabe No.2**	**Al-Kafreyat No.3**
Tomato	8	6	None
Onion	6	7	None
Potato	8	8	None
Cabbage	7	6	None
Cucumber	5	4	10

3.4. **Preparation of reagents and standard solution**:

a. NED reagent: 0.2 g N-(1-naphthyl) ethylenediamine.2HCl were dissolved in 150 ml 15% of (V/V) CH_3COOH. Filtered when it is necessary, and stored in glass-stoppered brown glass bottle.

b. Sulfanilamide reagent: 0.5 g sulfanilamide were dissolved in 150 ml of 15% CH_3COOH (V/V). Filtered if necessary and stored in glass-stoppered brown glass bottle.

c. Sodium nitrite standard solution: (1) stock solution (1000 ppm $NaNO_2$): 1 g $NaNO_2$ was dissolved in H_2O and diluted to 1L. (2) Intermediate solution (100 ppm $NaNO_2$): 100 ml of the stock solution were diluted to 1L with H_2O. (3) Working solution (1 ppm $NaNO_2$): 10 ml of the intermediate solution were diluted to 1L with H_2O.

3.5. **Nitrate and nitrite extraction**.

A fifty gram sample of the prepared crop was blended with 50ml distilled water in a home blender. The mixture was filtered through Whatman no.2 filter paper, and the filtrate was passed through a glass column fitted with a tape and filled with Activated alumina, in order to separate the green color (Chlorophyll) and get a transparent solution. Water was used as eluting solvent. The eluted solution filtered using 0.45um filter paper in order to eliminate the turbidity and get a clear solution.

3.6**. Quantitative determination**:

3.6.1. **Quantitative determination of nitrate**: 10ml of the transparent, clear solution was analyzed for the nitrate content using “HANNA Instrument” which gives the sample content of nitrate as NO_3-N. The concentration of NO_3 can be calculated by multiplying the nitrogen concentration by a factor of 4.43 to have the exact nitrate (NO_3) concentration in ppm of the sample.

3.6.2. **Quantitative determination of nitrite:** with the AOAC Official Method 973.31. A portion of solution containing nitrite was transferred into a 25 ml volumetric flask. Then 2.5 ml sulfanilamide were added, followed by addition of 2.5 ml NAD. The volume was completed with water and left for 15 minutes in order to give time for color development. The absorbance was measured at 545 nm against a blank solution. The nitrite concentration was determined using the calibration curve prepared as follows: 10, 20, 30, and 40 ml of nitrite working standard solution were transferred into 50 ml volumetric flasks in order to prepare standard solutions of 0.2, 0.4, 0.6, and 0.8 ppm $NaNO_2$. Then 2.5 ml of sulfanilamide reagent were added to each flask followed by 2.5 ml NAD reagent. The volumes were completed to the marks. The absorbances were measured after 15 minutes at 545 nm. The calibration curve was constructed by plotting the absorbance vs. the concentration.

3.7. Statistical analysis: Data were analyzed using SPSS package. One way ANOVA was conducted followed by a mean separation by LSD test.

Chapter Four

Results

Results

Nitrate and nitrite content in five vegetable crops were determined in three areas of Tulkarm district as shown in Table (7) for nitrate, and in Table (8) for nitrite.

It can be seen from Tables 7 and 8 that tomato fruits which were collected at different stages of growth, showed the lowest average of nitrate contents among other crops with an average of 17.95 and 15.96 mg/Kg, with a range of 0 - 34.12, and 0 - 58.14 mg/kg in locations 1 and 2, respectively. While the average nitrite content in tomato fruits were 0.13 and 0.16 mg/Kg, with a range of 0.01 - 0.37 and 0.04 - 0.28 mg/Kg for locations 1 and 2, respectively.

While the potato showed the highest levels of nitrate content, average of 231.84 mg/kg, with a range of 90.9 - 526.28 in location 1, and an average 274.42 mg/kg, with a range of 33.49 -562.28 mg/kg in location 2. While the average nitrite levels in potato were 0.54 and 0.84 mg/Kg, with a range of 0.09 - 1.29 and 0 - 1.83 mg/Kg for locations 1 and 2, respectively.

The dry onion results did not show high levels of nitrates. The average nitrate content were 49.79, and 49.88 mg/kg, with a range of 18.43 - 66.34, and 2.76 - 105.97 mg/kg for regions 1and 2, respectively. The average nitrite content in dry onion was 0.32 and 0.5 mg/Kg, with a range of 0.07 - 0.78 and 0.04 - 1.46 mg/Kg in locations 1 and 2, respectively.

The average nitrate levels in cabbage were 85.23, and 198.46 mg/kg, with a range of 0 - 306.2, and 115.68 - 273.88 mg/kg for locations 1 and 2 respectively. While the nitrite averages were 0.55 and 0.7 mg/Kg, with a range of 0 - 2.98 and 0.16 - 1.78 mg/Kg in locations 1 and 2, respectively.

Table (7): Average nitrate (NO_3) content in the vegetables surveyed in Tulkarm district.

Crops	location	No. of samples	Mean (NO_3) mg/kg ± S.E	Range (mg/Kg)
Tomato	1	8	17.95 ± 3.76	0 - 34.12
	2	6	15.96 ± 9.28	0 - 58.14
Onion	1	6	49.79 ± 6.99	18.43 - 66.34
	2	7	49.88 ± 13.77	2.76 - 105.97
Potato	1	8	231.84 ± 54.56	90.9 - 526.28
	2	8	274.42 ± 79.21	33.49 - 562.28
Cabbage	1	7	85.23 ± 39.49	0 - 306.2
	2	6	198.46 ± 25.52	115.68 - 273.88
cucumber	1	10	95.20 ± 17.11	26.78 - 124.4
	2	8	119.54 ± 18.79	53.56 - 235.84
	3	20	176.35 ± 19.71	44.05 - 328.26

*S.E: Standard Error of the mean.

Table(8): Average nitrite (NO_2) content in the vegetables surveyed in Tulkarm district.

Crops	Location	No. of samples	Mean (NO_2) mg/kg ± S.E*	Range (mg/Kg)
Tomato	1	8	0.11 ± 0.05	0.01 - 0.37
	2	6	0.16 ± 0.04	0.04 - 0.28
Onion	1	6	0.32 ± 0.11	0.07 - 0.78
	2	7	0.51 ± 0.18	0.04 - 1.46
Potato	1	8	0.54 ± 0.16	0.09 - 1.29
	2	8	0.84 ± 0.27	0 - 1.83
Cabbage	1	7	0.55 ± 0.41	0 - 2.98
	2	6	0.7 ± 0.25	0.16 - 1.78
Cucumber	1	10	1.78 ± 0.23	0.45 - 2.22
	2	8	1.47 ± 0.83	0.17 -5.45
	3	20	3.55 ± 0.69	0.15 - 8.05

*S.E: Standard Error of the mean.

The cucumber fruit samples were collected at different growth stages from three different locations at Tulkarm district. The samples were divided into two groups, in the first one the nitrate and nitrite content were determined in small and large size cucumber fruits. In the second group the nitrate and nitrite content were determined in the peel of the fruits and in the extract of the cucumber fruits.

Table (9) shows the distribution of nitrate contents between large size and small size cucumber samples.

Obtained results shows a higher nitrate levels in the small fruits than in the large ones. Where the average levels of nitrate in the large size cucumber were 53.03, 140.82, and 110.23 mg/kg, with a range of 24.19 - 82.93, 77.75 - 205.60, and 3.45 - 245.33 mg/Kg, from locations one, two, and three, respectively, while the average nitrate levels in small size cucumber were 83.12, 161.97, and 213.54 mg/kg, with a range of 29.37 - 165.86, 29.37 - 266.07, and 84.66 - 411.19 mg/Kg from locations 1, 2, and 3, respectively.

Table (10) shows the distribution of nitrite content in small size and large size cucumber, where the nitrite levels in large size samples were 1.13, 0.45, and 1.58 mg/kg, with a range of 0.54 - 1.48, 0.03 -1.37, and 0.02 - 4.80 mg/Kg from locations one, two, and three, respectively. The average nitrite levels in small size samples were 1.77, 2.7, and 4.48

mg/Kg, with a range of 0.37 - 2.95, 0.30 - 9.52, and 0.27 - 11.29 mg/Kg from locations 1, 2, and 3, respectively.

Table (9): Average nitrate (NO_3) levels in large size cucumber fruits.

Location No	No.of samples	Large size cucumber (>100mm)	
		Average NO_3 (mg/Kg) ± S.E*	Range (mg/Kg)
1	5	53.03 ± 9.53	24.19 - 82.93
2	4	140.82 ± 27.23	77.75 - 205.6
3	10	110.23 ± 25.88	3.45 - 245.33

*S.E: Standard Error of the mean.

Table (10): Average nitrate (NO_3) levels in small size cucumber fruits.

Location No	No.of samples	Small size cucumber (<100 mm)	
		Average NO_3 (mg/Kg) ± S.E*	Range (mg/Kg)
1	5	83.12 ± 24.53	29.37 - 165.86
2	4	161.97 ± 51.41	29.37 - 266.07
3	10	213.54 ± 25.88	84.66 - 411.19

*S.E: Standard Error of the mean.

Table (11): Average nitrite (NO_2) levels in large size cucumber fruits.

Location No	No.of samples	Large size cucumber (>100 mm)	
		Average NO_2 (mg/Kg) ± S.E*	Range (mg/Kg)
1	5	1.13 ± 0.16	0.54 - 1.48
2	4	0.45 ± 0.32	0.03 - 1.37
3	10	1.58 ± 0.56	0.02 - 4.8

*S.E: Standard Error of the mean.

Table (12): Average nitrite (NO_2) levels in small size cucumber fruits.

Location No	No.of samples	Small size cucumber (<100 mm)	
		Average NO_2 (mg/Kg) ± S.E*	Range (mg/Kg)
1	5	1.77 ± 0.50	0.37 - 2.95
2	4	2.7 ± 2.27	0.3 - 9.52
3	10	4.48 ± 0.56	0.27 - 11.29

*S.E: Standard Error of the mean.

Table (11) shows the distribution of the nitrate levels between the peel and the essences of cucumber. The results shows that the peel of the cucumber contains higher nitrate contents than the essence, where the average nitrate levels in the peel were 140.98, 104.52, and 205.6 mg/kg with a range of 91.57 - 236.69, 0 - 183.13, and 41.46 - 570.14 mg/Kg for locations one, two, and three, respectively, while the average of nitrate levels in the essence were 103.68, 70.835, and 176.05 mg/kg, with a range of 0 - 292.06, 24.19 - 114.03, and 81.20 - 502.76 mg/Kg for locations 1, 2, and 3, respectively.

Table (12) shows the distribution in nitrite levels in the peel and the essences of cucumber. The average nitrite content in the essences are 2.29, 0.14, and4.25 mg/Kg, with a range of 1.42 - 3.82, 0.08 - 0.21, and 0.07 - 21.12 mg/Kg for locations one, two, and three, respectively, while the average nitrite content in the peel were 1.91, 2.58, and 3.89 mg/Kg, with a range of 0.78 - 3.80, 0 - 10.25, and 0.26 - 10.04 mg/Kg for locations 1, 2, and 3, respectively.

Table (13): Average nitrate (NO_3) content in (mg/Kg) in the essence of cucumber fruits.

Location No	No. of samples	Nitrate in essences	
		Average NO_3 (mg/Kg) ± S.E*	Range (mg/Kg)
1	5	103.68 ± 49.57	0 - 292.06
2	4	70.84 ± 20.22	24.19 - 114.03
3	10	176.05 ± 38.79	81.2 - 502.76

*S.E: Standard Error of the mean.

Table (14): Average nitrate (NO_3) content in (mg/Kg) in the peel of cucumber fruits.

Location No	No. of samples	Nitrate in peels	
		Average NO_3 (mg/Kg)±S.E*	Range (mg/Kg)
1	5	140.98 ± 37.29	91.57 - 236.69
2	4	104.52 ± 40.65	0 - 183.13
3	10	205.6 ± 52.35	41.46 - 570.14

*S.E: Standard Error of the mean.

Table (15): Average nitrite (NO_2) content in (mg/kg) in the essence of cucumber fruits.

Location No	**No.of samples**	**Nitrite in essences**	
		Average NO_2 (mg/Kg) ± S.E*	**Range (mg/Kg)**
1	5	2.29 ± 0.42	1.42-3.82
2	4	0.14 ± 0.27	0.08-0.2
3	10	4.25 ± 1.96	0.07-6.57

*S.E: Standard Error of the mean.

Table (16): Average nitrite (NO_2) content in (mg/kg) in the peel of cucumber fruits.

Location No	**No.of samples**	**Nitrite in peels**	
		Average NO_2 (mg/Kg) ± S.E*	**Range (mg/Kg)**
1	5	1.91 ± 0.61	0.78 - 3.8
2	4	2.58 ± 2.55	0 - 10.25
3	10	3.89 ± 1.15	0.26 - 10.04

*S.E: Standard Error of the mean.

Chapter Five

Discussion

Discussion

Many studies were conducted to measure the nitrate and nitrite content in vegetables. The levels of the nitrate obtained in this study were compared with previous studies (Table 13).

It is clear that the result of our study shows a variation in the nitrate and nitrite levels in the different crop samples. The levels of nitrate in tomato were low compared to the other crops. However, for cucumber the level was normal, and for both potato and cabbage the level was high. Even with high levels obtained, the nitrate levels reported in our study does not exceed the pollution level (NO_3-N>325mg/Kg) [65]. More recent surveys have shown no correlation between nitrate levels and the incidence of methemoglobenemia until nitrate levels exceed 443 mg/Kg [66].

There are many factors that affect the nitrate level in crops, among these factors are the agricultural system and practices, for instance it was found that the nitrate level in vegetables grown under organic systems is higher than that grown under conventional system [39].

Another important factor that interferes with the nitrogen accumulation in plant tissues is the environmental and microclimate conditions. It was shown that nitrate level is affected by the high sunlight intensity [54], which increases the nitrate levels in plant tissues by increasing the nitrate reductase activity which converts the nitrogen in plant to nitrate.

Growing conditions like temperature, stress (drought), hot or dry winds affect the nitrate accumulation in plants [WHO,1995] [5, 15], it has been found that vegetables grown in winter, the time of year with low temperature and less sunlight, has a higher nitrate content [European Commission,1998] [27, 54].

Table (17): Comparison of nitrate (NO_3) content in the vegetables surveyed in Tulkarm district, and the nitrate content reported in other studies.

Nitrate content in the present work		**Reported nitrate content in other studies**	
Crops	**Nitrate content (mg/kg)**	**Nitrate content (mg/Kg)**	**Reference**
Tomato	16.955	5.3-144.3	Markowska.A,et al.(1995)
		4.43-119.61	T.M.Knight et al.(1987)
		20.8-191.9	Agopov et al.(1990)
Onion	49.835	0-1.9	Amr and Hadidi (2001)
		48	MAFF no.158 (1998)
Potato	253.13	118-273	Leszezynska (1996)
		219-350	MAFF no.91 (1996)
		75-283	MAFF UK no.163 (1998)
		136	MAFF UK no.137 (1994)
Cabbage	141.845	460.7-910	Knight et al (1987)
		338	MAFF no.158 (1998)
		165-351	MAFF no.91 (1996)
Cucumber	122.72	46.95-138.78	Amr.2000

*MAFF: Ministry of Agriculture, Fisheries & Food.

Table (18): Comparison of nitrite (NO_2) content in the vegetables surveyed in Tulkarm district, and the nitrate content reported in other studies.

Nitrite content in the present work		**Reported nitrite content in other studies**	
Crops	**Nitrite content (mg/kg)**	**Nitrite content (mg/Kg)**	**Reference**
Tomato	0.1475	0-0.31	Markowska et al.(1995)
Potato	0.69	0.44	Knight et al. (1987)
		ND-0.8	MAFF no. (163) (1997) and no.(137) (1994)
Cabbage	0.625	0.5	Knight el. al. (87)
Cucumber	2.02	0.78	Amr.2000

*ND: Not Detected.

Therefore, vegetables grown in heated greenhouses have higher nitrate contents than those grown in open fields or tunnels , because of the lower light intensity, and higher nitrogen mineralization [59, 41, 7].

The type of crop is also among the primary factors that affect the nitrate level [13]. Foods that tend to accumulate large amount of nitrate include leafy vegetables such as spinach, lettuce, and cabbage, and root vegetables such as carrots, beet, and broccoli, though at a much lower level than do the leafy vegetables [61].

The herbicide application may also affect the nitrate accumulation in plants [15, 63].

Other factors that may affect the nitrate concentrations in vegetables: shading rate which increased the nitrate content [72], and harvesting time [52], where late harvested vegetables had the lowest nitrate levels, although, the harvest date had no significant effect on either nitrate or nitrite content of the greenhouse grown vegetables [8].

All these factors as well as other factors related to local environmental conditions might be the reason behind the variation in the results we obtained with that of other results

The tomato fruits show the lowest nitrate content among all the other crops tested, the same results were found in all other studies conducted previously. This might be related to the types of the segments present and the lack of chlorophyll in the tomato crops. This may explains the high nitrate content in green vegetables and cucumber which shows a higher nitrate content than tomato.

Higher nitrate content was detected in small size cucumber. That might be due to the stage of growth. During initial growth, the nitrate taken up by the plant is used for root and shoots development. At this stage, the roots are able to take up more nitrate than required and it accumulates in the stems and leaves of the plants. As the plants develops, the leaves of the plant are able to convert more nitrate into plant protein, therefore less nitrate is found in the plant as it matures.[15, 63]

It was also reported by [12] that the NRA (Nitrate Reductase Activity) was greater in leaves (higher in young leaves). The NRA in leaf and root increased with increasing the nitrate supply.

Comparing the nitrate content in the essence and the peel of the cucumber fruits shows a higher nitrate content in the peel which may be due to the presence of the chlorophyll.

In a study on cucumber, it was reported that the highest concentrations of nitrate were found in the neck, and skin tissues of cucumber fruits.[31]

There are other factors that could affect the nitrate content in cucumber fruits such as the nutrient medium. The concentration of nitrate in all regions of the cucumber fruit was higher when nitrate constituted 75% or more of the total nitrogen in the nutrient medium, but was reduced by increasing the concentrations of the ammonium (NH_4) [31].

Comparison of nitrate content in underground stem samples (onion and potato) shows a lower nitrate content in onion, and this might be due to the fertilization program. Most of the farmers do not apply a specific fertilization program; they usually plant onion beside the greenhouse as a complementary crop.

Potato shows a high nitrate content; this is consistent with the previous studies that show high concentrations of nitrate, this might be due to the high application of nitrogen fertilization during the growing season.

The late application of nitrogen fertilizers results in higher accumulation in both leaves and potato tuber [20].

In a study, it was reported that lettuce and potato consumption make the greatest contribution to dietary intake of both nitrate and nitrite [59].

Cabbage shows high nitrate content in comparison with other studied and reported results.

The nitrate levels in leafy vegetables are higher than bulb, root, shoot, inflorescence, and tuber vegetables [58] [41].

Green leafy and root vegetables tend to accumulate large amount of nitrate [62] [61].

The nitrate and nitrite content in several crops collected from two different regions in Tulkarm district (Al-Sha'raweya and Thenabe) were analyzed to determine if the location has an effect on the nitrate and nitrite concentration in crops. The results show no significant effect of the region on the nitrate and nitrite content in different vegetable crops.

The nitrite levels generally were below the allowable range. The nitrite content must be lower than 1mg/Kg [13] [59]. The nitrite content results in this survey was lower than 1mg/Kg except in some cucumber samples which were higher than 1mg/Kg, but still in an acceptable range and propose no danger on human health. It was reported that the nitrite levels start to be dangerous if it is higher than 100 mg/Kg [54] which is not the case here.

Chapter Six

Conclusions

6. Conclusions and Recommendations

In spite of the miss use of fertilizer practiced by the farmers, the accumulation of both nitrate and nitrite content in samples of different cultivated crops shows that tomato fruits contain the lowest nitrate content, while potato and cabbage shows a relatively high nitrate levels, onion did not show high nitrate levels.

The study indicated that the size of fruit in cucumber has an effect on its nitrate content; large fruit contains less nitrate content.

In addition the result proved that the cucumber fruit peel contains high nitrate content than the pulp. The result also shows a high nitrate content in cabbage than other crops. Similar findings were shown with potato tuber samples.

The nitrite levels were below 1mg/Kg which is below the permitted limit of nitrite levels in vegetables, which agreed with all other previous studies.

The nitrate and nitrite levels in vegetables cultivated in Tulkarm district are below the pollution level number four, and does not pause the consumers to health risks.

In accordance of the results we obtained, we highly recommend that more work to be conducted on this subject. Both governmental and nongovernmental organizations have to focus on this issue and must consider it as part of their strategy. The education and research centers have to perform more comprehensive survey on other agricultural crops and on different production areas. The aim of this work was mainly to highlight this important and crucial issue which has a direct effect on our health and environment.

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Appendices:

Appendix (a): The area (dunum) cultivated with vegetable croups in Tulkarm district in (2003-2004).

Cluster	Protected vegetables			Unprotected vegetables		
	tomato	cucumber	others	tomato	cucumber	squash
Qafeen	7	55	0	2	0	0
Nazlet-essa	1	43	1.5	4	0	0
Al-Nazla Al-Sharqeya	1	26	0	7	0	5
Baqa As-Sharqeya	15	190	0	10	0	43
Al-Nzla Al-Wosta	0	0	0	0	0	3
Nazlat abu-Nar	0	0	0	0	0	5
Al-Nazla Al-Gharbeya	0	25	0	4	0	0
Zeata	30	196	0	12	0	18
Saida	1	55	0	8	0	41
Elar	18	116	0	0	0	38
Deir Alghoson	10	160	0	0	2	4
Bal'a	5	28	0	0	0	0
Thenaba	20	282	0	15	3	0
Tulkarm	20	253	0	16	12	43
Anabta	0	16	0	0	0	5
Rameen	0	2	0	0	0	7

Far'on	7	15	0	0	4	10
Shoofe	10	70	0	3	0	3
Kherbet Gbara	1	27	0	0	0	2
Beit Leed	0	0	0	0	0	8
Kofr Soor	0	2	0	0	0	0
Kofr Gammal	1	47	0	0	0	0
Kofor A'boush	20	0	0	0	0	0
A'teel	1	245	0	15	0	53
Kofr Al-Labad	168	21	0	0	0	0
Total	336	1874	1.5	96	21	288

*Ministry of agriculture in Tulkarm district

Appendix (b): The area (dunum) cultivated with unprotected vegetables and field crops in of Tulkarm district (2003-2004).

Cluster	Unprotected vegetables				Field crops			
	Leafy vegetables	Green legumes	tubers	others	cereals	tubers	Dry legumes	ornamental
Qafeen	21	25	5	7	280	5	16	0
Nazlet-essa	51	23	6	43	190	4	17	0
Al-Nazla Al-Sharqeya	10	12	11	10	95	3	0	0
Baqa As-Sharqeya	131	7	28	150	60	6	0	0
Al-Nzla Al-Wosta	8	6	4	2	30	0	0	0
Nazlat abu-Nar	0	3	0	0	23	9	0	0
Al-Nazla Al-Gharbeya	49	22	24	71	56	2	0	0
Zeata	25	55	39	31	294	20	21	0
Saida	47	53	41	128	78	35	10	0
Elar	184	32	27	280	271	66	0	0
Deir Alghoson	64	39	16	34	188	56	3	0
Bal'a	3	6	5	0	503	7	0	0
Kofr Roman	0	2	0	10	35	0	0	0
Thenaba	185	29	41	126	231	234	0	0
Tulkarm	140	141	138	86	1540	171	48	4
Anabta	2	26	6	7	455	50	7	3
Rameen	0	15	18	0	230	28	6	2
Far'on	20	14	2	15	271	6	0	0
Shoofe	25	22	0	0	165	22	0	0
Kherbet Gbara	0	0	0	5	140	0	0	0
Safareen	0	5	0	0	460	12	0	4

Beit Leed	0	23	22	0	340	6	9	2
Kofr Soor	0	0	0	0	50	0	1	4
Koor	0	0	0	0	130	0	0	0
Kofr Zeebad	0	0	0	0	190	0	5	3
Kofr Gammal	0	8	0	4	428	0	15	4
Kofor A'boush	0	0	0	0	500	0	11	5
A'teel	67	47	53	132	290	132	6	0
Kofr Al-Labad	0	11	4	0	620	0	0	0
Total	1032	628	490	1141	8143	874	175	31

*Ministry of agriculture in Tulkarm district

Appendix (c): The most cultivated trees and their areas (dunum) in Tulkarm district for years (2003-2004).

Cluster	Olive	Citrus	Stone fruits	Almond	Fig
Qafeen	8045	194.5	0	350	0
Nazlet-essa	993	129	0	50	0
Al-Nazla Al-Sharqeya	800	53	0	40	4
Baqa As-Sharqeya	785	192	4	20	0
Al-Nzla Al-Wosta	190	0	0	60	_
Nazlat abu-Nar	405	0	0	40	0
Al-Nazla Al-Gharbeya	95	74	0	50	0
Zeata	521	141	18	30	5
Saida	2570	0	1000	800	12
Elar	6570	369.5	400	2500	10
Deir Alghoson	9292	25.5	35	195	12
Bal'a	15100	0	1011	900	100
Kofr Roman	2200	36	0	50	5
Thenaba	402	49	5	30	3
Tulkarm	5741	795	22	285	24
Anabta	8930	22.5	5	650	35
Rameen	3408	0	55	250	75
Far'on	1634	294	5	30	0
Shoofe	5074	264	0	100	2

Kherbet Gbara	330	0	0	20	0
Safareen	2685	0	25	90	15
Beit Leed	10386	0	95	250	100
Kofr Soor	2565	0	5	80	2
Koor	1765	0	5	30	2
Kofr Zeebad	2850	0	5	40	2
Kofr Gammal	2800	259	6	120	4
Kofor A'boush	4497	0	2	60	0
A'teel	5259	103.4	3	150	5
Kofr Al-Labad	9293	0	4	250	4
Total	115185	3001.4	2710	7520	431

* Ministry of Agriculture in Tulkarm District.

In West Bank, the nitrate pollute a lot of the water sources. In Tulkarm the percentage of water not polluted with nitrate is 27%, while it is about 23% in Qalqelia. The average nitrate is higher than 50 mg/l in 14% of wells water (ground water) (55)

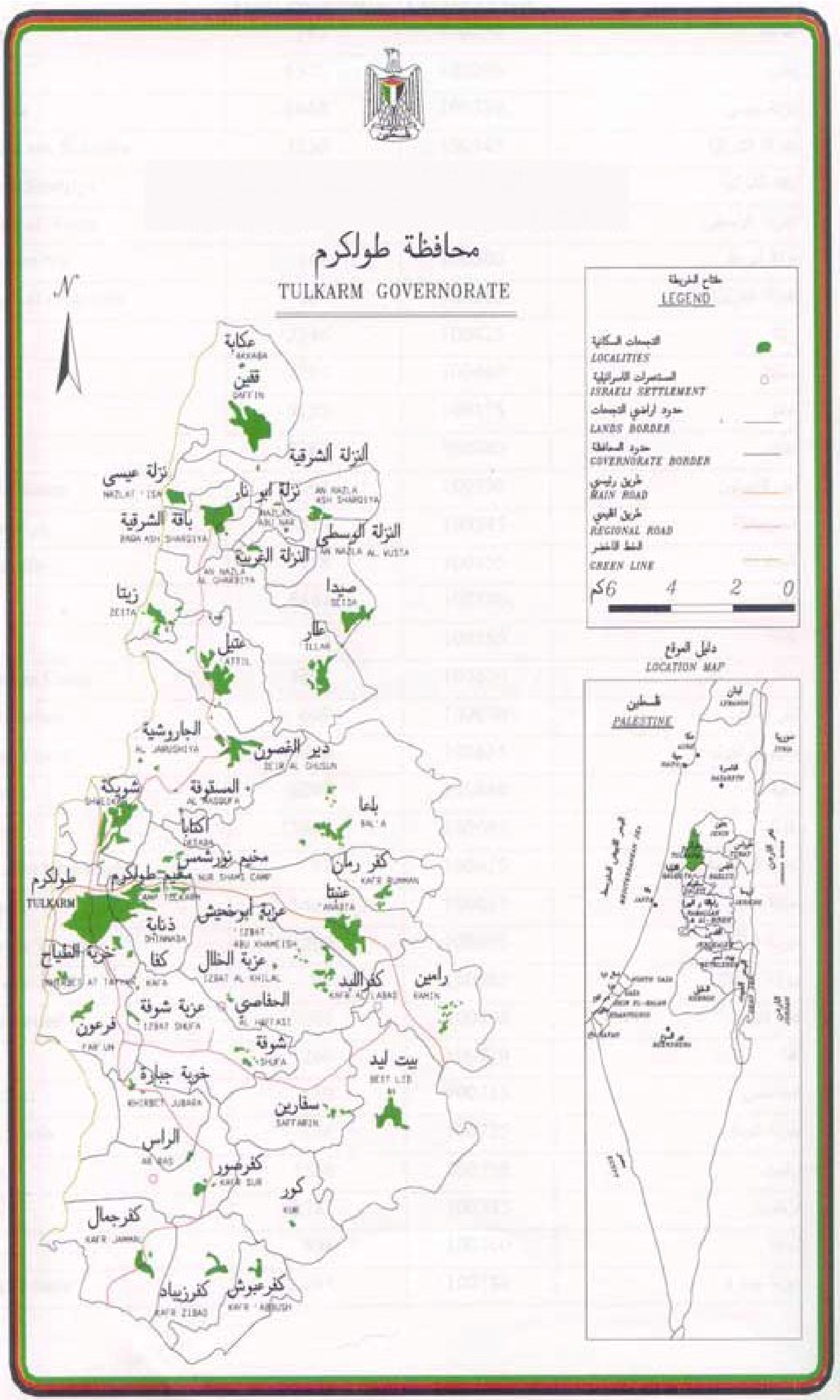
Appendix (d): Descriptive statistics of chemical and physical parameters of the wells studied in Tulkarm district [60].

Variable	Minimum	Maximum	Average	Std.Deviation
EC ($_M$S/cm)	209.00	2155.00	910.30	299.30
TDS (mg/L)	272.00	1194.00	508.60	.171.60
pH	7.22	8.25	7.73	0.27
Ca^{+2} (mg/L)	54.30	161.50	95.10	20.40
Mg^{+2} (mg/L)	7.60	40.90	22.60	7.70
Na^{+1} (mg/L)	11.30	140.00	54.10	240.20
K^{+1} (mg/L)	0.80	35.00	5.80	7.40
HCO_3^{-1} (mg/L)	130.20	526.80	308.40	69.90
SO_4^{-2} (mg/L)	4.80	143.30	25.80	21.60
Cl^{-1} (mg/L)	29.40	419.80	86.50	66.90
NO_3^{-1} (mg/L)	13.30	260.20	64.50	48.00

Appendix (e): Physical and chemical results obtained from the wells studied in Tulkarm district [60].

Name	Location	EC $_{M}$S/cm	pH	Ca^{+2} mg/L	Mg^{+2} mg/L	Na^{+} mg/L	K^{+} mg/L	HCO_3^{-} mg/L	SO_4^{-2} mg/L	Cl^{-} mg/L	NO_3^{-} mg/L
Said Jaber	Shwayka	783	7.80	66.1	38.6	27.0	8.5	314.0	16.5	61.5	46.0
M.Makkawi	'Allar	735	7.80	90.5	17.8	30.2	2.4	285.1	12.1	45.0	78.8
R.Samara	'Allar	1024	7.61	108.6	19.8	72.1	2.2	326.0	28.0	116.2	69.7
A.M.Qasem	"Attil	976	7.49	100.4	22.7	65.1	2.1	370.0	22.5	74.5	76.5
H.Amous	'Attil	965	7.37	120.1	16.8	48.8	5.9	296.3	19.1	86.2	127.0
J.Awartani	Anabta	1335	7.83	127.5	31.4	100.0	2.4	403.4	28.0	151.0	104.0
Municipality well1	Anabta	1063	7.23	109.4	23.7	61.0	22.1	238.0	39.1	97.0	205.7
N.Mousa	Anabta	696	7.54	88.9	13.8	30.2	2.8	269.0	19.3	59.8	32.6
Bal'a	Bal'a	594	8.00	56.9	24.3	24.0	2.4	266.5	6.8	38.8	13.3
S.Said	Baqa A-Sharqiyya	751	7.54	98.7	14.8	30.2	2.3	273.9	15.5	39.2	102.8
Municipality well	Deir Al Ghsun	706	7.62	87.2	17.8	27.9	1.3	200.8	13.3	50.9	125.6
Far'on	Far'on	634	7.55	75.7	23.2	13.6	2.4	275.2	10.7	38.2	35.3
I.Attir	Far'on	631	7.53	72.4	26.2	11.9	0.8	221.9	39.3	38.2	51.1

A.A.Shanab	Irtah	1070	7.86	112.3	32.2	43.4	22.0	430.1	23.6	78.1	52.6
R.Qubbaj	Nur Shams	1139	7.75	85.3	30.9	105.0	0.9	379.3	19.0	142.0	43.0
A.R.Mir'eb	Shwayka	892	7.98	104.7	23.1	38.6	4.4	371.8	15.4	64.4	42.0
Said Jaber	Shwayka	721	7.92	76.5	24.2	30.2	3.4	250.4	14.5	59.8	67.6
Asa'd Taffal	Thennaba	614	8.12	70.7	15.0	28.9	4.9	258.8	13.0	43.0	27.3
A.Q Quzmar	Tulkarm	989	8.03	97.2	20.0	65.1	22.0	347.7	36.6	93.7	51.6
Municipality well2	Tulkarm	1298	8.01	111.9	39.7	90.0	8.5	417.8	38.0	113.6	98.0
M.Said	Tulkarm	2155	8.10	161.5	36.4	240.0	2.4	526.8	96.0	359.7	26.0
A.J.Samara	Zeita	731	7.52	86.4	17.3	34.9	2.2	300.0	18.1	54.9	30.6


محافظة طولكرم
TULKARM GOVERNORATE
مفتاح الخريطة
LEGEND
التجمعات السكانية
LOCALITIES
المستعمرات الاسرائيلية
ISRAELI SETTLEMENT
حدود اراضي التجمعات
LANDS BORDER
حدود المحافظة
GOVERNORATE BORDER
طريق رئيسي
MAIN ROAD
طريق اقليمي
REGIONAL ROAD
الخط الاخضر
GREEN LINE
دليل الموقع
LOCATION MAP
فلسطين
PALESTINE
عكابة
قفين
النزلة الشرقية
نزلة عيسى
نزلة أبو نار
باقة الشرقية
النزلة الوسطى
النزلة الغربية
زيتا
صيدا
علار
عتيل
الجاروشية
دير الغصون
شويكة
السقوفة
بلعا
اكتابا
مخيم نورشمس
كفر رمان
طولكرم
TULKARM
مخيم طولكرم
عنبتا
عزبة أبوخميش
ذنابة
خربة الطياح
كفا
عزبة الخلال
كفراللبد
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الحفاصي
عزبة شوفة
فرعون
شوفة
بيت ليد
خربة جبارة
سفارين
الراس
كفرصور
كور
كفرجمال
كفرزيباد
كفرعبوش

تقدير محتوى النترات والنتريت لمختلف الخضروات في محافظة طولكرم

اعداد

عفاف غالب حافظ أبودية

اشراف

د.حسان أبو قاعود

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الملخص

تم في هذه الدراسة تقدير محتوى النترات (NO_3) والنتريت (NO_3) في بعض الخضروات المزروعة في محافظة طولكرم في الضفة الغربية. تم خلال الدراسة جمع 75 عينة من خمسة أنواع من الخضروات (البندورة، الخيار، البصل، البطاطا، الملفوف) من ثلاث مناطق مختلفة في محافظة طولكرم (الشعراوية، ذنابة، الكفريات) بطريقة عشوائية. ولقد تم تحليل محتوى النترات (NO_3) في المحاصيل بواسطة جهاز (HI93728-0) HANNA instrument، بينما تم تحليل محتوى النتريت (NO_2) باستخدام طريقة AOCA Official Method 973.31. أظهرت النتائج أن اختلاف نوع الخضروات له تأثير كبير على محتوى النترات (NO_3) فيها، حيث وجد أن أعلى محتوى من النترات (NO_3) موجود في عينات محصول البطاطا، حيث كان معدل النترات (NO_3) فيها حوالي 253.13 ملغم/كغم، بينما أقل محتوى من

النترات (NO_3) وجد في عينات ثمار البندورة بمعدل 16.95 ملغم/كغم. ولقد تبين أن حجم الثمار له تأثير على محتوى النترات (NO_3) في الثمار، حيث تبين أن ثمار الخيار صغيرة الحجم مستوى النترات (NO_3) فيها أعلى من مستواه في الثمار كبيرة الحجم. كما تبين أن وجود القشر في الثمار أيضا له تأثير على محتوى النترات (NO_3) في الثمار، حيث ظهر أن محتوى النترات في قشور ثمار الخيار كان أعلى منها في ثمار الخيار المقشرة (في اللب). بينما أظهرت نتيجة تحليل محتوى النتريت (NO_2) في الخضروات انه كان منخفضا (أقل من 1ملغم/كغم) وهي نسبة مقبولة وأقل من الحد المسموح به. أما اختلاف المواقع فلم يكن له تأثير يذكر على محتوى النترات (NO_3) أو النتريت (NO_2) في الخضروات. لقد سلطت هذه الدراسة الضوء على موضوع مهم لم يتم التطرق له بالسابق. تحت الظروف التي تمت بها هذه الدراسة، فان النتائج دلت على أن مستويات كل من النترات (NO_3) والنتريت (NO_2) في الخضروات مقبولة. على أية حال لا بد من القيام بالمزيد من الأبحاث والدراسات على هذا الموضوع وفحص عدد أكبر من العينات وأنواع أخرى من المحاصيل.