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FORWARD

Dear Colleagues,

I would like firstly to congratulate ourselves and all faithful and loyal persons who joined us during our journey to become here this year to celebrate the tenth anniversary of IJST. Since the journal ideal was a dream in 2005, we intended to do our best to move toward more promising steps, and we had succeeded to continue issuing IJST in four annual issues per year with non- stopping attempts and with all patience shown by the editorial board members, the advisory group and the editorial board secretary to keep IJST as a distinguished mark in the scientific journal world.

It is our day to celebrate the 10th anniversary of IJST by year 2015, with you all, and present the deepest appreciations for significant editorial board members who gave IJST their care, their suggestions and their evaluable time and efforts, those are: Prof. Jamal Ahmed Abbas and Dr. Abdullah Shaker Al- Shebani from the Faculty of Agriculture at Kufa University, Dr. Atheer Al- Douri from the College of Vateriaary Medicine at University of Baghdad, Dr. Mahmoud Othman Mattar from the College of Medicine at Al- Najah National University from beloved Palestine, and Prof. Waleed Al- Murrani from PlyMouth University in England. We can not also forget the one who did her best to expand IJST among academic media and gave us her time to support and encourage researchers to join us, this is Prof. Taghreed Al- Noor.

As we are moving to the tenth volume of IJST, 2015, I would like to present you all my deep wishes and hopes in most peaceful year, most prosperity times and best movements in our journey with IJST.

Finally, on behalf of the International centre, I would like to express my special thanking to the Editorial Board Secretary for her faithful efforts in managing the scientific, design, technical and administrative aspects of the Journal and for preparing this issue for final printing and publishing.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

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ENGLISH SECTION

Effects of media cultures and benzyladenine (BA) concentrations in micropropagation of three types of cherry Rootstock (Establishment and multiplication)

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ABSTRACT

The current study was carried out in plant tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, University of Duhok, Iraq, during the period from March to September 2014, using two types of explants (Shoot tips and nod segments) in the establishment stage. The establishment of new *in vitro* culture from mature rootstocks (Stockton Morello, F12/1 Mazzard, Mahaleb) is often a difficult task due to the miniature growth of initial explants. Since the explant source plays an essential role on both establishment and multiplication of the *in vitro* cultures, it was studied by using different culture media.

Best results during establishment were obtained with explants taken from micro propagated plants. The highest percentage of shoot /explant (100%) was obtained from Stockton Morello rootstock on both media, while (96.88%) was recorded with WPM (Woody plant medium) from nodal segment in F12/1 Mazzard and (87.5%) was observed from shoot tip in Mahaleb. While the highest shoot number with three rootstocks (24.50, 24.38 and 22.25 shoot /explant) with Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded from nodal segment with WPM. While the lowest number of shoots (19.63 shoots /explant) was formed when the shoot tips were cultured on MS medium. At the multiplication stage, the maximum number of shoot per explant (9.50 and 9.17 shoots /explant) in Stockton Morello rootstock was recorded with WPM and MS medium provided with 3mg l⁻¹ BA. The interaction of Stockton Morello with WPM and MS medium having 1.5 mg l⁻¹ BA produced an average of shoot length (3.58 and 3.52 cm) and the increased of shoot length was significantly compared with all treatment without the treatment in combination of Stockton Morello rootstock in WPM medium supplemented with 3 mg l⁻¹ BA. Also the highest length of shoot per explant with three rootstocks (3.58 and 2.98 cm) in Stockton Morello and F12/1 Mazzard were recorded in WPM having 1.5mg l⁻¹ BA and in Mahaleb rootstock (2.92 cm) was recorded on MS medium containing 1.5mg l⁻¹ BA.

Keywords: Micropropagation, Cherries Rootstock, Culture Medium Composition, BA Concentrations

الملخص باللغة العربية

أجريت هذه الدراسة في مختبر زراعة الأنسجة التابع لقسم البستنة، جامعة دهوك، إقليم كردستان العراق، خلال الفترة من شهر آذار/ مارس إلى شهر أيلول / سبتمبر من عام 2014، وقد تم استخدام نوعين من الاجزاء النباتية (القمة النامية والعقد المفردة) في مرحلة النشأة، وبشكل عام، فإن إنشاء نباتات جديدة في المختبر من الأصول الناضجة لـ (Stockton Morello, F12/1 Mazzard, Mahaleb) غالباً ما تكون مهمة صعبة بسبب النمو البطيء للأجزاء النباتية، حيث يلعب مصدر الجزء النباتي دوراً أساسياً في كل من مرحلة النشأة ومرحلة التضاعف لدراسة النباتات في المختبر باستخدام أوساط غذائية مختلفة.

وقد تم الحصول على أفضل النتائج في مرحلة النشأة، وكانت أعلى نسبة من الفروع / الجزء النباتي (100%) من أصل Stockton Morello. بينما تم تسجيل نسبة (96.88%) من العقد المفردة للأصل F12 / 1 Mazzard المزروعة على وسط WPM و تم تسجيل نسبة (87.5%) من القمة النامية للأصل Mahaleb المزروعة على نفس الوسط الغذائي، في حين أن أعلى عدد من الفروع لثلاثة أصول من الكرز (24.50، 24.38 و 22.25 فرعاً / جزء نباتي) Stockton Morello ، F12 / 1 Mazzard و Mahaleb على التوالي من العقد المفردة المزروعة على وسط WPM. في حين شكلت أقل عدد من الفروع (19.63 فرعاً / جزء نباتي) من القمة النامية المزروعة على وسط MS . أظهرت النتائج في مرحلة التضاعف أكبر عدد ممكن من الفروع / جزء نباتي (9.50 و 9.17 فرعاً / جزء نباتي) من أصل Stockton Morello المزروعة على وسط MS المحتوي على 3 ملغم/ لتر BA . كما أنتج التداخل بين Stockton Morello و الأوساط الغذائية WPM و MS المزود بـ 1.5 ملغم / لتر BA معدل طول الفروع (3.58 و 3.52 سم) وكانت الزيادة معنوية مقارنة بجميع المعاملات ما عدا المعاملة التي احتوت تداخل بين كل من أصل Stockton Morello المزروعة على وسط WPM المزود بـ 3ملغم/لتر BA . كذلك بلغ أعلى معدل لطول الفروع (3.58 و 2.98 سم) لأصل Stockton Morello و F12 / 1 Mazzard المسجلة على وسط WPM المزود بـ 1.5 ملغم / لتر BA و أصل Mahaleb الذي سجل طولاً للفروع بلغ (2.92 سم) على وسط MS المجهز بـ 1.5 ملغم/لتر BA .

INTRODUCTION

In Iraq, there are many rootstocks used for cherry production (1). However, they have many disadvantages. Rootstocks trees (Stockton Morello, F12/1 Mazzard, Mahaleb), can not grow satisfactorily in heavy wet soils, show weak compatibility between rootstocks and cultivars, are sensitive to nematodes and difficult propagation by traditional methods (2). The rootstocks of (Stockton Morello, F12/1 mazzard and Mahaleb) are most commonly used for sweet and sour cherries production and are effective in size-controlling; growing satisfactorily in heavy and wet soils, early and good production.

Cherry rootstocks are traditionally propagated either by relatively slow and labor-intensive vegetative methods (cuttings and suckers), or from seeds, which need long time for stratifications and often result in non-uniform materials. The application of plant tissue culture method for vegetative propagation of temperate fruit rootstocks is started in the mid-70s, and a considerable number of improved protocols were developed ever since. Generally, the goals of micro-propagation are obtaining rapid, large-scale and low-cost production of genetically identical, physiologically uniform and pathogen-free plant (3). Successful *in vitro* clonal propagation methods were reported in many rootstocks, including cherry (4), plum (5) and pear rootstocks (6).

The micropropagation method is applied as a useful method for propagation of vegetative rootstock, (7). There is no universal medium for *in vitro* culture, since plant species and cultivars are genetically specific with regard to different components of medium, which include not only organic substances, but also mineral elements (8). In 2001, Erbenova *et al.* reported a 50% increase of multiplication rate on the dwarf rootstocks of sweet cherries in MS medium containing 1.5 mg l^{-1} BAP (9). Ruzic *et al.* reported that the MS and MS (double macro-salts) media culture containing $4.4 \mu\text{M}$ BA, $0.5 \mu\text{M}$ NAA and $0.3 \mu\text{M}$ GA3 are a suitable culture media for propagation of cherry (10). In a study on micropropagation of *Prunus avium*, the combination of 0.5 mg l^{-1} BAP and 0.05 mg l^{-1} TDZ was suitable for multiplication and a culture media having 0.3 mg l^{-1} IBA is desirable for the rooting (11). Carolina *et al.* cultured nodal segments of *Prunus serotina* in MS medium culture supplemented with 4.4 Mm BA, $0.49 \mu\text{M}$ IBA and $0.29 \mu\text{M}$ GA3 (12).

The aim of the current study was to investigate the effects of different culture media and plant growth regulators on micropropagation of three types of cherry rootstocks depending on their roles in controlling size growth of cherry trees and solving the difficulty of propagation by common methods.

MATERIALS AND METHODS

Three contemporary cherry rootstocks, including 1-Stockton Morello, 2-F12/1 Mazzard, 3-Mahaleb, were collected from the field. The current study was carried out in plant tissue culture laboratory of the Horticulture Department, Faculty of Agriculture, University of Duhok, Iraq, during the period from March to September 2014.

Initial cultures of all rootstocks were established using actively growing shoots from the field. Shoots were packed in plastic and were transferred to the laboratory. Then, the explants were washed by tap water and dishwashed liquid to remove surface contamination. Then, they were divided into parts containing more than one node and were pre-sterilized by immersing in 70% ethanol for 30 seconds. The next step was doing a rinse in sterile distilled water, and 5 min soaking in 0.1% (W/V) of HgCl_2 mercuric chloride, followed by triple rinsing with sterile distilled water. (1-1.5cm) large shoots were isolated and placed onto WPM (13) and MS medium (14) containing 2 mg l^{-1} BA for 4 weeks to take disinfectant explants. After all, they were transferred to the different culture media for multiplications stage.

Upon establishing the aseptic culture, uniform single shoots were multiplied on MS and WPM media of constant hormonal composition. Shoot multiplication of cherry rootstock was monitored on the medium supplemented with different concentrations of BA (1 , 1.5 and 3 mg l^{-1}) + WPM and MS medium. All multiplication media contained 30 g l^{-1} sucrose and 8 g l^{-1} agar. The pH value was adjusted to 5.7 ± 0.1 before autoclaving at 121°C , 150 KPa for 20 min. Establishment and multiplication parameters i.e. Shoot percentage, Number of shoots, Number of leaves and Shoot length (cm) were calculated.

Shoot cultures were grown in 250 ml culture vessels containing 25ml of establishment and multiplication media at $23 \pm 1^\circ\text{C}$ and 16 hour photoperiods (Light intensity, 1000 Lux.).

The cultures were grown in growth room under 16 hours photoperiod and 8 hours darkness with a light intensity of 1000-1500 Lux on culture surface provided with cool white fluorescent tubes 40W, 6, 500 °K and temperature was $23 \pm 1^\circ\text{C}$.

The experiments were set up in a completely randomized design (CRD) and repeated three times. Each treatment included 6-8 replicates (with 4 explants in each 250 cm^3 jar). Data were analyzed by using SAS program (15). The differences between various treatment means were tested with Duncan Multiple Range test at 5% level (16).

RESULTS AND DISCUSSION

Establishment parameters:

Effect of various culture media on three types of cherry rootstock (1-Stockton Morello. 2-F12/1 Mazzard. 3-Mahaleb) from two types of explants:

1. Percentage of Shoot Proliferation:

The establishment of aseptic culture is the first critical step in *in vitro* propagation process. There are many reports on successful application of mercuric chloride at 0.1% at 5min for surface sterilized of initial explants (17, 18).

It is notable on table (1), figure (1) that the rootstocks were significantly various in their response on the percentages of shoot development and the highest percentage of shoot was from the Stockton Morello (100%) as compared with F12/1 Mazzard and Mahaleb rootstock. However, the mean value of different explants were recorded and the highest percentage of shoots was (93.23 %) from nodal segments compared with shoot tip (92.19%), but the increases were not significant. While the mean value of different media gave the highest percentage (93.75%), which was observed with WPM as compared with MS medium which gives (91.67%).

Regarding the combination between rootstocks and media, the highest percentage of shoots (100%) was obtained from Stockton Morello rootstock in WPM and MS media, while the lowest percentage was formed in Mahaleb rootstock (85.94%) in WPM and MS media. However, regarding the interaction between rootstock and explants, the nodal segments and shoot tip from Stockton Morello rootstock gave the highest shoot percentage (100%) compared with other two rootstocks and the lowest shoot percentage (84.38%) was produced from nodal segments for Mahaleb rootstock.

Interaction between media and explants showed that the explants in the WPM medium produced high percentage of (93.75%) from nodal segments and shoot tip. While the lowest percentage of shoots (90.63%) was produced from nodal segments when cultured on MS medium.

The results of the three combinations (different rootstocks, different culture media and different explants) showed that the combination of nodal explants and shoot tips from Stockton Morello rootstock with all media WPM and MS medium produced 100% as compared with all treatments, but the shoot percentage did not increase significantly. The highest percentage of shoot /explant (100%) was obtained from Stockton Morello rootstock on both media, while (96.88%) was recorded with WPM from nodal segment in F12/1 Mazzard and (87.5%) was observed from shoot tip in Mahaleb. While the lowest shoot percentage (84.38%) was obtained from nodal segment in Mahaleb rootstock. The findings of the current study are in conformity with other studies, where BA performed better than Kinetin as a synthetic cytokinin. BA has the

advantage over other Cytokinin in inducing *in vitro* shoot production in woody plant like Jak fruit (*Artocarpus heterophyllus*) it was found that the concentration of 1.5 mg l⁻¹ will have very effective results on the dwarf cherry rootstock (9, 20). Moreover, (20) found in their study on 9 kinds of pear that the concentration of 2mg l⁻¹ BAP was the best treatment.

2. Number of Shoot Proliferation:

Table (2) figure (1) showed an increase in the shoot number/ explant in all rootstocks in different media and different explants. The mean value of different rootstocks was not significantly affected. The highest number of shoot/explant (23.63) was obtained from Stockton Morello rootstock as compared with both other rootstocks (22.69 and 20.88) number of shoot/explant from F12/1 mazzard and Mahaleb respectively. On the other hand, the mean value of explants showed that the highest number of shoot/explant was formed from nodal segment (23.23) compared with shoot tip, which gave (21.56) shoot/explant. However, the mean value of media recorded the highest number of shoot on WPM compared with MS medium (22.85 and 21.94) shoot/explant respectively, but the increase of shoot was not significant.

The results of the interaction between rootstocks and media was not significant, where the highest number of shoots in Stockton Morello and F12/1 Mazzard (23.88 and 23.50 shoots /explant) were obtained on WPM respectively, while the lowest number of shoots was formed in Mahaleb when the explants were cultured the explants on MS medium. Regarding the interaction between rootstocks and explants, the nodal explant from Stockton Morello and F12/1 Mazzard rootstock produced the highest number of shoot (24.19 and 23.63 shoots/explant), while the lowest shoot number (19.88) shoot/explant was obtained from Mahaleb's shoot tip. In case of the interaction between different media and different explants, the nodal segments culture on WPM produced the highest shoot number (23.71shoots/explant) compared with all treatments but the increase was non-significant.

Regarding the combination between three factors, the results revealed that the shoots could be observed with used WPM containing fixed concentration of BA (2mg l⁻¹). The highest shoot number with three rootstocks (24.50, 24.38 and 22.25 shoot/explant) with Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded from nodal segment with WPM. While the lowest number of shoots (19.63shoots/explant) was formed when the shoot tips were cultured on MS medium.

Based on the obtained results, it may be concluded that there are differences in uptake of cytokinins, recognition by the cell, or mechanisms of action of the cytokinin compound. However, cytokinins in the current experiment can be divided into two groups: very active group only BA, which was more effective, i.e. more shoots of cherry cv. Lapins formed, whereas TDZ, 2ip and kin exhibited rather weak effects on multiplication. These results were in

competence with results obtained by (21) with pear (*Pyrus pyrifolia*), they suggested that BAP displayed more noticeable effect than TDZ and kintin, i.e. BA is more suitable for shoot multiplication of pear. It is well known that high

concentration of cytokine of adenine type is often necessary for growth and differentiation of tissue culture.

Table (1): Effect of different types of explants culture on different type of media on shoot response percentage of three cherry rootstocks after 4 weeks

Type of rootstocks	Type of media	Type of Explant		Rootstock X Media	Mean of Rootstock
		Node segment	Shoot tip		
Stockton Morello	WPM	100 a	100 a	100 a	100 a
	MS	100 a	100 a	100 a	
F12/1 Mazzard	WPM	96.88 a	93.75 a	95.31 b	92.19 b
	MS	87.5 a	90.63 a	89.06 b	
Mahaleb	WPM	84.38 a	87.5 a	85.94 b	85.94 b
	MS	84.38 a	87.5 a	85.94 b	
Rootstock X Explant	Stockton M.	100 a	100 a	Mean of Media	
	F12/1Mazzard	92.19 ab	92.19 ab		
	Mahaleb	84.38 b	87.5 b		
Media X Explant	WPM	93.75 a	93.75 a	93.75 a	
	MS	90.63 a	92.71 a	91.67 a	
Mean of explants		93.23 a	92.19 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16)

Table (2): Effect of different types of explants cultured on different types of media on shoot number of three cherry rootstocks after 4 weeks

Type of rootstocks	Type of media	Type of Explant		Rootstock X Media	Mean of Rootstock
		Node seg	Shoot tip		
Stockton Morello	WPM	24.50 a	23.25 a	23.88 a	23.63 a
	MS	23.88 a	22.88 a	23.38 a	
F12/1 Mazzard	WPM	24.38 a	22.63 a	23.50 a	22.69 a
	MS	22.88 a	20.88 a	21.88 a	
Mahaleb	WPM	22.25 a	20.13 a	21.19 a	20.88 a
	MS	21.50 a	19.63 a	20.56 a	
Rootstock X Explant	Stockton Morello	24.19 a	23.06 a	Mean of Media	
	F12/1 Mazzard	23.63 a	21.75 a		
	Mahaleb	21.88 a	19.88 a		
Media X Explant	WPM	23.71 a	22.00 a	22.85 a	
	MS	22.75 a	21.13 a	21.94 a	
Mean of explant		23.23 a	21.56 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16).

3. Number of leaves Proliferation:

Table (3) and figure (1) showed the effect of different media on different explants of three types of rootstocks of cherry in all rootstocks. Different culture media and different type of explants (shoot tip and node segment) resulted in an increase in the number of leaves per explant. The mean value of rootstocks showed that the highest number of leaves (28.34) was observed from Stockton Morello rootstock and (26.94) was found from F12/1 Mazzard rootstock and it was significantly increased as compared with the Mahaleb rootstock, which gave (24.75 leaves/ explant).

On the other hand, the mean value of the explant, the highest leaves number (27.08 leaves /explant) was obtained from nodal segment compared with shoot tip (26.27 leaves /explant), but the increased was not significantly. However, the mean value of media showed that the large number of leaves (27.31 leaves /explant) was observed on WPM compared with MS medium.

Regarding the interaction between rootstocks and media, the highest leaves number per explant (28.88) was obtained from Stockton Morello rootstock when the explant cultured on WPM and the increase was significantly as compared with Mahaleb rootstock, which gave (26.06 and 24.44 leaves /explant) when explants were cultured on WPM and MS medium respectively, but not significantly with F12/1 Mazzard when cultured on WPM and MS media (28.00 and 25.88 leaves /explant) respectively. For the combination between rootstocks and explant, the highest number of leaves per explant (28.81) was found from nodal segments of Stockton Morello and it was significantly increased with some treatments, while the lowest leaves number (24.50) was observed from nodal segments of Mahaleb.

In case of combination between media and explant, the nodal segment cultured on WPM medium produced an average of (28.21 leaves /explant) and shoot tip on WPM medium produced (26.42 leaves /explant), while the lowest leaves number (25.96 leaves /explant) was obtained from nodal segments when cultured on MS medium.

The results from interaction between three factors (type of rootstock, different culture media and different explant) revealed that more leaves could be obtained by using WPM and MS media supplemented with fixed concentration of BA (2 mg l⁻¹). The highest number of leaves per explant with three rootstocks (29.88, 29.50 and 25.25 leaves /explant) were obtained from nodal segment of Stockton Morello, F12/1 Mazzard and Mahaleb respectively, when the nodal explant cultured on WPM. On the other hand, the lowest number of leaves was formed on Mahaleb rootstock (23.75 leaves /explant), when shoot tip cultured on MS medium, while the highest number of leaves of Stockton Morello and F12/1 Mazzard (27.88 and 26.38 leaves /explant) respectively were recorded in MS medium when shoot tip and nodal segment were cultured on MS medium respectively. Reducing salt concentration in MS medium resulted in poor performance with regard to percent establishment, shoots number, leaves number and shoots length as compared with WPM. Some plants gave similar response with all media, while other showed preference for specific media for explants (22).

Table (3): Effect of different type of explant cultured on different type of media on leaves number of three cherry rootstocks after 4 weeks

Type of rootstocks	Type of media	Type of Explant		Rootstock X Media	Mean of Rootstock
		Node seg	Shoot tip		
Stockton Morello	WPM	29.88 a	27.88 abc	28.88 a	28.34 a
	MS	27.75 abc	27.88 abc	27.81 ab	
F12/1 Mazzard	WPM	29.50 ab	26.50 abc	28.00 ab	26.94 a
	MS	26.38 abc	25.38 abc	25.88 abc	
Mahaleb	WPM	25.25 abc	24.88 bc	25.06 bc	24.75 b
	MS	23.75 c	25.13 abc	24.44 c	
Rootstock X Explant	Stockton Morello	28.81 a	27.88 ab	Mean of Media	
	F12/1 Mazzard	27.94 ab	25.94 abc		
	Mahaleb	24.50 c	25.00 bc		
Media X Explant	WPM	28.21 a	26.42 a	27.31 a	
	MS	25.96 a	26.13 a	26.04 a	
Mean of explant		27.08 a	26.27 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16)



Figure (1): Rosety plant production of cherry rootstock. (1- stockton Morello, 2- F 12/1 Mazzard, 3- Mehlab) on proliferation media containing MS + 2 mg l⁻¹ BA after 4 weeks

4. Shoot length:

Results in table (4) revealed that the length of shootlets was formed on three rootstocks of cherry. The mean value of the rootstocks showed that the longest shoot (2.61cm) was recorded on Stockton Morello rootstock and gave significantly longer shootlets than F12/1 Mazzard and Mahaleb rootstocks (1.97 and 1.66 cm) respectively. While the mean value of explants, the longest of shootlets formed from shoot tips (2.21 cm) compared with the nodal segments which gave (1.95 cm). However the mean value of the different media, the high length of shootlets (2.10 cm) was observed on WPM than MS medium which gave (2.06 cm). Regarding the combination between rootstocks and media, the explant in the combination of WPM and MS medium produced the high length of (2.59 and 2.63 cm) respectively, from the Stockton Morello rootstocks, and which gave the significantly longer shootlets than F12/1 Mazzard and Mahaleb (2.00 and 1.78 cm) on MS medium and WPM medium respectively.

However, the interaction between rootstock and explant, the longest shoot/explant (2.74 and 2.47 cm) were obtained from Stockton Morello rootstock when shoot tip and nodal segment cultured on WPM medium. While the lowest length of shoot was found with Mahaleb rootstock from nodal segments. Concerning the combination between the rootstocks and explants, the nodal segment and shoot tip from Stockton Morello rootstock gave significant longer shootlet than the F12/1 Mazzard and Mahaleb rootstock on both two explants. In the case of interaction between media and explants, revealed that the length of shootlets on the media WPM and MS medium, shoot tip was inversely related to culture media at fixed concentration of BA (2mg l⁻¹). The longest shoot (2.30 and 2.12 cm) was recorded with WPM and MS medium from shoot tips respectively. While the lowest length of shootlet was formed (1.90 cm) from nodal segments with WPM medium.

The results of three combination (different type of rootstocks, different media and different explants), revealed that the more shoot length could be found with WPM and MS medium enriched with fixed concentration of BA at (2mg l⁻¹). The combination of Stockton Morello and F12/1 Mazzard with WPM medium produced an average of (2.81 and 2.24 cm) was obtained from shoot tips compared with (2.68 and 2.08 cm) was found from shoot tips in MS medium respectively. While the high length of shootlet in three rootstocks (2.68, 2.08 and 1.85cm) in Stockton Morello, F12/1 Mazzard and Mahaleb respectively, were recorded with WPM medium when used shoot tip compared with nodal segment on WPM and MS medium. While the lowest length of shootlets or shortest of shoot were found on Mahaleb nodal explant (1.49 cm) in MS medium containing fixed concentration of BA (2 mg l⁻¹), and the shortest of shoot were observed on Stockton Morello and F12/1 Mazzard (2.58 and 1.65 cm) in MS and WPM medium respectively, when used nodal explant for culture in the different media.

In general, the decrease in shootlets length in all rootstocks of cherries with different media containing fixed concentration of AB (2mg l⁻¹) are in agreement with the findings of (23) who also reported that the subculture improved shoot elongation at short-lasting incubation (30 or 45 day). In contrast, (24) recorded a decrease in shoot length and leaf size after several subcultures, which indicated that beside being affected by external factors, growth is highly influenced by genotype. In addition, the results obtained with *L. Corniculatus* shoots seedling showed that even in the lowest concentration (0.08 and 0.22μM), cytokinin retarded elongation (25). While cytokinin Kin mainly influenced shoot growth of cherry cv. Lapins, whereas it made little impact on multiplication. Some species, such as *Tabernaemontana fuschsiaefolia* L. (Apocynaceae), exhibited higher multiplication rate on media with Kin than BAP (26).

Table (4): Effect of different type of explants cultured on different types of media on shoot length of three cherry rootstocks rootstocks after 4 weeks

Type of rootstocks	Type of media	Type of Explant		Rootstock X Media	Mean of Rootstock
		Node seg	Shoot tip		
Stockton Morello	WPM	2.36 abc	2.81 a	2.59 a	2.61 a
	MS	2.58 ab	2.68 a	2.63 a	
F12/1 Mazzard	WPM	1.65 cd	2.24 a-d	1.94 b	1.97 b
	MS	1.93 bcd	2.08 a-d	2.00 b	
Mahaleb	WPM	1.70 cd	1.85 bcd	1.78 b	1.66 b
	MS	1.49 d	1.61 cd	1.55 b	
Rootstock X Explant	Stockton Morello	2.47 ab	2.74 a	Mean of Media	
	F12/1 Mazzard	1.79 cd	2.16 bc		
	Mahaleb	1.59 d	1.73 cd		
Media X Explant	WPM	1.90 a	2.30 a	2.10 a	
	MS	2.00 a	2.12 a	2.06 a	
Mean of explant		1.95 a	2.21 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16).

Multiplication parameters:

Effect of various culture media and BA concentration on three types of cherry rootstock (1-Stockton Morello. 2-F12/1 Mazzard. 3-Mahaleb):

1. Number of Shoot:

Table (5) showed the effect of different types of rootstocks, different types of media and different concentrations of BA, WPM and MS medium used initially to study the effect of basal media on shoot multiplication. According to our results, WPM proved to be better than MS medium since it showed a higher number of shoots, higher number of leaves and shoots length. results in table (5) clearly showed that in all rootstocks, different culture media and different concentration of BA resulted the increase in the shoots number but it was non-significantly only in some treatments.

The mean value of different rootstocks, and the highest shoot number (7.17 shoots/explant) were found from Stockton Morello compared with F12/1 Mazzard and Mahaleb, which gave (6.47 and 6.17 shoots/explant) respectively. While the mean value of concentrations showed that the (3mg l⁻¹ BA) added to the medium gave (8.58 shoots/explant) and it was significantly compared with treatment when using (1mg l⁻¹ BA), which gave (3.61 shoots/explant). However, the mean value of different culture media showed the highest number of shoots per explant (6.80 shoots/ explant) which

was found on WPM. This might be because the requisite concentration of BA differ greatly according to the culture features of plant (8).

For the combination between rootstocks and media, the maximum number of shoots (7.28 shoots/explants) was obtained from Stockton Morello rootstock when cultured on WPM medium but the increase was not significantly with all treatments. While the lowest number of shoot appeared from Mahaleb rootstock (5.83 shoots/explant) when using MS medium. The combination between rootstocks and different concentrations of BA, the larger shoots number had appeared from Stockton Morello rootstock (9.33 shoots/explant) when using 3mg l⁻¹ BA, while the minimum shoot number (3.42 shoots/explant) was observed from Mahaleb rootstock when the explant was cultured on medium containing 1mg l⁻¹ BA. The combination between different culture media and concentrations of BA resulted that the maximum shoots number (9.00 shoots/ explant) was found on WPM medium having 3mg l⁻¹ BA and the increase was significantly than the treatments used WPM and MS medium supplemented 1mg l⁻¹ BA, while the minimum shoots number (3.50 shoots/ explant) was obtained on MS medium containing 1 mg l⁻¹ BA.

The consequences of the three combinations (different type of rootstocks, different type of culture media and different concentrations of BA) revealed that more shoots could be observed with the use of WPM and MS medium supplemented with a higher BA concentration. The maximum number of shoot per explant (9.50 and 9.17

shoots/explant) was obtained from Stockton Morello rootstock and recorded with WPM and MS medium provided with 3mg l⁻¹ BA. However, the minimum number of shoots formed from Stockton Morrello (3.83 shoots/explant), when the explants cultured on MS medium supplemented with 1mg l⁻¹BA. Whereas, the higher number of shoots from F12/1 Mazzard and Mahaleb (8.67 and 8.83 shoots/explant) were observed on WPM containing 3mg l⁻¹ BA respectively. But the lowest number of shoots (3.17 shoots/ explant) was formed from F12/1 Mazzard when the explant cultured on MS medium having 1mg l⁻¹ BA. From the published reported on various systems, it is clear that the cytokinin is essential for multiple shoots induction from explant. BA-induced multiple shoot induction has been reported in cherry rootstock (PHL-A) (27). In addition, our results showed that the presence of cytokinin like BAP in the proliferation phase in necessary.

Arab *et al.* reported *in vitro* multiplication of G × N15 (hybrid of almond × peach) vegetative rootstock, showed (7.37 ± 0.35) 1mg l⁻¹ BAP is the most effective treatment (28). Sulusoglu and Cavusoglu (29) stated that the effective BAP concentration for Mahaleb rootstocks (K-KK1 and S-AB), F12/1 Mazzard and SL-64 are 1mg l⁻¹. The Increase of BA concentration in medium (MS + 0.5 mg l⁻¹ BA compared with MS + 1 mg l⁻¹ BA) resulted in increase of the shoot number and decrease of the shoot length on cherry rootstock (30). These findings confirmed our results.

Table (5): Effect of different type of rootstocks, different type of media and different concentrations of BA on number of shoot of cherry rootstock after 8 weeks

Type of rootstocks	Type of media	BA concentrations			Rootstock X Media	Mean of Rootstock
		0	1.5	3		
Stockton Morello	WPM	4.17 b	8.17 a	9.50 a	7.28 a	7.17 a
	MS	3.83 b	8.17 a	9.17 a	7.06 a	
F12/1 Mazzard	WPM	3.67 b	7.50 a	8.67 a	6.61 a	6.47 a
	MS	3.17 b	7.33 a	8.50 a	6.33 a	
Mahaleb	WPM	3.33 b	7.33 a	8.83 a	6.50 a	6.17 a
	MS	3.50 b	7.17 a	6.83 a	5.83 a	
Rootstock X Conce.	Stockton Morello	4.00 c	8.17 ab	9.33 a	Mean of Media	
	F12/1 Mazzard	3.42 c	7.42 ab	8.58 ab		
	Mahaleb	3.42 c	7.25 b	7.83 ab		
Media X Conce.	WPM	3.72 b	7.67 a	9.00 a	6.80 a	
	MS	3.50 b	7.56 a	8.17 a	6.41 a	
Mean of Conce.		3.61 b	7.61 a	8.58 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16).

2. Number of leaves:

Table (6) showed that the WPM proved to be better than MS medium, since it showed the highest number of leaves per explant. The mean value of different rootstocks showed that the highest number of leaves (15.28 leaves/ explant) was found on Stockton Morello rootstock, but the increase was not significant than the F12/1 Mazzard and Mahaleb rootstocks (14.36 and 13.89 leaves/ explant) respectively. While for the mean value of different concentrations of BA, the maximum leaves number per explant was (15.94 leaves/ explant) in 3mg l⁻¹ BA and there was significantly increase more than (1 and 1.5mg l⁻¹ BA). Regarding the mean value of

different culture media, the highest number of leaves (14.57 leaves/explant) was observed on WPM compared with MS medium which gave (14.44 leaves/ explant), but the increase was not-significant.

In case of the interaction between rootstocks and media, the explants from Stockton Morello rootstock cultured on MS medium produced the highest number of leaves (15.44 leaves/ explant). In addition, the interaction displayed that the greatest number of leaves were obtained on WPM medium and F12/1 Mazzard, which gave (14.72 leaves/ explant), while the lowest number of leaves per explant (13.89 leaves/ explant) were obtained from

Mahaleb rootstock when cultured on WPM medium. Nevertheless, the combination between rootstocks and different concentrations of BA, showed that the greatest number of leaves/explant (17.58 and 16.08 leaves/ explant) were found from Stockton Morello and F12/1 Mazzard rootstocks when the explant were cultured on medium having 3mg l⁻¹ BA and it was significantly increased compared with all treatments excepted the treatment containing 1.5mg l⁻¹ when Stockton Morello rootstock on culture medium. While the lowest number of leaves per explant (13.50 leaves/ explant) were observed from three rootstocks (Stockton Morello, F12/1 Mazzard and Mahaleb) when cultured on medium containing 1mg l⁻¹ BA and F12/1 Mazzard when cultured on medium having 1.5mg l⁻¹ BA. Whereas, the interaction between the culture media and different concentration of BA, the highest number of leaves per explants in both media (16.83 and 15.06 leaves/ explant) were observed on WPM and MS medium having 3mg l⁻¹ BA respectively. While the lowest number of leaves shows on WPM containing (1 and 1.5 mg l⁻¹ BA) (13.44 leaves/explant).

The results of interaction of three factors (different type of rootstocks, different type of culture media and different concentrations of BA) revealed that the major leaves could be observed on WPM than MS medium supplemented with high concentration of BA (table 6 and figures 2 and 3). The greatest number of leaves in three rootstocks (17.83, 17.50 and 15.17 leaves/explant) in the Stockton Morello, F12/1 Mazzard and Mahaleb rootstocks respectively, were recorded with WPM medium having 3mg l⁻¹ BA. As well as, the lowest number of leaves per explant were formed on Stockton morello explants (13.17 leaves/explant) in MS medium having 1mg l⁻¹ BA and formed on Mahaleb explants (13.17 leaves/ explant), was obtained on WPM and MS medium containing (1 and 3mg l⁻¹ BA). While the highest number of leaves per explant formed on Stockton Morello rootstock (17.33 leaves/explant) in MS medium having 3mg l⁻¹ BA and highest leaves number of leaves per explant formed on F12/1 Mazzard (14.67 leaves/explant) in MS medium supplemented with 3 mg l⁻¹ BA. Whereas, a promotive effect at low BAP concentration that was described in the literal is for explant of different citrus genotype (31). Similar results were reported by (27) who obtained the highest leaves number per explant by culturing the explants of cherry rootstocks on MS and DKW media, which containing (0.5 and 1 mg l⁻¹) BA.

3. Shoot length:

Results in table (7) revealed that the longest of shootlet formed on three rootstocks explants was inversely related to BA concentrations and different culture media. The mean value of different type of rootstocks, the lowest of shootlet (2.94 cm) was recorded on Stockton Morello rootstock and it was increased significant than the two other rootstocks (2.58 and 2.31 cm) were recorded on F12/1 Mazzard and Mahaleb rootstocks respectively, and the F12/1 Mazzard increased significantly than the Mahaleb

rootstock. As well as, the lowest shoot length (2.31 cm) was recorded on Mahaleb rootstock. Whereas, the mean value of different concentration of BA, show in the same table, the longest of shootlet (3.04 cm) was found on medium having 1.5mg l⁻¹ BA compared with the treatment contained (1 and 3 mg l⁻¹ BA) were recorded lowest shoot length (2.20 and 2.60 cm) respectively and the increase was significant between treatments. In addition, the treatment, which contained 3mg l⁻¹ BA showed significant increase on the shoot length than the treatment having 1mg l⁻¹BA, while the mean value of different type of culture media, the maximum shoot length (2.62 cm) was recorded on MS medium that the WPM medium which gave (2.60 cm), but the increase was not significant.

Concerning the interaction between the different type of rootstocks and different culture media, the longest shoot (3.02 and 2.87 cm) was found from the Stockton Morrelo rootstock, when the explants were cultured on WPM and MS medium respectively. Whereas, the lowest shoot length was formed in Mahaleb rootstock (2.22 cm) on WPM and the increase of the shoot length was significant between three rootstocks, where in the length of Stockton Morello rootstock increased significantly when cultured on both media than the F12/1 Mazzard and Mahaleb rootstock. The longest of shoot on F12/1 Mazzard and Mahaleb (2.59 and 2.40 cm) was formed from shoot on MS medium respectively, but the increase of shootlet was not significant. However, the combination between rootstocks and different concentrations of BA, showed that the longest of shootlets was produced in Stockton Morello (3.55 cm) when the explants were cultured on medium having 1.5mg l⁻¹ BA and the increase was significant than all treatments, while the lowest shoot length was produced from Mahaleb and F12/1 Mazzard (2.16, 2.18 and 2.19 cm) when the explants were cultured on media containing (1 and 3 mg l⁻¹ BA) respectively. The combination of BA showed that the explants in combination of MS medium with 1.5mg l⁻¹ BA were produced on an average length of (3.13 cm), and explants in WPM medium having 1.5 mg l⁻¹ BA produced an average length of (2.94 cm) and the shortest (2.18 cm) were produced when the explants were cultured on MS medium containing 1 mg l⁻¹ BA.

In case of the combination of three factors (different type of rootstocks, different type of media and different concentrations of BA), results revealed that more shoot length could be found with the use of WPM and MS medium enriched with BA concentration (Figures 2 and 3 and table 7). The interaction of Stockton Morello with WPM and MS medium having 1.5 mg l⁻¹ BA produced an average of shoot length (3.58 and 3.52 cm) and the increase of shoot length was significantly compared with all treatment without the treatment in combination of Stockton morello rootstock in WPM medium supplemented with 3mg l⁻¹ BA. The highest length of shoot per explant with three rootstocks (3.58 and 2.98 cm) in Stockton Morello and F12/1 Maazard were recorded in WPM having 1.5mg l⁻¹ BA and in

Mahaleb rootstock (2.92 cm) was recorded on MS medium containing 1.5mg l^{-1} BA. On the other hand, the shortest shoots were formed on Mahaleb rootstock (2.13 and 2.15 cm) in MS medium supplemented with (1 and 3mg l^{-1} BA), also the shortest of shoots on F12/1 Mazzard rootstock (2.17 cm) was found on MS medium contained 1mg l^{-1} BA.

The total decrease in shootlets length in all rootstocks with increasing BA concentration in the culture medium is in agreement with the findings of (17) on wild cherry (*Prunusaium* L.) and (6) on fruit rootstocks.

Hossini *et al.* (32) also reported that the different concentrations of BA improved shoot elongation at 1mg l^{-1} BAP when added to the MS medium and 1mg l^{-1} BAP when added to the LS medium. In contrast, (18) recorded a decrease in shoot length when adding a higher concentration of BA (2mg l^{-1}), but the longest of shoots were found in the current study when adding 0.2mg l^{-1} BA to the MS medium.

Table (6): Effect of different type of rootstocks, different type of media and different concentrations of BA on number of leaves of cherry rootstock after 8 weeks

Type of rootstocks	Type of media	BA concentrations			Rootstock X Media	Mean of Rootstock
		0	1.5	3		
Stockton Morello	WPM	13.83 a	13.67 a	17.83 a	15.11 a	15.28 a
	MS	13.17 a	15.83 a	17.33 a	15.44 a	
F12/1 Mazzard	WPM	13.33 a	13.33 a	17.50 a	14.72 a	14.36 a
	MS	13.67 a	13.67 a	14.67 a	14.00 a	
Mahaleb	WPM	13.17 a	13.33 a	15.17 a	13.89 a	13.89 a
	MS	13.83 a	14.67 a	13.17 a	13.89 a	
Rootstock X Conce.	Stockton Morello	13.50 b	14.75 ab	17.58 a	Mean of Media	
	F12/1 Mazzard	13.50 b	13.50 b	16.08 ab		
	Mahaleb	13.50 b	14.00 b	14.17 b		
Media X Conce.	WPM	13.44 b	13.44 b	16.83 a	14.57 a	
	MS	13.56 b	14.72 ab	15.06 ab	14.44 a	
Mean of Conce.		13.50 b	14.08 b	15.94 a		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16).

Table (7): Effect of different type of rootstocks, different type of media and different concentrations of BA on shoots length of cherry rootstock after 8 weeks

Type of rootstocks	Type of media	BA concentrations			Rootstock X Media	Mean of Rootstock
		0	1.5	3		
Stockton Morello	WPM	2.38 b-f	3.58 a	3.08 ab	3.02 a	2.944 a
	MS	2.17 f	3.52 a	2.98 abc	2.87 ab	
F12/1 Mazzard	WPM	2.10 f	2.98 abc	2.63 b-f	2.57 bc	2.58 b
	MS	2.28 c-f	2.95 a-d	2.53 b-f	2.59 bc	
Mahaleb	WPM	2.17 f	2.27 def	2.23 ef	2.22 c	2.311 c
	MS	2.15 f	2.92 a-e	2.13 f	2.40 c	
Rootstock X Conce.	Stockton Morello	2.25 c	3.55 a	3.03 b	Mean of Media	
	F12/1 Mazzard	2.19 c	2.97 b	2.58 bc		
	Mahaleb	2.16 c	2.59 bc	2.18 c		
Media X Conce.	WPM	2.22 d	2.94 ab	2.65 bc	2.60 a	
	MS	2.18 d	3.13 a	2.55 cd	2.62 a	
Mean of Conce.		2.20 c	3.04 a	2.60 b		

* Means followed by the same letter within each character (column) do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test (16).



Figure (2): Mass production of cherry rootstock (1- stockton Morello, 2- F 12/1 Mazzard, 3- Mehlab) on proliferation media containing the best concentration of BA + MS after 8 weeks.

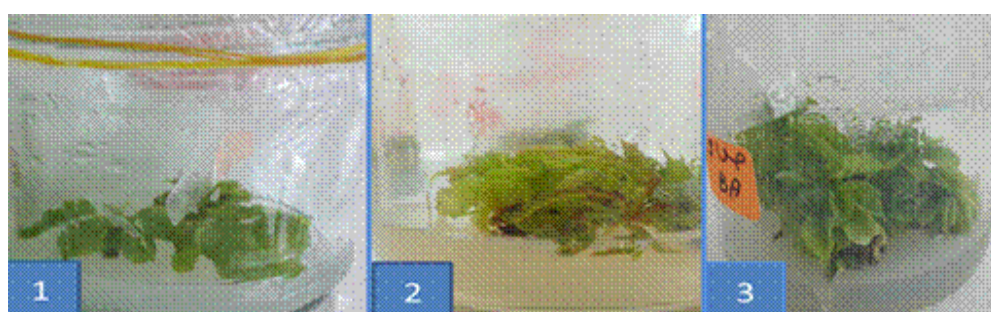


Figure (3): Mass production of cherry rootstock (1- stockton Morello, 2- F 12/1 Mazzard, 3- Mehlab) on proliferation media containing the best concentration of BA + WPM after 8 weeks.

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Effect of potassium and ascorbic acid on growth, yield, and quality of olive cv. Khadrawi

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ABSTRACT

The current study was carried out in a private olive orchard located near Kasara region during growing season 2014 in order to study the effect of spraying olive tree 8 years old with three concentrations of potassium fertilizer as potassium sulfate (0, 5 and 10 g.l⁻¹) and three concentrations of ascorbic acid (0, 200 and 400 mg.l⁻¹). The spraying of both potassium and ascorbic acid were carried out twice per season, first two weeks after growth began, and second after month. Results indicated that spraying potassium at 10 g.l⁻¹ had significantly increased leaf area, leaf dry weight, chlorophyll content, fruit weight, fruit flesh weight, and fruits quality including fruit length, fruit width and TSS %. Ascorbic acid at 400mg.l⁻¹ had significantly increased all vegetative growth, physical and chemical fruits properties except seed weight and TSS %. Interaction of 10 g.l⁻¹ potassium and 400 mg.l⁻¹ Ascorbic acid had significantly increased all vegetative growth, physical and chemical fruits properties.

Key words: olive, Khadrawi, potassium, Ascorbic acid

المخلص باللغة العربية

تم تنفيذ هذه الدراسة في بستان زيتون يتبع للقطاع الخاص و يقع في منطقة قسار في إقليم كردستان / العراق خلال موسم النمو 2014 بهدف دراسة تأثير رش أشجار الزيتون ذات عمر الثماني سنوات بثلاث تراكيز من سماد البوتاسيوم على شكل سلفات البوتاسيوم (0 و 5 و 10 غم. لتر⁻¹) وثلاث تراكيز من حامض الأسكوربيك (0 و 200 و 400 ملغ. لتر⁻¹). تم رش كل من البوتاسيوم وحامض الأسكوربيك مرتين خلال الموسم ، المرة الأولى كانت بعد أسبوعين من بدء النمو والثانية بعد شهر.

وأظهرت النتائج أن رش البوتاسيوم بتركيز 10 غم.لتر⁻¹ أدى إلى زيادة معنوية في مساحة الورقة والوزن الجاف للورقة ومحتوى الكلوروفيل ووزن الثمرة ووزن لحم الثمرة ونوعية الثمار من طول وعرض الثمرة، ونسبة المواد الصلبة الذائبة الكلية. كما أدى رش حامض الأسكوربيك بتركيز 400 ملغ.لتر⁻¹ إلى زيادة معنوية في جميع صفات النمو الخضري والصفات الفيزيائية والكيميائية للثمار، ما عدا وزن البذرة ونسبة المواد الصلبة الذائبة الكلية. وقد بين التداخل بين 10 غم. لتر⁻¹ بوتاسيوم و 400 ملغ. لتر⁻¹ حامض الاسكوربيك زيادة معنوية في جميع صفات النمو الخضري والصفات الفيزيائية والكيميائية للثمار.

INTRODUCTION

Olive belongs to the family *Oleaceae*. Olives are grown between two latitudes of 30° and 45° N from the equator. About 90% of olives in the world are produced for oil production and 10% are produced as table olives (1,2).

Olive trees are grown in some areas of central and northern of Iraq. Nineveh is the leading governorate in olive producing, its cultivation in Nineveh spreading in an area including village of Baashiqah, Bahzany, Fadiliya, Sheikh Uday, Dhecan, Sinjar and Aqrah, followed by Babylon, Diyala, Kirkuk, Baghdad, Erbil and Duhok (3). The Mediterranean region is native habitat (4).

However, current olive practices in Iraq largely ignored the mineral nutrition especially in arid and semi arid zones. Potassium is a major element with an important effect on fruit yield and quality. This element could be applied with different methods, the foliar application is helpful to satisfy plant requirement and has a high efficiency (5). Potassium is easily adsorbed and distributed through leaf tissues (6). A study was conducted in 2008 to investigate the effect of potassium fertilizer applied as fertigation in the form of potassium sulphate on olive (7). The obtained results revealed that high level of K fertigation (400 mg K.L⁻¹) improved the vegetative growth parameters, leaf water content especially at high level of irrigation water (7). Another study showed that the application of (NPK) fertilizer on cv. Ashrasi olive tree at two levels (15 and 30 Kg/Donum) caused significant increase in the nutritional element content of leaves such as nitrogen and potassium, also Spraying potassium nitrate at (15 and 30 g/L) had significant effect in the leaf content of nitrogen, potassium, leaf dry weight, the total chlorophyll content, potassium nitrate (8).

Nowadays, there is a prevalent use of the antioxidants especially ascorbic acid for enhancing growth and productivity of fruit trees as well as controlling the prevalence of most fruit disorders (9,10). Ascorbic acid as an antioxidant has auxinic action and has synergistic effect on growth, flowering and production of plants (9, 11, 12). A study was conducted and its results showed that application of ascorbic acid improved berry set, yield, cluster weight, berry weight, TSS and total sugar of grape, however acidity was reduced (13). Another study showed that using ascorbic acid alone or combined application with some micronutrient was positively affected on vegetative growth, yield and fruit quality of 'ANNA' apple trees (14). Recently, a study was conducted to determine the effect of Ascorbic acid and humic acid on vegetative growth of two olive (*Olea europaea*) cultivars (Khithairy and Sorany) (15). The transplants were sprayed with four levels (0, 500, 1000 and 2000 mg.l⁻¹) Ascorbic acid and four levels of humic acid (0, 20, 40 and 60 mg.l⁻¹). Results indicated when transplant treated with 500 mg.l⁻¹ Ascorbic acid significantly increased plant height, leaf fresh weight and leaf dry weight (15).

MATERIALS AND METHODS

The study was carried out during growing season 2014 in a private olive orchard located near Kasara region in order to investigate the effect of spraying olive trees of 8 years old with three concentrations of potassium fertilizer as potassium sulfate (0, 5 and 10 g.l⁻¹) and three concentrations of ascorbic acid (0, 200 and 400 mg.l⁻¹). The spraying of both potassium and ascorbic acid were carried out twice per season, first two weeks after growth began, and the second after month.

RBCD design was used for arranging the treatment and each treatment was replicated three times using one tree per experiment unit. The olive trees were irrigated with drip irrigation and planted at 5*5 m within the row and 5 m between the rows. The trees under taken in this study received all agricultural and horticultural practices that done in orchards.

A detergent powder as wetting agent at (1-2 g.L⁻¹) was added to all the spraying solution. The olive trees were sprayed with solutions until run off (2 L/tree). potential effects of potassium and ascorbic acid were evaluated in terms of the change in growth, leaf area was calculated by (leaf area meter AM 300), leaf dry weight, chlorophyll content as (SPAD), fruit weight, fruit flesh weight, seed weight, and fruits quality include fruit length, fruit width and TSS %. All results were analyzed statistically by using SAS programs (16). Duncan's multiple tests at 5% level of portability was to compare the treatment according to (17).

RESULTS AND DISCUSSION

Vegetative growth properties:

Results exhibited in table (1) showed that foliar application of both potassium and ascorbic acid had significantly increased vegetative growth properties represented in single leaf area, leaf dry weight and chlorophyll content, since spraying olive trees with 10 g.L⁻¹ and 400 mg.L⁻¹ resulted in highest value (35.74, 0.161 and 81.79) (35.06, 0.161 and 79.40) of single leaf area, leaf dry weight and chlorophyll content respectively.

Concerning the interaction, the same table showed that the interaction between potassium and ascorbic acid had significant effect of all vegetative growth properties undertaken in this study, maximum values (38.77 and 0.184) of single leaf area and leaf dry weight were obtained with the interaction between 10 g.L⁻¹ potassium and 400 mg.L⁻¹ Ascorbic acid respectively, whereas the maximum value of chlorophyll content was with the interaction between 10 g.L⁻¹ potassium and 200 mg.L⁻¹ Ascorbic acid compared to the minimum values resulted from control.

The significant effect of spraying potassium may be due to the main role of potassium in the synthesis of proteins and activates a number of enzymes and the promotion of normal cell division and growth, which are important components in the synthesis of

chlorophyll (18,19). Auxinic action of ascorbic acid on enhancing cell division and cell enlargement which reflected positively on leaf area was concluded by (10, 20, 21). On the other side, accumulation of dry matter production in canopy

and fruits can be assumed proportional to solar radiation intercepted by foliage resulting in more efficiency of photosynthesis process (22).

Table (1): Effect of potassium and Ascorbic acid on vegetative growth properties of olive cv. Khadrawi

Characters	Leaf area (cm ²)			Leaf dry weight (g)				Chlorophyll content (Spade)				
potassium (g.l ⁻¹)	Ascorbic acid (ml.L ⁻¹)			Mean Effect K	Ascorbic acid t (ml.L ⁻¹)			Mean Effect K	Ascorbic acid (ml.L ⁻¹)			Mean Effect K.
	0	200	400		0	200	400		0	200	400	
0	24.66 cd	25.87 cd	30.96 a-d	27.16 b	0.090 b	0.136 ab	0.143 ab	0.123 b	65.10 d	72.37 cd	75.40 abc	70.96 b
5	28.00 bcd	35.05 abc	35.44 ab	32.83 a	0.126 ab	0.144 ab	0.156 a	0.142 ab	74.30 bc	79.33 abc	83.33 ab	78.99 a
10	31.81 a-d	36.63 ab	38.77 a	35.74 aa	0.134 ab	0.165 a	0.184 a	0.161 a	81.77 ab	84.13 a	79.47 abc	81.79 a
Mean effect Ascorbic	28.16 b	32.52 ab	35.06 a		0.117 b	0.148 ab	0.161 a		73.72 b	78.61 a	79.40 a	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Fruit properties

Results in table (2) showed that fruit weight, fruit flesh weight and seed weight for olive trees sprayed with potassium at both concentration are superior significantly on that untreated trees. The highest fruit weight, fruit flesh weight and seed weight (50.49, 7.29, and 1.90) respectively, were given by spraying trees with potassium at 10 g.L⁻¹ compared with lowest values (34.54, 5.21 and 1.19) respectively at untreated trees. Various levels of potassium were also differed significantly among each other's. Data in the same table also shows that fruit weight and fruit flesh weight for trees sprayed with Ascorbic acid are superior significantly on that untreated. Highest values (47.05 and 7.03) respectively, were obtained in trees sprayed with Ascorbic acid at 400 mg.L⁻¹ compared with lowest values (37.75 and 5.25) respectively at untreated trees. Whereas Ascorbic acid had no effect on seed weight of olive fruit at both concentration.

For the interaction between potassium and Ascorbic acid, results in table (2) indicated that the interaction between 10 g.L⁻¹ potassium and 400 mg.L⁻¹ Ascorbic acid significantly overtopped most of other interaction and had highest value (60.32, 8.53 and 1.90) for the previous properties respectively.

Fruit weight, fruit flesh weight and seed weight of fruit were increased with increasing the concentration of application of potassium these increasing may be due to the role of potassium in activating meristematic growth, photosynthesis and activates a number of enzymes, including those involved in the synthesis of carbohydrates, and is also involved in the neutralization of organic acids and the promotion of normal cell division and growth (23- 25). Ascorbic acid act as antioxidants,

therefore expected increments of carbohydrates supply to fruits can explain improvements of yield fruit weight and flesh oil content obtained in this experiment (22, 26).

Fruit quality properties:

Results in Table (3) clearly showed that foliar application of potassium at 5 g.L⁻¹ and 10 g.L⁻¹ was accompanied with improving quality of the olive fruits in terms of increasing fruit length, fruit width and total soluble solids percentage. The best significant results were obtained by the addition of potassium via leaves at 10 g.L⁻¹. Ascorbic acid sprays also was of measurable influence on fruits quality in terms of increasing fruit width and total soluble solids percentage, while both concentration of Ascorbic acid had on significant effect on fruit length, the highest values of fruit width and total soluble solids percentage resulted in fruits of trees received Ascorbic acid at 400 mg.L⁻¹ compared to the lowest values obtained with control.

For the interaction, the best results were regarded when potassium was sprayed at 10 g.L⁻¹ and Ascorbic acid at 400 mg.L⁻¹, the highest values of fruit length, fruit width and total soluble solids percentage (3.12, 3.03, 1.26 and 16.25) respectively, were detected on olive trees received potassium at 10 g.L⁻¹ and Ascorbic acid at 400 mg.L⁻¹, compared with the lowest values (2.13, 1.75 and 12.83) respectively from control treatment. The increases in fruit quality traits may be due to the role of potassium influencing meristematic growth, photosynthesis and activates a number of enzymes, including those involved in the synthesis of carbohydrates, then increased food materials available to the fruits (23, 25).

Table (2): Effect of potassium and Ascorbic acid on fruit properties of olive cv. Khadrawi

Characters	fruit weight (g)				fruit flesh weight (g)				seed weight (g)			
potassium (g.l ⁻¹)	Ascorbic acid (ml.L ⁻¹)			Mean Effect K	Ascorbic acid (ml.L ⁻¹)			Mean Effect K	Ascorbic acid (ml.L ⁻¹)			Mean Effect K.
	0	200	400		0	200	400		0	200	400	
0	28.08 c	36.82 bc	38.72 b	34.54 c	3.49 c	6.21 b	5.91 b	5.21 c	0.91 c	1.34 b	1.33 b	1.19 c
5	39.85 b	41.10 b	42.11 b	41.02 b	56.77 b	6.91 b	6.66 b	6.78 b	1.63 ab	1.60 ab	1.69 ab	1.64 b
10	45.31 b	45.84 b	60.32 a	50.49 a	6.50 b	6.86 b	8.53 a	7.29 a	1.92 a	1.87 a	1.90 a	1.90 a
Mean effect Ascorbic	37.75 b	41.25 b	47.05 a		5.25 b	6.66 a	7.03 a		1.49 a	1.60 a	1.64 a	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

Table (3): Effect of potassium and Ascorbic acid on fruit quality properties of olive cv. Khadrawi

Characters	fruit length (mm)				fruit width (mm)				TSS (%)			
potassium (g.L ⁻¹)	Ascorbic acid (mL.L ⁻¹)			Mean Effect K	Ascorbic acid t (mL.L ⁻¹)			Mean Effect K	Ascorbic acid (mL.L ⁻¹)			Mean Effect K.
	0	200	400		0	200	400		0	200	400	
0	2.13 c	2.64 bc	2.80 ab	2.52 b	1.75 c	1.91 bc	1.98 bc	1.88 b	9.33 b	12.83 b	16.00 a	12.72 b
5	3.05 ab	3.23 ab	2.99 ab	3.09 a	2.17 bc	2.09 bc	2.07 bc	2.11 a	12.92 b	14.08 ab	14.83 ab	13.94 b
10	3.27 ab	2.70 bc	3.40 a	3.12 a	2.27 b	2.32 b	3.03 a	2.54 a	14.58 ab	15.92 a	16.25 a	15.58 a
Mean effect Ascorbic	2.81 a	2.91 a	3.01 a		2.06 b	2.11 ab	2.36 a		12.28 c	14.28 b	15.69 a	

Means with the same letter are not significantly different according to Duncan multiple ranges test at 5% level

The effects of Ascorbic acid on chemical quality of fruit may be due to the influence of Ascorbic acid on stimulating carbohydrate biosynthesis as a result of their effect on improving the vegetative of the plant, (15, 27), also Ascorbic acid serves as a co-factor for many enzymes and it contributes to the detoxification of reactive oxygen species (ROS) (28). Also vitamins with their anti oxidative properties play an important role in plant defense against oxidative stress induced by surfactants and selected pesticide (29).

CONCLUSION

According to the experimental results of this study, the most important conclusions can be expressed as follows:

1. Potassium markedly increased Leaf area, Leaf dry weight, total chlorophyll, fruit weight, flesh weight

and seed weight as well as fruit length, fruit width and total soluble solid.

2. Potassium at 10 g.L⁻¹ allowed maintenance of fruit physical properties without important loss in fruit quality.

3. Ascorbic acid noticeably increased Leaf area, Leaf dry weight, total chlorophyll, fruit weight and flesh weight as well as fruit length, fruit width.

4. Potassium was effective on fruit quality more than done Ascorbic acid.

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Effect of some organic and non organic fertilizers on growth, yield and quality of grape cv. Kamali

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ABSTRACT

The current study was conducted to determine the effect of using deferent mineral and organic fertilizers on the leaf area, leaf dry weight, total chlorophyll %, petiole mineral content and yield as well as physical and chemical properties of the grape cv. Kamali grown under drip-irrigated system during 2012 growing season. Results showed that Ammonium sulfate + Organic manure + Humic acid caused a remarkable stimulation on growth characters, yield as well as berries quality parameters compared to control. Total acidity percentage in the juice tended to reduce with using Ammonium sulfate + Organic manure + Humic acid treatments. Application of Ammonium sulfate + Organic manure, Ammonium sulfate + Humic acid or Organic manure + Humic acid caused a significant increase in most of the study parameters compared to the application of Ammonium sulfate, Humic acid and Organic manure alone.

Keywords: Ammonium sulfate, Organic manure, Humic acid, grape, Kamali

الملخص باللغة العربية

أجريت الدراسة الحالية لمعرفة تأثير استعمال أسمدة معدنية وعضوية مختلفة على مساحة الورقة والوزن الجاف للورقة ونسبة الكلوروفيل الكلي ومحتوى الأعناق من المعادن والحاصل، بالإضافة إلى دراسة الصفات الفيزيائية والكيميائية للعنب من صنف كمالي المزروع تحت نظام الري بالتنقيط خلال موسم النمو 2012.

وقد بينت النتائج بأن إضافة سماد سلفات الأمونيوم + السماد العضوي + حامض الهيوميك قد سببت تحفيزاً واضحاً في صفات النمو والحاصل، وكذلك الصفات النوعية للحبات مقارنة بالمقارنة، كما انخفضت نسبة الحموضة الكلية باستعمال سماد سلفات الأمونيوم + السماد العضوي + حامض الهيوميك. وقد سببت إضافة سلفات الأمونيوم + السماد العضوي و سلفات الأمونيوم + حامض الهيوميك أو السماد العضوي + حامض الهيوميك زيادة معنوية في معظم الصفات المدروسة مقارنة بإضافة سلفات الأمونيوم أو حامض الهيوميك أو السماد العضوي كل على حدة.

INTRODUCTION

Grape (*Vitis vinifera* L.) belongs to Vitaceae family, is perhaps the most widely cultivated fruit crop of the world in varying climatic zones extending from the temperate to the tropics. The berries are a good source of minerals and vitamins (B1, B2 and C). The fruits are consumed in fresh forms as a table grape and in the processed form as raisin and fresh juice (1).

Mineral fertilization causes the accumulation of harmful residual substances like nitrate and nitrite in the edible portion in berries or leaves of grapevines (2,3). So a great attention is focused on minimizing the intensive amounts of mineral fertilization (4). In this respect, the organic fertilization improved vegetative growth, nutritional status and reduced the residuals of nitrate and nitrite in grape berries and the continuous fertilization with organic fertilizer is hopeful in the long run for grapevine (5,6). Organic fertilization are beneficial for improving the efficiency of nutrients uptake and soil fertility (7). On the other hand, many commercial products containing humic acid (HA), including K-humate (KH) had been promoted for use on various crops (8).

Benefits attributed to the use of humic acid, particularly in low organic matter, alkaline soil, include increased nutrient uptake, tolerance to drought and temperature extreme, activity of beneficial soil microorganisms and availability of soil nutrients (9). Organic materials may also increase root growth in a manner similar to auxins (10, 11).

Hassan *et al.* (12) deliberated the effect of 15 nitrogen fertilization treatment on Thompson seedless grapevines, 18 year old. The best fruiting and leaf characteristics were obtained by the highest rate (100 g N/vine) from the following nitrogen sources urea + AM (Nitrification inhibitor), AN (Ammonium nitrate) and AN + AM. Gabara *et al.* (13) investigated the effect of varying N and Mg application ratios on growth, leaf chemical composition, yield as well as physical and chemical characteristics of Banaty grapes, results showed that there was an marvelous influence on growth characters, leaf N, Mg and K, yield as well as cluster weight, berry weight, TSS and total acidity. George *et al.* (14) investigated the influence of three levels of organic manures (10, 20, 40 T/ ha) of cow and sheep manures, and (5, 10, 20 T/ ha) of poultry manures, in addition to the control, on some qualitative properties of the grapevine's cultivar Al-Baladi. Results indicated that, the use of the low level of poultry manure (5 T/ ha) had the best results in the most of the studied parameters (14). Ferrara *et al.* (15) studied the effects of foliar applications of Humic acids and a compost on vegetative and qualitative parameters of 'Italia' table grape. At harvest, the application of Humic acids showed to have increased total soluble solids, TSS/acidity ratio and pH but decreased titratable acidity. Generally, treatments with Humic acids significantly increased berry size, and as a consequence, a general increase in the yield was observed (15). Abdel- Moneem *et*

al. (16) studied the minimizing of mineral nitrogen fertilization through using Humic acid (HA) on leaf mineral content, yield, fruit quality and the residual P, K, NO₃ and NO₂ in berry juice of Thomson seedless grapevines. Results indicated that Humic acid reduced N content in the leaves especially when existence with bio-fertilizer, whereas there were no differences between the other treatments, While, P and K content were not affected. On the other hand, results did not show any differences between treatments in respect with number of bunches/plant, bunch weight, TSS and acidity percentage compared with the control (100% mineral N) (16).

Therefore, the current study was conducted to evaluating mineral nitrogen, organic and Humic acid treatments on leaf mineral content, yield, fruit quality and the residual minerals in Kamali grapevine.

MATERIALS AND METHODS

This study was carried out during growing seasons 2012 on 12 years old kamali grapevine planted on clay soil under drip irrigation system in a private farm located at Bara-Buhar, Duhok governorate, Kurdistan region, Iraq. The vines were trained with T-trellis system, winter pruning was done at the second week of March, and vine load was 78 buds (7 fruiting canes each with 10 buds and four renewal spars × 2 buds).

Eight treatments were done to evaluate soil application of ammonium sulfate (100 g/vine) fertilization, Organic manure (sheep manure, 6 kg/vine) and humic acid (4 g/vine) as organic fertilization and their interaction. Therefore the treatment as the following:

- 1- T1 = Control.
- 2- T2 = 100 % Ammonium sulfate.
- 3- T3 = 100 % Organic manure.
- 4- T4 = 100 % Humic acid.
- 5- T5 = 50 % Ammonium sulfate + 50 % Organic manure.
- 6- T6 = 50 % Ammonium sulfate + 50 % Humic acid.
- 7- T7 = 50 % Organic manure + 50 % Humic acid.
- 8- T8 = 1/3 % Ammonium sulfate + 1/3 % Organic manure + 1/3 % Humic acid.

Each treatment was replicated three times with two vines per each and the randomized complete block design was arranged.

As for mineral fertilization treatment, 100 g N as ammonium sulfate (20.5% N) was added per each vine and placed 10 cm under the soil surface on both sides of the vine rows (30 cm from the trunk) at two equal doses (two week after bud burst and after berry set), while vines treated with Organic manure treatments received 6 kg was added per each vine and placed 10 cm under the soil surface on both sides of the vine rows (30 cm from the trunk), the O.M was add once at the first week of January. Humic acid was added as 4 g per each in the same way of mineral fertilization at two equal doses (two weeks before and after berry set). All vines under taken in this study received the same

horticultural practices that usually carried out in the vineyard. Data were analyzed by using SAS program (17).

Experimental measurements were as follows:

1. **vegetative characteristics:** Leaf area (cm²), Leaf dry weight (g), Leaf chlorophyll content (%) and leaf petiole mineral content.
2. **Yield characteristics:** Bunch weight (g), Number of bunches per vine and yield per vine (kg).
3. **Chemical characteristics:** Total soluble solid (TSS) %, Total sugars (%), Juice density (D.) and Total acidity (%).

RESULTS AND DISCUSSION

Vegetative growth characteristics:

Results in table (1) clearly showed that the best result obtained from plant received A. sulfate + Organic manure + Humic acid, it was significantly increased single leaf area, leaf dry weight and leaf chlorophyll percentage compared to the most of other treatments. The lowest value was with control. Application of each of the fertilizer alone also caused significant increase compared to the untreated vines.

Leaf mineral content:

Regarding leaf nutrients content, nitrogen, phosphorus and potassium percentage in the leaf-petiole, table (2) indicated that it was significantly affected by the treatments, where the application of Ammonium sulfate + Organic manure + Humic acid increased N content. All treatments were significantly differed with control. As for phosphorus and potassium percentage in the leaf-petiole, same table indicates that the highest value were obtained from the treatment of A. sulfate + Organic manure + Humic acid, which are significantly surpass most of other treatments.

Yield characteristics:

Table (3) showed that Bunch weight and number of clusters/vine was significantly affected by the fertilizer treatments. As for yield (kg)/vine, although there were significant differences between fertilizer treatments, no constant trend between them was detected in the study seasons. However (A. sulfate + Organic manure + Humic acid) recorded the highest value followed by treatment (A. sulfate + Organic manure) and treatment (Organic manure + Humic acid). Application of Organic manure or Humic acid alone also caused a significant increase in the yield characters compared to the un-fertilized.

Chemical characteristics:

Regarding berries chemical characteristics, TSS, total sugar and Juice density, table (4) clearly indicated that it was significantly affected by the fertilizer treatments, where the application of Ammonium sulfate + Organic manure + Humic acid increased TSS, total sugar and Juice density. All treatments were significantly differed with control. As for Total acidity percentage in the berry juice, same table indicates that the highest value obtained from the control so the application of A. sulfate + Organic manure + Humic acid significantly reduced the total acidity in the berry juice.

Application of A. sulfate + Humic acid and Organic manure + Humic acid also caused significant increase in the TSS, Total sugars and Juice density and reduced total acidity percentage.

The significant effect of ammonium sulfate may be due to the role of Nitrogen in the synthesis of protein and enzymes which are an important compounds in the synthesis of chlorophyll and cytochrome and their role in the processes of photosynthesis and respiration that lead to increase cell division and elongation (18-21).

The stimulation of growth aspects in response to application of Humic acid might be ascribed to the positive action of Humic acid in the increase of uptake of macro and microelements influenced by Humic substances which have been shown in different plant species (22). Moreover, Humic fertilizers activated the biochemical processes in plants such as Respiration, Photosynthesis and chlorophyll content (23,24). Furthermore, the growth promoting by Humic substances may be related to plant hormone-like material contained in the Humic substances (25), or may the presence of iron in the Humic acids or their colloidal nature have a positive effect on the growth of various groups of microorganisms which may excrete a range of vitamins, growth substances and antibiotics and these may promote plant growth (26-29).

CONCLUSION

In conclusion, the positive effect of organic manures on the vegetation growth and yield and its physical and chemical characteristics could be attributed to their effects on supplying the vines with their requirements of various nutrients as a relatively long times, as well as their effect on lowering soil pH in Rhizosphere which could aid in facilitating the availability of some nutrients in the soil and improving physical characters of soil in favor of root development (30).

Table (1): Effect of different mineral and organic fertilizers on some vegetative growth characteristics of grape cv. Kamali

Fertilizer's treatment	Vegetative growth characteristics		
	Leaf area (cm ²)	Leaf dry weight (g).	Leaf chlorophyll content (%)
Control	130.60 d	0.576 d	33.267 d
Ammonium sulfate	136.19 cd	0.613 c	39.667 bc
Organic manure	145.01 c	0.652 b	41.51 bc
Humic acid	140.81 c	0.608 c	37.733 cd
A. sulfate + Organic manure	155.13 b	0.655 b	39.84 b
A. sulfate + Humic acid	151.02 b	0.640 b	42.97 b
Organic manure + Humic acid	166.08 ab	0.697 a	43.07 b
A. sulfate + Organic manure + Humic acid	176.25 a	0.720 a	56.84 a

Means with same letter for each factor and interaction are not significantly different at 5% level based on multiple Rang Test

Table (2): Effect different mineral and organic fertilizers on some mineral content of grape cv. Kamali

Treatment	Mineral content		
	N (%)	P (%)	K (%)
Control	0.671 e	0.089 c	1.244 d
Ammonium sulfate	0.784 d	0.102 b	1.351 c
Organic manure	0.956 c	0.103 b	1.315 c
Humic acid	0.883 cd	0.120 ab	1.479 b
A. sulfate + Organic manure	1.058 b	0.138 a	1.489 b
A. sulfate + Humic acid	1.094 b	0.116 b	1.386 bc
Organic manure + Humic acid	1.127ab	0.137 a	1.606 a
A. sulfate + Organic manure + Humic acid	1.191 a	0.151 a	1.705 a

Means with same letter for each factor and interaction are not significantly different at 5% level based on multiple Rang Test

Table (3): Effect different mineral and organic fertilizers on some yield characteristics of grape cv. Kamali

Treatment	Yield characteristics		
	Bunch weight (g).	No. of bunches per vine	Yield per vine (kg).
Control	742.01 c	43.3 d	32.151 c
Ammonium sulfate	749.59 c	46.67 cd	34.983 c
Organic manure	968.76 b	50.33 bc	48.757 b
Humic acid	1004.1 ab	48.67 bc	48.867 b
A. sulfate + Organic manure	1032.4 a	54.00 ab	55.748 a
A. sulfate + Humic acid	958.86 b	52.3 ab	50.177 ab
Organic manure + Humic acid	983.99 ab	53.33 ab	52.476 ab
A. sulfate + Organic manure + Humic acid	1018.5 a	58.67 a	59.755 a

Means with same letter for each factor and interaction are not significantly different at 5% level based on multiple Rang Test

Table (4): Effect different mineral and organic fertilizers on some chemical characteristics of grape cv. Kamali

Treatments	Chemical characteristics			
	TSS (%)	Total sugars (%)	Juice density (D.).	Total acidity (%)
Control	14.96 bc	12.41 f	0.99 d	1.15 a
Ammonium sulfate	14.57 c	13.86 d	1.07 b	1.07 cd
Organic manure	15.35 b	14.75 cd	1.03 cd	1.03 bc
Humic acid	15.52 b	14.96 cd	1.03 cd	1.03 cd
A. sulfate + Organic manure	15.10 bc	15.59 bc	0.99 d	1.06 b
A. sulfate + Humic acid	15.28 b	16.61 b	1.06 b	0.99 d
Organic manure + Humic acid	16.10 a	16.05 b	1.05 bc	0.95 d
A. sulfate + Organic manure + Humic acid	16.08 a	18.46 a	1.15 a	0.99 d

Means with same letter for each factor and interaction are not significantly different at 5% level based on multiple Rang Test

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Post harvest treatment effect on fruits storage characteristics of local apricot cv. Zenjely

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ABSTRACT

The current study was conducted to evaluate the efficacy of package type (MAP), KMnO_4 and storage period in maintaining the post harvest quality of local apricot cv. Zenjely fruits. The fruits were harvested from the orchard of Horticulture Department/ college of agriculture/ University of Duhok, Duhok Governorate – Kurdistan region- Iraq. Fruits at the commercially mature stage were manual harvested carefully to avoid mechanical injury at 20 June 2010. Then fruits were separated randomly to groups according to their treatment to study the effect of [2 package type (perforated and non-perforated), 3 levels KMnO_4 (0, 20 and 30g/ bag) and 2 storage period (2 and 4 weeks)] on weight loss, ascorbic acid (V.C.), total soluble solids, total titratable acid, total sugars, and carotene content. MAP whether in non-perforated polythene bag significantly reduced fruit weight loss %, V.C. and flesh fruit total carotene content but increased total acid%. However, TSS% and total sugar% were not influenced significantly by the type of package. Higher levels of total acidity were observed among the fruits that were treated with 30g KMnO_4 . On other hand, fruits that were treated with KMnO_4 recorded lower level of fruit weight loss, TSS, total sugar and total carotene content. All of these parameters were significantly differed from 0 KMnO_4 , while there were no significant differences between treated and no treated fruits in ascorbic acid. When storage period was prolonged from 2 to 4 weeks, there was a significant effect on cumulative fruit weight loss and total carotene. On other hand, ascorbic acid and total acidity were significantly decreased, nevertheless TSS and total sugar were not impressed significantly when storage time progressed.

Keywords: Apricot, KMnO_4 , package type, Storability, storage period

الملخص باللغة العربية

أجريت الدراسة لتقييم فعالية نوع التعبئة وبرمنغنات البوتاسيوم ومدة التخزين في الحفاظ على نوعية ثمار المشمش المحلي صنف زنجيلي بعد الحصاد. تم جني الثمار من حقول قسم البستنة في كلية الزراعة والغابات - جامعة دهوك - دهوك - إقليم كردستان - العراق. في يوم 20 حزيران / يونيو 2010 تم جني الثمار يدويا بعناية لحمايتها من الأضرار الميكانيكية في مرحلة اكتمال النمو. وتم تقسيم الثمار عشوائيا تبعا لمعاملاتها إلى مجاميع لدراسة تأثير نوعين من التعبئة (عبوات مثقبة وغير مثقبة) و ثلاثة مستويات من KMnO_4 (0, 20 and 30g/ bag) ومدتي تخزين (أسبوعين وأربعة أسابيع) على نسبة فقدان وزن الثمار ونسبة المواد الصلبة الذائبة وفيتامين ج والحموضة الكلية والسكريات الكلية ومحتوى الكاروتين للثمار. وقد أظهرت النتائج أن نسبة فقدان في الوزن و وفيتامين ج ومحتوى لحم الثمار من الكاروتين قد انخفضت وزادت الحموضة الكلية بصورة معنوية في الثمار المعبئة في عبوات بولي إثيلينية غير مثقبة ، ولم تتأثر معنويا نسبة المواد الصلبة الذائبة والسكريات الكلية للثمار بنوع العبوة.

وقد لوحظت أعلى نسبة حموضة في الثمار المعاملة بـ 30g KMnO_4 ، ومن جهة أخرى، سجلت الثمار المعاملة بـ KMnO_4 انخفاضا في نسبة فقدان الوزن ونسبة المواد الصلبة الذائبة والسكريات الكلية والمحتوى الكاروتيني والانخفاض في هذه الصفات كان معنويا بالمقارنة مع الثمار غير المعاملة، ولم تلاحظ أي فروقات معنوية في فيتامين ج بين الثمار المعاملة وغير المعاملة. وقد ازدادت نسبة فقدان الوزن والمحتوى الكاروتيني بصورة معنوية عند إطالة مدة التخزين من أسبوعين إلى أربعة أسابيع، ولكن الحموضة الكلية وفيتامين ج انخفضت معنويا عند تقادم مدة تخزين الثمار، في حين لم تتأثر نسبة المواد الصلبة الذائبة والسكريات الكلية بإطالة مدة التخزين بصورة معنوية.

INTRODUCTION

Apricot (*Prunus armeniaca* L.) belongs to *Rosaceae* family, one of stone fruit (1). The reason of consider is that apricot fruit is one of the most important horticultural productions in preserving human health, because it contains carotenoids, antioxidant compounds such as phenols, vitamins, minerals, fibers, carbohydrates, and other bioactive compounds (2). Apricot fruit is not only consumed in fresh, frozen or dried forms, but also can be used for manufacturing pulp, juice, nectar, marmalade and jelly (3). Apricot fruits are considered as climacteric fruits. Climacteric is the name of a stage in the fruit ripening process, when respiration ascends and produce ethylene that causes change in color, flavor, and softening during ripening (4). Apricot fruit is characterized as a perishable fruit having short-life in storage. This property of fruit may be due to the smallness period between the commercial ripening and degradation processes like senescence (5).

The ration of refrigeration and control RH % is one of the most important methods used to delay the process of fruit senescence, but it will be more efficiency if it is combined with other techniques such as type of packaging and ethylene absorbent such as potassium permanganate to prolong post harvest life of fruit (6). In the current study, the fruits of apricot was characterized as sensitive to deterioration. The benefits of modified atmosphere package and potassium permanganate (KMnO₄) are a quite versatile, uncomplicated, and low price. In addition, these techniques can be applied to different types of fruits and vegetables (7).

Ethylene is synthesized by fruit during ripening and motive flesh softening, limiting long-term of cold storage, therefore, the oxidation of ethylene by KMnO₄ could extend the post harvest life of fruit (6). Water and CO₂ are formed from oxidation of ethylene by KMnO₄ (8). It was reported that KMnO₄ applications could delay fruit softening and increase post harvest life (9). Consequently, Potassium permanganate (KMnO₄) is the most effective system for absorbing ethylene, which produces ethylene oxide, acetate, and ethanol. During this process, the color will change from purple to brown and it is used to determine the capacity of absorbing the residual ethylene (10).

The application of potassium permanganate at ratios of 2 and 4 g /1 kg fruit, and fruit packed with non – perforated could result in lowering weight loss and decay percent compared with control treatment in bags with air hole (11). A study was conducted by (12), who reported that applying potassium permanganate alone resulted in restrain the weight loss, decrease in both titratable acidity (TA) and total soluble solids (TSS), enhancement of storability of apricot during storage (12). Another study conducted by (13) reported that, fruits treated with 10 g/kg KMnO₄ had the highest acidity in flesh and lower soluble solids after 7 days.

Fruit shelf life protraction is an important target to attain food sufficiency, such as storage techniques that were developed to prolong the marketing period and saving products after harvest. Different keeping methodologies were studied. One method of expanding post harvest life of fruits is coating (14),

as modified atmosphere packaging (MAP), which is a simple post harvest technology combined with low temperature storage to preserve freshness and safety of lowest processed fruit and vegetable products (15). MAP has two profitable technologies to prolong the shelf life of fresh agricultural and horticultural products. Simply stated, these technologies include storing a fruit or vegetable in a MAP usually consisting of reduced O₂, while elevating CO₂ concentrations compared to air. The modified atmosphere package causes decrease in respiration rates and production of ethylene and its action, which are often correlated with the usefulness of delaying physiological, pathological, and physical deteriorative processes occurred in the product (16) and slowing senescence and decay phenomena in product (17). After fresh fruit and vegetables are harvested, they continue its respiration. The respiration has an important role in protection the packed product fresh for long time, this already due to that respiration uses O₂ and produces CO₂, so generate decrease in respiration rate without lesion the quality of the product, through creating low level of O₂ and high level of CO₂ in the packaging atmosphere (18).

Physical, chemical and physiological mechanisms in fruits are either positively or negatively influenced by modified atmosphere (19). Some of the profitable effects of MAP include slowing action of ripening by retarding the synthesis of ethylene, lowering transpiration, delay of biochemical activities. All those effects are associated with ripening and increasing resistance to the aggression of post harvest pathogens (20).

Weight loss is one of the major problems in harvested fruit leading to loss of marketable weight and shrinkage, thereby affecting marketing ability and quality of the fruits. Water loss in harvested products is also correlated with loss of water-soluble vitamins including vitamin C (19). The MAP carries out satiate condition of water vapor pressure through reducing water loss of the tissues and the resultant weight loss (21). Conditions in MAP interpose with ethylene production by inhibiting activity of 1-Aminocyclopropane-1- carboxylic acid (ACC) oxidase, the enzyme that catalysis the alteration of ACC to ethylene (22).

Due to the lack of research about the effect of (package type and KMnO₄) on the post harvest quality of local apricot fruit cv. Zenjely during storage cultivated in Kurdistan region conditions - Iraq, the current study was conducted for the first time to find out the response of local apricot fruits cv. Zenjely to these two factors and to compare the effect of package type (polythene bags), 3 levels of KMnO₄ as post harvest treatments and storage period on post harvest characteristics of the Zenjely apricot fruits to prolong storage of fruit with best quality.

MATERIALS AND METHODS

Fruits Source of apricot (*Prunus armeniaca* L. cv. Zenjely) was from the orchard of Horticulture department/ college of agriculture / University of Duhok, Duhok Governorate – Kurdistan region-Iraq. Fruits at the commercially mature stage were manual harvested carefully to avoid mechanical injury in fruits at 20 June 2010. The fruits were

transported safe into the central laboratory of the college. Healthy fruits on the basis of size, color and absence of external injuries were put in cold room to perform pre cooling. On the next day, fruits were removed from the storage, then divided to groups according to the treatments.

Treatments:

1. Package: Perforated polyethylene bags and non-perforated polyethylene bags.
2. Storage period: 2 to 4 weeks.
3. KMnO_4 g / package (0, 20 g and 30 g).

After applying treatments, fruits were stored at 0 °C and 85-90 % relative humidity, then after each storage period, fruit characteristics were measured immediately after moving fruit from cold storage.

Measurements:

Fruit weight loss (%) was determined according to (23). On the other hand, Vitamin C ($\text{mg. } 100\text{ml}^{-1}$ juice), total soluble solid (%), Titratable acidity (%), total sugar (%) and total carotene (mg.cm^2 solution) were determined by using recommended method of A.O.A.C (24). Juice was extracted by juice extractor from 30 fruits, which were randomly taken from each treatment for analysis of every storage period.

Statistical analysis:

Data was statistically analyzed for analysis of variance in three-factor (2 packages*, 2 storage periods, and *3 levels of KMnO_4). Each treatment was divided to 4 replicates, each replicate was consisted of three polyethylene bags for studying physico - chemical properties. In addition, three replicates from each treatment were separated for weight loss and physiological injury. The experiment was laid out as factorial in Complete Randomized Design (CRD). Duncan's test at the significance level > 0.05 was used (25).

RESULTS

Fruit weight loss (%):

Results showed that fruit package in non-perforated polyethylene bags gave the lowest weight loss significantly as compared with fruit package in perforated polyethylene bags. While fruit weight loss was significantly increased with prolonged the storage period from (2- 4) weeks. Also fruit package with higher level of KMnO_4 (30g) had significantly lowest weight loss compared to 20g and 0g KMnO_4 . Regarding the interaction of package and storage period, results showed that the lowest fruit weight loss was recorded from interaction treatment of non-

perforated polyethylene bags and 2 weeks of storage. The minimum fruit weight loss was obtained from combination of treatment of perforated polyethylene bags and 30g KMnO_4 which significantly differed from all other interactions. Lowest fruit weight loss was obtained from the non-perforated polyethylene bags, 2 weeks of storage and 20g KMnO_4 (table 1).

Vitamin C ($\text{mg. } 100\text{ml}^{-1}$ juice):

Results in table (2) revealed that fruit vitamin C content of perforated polyethylene bags was significantly superior in fruit vitamin C content than non-perforated polyethylene bags. Fruit vitamin C content was significantly decreased with increasing storage period. The recorded data showed that there was no significant effect of KMnO_4 level on fruit vitamin C content. The highest vitamin C content was obtained from the interaction between perforated polyethylene bags and 2 weeks of storage. The interaction between perforate bags and 30g KMnO_4 appeared to be the most operative treatment, as it gave the highest fruit vitamin C content. The maximum fruit vitamin C. was obtained between 2 weeks of storage and 30g KMnO_4 . Regarding the interaction of the three studied factors, the highest fruit vitamin C was obtained from interaction between perforated bags, 2 weeks of storage, and 30g KMnO_4 .

Fruit Total Soluble Solids (TSS %):

Results in table (3) indicated that type of package and storage period had no significant effect on fruit TSS. The fruit TSS decreased significantly in fruit treated with the two levels of KMnO_4 compared to the control, but there were no significant difference between the two levels of KMnO_4 . Regarding the interaction between packaging and storage period, no significant effect was observed in fruit TSS. The interaction between perforated polyethylene bags and 0g KMnO_4 had significantly higher TSS. In addition, the results indicated that 4 weeks of storage and 0g KMnO_4 interaction had a significant effect on fruit TSS compared to the all other interactions between storage period and KMnO_4 levels. It was clear that TSS of fruit in the interaction between perforated polyethylene bags, 2 or 4 weeks of storage and 0g KMnO_4 was higher than fruit TSS in the all interactions of the three factors.

Titrateable acidity (TA%):

The results showed that fruit TA in the non-perforated polyethylene bags was significantly higher than fruit packaged in perforated polyethylene bags. Tabulated data declared that fruits stored 2 weeks had significantly decreased the reduction of fruit TA in comparison with 4 weeks. While TA of fruit was significantly increased with increase in the level of KMnO_4 from (0, 20g to 30g). Results indicated that, the better TA was obtained between the interaction of non- perforated polyethylene bags and 2 weeks of storage, which was significantly higher than other interactions between package and storage period. In addition, fruit packaged in perforated bags and 30g KMnO_4 were superior significantly from other interactions in TA. On the other hand, the interaction between 4 weeks of storage and 30g KMnO_4 preserved TA more than other interactions. Whereas the interaction treatment of non-perforated bags, 2 weeks of storage and (20g and 30g) KMnO_4 had significantly gave the highest TA than other interactions (table 4).

Table (1): Effect of package, post harvest treatment, storage period and their interactions on fruit weight loss (%) of apricot fruit cv. Zenjely stored at (0±1C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	7.92 ab	8.63 ab	9.72 a	8.755 a	8.19 a
	4	7.22 b	7.95 ab	7.75 ab	7.638 ab	
Non-perforated	2	8.28 ab	6.87 b	7.88 ab	7.677 ab	7.37 b
	4	7.05 b	6.97 b	7.22 b	7.077 b	
Mean effect of KMnO4 (g)		7.616 a	7.604 a	8.141 a	Storage period	
Package × storage	Perf.	8.10 ab	7.75 ab	8.80 a		
	Non- Perf.	7.13 b	7.45 ab	7.48 ab		
Treat. × storage	2	7.56 ab	8.29 ab	8.73 a	8.21 a	
	4	7.66 ab	6.91 b	7.55 ab	7.35 b	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

Table (2): Effect of package, postharvest treatment, storage period and their interactions on fruit vitamin C (mg. 100ml⁻¹ juice) of apricot fruit cv. Zenjely stored at (0 ± 1 C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	8.837 b	6.760 c	4.320 d	6.639 b	7.893 a
	4	12.347 a	8.303 b	6.790 c	9.147 a	
Non-perforated	2	1.417 e	0.443 e	0.833 e	0.898 c	1.020 b
	4	1.457 e	0.747 e	1.223 e	1.142 c	
Mean effect of KMnO ₄ (g)		6.014 a	4.063 b	3.292 c	store period	
Package × storage	Perf.	5.127 b	3.602 c	2.577 d		
	Non- Perf.	6.902 a	4.525 bc	4.007 c		
Treat. × storage	2	10.592 a	7.530 b	5.551 c	3.768 b	
	4	1.431 d	0.595 d	1.028 d	5.144 a	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

Table (3): Effect of package, postharvest treatment, storage period and their interactions on fruit total soluble solid (TSS %) of apricot fruit cv. Zenjely stored at (0 ± 1 C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	16.66 ab	15.00 bcd	14.33 def	15.333 a	15.250 a
	4	15.66 bc	15.50 bcd	14.33 def	15.167 a	
Non-perforated	2	17.000 a	13.667 f	14.00 ef	14.889 a	15.167 a
	4	17.000 a	13.667 f	15.667 bc	15.444 a	
Mean effect of KMnO ₄ (g)		16.58 a	14.45 b	14.58 b	store period	
Package × storage	Perf.	16.83 a	14.33 b	14.16 b		
	Non- Perf.	16.33 a	14.58	15.00 b		
Treat. × storage	2	16.16 b	15.250c	14.33 de	15.11 a	
	4	17.00 a	13.66 e	14.83 cd	15.30 a	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

Table (4): Effect of package, postharvest treatment, storage period and their interactions on fruit tetatable acidity (AT %) of apricot fruit cv. Zenjely stored at (0 ± 1 C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	0.616 d	0.713 bc	0.773 b	0.701 b	0.663 b
	4	0.513 e	0.633 d	0.733 bc	0.626 c	
Non-perforated	2	0.736 bc	0.87 a	0.936 a	0.847 a	0.786 a
	4	0.673 cd	0.746 bc	0.756 b	0.725 b	
Mean effect of KMnO4 (g)		0.635 c	0.740 b	0.800 a	store period	
Package × storage	Perf.	0.676 c	0.791 b	0.855 a		
	Non- Perf.	0.593 d	0.69 c	0.745 b		
Treat. × storage	2	0.565 d	0.673 c	0.753 b	0.774 a	
	4	0.705 bc	0.808 a	0.846 a	0.676 b	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

Total sugar (%):

It's obvious from table (5) that there were no significant effects of package and storage period on total sugar (%) of fruit, whereas KMnO₄ treatment in both level caused a significant effect in total sugar % and gave the lowest value of sugar. Results exposed that there were no significant interaction between package (perforate or non-perforate bag) and storage period (2 week or 4 week) on fruit total sugar. Results show that the highest fruit total sugar was obtained as a result of the interaction between

non - perforated bags and control (0g KMnO₄). On other hand the highest fruit total sugar was obtained as a result of the interaction between 0g KMnO₄ and 4 week storage which was significantly higher than all other interaction between the two factors. Substantially, the results displays that the interaction between non- perforated bags, 2 or 4 week storage and control (0g KMnO₄) gave the maximum value of fruit total sugar which were different significantly from most other interactions between the three factors under study.

Table (5): Effect of package, postharvest treatment, storage period and their interactions on fruit total sugar (%) of apricot fruit cv. Zenjely stored at (0 ± 1 C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	14.45 ab	12.90 cde	12.28 def	13.21 a	13.13 a
	4	13.52 bc	13.36 bcd	12.28 def	13.05 a	
Non-perforated	2	14.76 a	11.66 f	11.97 ef	12.79 a	13.05 a
	4	14.76 a	11.66 f	13.52 bc	13.31 a	
Mean effect of KMnO4 (g)		14.37 a	12.39 b	12.51 b	store period	
Package × storage	Perf.	14.605 a	12.28 b	12.125 b		
	Non- Perf.	14.14 a	12.511 b	12.900 b		
Treat. × storage	2	13.985 b	13.131 c	12.28 e	13.003 a	
	4	14.76 a	11.66 de	12.745 cd	13.183 a	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

Total carotene (mg.cm² solution):

Results in table (6) revealed that total carotene was affected significantly by package, so fruit packed in perforated polyethylene bags gave the highest value of fruit total carotene compared with fruit of non-perforated bag. The same table showed that the increase of storage period was affected significantly in fruit total carotene, where the maximum total carotene was recorded at 4 weeks of storage. It is clear from the same table that apricot fruit treated with 30g KMnO₄ showed significantly decrease in

fruit total carotene compared to fruit treated with 0 or 20 g KMnO₄. The results indicated that the combination between perforated polyethylene bags and 4 weeks of storage gave the highest value of fruit total carotene. Whereas the interaction between perforated polyethylene bags and 0g KMnO₄ was the most effective treatment as it gave the highest value of fruit total carotene. The combination between 0g KMnO₄ and 2 weeks of storage appeared to be the most operative treatment, as it gave the highest fruit total carotene. The highest fruit total carotene was obtained from the interaction of perforated bags, 0g KMnO₄ and 4 weeks of storage, while the lowest fruit total carotene was

showed at the interaction between non-perforated bags, 20 or 30g KMnO₄ and 2 weeks of storage (table 6).

Table (6): Effect of package, postharvest treatment, storage period and their interactions on fruit total carotene (TSS %) of apricot fruit cv. Zenjely stored at (0 ± 1 C)

Package type	Storage period (week)	Treatment			Package × storage	Package
		KMnO ₄ (g)				
		0	20	30		
Perforated	2	1.59 ab	1.60 ab	1.52 bc	1.57 b	1.60 a
	4	1.68 a	1.66 a	1.56 bc	1.63 a	
Non-perforated	2	1.53 bc	1.47 c	1.47 c	1.49 c	1.50 b
	4	1.55 bc	1.53 bc	1.46 c	1.52 c	
Mean effect of KMnO4 (g)		1.59 a	1.56 a	1.51 b	store period	
Package × storage	Perf.	1.57 abc	1.57 bc	1.50 c		
	Non- Perf.	1.61 a	1.59 ab	1.52 c		
Treat. × storage	2	1.64 a	1.63 a	1.54 b	13.00 a	
	4	1.54 bc	1.50 bc	1.48 c	13.18 a	

Means of each factor and their interactions followed by the same letters are not significantly different from each other according to Duncan's multiple ranges test at 5% level

The loss of weight is one of the most important factors responsible for producing quality deterioration. Transpiration and respiration had induced weight loss in harvested fruits, which are still persistent living and the processes were normal (26). The decrease in gas and water penetration of the polyethylene bags being used in MAP decrease the rate of water loss through transpiration (27). Because the inner atmosphere package becomes filled with water vapor pressure, under this conditions transpiration by the commodity stored under MAP leading to reduced weight losses as notice in this study. In addition, reduced respiration is one of the initial metabolic responses to low O₂. Minimization in respiration in hinders the breakdown in stored energy. Therefore fruit weight loss packaged in non- perforated polyethylene bags decreased in the present study. Packaged fruits by non- perforated significantly protect its total acidity (TA) compared with packaged fruits by perforated bags. Low level of O₂ in MAP associated with lower respiration rate could clarify why non-perforated packaged fruits remained higher TA during the storage. Similar results were showed by (28) on strawberry packed at low level of O₂. MAP significantly delayed carotene changes in flesh of fruit, with the packaged in non- perforated bags during storage. Other studies showed that color changes associated with the ripening process were generally delayed under MAP conditions. This had been previously reported in fruits such as mango (29). MAP affects carotene changes, which were shown in the present study, could be due to the low O₂ conditions that may have resulted in decrease ethylene biosynthesis. Ethylene is known to generate the activities of enzymes including color changes through ripening. These contain oxidase enzyme that causes degradation of chlorophyll and carotene appearance (30). In addition, fruit package by MAP produces required condition to inhibit biosynthesis activities of metabolic processes of carotenoids (31).

The lowest weight loss was recorded in the fruit treated with KMnO₄. Reason of this result might be due to the absorption of ethylene by KMnO₄, as

known that ethylene plays a role in increasing the respiration process, which increases fruit weight loss and a rise in temperature that in turn increases water loss from fruits. A higher content of soluble solids (TSS) and total sugar were observed in the fruit without KMnO₄; imply to accelerated ripening processes, while in treated fruit, KMnO₄ significantly showed decrease of the total soluble solids content in fruits. These results are in line to the result obtained by (9).

The absorption of ethylene by KMnO₄ performs to prevention of enzyme activity, then starch dose can not be converted into sugar and TSS content remains in low level (32). The lower amount of TSS in response of potassium permanganate was reported in apricot (33). TSS lower level by KMnO₄ could be due to decrease in the rate of respiration and processes of ripening (34). The lowest color change had appeared in 30 g/ bag KMnO₄. Thus, it means that peel color change is influenced by KMnO₄. Apricot fruit is one of climacteric fruits that produce ethylene during maturity, which causes protein synthesis and promotes chlorophyllase enzyme. The efficiency of chlorophyllase befalls in chlorophyll analysis and peel color is converted from green to yellow. Therefore, KMnO₄ absorbs ethylene, which is produced by fruits and retards the activity of chlorophyllase, which has an important role in degradation of chlorophyll. Results of the present study were in harmonious with (35), who reported that fruits of pear treated with potassium permanganate had more green peel color compared with the untreated fruits.

CONCLUSION

Packaging apricot fruit in non-perforated polyethylene bags after harvest at commercial maturity stage was effective in delaying most of the ripening related changes in apricot fruits in the term of weight loss, V.C., total acidity and total carotene during storage. In addition, fruits treated with KMnO₄ maintained quality of the fruits better than control through conversion processes occurring in fruit with advancing fruit in storage.

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Effect of cadmium in cord blood on intrauterine growth in smoker pregnant women

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ABSTRACT

The current study was performed to assess the effect of smoking during pregnancy on cord blood concentration of cadmium. The dose of smoking was referred to as active Vs passive smoking environment, also to assess the effect of smoking and its dose during pregnancy on selected foetal growth indices (nutritional anthropometric indices measured at birth, namely: Height for age z score, weight for height z score, weight for age z score and head circumference for age z score) as well as to assess the magnitude of effect for cadmium on foetal growth indices.

A cross sectional study was conducted from July 2013 till June 2014. Sample of 80 mothers and their newly born babies were selected through convenient sampling technique taken from pregnant women divided to three groups: 30 non-exposed non-smoker pregnant (control group), 30 exposed non-smoker pregnant (passive smoker group) and 20 active smoker pregnant group, their age ranged between (18- 45years). Cord blood were tested for cadmium by using of (Flameless Atomic Absorption Spectrophotometer).

Results showed that the mean cord blood cd was highest in active (current smoker) group (0.306 ± 0.031) and lowest in the no smoking environment group (0.167 ± 0.016), there was a statistically significant strong positive (direct) linear correlation between blood Cd and dose of smoking. There was no obvious or statistically significant difference in mean HAZ between the three study groups. On the other hand, there was statistically significant difference in mean WAZ between the three study groups, also, there was statistically significant difference in mean WHZ between the three study groups. In present study, there was no obvious or statistically significant difference in mean head circumference between the three study groups.

Keywords: cadmium, cord blood, smoking, pregnant, weight for age Z-Score (WAZ), Height for age Z-score (HAZ), Head circumference for age Z-score (HAZ), Weight for Height Z-score (WHZ)

الملخص باللغة العربية

سعت الدراسة الحالية لتقييم تأثير التدخين خلال الحمل على تركيز الكاديوم في دم الحبل السري . حيث تشير جرعة التدخين إلى بيئة التدخين الفعال و السلبي، وأيضا لتقييم تأثير التدخين وجرعته لها أثناء الحمل على مؤشرات النمو المنتقاة) قياس مؤشرات الجسم البشري الغذائية عند الولادة، وهما: الارتفاع بالنسبة للعمر، الوزن بالنسبة للطول، الوزن بالنسبة للعمر ودرجة محيط الرأس بالنسبة للعمر (وكذلك لتقييم حجم تأثير الكاديوم على مؤشرات النمو للجنين).

وقد أظهرت النتائج أن متوسط كاديوم دم الحبل السري كان أعلى في (المدخن الحالي) المجموعة الفعلية (0.306 ± 0.031) والأدنى في المجموعة ليست بيئة التدخين (0.167 ± 0.016)، كما كان هناك إيجابية قوية ذات دلالة إحصائية (مباشرة) الارتباط الخطي بين الكاديوم في الدم وجرعة التدخين، في حين لم يكن هناك اختلاف واضح أو ذو دلالة إحصائية في متوسط HAZ بين مجموعات الدراسة الثلاثة. من ناحية أخرى، كان هناك فروق دالة إحصائية في متوسط WAZ بين مجموعات الدراسة الثلاثة، أيضا، كان هناك فروق دالة إحصائية في متوسط WHZ بين مجموعات الدراسة الثلاثة. في حين لم يكن هناك اختلاف واضح أو ذو دلالة إحصائية في متوسط محيط الرأس بين مجموعات الدراسة الثلاثة، وبذلك فإن نتائج الدراسة تخلص إلى تأثير بيئة التدخين على المؤشرات الغذائية التي وضحت عن طريق الوزن بالنسبة للعمر، الوزن بالنسبة للطول ومحيط الرأس للعمر بالنسبة للنمط القياسي يفسره تأثير الكاديوم على مؤشرات الجنين.

INTRODUCTION

Tobacco use is a global public health problem that leads to over 5 million deaths per year and shortens life expectancy by an average of 15 years (1). The exposure to environmental tobacco smoke (ETS), also known as passive smoke, secondhand smoke or involuntary smoke, has been an important public health hazard (2). Environmental tobacco smoke (ETS) is a mixture of the smoke given off by the burning end of tobacco products (side stream smoke) and the smoke exhaled by smokers (mainstream smoker (3). Maternal smoking during pregnancy has long considered as an important risk factor for intrauterine growth retardation (4). A remarkably constant 100-300g differences in baby birth weight between cigarette smokers' and nonsmokers' has been determined in many studies since 1957 (5). There is evidence that other growth measures, such as length and head circumference, are also reduced in infants of smoker (6). Cadmium Intake From Cigarette Smoking - Smokers absorb amounts of cadmium comparable to those from food, about 1 to 3 µg of cadmium per day, from the smoking of cigarettes. Direct measurement of cadmium levels in body tissues confirms that smoking roughly doubles cadmium body burden in comparison to not smoking. It has been reported that one cigarette contains about 1 - 2 µg of cadmium and that about 10% of the cadmium content is inhaled when the cigarette is smoked (7,8). The national geometric mean blood cadmium level for adults is 0.376 µg/L. Mean blood cadmium levels for heavy smokers had been reported as high as 1.58µg/L (9). Cd accumulates in human placenta, but the placenta is not a complete barrier, and Cd concentrations in cord blood increase with maternal exposure (10). There is increasing evidence of associations between maternal Cd exposure and adverse pregnancy outcomes, such as reduced size at birth (11-14) and preterm delivery (15, 16).

PATIENTS AND METHODS

Between July 2013 and June 2014, a prospective study was conducted, which included 90 mothers and their newly born babies selected through convenient sampling technique taken from pregnant women attending the hospital for delivery in the delivery room of Al-Emamian AL-Kademian medical city, Baghdad, Iraq. The study population included 20 active smoker pregnant women, 30 passive smoker pregnant women and 30 control pregnant women with range 18 – 45 years. Inclusion criteria for selection included woman who smoke at least 10 cigarettes per day, as well as women exposed to ETS from her husband or relatives at the same home or from work. Exclusion criteria included all mothers exposed to other types of smoke as Argil and pipe. Mothers with hypertension or diabetes were also excluded. Plan of work included measuring the weight, length and head circumference of the baby in labor ward, Blood samples were taken from the cord at the time of delivery for measurement of cadmium levels. Chemical analysis of cadmium was carried out at the poisoning Consultation center / Specialized Surgeries Hospital by using of flameless atomic absorption spectrophotometer.

Statistical analysis:

Statistical analysis was performed by using SPSS version 17 Microsoft excel 2010. For all tests p-value < 0.05 was considered statistically significant and 0.001 was considered highly significant.

RESULTS

The results presented in this study were based on the analysis of three groups of pregnant women classified based on the smoking environment. The no smoking environment was considered control group, the passive smoking environment and finally the active (current smokers) smoking environment, which is associated with the highest exposure to smoking chemicals. As shown in table (1), the mean age for active (current smoker mothers) was significantly higher (35.2 years) than both passive smoking and non-smoking environment (23.7 and 24.8 years) respectively. Age qualifies as a confounder in the study.

Table (1): The difference in mean age among 3 studied groups

Mother age	Smoking environment			P ANOVA
	No smoking environment	Passive smoking	Active (current smoker)	
Range	(18- 36) years	(18- 35) years	(26- 42) years	< 0.001
Mean	24.8	23.7	35.2	
SD	4.19	4.44	3.76	
SE	0.77	0.81	0.84	
N	30	30	20	

P (Bonferroni t-test) for difference in mean between

Passive smoking x No smoking environment = 0.85[NS]

Active (current smoker) x No smoking environment <0.001

Active (current smoker) x Passive smoking <0.001

As shown in table (2) and figure (1), the mean cord blood cd was highest in active (current smoker) group (0.306 µg/dl ± 0.031) and lowest in the no smoking environment group (0.167 µg/dl ± 0.016). There was a statistically significant difference in mean blood cd between all possible period combinations of groups and a statistically significant very strong positive (direct) linear correlation between blood cd and dose of smoking. The weight, body length and head circumference of the newborns were measured and then compared to age and gender matched standard population reported (17).

Table (2): The differences in mean blood cadmium concentrations among 3 studied groups

Blood Cd conc. (µg/dl)	Smoking environment			P ANOVA
	No smokng environment	Passive smoking	Active (current smoker)	
Range	(0.131-0.194)	(0.189- 0.216)	(0.252- 0.362)	< 0.001
Mean	0.167	0.206	0.306	
SD	0.016	0.007	0.031	
SE	0.003	0.0013	0.0068	
N	30	30	20	

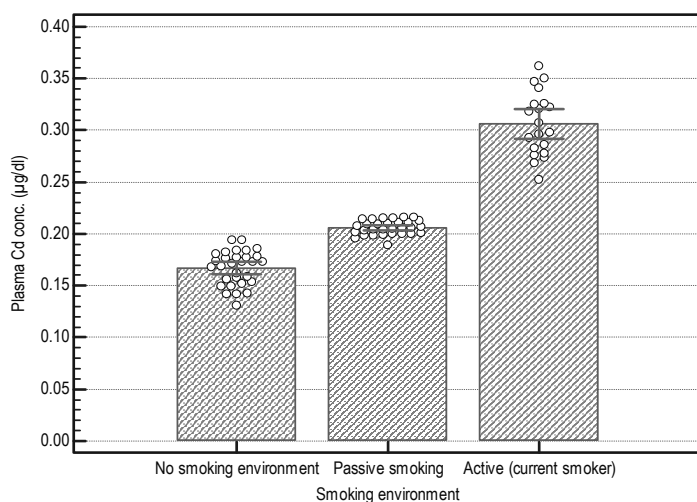
$r=0.936$, $P<0.001$

P (Bonferroni t-test) for difference in mean between

Passive smoking x No smoking environment = <0.001

Active (current smoker) x No smoking environment <0.001

Active (current smoker) x Passive smoking <0.001

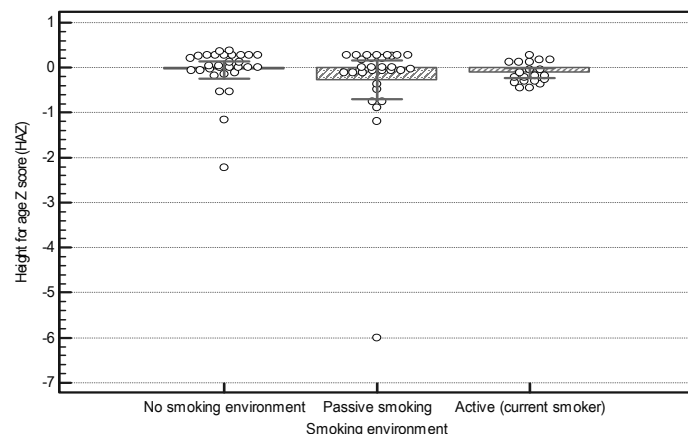
**Figure (1): Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) blood Cd conc. among 3 studied groups**

As shown in table (3) and figure (2), there was no obvious or statistically significant difference in mean of HAZ between the three studied groups. The dose of smoking showed a statistically significant weak negative (inverse) linear correlation with HAZ.

Table (3): The differences in mean height for age Z score among 3 studied groups

height for age Z score (HAZ)	Smoking environment			P ANOVA
	No smokng environment	Passive smoking	Active (current smoker)	
Range	(-2.22 - 0.38)	(-6.01 - 0.28)	(-0.45- 0.28)	0.53[NS]
Mean	- 0.1	- 0.3	- 0.1	
SD	0.52	1.15	0.23	
SE	0.09	0.21	0.05	
N	30	30	20	

$r=-0.253$, $P=0.024$

**Figure (2): Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) height for age Z score among 3 studied groups**

Results in table (4) and figure (3) showed that there were statistically significant differences in mean of WAZ among the three studied groups.

Table (4): The difference in mean weight for age z score among 3 studied groups

weight for age z score (WAZ)	Smoking environment			P ANOVA
	No smokng environment	Passive smoking	Active (current smoker)	
Range	(- 2.51- 2.94)	(- 2.21- -0.59)	(- 2.02- -1.71)	< 0.001
Mean	- 0.8	- 1.2	- 1.9	
SD	0.86	0.47	0.08	
SE	0.16	0.09	0.02	
N	30	30	20	

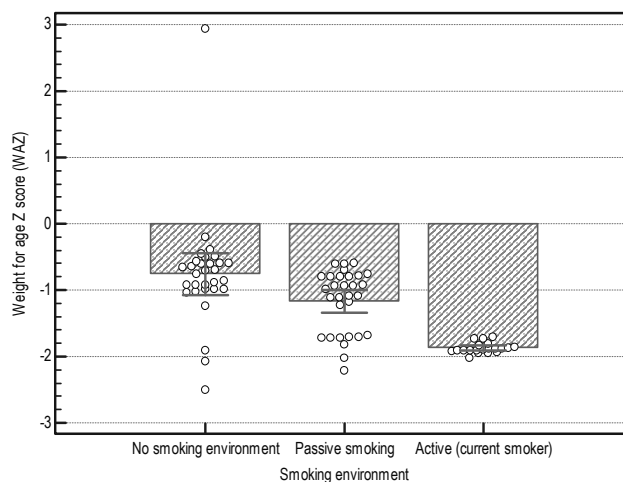
$r=-0.643$, $P<0.001$

P (Bonferroni t-test) for difference in mean between

Passive smoking x No smoking environment = 0.03

Active (current smoker) x No smoking environment <0.001

Active (current smoker) x Passive smoking <0.001

**Figure (3): Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) weight for age Z score among 3 studied groups**

Results in table (5) and figure (4) indicated that there were statistically significant differences in mean WHZ among the three studied groups.

Table (5): The differences in mean weight for height Z score among 3 studied groups

weight for height Z score (WHZ)	Smoking environment			P ANOVA
	No smoking environment	Passive smoking	Active (current smoker)	
Range	(- 1.38 – 2.74)	(-2.59 – 0.00)	(-2.74- 2.24)	< 0.001
Mean	- 0.6	- 1.2	- 2.5	
SD	0.78	0.51	0.16	
SE	0.14	0.09	0.04	
N	30	30	20	

$r = -0.766$, $P < 0.001$

P (Bonferroni t-test) for difference in mean between

Passive smoking x No smoking environment = 0.002

Active (current smoker) x No smoking environment <0.001

Active (current smoker) x Passive smoking <0.001

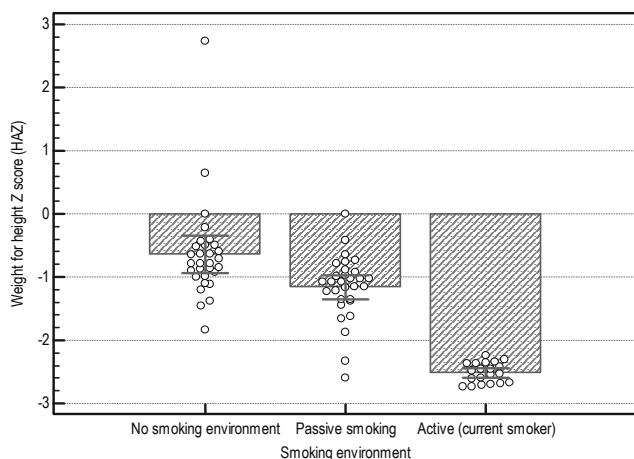


Figure (4): Dot diagram with error bars showing the difference in mean (with its 95% confidence interval) weight for height Z score among 3 studied groups.

As shown in table (6) and figure (5) there was no obvious or statistically significant difference in mean of head circumference among the three studied groups. The dose of smoking showed a statistically significant weak negative (inverse) linear correlation with head circumference.

Table (6): The differences in mean of head circumference for age Z score among 3 studied groups

weight head circumference Z score	Smoking environment			P ANOVA
	No smoking environment	Passive smoking	Active (current smoker)	
Range	(- 1.47 – 1.5)	(-1.47 – 0.24)	(-0.89- 0.18)	0.009
Mean	0.0	- 0.3	- 0.5	
SD	0.64	0.43	0.36	
SE	0.12	0.08	0.08	
N	30	30	20	

$r = -0.38$, $P < 0.001$

P (Bonferroni t-test) for difference in mean between

Passive smoking x No smoking environment = 0.22[NS]

Active (current smoker) x No smoking environment = 0.008

Active (current smoker) x Passive smoking = 0.42[NS]

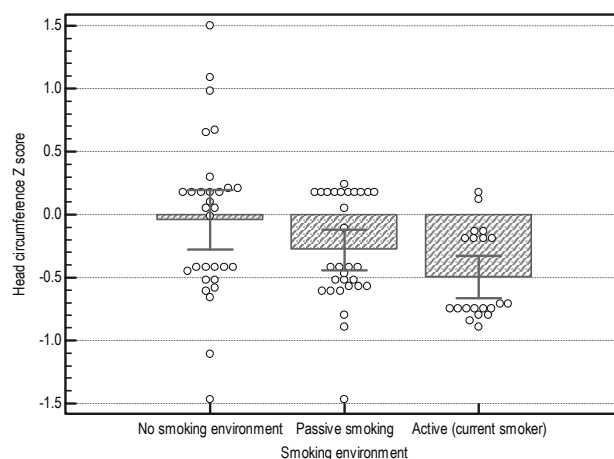


Figure (5): Dot diagram with error bars showing the differences in mean (with its 95% confidence interval) head circumference for age z score among 3 studied groups

The associations between cord blood cadmium and nutritional indices in three study groups are shown in tables (7, 8 and 9).

Table (7): Linear correlation coefficient for No smoking environment group.

	Height for age Z score (HAZ)	Weight for age Z score (WAZ)	Weight for height Z score (WHZ)	Head circumference Z score
Blood Cd conc. (µg/dl)	$r = -0.292$ $P = 0.12$ [NS]	$r = -0.526$ $P = 0.003$	$r = -0.496$ $P = 0.005$	$r = -0.516$ $P = 0.004$

Table (8): Linear correlation coefficient for Passive smoking group

	Height for age Z score (HAZ)	Weight for age Z score (WAZ)	Weight for height Z score (WHZ)	Head circumference Z score
Blood Cd conc. (µg/dl)	$r = -0.167$ $P = 0.38$ [NS]	$r = -0.741$ $P < 0.001$	$r = -0.553$ $P = 0.002$	$r = -0.795$ $P < 0.001$

Table (9): Linear correlation coefficient for Active (current smoker) group

	Height for age Z score (HAZ)	Weight for age Z score (WAZ)	Weight for height Z score (WHZ)	Head circumference Z score
Blood Cd conc. (µg/dl)	$r = -0.893$ $P < 0.001$	$r = -0.67$ $P = 0.001$	$r = 0.11$ $P = 0.64$ [NS]	$r = -0.893$ $P < 0.001$

DISCUSSION

Smoking is an important hazard to health. It is frequently implicated in the pathogenesis and causality of selected chronic diseases of human like chronic obstructive airway disease, cancers and cardiovascular diseases (18). During pregnancy, smoking adversely affects the health of fetus and is considered an important risk factor for intrauterine growth retardation (4).

The current study followed a meticulous methodology to measure the magnitude of exposure to cigarette smoking for mothers during pregnancy. These exposure levels were defined as: passive (side stream) smoke given off directly from the burning end of the cigarette and active (main stream) smoke. Analysis of cord blood for heavy metals and nicotine was used to assess the direct exposure of foetus to cadmium instead of relying on venous blood of his mother. In this way the barrier between mother and foetus made by the placental circulation is bypassed.

The current study showed that the cord blood cadmium levels in smoking environment are much higher than those for no smoking environment. The dose of smoking is directly reflected in the observed increase in cord blood Cd concentration. Published articles consistently showed that tobacco is an important source of cadmium uptake in smokers. Bernhard D, 2005 has agreed that cigarette smoking may be one of the most important sources of Cadmium (Cd) exposure affecting the general population. Smoking a pack of cigarettes daily can result in the inhalation of 2-4 µg Cadmium per day depending on the type of tobacco smoked (19). Direct measurement of cadmium levels in body tissues showed that smoking roughly doubles cadmium body burden in comparison to those not smoking (8). Unlike the passive diffusion of lead to the placental circulation, being almost unaffected by placental barrier, the Cd accumulates in human placenta, but the placenta is not a complete barrier, and Cd concentrations in cord blood increase with continuous maternal exposure (9). It was reported that the mean whole blood Cd content in smokers was 1.9 times higher than in non-smokers (0.267 ± 0.121 µg/dl and 0.137 ± 0.045 µg/dl, respectively) (20).

Tobacco smoke has a harmful influence on the development of the fetus not only when the mother is an active smoker, but also when a pregnant woman is exposed to tobacco smoke in the environment (passive smoking) (21, 22).

Heavy metals like: lead and cadmium are toxicants, which are well known to cross the placenta and to accumulate in fetal tissues. These metals adversely affect placental functions. Both metal-specific placental transfer and impairment of placental function can explain the relationship between prenatal metal exposures and adverse effects on intrauterine growth and neuro-development. A previous study was conducted by (23) indicated that cord blood cadmium level was negatively correlated with fetus development. Low birth weight (less than 2,500 g) occurred significantly more frequently in infants with higher cord blood cadmium than in those exposed to lower levels of cord blood cadmium.

Another study conducted by (24) showed that cord blood cadmium level, but not maternal blood cadmium and placenta cadmium, were negatively associated with neonatal birth height. Compared with lower cord blood cadmium level, higher level of cord blood cadmium was associated with 2.24cm decrease in neonatal birth height. There was no significant association between cadmium exposure and birth weight in that study.

The current study showed direct evidence of adverse effect of cadmium on indices of foetal growth. The cord blood concentration of cadmium showed a statistically significant moderately strong to strong negative (inverse) correlation with weight for age and head circumference for age z scores. The height for age on the other hand was less strongly affected by blood concentrations of cadmium.

CONCLUSION

The assessment of cadmium in cord blood of pregnant woman could be considered as a useful marker in predication of smoking environment. The current study showed elevation significantly in cord blood cadmium in smoking environmental. The current study showed effect of smoking environment on nutritional indices presented by weight for age, weight for height and head circumference for age Z score is explained by the effect of cadmium on fetal indices.

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Estrogen and progesterone levels in the blood samples of breast cancer Iraqi patients and its relation to breast cancer risk

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ABSTRACT

Breast cancer is considered the most common cancer diagnosed in women worldwide.

Estrogen and progesterone show contrast in their levels in women with breast cancer, indicating that estrogen and progesterone levels might be considered as breast cancer risk factors.

The demographic and hormonal study was conducted and included 65 breast cancer patients, 19 patients were diagnosed to have benign breast tumors, and 10 were apparently healthy volunteering females as normal control group. Estrogen and progesterone concentrations were detected in the peripheral blood of both patients and control groups.

The results revealed a highly significant association ($p < 0.01$) between breast cancer risk and elevated estrogen levels. On the other hand, the decrease in progesterone levels was significantly ($p < 0.01$) associated with the risk of breast cancer, since elevated level of estrogen was observed in 62% of the studied cases, and decrease in progesterone level was observed in 59% of them

Keywords: Breast Cancer, Estrogen, Progesterone, Hormones

الملخص باللغة العربية

يعتبر سرطان الثدي من أكثر أنواع السرطان المنتشرة بين النساء في جميع أنحاء العالم. ويظهر هرمونا الأستروجين والبروجسترون تباينا في مستوياتهما لدى النساء المصابات بسرطان الثدي، مما يجعل مستويات الأستروجين والبروجسترون تمثل عامل خطورة للإصابة بسرطان الثدي.

تم تنفيذ الدراسة الحالية (الديموغرافية والهرمونية) لتشمل 65 مصابة بمرض سرطان الثدي، 19 مريضة لديهن أوراما حميدة في الثدي ، و 10 متطوعات بحالة صحية سليمة كمجموعة سيطرة. وتم الكشف عن تركيز هرمون الأستروجين والبروجسترون في الدم المحيطي لكل من مجموعتي المرضى والسيطرة.

أظهرت النتائج وجود علاقة معنوية عالية ($P < 0.01$) بين خطر الإصابة بسرطان الثدي ومستويات هرمون الأستروجين المرتفعة، و من ناحية أخرى، أظهرت النتائج انخفاضا ذا معنوية عالية في مستويات هرمون البروجسترون ($P < 0.01$) المرتبطة بخطر الإصابة بسرطان الثدي ، وقد لوحظ ارتفاع مستويات هرمون الأستروجين في 62% من الحالات التي تمت دراستها، ولوحظ انخفاض في مستويات هرمون البروجسترون في 59% منها.

INTRODUCTION

Breast cancer is by far the most common cancer diagnosed in women worldwide, with an estimated 1.38 million new cases diagnosed in 2013 (1). Globally, more women are developing breast cancer (BC) and more women are dying from it than ever. Between 1980 and 2013, the number of breast cancer cases steadily increased more than two and a half times. The rise in BC cases is happening in every region and in every country, with the number of cases in some countries increasing much faster than the global trend. Four hundred and twenty five thousands women died from breast cancer in 2013. In developing countries, 68,000 of those women were in their reproductive years, aged 15 to 49. In Iraq, BC is the most common cancer among females (2- 4).

Estrogen is a potent hormone, produced in the ovaries of females. A smaller amount is present in males as well. The normal estrogen levels range vary widely depending on a person's age. For those between 20-29 years, the average is 149 pg/ml and shall increase to 210 pg/ml for females between 30-39 years. The level falls back to 152 pg/ml for women ≥ 40 who are not yet in menopause. These levels are generally the exact level varies on a daily basis and are closely related to the various phases of the menstrual cycle (5,6). Progesterone is produced mainly by the corpus luteum, the remnant of the follicle that contains the egg released from the ovary. Progesterone levels are less than 1.5 ng/ml before ovulation and rise to more than 15 ng/ml after ovulation, according to fertility plus female hormone level charts. Progesterone continues to rise if pregnancy occurs to 300 ng/ml or higher, if no pregnancy occurs, levels drop back to 1.5 ng/ml or less (5,6).

Estrogen and progesterone work to balance each other in the body. When estrogen levels increase for any reason, progesterone levels may drop. Because high estrogen levels suppress progesterone production, the problem becomes cyclical. Many of the cells throughout the body — both healthy cells and potentially cancerous ones — contain estrogen receptors. These receptors are type of protein molecules that stimulate cell growth when they come in contact with estrogen. As estrogen circulates through the bloodstream, it attaches to the estrogen receptors in cancerous cells, causing them to divide and accumulate in the body. In the absence of estrogen, these same cells would stop growing and eventually die (7).

As Iraq had faced several and consequent wars since 1990 until now, Iraqi women had exposed to many types of environmental pollution and stress factors, which had a critical impact on their hormonal status, age at menarche, and age at menopause. Thus, the current study aimed to evaluate the variation of serum estrogen and progesterone levels as risk factors for breast cancer among selected sample of Iraqi women.

PATIENTS AND METHODS

Patients:

Eighty seven patients (with operable breast tumor) were enrolled before the surgical removal of the tumor and before adjuvant chemotherapy, 65 breast cancer patients (43 patients with metastatic breast cancer, 22 patients with early diagnosed breast cancer tumors), 19 patients with benign tumors, and 10 apparently healthy females as control group with age ranged 15-74 years.

The patients had all diagnosed with breast cancer or benign lesions of breast at Iraqi hospitals and breast cancer centers including Hospital of Saint Raphael, Baghdad Red Crescent Authority, Yarmouk Hospital Teaching, Baghdad Hospital, and National Center for Early Detection of Tumors

Methods:

1. Demographic study:

Information were taken from the patients enrolled in this study, which included name, age, medical history, marital status, children number, lactation status, age at menarche, and age at menopause.

2. Hormonal study:

Five ml of venous blood were collected from 84 patients and 10 healthy voluntaries. Blood samples were drawn between 9 a.m and 6 p.m. For each case, blood samples were left to stand at room temperature (18-22°C) to allow clotting, then blood sample tubes were centrifuged at 3000 rpm for 5 minutes, then the serum was separated to be stored in -20°C for use in measuring estrogen and progesterone levels.

2.1 Estrogen level measuring assay: Radio Immuno Assay Estradiol Kit from Beckman coulter / Germany was used. For coated tubes, 100 μ l of calibrator were added to both control and sample groups, then 500 μ L of tracer were added, and mixed briefly. The mixture was incubated for 3 hours at 18-22 C° with shaking. Then the contents of the tubes were aspirated carefully, placed in Gamma counter, and then the bound cpm and total cpm were counted.

2.2 Progesterone level measuring assay: Radio Immuno Assay Progesterone Kit from Beckman coulter / Germany was used. For coated tubes, 50 μ l of calibrator, control or sample were sequentially added, then 500 μ L of tracer were added and mixed briefly. The mixture was incubated for 1 hour at 18-22 C° with shaking. Then the contents of the tubes were aspirated carefully, placed in Gamma Counter, and then the bound cpm and total cpm were counted.

RESULTS AND DISCUSSION

1. Demographic study:

1.1 Patients age group distribution:

Patients were classified into six groups according to their age (Table 1). Fifteen individuals (17.85%) of the studied patients were 15-24 years old, within this age group, 20% of members were suffered from early diagnosed breast cancer, 46.7% of members were suffered from metastasis breast cancer, and 33.3% of members were suffering from benign breast tumors.

A percentage of 9.52 of the second age group were 25-34 years old, within this age group 25% of members were presented with early diagnosed breast cancer, 75% of members were presented with metastasis breast cancer, and there were no patients with benign breast tumors within this age group members.

Twenty patients (23.82%) were 35-44 years old, within this age group, 25% of members were suffering from early diagnosed breast cancer, 50% of members were suffering from metastasis breast cancer, and 25% of members were suffering from benign breast tumors.

Twenty five percentage 25% of patients were within third age group 45-54 years old, within this age group, 33.3% of members diagnosed with early breast cancer, 42.9% of members were suffering from metastasis breast cancer, while 23.8% of members had benign breast tumors.

Fifteen patients (17.86%) were 55-64 years old, within this age group, 26.7% of members presented with early diagnosed breast cancer, 46.6% of members presented with metastasis breast cancer, and 26.7% of members presented with benign breast tumors.

Five patients (5.95%) were ≤ 65 years old, within this age group, 20% of members were suffering from early diagnosed breast cancer, 80% of members had metastasis breast cancer, and there were no patients with benign breast tumors within this age group.

The (45-54 years) group has the highest percentage with 25% followed by (35-44 years) group with 23.82%, the lowest percentage with only 5.95% for (≤ 65) group.

Significantly, most cases enrolled in this study were women within their reproductive age (25-44) years old, which revealed the importance of early diagnosis and case follow, since breast cancer is by far the most common cancer diagnosed in women worldwide (8).

Table (1): Distribution of patients age groups according to disease cases

Age	Malignant tumor				Benign tumor		Total	
	Early stage		Late stage		No.	%	No.	%**
	No.	%*	No.	%				
15-24	3	20.00	7	46.70	5	33.30	15	17.85
25-34	2	25.00	6	75.00	0	0	8	9.52
35-44	5	25.00	10	50.00	5	25.00	20	23.82
45-54	7	33.30	9	42.90	5	25.00	21	25.00
55-64	4	26.70	7	46.60	4	26.70	15	17.86
65<	1	20.00	4	80.00	0	0	5	5.95

* The proportion of disease cases per age group

** The proportion of age group from total disease cases enrolled in the study

The results obtained from the current study are in agreement with the results obtained from several previous studies conducted in Iraq, Arabic countries, USA, and Europe, which pointed that the incidence of breast cancer had occurred after age of 40. Al-Alwan (9) confirmed that approximately one third of the patients were diagnosed in their forties age, where the peak frequency had occurred, while an obvious decline was displayed after the age of 60 years.

Moreover, recent studies conducted by Al-Thwani and Ayad (10), Al-Bayati (2) had confirmed the same results. Furthermore, in 2007, the Iraqi Cancer board mentioned that there is an increase in the incidence in younger age groups, and the peak frequency was found in the age ranged 40-49 years (11).

Another study was conducted on Arabic women population, which results showed that breast cancer incidence increased in (40 - 44) years old among Arabic women (3).

A study carried out in Europe explained that Age-specific incidence rates for breast cancer rise steeply from around age 30-34, and the peaks of incidence for women aged 40-50 (12).

A statistical breast cancer study shows that in USA overall breast cancer incidence rates increased during the most recent time period (2006-2010) among women aged 30 years to 49 years (13).

1.2 Marital status and gynecological and obstetrical history:

The majority of the examined female groups were married with a total number of 61 and a percentage of 72.6% , while 23 (27.4%) of the examined females were unmarried.

The mean age at menarche was (12.6 \pm 0.7) years, the lowest age was 11years and the highest was 14 years, 77 (91.6%) cases of the patients started their menarche at age less than 14 years, while 7 (8.4%) cases at age of 14 years. Parous women represented (75%) of married cases, vs. (25%) nulliparous. Parous group was lactating (Table 2).

Distribution of those patients according to age group, marital status, and lactation status showed no statistically significant association ($P>0.05$) with any of these variables, in all comparison.

The age at menopause of 21 cases who got menopause was ranging from 47 – 58 years with a mean of (49.4 ± 1.25) years, almost 13.2% of cases were in menopause at age (47-48 years), (77.6%) at age (49-50) years and only (9.2%) at age more than 50 years.

Table (2): Distribution of gynecological and obstetrical history among study women population

Variables	No.	%
Marital status :		
Married	61	72.6
Unmarried	23	27.4
Age at menarche (years):		
11	3	3.8
12	36	42.6
13	38	44.9
14	7	8.7
Total	84	100
Mean \pm SD	12.6 \pm 0.7	
Range	11 – 14	
Lactation :		
Yes	47	56
No	14	16.6
Total	61	72.6
Age at menopause (years):		
47-48	5	5.9
49-50	12	14.3
> 50	4	4.8
Total	21	25
Mean \pm SD	49.4 \pm 1.25	
Range	47 – 58	

The statistical results showed that age at menarche was a risk factor among both pre-menopausal and post-menopausal women (statistically significant $p < 0.01$). It was found that a delay in age at menarche is corresponding to a reduction in breast cancer risk, also age at menopause was also considered as a breast cancer risk factor (statistically significant $p < 0.01$). Women with menopause at older age have a higher risk of breast cancer.

It can be said that the early age at menarche, and the late age at menopause are linked to a modest increase in the risk of developing breast cancer. Different studies agreed with these results. An epidemiological study from the year 1966 to 2005 carried out on thousands of women with breast cancer and its results revealed that both the late age at menarche, and the early age at menopause decreased the risk of breast cancer (14).

Another epidemiological study showed that women at the oldest age in the menarche category were at a 34% lower risk of breast cancer, and women at the youngest age in the menopause category were at a 31% lower risk of breast cancer (15).

2. Hormonal study:

2.1 Patient's hormonal status (Estrogen and progesterone):

The results of the current study revealed that elevated levels of estrogen were observed in 52 (62%) cases (Table 3) . these results indicated a highly significant association ($p < 0.01$) between breast cancer risk and elevated estrogen levels.

Forty five cases out of 52 cases whom showed elevation on estrogen levels reflected a positive estrogen receptor status, and thus confirming the role of high level of estrogen in monitoring breast cancer tumor growth. Several studies worldwide had concerned in the concept of estrogen effect on the risk of breast cancer, and, in consistent with the results of this study; they indicated a positive relationship between high estrogen level and the risk of breast cancer (16,17).

Table (3): Estrogen and Progesterone levels in the cases enrolled in the study

Variables	Elevated		Decreased		Normal		Total	
	No.	%	No.	%	No.	%	No.	%
Estrogen	52	62	10	12	22	26	84	100
Progesterone	9	11	50	59	25	30	84	100

In the present study , 10 cases (12%) have had a decreased plasma estrogen (Table 3) , all the 10 cases have had irregular menses , and this seems difficult to be correlated to breast cancer risk, but in fact, an increase in breast cancer risk may be seen after a modest reduction in circulating estrogens such as that produced by unilateral ovariectomy , oral contraceptives or contraceptive depot use [both of which inhibit ovulation and ovarian estrogen production], or low body weight and low fat intake (6). Moreover, 19 cases were presented with benign breast tumors, but none of them showed abnormal estrogen levels.

In addition, results had shown that only 9 cases (11%) had elevated levels of progesterone. Thus, there was a weak association between progesterone elevation and breast cancer risk. This result actually agreed with results of other epidemiological studies which had shown that there is no statistically significant association between elevated progesterone and breast cancer risk (16 , 18).

On the other hand, the results indicated that decrease in progesterone levels was observed in 50 (59%) cases out of the 87 studied cases, which explained that the decrease in progesterone levels is significantly ($p < 0.01$) associated with the risk of breast cancer, and none of the 19 benign breast tumors cases showed abnormal progesterone levels. These findings are generally supported by results of other studies conducted by (16), which involved 30 patients with approved diagnosis of carcinoma and had metastasis. The results showed that high levels of estrogen were observed in 19 (63%) cases, which in turn indicated a significant association between

breast cancer risk and elevated estrogen levels. Results also indicated that decrease in progesterone levels was observed in 16 (54%) cases, which explained that the decrease in progesterone levels is significantly associated with the risk of breast cancer (16). In another study, it was observed that there was a statistically significant direct association between the endogenous levels of each steroid hormone evaluated and the risk of breast cancer, with the exceptions of the endogenous levels of progesterone (18).

Among the 52 cases with elevated estrogen levels, the most important cases were those who had additional decreased levels of progesterone. They represented 41 cases (all were suffering from breast cancer relapse) from the total number of cases (87) and these results are in agreement with those shown by (16).

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Genetic distance analysis of dermatophytes fungi using the RAPD-PCR

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ABSTRACT

The molecular technique random amplified polymorphic DNA (RAPD) was employed for identification of nine species of dermatophytes isolated from swimming pools, to determine the genetic diversity, and relationships. RAPD in which four universal primers are using molecular marker techniques showed considerable potential for identifying and discriminating dermatophytes species and the obtained results confirmed identification based on conventional morphological methods. The genetic distance (RAPD-GD) among nine species varieties was calculated using genetic program (Numerical Taxonomy and Multivariate Analysis System Version 1.80 package) depending on shared bands between each variety.

Four universal primers (OPAA11, OPD18, OPAA17 and OPU15) used in this study produced 38 bands across nine isolates. Of these bands, 31 bands were polymorphic. The sizes of the amplified bands ranged between 100-1,100 bp. The genetic polymorphism value of each primer was determined and ranged between 50-90%. Genetic distances ranged from 0.23240 to 0.78235 among dermatophyte isolates. Cluster analyses were performed to construct a dendrogram among studied dermatophyte isolates.

Keywords: RAPD, genetic distance, dermatophyte

الملخص باللغة العربية

تم استخدام مؤشرات التضاعف العشوائي للحمض النووي (DNA) المتعدد الأشكال لتشخيص تسعة أنواع من الفطريات الجلدية التي عزلت من أحواض السباحة، لتحديد التغيرات الوراثية ودراسة العلاقة بينها، وقد حظي استخدام التقنية الجزيئية المعتمدة لمؤشرات التضاعف العشوائي للحمض النووي مع البادئات الشاملة بجهد هام وكبير لتشخيص وتمييز أنواع الفطريات الجلدية، كما حققت نتائج تشخيصية مثبتة تفوقت على طريقة التشخيص المظهرية الاعتيادية.

وقد تم حساب تحليل البعد الجيني للفطريات الجلدية باستخدام مؤشرات التضاعف العشوائي للحمض النووي بين تسعة أنواع متضاربة باستعمال البرنامج الجيني (التصنيف العددي ونظام التحليل متعدد المتغيرات ذي المعدل 1.80 رزمة) اعتماداً على تقاسم الحزم بين المجاميع المتنوعة. استخدمت في هذه الدراسة أربع بادئات شاملة (OPAA11, OPD18, OPAA17, OPU15) أنتجت 38 حزمة عبر 9 عزلات، وكان من بين هذه الحزم 31 حزمة متباينة. وقد تراوح الوزن الجزيئي للحزم المتضاعفة بين 100-1,100 زوجاً قاعدياً. وتراوحت قيمة المتغيرات الوراثية لكل حزمة بين 50-90%. أما قيمة تحليل البعد الوراثي فقد تراوحت بين 0.23240 - 0.78235 بين عزلات الفطريات الجلدية. وقد تم تنفيذ التحليل العنقودي وتم رسمه بشكل مخطط شجري بين عزلات الفطريات الجلدية المدروسة.

INTRODUCTION

Conventional methods for identification of fungi rely on macro and micro morphological features of the colonies on general and specific culture media can greatly influence the phenotypic characteristic and consequently can make the identification more difficult (1). Recently, molecular marker approaches, such as nested-polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD)-PCR were adapted for detection of dermatophytes from water (2,3). RAPD methods had frequently been used for phylogenetic analysis and identification of dermatophytes (4). The random amplified polymorphic DNA primers is used to recognize *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton interdigitale*, *Trichophyton mentagrophytes* and *Trichophyton tonsurans*. The amplification patterns in RAPD is done by using four primers.

A better understanding of the role of environmental sources of dermatophyte isolates causing infection would facilitate prevention strategies (5). Environmental isolates could contribute to the solution of several relevant clinical problems such as the identification of the environmental source of dermatophyte isolates causing dermatophytosis, determining the existence of pathogenic isolates and the role of natural habitants.

The aim of the current study was to investigate the genetic diversity, and study the relationships among dermatophyte species.

MATERIALS AND METHODS

Fungal culture media:

Nine fungal strains were isolated from different swimming pools in Baghdad city (Alrafedain – Alyarmok – Aladel – Aljadria). Conventional morphological methods were employed for identification of these fungi, where dermatophytes were cultured on Sabouraud's Dextrose agar (SDA) and autoclaved at 121°C and pressure 1.5 kg/cm² for 15 min. After cooling the media, 50 mg of chloramphenicol was added according to (6). Dermatophytes were identified by using classification and identification of fungal species described in (7,8) to be used for DNA extraction.

Genomic DNA isolation:

Chitin is one of the most abundant compounds in the cell wall of the most fungi. Because chitinase was not available for the current study, DNA extraction was used. For this purpose, Grind cell pellets were collected from 3 ml fungi culture (isolated from pools on SDA for 10 days) and centrifuged, freeze-thaw under liquid nitrogen was done to produce a rapid extraction and high quality of extracted DNA. Purity and concentration of DNA were measured by spectrophotometer according to

(9). Genomic DNA integrity was detected by running on 1% agarose gel electrophoresis followed by staining with ethidium bromide and visualized under UV light according to (10). DNA samples were diluted to concentration of 50 ng/μl in order to be used in the RAPD-PCR experiments.

Primer selection and RAPD assay:

According to (11), the following procedure was done:

1. Four decamers of oligonucleotides primers in random sequence were used (Operon Technologies) in a lyophilized form and were dissolved in sterile deionized distilled water to give a final concentration of (10pmol/μl) as recommended by provider. The primers tested in this study were (OPAA11, OPU-15, OPAA17 and OPD18).
2. Amplification reactions were performed in a volume of 25 μl containing 5 μl of PCR Master Mix (Bioneer), with concentration (1X) containing (10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM each deoxynucleotide triphosphate (dNTP) and 1 unit DNA polymerase), 10pmol of the primer, and 50 ng of template DNA.
3. Amplification was carried out using a thermocycler (Eppendorf-Germany), using the following program: 1 cycle of 5 min at 94°C for initial strand separation, followed by 45 cycles of 1 min at 94°C for 72°C for primer extension.
4. Finally, 1 cycle of 10 min at 72°C was used for the final extension, followed by a hold at 4°C.
5. Each PCR amplification reaction was repeated twice to ensure reproducibility.
6. Four microliters of PCR products were analyzed by electrophoresis in a 1.2% agarose gels at 5 Volt/cm for 2 hours in 0.5x TBE buffer. Agarose gels were stained with ethidium bromide 0.5 μg/ml for 20-30 minutes. The 100bp DNA ladder (100-2,000) bp (Bromiga) was used as a molecular size marker. After electrophoresis, images of gels were captured using Gel Documentation System (Consort – Belgium).

Data analysis:

1. molecular weight estimation: Molecular weight was calculated by using computer software M.W. detection program, Photo-Capture M.W. program from Consort, based on comparing the RAPD-PCR products with the known size of DNA fragments of a 100bp DNA ladder (which consist of 13 bands from 100 to 2,000 bp Bromiga) was used.

2. estimation of genetic distance: The banding patterns were compared to determine the genetic relatedness of species. Separate data matrix was constructed for each primer by scoring each isolate for the presence or absence of each band. The results obtained for "4" primers were pooled and these data were clustered using the unweighted pair-group method arithmetic average (UPGMA) Data

generated from the detection of polymorphic fragments were analyzed.

The presence of band scored as "1" and the absence of the same band of the same size in other isolate scored as "0". Only clear and reproducible amplified fragments were considered for genetic relationship analysis. Estimates of genetic distance (G.D) were calculated between all pairs of the varieties according to (12), based on following formula:

$$G.D = 1 - \{2Nab / (Na + Nb)\}$$

Where,

Na: the total number of fragments detected in individual 'a',

Nb: the total number of fragments shown by individual 'b',

and Nab: the number of fragments shared by individuals 'a' and 'b'.

Cluster analysis was performed to construct genetic relationship tree diagrams among studied *A.fumigatus* isolates using an Unweighted Pair-Group Method with Arithmetic Average (UPGMA). All computations were carried out using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), Version 1.8 package (13). The percentage of polymorphic bands was defined as ratio of the number of polymorphic bands amplified by a single primer to that of the total number of bands produced by the same primer.

RESULTS AND DISCUSSION

Analysis of genetic distance:

The genetic distance (RAPD-GD) among nine barley varieties was calculated using genetic program (Numerical Taxonomy and Multivariate Analysis System Version 1.80 package) depending on shared bands between each variety when increasing bands number that lead to decreasing of genetic distance and vice versa. Table (1) illustrates the values of genetic distance of *Dermatophytes* isolated species. The genetic diversity and the relationships among of *Dermatophytes* species isolates were evaluated using RAPD markers amplified from four universal primers, the primers varied greatly in their ability to resolve variability among varieties. Some primers generated several bands, while others generated only a few bands.

Genetic distances:

The ratio of genetic similarity among the *Dermatophytes* isolated species ranged from 0.38 to 0.76 as shown in table (1). The highest similarity (0.76) 76% was obtained between the isolate number '1' and '8'. This was followed by (0.70) 70% similarity between a

pair of the isolates number '4 and 6'. The lowest level of similarity (0.38) 38% was obtained between the isolates number '1' and '7'.

Cluster analysis:

Dendrogram was constructed for genetic distance using UPGMA according to (12). Cluster analysis and depicted genetic relationships among nine isolates of *Dermatophytes* species were done. Figure (1) showed the major clusters 1, 11,111.

These main groups were linked together finally as shown in the figure (1), and expressed as follows:

The first main group: included one subgroup, the first subgroup contains the isolates '1 and 8' and the subgroup contain the isolate number '2'.

The second main group: included three subgroups, the first subgroup contains the isolates number '3,5 and 7'.

The third main group: included two subgroups, the first subgroup contains the isolates number '4' and '6', while the second subgroup contains the isolates number '9 and 4',6.

Cluster analysis had placed most of the dermatophyte species isolated from water, showing a high level of genetic relatedness and these were distinct from those isolates isolated from another government.

Table (1): Values of genetic distance between *Dermatophytes* species isolates calculated according to (12) data matrix

	1	2	3	4	5	6	7	8	9
1	0.00000								
2	0.30399	0.0000							
3	0.48627	0.6346	0.00000						
4	0.37884	0.46926	0.49041	0.00000					
5	0.43078	0.3781	0.31921	0.37884	0.00000				
6	0.71725	0.78235	0.72346	0.29758	0.42957	0.00000			
7	0.61189	0.66457	0.40501	0.70305	0.51658	0.75377	0.00000		
8	0.23240	0.37388	0.55615	0.38419	0.29302	0.68177	0.49945	0.00000	
9	0.46825	0.3465	0.59784	0.30809	0.33472	0.46110	0.62815	0.34397	0.00000

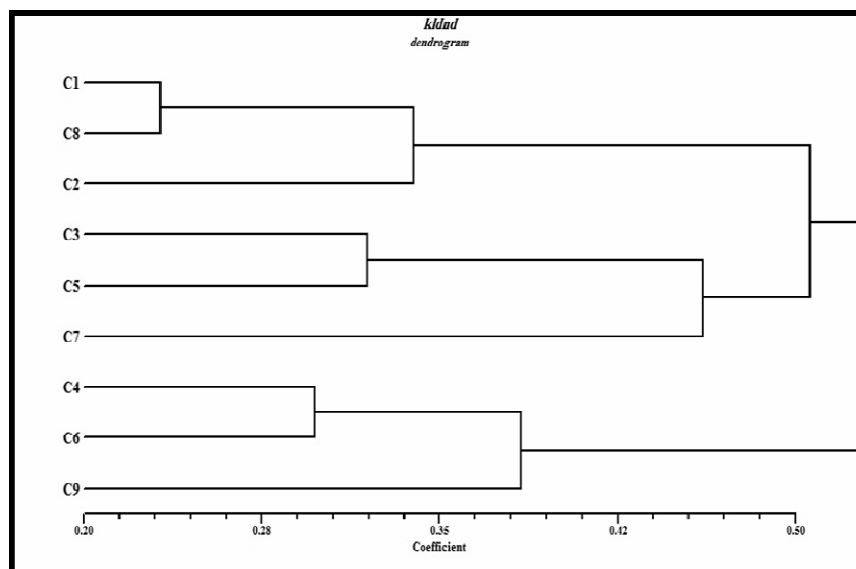


Figure (1): Dendrogram illustrated genetic fingerprint and relationships between *Dermatophytes* species isolates developed from RAPD data

According to the obtained results, it seems that RAPD-PCR using aforementioned primers is a sensitive method for rapid differentiation of dermatophytes (table 1) is calculated from genomic similarity group average of each species of dermatophyte. The similarity of *T. rubrum* with *T. violaceum* was nearly and the similarity of *T. tonsurans* with *T. mentagrophytes* and the similarity of *M. canis* with *M. audouinii*. These results provide the basis for the rapid identification of dermatophytes at the genetic level, in additions to the existing laboratory methods. The phylogenetic relationships among different dermatophyte species are estimated by determining the degrees of similarity between their DNA sequences (Figure 1).

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Investigation of some pesticide residues in full cream milk from Baghdad's markets

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ABSTRACT

The current study aimed to test and analyze of 18 samples, involved from liquid and powdered of full cream milk, to detect a residue of organic chlorinated pesticides. Standard curves of detected pesticides then got the equation of straight line for each type of pesticide separately by using the application of equation on the concentration of the pesticide in contaminated form.

Results showed, eight samples of contaminated milk, one sample trademark of Almarai milk contaminated by (5.92) ppb of Lindane pesticide, two samples of milk trademark Alray and Almudhish, contaminated with (4.61, 2.60) ppb PP-DDD respectively. For trademark Pinar milk we found a concentration of (9.687) ppb. From Endrine pesticide. And Nido milk trademark showed a contamination of (2.94) ppb. With Heptachlor. In addition, Alrai milk contained a (20.53) ppb. of PO-DDT. as well as for Canon and milko milk, contaminated by (5.94 and 12.70) ppb respectively with Aldrin.

According to the results of research, which showed a presence of organic chlorinated pesticide residues concentrations in full cream milk samples most commonly used by consumers, over time residue of organic chlorinated pesticide will accumulate, which leads to high concentration in the body.

Keywords: Organic chlorinated pesticide residues, full cream milk

الملخص باللغة العربية

هدفت الدراسة الحالية إلى فحص وتحليل 18 عينة من الحليب كامل الدسم السائل والمجفف ، للكشف عن بقايا المبيدات الكلورية العضوية. تم الكشف عن المنحنيات القياسية للمبيدات ومن ثم الحصول على معادلة خط مستقيم لكل نوع من المبيدات وذلك للحصول على تركيز المبيد في شكله الملوث.

أظهرت النتائج تلوث ثمانية نماذج من الحليب ، عينة من حليب العلامة التجارية المراعي ملوثة (5.92) جزء من البليون من مبيد Lindane، عينتين من الحليب العلامة التجارية الراي والمدهش ملوثة (4.61، 2.60) جزء في البليون من مبيد PP-DDD على التوالي، وعينة من حليب العلامة التجارية بينار تركيز (9.687) جزء في البليون مبيد الإندرين (Endrine). كما أظهر حليب العلامة التجارية نيدو تلوث بنسبة (2.94) جزء في البليون بمبيد Heptachlor. ، بالإضافة إلى تلوث حليب علامة الراي بمقدار (20.53) جزء من البليون من مبيد PO-DDT، وكذلك حليب علامة كانون وميلكو الملوثين (5.94 و 12.70) جزء في البليون على التوالي مع الدرين.

طبقاً لنتائج الدراسة التي أظهرت وجود تراكيز بقايا المبيدات العضوية الكلورية في عينات الحليب كامل الدسم الأكثر استخداماً من قبل المستهلكين، فإنه ومع مرور الوقت، تتراكم بقايا المبيدات الكلورية العضوية، الأمر الذي يؤدي إلى تركيز عال في الجسم.

INTRODUCTION

The organic chlorinated pesticides are considered as one varieties of highly toxic pesticides which resist degradation due to environment because of high stability and their ability to move in the environment therefore become a part of food chain (1). Organic chlorinated pesticides were used in fields of agriculture, to eliminate pests such as insects and harmful plants like fungi or rodents (2). A plants which usually sprayed with pesticides, absorb these pesticides from many parts of them and store it in their tissues, then followed by direct pesticide transmission to the all animals bodies which feeds on the contaminated plants. (3), these toxic materials, accumulate in the fatty tissues of animals, because of high ability of soluble in fats (4). Thus, exactly what indicated from the results when fatty tissues of animals analyzed and found sensible concentrations of Aldrin pesticide arrived (11000ppb). This is a result of an accumulation for these residues over time and another part of pesticide can be move through the circulatory system of animals to udder factory where the milk composition (5-7). Researchers has detected residues of organic chlorinated pesticides mainly such as DDT, Aldrin, Lindane and others (8-10). As detected in (11) of some types of organic chlorinated pesticides in maternal milk samples. However, striking that concentration of pesticides in milk exceeds the concentration in animal's fodder about ten times (12, 13). Then an output milk involves a serious threat to consumers, naturally, moving residue of pesticides eventually to the head of food pyramid, stores in fatty tissues (14). Generally, organic chlorinated pesticides considers as dangerous to human health because they impact Biosynthesis and causes a kidney failure and liver, also cause disease, infertility and disorders in growth, some cancers, weakening the immune system, an imbalance in the growth of the nervous system, disorders of behavior and an imbalance in the system, metabolism, such as diabetes. Also caused trace amounts of pesticides in dysfunctional and growth of the nervous system when the fetus, child and the elderly and possibly death (15, 16). Thus, this study aimed to detect pesticide residues in whole milk, and what consumed of large quantities of contaminated milk with residual organic chlorinated pesticides from humans, these quantities will accumulate in human's bodies of such quantities; even if it's a small remnant and the consequent consequences of pathogens then impact human health.

MATERIALS AND METHODS

Identification and qualification of organic chlorinated pesticides:

Gas Chromatography GC 2010 model type with Shimadzu detector sniping mail ECD were used with the following analytical program:

- Column Type: 5 MS 30m × 0.25 mm ID, P.size 0.25 µm
- DeT.E.C.D
- Temp. program :
- Inj: 280°C, Oven: 250 °C, DeT: 310°C

Sampling :

A sample composed of 18 different trademarks of full cream milk (canned liquid and powder) were examined, which were withdrawn randomly from the local markets at city of Baghdad during 4-10th of February 2014, with detailed information of examined types of milks as shown in Table (1).

Table (1) manufacturing information for the selected samples

No.	Sample of milk	Origin	Production date	Expiration date
1	Almarai	Saudi Arabia	24/12/2013	22/6/2014
2	KDD (liquid)	Kuwait	9/12/2013	8/6/2014
3	Alary(liquid)	Saudi Arabia	30/9/2013	29/3/2014
4	Alsafy (liquid)	Turkey	13/10/2013	12/3/2014
5	Pienaar (liquid)	Turkey	10/11/2013	10/5/2014
6	Mersin (liquid)	Turkey	26/11/2013	26/05/2014
7	Beyti (liquid)	Egypt	17/12/2013	Potable during six months
8	Nadi (liquid)	Saudi Arabia	19/12/2013	19/6/2014
9	Nadi banana flavor (liquid)	Saudi Arabia	18/12/2013	18/6/2014
10	Nido (Powder)	Switzerl and	27/3/2013	26/3/2014
11	Landuze Milk (Powder)	Malaysia	10/6/2013	9/6/2014
12	Abu Alques milk (Powder)	Netherla nds	24/6/2013	23/12/2014
13	Dielac Milk (Powder)	Vietnam	4/2013	9/2014
14	Almudhish milk (Powder)	Sultanate of Oman	26/6/2013	27/12/2014
15	Iraqi Cow's milk (Powder)	Iraq	-----	-----
16	Canon strawberry flavored milk (liquid)	Saudi Arabia	13/11/2013	13/5/2014
17	Alraiyl milk (liquid)	Kuwait	13/12/2013	12/6/2014
18	Milko Milk (Powder)	New Zealand	2/11/2013	1/5/2015

Extraction process to investigate chlorinated organic pesticide residues:

A volume of (50 ml) from liquid milk sample and weight from powder milk (5 gm add to 50 ml water) took and put in a separating funnel, then added (5 ml) of Methanol with 0.5 g of Sodium Oxalate and left 1min for homogenizing. In addition, a mixture of 20 ml from diethyl ether and 25 ml petroleum ether added and left 1 min. The mixture of milk and solvents centrifuged (1500 rpm for 5 min.). Separated upper organic layer, taken and added to it 50 ml of petroleum ether and diethyl ether with ratio of (1:1), then filtered with filter paper contained sodium sulphate, the filtrate mixture of milk and solvent evaporated by rotary at 35 °C. The extracts analyzed by chromatography gas GC. To estimate residues of organic chlorinated pesticide by using a detector of Electron Capture Detector (ECD) according to the analysis conditions. Results were compared with standards compounds (9).

RESULTS AND DISCUSSION

Estimated concentrations of organic chlorinated pesticide in 18 types of full cream milk (liquid and powder) by using the equation of a straight line pesticides standard curve for each type of detected organic chlorinated pesticide (PolyScience Corporation) separately as shown in figures (1 , 2).

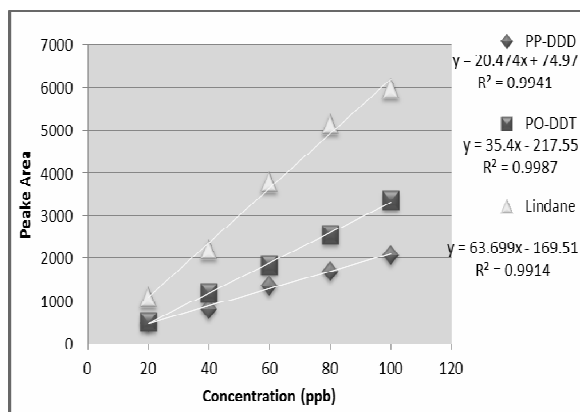


Figure (1): pesticide standard curve (PP-DDD, PO-DDT and Lindane)

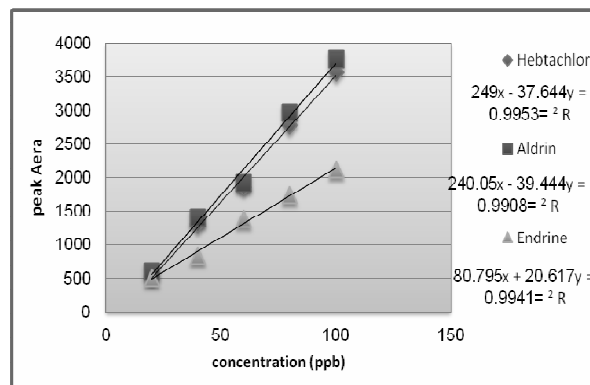


Figure (2): pesticide standard curve (Heptachlor, Aldrin and Endrine)

Results of invasive screening device Chromatography (GC) are shown in Table (2). This indicates contamination No.1. (Milk pasture) Lindane nets (Figure 3) presents Chromatography and GC-invasive as a concentration of (5.92) ppb. within allowed limits to presence in milk, which bound by Food Agriculture Organization and World Health Organization (14).

Figure (4) showed contamination in the samples No. (3 and 14) PP-DDD as concentration (4.61) and (2.60) ppb, respectively, in the limits permitted (FAO / WHO). While the results gave the contamination sample No.5 (Pienaar liquid milk) pesticide Endrine (Figure.5) concentration (9.687) ppb higher than the limit permitted by (FAO /WHO), a (0.8) ppb. and the sample No.10 (Nido milk) pesticide contamination of Heptachlor (Figure.6) concentration (2.94) ppb within the limits permitted by (14). No.17 contaminated with nets (PO-DDT) (Figure 7) concentration of (20.53) ppb higher than. Which are the allowed limits according to the (14).

In addition, results showed that two Samples, No. (16 and 18) were contaminated by Aldrin (Figure. 8), with concentrations (5.94 and 12.70) ppb. While sample No. 18 concentration is higher than the allowed limits. 10 samples were contaminated with low concentrations of organic chlorinated pesticides remains, than detection limits (UDL).

Table (2): Results of detecting chlorinated organic pesticide residues in samples of whole milk

No.	Samples name	Pesticides concentration (ppb)						EMRL (ppb) WHO/FAO in milk
		Lindane	PP-DDD	Heptachlor	PO-DDT	Endrine	Aldrin	
1	Almarai	5.9	*	*	*	*	*	10
2	KDD (liquid)	*	*	*	*	*	*	-
3	Alary (liquid)	*	4.6	*	*	*	*	20
4	Alsafy (liquid)	*	*	*	*	*	*	-
5	Pienaar (liquid)	*	*	*	*	9.7	*	8
6	Mersin (liquid)	*	*	*	*	*	*	-
7	Beyti (liquid)	*	*	*	*	*	*	-
8	Nadi (liquid)	*	*	*	*	*	*	-
9	Nadi banana flavor (liquid)	*	*	*	*	*	*	-
10	Nido (Powder)	*	*	2.9	*	*	*	6
11	Landuze Milk (Powder)	*	*	*	*	*	*	-
12	Abu Alques milk (Powder)	*	*	*	*	*	*	-
13	Dielac Milk (Powder)	*	*	*	*	*	*	-
14	Almudhish milk (Powder)	*	2.6	*	*	*	*	20
15	Iraqi Cow's milk (Powder)	*	*	*	*	*	*	-
16	Canon strawberry flavored milk (liquid)	*	*	*	*	*	5.9	6
17	Alraiyy milk (liquid)	*	*	*	20.5	*	*	20
18	Milko Milk (Powder)	*	*	*	*	*	12.7	6

* : UDL Below the detection limits ppb: part pair billion ($\mu\text{g}/\text{kg}$ or $\mu\text{g}/\text{L}$)

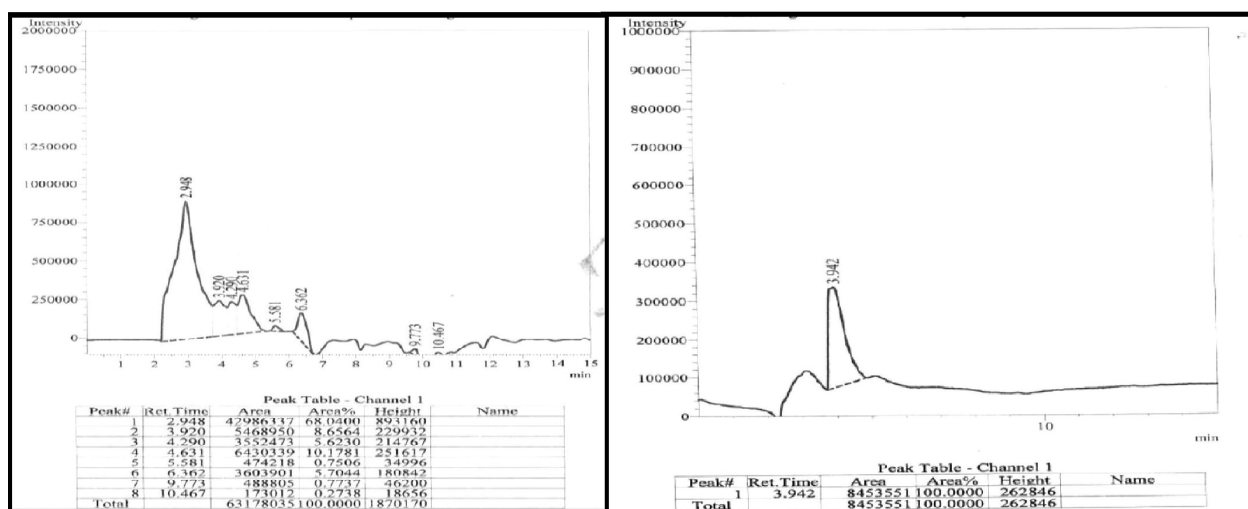


Figure (3): chromatogram GC sample number 1 (milk Almarai) shows the pesticide contamination and standard pesticide (Lindane)

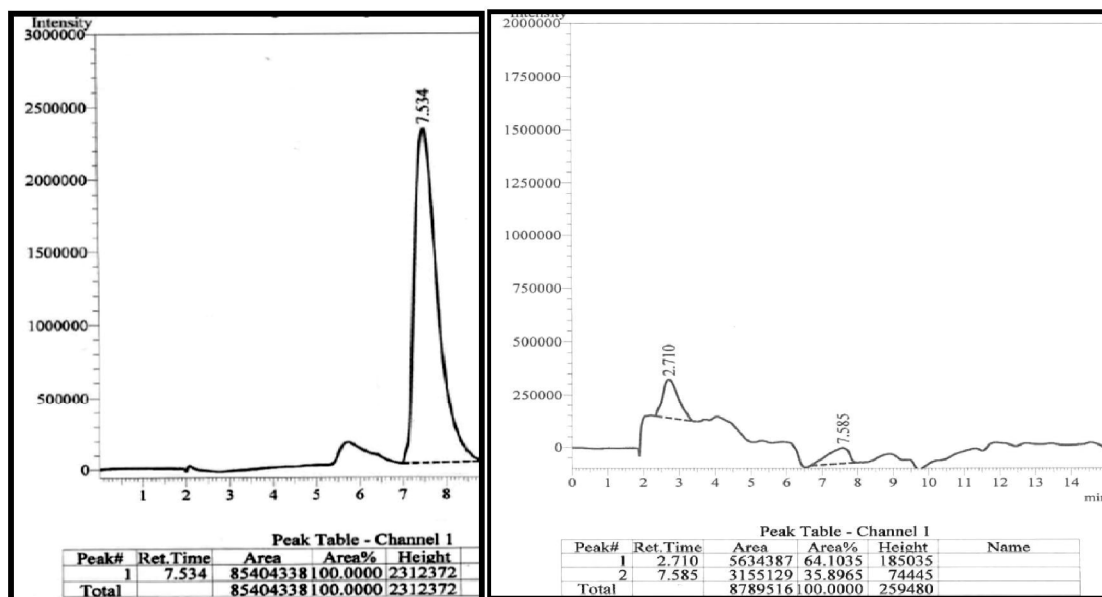


Figure (4): chromatogram GC sample number 3 (milk Airy) shows the pesticide contamination (PP-DDD)

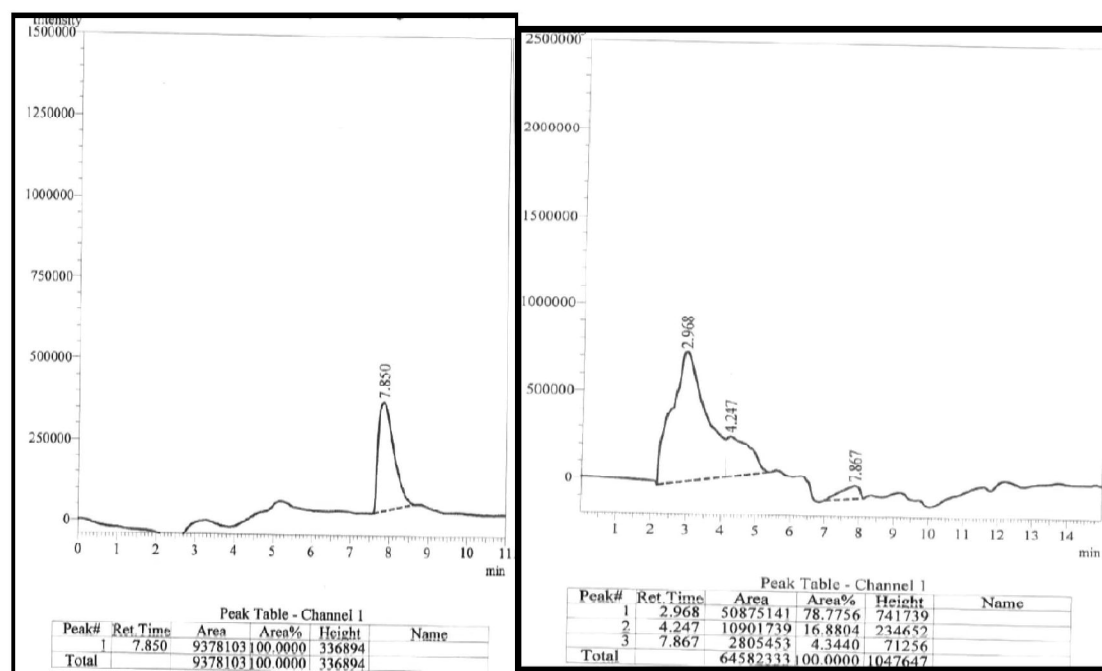


Figure (5): chromatogram GC sample number 5 (milk Pinaar) shows the pesticide contamination (Endrine)

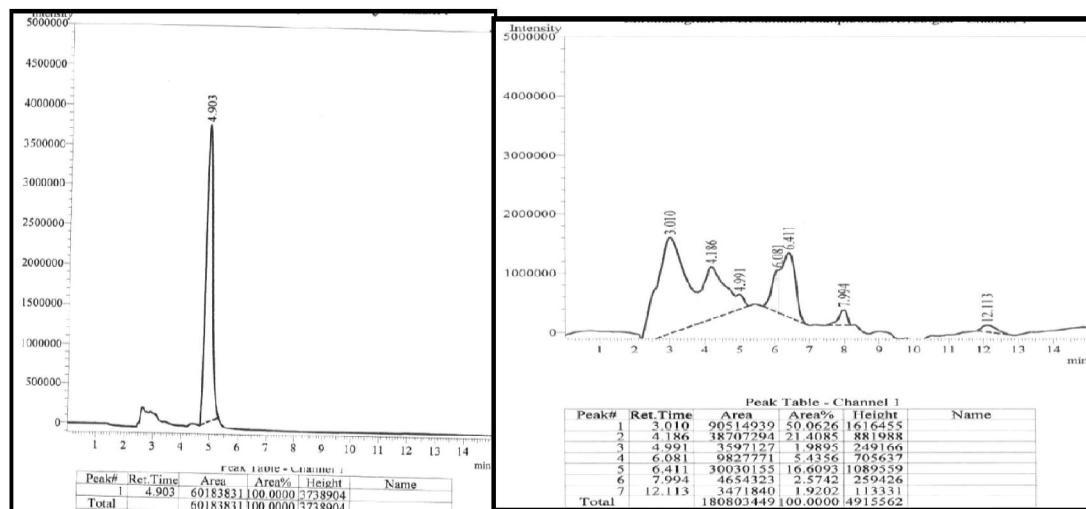


Figure (6): chromatogram GC sample 10 (milk Nido) shows the pesticide contamination (Heptachlor)

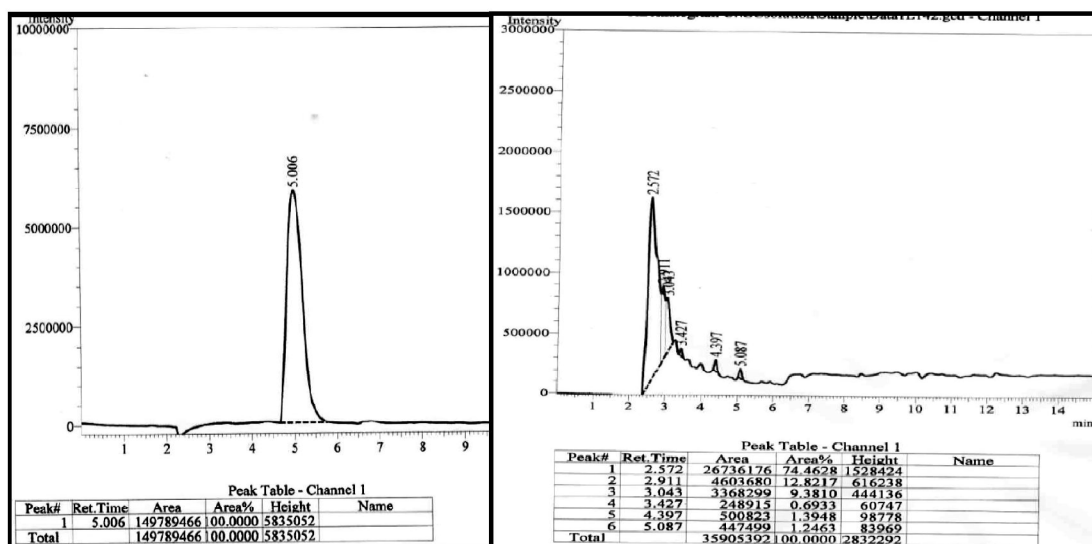


Figure (7): chromatogram GC sample16 (milk canon by strawberry flavor) shows the pesticide contamination (Aldrin)

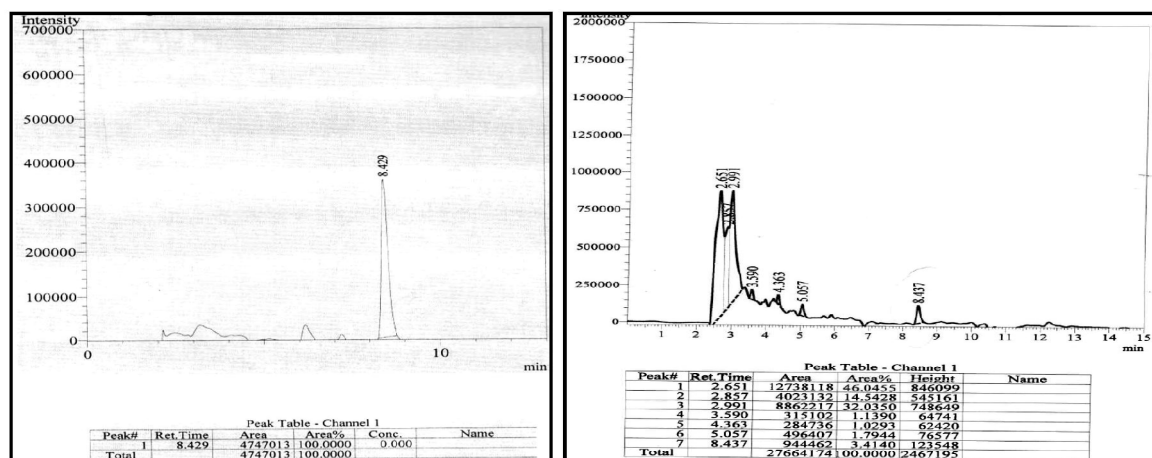


Figure (8): chromatogram GC model 17 (milk Alraay) shows the pesticide contamination (PO-DDT)

Regarding the obtained results, the existence of contamination with organic chlorinated pesticides in some trademarks of whole milk are within the allowed limits. However, even if the concentrations are relatively under detection limits, but they have ability to accumulate in the body to become effective after a period of time, which lead to the occurrence of several medical problems to human health.

RECOMMENDATIONS

- 1- There must be a scientific method depending on needs to use of organic chlorinated pesticides in pest control in agricultural land according to scientific standards.
- 2- Using modern scientific methods to treat cows as milk producing animals from insects and parasitic diseases.
- 3- Control provisions of general stores and factories shopping whole milk in terms to validate food for consumption.
- 4- Introduction of modern analysis methods to regulate a monitor and detect a possibility of concentration for Organic chlorinated pesticides in imported milk or produced locally.

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Changes in humoral immunity in children suffering from chronic tonsillitis before and after tonsillectomy

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ABSTRACT

Immunoglobulin and complements represent the humoral immunity. These parameters (IgA, IgM, IgG, C3 and C4) had been measured in serum of forty children, aged 6 – 10 years, who were suffering from chronic tonsillitis before and two months after tonsillectomy. These patients were attending the ENT department in Baghdad Teaching Hospital and diagnosed by an ENT specialist surgeon. Thirty apparently healthy children were enrolled in this study as a control group, and submitted to investigations of the serum levels of the mentioned factors as well. The present study extended from March 2014 to September 2014.

Results revealed a statistically significant increase in serum levels of IgA, IgM, IgG, C3 and C4 of the patients group as compared to those of the control group. Furthermore, in this study it was found that tonsillectomy renders the immunoglobulin and complements normal; a finding attributed to the disappearance of constant antigenic stimulus from infected tonsils. In conclusion, the immunoglobulin and complements are elevated in serum of children suffering from chronic tonsillitis, and later they significantly dropped to their reference ranges after tonsillectomy.

Keywords: immunoglobulin, complements, adenotonsillitis, post-tonsillectomy

الملخص باللغة العربية

تمثل الأجسام المضادة (الجلوبيينات المناعية) والبروتينات المكملية المناعية الخلوية. في هذه الدراسة، تم قياس هذه المعايير (IgA, IgM, IgG, C3 and C4) في مصل الدم لأربعين طفلاً، تراوحت أعمارهم بين 6-10 أعوام، والذين كانوا يعانون من التهاب اللوزتين المزمن، حيث تم القياس قبل عملية استئصال اللوزتين وبعد شهرين من ذلك. هؤلاء المرضى كانوا يراجعون قسم الأنف والاذن والحنجرة في مستشفى بغداد التعليمي وتم تشخيصهم من قبل طبيب جراح اختصاصي بالأنف والحنجرة. كما تم تضمين ثلاثين طفلاً أصحاء في هذه الدراسة بمثابة المجموعة الضابطة، وخضعت إلى قياس مستويات العوامل المذكورة أيضاً. امتدت هذه الدراسة من شهر آذار/ مارس 2014 إلى أيلول/ سبتمبر 2014. وقد بينت النتائج زيادة ذات دلالة إحصائية في مستويات (IgA, IgM, IgG, C3 and C4) لمجموعة المرضى مقارنة مع المجموعة الضابطة. وعلاوة على ذلك، بينت هذه الدراسة أن استئصال اللوزتين يعيد الجلوبيينات المناعية والبروتينات المكملية إلى مستوياتها الطبيعية، وهو ما يمكن أن يعزى إلى اختفاء التحفيز المتواصل ضد المستضدات المتأثرة من اللوزتين المصابة. كاستنتاج، يمكن القول أن الأجسام المضادة (الجلوبيينات المناعية) والبروتينات المكملية تكون ذات مستويات مرتفعة في مصل الأطفال الذين يعانون من التهاب اللوزتين المزمن، وتنخفض إلى مستوياتها الطبيعية بشكل ملحوظ بعد استئصال اللوزتين.

INTRODUCTION

The tonsillar ring (Waldeyer's ring) is composed of a series of lymphoepithelial "organs" called the tonsils. This tissue is structurally similar to lymph nodes but lacks afferent lymphatic vessels. The tonsils are named for their location, consisting of a *pharyngeal tonsil*, the paired *palatine tonsils*, and the unpaired *lingual tonsil* at the base of the tongue (1).

Palatine tonsils are patches of lymphatic tissue located in a ring about the pharynx (2). They appear to function as the host's first line of defense against exogenous microorganisms (3).

The tonsils and adenoid are lymphoid organs that participate in the mucosal immune system of the pharynx. They are positioned strategically at the entrance of both the respiratory and gastrointestinal tracts, where they serve to initiate immune responses against antigens entering the body through the mouth or nose. As such, myriad infectious and inflammatory disorders are manifest in the tonsils and adenoid. This entry serves to review some of those disorders that are of the greatest importance to the otolaryngologist (4). The tonsils are lined with squamous epithelium. This lining forms crypts that extend well into the body of the tonsil, where pus and debris can collect. Both adenoids and tonsils are especially well developed in children and reduce in size as the child gets older (5).

Tonsillitis is the inflammation of the tonsils most commonly caused by viral, bacterial infection, allergies and respiratory problems (6). This process usually begins with a sudden sore throat, painful swallowing and tonsils cause's throat tissues to swell obstructing air from passing in and out of the respiratory system. When inflamed, tonsils become swollen and red with a grayish or yellowish coating on its surface (7).

Clinical features:

- high temperature (fever) and chills
- coughing
- headache, tiredness and a general sense of feeling unwell (malaise)
- white pus-filled spots on the tonsils
- swollen lymph nodes (glands) in the neck
- pain in the ears or neck
- weight loss and difficulty ingesting and swallowing meal/liquid intake

Less common symptoms include:

- nausea, vomiting and fatigue
- stomach ache
- furry tongue, bad breath (halitosis) and voice changes
- difficulty opening the mouth (trismus)
- loss of appetite (anorexia) and anxiety/fear of choking (10, 11).

Several studies showed high serum levels of immunoglobulin, particularly IgG and IgA, in patients with chronic and chronic tonsillitis (12,13). On the other hand, other reports demonstrated that inflammation and/or hypertrophy of adenoids and tonsils are caused by hypofunction of local and systemic immunity (12, 13).

The aim of this study was to demonstrate the humoral immunity state (IgA, IgM, IgG, C3 and C4 serum levels) in children suffering from chronic tonsillitis, before and two months after tonsillectomy, and to monitor the effect of tonsillectomy on these parameters

PATIENTS AND METHODS

A total of forty (40) patients (22 males and 18 females, aged 6 - 10 years, with mean age of 7.52 ± 1.24 years) with chronic tonsillitis, attending ENT department in Baghdad Teaching Hospital were enrolled from March 2014 through September 2014. They had been clinically diagnosed as having chronic tonsillitis and tonsillar bacterial infection was confirmed using standard culture methods. The ENT specialist surgeon planned to go on tonsillectomy for them. Besides, thirty (30) apparently healthy age-matched children were enrolled as a control.

Immunologic analysis:

A volume of 5 ml venous blood samples were aspirated from patients one day prior to surgery (pre-operative), and 2 months after (post-operative) in a disposable dry plane tubes. Sera were obtained after clotting and separation of centrifuged blood samples. They were immediately freezed at -20°C up to the time of immunologic analysis. Investigations of serum levels of IgA, IgM, IgG and complements (C3 and C4) were done for patients and control groups using the commercially available kits for quantitative measurement, namely the radial immunodiffusion plates (LTA - Italy). These tests were performed according to the procedure protocol included within the kit packing as issued from the manufacturer company.

Statistical analysis:

Sciences (SPSS) version (15.1) and *STSTATISTICA* program version (10.1). A comparison between case and control group was done using a one-way Anova test in regard to immunoglobulin levels and complements, while a comparison among the cases group was done using an independent – sample T test. A probability value (P) of < 0.05 was considered significant and used in the study.

RESULTS

When a comparison was done between pre-operative patients' serum levels of IgA, IgG, IgM, C3 and C4 and those of control group, statistically strong significant differences were noticed, as shown in tables (1-5) respectively. It was observed that the mean pre-operative values of the mentioned immunologic parameters were significantly higher in patients before surgery than those of the control group ($P < 0.05$).

Table (1): Comparison of the immunoglobulin levels (IgA) between children suffering from chronic tonsillitis and control group

Case- control groups	Immunoglobulin levels (IgA)	
	Mean \pm St. Error	St. Deviation
Pre- operation	91.65 \pm 3.128	19.785
Post- operation	27.40 \pm 1.411	8.926
Controls	18.60 \pm 1.701	9.317

$F = 305.0001$, $df = 2$, $P = 0.000$

Table (2): Comparison of the immunoglobulin levels (IgG) between children suffering from chronic tonsillitis and control group

Case- control groups	Immunoglobulin levels (IgG)	
	Mean \pm St. Error	St. Deviation
Pre- operation	1458.00 \pm 43.315	273.946
Post- operation	415.88 \pm 26.333	166.544
Controls	529.60 \pm 40.862	223.809

$F = 248.248$, $df = 2$, $P = 0.000$

Table (3): Comparison of the immunoglobulin levels (IgM) between children suffering from chronic tonsillitis and control group

Case- control groups	Immunoglobulin levels (IgM)	
	Mean \pm St. Error	St. Deviation
Pre- operation	204.98 \pm 24.939	157.730
Post- operation	55.50 \pm 4.306	27.233
Controls	52.70 \pm 4.288	23.486

$F = 30.462$, $df = 2$, $P = 0.000$

Table (4): Comparison of the complement levels (C3) between children suffering from chronic tonsillitis and control group

Case- control groups	Complement levels (C3)	
	Mean \pm St. Error	St. Deviation
Pre- operation	185.18 \pm 2.817	17.814
Post- operation	118.88 \pm 2.304	14.571
Controls	112.57 \pm 5.440	29.795

$F = 140.530$, $df = 2$, $P = 0.000$

Table (5): Comparison of the complement levels (C4) between children suffering from chronic tonsillitis and control group

Case- control groups	Complement levels (C4)	
	Mean \pm St. Error	St. Deviation
Pre- operation	86.05 \pm 2.093	13.239
Post- operation	33.67 \pm 1.246	7.879
Controls	29.57 \pm 1.263	6.917

$F = 376.410$, $df = 2$, $P = 0.000$

Concerning the differences between pre- and post-operative patients' serum levels of IgA, IgG, IgM, C3 and C4, statistically strong significant differences were observed in this study. IgA, IgG, IgM, C3 and C4 significantly decreased two month after tonsillectomy ($P < 0.05$). Nevertheless, they were within the reference range levels for each (Tables 6-10) respectively, where we can notice the considerable mean differences.

Table (6): Comparison of the immunoglobulin levels (IgA) in children suffering from chronic tonsillitis before and two months after tonsillectomy

Case groups	Immunoglobulin levels (IgA)		
	Mean \pm St. Error	St. Deviation	Mean difference
Pre- operation	91.65 \pm 3.128	19.785	64.250
Post- operation	27.40 \pm 1.411	8.926	

$t = 18.721$, $df = 78$, $P = 0.000$

Table (7): Comparison of the immunoglobulin levels (IgG) in children suffering from chronic tonsillitis before and two months after tonsillectomy

Case groups	Immunoglobulin levels (IgG)		
	Mean \pm St. Error	St. Deviation	Mean difference
Pre- operation	1458.00 \pm 43.315	273.946	1042.125
Post- operation	415.88 \pm 26.333	166.544	

$t = 20.558$, $df = 78$, $P = 0.000$

Table (8): Comparison of the immunoglobulin levels (IgM) in children suffering from chronic tonsillitis before and two months after tonsillectomy

Case groups	Immunoglobulin levels (IgM)		
	Mean \pm St. Error	St. Deviation	Mean difference
Pre- operation	204.98 \pm 24.939	157.730	149.475
Post- operation	55.50 \pm 4.306	27.233	

$t = 5.906$, $df = 78$, $P = 0.000$

Table (9): Comparison of the complement levels (C3) in children suffering from chronic tonsillitis before and two months after tonsillectomy

Case groups	Complement levels (C3)		
	Mean \pm St. Error	St. Deviation	Mean difference
Pre- operation	185.18 \pm 2.817	17.814	66.300
Post- operation	118.88 \pm 2.304	14.571	

$t = 18.220$, $df = 78$, $P = 0.000$

Table (10): Comparison of the complement levels (C4) in children suffering from chronic tonsillitis before and two months after tonsillectomy

Case groups	Complement levels (C4)		
	Mean \pm St. Error	St. Deviation	Mean difference
Pre- operation	86.05 \pm 2.093	13.239	52.375
Post- operation	33.67 \pm 1.246	7.879	

$t = 21.501$, $df = 78$, $P = 0.000$

DISCUSSION

Tonsillitis is an infectious process causing fever, sore throat, odynophagia, and malaise. Oropharyngeal erythema, edema, exudates, associated rash or lymphadenopathy may be present. Children who experience recurrent episodes of acute tonsillitis may be considered for surgical treatment (14).

The tonsils represent the first site of contact with a variety of microorganisms and other antigenic substances present in food and inhaled air (15). Hence, they have an important role in body defense, a defense that can be elicited by immunoglobulin and various plasma proteins. As secondary lymphatic organs, tonsils are concerned with antigen processing. Small amounts of antigen are transported through the reticular cell epithelium by M cells and antigen-presenting cells (APC), with the pronounced role of T (helper and cytotoxic T cells) and B lymphocytes. They probably play a major role in local immunity (16-18).

Tonsillar tissue has the ability to mount specific immune reactions in response to various antigens. The activity of this lymphatic organ is especially pronounced during childhood, when immunologic challenges from the environment induce hyperplasia of the palatine tonsils (1). That immune response in such cases was monitored by some researchers (10, 11, 19, 20).

In this study it was observed that the serum levels of IgA, IgG, IgM, C3 and C4 of pre-operative patients were significantly elevated as compared to those of control group (Tables 1, 2, 3, 4 and 5 respectively). Clearly, this finding is in agreement with some other reports when they studied immunoglobulin level alteration in chronic tonsillitis in children (10, 11, 20) and in cases of hypertrophy of adenoids and tonsils compared with those of the control group before operation (16). That increment can be attributed to ongoing antigenic stimulation in infected tonsils. This explanation is consistent with that of some studies (18, 21).

In sick tonsils, increased presence of antigens leads to proliferation of B lymphocytes, a subset of lymphocytes, which are capable of migrating to adjacent tissues and producing antibodies (22).

In patients enrolled in this study, an important comparison between pre-operative and 2 months post-operative status of their serums immunoglobulin and complements was held. It was obvious that there were statistically significant differences between the two occasions. IgA, IgG, IgM, C3 and C4 were significantly reduced after two month of operation. Though, they were within the reference ranges (Tables 6, 7, 8, 9 and 10 respectively). As an interpretation of these findings, the decreased stimulation of the immune system after surgical removal of the tonsils is the feature. The immunity parameters retained normal probably due to the cessation of the offending process, i.e., the recurrent infection of the tonsils.

These results can be considered as consistent with some other studies (16, 19, 20, 23). A number of researchers showed that there is a significant decrease in values of IgM, IgG, IgA in sera of children postoperatively. They assured that those values have retained normal at least 2-4 months after surgery (24-26).

Complements are considered as the major effector of the humoral branch of the immune system. They clearly play a key role in both innate and adaptive immunity and they are important mediators of the acute inflammatory response (27).

Regarding the postoperative complement values, M. Sainz *et al.* (24) disagreed with the finding of the present study. They concluded that there were no change in the levels of serum C3 and C4 before and after tonsillectomy.

However, some investigators reported that patients with chronic tonsillitis have high levels of IgG and IgA with unsignificantly altered IgM levels in their serums, and the following two months after surgical removal of tonsils revealed significant reduction in levels of serum IgG and IgA (21). Other studies disagreed with the present study. The authors concluded that there is no significant differences between pre and postoperative in immunoglobulin values and were thought that the ending of continuous bacterial antigen stimulation of tonsillar tissue might be responsible for slightly decrease in serum values of IgM, IgG and IgA (28-30).

CONCLUSION

The parameters of the humoral immunity, namely the immunoglobulin (IgA, IgM and IgG) and complement proteins (C3 and C4), are found elevated in serum of children suffering from chronic tonsillitis. After tonsillectomy, those parameters significantly dropped to their reference ranges, due to the disappearance of the continuous stimulation caused by chronic infection of tonsillar tissue. This change obviously does not seem to affect the competency of the immune system. Hence, we can consider the elevated serum levels of the humoral immunity factors as potentially useful monitors in evaluation of the chronic tonsillitis cases in regards to severity and indication of surgical removal of the affected tonsils.

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Serological detection of *Toxoplasma Gondii* antibodies and its effect on thyroid function in non- pregnant women

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ABSTRACT

The current study was designed to detect the toxoplasma infection in non- pregnant women in Al-Diwaniya province and to investigate the effect of toxoplasma infection on thyroid function. One hundred thirty eight blood samples were collected from non- pregnant women (15 ≥ 35 years old) who visited maternity and children teaching hospital in Al-Diwaniya province.

Samples were tested for the presence of toxoplasma IgG and IgM by ELISA and the results showed that : (57.2%) seventy nine samples gave positive to anti-toxoplasma IgG, while 16 samples (11.6%) were positive to anti-toxoplasma IgM. The highest IgG and IgM seroprevalence were among participants age (≥30) years. Infected women with toxoplasmosis had significant lower serum level of TSH ($0.52 \pm 0.06 \mu\text{IU/L}$) Compared to control group ($4.45 \pm 0.07 \mu\text{IU/L}$) while there is non-significant increment in (T3 and T4) concentration compared with control group at ($P \leq 0.05$).

Keywords: Toxoplasmosis, thyroditis, thyroid hormones, ELISA

المخلص باللغة العربية

صممت الدراسة الحالية للكشف عن الإصابة بداء المقوسات عند النساء الغير حوامل في محافظة الديوانية والكشف عن تأثير داء المقوسات على وظيفة الغدة الدرقية.

تم جمع 138 عينة دم من النساء الغير حوامل تراوحت أعمارهن ما بين (15 إلى 35) سنة فأكثر، واللاتي راجعن مستشفى الولادة والأطفال التعليمي في محافظة الديوانية، وقد خضعت جميع العينات لفحص الإليزا للتحري عن وجود الجلوبيولينات المناعية (G و M) وأظهرت النتائج أن تسعة وسبعون عينة بنسبة (57.2%) أعطت نتيجة موجبة للجلوبيولين المناعي (G) بينما كانت ستة عشر عينة بنسبة (11.6%) ذات نتيجة موجبة بالنسبة للجلوبيولين المناعي (M). كما أظهرت النتائج أن أعلى نسبة انتشار مصلي للجلوبيولينات المناعية (G و M) كانت في الفئات العمرية من (30) سنة فأكثر. لوحظ انخفاض معنوي في هرمون TSH في مصل النساء المصابات بداء المقوسات بنسبة (0.52 ± 0.06 ميكرو وحدة دولية/ لتر) مقارنة مع مجموعة السيطرة (4.45 ± 0.07 ميكرو وحدة دولية/ لتر)، بينما لم تسجل أي زيادة معنوية في تراكيز (T3 و T4) مقارنة مع مجموعة السيطرة بمستوى احتمالية ($P \leq 0.05$).

INTRODUCTION

Toxoplasmosis, one of the most common zoonotic diseases worldwide, can induce various hormonal and behavioral alterations in infected hosts(1). It has a complex life cycle and causes a wide variety of symptoms in infected individuals. Infection in humans follows course of active replication of the parasite and dissemination of the infection throughout the body followed by encystment in the brain, retina, and other organs as latent tissue cysts(2).

Feline including domestic cat act as definitive host and various warm-blooded animals as well as human, act as an intermediate host. The infection in human generally occurs through consuming food or drink contaminated with oocysts or tissue cysts(3).

Several techniques employed for diagnosis of toxoplasmosis including coprological (feces), histological (tissues), bioassay (inoculation of cat and mice) and serological tests including dye test, indirect haemagglutination (IHA), latex agglutination test (LAT), Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) (4,5).

Serological diagnosis of acute toxoplasmosis is based on the demonstration of a significant increase in specific IgG antibody levels and / or the presence of specific IgM antibodies. However, the prevalence of high toxoplasma IgG antibody titers among normal individuals in most population and the sustained persistence of specific IgG antibodies in some persons have complicated the interpretation of serological tests when acute toxoplasmosis is suspected (6). The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery (7).

Toxoplasma infection is complex and depends on the genetic background of the host, his immune status and also parasite factors including virulence. Toxoplasma have the capacity to spread in all the tissues and each tissue compartment has its own specific immune response (8).

Thyroid is an endocrine gland, located immediately below the larynx on either side of and anterior to the trachea. The principal hormones of thyroid gland are Thyroxine (T4) and Triiodothyronine (T3) and their concentrations are 93% and 7% respectively (9).

Inflammation of the thyroid tissue is called thyroiditis. According to its etiology, thyroiditis can be classified into autoimmune diseases) and non-autoimmune (including a cute, subacute and chronic thyroiditis). Subacute thyroiditis is caused by the action of infectious agents clinically, goiter, dysphonia and dysphagia can be observed. In human, each of these types can be characterized by its clinical development and the causative aetiological agent (10). A study was conducted involving 1248 pregnant women, had provided an evidence for the existence of an association between latent toxoplasmosis and thyroid autoimmunity and thyroid function in pregnancy (1). In addition, the

impairment of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) secretion as well as decreased serum thyroxine (T4) had been reported in *T. gondii* -infected mice (11). Another study concluded that toxoplasmosis affects the thyroid morphology, being able to alter its function with the development of autoimmune thyroiditis in susceptible individuals (10).

In Iraq, until now, no study had addressed the effect of toxoplasmosis on thyroid hormone levels in non-pregnant women. Thus, the current study aimed to investigate the effect of toxoplasma infection on thyroid function.

PATIENTS AND METHODS

The current study was carried out on 138 women attending outpatient gynecology of the Maternity and Children Teaching Hospital in Al-Diwaniya Province during the period from May to October 2014.

The study group comprised 138 suspected women who attended the Maternity and children teaching hospital in Al-Diwaniya. Their ages ranged from (15-40) years and 25 control normal women who had no history of toxoplasmosis. Their ages ranged from (17-39) years.

Statistical analysis:

Statistical package for social sciences (SPSS) software was used to analyze data. T-test of independence was used to find out the significant differences between means of the two groups (infected women and control). TSH, T3 and T4 concentrations in the serum were expressed as Mean \pm Standard deviation (SD). Statistical significance was set at a P value ≤ 0.05 .

Serological testing:

Blood samples were obtained and serum was separated and stored at -20°C until processing. The sandwich ELISA kits were as follows:

1. Toxoplasma IgG ELISA kit from BIOTEC Laboratories Ltd. (UK).
2. Toxoplasma IgM ELISA kit from BIOTEC Laboratories Ltd. (UK).

Instructions supplied by the manufacturers were followed exactly. The cut-off value of the assay was calculated and results were expressed in an index by dividing sample absorbance by the cut-off value. The test was considered negative if the index was < 7.2 , the result was equivocal, when index was from 7.2 to ≤ 8.8 , while the positive result was if index was > 8.8 . A negative reaction indicates absence of significant *Toxoplasma* antibodies. A positive *Toxoplasma* IgG reaction was interpreted as an indication of either a past or recent infection.

Biochemical kit:

All patients and 25 healthy control group were sent for thyroid function tests assay by IRMA (immunoradiometric assay) methodology in Radio Active isotope clinical Laboratory/ Baghdad – Harthia.

TSH : the p^{25} labeled signal – antibody binds to an epitope of (TSH) molecule especially different from that recognized by biotin –capture- Ab , the formation of a capture- Ab –Ag signal (Ab-Complex) also referred to as a sandwich "that measured the concentration of (TSH) in gamma counter (12).

Thyroxine "T4" is measured by I^{125} RLA system , this assay is based on competition between unlabeled "T4" and fixed quantity of I^{125} labeled "T4" for limited number of binding sites or "T4" specific Abs. Triiodo thyroxine "T3" Level was determined by using I^{125} RIA system with the same principle (13).

RESULTS AND DISCUSSION

Out of the 138 non- pregnant women, 79(57.2%) were T.gondii IgG seropositive (Table1). Twenty one of them, demonstrated strong responses manifested by high IgG concentrations (>240 IU/ml), whereas the remaining 44 women gave only weak IgG responses (8.8-100 IU/ml).

Table (1): Toxoplasma seropositivity rates (IgG, IgM) in non- pregnant women

Age group (yr)	No. of women tested	Immunoglobulin			
		IgG		IgM	
		No.	%	No.	%
15-19	10	6	7.6	0	0
20-24	17	10	12.6	1	6.3
25-29	28	13	16.5	3	18.6
30-34	38	24	30.4	6	37.5
≥35	45	26	32.9	6	37.5
Total	No.	138	79	16	-
	%	100	57.2	11.6	-

The obtained results were not much differed from that revealed by (14) in Kuwait and (15) in Jordan, which were 58% and 54% respectively. Moreover, lower figures were reported in Saudi pregnant women by (16) and in UAE by (17) (35.6%, 24.2%) respectively.

Mahdi *et al.* (18) in Iraq found that percentage of toxoplasmosis was (49.2%), Abbas *et al.* (19) in Saudi Arabia and Elmansouri *et al.* (20) in Morocco, all of them showed rates of 51.2% and 50.6% respectively.

As the current study employed quantitative ELISA assay, it supplied reliable evidences on the bad role of toxoplasmosis on women.

Forty-four (31.9%) women presented with weak IgG responses. Low IgG titers indicated either an old infection or waned antitoxoplasma immune responses. Twenty-one (15.2%) women showed

high IgG seropositivity, probably because a recent or a chronic illness reactivated infection. Of the high responders, ten women were IgM positive indicating that the infections were indeed recent ones. The rest women were IgM negative and probably indicated a chronic infections.

The prevalence rate of *T. gondii* infection is highly variable according to the group of people if they are adults, children, infants, women, men and pregnant or non- pregnant. Even within each of these groups, the variation in the prevalence rate of *T. gondii* infection is also high which is largely affected by many factors such as the geographical distribution, climatic condition, eating habits, contacts with animals, hygienic condition, sensitivity and specificity of various employed serological methods and/or researchers differences in interpretation of a serological techniques (21).

Results in table (1) showed that T.gondii IgG seropositivities were found to increase with age. Indeed, in the 15-19 years age group, only six (7.6%) were T. gondii IgG seropositive. In the 20-24 years age group, 10 (12.6%) were seropositive and in the 25-29 years age group ,13 (16.5%) were seropositive, whereas in the 30-34, ≥ 35 years age group, 24(30.4%), 26(32.9%) were seropositive respectively . In respect to IgM profiles depicted in table (1), sixteen (11.6%) of the recruited women were T.gondii IgM seropositive. All of them were T.gondii IgG positive with different concentrations. IgM seroprevalences in relation to age indicated that one (6.3%) out of the 16 positive women were between 20-24 years. Another three (18.6%) ranged between 25-29 years, whereas the other six (37.5%) belonged to both age groups 30-34 and ≥35 years. These results are similar to many earlier results (22-25). With increasing age, risk of acquiring toxoplasmosis as well its harmful pathological sequelae become higher and more possible.

In the current study, the Mean±SD for TSH was 0.52 ± 0.06 μ IU/L with range of 0. 1- 1.2 μ IU /L. The Mean±SD for T3 was 3.48 ± 0.14 ng/dl with range of 2.3-4.8 ng/dl and for T4 it was found to be 1.88 ± 0.32 ng/dl with range of 0.8-3 ng/dl in infected women, while Mean±SD for TSH was 4.45 ± 0.07 μ IU/L with range of 3.8-5.1 μ IU /L. The Mean±SD for T3 was 3.12 ± 0.11 ng/dl with range of 2.1-4.1 ng/dl and for T4 it was found to be 1.4 ± 0.05 ng/dl with range of 0.8-1.8 ng/dl in control women . Infected women with toxoplasmosis had significant lower serum level of TSH (0.52 ± 0.06 μ IU/L) Compared to control group (4.45 ± 0.07 μ IU/L) but no significant increase in value T3 and T4 when compared with control group at ($P \leq 0.05$) (Table 2).

Table (2): Effect of infection on serum concentration of thyroid hormones in non- pregnant women

Hormone	Infected women	Healthy control
T3	3.48±0.14 A	3.12± 0.11 A
T4 (pmol/L)	1.88±0.32 A	1.4± 0.05 A
TSH (ng/mL)	0.52±0.06 A	4.45±0.07 B

*No. of tested samples = 25 for each group

A study of the thyroid function reveals crucial low concentration of thyrotropine (TSH), normal or slightly high levels of thyroxine (T4) and (T3). This may be caused by therupture or loss of thyroid follicles, thus depleting any reserve of hormones stored in the colloid of the thyroid follicles (10). Being a pathology provoked by an infectious agent of intracellular location, the immunologic response is mainly cell mediated (26), triggering via the TCD8 lymphocyte cytotoxicity) as well as the TCD4. The production of interleukin 12 (IL-12) by the macrophage activates the T helper 1 (Th1) lymphocyte which participates in the cell mediated immunity. In turn, the Th1 releases gamma interferon (Inf- γ) and IL-10, contributing to this type of response (27). Both IL-12 and Inf- γ are released during the acute phase of toxoplasmosis. The release of other interleukins, such as IL-6 as well as tumour necrosis factor alpha (TNF- α) is also described. These cytokines have been claimed to affect thyroid glands, causing a reduced gland function (28,29). Also studies in Nylar female mice infected with *T. gondii*, exhibited hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction (30,31). These mice infected with *T. gondii* Cornell strain, present atrophy in the thymus, ovaries, and uterus, cessation of cycling, anovulation, and decline of serum thyroxine (T4) levels (30).

CONCLUSION

From the current study, it can be concluded that the toxoplasmosis in non-pregnant women have crucial role on thyrotropine (TSH), and normal or slightly appreciable effects on the level of (T3) and (T4).

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Study the role of Th-17 cells in the development of bladder cancer in sample of Iraqi patients

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ABSTRACT

The contributions of Th17 involvement in bladder tumour progression are the subject of intense investigation in Iraqi patients with bladder cancer. The aim of current study is to investigate the tumour-infiltrating inflammatory cells in bladder tumours, focusing on transcription factors defining effector [Th17], on archived bladder tumour tissue (before treatment), and compared it with benign tumour condition. Eighty six paraffin-embedded bladder tissue were obtained from patients with malignant tumour (transitional cell carcinoma). Beside twenty control bladder tissue were obtained from patients with benign bladder tumour. Antigen retrieval from deparaffinized bladder tissue was obtained through the combined use of high temperature and appropriate buffers. Expression of Th17 cells, was determined using a previously defined semi-quantitative immunological scoring system using a polymeric conjugate system (EnVision, Dako). Results indicated that prominent Th17 positivity were present in the bladder tissue of both malignant and benign forms. However, Th17 positive cells were significantly higher in patients with malignant tumours than in benign forms ($p \leq 0.0001$). Also the study demonstrated that Th17 positive cells were significantly higher in patients with high grade than in low grade bladder carcinoma. In conclusion, higher expression of Th17 infiltrated cells in malignant indicated that Th-17 pro inflammatory was involved in pathogenesis of bladder cancer.

Keywords: Immunohistochemistry , Bladder cancer, Th-17

المخلص باللغة العربية

إن تواجد الخلايا التائية المساعدة من نوع 17 (Th-17) يمكن أن يساهم في تطور ورم المثانة ، وفي إطار التحري عن هذا النوع من الخلايا ، فقد جرى تنفيذ الدراسة الحالية على عينات من المرضى العراقيين المصابين بسرطان المثانة. هدفت الدراسة الحالية إلى التحري عن الخلايا الالتهابية المترشحة في الورم بشكل مركز على الخلايا التائية المساعدة من نوع 17 في ورم المثانة (قبل العلاج) ، ومقارنة الدراسة مع الورم من النوع الحميد . تم الحصول على 86 عينة نسيجية مغمورة في شمع البارافين للمرضى الذين يعانون من ورم خبيث (سرطان المثانة الانتقالي) ، كما تم الحصول على عشرين عينة لمرضى يعانون من ورم حميد وذلك لغرض المقارنة. تم إظهار الانتجينات الموجودة على أنسجة المثانة من خلال معاملتها بحرارة عالية ومحلول معادل مناسب . وقد تم تحديد التعبير عن هذه الخلايا بطريقة مناعية شبه كمية وهي طريقه نظام البوليمرات المقيدة ، حيث أوضحت النتائج تواجد هذا النوع من الخلايا المناعية في كلا النسيجين (الخبيث و الحميد) ، ولكن على الرغم من ذلك ، فإن أعداد هذه الخلايا المتواجدة في النسيج الخبيث كانت أعلى بكثير من النسيج الحميد بفرق معنوي عال ($p \leq 0.0001$). كما تبين أن أعداد هذه الخلايا في نسيج الورم الخبيث المتقدم تتواجد بشكل أكبر من نسيج الورم الخبيث البدائي ، حيث تم الاستنتاج أن تواجد الخلايا التائية المساعدة من نوع 17 (Th-17) المترشحة في الورم الخبيث بشكل كبير يعطي دليلا على أن هذه الخلايا تتواجد في بداية الالتهاب وقد تشترك في الإمبراضية لسرطان المثانة.

INTRODUCTION

Bladder cancer is the second most common urologic malignancy amongst the males after prostate cancer with most of the cases being diagnosed as urothelial carcinoma(1). Bladder cancer besides several physiological changes in and around bladder is also accompanied by immunological changes.

Th17 cells are defined as CD4+ T helper cells that secrete the cytokine IL-17 and whose developmental program is controlled by multiple cytokines (2) and the transcription factor retinoic acid receptor-related orphan receptor gamma T.

CD4 + T helper (Th) cells play a central role in orchestrating host immune responses through their capacity to help other cells of the immune system. More recently, a novel CD4 + T cell subset termed Th17 cells has been identified, which expresses the transcription factor retinoid-related orphan receptor (ROR)-gt and produce the pro inflammatory cytokine interleukin (IL)-17 (3,4). Although Th17 cells play a critical role in the pathogenesis of many inflammatory and autoimmune diseases (5,6) their prevalence among tumor-infiltrating lymphocytes (TILs) and function in human tumor immunity remain largely unknown. The results from two studies in prostate and ovarian cancer patients have suggested both beneficial and harmful implications of Th17 cells in tumor development (7,8). Apart from its pro inflammatory role, IL-17 up-regulates the production of a variety of proangiogenic factors, thus contributing to tumor angiogenesis and development. The basis for this discrepancy is not yet understood, and the presence or absence of the adaptive immune system has been suggested to account for it (9).

RORgt, member of the retinoic acid receptor-related orphan nuclear hormone receptor family, is a transcription factor expressed specifically in Th17 cells which plays an important role in directing Th17 differentiation and cytokine production (10).

Major Cytokines associated with Th17 cells are IL17A, IL-23 and IL-6. IL-17A is a pro inflammatory cytokine secreted by activated T cells. There are several roles attributed to this cytokine which includes regulation of the activity of NF-kB and mitogen-activated protein kinases (11-12).

Since discovery the role of the Th17 only 5 years ago, these cells have risen to prominence in studies of virology, autoimmune disease, inflammation, and immune responses to various parasites and fungi. Although their role in the pathogenesis of many of these conditions is rather well defined, their functions in the context of tumor immunology remains controversial. To begin with, it is imperative to stress that the cytokine interleukin (IL)-17 and the T-cell subset Th17. Recent studies indicated that Th17 cells are also implicated in tumor immunology. Significant increase in the number of Th17 cells has been reported in peripheral blood and

tumor tissues in various human malignancies including gastric and pancreatic cancer (13,14). Few studies have focused on primary Th17 cells in the human tumor microenvironment, so it is difficult to deduce the exact roles they may have in cancer patients. In the more exhaustive studies of patients with established epithelial cancer, Th17 presence and function have correlated with reduced tumor progression and improved patient survival (15).

PATIENTS AND METHODS

Patients:

The study included 106 patients (67 male

and 39 female) were classified in to two groups:-

Group 1 : 86 Urinary bladder carcinoma(UBC) patients (57 males and 29 females)with an average age of 53 years and a ranged of 26 to 80 years.

Group 2 : 20 patients with urinary bladder diseases (UBD) other than cancer were considered as control group (10 males and 10 females)with an average age of 52 years and a ranged of 32 to 78 years.

Patients were under suspicion for bladder cancer who were undergone cystoscopy over the period of study from May-2012 to May-2013. They were diagnosed clinically by consultant urologists at Ghazi Al-Harery Hospital / Medicine city / Baghdad based on a clinical evaluation and a histopathological examination.

Tissue biopsies:

Tissue biopsies from cases of bladder cancer, and cases of pathological urothelium control were fixed in 10 % buffered formalin and embedded in paraffin wax, and stained with hematoxylin-eosin. Urothelium samples were taken from patients who do not suffer from bladder carcinoma in the past during cystoscopy (without treatment) and they were taken as control (16).

Preparation of tissue sections:

Paraffin embedded sections of bladder cancer tumor or UTI tissues were cut into 4 µm thicknesses using a microtome. The sections were applied on Fisher-brand positively charged slides and left overnight to dry at room temperature (16).

Principle of the Assay:

This part of the study was performed at institute of Liver studies in King's College Hospital /London /UK as a part of research scholarship funded by the Iraqi Higher Education and Scientific Research.

The immunohistochemical staining techniques was used for visualization of tissues antigens by sequential reaction of a specific antibody (primary antibody) to its corresponding antigen (Th-17) tissue samples; then a secondary antibody was

added to the primary antibody and an enzyme complex with a chromogenic substrate, interposed by washing steps. The enzymatic activation of the chromogene results in a visible reaction product at the antigen site. The specimen counterstained and cover slipped, results are interpreted using light microscope (17) as shown in figure (1).

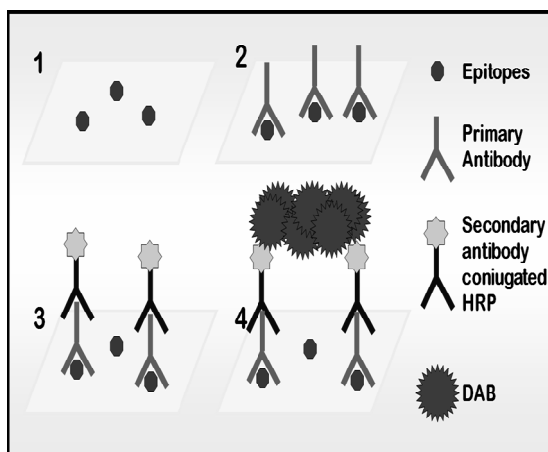


Figure (1): NovaLink™ Polymer Detection system (17)
DAB:- 3,3-diaminobenzidine, liquid and substrate chromogen system

Statistical analysis:

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage and Least significant difference –LSD test was used to significant compare between means in this study (18).

RESULTS AND DISCUSSION

Th-17 infiltrating cells were detected in the bladder of patients with cancer. The pattern of positive staining of Th-17 cells was cytoplasmic. The result of the positive staining of Th-17 was confirmed by using control positive staining in tonsils when applied the technique (avidin-biotin technique). Th-17 cells identified by positive anti-Th-17 reaction which is demonstrated at the lower part of the panel (Figure 2). The score of the positivity of the Th-17 staining cells ranged between 1-3 score. Various of score of positivity staining were shown in table (1). The number of the patient with UBC with immunohistochemistry staining of Th-17 cells (9/10 (90%) was higher than in UBD patients 1/9 (10%) with high significant difference ($p \leq 0.0001$) (Table 2). Interestingly the present study demonstrated that the frequency of Th-17 cells was prominently increased in tissue in patients with high grade with bladder cancer compared with patient with low grade disease.

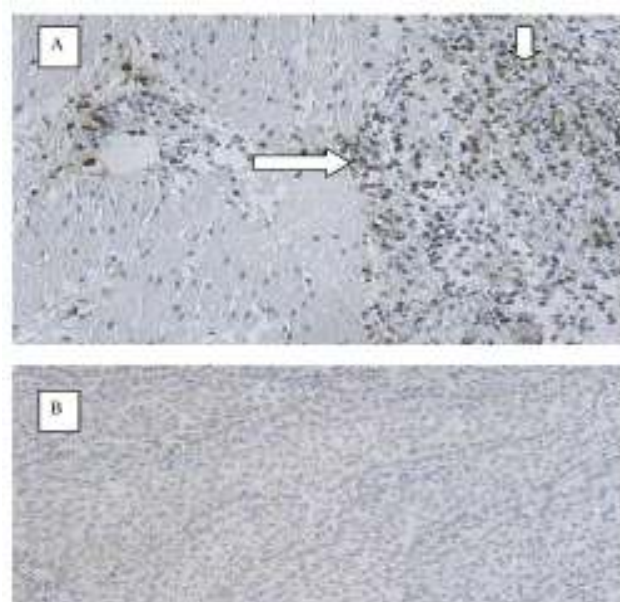


Figure (2):A /Invasive transitional cell carcinoma , poorly differential (Grade II) showing positive Th17 immunostaining (Score +++,brown)(arrow) (20X).

Figure (2):B /Invasive transitional cell carcinoma , poorly differential(Grade II)Showing negativeTh17 immunostaining (No Score) (10X)

Table (1): Frequency of Th17cells IHC scores in bladder patients groups

Th-17	Malignant		Benign (Control)	
	No.	%	No.	%
*0 (N)	9	10.47	13	65.00
**1 (#)	26	30.23	6	30.00
***2 (##)	34	39.53	1	5.00
****3 (###)	17	19.77	0	0.00

$P < 0.0008$ High significant

*0 (n) :- Negative: No stained cells

**1 (#):-The positive cells (stained with brown color) represented more than 10% of total cells.

***2(##):-The positive cells (stained with brown color) represented more than 30% to 50% of total cells.

****3 (###):-The positive cells (stained) represented more than 50% of total cells.

Table (2):IL17-cells production in bladder patients groups

Study groups		Th17 cells		Total
		Positive	Negative	
UBC	No.	77	9	86
	%	90	10	100
UBD	No.	7	13	20
	%	35	65	100
Total	No.	84	22	106
	%			100

$P < 0.001$ high significance

In terms of scores, UBC patients with the score +++ represented the frequency (22%), while the score ++ represented the frequency in UBD (26%). But the score + represented higher frequency in UBC (44%). However, table (3) showed the frequency distribution of Th17 cells IHC scores in group subjects. Microstate test showed that there was a high statistical difference ($p < 0.0008$) between urinary bladder carcinoma and other urinary bladder disorders for Th17 cells IHC scores in tissue samples taken from each case.

Table (3): Association between Th17 cells IHC expression with tumor grade

Parameters		TH-17 cell expression		Total
		Positive	Negative	
Grade of UBC	Low grade ($G \leq 1$)	30(61.5%)	18(27.5%)	48
	High grade ($G \geq 2$)	35(92 %)	3(8%)	38
	Total	41(82%)	9(18%)	86

** ($P \leq 0.01$). High significant

To explain the role of Th17 cells in the development of bladder cancer, one must first understand the distribution of Th17 cells in bladder cancer patients. In this study, we detected Th17 cells in bladder tumor tissues using immunohistochemistry technique and found that the frequency of Th17 cells was significantly higher in urinary bladder cancer (malignant) than in urinary bladder disease (benign).

Accumulating data have suggested that Th17 cells play an important role in host defense against microbial infections and appear to be important mediators in the pathogenesis of inflammatory and autoimmune diseases (19). However, the distribution, phenotype and cytokine profile of Th17 cells in human tumors still remain poorly defined.

Result of present study are in agreement with (1) using flow cytometric analysis to count Th17 cells subpopulation, who found that Th17 cells were significantly increased in peripheral blood of bladder cancer patients as compared to control, who suggested that is possible involvement of Th17 cells in urothelial carcinoma of bladder. On other hand this result is disagreement with (20) who was show that tumor growth and metastasis were enhanced in IL17- deficient mice, they demonstrate that tumor growth in subcutaneous and lung metastasis are enhanced in IL-17-deficient mice and accompanied with reduced IFN- γ NK cells and tumor-specific IFN- γ T cells in the tumor-draining lymph nodes and tumors. They suggested that endogenous IL-17 positively impacts on tumor immunity. In contrast, Th17 cells have been reported to have tumor-promoting effects (21, 22). One study showed that IL-17, secreted by Th17 cells, positively affected tumors STAT3 activity and tumor growth in some transplant models (21). An enterotoxigenic *Bacteroides fragilis* (ETBF) colon

tumor genesis model also indicated the importance of an IL-17-STAT3 feed-forward loop in tumor development; in this model, both IL-17 production and STAT3 activity were increased. Antibody-mediated blockade of IL-17 abrogated most of the colon tumor genesis induced by ETBF colonization (22). In another study of colorectal cancer patients, immunohistochemical analysis showed that IL-17-producing cells were more highly expressed in the colonic mucosa of cancer patients than in those with normal colonoscopy findings (23).

More recently, a genetic and immunohistochemical analysis of 125 colorectal cancer specimens found that patients with high expression of the Th17 cluster (RORC and IL17A) had a poor prognosis (24).

Kryczek *et al.* (8) demonstrated that the levels of Th17 cells were significantly increased in peripheral blood, malignant ascites fluid and tumor tissues in human ovarian, renal and pancreatic malignancies.

our study demonstrated that the frequency of Th17 cells was prominently increased in tissue in patients with high grade with bladder cancer compared with patients with low grade disease, patients with advanced disease exhibited a significantly higher percentage of the Th17 cells. The majority of high grade tumor cases showed positive immunohistochemical Th17 cells expression 35 (92%), while only 30 cases (62.5%) of low grade tumor showed positive immunohistochemical Th-17 cells expression (Table 3).

These results were compatible with Chen *et al.* (25), who study on 207 breast carcinoma specimens were assessed by immunohistochemistry and they found increased in the number of Th17 producing cells correlated with high grade of the tumor and they suggest this correlation of IL-17 producing cells with high histological grade may display a pathogenic link between IL-17 producing cell infiltration, hormonal, receptors, and tumor differentiation.

Th17 cell infiltration gradually increasing with disease progression. This finding is disagreement with a report on prostate cancer in which an inverse correlation was noted between Th17 levels and tumor grade (26).

CONCLUSION

Higher expression of Th17 infiltrated cells in malignant indicates that Th-17 pro inflammatory is involved in pathogenesis of bladder cancer. The higher expression of Th17 in patients with malignant tumour suggests that they are contributed in progression of tumour.

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The role of humoral and cellular (CD4 and CD8) immunity against toxoplasmosis in abortive women

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ABSTRACT

CD8+ and/or CD4+ T cells play synergistic role in the acquisition of protective immunity and involved in the protection against *Toxoplasma gondii*. Blood from 60 abortive women and 25 apparently healthy pregnant women with no history of abortion as control group were taken to evaluate humoral and cellular immunity against *T. gondii*. The result of IgM & IgG ELISA indicated that 5(8.3%) IgM+ IgG-, 2(3.3%) IgM +IgG+, 53(88.3%) IgM- IgG +, and the serum of apparently healthy pregnant women with no case of abortion (control group) indicated that 25 (100 %) women were negative for anti-*T.gondii* antibodies. Comparison between patients and control subjects using Pearson Chi-square analysis showed highly significant difference. The result of CD4 and CD8 ELISA test indicated that there was highly significant differences ($P \leq 0.01$) in serum concentrations of CD4 between patients and control in all age group and the highest concentration in age group 22-25 and 26-29 respectively. Whereas in case of CD8 there was significant difference ($P \leq 0.05$) in these two groups.

Keywords: CD8+, CD4+ T cells, *Toxoplasma gondii*

الملخص باللغة العربية

تلعب الخلايا التائية نوع (CD4 و/أو CD8) دوراً متعاضداً في المناعة الوقائية وتساهم في الحماية من الإصابة بالمقوسات الكوندية (*Toxoplasma gondii*). وفي هذه الدراسة، تم جمع 60 عينة دم من نساء عانين من الإجهاض وأخريات (25) امرأة حامل سليمة لم يسبق لها أن تعرضت للإجهاض لتمثل مجموعة سيطرة لتقييم المناعة الخلوية والخلوية ضد (*Toxoplasma gondii*). أظهرت نتائج فحص الـ IgM و IgG أن 5 (8.3%) تحتوي على IgM ولا تحتوي على IgG، و 2 (3.3%) تحتوي على كلاهما و 53 (88.3%) تحتوي على IgG فقط. ولم يسجل وجود أي من الجلوبيولينات المناعية في (25 امرأة) من النساء اللواتي لم يتعرضن للإجهاض وكن بحالة سليمة. بينت النتائج الإحصائية باستخدام تحليل بيرسن (مربع كاي) فروقات نوعية بين مجموعتي الدراسة، كذلك فإن استخدام الخلايا التائية (CD4 و CD8) باستخدام فحص الـ ELISA، قد أظهر نتائج ذات فروقات معنوية ($P \leq 0.01$) في أمصال النساء بالنسبة إلى (CD4) المجهضات والسليمة عند جميع الأعمار، وكان أعلى مستوى عند المجموعة بين 22-25 و 26-29 على التوالي، بينما لم توجد فروقات معنوية على مستوى ($P \leq 0.05$) بين مجموعتي الدراسة في الأمصال التي تحتوي على (CD8).

INTRODUCTION

Toxoplasmosis is caused by infection with the obligate intracellular protozoan parasite *T. gondii*. It is one of the most prevalent infectious affecting one third of the world human population (1).

Toxoplasma is a very common parasite both in developed and developing countries, and some forms of diseases caused by *Toxoplasma* infection have very serious impacts on human health; taken together, all forms of toxoplasmosis are a serious socio-economic burdens throughout the world (2,3). The majority of *T.gondii* infection in most host species are subclinical or asymptomatic, and chronic. The parasite remains dormant in the tissues of the host until reactivation or until it is eaten by another host, thus most host survive the infection and acquire life-long immunity, while likely harboring the parasite in their tissues for the rest of their lives (4). The immune response of the host is important for the parasite as well without it the infections would become fulminant. Chronic latent *T.gondii* infection can generally be detected indirectly by screening for the presence of specific antibodies against the parasite (5,4).

It is well established that both Humoral and cell-mediated immune response, include T-cell mediated immune response that involves both CD4+ and CD8+ T lymphocytes, and antibodies, are important in on ferrying immunity to *T.gondii* infection (6). Immune CD8+ T cells from both infected mice or human secretes interferon gamma (IFN- γ) and exhibit *invitro* cytotoxicity towards infected cells (7). T-helper (Th-1) CD4+ T cells produce IFN- γ and IL-12, while T-helper (Th-2) cells produce IL-4, IL-5 and IL-10 which are associated with down regulation of parasitic cell mediated immune response (8).

The current study aimed to evaluate the role of humoral and cellular (CD4 and CD8) immunity in infection with toxoplasmosis in sample of Iraqi abortive women.

PATIENTS AND METHODS

The current study was carried out on three teaching hospitals at Baghdad area: Al-Alwai Teaching Hospital, Fatima Al-Zahra Teaching Hospital, Eben Al Balady Teaching Hospitals, between September 2013 to the end of June 2014.

Blood Samples were collected from 60 aborted Iraqi women and 25 apparently healthy pregnant women. The age of studied groups were between 18-34 years.

Blood sample and serological diagnosis:

Three ml of venous blood sample were obtained from studied group by sterile disposable syringe under sterile condition, Serum separated by centrifugation at 3000 rpm / for minutes and stored at -20 until used. Serologic determination were

performed with the kit *Toxoplasma* antibody (IgG) Enzyme Immunoassay Test Kit and *Toxoplasma* (IgM) Enzyme Immunoassay Test Kit (Bio Check /U K). In regard to CD4 and CD8 Human Cluster of Differentiation 4 (CD4) ELISA Kit and Human Cluster of Differentiation 8 (CD8) ELISA Kit Lifeome / China.

RESULTS AND DISCUSSION

Sixty positive patient with toxoplasmosis were used in this current work. All subjects (patients and control) were re tested by ELISA and the result revealed that all patients (abortive women) gave positive to ELISA test, while the control subjects (women without abortion) gave negative to this test. Pearson Chi-square analysis revealed highly significant difference ($P \leq 0.01$) between patients and control group as shown in table (1).

Table (1): Frequency distribution of *T. gondii* antibodies according to the abortion

ELISA results	Patients		Control		Total	
	No.	%	No.	%	No.	%
ELISA positive	60	100	0	0.00	60	70.59
ELISA negative	0	0.00	25	100	25	29.41
Total	60	100	25	100%	85	100 %
Chi-square- χ^2	---	15.75 **	---	15.75 **	---	11.69 **

** ($P \leq 0.01$)

Antibody pattern detection by ELISA for 60 sample of aborted women sera had been tested for specific IgG and IgM antibodies showed that 5 (8.3%) IgM+ IgG-, 2 (3.3%) IgM +IgG+, 53 (88.3%) IgM- IgG +, and the serum of apparently healthy pregnant women with no case of abortion (control group) indicated that 25 (100 %) women were negative for anti-*T. gondii* antibodies. Comparison between patients and control subjects using Pearson Chi-square analysis showed highly significant difference ($P \leq 0.01$) as shown in table (2).

Table (2): Distribution of patients and control according to pattern of antibodies by ELISA

Pattern of antibody	Patients		Control		Chi-square- χ^2
	No.	%	No.	%	
IgM ⁺ IgG ⁻	5	8.3	0	0.00	4.26 *
IgM ⁺ IgG ⁺	2	3.3	0	0.00	0.072 NS
IgM ⁻ IgG ⁺	53	88.3	0	0.00	14.69 **
IgM ⁻ IgG ⁻	0	0.00	25	100.00	15.75 **
Total	60	100 %	25	100 %	---
Chi-square- χ^2	---	16.835 **	---	15.750 **	---

* ($P \leq 0.05$), ** ($P \leq 0.01$). NS: Non-significant

In the current study, IgM antibody levels 5 (8.3%) can be used to confirm an acute exposure, although IgM antibody are almost always present following an acute exposure. IgM is primary response appears within the first 10-14 days and disappear and elevating again if the Ag trigger again with non-detectable titer, leading to an inaccurate assessment of when the exposure occurred. This situation can be problematic because congenital toxoplasmosis occurs when the mother is infected during her pregnancy, and the severity of the disease is determined by a screening in the pregnancy when the infection occurred. A significant increase in specific antibody titers or seroconversion during pregnancy is usually considered diagnostic of a recent exposure (9).

The results represented 2(3.3%) patients positive for both IgM and IgG this indicates either a recent infection or a false – positive test result (10). If acute infection is suspected, repeat testing is recommended within 2 to 3 weeks (11,12).

Elevated IgG levels in 53(88.3%) patients confirmed that women were exposed to the *Toxoplasma gondii*. Specific IgG antibodies to *Toxoplasma* rise gradually and reach to peak two to five months after the onset of infection. Therefore, the presence of IgG is useful in distinguishing subjects who have acquired the disease from those who have not. This is particularly important to identify susceptible women of childbearing age (13) In control group, both IgM and IgG were negative, which indicated the absence of infection or extremely recent acute infection.

The cellular immunity and response against *T. gondii* infection was investigated by estimating the concentration of CD4 and CD8 T cells in serum sample by ELISA. The current study showed that the age groups of 22-25 years had the highest mean concentration of CD4 (187.24 ± 22.70) and CD8 (53.63 ± 4.17) followed by age group 26-29 who had CD4 (172.54 ± 16.37) and (44.24 ± 2.06) CD8 with significant difference ($P \leq 0.05$), then the age groups 18-34 years CD4 represent (153.58 ± 18.61), CD8 (25.82 ± 1.47). While the lowest mean concentration of CD4 (138.01 ± 14.49) and CD8

(22.04 ± 2.15) found in the age group 30->34 years as shown in table (3).

There were highly significant differences ($P \leq 0.01$) in serum concentrations of CD4 between patients and control in all age group and the highest concentration in age group 22-25 and 26-29 respectively. whereas in case of CD8 there was significant difference ($P \leq 0.05$) in that two groups when compared with other groups who represented the higher percentage of infection with *T. gondii* and this lead to stimulate strong cellular immunity as mentioned by (14).

According to the results in table (3), the serum concentration of CD4 was found to be relatively higher among all age groups when compared with the serum concentration of CD8 this may be due to that host resistance to *T. gondii* infection is primarily dependent on T-cell-mediated immunity, and most attention has been focused on IFN γ -producing CD4⁺ T helper type 1 (Th1) and CD8⁺ T effector lymphocytes that are critical for the resolution of acute illness and to prevent reactivation of latent infection (15).

Table (3): Distribution of CD4 and CD8 according to age for patients and control

Age (year)	CD4 conc. (ng/ml)		T-test	CD8 conc. (ng/ml)		T-test
	Control	Patients		Control	Patients	
18-21	55.92 ± 2.75	153.58 ± 18.61	32.58 **	21.37 ± 1.59	25.82 ± 1.47	6.84 NS
22-25	58.70 ± 3.81	187.24 ± 22.70	29.81 **	13.35 ± 0.86	53.63 ± 4.17	11.72 *
26-29	58.09 ± 3.64	172.54 ± 16.37	36.64 **	19.53 ± 1.04	44.24 ± 2.06	9.64 *
30 - > 34	56.37 ± 3.52	138.01 ± 14.94	23.03 **	14.79 ± 0.76	22.04 ± 2.15	9.40 NS
LSD value	16.48 NS	30.66 *	---	8.93 NS	12.57 *	---

* ($P \leq 0.05$), ** ($P \leq 0.01$), NS: Non-significant

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Biofilm forming bacteria isolated from chronic tonsillitis

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ABSTRACT

Bacteria in biofilm niches resist host defenses and so that the presence of bacterial biofilms within the tissue and crypts of inflamed tonsils may explain the chronic tonsillitis. The goal of this study was to show the presence of bacterial biofilm on the tonsils which were resected from patients with chronic tonsillitis and on other hand to study the ability of isolated bacteria to form biofilms *in vitro*. This prospective study was conducted in Ramadi Teaching Hospital/ENT branch and Microbiology Department in Anbar Medical College, West of Iraq. Tonsils from Twenty five patients with chronic tonsillitis were tested microbiologically post tonsillectomy during the period from 1st October 2012 to 31st December 2012. Out of 25 patients, 21 (84%) were showing biofilms on tonsils surfaces and crypts, 2 (8%) patients were showing biofilms on tonsillar surface, and 2 (8%) showed biofilms on crypts. Out of the 109 of the bacterial isolates from resected tonsils due to chronic infection, 53 (48.6%) of microorganisms were strong biofilm producer, 26 (23.9%) were weak producer and 30 (27.5%) were non-producer. The most common organisms producing biofilm was *Streptococcus pyogenes* 35 / 32.1 %, and the least one was *Pseudomonas aeruginosa*: 2 / 1.8 %. The study confirms the ability of multiple micro-organisms to form biofilms on the surfaces and deep structures of the resected chronically infected tonsils. Prevention and treatment of many cases of chronic tonsillitis can be achieved by understanding that chronic tonsillitis is biofilm related.

Keywords: Bacterial biofilm, Tonsillitis, Bacterial adhesion test

الملخص باللغة العربية

تبدى الجراثيم في بيئات الأغشية البيولوجية مقاومة للوسائل الدفاعية الموجودة لدى المضيف وعليه، فإن وجود الأغشية الجرثومية في أنسجة واخاديد اللوزتين يجعل خمجها مزمنًا. وقد سعت الدراسة الحالية لتوضيح وتحديد وجود الغشاء البيولوجي الجرثومي على اللوز المستأصلة جراحيا من المرضى المصابين بخمج اللوزتين المزمن، وبيان قابلية بعض العزلات الجرثومية المعزولة من اللوز المستأصلة جراحيا من المرضى المصابين بخمج اللوزتين المزمن على تكوين الغشاء البيولوجي في الزجاجية. أجريت هذه الدراسة في مستشفى الرمادي التعليمي / شعبة الأنف والأذن والحنجرة وفي فرع الأحياء المجهرية الطبية / كلية الطب / جامعة الأنبار / غرب العراق. وقد تضمنت هذه الدراسة خمسة وعشرين مريضاً مصاباً بخمج اللوزتين المزمن والذين أجريت لهم عملية استئصال اللوزتين خلال الفترة من الأول من شهر أكتوبر / تشرين الأول إلى نهاية شهر ديسمبر / كانون الأول من عام 2012. وقد أجريت الفحوصات الميكروبيولوجية لهذه اللوز، حيث لوحظ وجود الأغشية البيولوجية على أسطح اللوزتين لواحد وعشرين (21، 84 %) مريضاً، ولوحظ وجود الأغشية في أخاديد اللوز في مريضين فقط (2، 8 %)، وكانت 53 (48 %) عزلة جرثومية من أصل 109 عزلة منتجة للغشاء البيولوجي بدرجة عالية، بينما كانت 26 (23.9 %) عزلة أخرى منتجة للغشاء الجرثومي بدرجة ضعيفة وكانت ثلاث عزلات أخرى غير منتجة للغشاء. وكانت المسببات القححية الأقوى في قدرتها على إنتاج الغشاء البيولوجي في حين كانت الزوائف الزنجارية أضعفها. وقد أثبتت هذه الدراسة قدرة العديد من الجراثيم على إنتاج الغشاء البيولوجي على أسطح وداخل أنسجة اللوز المستأصلة من أشخاص مصابين بخمج اللوزتين المزمن، وبهذا، فإن الوقاية وعلاج خمج اللوزتين المزمن يعتمد على مدى إدراك وفهم تكون الأغشية البيولوجية.

INTRODUCTION

Tonsillitis is one of the most common childhood disease and represents a real challenge because of its sequences may lead to antibiotics resistance (1-4).

Chronic tonsillitis is defined as persistent sore throat, anorexia and tonsillar erythema. It is also characterized by the presence of halitosis and palpable jugulodiaphragmatic lymph nodes. Both aerobic and anaerobic microorganisms with predominance of streptococci are usually involved (5). These organisms are able to form biofilms leading to the failure of antibiotics to eradicate them. For example, despite adequate treatment of acute tonsillitis due to β -hemolytic streptococci with antibiotics particularly penicillin, 20% of cases was bacteriologically positive after treatment (6).

The participation of the extracellular-matrix and cell-surface molecules, including membrane proteins is involved in the formation of biofilms. In addition, a huge quantity of bacterial energy and resources are required for biofilms formation. Bacterial cells attach to an appropriate surface, replicate, spread, and mature to form biofilms (6). Laser Doppler flowmetry was recently employed for biofilm analysis (7).

Limited studies were reporting the formation of biofilms on the surface of the tonsils (8-10).

The aim of the current study was to confirm the presence of bacterial biofilms in the resected chronic infected tonsils and which organisms forming biofilms *in vitro*.

PATIENTS AND METHODS

This prospective study was conducted at the Ear, Nose, and Throat (ENT) Department in Ramadi Teaching Hospital, Ramadi city, West of Iraq, from 1st October 2012 to 31 December 2012. Twenty-five patients with chronic tonsillitis were included in the study. Diagnosis of cases was based on the presence of two or more of the following: 1. Congested anterior pillars, 2. Palpable jugulodiaphragmatic lymph nodes in absence of acute infection and 3. Positive spatula test (pus extruding from tonsil on pressure either pre- or intra-operatively).

All patients were free from antibiotics for one month or more before surgery. All patients were admitted to the surgical ward in Ramadi Teaching Hospital, and tonsillectomy was done by senior surgeon after taking an informed consent from patients or their parents. Tonsillectomy was performed by cold snare technique under general anesthesia. The resected tonsil was taken aseptically in clean and dry disposable plastic cup within one hour for bacteriological investigation at Bacteriology lab at College of Medicine/ University of Anbar. Swabs from the surface and crypts of each swab were taken and cultured on blood, chocolate agar and MacConkey agar and incubated at 37 °C for 48 hours aerobically. Bacterial isolates were identified using cultural and biochemical

investigations. Selected bacterial isolates were used for biofilm production assay by Microtiter plate adhesion test. Biofilm formation was assessed by using a spectrophotometric technique. The optical densities (OD) of each was measured at 630 nm by using Stat Fax 3200 ELISA Reader. Isolates were classified according to biofilm production depending on the criteria used by Lama et al (2012) (11), as the following: non-adherent (OD < 0.041), weakly-adherent (0.041 < OD < 0.082), or strongly adherent (OD > 0.082).

RESULTS

Twenty-one (21.84%) out of 25 patients included in this study were showing biofilm on tonsil surface and crypts. While only two patients were showing biofilm on tonsil surface only and other two patients were showing biofilm on crypts only (Figure 1). Regarding type of bacterial isolates, *Streptococcus pyogenes* took the first rank of isolation from both tonsil surface and crypts, 19 (54%) and 16 (45.7%) for each respectively. *Staphylococcus aureus* species became next, 26 isolates were *Staphylococcus aureus*, which were isolated equally from tonsil surface and crypts (Table 1). Fourteen (14) isolates of *Moraxella catarrhalis* were isolated from tonsil surface and only four (4) isolates of *Haemophilus influenzae* type b were isolated from tonsil surface only. Other bacterial types like aerobic bacillus species, Diphtheroid (non Diphtheria *Corynebacterium*), *Pseudomonas aeruginosa* and *E. coli* were showing the lowest ratio of isolation (3,2,2,1) for each respectively as shown in table (1). Gram-positive bacteria showed the highest rate of isolation (88) isolates. Forty-four (44) isolates were from tonsil surfaces and crypts (Table 2). Thirty (30, 68.2%) of them were biofilm forming while (14, 31.8%) of them were non-biofilm former. No significant difference ($P < 0.5$) was found between Gram-negative bacterial isolates from tonsil surface and crypts as shown in table (2). Significant differences ($P < 0.5$) were found between number of gram positive and Gram-negative biofilm producing bacterial isolates (Table 2). Figure (2) showed that the number of strong biofilm forming bacterial isolates was more than that of weak and non-biofilm producer ($P < 0.05$).

Regarding degree of biofilm formation, no significant difference ($P < 0.5$) was found between isolates from tonsils crypts and surface within each degree (Table 3 and figure 2). *Streptococcus pyogenes* showed the highest rate among biofilm forming bacteria from tonsil surface and crypts, *Staphylococcus aureus* became next as shown in table (3).

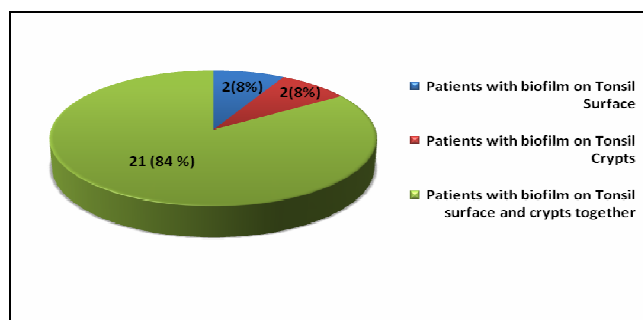


Figure (1): Number of tonsillitis patients

Table (1): Bacteria isolated from tonsillar surface and crypts

Bacterial isolates		No. of bacterial isolates		Total
		Surface Tonsil	Tonsil Crypts	
1	<i>Staphylococcus aureus</i>	8 (50%)	8 (50%)	16
2	<i>Staphylococcus epidermidis</i>	5 (50%)	5 (50%)	10
3	<i>Streptococcus pyogenes</i>	19 (54.3%)	16 (45.7%)	35
4	<i>Pneumococci</i>	3 (30%)	7 (70%)	10
5	<i>Viridans Streptococci</i>	6 (50%)	6 (50%)	12
6	<i>Haemophilus influenza</i>	4 (100%)	0	4
7	<i>Diphtheroides</i>	2 (100%)	0	2
8	<i>Aerobic Bacillus spp.</i>	1 (33.3%)	2 (66.7%)	3
9	<i>Escherichia coli</i>	0	(100%)1	1
10	<i>Pseudomonas aeruginosa</i>	1 (50%)	1 (50%)	2
11	<i>Niesseriacatarrhalis</i>	10 (71.4%)	4 (28.6%)	14

Table (2): Gram positive versus Gram negative bacterial isolates from tonsil surface and crypts regarding with biofilm production

No. of Gram positive isolates				No. of Gram negative isolates			
Surface		Crypts		Surface		Crypts	
44 (50 %)		44 (50 %)		11 (52.4 %)		10 (47.6 %)	
Biofilm producer	Non-biofilm producer	Biofilm producer	Non-biofilm producer	Biofilm producer	Non-biofilm producer	Biofilm producer	Non-biofilm producer
30 (68.2%)	14 (31.8%)	32 (72.7%)	12 (27.3%)	9 (81.8%)	2 (18.2%)	8 (80.0%)	2 (20.0%)

Table (3): Scoring of biofilm production on tonsillar surface and crypts by all study bacterial isolates

No	Bacterial isolates	Results of biofilm production according to depended criteria.					
		Tonsil surface			Tonsil crypts		
		Biofilm producers		Non-biofilm producers	Biofilm producers		Non-biofilm producers
		SP	WP	NP	SP	WP	NP
1	<i>Staphylococcus aureus</i>	6	1	1	7	1	0
2	<i>Staphylococcus epidermidis</i>	2	1	2	3	1	1
3	<i>Streptococcus pyogenes</i>	11	7	1	10	4	2
4	<i>Pneumococci</i>	0	1	2	4	2	1
5	<i>Viridans Streptococci</i>	0	1	5	0	0	6
6	<i>Haemophilus influenza</i>	0	0	0	2	1	1
7	<i>Diphtheroides</i>	0	0	2	0	0	0
8	<i>Aerobic Bacillus spp.</i>	0	0	1	0	0	2
9	<i>Escherichia coli</i>	0	0	0	1	0	0
10	<i>Pseudomonas aeruginosa</i>	1	0	0	1	0	0
11	<i>Niesseriacatarrhalis</i>	4	4	2	1	2	1

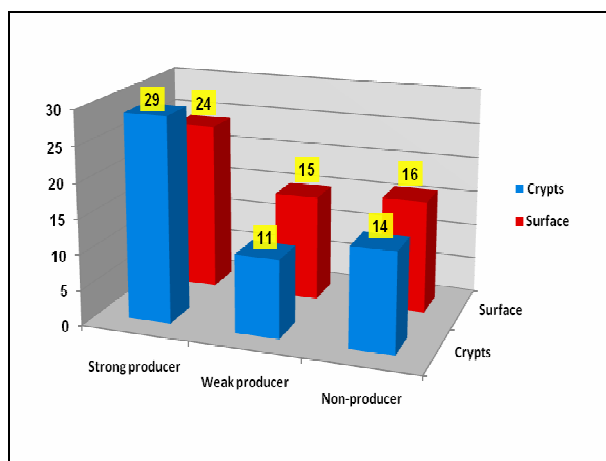


Figure (2): number of strong biofilm forming bacteria isolates compared with weak and non- biofilm producer

DISCUSSION

In the last 20 years, biofilm have been recognized as a considerable cause of chronic infections such as chronic cystitis, osteomyelitis and otitis media (3, 12). It appears likely that the first step in biofilm formation is the binding of bacteria to the tonsillar epithelium. Attached bacteria form large colonies of mixed bacteria (6, 13). This biofilm in addition to protect the bacteria from host defense mechanisms and antibiotics, they help them to continue to metabolize and form endotoxin. The release of local endotoxin may lead to chronic tonsillitis. Furthermore, when local circumstances are appropriate, bacteria within the biofilm may become motile, causing acute infection to appear (8, 12). High rate of biofilm formation on tonsillar surface and crypts of patients was in accordance with the findings of others (8, 14, 15). This was ought to the chronicity and recurrence of tonsillitis which leads to the biofilm formation (11, 14, 15). In addition to that the antimicrobial resistance of tonsillitis causative agents leading to the difficult eradication of bacterial agents ends with biofilm formation (11, 16). Gram positive bacteria were showing high rate of isolation than Gram negative bacteria particularly Enteric bacteria, this was in agreement with results of other studies (1, 17). This was due to the low incidence rate of enteric bacterial infections in the upper respiratory tract except those with immunocompromised individuals and those with bad oral hygiene (17).

Regarding types of bacterial isolates, *Streptococcus pyogenes* reported the first rank of isolation from tonsillar surface and crypts followed by *Staphylococcus* species. This was in agreement with results of (18-20). In other hand these results were disagreed with the findings of (11, 16, 21) who found that *Staphylococcus* species were most frequently isolated from patients followed by *Streptococcus* species. This difference in results was might be due

to difference in age groups of patients as well as social status. No significant difference was found between biofilm forming bacteria both in tonsil surface and crypts, this was not agreed with the results of previous study (8). This was attributed to the available chance and environment for biofilm formation in tonsil surface and cryptslike food and Ph (17). In addition to factors related to bacterial pathogen (15). *S. pyogenes* showed the highest rate of biofilm formation among bacterial isolates from tonsil surface and crypts, *Staphylococcus aureus* became next. This was attributed to the ability of these organisms to produce slime material which is polysaccharide constituents of capsules of these bacteria (13, 17, 22, 23). This study concluded that the biofilm forming bacteria can be isolated from tonsil surface and crypts with different degrees of biofilm forming capacities depending on bacterial species so we recommend surfactant use beside antibiotic in chronic tonsillitis to prevent biofilm formation.

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Correlation of Biofilms-Pyocyanin producing *Pseudomonas aeruginosa* with the antibiotics resistant profiles A.

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ABSTRACT

In order to detect biofilm-pyocyanin producing and multidrug resistant isolates of *Pseudomonas aeruginosa* in some raw dairy products, a total of 73 samples of locally produced Cows raw milk (33 samples) and soft cheese (40 samples) were collected from different markets in Baghdad during February till August 2014, in which they processed (at Zoonoses Unit) according to standard and modified reference food and dairy microbiology procedures by using of gold standard, Cefrimide-Nalidixic acid *Pseudomonas aeruginosa* Chromogenic agar (CNP), rapid biochemical panel identification system (Microbact) and polyclonal latex agglutination kit. All isolates were tested for biofilm formation by standard Microtiter plate and Congo red agar assay and pyocyanin production on CNP agar, then correlate among biofilm-pyocyanin producers and their ability to resist selected antibiotics by standard Kirby-Bauer disc diffusion method. The results showed isolation of 47 isolates of *Pseudomonas aeruginosa* out of 73 samples (64.38%): 17 (23.28%) isolates from raw milk samples, in which 7 (9.59%) isolates were biofilm-pyocyanin producers and multidrug resistant; and 30 (41.1%) isolates from soft cheese samples, in which 22 (30.13%) isolates were biofilm-pyocyanin producers and multidrug resistant. These findings suggest presence of strong correlation among biofilm-pyocyanin producing isolates, multidrug resistant profiles and environment (contamination) in Baghdad, thus recommend to monitoring these dairies periodically.

Keywords: *Pseudomonas aeruginosa*, biofilm, pyocyanin, antibiotics resistance, raw milk and soft cheese

الملخص باللغة العربية

لغرض التحري عن الزوائف الزنجارية المنتجة للأغشية الحيوية وصبغة البايوسيانين ومدى مقاومتها للمضادات الحيوية في بعض منتجات الألبان الخام للأبقار، تم جمع 73 أنموذجاً عشوائياً من مناطق مختلفة في أسواق بغداد خلال الفترة من شهر فبراير / شباط وحتى أغسطس / آب من 2014، حيث تمت معاملة النماذج في وحدة الأمراض المشتركة حسب الطرائق القياسية لميكرو بيولوجيا الأغذية والألبان مع بعض التحويرات باستخدام الوسط الصباغي المتخصص أجار السترمبايد نالديكسك، والعدة الكيموحيوية الالكترونية السريعة ونظام المايكروباكت وعدة التلازن النوعي المتعددة النسيلة، إضافة إلى دراسة قابلية العزلات على إنتاج الأغشية الحيوية بواسطة طبق المقايسة الدقيقة المحور وصبغة الكونغو الحمراء، وقدرتها على إنتاج وإفراز صبغة البايوسيانين الزرقاء-الخضراء (حبر الأخطبوط) على الأجار المتخصص، ودراسة ارتباطها بقدرتها على مقاومة المضادات الحيوية المنتجة بواسطة طريقة انتشار أقراص المضادات الحيوية في الطبق (تقانة كيربي باور). أظهرت النتائج عزل وتشخيص الزوائف الزنجارية بنسبة 47 (64.38%) عزلة من 73 أنموذجاً: 17 (23.28%) عزلة من الحليب الخام، كان منها 7 (9.59%) عزلات منتجة للأغشية الحيوية وحبر الأخطبوط ومقاومة للمضادات الحيوية؛ و 30 (41.1%) عزلة من الجبن الطري المحلي الخام، كان منها 22 (30.13%) عزلة منتجة للأغشية الحيوية وصبغة البايوسيانين ومقاومة للمضادات الحيوية. وتشير هذه النتائج إلى وجود علاقة ارتباطية قوية بين قابلية العزلات على إنتاج الأغشية الحيوية-حبر الأخطبوط، ومقاومتها للمضادات الحيوية المنتجة والمحيطة (التلوث) في بغداد وبالتالي توصي الدراسة بمراقبة هذه المنتجات بشكل دوري للحفاظ على الصحة العامة للمستهلك.

INTRODUCTION

Pseudomonas aeruginosa was genetically well equipped to survive food processing technologies and host defense strategies. *P. aeruginosa* was one of the major bacterial pathogens which cause serious clinical infection and food poisoning cases that is also an emerging concern in veterinary medicine and animal agriculture (1,2). *P. aeruginosa* has emerged globally as a significant public health / antimicrobial resistance problem in both human and veterinary medicine due to quorum-sensing alginate-biofilm formation and pyocyanin production (1-4). It is known that food was a cause of some diseases and has an important role for spreading these diseases. Food originated diseases can be depended on the factors of microbial, chemical, herbal and animal. *Pseudomonas* spp. also plays an important role in milk spoilage.

During the storage of raw milk they produce many thermo-tolerant lipolytic and proteolytic enzymes that reduce both the quality and shelf life of processed milk (5,6)

Pseudomonas infections can involve any part of the body i.e. respiratory tract infections, bacterial keratitis, bones and joints, gastrointestinal infection, hematological malignancies, meningitis and brain damage in some patients, chronic otitis, urinary tract infection etc. *P. aeruginosa* has an intrinsic high resistance against most antibiotics, which significantly contributes to eradication failure. Different mechanisms account for this inherent multi drug resistance against a range of structurally and functionally different antibiotics such as penicillin, gentamycin, third generation cephalosporins, carbapenems like imipenem and meropenem, fluoroquinolones like ciprofloxacin, norfloxacin and tetracycline etc. (3).

Biofilms are multicellular communities held together by self-produced extracellular matrix. The mechanisms that different bacteria employ to form biofilms vary, frequently depending on environmental conditions and specific strain attributes (7). Bacterial cells in the biofilm often display a variety of phenotypic differences from those in the planktonic culture. These include some phenotypic changes such as motility, production of extracellular polysaccharide and increased resistance to antibiotic and host defense system. *P. aeruginosa* also forms biofilm readily, which may be the most important reason why the infections cannot be effectively treated and cured (3).

P. aeruginosa produces a characteristic blue-green phenazine pyocyanin (5-N-methyl-1-hydroxyphenazine), which was associated with the majority of *P. aeruginosa* isolates. Phenazines were redox-active compounds capable of undergoing oxidation-reduction transformations and a so-called chameleon phenomenon, in which cultures of *P. aeruginosa* change the color of the medium depending on the pH value and aeration as well as

low iron conditions. Pyocyanin production was regulated by acyl homoserine lactone quorum sensing genes, in which these octopus ink like blue-green pigments and their derivatives with recalcitrant biofilm protect *P. aeruginosa* from toxic competitive invaders like *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus*, antibiotics, antibodies, macrophages, etc. (2).

The current study was aimed to identify and correlate between production of biofilm-pyocyanin complex barrier matrix and the ability of isolates to resist multiple selected antibiotics in some raw dairy products in Baghdad.

MATERIALS AND METHODS

Collection and Processing of Samples:

A total of 73 samples of locally produced Cows raw milk (33 samples) and soft cheese (40 samples) were collected from different markets in Baghdad during February till August 2014. All samples were collected aseptically in sterile non-permeable & non-durable plastic bags (500 ml) and transported to Zoonoses laboratory as soon as possible, then processed by a standard and modified isolation procedures of dairy and food microbiology, in which they refrigerated for 48 hrs. at 4°C before culturing in order to increase chance for isolation of these psychrotrophic pathogens (8-11).

Isolation and Identification procedure:

At Zoonoses Unit samples were warmed and homogenized at lab temperature, after that processed as two separate unit (Directly and Indirectly) wholly concentrated and decimally diluted samples as follow: directly milk samples were streaked on a selective and differential Cetrimide-Nalidixic acid *P. aeruginosa* Chromogenic agar (CNP) by transferring 2ml of sample on duplicate agars by sterile inoculating loops & cotton swabs, then incubated at 35-37°C for 24-48 hours and monitored for production of fluorescent pigments (chameleon phenomenon), changes in colony morphology (polymorphism) and alginate-biofilm formation; and indirectly inoculated on double strength power tryptone soya yeast extract broth (TSB-YE) as 1 part sample (10ml) to 9 parts (90ml) broth (1:10 dilution food standard formula), then mixed well for (3-5) minutes, then incubated at 35-37°C for 24 hours for resuscitation of stressed or sub-lethally damaged cells, then streaked on CNP at same temperature and incubation period above (8-13).

Cheese samples were macerated manually with its own whey (original sample), after that processed as two separate unit (directly and indirectly) as follows: directly a 1 part (25 gm) sample from a whole homogenized cheese lobes (original sample) were diluted decimally with 9 parts (225 ml) 2%

buffered sodium citrate solution (emulsifying cheese lobules to a tiny portions to extract hidden cells), then homogenized with stomacher for (3-5) minutes, after that transferring 1 ml of suspension on duplicate CNP by sterile inoculating loops and cotton swabs, then incubated and monitored as above; and indirectly inoculated on double strength power TSB-YE as 1 part sample (25 gm) to 9 parts (225 ml) broth (1:10 dilution food standard formula), then homogenized with stomacher for (3-5) minutes, then incubated at 35-37°C[°] for 24 hours for resuscitation of stressed or sub-lethally damaged cells, then streaked on CNP at same temperature and incubation period above (8-13).

According to dairy and food microbiological procedures; pure isolated single colonies on CNP agar with standard criteria of oxidase and catalase positive *P. aeruginosa* produce polymorphic blue-green pigmented swarming colonies with fruity odor, were picked up and recultured on double strength power TSB-YE at 35-37°C[°] for 24 hours, then transferred to double strength power tryptone soya yeast extract agar (TSA-YE) at 35-37°C[°] for 24 hours, after that inoculated universal and bijoux slant bottles preserved inside a refrigerator as pure seeds or nucleus for further identification. Purification and Identification (Confirmation) procedures were done via computerized Microbact biochemical panel identification system and polyclonal latex agglutination kit (8-13).

Biofilm Assay:

The microtiter plate assay described by (3,14) was most widely used and considered as a gold standard test for detection of biofilm formation. At Zoonoses unit, a modified procedure was done by using double-strengthen power TSB-YE for overnight growth of biofilm-producing isolates and a wide 24 wells microtiter plate, in which they filled with 1 ml fresh inoculated and control broths. The microtiter plates were incubated for 24-48 hrs. at 37°C[°]. After incubation, content of each well was gently removed by tapping the plates. The wells were washed three times with 1 ml of Phosphate Buffer Saline (PBS pH 7.2). Biofilms formed by adherent isolates in plate were fixed with sodium acetate (2%) and stained with crystal violet (5%). Excess stain was rinsed off by thorough washing with distilled water and plates were kept for drying.

An alternative method of screening biofilm formation was described by (3,14), which required the use of a specially prepared solid medium-brain heart infusion (BHI) agar supplemented with 5% sucrose (5 gm\ 100 ml) and Congo red stain (10 gm/L). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C[°] for 15 minutes, filtered, separately from other medium constituents and was then added when the agar had cooled to 55°C[°]. Plates were inoculated and incubated for 24 to 48 hrs. at 37°C[°]. Positive result was indicated by black colonies with a dry crystalline consistency. Weak slime producers

usually remained pink, though occasional darkening at the centers of colonies was observed. A darkening of the colonies with the absence of a dry crystalline colonial morphology indicated an intermediate result. A modification was also done by using alternative double-strengthen TSA-YE.

Antibiotic Susceptibility test:

Pure isolates tested for their susceptibility to selected antibiotics according to standard methods of Clinical Laboratory Standards Institute CLSI (National Committee for Clinical Laboratory Standards NCCLSs, 15) and followed in this account of the Kirby-Bauer disc diffusion method (15,16). Selected antibiotic discs from Bioanalyse[®](Turkey): Cefepime (FEP, 30µg), Ciprofloxacin (CIP, 10µg), Erythromycin (E, 10µg), Aztreonam (ATM, 30µg), Azithromycin (AZM, 15µg), Gentamicin (CN, 10µg), Imipenem (IPM, 10µg), Meropenem (MEM, 10µg), Piperacillin (PRL, 30µg), Tobramycin (TOB, 10µg) and Ceftazidime (CT, 30µg) with standard antibiotics zone of inhibition tables as a control guidelines.

Statistical Analysis:

The data were analyzed according to statistical software, Statistical Package for the Social Sciences (SPSS, version 21, 2013), including Chi-square analysis and ANOVA in accordance with (17).

RESULTS AND DISCUSSION

Contamination of locally produced dairy products in Baghdad markets with multidrug resistant *P. aeruginosa* (MDR-PA) represent dangerous environmental source for spreading of these zoonotic foodborne strains as community, nosocomial and livestock associated invaders. The results showed isolation of 47 isolates of *P. aeruginosa* out of 73 samples (64.38%): 17 (23.28%) isolates from Cows raw milk samples, in which 7 (9.59%) isolates were biofilm-pyocyanin producers and multidrug resistant; and 30 (41.1%) isolates from soft cheese samples, in which 22 (30.13%) isolates were biofilm-pyocyanin producers and multidrug resistant. All MDR-PA isolates showed dual growth phases: large mucoid morphotypes and small colonial variants. Most isolates and their morphotypes were susceptible to ciprofloxacin. Results were represented by tables (1, 2, 3) and figure (1).

Table (1): Isolation percentages of biofilm-pyocyanin producing and multidrug resistant *P. aeruginosa* (MDR-PA) from Cows Raw Milk and Soft Cheese in Baghdad

Sample Type	Numbers	Positive <i>P. aeruginosa</i>	Positive MDR-PA
Raw Milk	33	17 (23.28%)	7 (9.59%)
Soft Cheese	40	30 (41.1%)	22 (30.13%)
Total	73	47 (64.38%)	29 (39.72%)

Table (2): Percentages of Resistance and Susceptible MDR-PA isolates in Raw Milk

Antibiotics Profile	Colony Morphotypes of MDR-PA Isolates in Raw Milk			
	Large Muroid Morphotypes		Small Colonial Variants	
	Resistance %	Susceptible %	Resistance %	Susceptible %
Meropenem	7 (100) ^{Aa}	None (0) ^{Ab}	7 (100) ^{Aa}	None (0) ^{Ab}
Cefepime	4 (57.14) ^{Ba}	3 (42.86) ^{Ba}	3 (42.86) ^{Ca}	4 (57.14) ^{Ca}
Imipenem	7 (100) ^{Aa}	None (0) ^{Ad}	5 (71.43) ^{Bb}	2 (28.57) ^{Bc}
Aztreonam	4 (57.14) ^{Ba}	3 (42.86) ^{Ba}	3 (42.86) ^{Ca}	4 (57.14) ^{Ca}
Azithromycin	7 (100) ^{Aa}	None (0) ^{Ab}	7 (100) ^{Aa}	None (0) ^{Ab}
Piperacillin	7 (100) ^{Aa}	None (0) ^{Ab}	7 (100) ^{Aa}	None (0) ^{Ab}
Erythromycin	7 (100) ^{Aa}	None (0) ^{Ab}	7 (100) ^{Aa}	None (0) ^{Ab}
Gentamicin	7 (100) ^{Aa}	None (0) ^{Ac}	3 (42.86) ^{Cb}	4 (57.14) ^{Cb}
Ciprofloxacin	2 (28.57) ^{Cc}	5 (71.43) ^{Cb}	None (0) ^{Dd}	7 (100) ^{Da}
Ceftazidime	7 (100) ^{Aa}	None (0) ^{Ab}	7 (100) ^{Aa}	None (0) ^{Ab}
Tobramycin	7 (100) ^{Aa}	None (0) ^{Ad}	5 (71.43) ^{Bb}	2 (28.57) ^{Bc}

A,B,C,D: Indicate significant differences among antibiotics for isolates vertically at level ($P \leq 0.05$).

a,b,c,d: Indicate significant differences among isolates for antibiotic horizontally at level ($P \leq 0.05$)

**Figure (1): Antibiotic disc diffusion method and diffusible green pyocyanin**

Table (3): Percentages of Resistance and Susceptible *MDR-PA* isolates in Soft Cheese

Antibiotics Profile	Colony Morphotypes of MDR-PA Isolates in Soft Cheese			
	Large Mucoïd Morphotypes		Small Colonial Variants	
	Resistance %	Susceptible %	Resistance %	Susceptible %
Meropenem	22 (100) ^{Aa}	None (0) ^{Ab}	22 (100) ^{Aa}	None (0) ^{Ab}
Cefepime	11 (50) ^{Cb}	11 (50) ^{Cb}	15 (68.18) ^{Ba}	7 (31.82) ^{Bc}
Imipenem	22 (100) ^{Aa}	None (0) ^{Ab}	22 (100) ^{Aa}	None (0) ^{Ab}
Aztreonam	11 (50) ^{Ca}	11 (50) ^{Ca}	11 (50) ^{Ca}	11 (50) ^{Ca}
Azithromycin	22 (100) ^{Aa}	None (0) ^{Ad}	15 (68.18) ^{Bb}	7 (31.82) ^{Bc}
Piperacillin	15 (68.18) ^{Ba}	7 (31.82) ^{Bb}	15 (68.18) ^{Ba}	7 (31.82) ^{Bb}
Erythromycin	22 (100) ^{Aa}	None (0) ^{Ab}	22 (100) ^{Aa}	None (0) ^{Ab}
Gentamicin	7 (31.82) ^{Db}	15 (68.18) ^{Da}	7 (31.82) ^{Db}	15 (68.18) ^{Da}
Ciprofloxacin	7 (31.82) ^{Dc}	15 (68.18) ^{Db}	None (0) ^{Ed}	22 (100) ^{Ea}
Ceftazidime	22 (100) ^{Aa}	None (0) ^{Ab}	22 (100) ^{Aa}	None (0) ^{Ab}
Tobramycin	22 (100) ^{Aa}	None (0) ^{Ab}	22 (100) ^{Aa}	None (0) ^{Ab}

A,B,C,D,E: Indicate significant differences among antibiotics for isolates vertically at level ($P \leq 0.05$).

a,b,c,d: Indicate significant differences among isolates for antibiotic horizontally at level ($P \leq 0.05$).

Versatile features of study isolates indicate frequency and distribution of *P. aeruginosa* in some Baghdad. Increased or decreased microbial load of *MRAP* among food samples indicate significant differences (12), which may be referred to unhygienic processing strategies (no-thermal treatment of raw milk, contaminated milk equipment especially milk cans, asymptomatic milkers' carriers, flies, insects, polluted water, retailing situations, etc.) as well as climatic factor, presence of infectious foci (biofilm problems) in animals fomites and the role of hospitals as a nosocomial factor in a disease history.

Milk and soft cheese were ideal growth media for most pathogens (12,18) but, cheese represents the major reservoirs of *P. aeruginosa* may be due to its sequestered tropism nature especially its whey, pre- and post-contamination and cross-contamination processing. The coincidence of *P. aeruginosa* isolation may reveal the selective new technology in resuscitation of sub-lethally damaged cells from samples of raw milk and soft cheese. From scientific and hygienic points of view, the isolation percentages were higher than expected in accordance with the similar researches in nearby countries, which may reflex high level of contamination and development of resistancy in these pathogens due to partially misuse. These results were unaccepted in modern world, and the

ratio of isolation if reached further than 5% this may indicate a red-line risk with forcing banding laws about products from these epidemic countries (12,19).

The colonies of *P. aeruginosa* on CNP agar showed development of versatile morphotypes and appeared as blue-green, shiny, convex, flat, strongly cohesive mucoid-large or smooth-small colonial variants or tiny or wrinkled or rough as fried-eggs with entire circular or corroded margins. They secrete four pigments: pyocyanin, pyoverdine, pyorubin and pyomelanin (Chameleon phenomenon), this may aid in diagnosis and indicate hyperactive virulence strategies (4,20). Recent reports indicate that different types of environmental pressures select for colony morphology variants of *P. aeruginosa*, and many of these variants have biofilm-related phenotypes. The literatures describes the appearance of these variants on solid growth medium by several names: small-colony variants, rough small-colony variants, wrinkled variants, auto-aggregating cells, and rugose colonies (21).

Cell-to-cell communication by means of diffusible signaling molecules (quorum sensing alginate-biofilm architecture and pyocyanin-phenazine derivatives) allows bacteria to trigger coordinated responses to achieve outcomes that would otherwise remain impossible for individual cells. During the

past two decades, much attention has been given to bacterial communication systems due to their involvement in acute and chronic infections and multidrug resistant problems. Analyses of the molecular mechanisms of cell-to-cell communication may help scientists to develop specific antimicrobial agents that will decrease both the defensive and offensive traits of pathogens. The signaling network of *P. aeruginosa* was perhaps one of the most complex systems known and to date the best studied among all microorganism systems. It consists of multiple interconnected signaling layers that coordinately regulate virulence and persistence, driving the emergence of *P. aeruginosa* from the enormous number of species that comprise the biodiverse bacterial domain to join an elite group of a few dozen that pose a major threat to humans and animals (4,22).

Acyl-homoserine lactone based quorum-sensing system was important for *P. aeruginosa* virulence and biofilm formation. The genetic diversity within a bacterial species is determined by the number and size of chromosomal and extra chromosomal elements, rates of nucleotide substitution, recombination, genome rearrangements and gene flow, and both the size and growth of the bacterial population (23,24).

P. aeruginosa has become a model organism for the study of biofilms due to its metabolic versatility and variability in its response to environmental signals, which promotes successful colonization of different habitats and growth under varying environmental conditions (25). In this study, alginate-biofilm formation by biofilm-producing morphotypes were detected by two assay, in which formation of mucoid, grayish-black colonies was noticed obviously in modified Congo-red agar with a dry crystalline consistency. The exact mechanism of the Congo red phenomenon was yet unknown suggesting that a secondary product may be involved. In Microtiter plate assay, a positive result was indicated by the presence of an adherent blue layers, flakes and dots of stained material on the inner surface of the wells, these observations suggested a strong dependence between growth condition and biofilm formation in *P. aeruginosa* (3,14,26).

Obviously noticed strong and diffusible mucoid secretion of green pyocyanin with profuse biofilm and grape-apple odour in Muller-Hinton agars as protecting barriers from antibiotics especially from cheese isolates, this may indicate indirectly coordinated response of quorum sensing mechanism under stressed conditions. Most isolates and their morphotypes were susceptible to ciprofloxacin. These findings suggest presence of strong correlation among biofilm-pyocyanin producing isolates, multidrug resistant profiles and environment (contamination) in Baghdad, thus recommend to monitoring these dairies periodically and using of ciprofloxacin for treatment of nosocomial, community and livestock cases with caution from development of resistance against it.

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Isolation of Methicillin Resistant *Staphylococcus aureus* phage from mastitic cows

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ABSTRACT

One hundred and fifteen milk samples collected from cows presented with mastitis found at the Faculty of Veterinary Medicine fields as well as the cities of Abu Ghraib, Al-Shulaa, Al-Kassem and Al-Dorah. All samples were cultured on Blood agar, Manitol salt agar and Staph 110. After purification of bacteria and conducting biochemical tests in addition to API Staph System, the results revealed that 30 isolates were identified as *S. aureus*. Antibiotic susceptibility indicated that 50% of the isolates were resistant to methicillin. *S. aureus* phage was isolated from milk sample by agar over lay method. The isolated phage (named SP9) developed clear and circular plaques ranged between 2-3 mm in diameter. SP9 showed icosahedral head and a long contractile tail when examined by scanning electron microscope (SEM). Furthermore, it was resistant to lipid solvents and having a wide host range, 5 isolates showed sensitivity to the phage.

Keywords: Methicillin Resistant *Staphylococcus aureus*, mastitic cows, phage

الملخص باللغة العربية

جمعت 115 عينة حليب من حقل كلية الطب البيطري و مدن أبو غريب والشعلة والقاسم والدورة من أبقار مصابة بالتهاب الضرع، وتمت زراعة جرثومة المكورات العنقودية الذهبية على وسط أجار الدم ووسط المانيتول الملحي الصلب ووسط المكورات العنقودية 110 ، وبعد تنقية البكتيريا وعمل الاختبارات الكيموحيوية وإجراء اختبار (API Staph System)، أظهرت النتائج أن 30 عزلة كانت بكتيريا *S. aureus* ، وبعد إجراء اختبارات الحساسية وجد أن 50% من العزلات مقاومة للميثيسيلين، وقد تم عزل عاثي المكورات العنقودية من عينات الحليب باستعمال طريقة الكساء، إذ تميزت بقع العاثي بكونها دائرية وواضحة وتراوح حجمها بين 2-3 ملليمتر، وقد تميز العاثي بامتلاكه رأساً سداسياً وذيلاً متقلصاً عند تشخيصه بالمجهر الإلكتروني ، كما تميز بكونه مقاوماً للمذيبات الدهنية وامتلاكه مدى إصابة واسعاً، إذ أظهرت 5 عزلات حساسيتها للعاثي.

INTRODUCTION

Staphylococcus aureus is a gram-positive pathogen that causes a variety of animal diseases including mastitis in dairy cattle and brought substantial losses to dairy industry including reduced milk yield, milk quality and increased production costs (1). Methicillin Resistant *Staphylococcus aureus* (MRSA) is resistant to a large group of antibiotics called the beta-lactams, which include penicillins and cephalosporins. It has evolved an ability to survive treatment with beta-lactamase resistance beta-lactam antibiotics including methicillin, dicloxacillin, and oxacillin (2). The incidence of antibiotic resistant bacteria has increased in recent years (3). As a result, there has been renewed interest in the use of other natural or engineered antimicrobial agents as an alternative to antibiotics for treatment staphylococcal diseases such as mastitis (4). Phage therapy can serve as one of the stand-alone approaches for treating staphylococcal infections refractory to the action of commonly deployed antibiotics (5). Phage therapy has several characteristics that make it a very attractive alternative to chemical antibiotics, phages infect target cells very specifically. This would enable treatment of the infecting bacteria only, without disrupting the natural flora (6). Phages can still target and kill multi-drug resistant bacteria (7). Phages have specific properties which give them advantages as therapeutic agents; they are self-replicating as well as self limiting, they continue to multiply and penetrate deeper as long as local infection is present (8). The present study aims for isolation and identification of *S. aureus* phage from mastitic cows and determine their ability for lysis MRSA.

MATERIALS AND METHODS

S. aureus strains:

One hundred and fifteen distinct raw milk samples were collected from cows suffered from symptoms of mastitis. They were provided from Faculty of Veterinary Medicine field, and the cities of Abu Ghraib, Al-Shulaa, Al-Kassem and Al-Dorah. All samples were collected in sterile tubes, kept on ice, and then stored at 20°C during transport to the laboratory where an analysis was performed.

Specimens processing:

All samples were centrifuged at 3000 rpm for 15 minutes, and the precipitate was cultured on blood agar and nutrient agar, incubated at 37 °C for 24 hrs. colonies with hemolysis were recultured on mannitol salt agar as selective medium for *S.aureus* and incubated at 37 °C for 24 hrs. (9). *S. aureus* were identified depending on the morphological features on culture media and biochemical tests

according to Bergey's manual (10). Identification results were confirmed using API system.

Antibiotic susceptibility test:

Antibiotic susceptibility test was performed using a disc diffusion method on Mueller –Hinton Agar (11).

Isolation of Phage:

10 ml of milk sample centrifuged at 3500 rpm for 15 minutes, the supernatant was filtered through 0.22 µm Millipore filter, Thereafter, the filtrate was assayed for plaque by double agar layer technique. A log phase cultures of *S. aureus* prepared by overnight culture at 37 °C.

Milk filtrate (0.1 ml) and 0.1 ml of *S. aureus* suspension were added to 3 ml of top agar, mixed and poured over a plate of bottom agar. Afterward, the plate allowed to be harden. Subsequently, the plate was inverted and incubated overnight at room temperature.

Phage single plaque on bottom agar plate harvested by stabbing the center of a plaque with a sterile needle and rinse in broth, this constitutes phage stock (12).

Preparation of phage stock:

0.1 ml of diluted phage solution was added to 3 ml of top agar, add one or two drops of host *S. aureus* suspension were added and mixed, pour over a plate of bottom agar, solidified, inverted and incubated overnight at room temperature.

To each plate, 10 ml of nutrient broth was added and incubated at room temperature for 3 hrs. Afterward, the broth was gently aspirated from the plate and centrifuged at 3500 rpm for 15 minutes to remove cells and debris. Subsequently, the supernatant was filtrated through 0.22 µl Millipore filter and stored at 4°C (13).

Determining titer of phage:

In a 47°C water bath, 3 ml of hot, melted top agar were placed in three 13x100 mm test tubes. To each tube 0.1 ml of *S. aureus* was added and 0.1 ml of the 10⁸ phage dilution was quickly pipetted into a tube of top agar containing host bacteria, mixed well and the mixture was poured immediately onto the surface of bottom agar. However, plates were tilted rapidly to distribute the agar over the surface of the bottom layer. The same protocol was repeated with 10⁷ and others phage dilutions.

The top agar was allowed to solidify and became firmly attached to the hard agar bottom, all the plates were inverted and incubated at room temperature for 24 hrs. The number of plaques seen were counted in the plate. Hence the phage titer could be calculated in plaque forming unit (pfu) by applying the following formula:

Phage titer = number of plaques X final dilution X plating dilution (14)

Plaque morphology:

Full characterization of phage plaques including size and shape was achieved by using agar overlay method, According to (15).

Sensitivity to lipid solvent:

9 ml of chloroform and 9 ml of ether was added to 1 ml of *S. aureus* phage and incubated diagonally in a sterilized glass tube for 1 h. at room temperature with gentle shaking. The mixture was centrifuged at 3500 rpm for 5 minutes. Supernatant was separated in case of chloroform and precipitated in case of ether. The number of PFU were determined with 1 ml of phage as a control, according to (16).

Scanning Electron Microscope (SEM):

Bacteriophage pellet obtained by centrifugation for 1 h. at 25000 rpm was resuspended in 0.1 M ammonium acetate (pH 7), phage particles were deposited on carbon-coated copper grids, stained with 2% (w/v) potassium phosphotungstate (pH 7) and examined in Tescan EM (17).

Host range determination:

A spot test was used to determine the sensitivity of *S. aureus* isolates to phage infection, plating bacteria of each of the test *S. aureus* was prepared and overlaid of bottom agar. After overlay (approximately 30 minutes) a drop of phage suspension (10^8 PFU/ml) was carefully spotted on marked areas of the plates and incubated for 24 h. at room temperature, *S. aureus* isolates was classified as sensitive only if a distinct clear zone appeared in the spotted area against the background lawn of the bacteria (18).

RESULTS AND DISCUSSION

A total of 115 milk samples, 30 milk samples showed positive results for the presence of *S. aureus* after culturing on different media, on blood agar most colonies were produce Beta or Alpha hemolytic, On selective media which is mannitol salt agar, the colonies appeared as rounded, smooth convex, mucoid, yellowish in color due to fermentation of mannitol. These isolates were catalase positive, coagulase positive, oxidase negative. *S. aureus* is a major pathogen of bovine mastitis worldwide. Despite implementing intensive control measures, it is difficult to eradicate the intra mammary infections caused by this pathogen and it remains a substantial economic problem (19).

Antibiotics susceptibility:

Table (1) summarizes the antibiotic susceptibility results of 7 different antibiotics by disc diffusion test against 30 *S. aureus* isolates. In the present study, The antimicrobial susceptibility of *S. aureus* isolates revealed that all isolate were highly sensitive to Ciprofloxacin (90%), Gentamycin (83.3%) A moderate sensitivity was developed against Vancomycin (60%). Nevertheless less sensitivity 33.3% and 36.6% was noticed towards Penicillin and Ampicillin, respectively. Moreover, 50% of isolates were sensitive to Methicilline and Cefoxitin. The higher resistance of most *S. aureus* isolates to Methicillin, Penicillin and Ampicillin are coming from increased and frequent use of β -lactam antibiotics which lead to development of resistance due to production of Pencillinase enzymes (20).

Table (1) : Antibiotic susceptibility of of *S.aureus* isolates

Antibiotics	Resistant %	Intermediate %	Susceptible %
Ampicillin	56.6	6.6	36.6
Cefoxitin	43.3	6.6	50
Ciprofloxacin	10	0	90
Gentamycin	13.3	3.3	83.3
Methicilline	43.3	6.6	50
Penicillin	63.3	3.3	33.3
Vancomycin	23.3	16.6	60

Isolation of phage:

Bacteriophage specific to *S. aureus* associated with mastitis has been isolated from milk samples which is named (SP 9), phage showed the ability to produce a plaques after plated on bottom agar, as shown figure (1).



Figure (1): Plaques formed after agar overlay method

Morphological characterization and classification of phages:

The plaques were clear, round zones and their sizes were about 2–3 mm in diameter. Agar overlay method was used for preparation of *S. aureus* phage stocks and obtain a titer between 10^7 to 10^{12} PFU/ml. The SEM showed that phage structure composed of iso-metric head and contractile tail, these morphological characteristics indicated that the phage belong's to the family *Myoviridae*. (Figure 2).

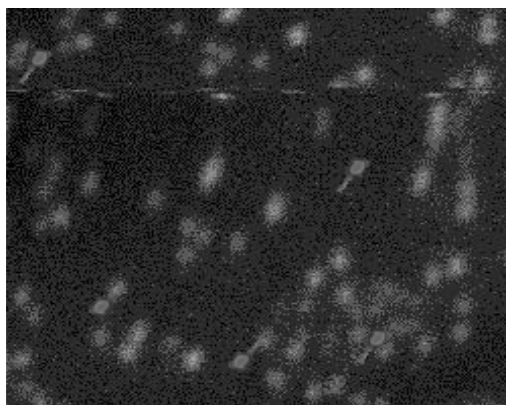


Figure (2): Scanning electron micrograph of phage. The magnification 85,94 Kx

Phage Sensitivity to lipid solvent:

Table (2) demonstrates data related to resistance of *S. aureus* phage to chloroform and ether, the survival rate of the virus was more than 95%, that's mean the phage belong's to *Myoviridae* family. That is mean the phage does not contain an envelope in its composition, therefore chloroform and ether might give a protection to *S. aureus* phage (21).

Table (2): sensitivity of phage to lipid solvent

Solvent	Phage PFU/ml	Survival %
Control	4.6×10^8	100
Chloroform	4.4×10^8	95.6
Ether	4.5×10^8	97.8

Determination of host range:

Spot test was used to determine multiplicity of host range, a total of 30 strains, 5 isolates (S8, S9, S10, S11, S13) were successfully lysed by phage, forming a clear lyses zone and inhibition bacterial growth at site of dropped phage (Figure 3). These *S. aureus* isolates have specific receptor on its cell wall enabling phage to be attached (12).



Figure (3): Spot lysis zone

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Impact of type 1 diabetes on quality of life of children and adolescents in Erbil City

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ABSTRACT

Type 1 Diabetes Mellitus is a chronic condition that may have an impact on children and adolescents health status. It's a highly prevalent, irreversible and disabling disease which will stay with the patients for life and affecting whole quality of life domains. This study aimed to assess the impact of Type 1 Diabetes on quality of life of children and adolescents with Type 1 Diabetes and its association with some variables.

A descriptive correlation study was conducted in Leila Qasim Center for Diabetes Care in Erbil City of Kurdistan region in Iraq from 5th Feb. 2012 to 5th Feb. 2013. Non probability (purposive) sample was selected among 120 children and adolescents having Type 1 Diabetes according to criteria of the study. A questionnaire format was constricted, and it consisted of three main parts (socio-demographic characteristics, medical findings of Type 1 Diabetes and assessing quality of life of children and adolescents having Type 1 Diabetes. Correlation coefficient was used to determine the reliability of the instrument 0.80. Validity of tool was applied through panel 14 experts. Data were collected by interview technique of children and adolescents, and analyses through using of SPSS, version 19.

the obtained results indicated that most children and adolescents were female, their mean age were 11.80 most of them were students and living low socio economic level. The domains of quality of life affected by disease including a physical, treatment, and snap finger. While worry and communication domains less affected.

Study recommended that health education should be a part of diabetes management in all diabetic clinics and hospitals rather than a set of instructions given once at the beginning of the follow-up.

Keywords: Type 1 Diabetes Mellitus, quality of life

الملخص باللغة العربية

يعتبر داء السكري من الأمراض المزمنة التي قد يكون لها تأثير على صحة الأطفال والمراهقين. إنه مرض واسع الانتشار، وله تأثير على نوعية الحياة بمختلف أوجهها لدى المرضى الذين يعانون منه.

تهدف الدراسة لتقييم جوانب نوعية الحياة لدى عينة من الأطفال والمراهقين المصابين بداء السكري من النوع الأول وارتباطها ببعض المتغيرات، ومن أجل ذلك، فقد صممت الدراسة اعتماداً على المنهج الوصفي ليتم تنفيذها في مركز ليلي قاسم لرعاية مرضى السكري في مدينة اربيل / إقليم كردستان - العراق، خلال الفترة من 5 فبراير / شباط عام 2012 إلى 5 فبراير / شباط عام 2013. وتم اختيار عينة غير احتمالية (غرضية) من 120 طفلاً ومراهقاً من المصابين بداء السكري من النوع الأول وفقاً لمعايير الدراسة، وقد تم إعداد وتطوير استبانة تضمنت ثلاثة أجزاء (المعلومات الديموغرافية والاجتماعية و المعلومات الطبية للأطفال والمراهقين، وأسئلة لتقييم نوعية الحياة لدى المصابين بداء السكري). تم تحديد الثبات والمصداقية للأسئلة من خلال حساب معامل الارتباط ألفا (0.80) والتي أجريت على (10) من الأطفال والمراهقين، كما تم تحقيق المصداقية للأسئلة من خلال عرضها على مجموعة من الخبراء ذوي الاختصاص وعددهم (14) خبيراً. تم جمع البيانات عن طريق المقابلات مع عينة الأطفال والمراهقين، وتحليلها من خلال استخدام (SPSS، النسخة 19).

دلت نتائج الدراسة أن معظم الأطفال والمراهقين كانوا من الإناث، بمتوسط أعمار (11.80) سنة، معظمهم من الطلاب وأنهم يعانون من مستوى متدن من الناحية الاقتصادية والاجتماعية. واستنتجت الدراسة بأن هناك مجموعة من العوامل لها تأثير كبير على الجوانب الحياتية لدى الأطفال والمراهقين المصابين بداء مثل العلاج ووخز الاصبع، بينما كانت عوامل أخرى مثل القلق والاتصالات أقل تأثيراً في حياتهم. أوصت الدراسة بضرورة أن يتم تعزيز مستوى التنقيف الصحي لدى المصابين بالسكري وأن يكون ضمن استراتيجيات إدارة المرض في جميع العيادات والمستشفيات المتخصصة بدلاً من أن يقتصر الأمر على تقديم مجموعة من التعليمات التي تقدم للمرضى في أول متابعة لهم.

INTRODUCTION

Diabetes is a killer disease in many part of the world, especially in childhood or adolescence, because their parents and families members do not recognize the beginning symptoms (1). It may lead to serious multi-system complication that often require additional intrusive treatments. It appears that T1Ds has a greater negative impact on Quality of Life (QOL) than Type 2 Diabetes (2).

Type 1 Diabetes Mellitus is one of the most common chronic childhood illnesses, affecting approximately 1 in every 400–600 children and adolescents (3). The incidence of T1Ds continues to increase by 3-5% per year, which associated with severe morbidity, mortality and enormous health care expenditures, makes T1Ds a prime target for prevention (4,5). The main aims of diabetes care in children and adolescents are to achieve optimal glycemic control, normal psychosocial development, a major challenge is to maximize QOL for the adolescent (6). It is the most common disease, which affects the QOL of children in different aspect of life including emotionally, physically, psychologically, socially and school achievement (2). Generally, lower QOL scores were associated with old age, poor glycemic control, an increasing number of hypoglycemic episodes, complications, low levels of education and outcome, self-reported depression, and female gender (7). In children has been given less attention, and many children that die early with complication (8).

In Kurdistan Region Iraq, Leila Qasim Center for Diabetes Care between the periods 1st December 2007 to 15th November 2008, there were 62 registered within the pediatrics age group (9). According to the statistical record of Leila Qasim Center for Diabetes Care in 2012, the number of diabetic pediatric registration was 230 patients from birth to 15 years old and 50 patients at age 16-18 years old. Also there were no studies in Kurdistan Region about QOL of Type 1 Diabetes Mellitus of children and adolescent and for that we need to study their QOL to improve their life through improving their management.

The aims of the study:

1. To assess socio demographic characteristics of children and adolescents having T1Ds.
2. To estimate the impact of T1Ds on the quality of life of those children and adolescents.
3. To identify the association between socio demographic characteristics (age, gender, parent's level of education, parent's occupation and economic status) of those and their quality of life.
4. To assess general impact of quality of life of children and adolescents having T1Ds.

MATERIALS AND METHODS

Design of the study:

Descriptive correlation design was used to assess the impact of Type 1 Diabetes on Quality of Life of children and adolescents in Erbil City.

Setting of the study:

The study was carried out in Leila Qasim Center for Diabetes Care. The Center was established in 1st Dec 2007, located in Ronaky Street, Erbil City/ Kurdistan Region the study period extended from 5th Feb 2012 to 5th Feb 2013.

Ethical Consideration:

When data collection was done, they were kept confidentially. The form for data collection was applied without mentioning the name of patients and their addresses, the purpose of the study was explained to all participants; explaining ethical aspects of the study and a verbal agreement was obtained from the involved participants.

Sample of the study:

A non- probability (purposive) sample of (120) children and adolescents were selected, attending Leila Qasim Diabetes Center for treatment and follow up. The sample was selected according to the following inclusion and exclusion criteria:

A: Inclusion criteria : Children and adolescents who are diagnosed by a physician with Type 1 Diabetes Mellitus for both genders. Their ages were between 6-18 years, under treatment for more than one year, and registered in this center, visiting for treatment and follow up were accepted in the study.

B: Exclusion criteria: Children and adolescents with Type 2 of diabetes ,gestational diabetes , ages above 18 and less than 6 years old and with newly diagnosed T1Ds were excluded.

Instrument of the study:

Through the review of related literatures studies and reliable QOL questionnaire (PedQL™ Diabetes Module version 3.0, available from: <http://www.pedsq.org/>) was selected to assess the impact of disease on QOL patients with Type 1 Diabetes, modified regarding purposive of the study and manipulation. The questionnaire form consisted of three parts:

Part I: Socio demographic characterizations of T1Ds of children and adolescents: which consisted general information about study sample (age, gender, occupation, duration of illness and socio-economic status) according to (WHO) includes (parent level education, parent occupation, crowding index and property) and measured by

special scale composed of 150 scores ranged from low 90 score, middle score 90-120 and high 121-150 score.

Part II: Children and adolescent's medical findings about T1DM: includes three parts: first part included signs and symptoms consisting of (6) items, second part included risk factors of disease and some questions related to family history of DM of parents, sibling, first degree of relative or none and pattern during 1st six months of life. Third part included complication of disease.

Part III: QOL of children and adolescent having T1DM: consisting of four domains; (physical, treatment, worry, communication) and snap finger each domain has some items, physical has (11) items, treatment has (7) items, worry has (4) items, and (3) items for communication. Each domain has three liker scales, (1) for not at all, (2) for some time, (3) for a lot.

Validity of study was done by (14) experts of different specialties related to the field of the present study, their responses were positive towards the study instrument. Correlation coefficient of the study was used to determine the reliability of the study instrument. Correlation coefficient was (0.80), which indicated that the scales were adequately reliable. Data were collected by using questionnaire format and filled out by the investigator, direct interview techniques used and those who kindly accepted to participate in the study. Each interview session took approximately (15- 20) minutes. In the present study, data were analyzed through the use of statistical package for social sciences SPSS version 19th. All statistical produces were tested on a probability of P. value, considering in the following ≤ 0.01 High significant (HS), ≤ 0.05 Significant (S) and >0.05 Non significant (NS).

RESULTS

Table (1) showed that the age of patients ranged between (6-18) years, the mean age and standard deviation were (11.80 \pm 3.129), (53.3 %) of them were females ,(85.8) were students and the lowest percentage (14.2%) were unemployed, (45%) of mothers were illiterate, while the lowest percentage (7.5%) was institute or college graduates .Father's education (26.7%)were graduated from primary school, more than two third of mother's occupation (91.7%) were unskilled workers, while the lowest percentage (3.3%) were lower professional and semi skilled workers. Fathers' occupation more of them (59.2%) were unskilled workers but the lowest percentage (8.3%) was high professional ones. Children and adolescents coming from urban, rural areas were nearly equal (55.8%, 44.2%) respectively. The socio-economic status, the highest proportion (88.3%) of children and adolescents reported low socioeconomic status (less than 90) score and the lowest percentage revealed high socioeconomic status (121-150) score.

Table (1): socio demographic characteristics of T1Ds patients

Socio demographic characteristics	N = 120	
	F	%
Age Group		
6-9	34	28.3
10-12	31	25.8
13-15	42	35.0
16-18	13	10.8
Mean \pm SD	11.80 \pm 3.129	
Gender		
Male	56	46.7
Female	64	53.3
Patient occupation		
Employed	0	0
Unemployed	17	14.2
Student	103	85.8
Education of Mother		
Illiterate	54	45.0
Read and write	18	15.0
Primary school graduate	28	23.3
Secondary school graduate	11	9.2
Preparatory school graduate	0	0
Institute or college graduate	9	7.5
Education of father		
Illiterate	23	19.2
Read and write	22	18.3
Primary school graduate	32	26.7
Secondary school graduate	21	17.5
Preparatory school graduate	7	5.8
Institute or college graduate	15	12.5
Occupation of Mother		
High professional jobs	6	5.0
Lower professional, skilled& semiskilled workers	4	3.3
unskilled workers	110	91.7
Occupation of father		
High professional jobs	10	8.3
Lower professional, skilled& semiskilled workers	39	32.5
unskilled workers	71	59.2
Residence area		
Urban	67	55.8
Rural	53	44.2
Socio economic status/score		
Low (<90)	106	88.3
Middle (90-120)	8	6.7
High (121-150)	6	5.0

Table (2) shows that the duration of illness (56.7%) of patients were ranging from (1-3) years and those having disease from (10-11) years showing the lowest percentage (2.5%).

Table (2): Duration of illness

Duration of Illness (years)	F	%
1-3	68	56.7
4-6	32	26.7
7-9	17	14.2
10-11	3	2.5

Table (3) shows that the (83.3%) were having frequent thirst ,half of them (86.7%) were having infrequent hypoglycemia but the lowest percentage (16.7%) having infrequent of fatigue.

Table (3): Signs and symptoms of T1Ds

Signs and symptoms items	Frequent		Infrequent	
	F	%	F	%
Fatigue	81	67.5	39	32.5
Thirst	100	83.3	20	16.7
Frequent need to urinate	82	68.3	38	31.7
Felt unusually hungry	99	82.5	21	17.5
Nocturnal enuresis	52	43.3	68	56.7
Symptom of low blood sugar	16	13.3	104	86.7

Table (4) revealed that (63.3%) of them were not having family history of the disease, while (6.7%) have a sibling with T1Ds, and (58.3%) was received breast feeding (14.2%) was received bottle feeding during childhood period.

Table (4): Risk factors of T1Ds

Risk factors	Parent		Sibling		Relative		Non	
	F	%	F	%	F	%	F	%
Family history	24	20.0	8	6.7	12	10.0	76	63.3
Patterns of feeding	Breast		Bottle		Mixed			
	F	%	F	%	F	%		
	70	58.3	17	14.2	33	27.5		

Table (5) shows that few patients were complaining from the following T1Ds complications, retinopathy (14.2%) and nephropathy with neuropathy (8.3%, 3.3%) respectively.

Table (5): Complication of T1Ds

Compilation	Yes		No	
	F	%	F	%
Retinopathy	17	14.2	103	85.8
Nephropathy	10	8.3	110	91.7
Cardiovascular disease	0	0	120	100.0
Diabetic foot	0	0	120	100.0
Neuropathy	4	3.3	116	96.7

Table (6) shows rank order of the overall domains of quality of life in children and adolescents with T1Ds. It has shown that (physical and treatment) domains were affected by the disease, it means they reported high means score 2.21, 1.92, and 1.87 respectively while the worry and communication domains were less affected, it means they reported low means score 1.57, 1.69 respectively.

Table (6): Rank order of overall quality of life of diabetes patients

Quality of life domains	Mean values	Standard deviation
Physical	1.92	0.24
Treatment	1.87	0.43
Worry	1.57	0.34
Communication	1.69	0.58
Shape (snap finger)	2.21	0.79

Table (7) shows that (46.7%) of patients had moderate level of quality of life and (25%) of them had low level of quality of life while (28.3%) had high level of quality of life.

Table (7): Level of quality of life domains

Level of quality of life /score	F	%
High (5.84-8.14 score)	34	28.3
Moderate (8.15-10.45 score)	56	46.7
Low (10.46-12.73 score)	30	25.0
Total	120	100

Table (8) shows that there was no significant association between the level of quality of life and socio demographic status.

Table (8): Level of quality of life with socio demographic characteristics

Level quality of life		High	Moderate	Low	X ² P. Value
Socio demographic		F	F	F	
Age	6-9	10	10	14	0.06 NS
	10-12	9	13	9	
	13-15	11	26	5	
	16-18	4	7	2	
Gender	Male	17	27	12	0.69 NS
	Female	17	29	18	
Occupation	Employ	0	0	0	0.13 NS
	Unemployed	2	8	7	
	Student	32	48	23	
Residence are	Urban	19	31	17	0.99 NS
	Rural	15	25	13	
Socio economic	High	27	50	29	0.26 NS
	Middle	4	4	0	
	Low	3	2	1	

Table (9) shows that there was no significant association between the level of quality of life and duration of illness at $p=0.16$.

Table (9): Level of quality of life with duration of illness

Level QOL \ Duration of illness	High	Moderate	Low	X ² P. Value
1-3	21	30	17	0.16 NS
4-6	6	15	11	
7-9	5	11	1	
10-11	2	0	1	

Table (10) shows that there was significant association between the level of quality of life and signs and symptoms including fatigue at $P= 0.03$, frequent urination at $P=0.003$ and nocturnal enuresis at $P= 0.00$, while there was no significant association between the level of quality of life and other signs and symptoms such as at $P=0.10$, unusual hunger at $P= 0.06$ and hypoglycemia at $P= 0.69$ respectively.

Table (11) shows that there was no significant association between the level of quality of life with family history at $P= 0.96$ and feeding during 1st six months of life of study sample at $P=0.39$.

Table (12) shows that there was significant association between the level of quality of life and neuropathy at $P=0.048$ complication, while there was no significant association between the level of quality of life and retinopathy and nephropathy complications respectively.

Table (10): Association between level quality of life and signs and symptom of diabetes patients

Signs and symptom \ Level of QOL		High	Moderate	Low	X ² P. Value
Fatigue	Frequent	20	35	26	0.03 S
	Infrequent	14	21	4	
Thirst	Frequent	25	47	28	0.10 NS
	Infrequent	9	9	2	
Frequent need to urinate	Frequent	19	35	28	0.003 S
	Infrequent	15	21	2	
Felt unusually hungry	Frequent	26	44	29	0.06 NS
	Infrequent	8	12	1	
Nocturnal enuresis	Frequent	11	18	23	0.00 HS
	Infrequent	23	38	7	
Hypoglycemia	Frequent	4	9	3	0.69 NS
	Infrequent	30	47	27	

Table (11): Association between level of quality of life and risk factors

Risk factors \ Level of QOL		High	Moderate	Low	X ² P. Value
Family history of DM	Parent	5	11	8	0.94 NS
	Sibling	2	4	2	
	Relative	4	5	3	
	Non	23	36	17	
Patterns of feeding during 1 st six month of life	Breast feeding	24	30	16	0.39 NS
	Bottle feeding	5	8	4	
	Mixed feeding	5	18	10	

Table (12): Association between level of quality of life and complication

complication \ Level of QOL		High	Moderate	Low	X ² P. Value
Retinopathy	Yes	4	6	7	0.24 NS
	No	30	50	23	
Nephropathy	Yes	1	4	5	0.12 NS
	No	33	52	25	
Neuropathy	Yes	1	0	3	0.048 S
	No	33	56	27	

DISCUSSION

The current study shows in (table 1) patient's aged at group of 13- 15 years old and the mean age and standard deviation of them were (11.80±3.129). These findings were in agreement with the results of the study done on 84 Bahraini patients under 15 years of age diagnosed with T1Ds during a study period from the years, 1998 to 2000 (10,11). The current study agreed with another study done in Poland (12) found that the highest incidence ratio was observed in the group of adolescents' age. While a study (13) added that the incidence rates for T1Ds were largely based on observations in children under the age of 15 years.

The majority of them were females rather than males, which was supported by (4) who conducted a study in Basrah on 96 patients who had been admitted to pediatric hospital and the study found that the majority of them were females 65.6%. another study (14) in Shiraz/ Southern Iran of 94 children and adolescents having T1Ds mentioned that there were 56.4% female and 43.6% male patients. Most of the study samples were students in school, this was supported by the study (3,15), and supported that most of children and adolescents having T1Ds were students.

High percent of patients living in urban area more than in rural area that was in agreement with a study (16) who mentioned that the possible causes of lower prevalence of diabetes in Saudi Arabia probably due to more individuals live in rural areas with more physical activity and lower rates of obesity. Most of patients were from low socioeconomic status. This result is in agreement with the study (7) who showed that people at a low socioeconomic status groups 39.1% had more problems than people in the high socioeconomic status groups.

Table (2) shows that the duration of illness in most of patients were (1-3) years. This result was near to a study (9) on 99 patients with T1Ds, the highest percentage 51.1% of their duration of disease was from (1-5) years.

Results obtained from table (3) showed that most patients with T1Ds had many signs and symptoms. They complained from fatigue, frequency of urination, excessive thirst and hunger. These results were supported by the (13), who mentioned that most symptoms of T1Ds were frequency of urination excessive thirst, hunger and fatigue.

The current study found that there was no family history of DM(table 4), thus, it agreed with studies (14,15), who stated that most patients 60.5% didn't have family history of diabetes and 56.6% patients with positive family history of diabetes.

The patients with advanced T1Ds developed many complications. Patients in the present study had some of these complications such as retinopathy, nephropathy and neuropathy as shown in table (5). These findings were in agreement with the study done in Kilimanjaro/Tanzania which found a

prevalence of retinopathy 14% in Type 1 Diabetes (16).

Results in table (6) showed the rank order of overall of domains of quality of life of study sample with T1Ds. It has shown that snap finger, physical, treatment, communication and worry domains were affected by the disease also shows that snap finger and physical domains were more affected by the disease while treatment and communication domains were less affected by the disease than other domains. Present study was supported by the study that reported that physical and communication domains were more affected by the disease than other domains, and also found that worry domain in quality of life was less affected by the disease (17).

Results in table (7) showed that the highest percentage of patients were in moderate level of quality of life. This result was supported by the study (7) who found that most of children and adolescents were in moderate level of quality of life in T1Ds.

Results in table (8) found that there was no relation between age groups and physical , treatment and worry domains. Thus, these results were disagreed with the study conducted on a respective sample of 684 patients with T1Ds in Heinrich-Heine University in Germany mentioned that there was significant relation between physical complain and age (18). Another study found that there was no significant relation between age and treatment domain of diabetic patients while there was significant relation between age and worry domain of quality of life (19), while there was significant relation between age and communication domain and this result was in agreement with a study done in 89 children and adolescents with T1Ds in Aristotle university of Thessaloniki in Greece, which mentioned that there was significant relation between age and communication domain of quality of life (20). In general, there were no significant correlations at baseline total quality of life reported by their ages (19).

Results shown in table (9) represented the duration of illness in study sample and reflected that physical and treatment domains were not affected by diabetics. A study determined that the impact of quality of life of diabetic patients had no effect on duration of diabetes (6). The current study disagreed with study that found that there was significant relation between the duration of disease and treatment domain of diabetic patients (20). In general, the current study found that there was no significant relation between duration of disease and quality of life domains, which were supported by (21, 22), who mentioned that there was no significant association between quality of life and disease duration.

As shown in table (10), there was highly significant relation between physical domain and the symptoms of disease such as hungry, thirsty, fatigue and urination. These results were in agreement with studies (23,24) which emphasized that there was significant relation between physical domain and

hungry and thirsty. However, there was no significant relation between hypoglycemia and physical domain in the current study, which was supported by a study (24), while there was a significant relation between treatment domain and signs and symptoms such as thirsty, excessive urination and others of T1Ds, these results were supported by (19). These results were in agreement with a study who found that the T1Ds has less impact on the quality of life (24).

Results in table (11) revealed that there was significant relation between family history of DM and physical domain of quality of life, which were supported by a study findings that revealed that there was highly significant relation between family history of DM and physical domain of quality of life (26,27), while there was no relation between treatment, worry and communication domains with quality of life. These results were supported by study that mentioned that there was no significant impact of quality of life on family history of diabetic patients (2).

In table (12), results reported that the physical and worry domains of quality of life didn't appear significant relation with complication of T1Ds but worry domain was significant with neuropathy. This result was disagreed with the study that found that worry domain appeared less sensitive to differences in severity of complications (7). These results were comparable to a study that found an association between nephropathy and reduced health related QOL in the domain of physical functioning and general health perceptions.

CONCLUSION

The study showed that mean age of patients was (11.80), most of them were females, students and coming from urban, more than half of them were from low socioeconomic status. The duration of illness most of patients was range from 1-3 years. Many patients complained of signs and symptoms such as fatigue, thirst, frequent urination and hunger. Most of them did not have positive family history of T1DM but a little of them had it, and few of patients complained of retinopathy, nephropathy and neuropathy. Furthermore concerning domains of quality of life in snap finger, physical domain and treatment domain were affected by the disease, while worry and communication domains were less affected by the disease, in general children and adolescent's quality of life was at moderate level.

RECOMMENDATIONS

1. Continuous training of health staff (doctors, nurses, and lab workers) is needed for the implementation of educational interventions to raise awareness about the importance of health education among children and adolescents.

2. Health education can be an integral part of diabetes management in all diabetic clinics and hospitals rather than a set of instructions given once at the beginning of the follow-up. Education needs to be supported by psychosocial and possibly family therapy interventions.

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قسم الدراسات والبحوث العربية

***ARABIC STUDIES AND RESEARCHES
SECTION***

تأثير إضافة السماد العضوي Azomine و النيتروجيني على نمو و إنتاجية صنفين من نبات الريحان

Ocimum basilicum L.

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الملخص باللغة العربية

أجري البحث في كلية الزراعة / جامعة الكوفة خلال الموسم الزراعي 2013 لدراسة تأثير رش السماد العضوي Azomine بتركيز 5 مل. لتر⁻¹ و السماد النيتروجيني بتركيز 400 ملغم . لتر⁻¹ على صنفين من نبات الريحان هما الريحان الحلو *Ocimum basilicum* L. و الريحان الأحمر *Ocimum basilicum* L. var Purple واستعمل تصميم القطاعات العشوائية الكاملة R.C.B.D وبثلاثة مكررات ، وفورنت المتوسطات باستعمال اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05.

وأظهرت النتائج أن نبات الريحان الحلو تفوق معنوياً في زيادة كل من ارتفاع النبات إلى 18.50 سم و عدد الأوراق إلى 11.46 ورقة. نباتات¹ والمساحة الورقية إلى 20.57 سم² قياساً مع نبات الريحان الأحمر الذي انخفض إلى 15.92 سم و 9.30 ورقة. نباتات¹ و 19.19 سم² لكل من ارتفاع النبات وعدد الأوراق والمساحة الورقية على التوالي، إلا أنه أظهر تفوقاً في زيادة محتوى الأوراق من الكلوروفيل الكلي إلى 48.68 ملغم. 100غم⁻¹ مقارنة مع نبات الريحان الحلو الذي انخفض إلى 43.43 ملغم. 100غم⁻¹. فيما تفوق الرش بالسماد النيتروجيني و العضوي Azomine معنوياً لأغلب الصفات المدروسة، حيث بلغ ارتفاع النبات 21.50 و 20.94 سم و الوزن الجاف للمجموع الخضري 11.55 و 11.45غم. نباتات¹ و محتوى الأوراق من الكلوروفيل الكلي 50.30 و 49.48 ملغم. 100غم⁻¹ و كمية الإنتاج لوحدة المساحة إلى 3.02 و 2.94 طن. هكتار⁻¹، على التوالي، فيما تفوق السماد العضوي Azomine معنوياً في زيادة كل من عدد الأوراق الكلية إلى 13.49 ورقة. نباتات¹ و المساحة الورقية إلى 21.97 سم² قياساً مع معاملة المقارنة والتي أظهرت انخفاضاً في الصفات المدروسة، وكان للتداخل بين عاملي التجربة التأثير المعنوي على مؤشرات النمو الخضري.

الكلمات المفتاحية: السماد العضوي ، السماد النيتروجيني، الريحان الحلو، الريحان الأحمر

Effect of Azomine and nitrogen spraying on growth and yield of two Basil Cultivars *Ocimum basilicum* L

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ABSTRACT

The research was conducted in a department of Horticulture /Agriculture faculty /Kufa university during the spring growing season of 2013. The aim was to study the effects of nitrogen with 400 mg. L⁻¹ and Azomine spraying with 5 ml.L⁻¹ on sweet basil *Ocimum basilicum* L. and *Ocimum basilicum* L. var. Purple on plant growth parameter. Randomized Complete Block Design (R.C.B.D.) was used with three replications , using Duncans multiples range test to compare means with probability of 0.05 level.

The sweet basil significantly increased of plant length to 18.50 cm , leaf area to 20.57 cm² compare with red basil which decreased of plant length to 15.92cm , leaf area to 19.19 cm² but significantly increased of leaves content of chlorophyll pigments to 48.68 mg.100g⁻¹ while reduced in sweet basil to 43.43 mg.100g⁻¹. While nitrogen and Azomine spraying significantly increased of plant length to 21.50 and 20.94 cm ,dry matter to 11.55 and 11.45g. plant⁻¹ and leaves content of chlorophyll pigments to 50.30 and 49.48 mg.100g⁻¹ and yield to 3.02 and 2.94 t.ha⁻¹ respectively,while Azomine spraying significantly increased of leaf number to 13.49 leaf.plant⁻¹ and leaf area to 21.97 cm² compare with control treatment which decreased all vegetative growth parameter and yield. The interaction between two factors were significantly for most vegetative growth parameter and yield.

المقدمة

جدول رقم (1) : بعض الصفات الفيزيائية والكيميائية لتربة الحقل لموسمي التجربة قبل الزراعة

نوع التحليل	وحدة القياس	القياس
نسجة التربة	-	طينية رملية
الرمل	غم . كغم ⁻¹	340
الغرين	غم . كغم ⁻¹	250
الطين	غم . كغم ⁻¹	300
درجة تفاعل التربة pH	-	7.8
التوصيل الكهربائي EC	ديسيمينز متر ⁻¹	2.9
المحتوى العضوي Organic Matter(O.M.)	غم . كغم ⁻¹	9.3

تكون الوسط الزراعي من بيت الموس والتربة النهرية (مزيج) بنسبة 1:1 ، زرعت البذور بتاريخ 2013/3/15 واستمرت التجربة لغاية 2013/7/15. تضمنت التجربة دراسة تأثير عاملين ، العامل الأول صنفين من نبات الريحان الحلو والاحمر، أما العامل الثاني فكان نوعين من التسميد هما رش السماد العضوي Azomine بتركيز 5 مل لتر⁻¹ والسماد النيتروجيني (اليوريا) بتركيز 400 ملغم لتر⁻¹ وبدون تسميد (معاملة المقارنة). رشبت النباتات مرتين، الرشوة الأولى بعد تكون أربعة أوراق حقيقية، أما الرشوة الثانية فكانت بعد عشرة أيام من الرشوة الأولى بحسب طريقة (10) ، فيما رشبت معاملة المقارنة بالماء المقطر فقط، وقد أضيفت مع كل تركيز من التراكيز المحضرة 5 غم / 10 لتر من مسحوق الغسيل (Sodium Tripolyphosphate) كمادة ناشرة وذلك لتقليل الشد السطحي للمحلول، واستعملت الرشوة الظهرية سعة 10 لتر في رش المعاملات عند وقت الغروب وحتى درجة الليل الكامل ونزول أول قطرة ، وبفارق 24 ساعة بين محلول وآخر، كما أجريت عمليات الخدمة كالري والعرق وإزالة الأدغال وغيرها وفقاً للموصى به وحاجة النباتات (11). نفذت التجربة باستعمال تصميم القطاعات العشوائية الكاملة Randomized Complete Block design وبثلاث مكررات ، وبذلك كان عدد الوحدات تجريبية 18 وحدة تجريبية . وتمت المقارنة بين المتوسطات حسب اختبار دنكن متعدد الحدود Duncans Multiple Test عند مستوى احتمال 0.05. وقد شملت الصفات المدروسة ما يلي:

1. ارتفاع النبات (سم): تم قياس ارتفاع النبات بواسطة شريط القياس المعدني من سطح التربة ابتداءً وحتى أعلى قمة النبات.
2. عدد الأوراق الكلية (ورقة نبات⁻¹): تم حساب عدد الأوراق بعد اخذ عشر نباتات بصورة عشوائية من كل وحدة تجريبية.
3. المساحة الورقية (سم²): تم حساب المساحة الورقية على أساس الوزن الجاف كما ذكرها Watson و (1953) Watson بتطبيق المعادلة الآتية:

$$\text{المساحة الورقية (سم}^2\text{)} = \frac{\text{مساحة 30 قرصا} \times \text{الوزن الجاف الكلي لأوراق النبات (غم)}}{\text{الوزن الجاف لـ 30 قرصا (غم)}}$$

4. عدد الأغصان الجانبية (فرع نبات⁻¹): تم حساب عدد الأغصان الجانبية بعد اخذ عشر نباتات بصورة عشوائية من كل وحدة تجريبية.
5. الوزن الجاف للمجموع الخضري (غم نبات⁻¹): قُلعت النباتات ثم قطعت بالقرب من منطقة التاج لفصل المجموع الخضري عن المجموع الجذري ثم وزنت بالميزان الحساس Sensitive balance بعدها وضعت في أكياس ورقية مثقبة وجففت في الفرن الكهربائي Oven على درجة حرارة 70 - 72 م° ولحين ثبوت الوزن (12).

يعد نبات الريحان *Ocimum basilicum* L. أحد نباتات العائلة الشفوية Lamiaceae التي تضم عددا كبيرا من النباتات العطرية الواسعة الانتشار في العالم (1) ، إذ يضم هذا الجنس حوالي 200 نوع ، بالإضافة إلى عدد من الأصناف التي تنتشر طبيعياً في المناطق الاستوائية لقارتي آسيا وأفريقيا والنتين تعدان الموطن الأصلي لهذا النبات (2، 3). يتميز نبات ريحان بأنه نبات عشبي قوي النمو و ذو رائحة عطرية قوية شبيهة برائحة القرنفل أو الينسون أو الحمضيات ، سيقانه منتصب يصل ارتفاعها من 40 إلى 60 سم تقريباً غزيرة التفريع، خاصة الأجزاء العلوية منها، وتتخذ الأوراق الشكل البيضاوي أو الرمح وتكون حوافها مسننة تسنينا بسيطاً ذات لون أخضر غامق (4) ، أما نبات الريحان الأحمر فهو يشبه الريحان الحلو من حيث النمو، إلا أنه يتميز بأن أوراقه وسيقانه تكون باللون الأحمر (5).

ونظراً لأهمية النباتات الورقية والعطرية عند الشعوب، فقد انتشرت زراعتها في كثير من بقاع العالم ومنها العراق وتنوعت استعمالاتها وازداد الطلب عليها، إذ شهد العالم العودة إلى الطبيعة والغذاء الطبيعي، وهذا ما أكدته منظمة الصحة العالمية (6) في تقريرها الذي أشار إلى أن الكثير من السكان يفضلون تناول الغذاء الطازج واستعمال الطب الشعبي المتمثل بالنباتات الطبية والعطرية لعلاج الكثير من الأمراض، إذ إن من أهم المميزات التي شجعت على استعمال هذه النباتات بوصفها غذاء وعلاجاً لقلّة تأثيراتها الجانبية وهضمها بصورة جيدة دون أن تحدث آثاراً جانبية على الجسم.

وتعد الزراعة العضوية وسيلة للتوازن الطبيعي للبيئة بجميع عناصرها ومكوناتها ، فهي نظام زراعي متكامل لإنتاج غذاء ذي قيمة ونوعية جيدة ، وبمواصفات صحية عالية (7) ، وتعتبر الأسمدة الدبالية Humus Fertilizers كاملة التحلل من تلك الوسائل الهامة التي تجنب الآثار السلبية الناجمة من استعمال الأسمدة الكيميائية المصنعة التي تعمل على إحداث مشاكل كثيرة أهمها تلوث المياه الجوفية ببقايا تلك الأسمدة، فضلاً عن زيادة محتوى منتجات الخضار من النترات وما تتركه من آثار سلبية على صحة الإنسان والحيوان (8).

إن الأسمدة الدبالية تتضمن عدداً من الأحماض العضوية مثل Humic و Fulvic التي لها أثر فعال في جاهزية العناصر الغذائية الصغرى للنبات، وبالتالي تأثيرها على نموه ، فهي تسهل حركة العناصر المعدنية وانقسام الخلايا في النبات مما له انعكاس كبير في تحسين مؤشرات النمو الخضري والصفات النوعية للثمار (9).

ونظراً لقلّة الدراسات على نبات الريحان الأحمر في العراق وعدم إدخاله كصنف مزروع، فقد أجري هذا البحث لدراسة ما يلي:

1. معرفة ظروف زراعة نبات الريحان الأحمر ومدى نجاحه في منطقة الفرات الأوسط من العراق مقارنة مع نبات الريحان الحلو .
2. تأثير رش الأسمدة العضوية على مؤشرات النمو الخضري لنباتات قياساً مع السماد النيتروجيني الكيميائي.

المواد وطرق العمل

أجريت التجربة خلال الموسم الزراعي 2013 في كلية الزراعة /جامعة الكوفة، أخذت عشرة عينات من التربة ومن عدة أماكن مختلفة لكلا موسمي الزراعة و بأعماق مختلفة 0 - 30 سم ثم خلطت العينات خلطاً متجانساً وتم تعريضها لأشعة الشمس لمدة 24 ساعة ثم طحنت ونخلت بمنخل ذي فتحات 2 ملم بعد ذلك أخذت منها عينة واحدة عشوائية لغرض تحليل بعض الصفات الفيزيائية والكيميائية للتربة في مختبر البحوث التابع لكلية الزراعة / جامعة الكوفة، ويوضح الجدول رقم (1) نتائج تحليلات تربة الحقل.

جدول رقم (2): تأثير صنف النبات ونوع السماد والتداخل بينهما في ارتفاع النبات (سم)

الصنف	نوع السماد			معدل الصنف
	سماد بدون تسميد	سماد النتروجيني 400 ملغم/لتر ¹	سماد العضوي Azomine 5 مل/لتر ¹	
ريحان الحلو	9.66d	24.03a	21.81b	18.50a
ريحان الاحمر	8.73d	18.97c	20.07b	15.92b
معدل نوع السماد	9.19b	21.50a	20.94a	

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها معنوياً حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

2. عدد الأوراق الكلية (ورقة نبات¹): يتضح من الجدول رقم (3) ان الريحان الحلو قد تفوق معنوياً في عدد الأوراق الكلية نبات¹ على الريحان الأحمر، فقد بلغ 11.46 ورقة نبات¹، أما ريحان الأحمر فقد بلغ 9.30 ورقة نبات¹. ويلاحظ من الجدول نفسه أن الرش بالسماد العضوي Azomine قد تفوق معنوياً في زيادة عدد الأوراق الكلية إلى 13.49 ورقة نبات¹، فيما وصل في السماد النتروجيني إلى 9.55 ورقة مقارنة بالنباتات غير المعاملة التي أعطت أقل معدل في عدد الأوراق الكلية بلغ 8.09 ورقة نبات¹، أما بالنسبة للتداخل بين صنف النبات ونوع السماد، فقد أظهرت النتائج أن الريحان الحلو مع السماد العضوي Azomine قد تفوق معنوياً في زيادة عدد الأوراق الكلية والتي وصلت إلى 15.33 ورقة نبات¹، بينما انخفض في نباتات الريحان الأحمر وغير المسمدة بأي نوع من الأسمدة إلى 7.85 ورقة نبات¹.

جدول رقم (3): تأثير صنف النبات ونوع السماد والتداخل بينهما في عدد الأوراق الكلية (ورقة نبات¹)

الصنف	نوع السماد			معدل الصنف
	سماد بدون تسميد	سماد النتروجيني 400 ملغم/لتر ¹	سماد العضوي Azomine 5 مل/لتر ¹	
ريحان الحلو	8.33cd	10.72 b	15.33 a	11.46 a
ريحان الاحمر	7.85 d	8.39 c	11.66 b	9.30 b
معدل نوع السماد	8.09 c	9.55 b	13.49 a	

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها معنوياً حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

3. المساحة الورقية (سم²): تشير نتائج الجدول رقم (4) على أن الريحان الحلو قد تفوق معنوياً في زيادة المساحة الورقية إلى 20.57 سم² قياساً مع الريحان الأحمر الذي بلغ 19.19 سم². ومن الجدول نفسه نلاحظ تفوق السماد العضوي Azomine معنوياً في زيادة المساحة الورقية إلى 21.97 سم² مقارنة مع معاملة المقارنة التي أعطت أقل معدل بلغ 17.49 سم². أما عن التداخل بين عاملي التجربة فيشير الجدول رقم (4) إلى أن تسميد الريحان الاحمر مع السماد العضوي Azomine قد تفوق معنوياً في إعطاء أعلى معدل من المساحة الورقية بلغت 21.99 سم²، فيما بين نفس النبات أن عدم رشه بأي نوع من السمادين المذكورين قد خفض المساحة الورقية إلى 16.34 سم².

6. محتوى الأوراق من الكلوروفيل (ملغم/100غم¹): أخذت خمس عينات عشوائية من أوراق نباتات لكل وحدة تجريبية (ضمن المربع المعلم)، إذ تم اختيار الورقة الخامسة من القمة النامية (الصحاف، 1989) لكل نبات وغسلت بالماء جيداً ثم أخذ 5 غم من النسيج الورقي، بعد ذلك تم تقطيعه لتسهيل عملية الاستخلاص أضيف لكل عينة 10 مل أسيتون تركيز 85% وسحق النسيج بهاون خزفي ثم رشح محلول الصبغات باستعمال ورق الترشيح نوع (Whatman No.1) أعيدت العملية مرة أخرى لاستخلاص المتبقي من الصبغات مع 10 مل أخرى من الأسيتون، حتى ابيض النسيج ثم أكمل حجم الراشح الكلي بالأسيتون إلى 100 مل (13). استعمل جهاز المطياف الضوئي Spectrophotometer لقياس الامتصاص الضوئي بالطولين الموجين 645 و 663 نانومتر في مختبر الدراسات العليا في كلية الزراعة / جامعة الكوفة وحُسبت كمية الصبغات الكلوروفيل الكلي وفق المعادلة الآتية:

$$\text{Total Chlorophyll} = 20.2 \times D_{645} + 8.02 \times D_{663} (V/W \times 1000)$$

7. كمية الإنتاج لوحدة المساحة (طن/هكتار¹): تم حساب معدل الإنتاج لوحدة المساحة وذلك بأخذ عينة عشوائية من الوحدات التجريبية لتحصد النباتات بارتفاع 2 سم من سطح الأرض (14)، لتوضع بعد ذلك النباتات في أكياس ورقية ووزنت مباشرة في الحقل بعد حصاها لغرض استخراج الوزن الرطب لكل عينة ليمثل الإنتاج (بعد أن استخراج وزن الكيس).

8. النسبة المئوية للنيتروجين: قدر محتوى النيتروجين الكلي حسب طريقة كيدال Kjeldahl باستعمال جهاز Micro Kjeldahl كما ذكرها الصحاف (12) بأخذ 10 مل من كل عينة وأضيف لها 10 مل من هيدروكسيد الصوديوم NaOH تركيز 40% ثم أجريت له عملية التقطير، وجمعت الأمونيا المتحررة في دورق زجاجي يحتوي على 25 مل حامض البوريك تركيز 2% مع قطرتين من خليط دليلي Methyl Red و Bromocresal Green، وسحقت الأمونيا التي تم جمعها مع HCl وطبقت المعادلة التالية:

$$N\% = \frac{\text{حجم الحامض المستهلك بالتسحيح} \times \text{عيارية الحامض} \times 14 \times \text{حجم التخفيف}}{\text{حجم العينة المأخوذة عند تقطير} \times \text{وزن العينة المهضومة} \times 1000}$$

النتائج والمناقشة

1. ارتفاع النبات (سم): تظهر نتائج الجدول رقم (2)، تفوق نبات الريحان الحلو تفوقاً معنوياً في زيادة ارتفاع النبات إلى 18.50 سم قياساً مع نبات ريحان الاحمر الذي بلغ 15.92 سم. أما عن تأثير نوع السماد فيتضح من الجدول نفسه تفوق التسميد النتروجيني والعضوي معنوياً في زيادة ارتفاع النبات إلى 21.50 و 20.94 سم على التوالي قياساً مع معاملة المقارنة التي أظهرت انخفاضاً في هذه الصفة المدروسة إلى 9.19 سم. فيما كان للتداخل ما بين صنف النبات والتسميد فقد أظهرت النتائج ان رش السماد النتروجيني على نبات الريحان الحلو قد تفوق معنوياً في زيادة ارتفاع النبات إلى 24.03 سم مقارنة مع النباتات غير المسمدة والتي أنخفضت فيها الصفة المدروسة إلى 9.66 و 8.73 سم لكل من الريحان الحلو والاحمر على التوالي (جدول رقم (2)).

جدول رقم (6): تأثير صنف النبات ونوع السماد والتداخل بينهما في الوزن الجاف لمجموع الخضري (غم. نبات⁻¹)

معدل الصنف	نوع السماد			معدل الصنف
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد	
10.83 a	11.60 b	12.44 a	8.45 d	ريحان الحلو
10.25 a	11.30 b	10.67 c	8.80 d	ريحان الأحمر
	11.45 a	11.55 a	8.62 b	معدل نوع السماد

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها
معنويًا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

جدول رقم (4): تأثير صنف النبات ونوع السماد والتداخل بينهما في المساحة الورقية (سم²).

معدل الصنف	نوع السماد			معدل الصنف
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد	
20.57 a	21.96 a	21.11 a	18.65c	ريحان الحلو
19.19 b	21.99 a	19.26 b	16.34d	ريحان الأحمر
	21.97 a	20.18 b	17.49c	معدل نوع السماد

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها
معنويًا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

6. محتوى الأوراق من الكلوروفيل الكلي (ملغم. 100 غم⁻¹): تبين النتائج في الجدول رقم (7) أن الريحان الأحمر تفوق معنويًا في زيادة محتوى الأوراق من الكلوروفيل التي بلغت 48.68 ملغم. 100 غم⁻¹ قياسًا مع الريحان الحلو الذي انخفض إلى 43.43 ملغم. 100 غم⁻¹، كما تشير النتائج إلى أن التسميد النتروجيني والعضوي تفوقا معنويًا في زيادة محتوى الأوراق من الكلوروفيل بلغ 50.30 و 49.48 ملغم. 100 غم⁻¹ مقارنة بالنباتات غير المعاملة التي أعطت أقل معدل بلغ 38.39 ملغم. 100 غم⁻¹. أما بالنسبة للتداخل بين الصنف مع نوع السماد فقد أظهر التسميد ريحان الأحمر مع السماد النتروجيني تفوقًا معنويًا في محتوى الأوراق من الكلوروفيل بلغ 55.06 ملغم. 100 غم⁻¹، فيما أظهرت نباتات الريحان الحلو غير المسمدة انخفاضًا في هذه الصفة إلى 37.98 ملغم. 100 غم⁻¹.

جدول رقم (7): تأثير صنف النبات ونوع السماد والتداخل بينهما في محتوى الأوراق من الكلوروفيل الكلي (ملغم. 100 غم⁻¹)

معدل الصنف	نوع السماد			معدل الصنف
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد	
43.43b	46.78c	45.55c	37.98d	ريحان الحلو
48.68a	52.19b	55.06a	38.80d	ريحان الأحمر
	49.48a	50.30a	38.39c	معدل نوع السماد

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها
معنويًا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

4. عدد الأفرع الجانبية (فرع. نبات⁻¹): من خلال النتائج الظاهرة في الجدول رقم (5)، نلاحظ أن الريحان الأحمر قد تفوق معنويًا في زيادة عدد الأفرع الجانبية إلى 7.95 فرع. نبات⁻¹ قياسًا مع نبات الريحان الحلو الذي بلغ 6.72 فرع. نبات⁻¹. أما عن تأثير السماد فيتضح من الجدول نفسه أن التسميد النتروجيني والعضوي قد تفوقا معنويًا في زيادة عدد الأفرع الجانبية إلى 7.80 و 8.26 فرع. نبات⁻¹ على التوالي، فيما بلغ في نباتات المقارنة 5.95 فرع. نبات⁻¹. أما بالنسبة للتداخل ما بين صنف النبات والسماد، فقد أظهر التداخل ما بين نبات الريحان الأحمر ورش السماد النتروجيني والعضوي تفوقًا معنويًا في زيادة عدد الأفرع الجانبية إلى 8.42 و 8.86 فرع. نبات⁻¹ على التوالي، مقارنة مع نباتات الريحان الحلو غير المسمدة والتي انخفضت إلى 5.33 فرع. نبات⁻¹.

جدول رقم (5): تأثير صنف النبات ونوع السماد والتداخل بينهما في عدد الأفرع الجانبية (فرع. نبات⁻¹)

معدل الصنف	نوع السماد			معدل الصنف
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد	
6.72b	7.66b	7.19bc	5.33d	ريحان الحلو
7.95 a	8.86a	8.42a	6.57c	ريحان الأحمر
	8.26a	7.80a	5.95 b	معدل نوع السماد

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها
معنويًا حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

7. كمية الإنتاج لوحدة المساحة (طن. هكتار⁻¹): نلاحظ من الجدول رقم (8) أن كمية الإنتاج لوحدة المساحة في نباتات ريحان الحلو قد تفوقت معنويًا على نباتات ريحان الأحمر فقد بلغت 2.69 طن. هكتار⁻¹ أما الريحان الأحمر فقد بلغ 2.54 طن. هكتار⁻¹، ويتضح من الجدول نفسه أن السماد النتروجيني تفوق معنويًا في زيادة كمية الإنتاج لوحدة المساحة إلى 3.02 طن. هكتار⁻¹ فيما وصلت كمية الإنتاج لوحدة المساحة عند رش السماد العضوي Azomine 2.94 طن. هكتار⁻¹ أما معاملة المقارنة فقد أعطت أقل معدل من الإنتاج بلغ 1.89 طن. هكتار⁻¹. أما بالنسبة للتداخل بين الصنف ونوع السماد فقد تبين أن تسميد ريحان الحلو بالسماد النتروجيني تفوقًا معنويًا في كمية الإنتاج لوحدة المساحة بلغ 3.21 طن. هكتار⁻¹ فيما أعطت نباتات ريحان الأحمر غير المعاملة انخفاضًا في الصفة المدروسة إلى 1.75 طن. هكتار⁻¹.

5. الوزن الجاف للمجموع الخضري (غم. نبات⁻¹): يتضح من الجدول رقم (6) عدم وجود فروق معنوية بين صنفَي الريحان الحلو والأحمر في الوزن الجاف لمجموع الخضري، فيما أظهر رش السمادين النتروجيني والعضوي Azomine أثرًا معنويًا في زيادة الوزن الجاف للمجموع الخضري إلى 11.55 و 11.45 غم. نبات⁻¹ على التوالي قياسًا بالنباتات المقارنة التي أعطت أقل معدل بلغ 8.62 غم. نبات⁻¹، وكان للتداخل بين صنف النبات مع نوع السماد تأثيرًا معنويًا على الصفة المدروسة، إذ تفوق تسميد الريحان الحلو بالسماد النتروجيني معنويًا في زيادة الوزن الجاف لمجموع الخضري إلى 12.44 غم. نبات⁻¹ مقارنة مع نباتات الريحان الحلو والأحمر غير المعاملة بالتسميد فقد انخفضت إلى 8.45 و 8.80 غم. نبات⁻¹، على التوالي.

أعلى المعدلات من المادة الجافة فيهما (18). ومن ناحية أخرى قد يعود سبب زيادة محتوى الكلوروفيل في أوراق النباتات المضاف إليها النتروجين (جدول 7) وذلك لدوره في تصنيع Prophyryns التي تدخل في بناء جزئية الكلوروفيل (19)، فيما يمكن أن يعزى سبب تفوق إضافة الأسمدة العضوية السائلة عن طريق الأوراق في تأثيرها على صفات النمو الخضري وذلك لكون الأوراق تعد مركزاً مهماً تحدث فيها العديد من العمليات الفسلجية والحيوية (20) فضلاً عن كونها طريقة فعالة في انتقال المغذيات بشكل أفضل داخل النبات (21). وتكمن أهمية الأسمدة العضوية السائلة في تحسين صفات النمو الخضري لما تحويه هذه الأسمدة من العناصر المغذية مثل الكاربون والنتروجين بالإضافة إلى الأحماض الأمينية والأحماض العضوية المختلفة وقد يعزى السبب إليها في زيادة الفعاليات الحيوية للنبات وتنشيطها من خلال تحفيز الأنظمة الأنزيمية وزيادة تكوين الأحماض النووية RNA و DNA (22) وتحفيزها في إنتاج الهرمونات النباتية كالأكسينات والسايونكانينات، مما يشجع عمليات الانقسام الخلوي واستطالة الخلايا وانعكاس ذلك في زيادة ارتفاع النبات وعدد الأوراق والمساحة الورقية (الجدول 2، 3، 4) (23)، وكذلك قد يعود التأثير إلى امتصاص العناصر المغذية والأحماض الأمينية المتواجدة في السماد العضوي السائل والتي تمتص مباشرة داخل خلايا الورقة لتسهل في زيادة صنع الغذاء من خلال زيادة كفاءة عمليتي البناء الضوئي والتمثيل الكربوني، مما يؤدي إلى ازدياد المواد الغذائية المصنعة في النبات وتراكمها كالكربوهيدرات والبروتينات (24)، والذي انعكس بشكل إيجابي في زيادة الوزن الجاف للنبات (جدول رقم 6)، مما انعكس بدوره مجملًا في زيادة النمو الخضري للنبات وكبر حجمه والتمثل في زيادة ارتفاع النبات (جدول رقم 2) والمساحة الورقية (جدول رقم 3)، وبالتالي زيادة كمية الانتاج الخضري في وحدة المساحة، أما زيادة الوزن الجاف للنمو الخضري في جميع معاملات الرش بتركيز السماد العضوي السائل والنتروجيني يعزى إلى زيادة ارتفاع النبات وعدد الأوراق وعدد الأفرع الجانبية (جدول 5) مما أثر إيجاباً في زيادة هذه الصفة (3).

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جدول رقم (8): تأثير صنف النبات ونوع السماد والتداخل بينهما في كمية الانتاج لوحدة المساحة (طن . هكتار⁻¹)

الصنف	نوع السماد		
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد
ريحان الحلو	2.69 a	2.83 bc	2.04 c
ريحان الأحمر	2.54 b	3.05 b	2.83 bc
معدل نوع السماد	2.94 a	3.02 a	1.89 b

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها مغنوياً حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

8. النسبة المئوية للنتروجين في الأوراق: يتضح من نتائج الجدول رقم (9) عدم وجود فروق معنوية في نسبة المئوية للنتروجين في الأوراق لكل من صنفَي الريحان الحلو والأحمر، في حين أعطى رش السماد النتروجيني والعضوي Azomine ارتفاعاً في النسبة المئوية للنتروجين في الأوراق إلى 2.43 و 2.35% على التوالي مقارنة بالنباتات غير المعاملة التي انخفضت النسبة إلى 1.45%.

وكان للتداخل بين عاملي التجربة تأثيراً معنوياً على الصفة المدروسة فقد تفوق رش نبات ریحان الحلو بالسماد النتروجيني تفوقاً معنوياً في زيادة النسبة إلى 2.73%، فيما أعطت نباتات غير المعاملة (المقارنة) انخفاضاً في الصفة المدروسة بلغت 1.57 و 1.33% لكل من الريحان الحلو والأحمر، على التوالي.

جدول رقم (9): تأثير صنف النبات ونوع السماد والتداخل بينهما في نسبة المئوية للنتروجين في الأوراق

الصنف	نوع السماد		
	سماد العضوي Azomine 5 مل.لتر ⁻¹	سماد النتروجيني 400 ملغم.لتر ⁻¹	بدون تسميد
ريحان الحلو	2.17 a	2.21 b	c 1.57
ريحان الأحمر	1.98 b	2.49 ab	1.33 c
معدل نوع السماد	2.35 a	2.43 a	1.45 b

*المعدلات التي تحمل الحروف الأبجدية نفسها لا تختلف عن بعضها مغنوياً حسب اختبار دنكن متعدد الحدود عند مستوى احتمال 0.05

من خلال النتائج، يمكن ملاحظة حصول تباين في معدل النمو الخضري وكمية الإنتاج بين نباتي ریحان الحلو وریحان الأحمر، ربما يعزى سبب ذلك إلى الاختلاف الوراثي بين الأصناف الناتج من تباين العوامل الوراثية المسؤولة عن صفات النمو الخضري أو ربما إلى اختلاف محتوى هذه الأصناف من الهرمونات كالأكسينات والجبرلينات والسيونكانينات (15).

أما عن دور النتروجين ربما يعود السبب إلى أهميته في تنشيط العديد من الأنزيمات والمرافقات الأنزيمية التي تدخل في عدة عمليات حيوية مؤدية إلى زيادة انقسام الخلايا المكونة للأنسجة المرستيمية وزيادة حجم خلايا الورقة (16) وبالتالي رفع معدل نمو النبات مما يؤثر إيجاباً على زيادة عدد وحجم خلايا الورقة مما ينتج عنه زيادة في نمو وحجم المجموع الخضري المتمثل بارتفاع النبات والمساحة الورقية (17) مما ينعكس على زيادة امتصاص الماء والعناصر المغذية وكفاءة فعالية الأوراق في عملية البناء الضوئي وينتج عن تلك العمليات تراكم المواد الغذائية المصنعة وتوفر المواد اللازمة لنمو المجموع الخضري والجذري وإعطاء

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التحري عن بقايا المبيدات العضوية الكلورية في نماذج الرز في بغداد

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الملخص باللغة العربية

تضمن البحث فحص وتحليل (21) عينة رز مسحوبة من الاسواق المحلية لمدينة بغداد تعود لمناشئ عالمية مختلفة للتحري عن بقايا المبيدات العضوية الكلورية فيها. حلت العينات بواسطة جهاز كروماتوغرافيا الغاز باستخدام كاشف القنص الالكتروني وبطريقة العينة القياسية الخارجية. اظهرت نتائج التحليل تلوث خمس عينات ببقايا مبيد DDT وايزومراته، ثلاثة منها ملوثة ببقايا مبيد P.P-DDE ، واثنان منها ملوثة ببقايا مبيد P.O-DDT وكلها ضمن تراكيز الحدود المسموح بها وهي (0.1) جزء بالمليون، وفقا لمنظمة الصحة العالمية (WHO)، ما عدا عينة الرز الذهبي الذي اظهر تلوثا مقداره (0.348) جزء بالمليون وهو أعلى من الحد المسموح به.

الكلمات المفتاحية: مبيدات الكلور العضوية ، رز ، كروماتوغرافيا الغازية

Detection of organo chlorinated pesticides in samples of rice at Baghdad Markets

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ABSTRACT

The current study was conducted to examine and analyze (21) samples of rise collected from local markets of Baghdad City that were manufactured in international factories to detect the existence of organo chlorinated pesticides.

Samples were analyzed by Gas chromatography (GC) with electron capture detector (ECD) and external standard method. Results of analysis showed contamination of five samples by DDT and its isomers. Three of these samples were contaminated with residue of P,P-DDE pesticide , two of samples are contaminated with residue of P,O-DDT pesticide, all of concentrations were within allowed limits which is (0.1) part per million according to world health Organization (WHO), except that with trade mark (Golden Rice), which has a concentration of (0.348) part per million , which was higher than the allowed limits.

المقدمة

المواد وطرق العمل

جهاز التحليل:

استخدم جهاز كروماتوغرافيا الغازية GC موديل 2010 نوع Shimadzu مع كاشف القنص الالكتروني ECD لغرض كشف وتقدير مبيدات الكلور العضوية وحسب البرنامج التحليلي التالي:
- Column Tupe : 5 MS 30m × 0.25 mm ID, P.size 0.25 μm

- Temp.programme:

Temp. Inj. 280C°,

Oven : 250 C°,

DeT –ECD : 310C°

-Vol . Inj. : 1 μl

النمذجة:

شمل البحث على 21 عينة من الرز سحبت عشوائيا من الاسواق المحلية لمدينة بغداد للفترة من 17-26 أغسطس / آب عام 2014 (جدول رقم 1).

يشكل الرز والخبز وخاصة في العراق حوالي 50% من الغذاء اليومي للإنسان وتستهلك كميات كبيرة جداً من الرز كونه مصدر رئيسي للطاقة التي يحتاجها جسم الإنسان سيما غذاء الاطفال كونه غني بالمواد الكربوهيدراتية، الدهنية، الألياف، الاحماض والمعادن (1).

تستخدم المبيدات لمكافحة الآفات الزراعية والحشرات والفطريات والقوارض. وقد انحصر استخدام المبيدات العضوية الكلورية مثل (Endrin, Aldrin, HCH, DDT) في البلدان المتقدمة لطبيعتها المقاومة وسمية نتائج ايضها باستثناء الاندوسفان (Endosulfan) الذي لازال يستعمل عالمياً وبشكل واسع، وفي الهند لازال يستعمل مبيد (DDT) في مجال الصحة العامة لانتشار البعوض والملاريا. وتشير التقارير الى وجود بقايا هذه المبيدات في الاغذية كالحليب، الاطعمة الحيوانية، والخضراوات (2).

وقد وجدت إحدى الدراسات أن بقايا المبيدات العضوية الكلورية في المنتجات الزراعية والاسماك واللحوم وحليب الامهات في الصين قد انتقلت من التربة وان وجودها في هذه المنتجات دلالة على استخدامها سابقاً في البيئة الصينية (3)، وتشكل بقاياها خطورة عالية على صحة الإنسان لقدرتها على مقاومة التحلل البيئي وامكانية تجمعها في الأنسجة الدهنية والانتقال عبر السلسلة الغذائية الى جسم الإنسان (4).

جدول رقم (1): نماذج الرز المدروسة

ت	اسم النموذج	المنتج	رقم الوجبة	تاريخ الإنتاج	تاريخ الانتهاء
1	رز بسمتي العربية (ذهب)	الهند	JRA/0206	5/2014	4/2016
2	كونز (روزانا)	الهند	SS-17267	4/2014	3/2016
3	الربيع الأخضر	الهند	1011	2/2014	3/2016
4	رز خضراء	الهند	---	3/2014	3/2016
5	رز محمود	الهند	1070	1/2014	12/2015
6	الذهبي 727	الهند	---	17/12/2013	16/12/2015
7	نورس	الهند	---	1/2013	10/2015
8	Maharani لمرضى السكري	الهند	CLD-MG 11 crop2012	20/1/2013	19/12/2015
9	رز عنبر نجفي	العراق	---	2014	2016
10	الربيع الأخضر سيل 1211	الهند	1011	3/2014	3/2016
11	رز أمريكي	امريكا	NY 10591	2014	2016
12	رز أرجنتيني	ارجنتين	GB/RA/1/2014	2014	2016
13	رز R	امريكا	---	2014	2016
14	رز كلستد	الهند	---	---	---
15	رز eagle star	ارغواي	---	---	---
16	رز دعوة	الهند	A2/9000 0 201	3/2014	2/2016
17	رز أحمد	الهند	---	12/2013	11/2015
18	رز الدرة	الهند	0101140510-0300	4/2014	3/2016
19	رز كوهينور	الهند	NO228JE01	2/2014	1/2016
20	رز maxims	امريكا	---	10/2013	10/2015
21	رز عنبر ديوانية	العراق	---	5/2014	4/2016

الكروماتوغرافي الغازي واستخدام كاشف القنص الالكتروني (ECD) (5).

النتائج والمناقشة:

اظهرت نتائج التحليل بواسطة جهاز كروماتوغرافيا الغاز GC- (ECD) ثلوث قسم من عينات الرز ببقايا المبيدات العضوية الكلورية كما في الجدول رقم (2).

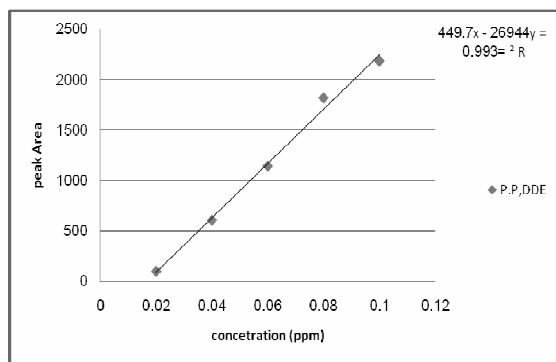
طريقة العمل: حضرت المحاليل القياسية لمبيدي (P,P-DDT, P,P-DDDE) بالتركيزات (0.02, 0.04, 0.06, 0.08, 0.1) جزء بالمليون (ppm) لغرض التحليل بواسطة GC-ECD ورسم منحني قياسي لكل مبيد.

عملية الاستخلاص: تم أخذ (10) غم من الرز المطحون ويضاف له (15) مل من الماء المقطر وينقل الى انبوبة مناسبة ثم اضيف (10) مل من مذيب الايثيل استيت وكبريتات الصوديوم اللامائية والمجانسة لمدة (10) دقائق على جهاز vortex ثم تم الفصل بجهاز الطرد المركزي بسرعة 2000 rpm ولمدة 5 دقائق، بعد ذلك تم اخذ طبقة الايثيل استيت وجرى تبخيرها لحد الجفاف ثم اضيف (2) مل من مذيب الهكسان لغرض التحليل بواسطة جهاز

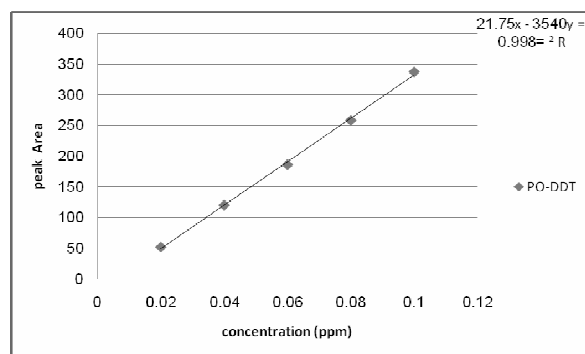
جدول رقم (2): تراكيز بقايا المبيدات العضوية الكلورية (ppm) في نماذج الرز

ت	اسم العينة	تراكيز المبيدات العضوية الكلورية (ppm)								
		methoxychloro	Dursban	O,P-DDD	P,P-DDE	P,P-DDD	P,O-DDT	Endrine	Heptachloro	Aldrine
1	رزبسمتي العربية(ذهب)	--	--	--	--	--	--	--	--	--
2	كنوز(روزانا)	--	--	--	--	--	--	--	--	--
3	الربيع الاخضر	--	--	0.117	--	--	--	--	--	--
4	رز خضراء	--	--	--	--	--	--	--	--	--
5	رز محمود	--	--	--	--	--	--	--	--	--
6	الذهبي727	--	--	0.348	--	--	--	--	--	--
7	نورس	--	--	--	--	--	--	--	--	--
8	Maharani لمرضى السكري	--	--	--	--	--	--	--	--	--
9	رز عنبر نجفي	--	--	--	--	--	--	--	--	--
10	الربيع الاخضر سيل1211	--	--	--	--	--	--	--	--	--
11	رز امريكي	--	--	--	--	--	--	--	--	--
12	رز ارجنتيني	--	--	0.076	--	--	--	--	--	--
13	رزR	--	--	--	--	--	--	--	--	--
14	رز كليسد	--	--	--	--	--	--	--	--	--
15	رز Eagle star	--	--	--	--	--	--	--	--	--
16	رز دعوة	--	--	--	--	0.089	--	--	--	--
17	رز احمد	--	--	--	--	0.048	--	--	--	--
18	رز الدره	--	--	--	--	--	--	--	--	--
19	رز كوهينور	--	--	--	--	--	--	--	--	--
20	رزMaxims	--	--	--	--	--	--	--	--	--
21	رز عنبر ديوانية	--	--	--	--	--	--	--	--	--

part per million: ppm*



شكل رقم (1): المنحنى القياسي للمبيد (P,P-DDE)

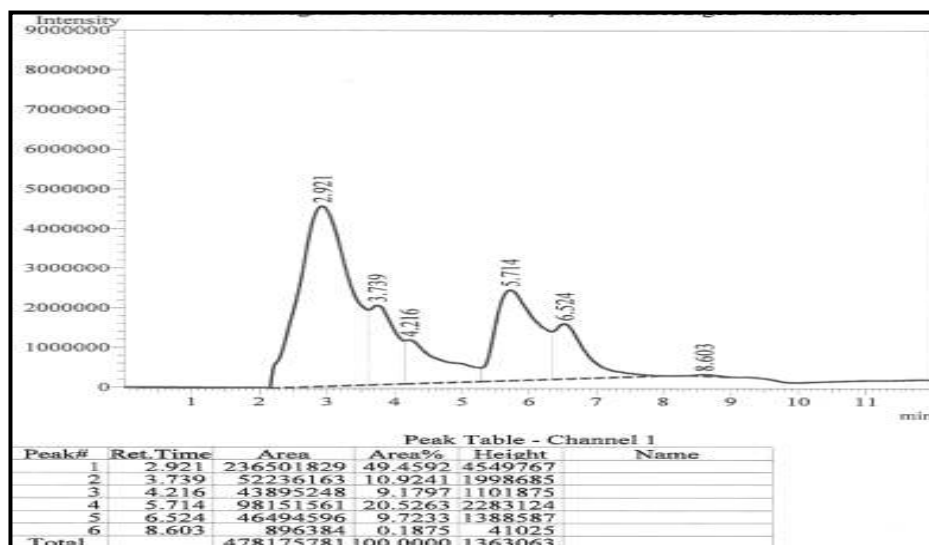


شكل رقم (2): المنحنى القياسي للمبيد (P,O-DDT)

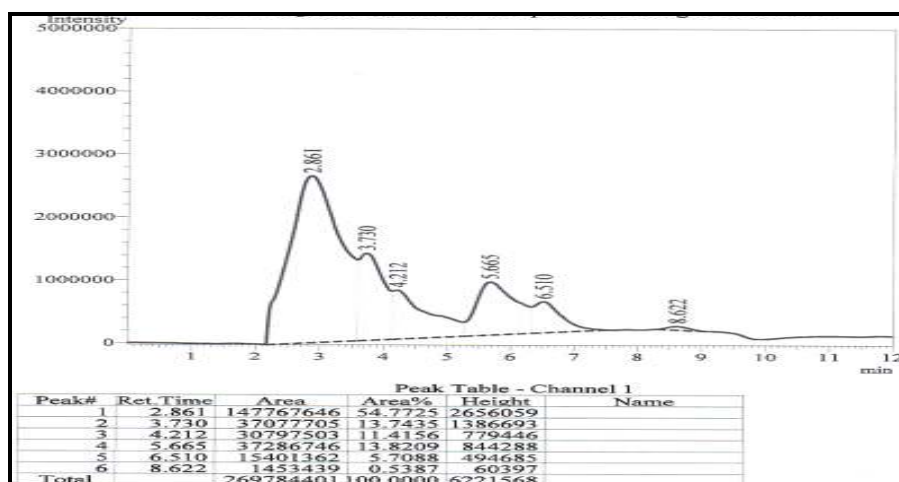
تم احتساب تراكيز بقايا المبيدات من خلال تطبيق معادلات الخط المستقيم التي تم الحصول عليها من المنحنى القياسي لمبيد (P,P-DDE , P,O-DDT) كما في الشكلين (2،1) على التوالي. و يتبين من نتائج التحليل بواسطة GC-ECD ثلوث نموذج الرز الذهبي (تسلسل 6) بمبيد (P,P-DDE) بتركيز (0.348 ppm) كروموتوغرام (1) وهو أعلى من الحد المسموح به (0.1 ppm) (6)، بينما اظهرت العينات: رز بسمتي الربيع الاخضر (تسلسل 3)، رز حصة تموينية ارجنتيني (تسلسل 12) ثلوثهما بنفس المبيد (P,P-DDE) ولكن ضمن الحدود المسموح بها (0.117, 0.076 ppm) على التوالي كما في كروموتوغرام (2، 3).

كما اظهرت العينات رز دعوة (تسلسل 16)، رز احمد (تسلسل 17) ثلوثهما بمبيد (P,O-DDT) بتركيز (0.089, 0.048 ppm) على التوالي كروموتوغرام (4، 5) على التوالي وهو ضمن الحدود المسموح بها.

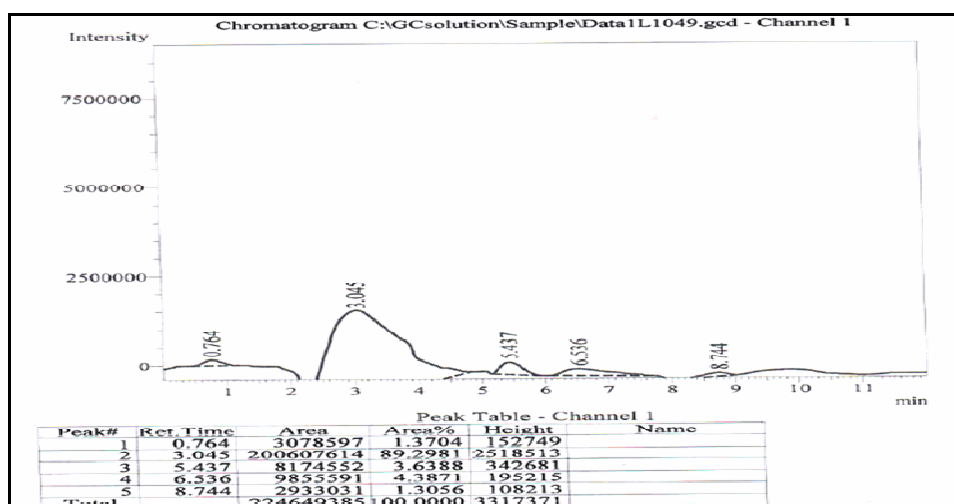
ان ثلوث بعض عينات الرز التي تعد من اكثر الانواع استهلاكاً من قبل الانسان ببقايا مبيدات الكلور العضوية نتيجة استخدام هذه المبيدات بطريقة غير صحيحة ومفرطة في رش المزروعات للقضاء على الحشرات مما يؤدي الى انتقال المبيدات من خلال السلسلة الغذائية الى المستهلك ولقدرة هذه المبيدات على التراكم في جسم الانسان فان كميات ضئيلة منها تصبح مؤثرة بمرور الزمن مما تسبب تشوهات خلقية وامراض سرطانية (7).



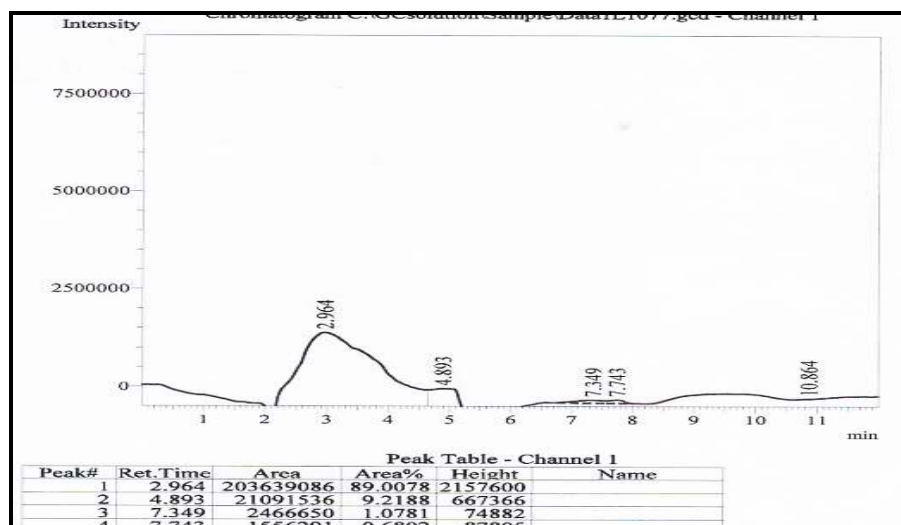
كروماتوغرام (1): تحليل عينة الرز الذهبي بواسطة GC-ECD



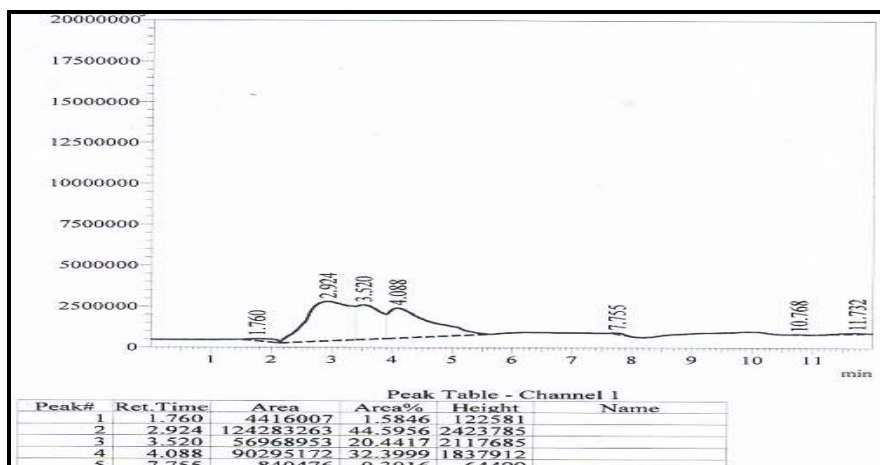
كروماتوغرام (2): تحليل عينة رز الربيع الأخضر بواسطة GC-ECD



كروماتوغرام (3): تحليل عينة رز أرجنتيني (تموينية) بواسطة GC-ECD



كروماتوغرام (4): تحليل عينة رز دودة بواسطة GC-ECD



كروماتوغرام (5): تحليل عينة رز أحمد بواسطة GC-ECD

المصادر

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عزل وتشخيص الفطريات الملوثة للتوابل والكشف عن سموم الأفلاتوكسين فيها

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الملخص باللغة العربية

سعت الدراسة الحالية إلى عزل وتشخيص الفطريات الملوثة للتوابل المنتجة محليا في بغداد والكشف عن سموم الأفلاتوكسين فيها. تم جمع (25) منتجا من التوابل بصورة عشوائية من الأسواق المحلية لمدينة بغداد وتم التحري عن الفطريات باستخدام طريقة الصب بالأطباق والوسط الزراعي (Rose Bengal Agar) والكشف عن سموم الأفلاتوكسين (B1, B2, G1, G2) باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة وتقنية الكروماتوغرافيا السائلة ذات الأداء العالي.

بينت النتائج تلوث عينات التوابل بأعداد وأنواع مختلفة من الفطريات هي *Rhizopus* spp., *Penicillium* spp., *Byssoschlamys* spp., وكانت أعلى نسبة لتواجد الفطريات للنوع *Cladosporium* spp., *Alternaria* spp., *Paecilomyces* spp., *Fusarium* spp. *Aspergillus niger* (92%) في حين كانت نسبة تواجد الفطريات المنتجة لسموم الأفلاتوكسين (*Aspergillus flavus* و *Aspergillus parasiticus*) قليلة نسبيا ولا تتجاوز (12%) ، كما بينت نتائج الفحوص الكيميائية خلو عينات التوابل من سموم الأفلاتوكسين.

الكلمات المفتاحية: التوابل ، فطريات ، افلاتوكسين

Isolation, identification and detection of aflatoxins in local produced spices contaminated with fungi

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ABSTRACT

The current study aimed to isolate and diagnose fungi contaminated spices produced locally and to investigate the presence of aflatoxins. A total sample of 25 different types of spices were collected randomly from some local markets in Baghdad City. The investigation of fungi was done by using the method of pouring plate and culture media Rose Bengal Agar, where the investigation of aflatoxin (B1 , B2, G1, G2) was done by using thin layer chromatography technique (TLC) and high performance liquid chromatography technique (HPLC). The results showed that the tested samples of spices were contaminated with fungi (*Rhizopus* spp. , *Penicillium* spp. , *Byssoschlamys* spp. , *Cladosporium* spp. , *Alternaria* spp. , *Paecilomyces* spp. , *Fusarium* spp.) Fungi type *Aspergillus niger* recorded the highest rate of contamination with (92%), while the percentage of presence of fungi producing aflatoxin (*Aspergillus flavus* and *Aspergillus parasiticus*) was not more than (12%). The results of the chemical tests showed that spices samples were free of aflatoxin.

المقدمة

بهارات مخللات)، حيث سحبت تلك العينات بصورة عشوائية من الأسواق المحلية لمدينة بغداد خلال الفترة من الأول من شهر حزيران / يونيو إلى الأول من شهر كانون أول / ديسمبر عام 2013.

الكشف عن الفطريات في التوابل:

تم التحري عن الفطريات بإضافة (10غم) من كل عينة من التوابل على حدة إلى ما مقداره (90 مليلتر) من المحلول الملحي المعقم (4)، وزرعت العينات باستخدام طريقة النشر (Plate Spread method) على وسط (Rose Bengal Agar HIMEDIA) الذي حضر حسب التعليمات المثبتة على العبوة، ثم عقم بالمؤودة بدرجة حرارة 121 درجة مئوية وضغط 1 بار لمدة 15 دقيقة.

حضنت الأطباق بعد الزرع في درجة حرارة تتراوح (25 - 28م) لفترة (3 - 5) أيام، بعدها تم تفريق المستعمرات الفطرية النامية بشكل منفصل على وسط (RBA) وتم احتضانها مرة أخرى للحصول على عذلة نقية لغرض التشخيص، وتم تشخيص الفطريات المعزولة من عينات التوابل حسب المفتاح التشخيصي (5).

حساب أعداد الفطريات:

تم حساب أعداد المستعمرات الفطرية في عينات التوابل بحسب الدستور البريطاني، وذلك بإجراء تخفيف عشرية لكل عينة على حدة، وبتابع طريقة الصب بالأطباق (Pour Plate Method) وقد تم استخدام الوسط التجميعي Standar Plate Count (SPC) لحساب عدد المستعمرات/ الغرام الواحد (CFU / gm) بواسطة جهاز عد المستعمرات (Colony Counter)، ومن ثم تم حساب معدل أعداد الفطريات لثلاث مكررات من كل عينة.

الاستخلاص العضوي للسموم الفطرية (الأفلاتوكسينات):

تم التحري عن سموم الأفلاتوكسين باستخدام طريقة الصفائح الرقيقة (6) وكما يلي:

- 1- وزن 50 ملغم من العينة
- 2- أضيف ما مقداره 250 مليلتر من (Acetone : Water) بنسبة (15 : 85) ثم نقل المزيج إلى الخلاط لمدة ثلاث دقائق، بعدها رشح من خلال ورق ترشيح نوع (Watman No. 4).
- 3- تم أخذ ما مقداره 150 مليلتر من الراشح ونقل إلى دورق بحجم 400 مليلتر.
- 4- أضيف ما مقداره 170 مليلتر من Sodium hydroxide (0.02 N) مع 30 مليلتر من Ferric chloride مع 3 غرام من Basic copper carbonate ومزجت جيدا، ثم نقل المزيج إلى دورق بسعة 600 مليلتر ليعاد مزجه، ومن ثم رشح المزيج من خلال ورق ترشيح نوع (Watman No. 4).
- 5- أخذ ما مقداره 150 مليلتر من الراشح ونقل إلى قمع فصل بحجم 500 مليلتر.
- 6- أضيف إلى المزيج 150 مليلتر من حامض Sulfuric acid بتركيز 0.03% ورج بهدوء، ثم أضيف 10 مليلتر من Chloroform، ونقلت الطبقة السفلى إلى قمع فصل ثان.
- 7- أضيف إلى قمع الفصل الثاني (الذي يحوي الطبقة السفلى) 100 مليلتر من Potassium hydroxide (0.02 N)، ورج بهدوء لمدة 30 ثانية، ثم ترك ليتم فصل طبقة Chloroform السفلى، وجمع مستخلص Chloroform في قنينة زجاجية، ثم أخذ ما مقداره 8 مليلتر ليتم تبخيره بدرجة حرارة 45 مئوية تحت بخار النيتروجين باستخدام اللوح الساخن.

أصبحت المضافات الغذائية والمنكهات ومنها التوابل شائعة في العديد من المجتمعات، ويعود استخدام التوابل إلى عصور قديمة، وقد تزايد الاهتمام بجودة ونوعية التوابل مما دفع بالكثير من الدول إلى توجّه مكثف حول العناية بمصادرها وتطوير طرق تعبئتها ووسائل تسويقها.

تتعرض التوابل خلال مراحل الإنتاج والتخزين والتسويق إلى العديد من الملوثات وخاصة الإحيائية منها، مما يؤدي إلى تلفها إذا لم يتم اتباع الإرشادات الصحية ل تخزينها وتداولها وتعبئتها، ومن أهم ملوثات التوابل الإحيائية الفطريات، التي من الممكن انتقالها إلى الإنسان عن طريق الغذاء مسببة له الكثير من الأمراض (1). تعد الفطريات من أكثر الأحياء المجهرية تأثيرا على المحاصيل الزراعية قبل وبعد الحصاد، ويعد نموها وإنتاجها للسموم الفطرية من المشاكل الرئيسة التي تواجه منظومة سلامة الغذاء في جميع أنحاء العالم، وقد أكد بعض الباحثين أن الحصاد المتأخر قد يسبب زيادة في التلوث بالسموم الفطرية وبالأخص سموم الأفلاتوكسين ذات التأثير المباشر على الإنسان، فهي تسبب ضررا بالكبد والكلية والأوعية الدموية الشعرية. ومن خصائص سموم الأفلاتوكسين أنها من السموم الفعالة بيولوجيا وذات أوزان جزيئية صغيرة، لذا تكون مقاومة للعوامل البيئية وغير محفزة للجهاز المناعي للإنسان على إنتاج أجسام مضادة، كما أنها مقاومة لدرجة حرارة الطبخ، ومن الفطريات المنتجة لسموم الأفلاتوكسين (*Aspergillus flavus* و *Aspergillus parastictus*).

يتأثر نمو الفطريات وإنتاجها للسموم في معظم المنتجات الغذائية بمجموعة من العوامل الطبيعية والبيولوجية والكيميائية من أهمها: محتوى الرطوبة في المحصول المخزون، مدة ودرجة حرارة الخزن، التهوية، سلامة الفطر والمادة الغذائية وطبيعتها (2). ويمكن الوقاية من التلوث الفطري ومنع تكوين السموم الفطرية في المنتجات الغذائية باتباع تدابير الوقاية في المستوى الأول، فالوقاية الأولية تكون عن طريق السيطرة على الإصابات الفطرية قبل غزو الفطريات للمحاصيل وإحداث التلوث بالسموم الفطرية، وذلك بتطوير أصناف ذات أصول مقاومة للأمراض النباتية، واتباع أنظمة مكافحة ضد الفطريات المرافقة للمحاصيل الزراعية مع مراعاة خفض محتوى الرطوبة في المحاصيل أثناء وبعد الحصاد وأثناء الخزن، واستخدام مبيدات الفطريات والمواد الحافظة. أما المستوى الثاني من الوقاية فينبع في حالة غزو بعض الفطريات للسلع الغذائية الأساسية في مرحلة مبكرة، لذا يجب محاولة إيقاف نمو الفطريات من خلال إعادة تجفيف المنتجات ثم عزل البذور الملوثة بالفطريات وإتلافها للحد من إنتاج الأفلاتوكسين.

وعادة ما يلجأ المختصون إلى مستوى الوقاية المتقدمة عند ثبات وجود تلوث عال بالفطريات ووجود تراكيز عالية من السموم الفطرية في المادة الغذائية، وذلك بتدمير المنتجات الملوثة بأكملها وإزالة أو تحطيم السموم الفطرية.

وللحفاظ على صحة الإنسان وسلامة البيئة، قدمت بعض الدراسات طرقا بديلة لحفظ الأغذية من التلوث الفطري. وبسبب احتواء بعض النباتات على مركبات ذات تأثير تثبيطي على بعض الفطريات، فقد أدخلت في عالم الطب البديل لعلاج بعض أمراض الإنسان (3).

ولندرة الدراسات حول هذا الموضوع، فقد جاءت الدراسة الحالية بهدف التحري عن الفطريات الملوثة للتوابل والكشف عن سموم الأفلاتوكسين فيها.

المواد وطرق العمل

تم التحري عن الفطريات وسموم الأفلاتوكسين في (25) عينة من التوابل المنتجة محليا في بغداد وهي (كمون، كبابية، جوزة بوة، قرفة، قرنفل، كزبرة، فلفل احمر، حبة حلوة، زنجبيل، كركم، فلفل اسود، بهارات شاورما لحم، بهارات باسطرمة و بهارات كبة، بهارات برياني، بهارات ملفوف، بهارات كفتة، بهارات همبرجر، بهارات كاري سمك، بهارات دجاج، بهارات فلفل ابيض، بهارات كاري، بهارات مرق لحم، بهارات عنبية،

إفراز العديد من السموم الخطرة على صحة الإنسان، منها (Malformin, Nigragillin)، أما أقل نسبة توجد للفطريات فكانت للجنسين (Cladosporium spp., Alternaria spp.)، إذ بلغت نسبة وجود كل منهما (4%)، وكلاهما يسبب حساسية الجهاز التنفسي والربو التحسسي الذي تشمل أعراضه تخرش واحتقان الحنجرة، بالإضافة إلى التهاب الجيوب الأنفية، وهي مفرزة لأنواع خطيرة من السموم الفطرية في حال توفر الظروف الملائمة كسموم (Alternations, Altertoxin). وقد تراوحت نسب تواجد الأنواع الأخرى من الفطريات بين الحدين الأعلى والأدنى، كما بينت نتائج الفحص الكيميائي خلو عينات التوابل من سموم الأفلاتوكسين كما في الشكل رقم (2).

جدول رقم (2): أعداد الفطريات في عينات التوابل

ت	المنتج	مستعمرة / غرام
1	كمون	3050
2	كبابة	11266
3	جوزة بوة	7500
4	قرقة	-ve
5	قرنفل	250
6	كزبرة	1950
7	فلفل احمر	225
8	حبة حلوة	2100
9	زنجبيل	1060
10	كركم	315
11	فلفل اسود	4800
12	بهارات شاووما لحم	17433
13	بهارات باسطرمة	433
14	بهارات كبة	1746
15	بهارات برياني	1400
16	بهارات ملفوف	25000
17	بهارات كفتة	3200
18	بهارات همبركر	890
19	بهارات كاري سمك	2976
20	بهارات دجاج	906
21	بهارات فلفل ابيض	13000
22	بهارات كاري	200
23	بهارات مرق لحم	120
24	بهارات عنبية	15
25	بهارات مخللات	-ve

*فترة الحضانة (5) أيام

8- أضيف إلى باقي طبقة Chloroform ما مقداره 200 مليلتر من Acetonitrile : Benzene بنسبة (2 : 98) و تم تحليله بتقنية الصفائح الرقيقة TLC وتقنية الكروماتوغرافيا السائلة عالية الأداء HPLC.

الكشف النوعي عن سموم الأفلاتوكسين:

استخدمت تقنية كروماتوغرافيا الطبقة الرقيقة (TLC) لغرض الفصل والكشف النوعي عن السموم الفطرية وفق الظروف التالية (Mobile phase) (Toluene : Ethyl Acetate : Formic acid) بنسبة (60 : 30 : 10) على التوالي ومقارنتها بالمرکبات القياسية، وجفف المذيب وأخذ النموذج وأضيف له اسيتونتريل (Acetonitrile) كمذيب مناسب للفحص بجهاز HPLC، لغرض التحليل، حيث تم استخدام تقنية كروماتوغرافيا السائل ذات الأداء العالي (HPLC) للتقدير الكمي لمرکبات السموم الفطرية (الأفلاتوكسين) في عينات التوابل وفق الظروف كما هو موضح في الجدول رقم (1).

جدول رقم (1): ظروف تقدير مركبات السموم الفطرية (الأفلاتوكسين) في عينات التوابل بجهاز (HPLC)

ظروف تشغيل جهاز تقنية HPLC	
H2O : CH3CN	الطور الناقل
60 : 40	
ml / min	سرعة الجريان
UV 365 nm	الكاشف
ODS	العمود
100µl	حجم الحقن

النتائج والمناقشة

أظهرت نتائج التحري عن الفطريات في عينات التوابل احتواءها أنواعاً عديدة من الفطريات، وبأعداد تراوحت ما بين (0 - 25000) مستعمرة/غرام كما هو موضح في الجدول رقم (2) والشكل رقم (1)، وكان أعلى معيار لتواجد الفطريات في أربع عينات من التوابل هي على التوالي (الملفوف، شاووما لحم، فلفل ابيض، كبابة) حيث فاق الحدود المسموح بها في المواصفة القياسية العراقية للأغذية والبالغة (10 مستعمرة/غم)، فيما أظهرت نتائج العينتين (قرقة والمخللات) خلوهما من الفطريات، وكانت أعلى نسبة تواجد للفطر (Asp. niger) في عينات التوابل، إذ بلغت (92%) كما هو موضح في الجدول رقم (3)، وهو من الفطريات التي تسبب العديد من الأمراض للإنسان بدءاً من الحساسية الشعبية الرئوية (ABPA)، والإصابة الاسبرجولوسية الجلدية (Cutaneous Aspergillosis) (7)، وعند توفر الظروف الملائمة من درجة حرارة ورطوبة تصبح له القدرة على



شكل رقم (1): الفطريات المعزولة من التوابل

جدول رقم (4): السموم المفترزة من الفطريات

ت	نوع الفطر	السموم التي يفرزها
1	<i>Asp. niger</i>	Malformin , Nigragillin
2	<i>Asp. flavus</i>	Aflatoxin , Aspergillic acid
3	<i>Asp. Parasiticus</i>	Aflatoxin
4	<i>Byssoschlamys nivea</i>	Patulin , Byssoschlamic acid
5	<i>Paecilomyces</i>	Patulin
6	<i>Alternaria alternata</i>	Altertoxin , Alternarios
7	<i>Fusarium sporotrichioides</i>	Trichothecene , Zearalenone
8	<i>Asp. Ochraceus</i>	Ochratoxin , Kojic acid
9	<i>Rhizopus spp.</i>	Isofumigaclavine A
10	<i>Mucor spp.</i>	Unknown
11	<i>Cladosporium spp.</i>	Unknown
12	<i>Penicillium spp.</i>	Citrinin , PR-toxin

التوصيات

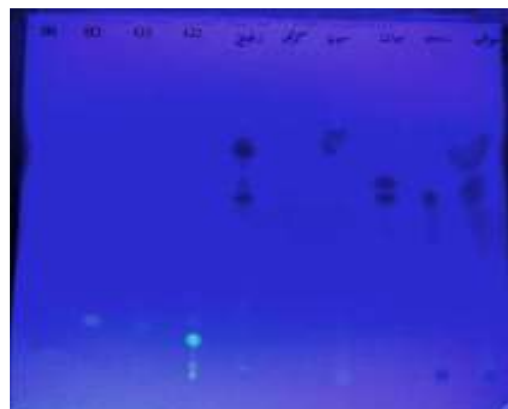
- (1) التأكد من خلو التوابل من الملوثات البيولوجية والكيميائية بإجراء الفحوصات المخبرية قبل تسويقها .
- (2) الاهتمام بعملية التعبئة، والحرص على اختيار عبوات ذات مواصفات جيدة وقابلة للخرن .
- (3) تأمين مخازن ذات ظروف خزن جيدة لضمان عدم حدوث تلوث خلال فترة الخزن .
- (4) تثبيت تاريخ الإنتاج والانتهاك بشكل واضح على العبوة .
- (5) عدم الإفراط في تناول التوابل لأنها سلاح ذو حدين .

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جدول رقم (3): النسب المئوية لتواجد الفطريات في عينات التوابل

ت	نوع الفطر	النسبة المئوية
1	<i>Asp. niger</i>	92
2	<i>Mucor hiemalis</i>	44
3	<i>Rhizopus oryzae</i>	44
4	<i>Penicillium aurantiogriseum</i>	16
5	<i>Byssoschlamys nivea</i>	20
6	<i>Asp. Ochraceus</i>	12
7	<i>Asp. Parasiticus</i> + <i>Asp. flavus</i>	12
8	<i>Paecilomyces variotii</i>	8
9	<i>Fusarium sporotrichioides</i>	8
10	<i>Cladosporium cladosporioides</i>	4
11	<i>Alternaria alternata</i>	4



شكل رقم (2): كروماتوغرافيا الطبقة الرقيقة (TLC) لعينات التوابل

إن التباين في نسب معيار وتواجد الفطريات يعود لأسباب كثيرة منها اختلاف ظروف وفتحات الخزن في المخازن والمتاجر، واختلاف كفاءة طرق التعبئة والتغليف للمنتج، كذلك فإن احتواء بعض التوابل على مركبات فعالة ذات تأثير تثبيطي على نمو بعض أنواع الفطريات قد يفسر خلو توابل (القرقة والمخللات) من الفطريات واحتواء بهارات (القرنفل والكرم والكاري والعنبية) على أعداد قليلة منها.

وبين الجدول رقم (4) قدرة الفطريات على إفراز السموم في حال توفر الظروف الملائمة لها من درجة حرارة ورطوبة، و لا يعني إفرازها لهذه السموم في عينات التوابل التي تم فحصها.

تقييم بعض الخصائص الكيميائية لبعض أهوار جنوب العراق بين عامي (2006، 2014)

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الملخص باللغة العربية

سعى البحث لدراسة بعض الخصائص الكيميائية لأهوار الجبايش والحمار وأبو زرك والسناف والعدل في جنوب العراق خلال الفترة من عام 2006 إلى عام 2014، حيث ركز البحث على الخصائص التالية: التوصيل الكهربائي EC، درجة الحموضة pH، الأملاح الذائبة الكلية TDS، القلوية Alk، الحموضة Acid درجة العسرة T.H، الأوكسجين الذائب DO، درجة التعكر Tr، كما تم تحديد وجود بعض الأيونات: الكالسيوم، المغنيسيوم، والكلورايد. أوضحت النتائج أن أغلب الخصائص المدروسة سجلت تغيراً مع الزمن بالنسبة لجميع الأهوار المختارة نتيجة استمرار تدفق المياه لإنعاشها، فضلاً عن تحويل مجرى نهر المصب العام (المالح) إلى الأهوار لتعويض النقص الحاد في المياه بعد عام 2010، وقد كانت مياه الأهوار متوسطة الملوحة وضمن الاتجاه القاعدي عام 2006 وبلغت 3.2 ملي سمنز.سم⁻¹ كمعدل ولجميع الأهوار المدروسة، في حين ارتفعت وبشكل ملحوظ عام 2014 وبلغت 7.9 ملي سمنز.سم⁻¹ كمعدل، إذ ارتفعت قيم الايصالية الكهربائية مع الزمن وبلغ أعلاها في هور السناف مقارنة ببقية الأهوار المدروسة (4.7 و 10.9 ملي سمنز.سم⁻¹) لعامي 2006 و 2014 على التوالي. وسجل أيون الكلورايد ارتفاعاً حاداً، وخاصة لهور الجبايش حيث بلغ تركيزه (145.3 و 200.3 ملي مول.لتر⁻¹) لعامي 2006 و 2014 على التوالي، ويعود هذا إلى تأثيرها وبصورة رئيسة بنهري دجلة والفرات وتذبذب كميات ونوعية المياه فيهما.

الكلمات المفتاحية: الخصائص الكيميائية، الأهوار.

Evaluation of some chemical properties of some Marshlands in south of Iraq between (2006-2014)

Ameer K. Yasear

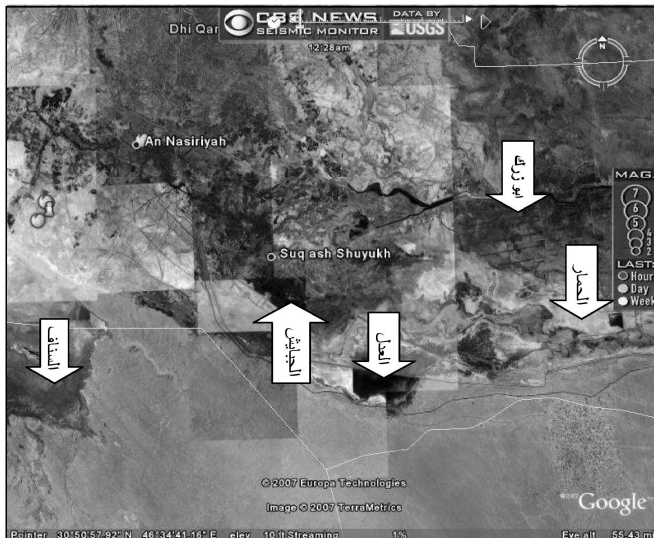
Dept. of soil sciences and water resource/ Faculty of Agriculture / Kufa University / Republic of Iraq

ABSTRACT

The current research was conducted to study some chemical properties of the marshlands located at the south of Iraq (Al-Jbaish, Al-Hammar, Abu-Zerk, Al-Sanaff and Al-Adel) during the period from 2006 to 2014. The research focused mainly on the following properties: electrical conductivity EC, pH, total dissolved salts TDS, alkalinity Alk, acidity, hardness degree TH, dissolved oxygen DO, and turbidity degree Tr. In addition, the presence of ions of Calcium, Magnesium, Chloride were studied.

Results revealed that most of studied properties had recorded changes with time as for all of selected Marshlands. These results can be justified by water constancy flow to marshlands, and due to the transfer of the riverbed (salty) to the marshlands to compensate the sharp lack of water after year 2010. Water of marshlands were relatively salt and in alkaline status in 2006, which reached 3.2 mS.cm⁻¹ as average for all studied marshlands, whereas increased appreciably in year 2014 that became 7.9 mS.cm⁻¹ as average that increased value of electric conductivity with time and it was higher in Al-Sanaff marshland compared with the other marshlands (4.7 and 10.9 mS.cm⁻¹) in year 2006 and 2014 respectively. Chloride ion recorded a significant increase at Al-Jbaish marshland (145.3 and 200.3 Mmol.l⁻¹) for 2006 and 2014 respectively and that was due to the impact of quantity and quality of water of Euphrates and Tigris rivers.

الأهوار وبواقع 3 مكررات . الصورة رقم (1)، وعينات من نهر الفرات ونهر الخراف الذي ينبع من نهر دجلة في مدينة الناصرية قبل تزويدهما الأهوار بالمياه للعامين 2006، 2014. وتم حفظ العينات في حاويات مصنوعة من البولي إيثيلين بسعة 1 لتر تم تهيئتها مسبقاً ، وبعد وضع العينات في الحاويات أضيف إليها بضع قطرات من الكلوروفورم لغرض المحافظة على ثبات التركيز للعينات.



صورة رقم (1): موقع جمع النماذج من الأهوار في جنوب العراق (4)

تقدير الخصائص الكيميائية:

تم قياس التوصيل الكهربائي (EC) ودرجة الحموضة (pH) والأملاح الذائبة الكلية (TDS) ودرجة الحرارة بواسطة جهاز TDS / EC/PH Meter نوع HANNA HI 8733 ، وهذه القياسات تمت موقعياً وتم قياس درجة التعكر باستخدام جهاز قياس التعكر Hach 2100A ، وقدرت كاتيونات الكالسيوم والمغنيسيوم بطريقة التسحيح مع (EDTA) 0.01 عياري، والكلوريد بالتسحيح مع نترات الفضة 0.01 عياري ، أما القلوية فقد تم قياسها بشكل كاربونات بالتسحيح مع حامض الكبريتيك المركز واستخدام المثل الأحمر كدليل ، و قدرت الحامضية بالتسحيح مع هيدروكسيد الصوديوم ، أما العسرة الكلية فقد تم قياسها بواسطة جهاز Oxfam water testing ، وتم قياس الأوكسجين الذائب DO بواسطة جهاز DO meter وحسب الطريقة الموصوفة في (5).

النتائج والمناقشة

بينت النتائج أن أغلب الخصائص الكيميائية المدروسة قد تغيرت خلال الفترة الزمنية الممتدة بين عام 2006 إلى 2014 ، إذ سجلت ارتفاعاً واضحاً في قيم الإيصالية الكهربائية والذي يعد مهماً لنمو وكثافة النباتات، وأيضاً بالنسبة إلى الأحياء المائية والإنسان، إذ سجلت أقل قيمة في هور أبو زرك (1.6) ملي سمينز .سم⁻¹ ، بينما سجل هور السناف أعلى قيمة بلغت (4.7) ملي سمينز .سم⁻¹ ، وبالنسبة إلى كل من هور الجبايش و الحمار والعدل فبلغت (3.3، 2.0، 4.4) ملي سمينز .سم⁻¹ ، وهذا يتفق مع دراسة (3) ، لكنها أعلى من القيم التي وجدها (6) ، بينما سجل هور السناف أعلى قيمة pH (8.1) ، وكما هو موضح بالجدول رقم (1).

المقدمة

تشكل الأهوار في جنوب العراق الحوض الطبيعي لأنهار العراق، كونها مناطق منخفضة يصل منسوب قسم منها إلى مستوى أخفض من منسوب سطح البحر، تكونت منذ آلاف السنين من تغذية نهري دجلة والفرات، وأهم هذه الأهوار "الحمار، الحويزة، السنية والسناف"، إضافة إلى مجموعة أخرى من الأهوار والمستطحات المائية المتصلة تقريباً مع بعضها مثل الجبايش ، أبو زرك، والعدل ، موزعة على مساحة محافظات البصرة وذي قار وميسان، وتزيد مساحتها على 3 مليون دونم (1).

تعد بيئة الأهوار من المناطق الطبيعية المهمة، لاحتوائها على ثروات كثيرة ومتنوعة، ساعدت على إنشاء وتكوين التجمعات السكانية على أطرافها وفي عمقها.

تقع منطقة الأهوار في الجزء الجنوبي أو السفلي من وادي الرافدين، وتضم أهوار جنوب العراق المنطقة المثلثة الواقعة بين مدينتي العمارة شمالاً، والبصرة جنوباً وشرقاً، وسوق الشيوخ غرباً، وتضم بينها جزراً كثيرة ، وعرفت المنطقة أيضاً بجنة عدن قديماً.

تعد الأهوار عاملاً مهماً في تحسين مناخ المنطقة ، وتمثل أكبر مصفى للمياه الموجودة لوجود القصب والبردي فيها (2)، كما تعرضت لأعمال التجفيف والتي شكلت أكبر كارثة حلت بها حيث أسفرت عن تغيير ملحوظ في معالم الطبيعة والحياة فيها في أوائل التسعينات من القرن الماضي.

وبحسب برنامج الأمم المتحدة ، فقد أظهرت صور عبر الأقمار الصناعية للأهوار عودتها لتغطي تقريباً نحو 40 % من مساحتها في شهر آب / أغسطس من عام 2005 ، وقد تزايدت النسبة لتصل إلى ما يقارب 50% في شهر آذار/ مارس من عام 2006 نتيجة أمطار الشتاء وذوبان الجليد عند منابع دجلة والفرات، وأظهر تقرير البرنامج في بيان تضمن صوراً جديدة عبر الأقمار الصناعية زيادة سريعة في الغطاء المائي والخضري في عامي 2004 و 2005 ، لكن هذا الأمر يحتاج إلى تحليلات ميدانية مفصلة لنوعية التربة والمياه لتحديد الحالة التي وصلتها الأهوار مؤخراً، هذا وقد أشارت نتائج برنامج الأمم المتحدة في نيروبي أن أحدث صور الأقمار الصناعية توضح معدلاً (غير عادي) للتعافي في الأهوار الجنوبية في عام 2005 ، حيث عادت مساحتها إلى نحو 3500 كم مربع بعد تراجعها إلى 760 كم فقط في عام 2002.

ولأجل ذلك، يأتي البحث الحالي بهدف دراسة التغيرات في الخصائص الكيميائية لمياه الأهوار ومقارنتها بالدراسات السابقة والحالية واستنتاج تلك المستجدات والمتغيرات خلال الأعوام من 2006 إلى 2014.

المواد وطرق العمل

وصف الموقع الجغرافي لأهوار جنوب العراق:

تقع هذه المنطقة بين خطي عرض 30 35 و 32 45 شمالاً وخطي طول 48 13 شرقاً ، وتبلغ مساحتها 35000 كم² ، منها 9000 كم² أهوار دائمة والباقي أهوار موسمية ، ويبلغ طول المنطقة من الشمال إلى الجنوب 210 كم وعرضها 170 كم (3) ، وتقع الأهوار المدروسة في هذا البحث ضمن المنطقة الثانية من الأهوار الجنوبية وتشمل أهوار الجبايش والحمار وأبو زرك والسناف والعدل ، ويحدها كرمه بني سعيد غرباً، ونهر الفرات وشط العرب شمالاً، وسكة حديد بغداد جنوباً، والكرمة شرقاً ، وتصب في شط العرب بواسطة نهر كرمه علي.

جمع النماذج:

تم جمع نماذج من 5 عينات من مياه أهوار جنوب العراق شملت كلا من: الجبايش والحمار وأبو زرك والسناف والعدل عام 2006 ، وفي عام 2014 تم أخذ نفس العدد من العينات من جميع

مول.لتر⁻¹ مع الزمن (الجدول رقم 1) ، وهذا يتوافق مع النتائج التي وجدها (7 ، 8).

أما بالنسبة إلى قيم الكلوريد ، فإنها تباينت في أغلب الأهوار ماعدا هور الجبايش الذي سجل ارتفاعا ملحوظا (145.3) ملي

جدول رقم (1): قيم بعض الخصائص الكيميائية للأهوار المختارة عام 2006

اسم الهور	pH	EC ملي سم.سم ⁻¹	T.D.S	Alk	Acid	T.H	DO	التعكر	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻
	-					ppm					ملي مول.لتر ⁻¹
الجبایش	7.7	3.3	1850.0	306.7	0.0	360.0	6.2	50.0	2.7	1.2	145.3
الحمار	7.9	2.0	1076.7	336.7	0.0	323.3	6.2	6.7	2.6	0.9	14.9
العدل	7.8	4.4	2450.0	290.0	0.0	380.0	6.6	48.3	3.0	1.2	35.0
ابوزرك	7.6	1.6	866.7	313.3	0.0	340.0	5.7	33.3	2.5	1.3	16.4
السناف	8.1	4.7	2600.0	300.0	0.0	333.3	6.3	11.0	2.7	1.2	42.5
المعدل	7.8	3.2	1768.7	309.3	0.0	347.3	6.2	29.9	2.7	1.2	50.8

في هور الجبايش ، نتيجة لكون المصدر الرئيسي للمياه من نهر الفرات والذي يمتاز بارتفاع قيم أيون الكلوريد نتيجة نسبة الأملاح العالية التي يحملها هذا النهر أثناء مروره بأراض تشتهر بزراعة الرز في النجف والديوانية، وبالتالي فإن أغلب مياه البزل ترجع إلى نفس النهر حاملة تراكيز مرتفعة من الأيونات الملحية وخاصة الكلوريد ، فضلا عن مروره بأراض تسمى بالمالحة في السماوة تشتهر بارتفاع ملوحة تربتها ووجود عيون ملحية تصب في النهر وهذا يتفق مع ما وجده (9 ، 10).

وفي عام 2014 بلغت أقل قيم للملوحة 4.5 ملي سيم.سم⁻¹ لهور الحمار في حين بلغت 10.9 ملي سيم.سم⁻¹ في هور السناف وبلغت قيم pH 7.3 لهور العدل وأعلىها بلغت 8.2 في هور السناف ، وسجلت قيم الكلوريد انخفاضا في هور أبو زرك، إذ بلغت 22.7 ملي مول.لتر⁻¹ (جدول رقم 2)، في حين سجلت ارتفاعا بلغ 200.3 و 145.3 ملي مول.لتر⁻¹ في هور الجبايش لعام 2014 و 2006 على التوالي، وهي أعلى من قيم الكلوريد لبقية الأهوار، و ربما يرجع ذلك إلى وجود مصدر لأيون الكلوريد

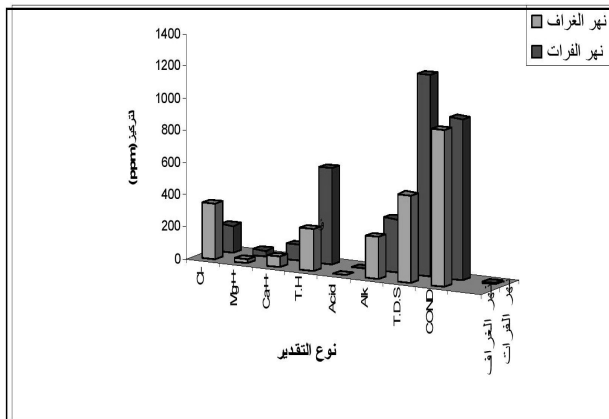
جدول رقم (2): قيم بعض الخصائص الكيميائية للأهوار المختارة عام 2014

اسم الهور	pH	EC ملي سم.سم ⁻¹	T.D.S	Alk	Acid	T.H	DO	التعكر	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻
	-					ppm					ملي مول.لتر ⁻¹
الجبایش	7.8	8.5	6256.4	1245.3	0.0	389.4	5.3	62.3	14.2	10.7	200.3
الحمار	7.5	4.5	4568.3	444.2	0.0	489.7	4.2	45.7	8.6	5.1	20.8
العدل	7.3	9.5	8368.1	456.9	0.0	789.6	3.8	59.7	20.1	15.7	60.7
ابوزرك	7.9	6.3	2154.3	635.1	0.0	473.6	5.4	23.6	5.8	6.3	22.7
السناف	8.2	10.9	6389.7	236.9	0.0	659.8	5.9	20.7	12.4	8.4	65.3
المعدل	7.7	7.9	4947.3	603.6	0.0	560.4	4.9	43.2	12.2	9.2	73.9

منخفض في مياهه والتي بلغت (11.0 و 20.7 ppm) مقارنة ببقية الأهوار المدروسة وفي عامي 2006 و 2014 على التوالي . يرجع السبب الرئيس في ارتفاع تركيز الأملاح في عام 2014 مقارنة بعام 2006 وبالتالي زيادة تركيز الأيونات ومنها TDS و Ca⁺⁺ و Mg⁺⁺ و Cl⁻ إلى زيادة تركيز الأملاح في عام 2014 وخاصة في مياه نهر الفرات والتي بلغت 6.3 ملي سيم.سم⁻¹ مقارنة بقيم EC ، والتي بلغت 2.4 ملي سيم.سم⁻¹ عام 2006 نتيجة النقص الحاد في كمية المياه الواردة إلى نهر الفرات عند دخولها إلى الحدود العراقية بسبب السدود التي أنشئت من الجانب التركي على روافد هذا النهر في أراضيها، فضلا عن قلة سقوط الأمطار وخاصة في الأعوام الممتدة من 2006 إلى 2012 (12) ، ومرور نهر الفرات بأراض مالحة في منطقة بادية السماوة (المالحة) ، والذي انعكس بدوره على زيادة تركيز الأملاح في مياه نهر الفرات ، فضلا عن تحويل مجرى المصب العام في عام 2012 إلى الأهوار نتيجة انخفاض مستوى المياه بعد عام 2007 ، وبالتالي فإن مياه نهر المصب العام تعد مياه بزل وذات

أشارت نتائج التحليل الاحصائي إلى وجود فرق معنوي في قيم EC و TDS و Ca⁺⁺ و Mg⁺⁺ لهور السناف مقارنة بالأهوار الأخرى، فضلا عن وجود الفرق المعنوي عام 2014 لنفس الصفات الكيميائية مقارنة بعام 2006 ، وهذا يرجع إلى تراكم الملوحة في هذا الهور مقارنة ببقية الأهوار الأخرى، ومع مرور الزمن، إذ بين معامل الارتباط r² بين قيم الملوحة EC وتركيز الكاتيونات Ca⁺⁺ و Mg⁺⁺ بلغ (0.54 و 0.22) للأيونين على التوالي، وهذا يشير إلى أن أيون Ca⁺⁺ له ارتباط أقوى من Mg⁺⁺ في زيادة تركيز الأملاح في مياه الأهوار ، فضلا عن أن معامل الارتباط r² بلغ 0.61 بين قيم EC و Alk ، إذ إن للملوحة تأثيرا على زيادة القلوية لمياه الأهوار، وهذا ناتج من كون المصدر الرئيس للمياه هو نهر الفرات (11) ، وبالتالي فإن نوعية المياه عام 2014 تتصف بارتفاع الملوحة والتي بلغت 6.3 ملي سيم.سم⁻¹ مقارنة بنهر دجلة ، والتي بلغت 1.1 ملي سيم.سم⁻¹ ، فضلا عن أن التيار المائي لهذا الهور يتباطأ تدريجيا مما يسبب تراكم العوالق في مياه الهور، وهذا يفسر أن التعكر

عنصر مهم في عسرة مياه الأهوار. وتسهم ضحالة هور الحمار في حدوث تبادل للمغذيات بين القاع وعمود الماء ، مما يؤدي إلى زيادة تركيز المغذيات في المياه، وبشكل عام، تتميز المياه الداخلية العراقية ومن ضمنها الأهوار بارتفاع نسب الأيونات الموجبة والسالبة الأساسية عن مثيلاتها في العالم ما عدا البوتاسيوم، وهذا ما جاء في دراسة (16).

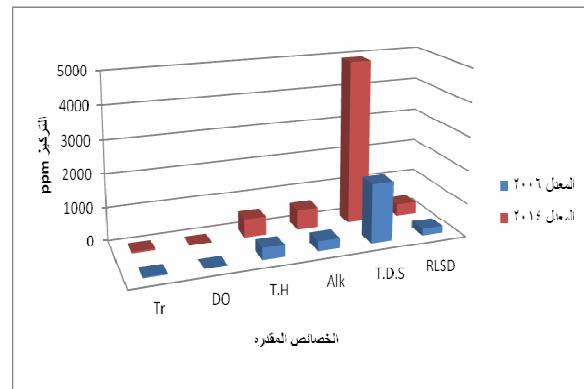


شكل رقم (3): الخصائص الكيميائية لنهري الفرات والفرات (دجلة)

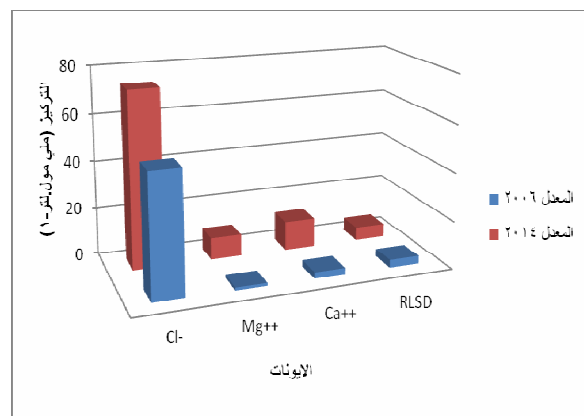
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قيم ملوحة تصل إلى 10.5 ملي سيمنز.سم⁻¹ ، وهذا ينعكس بدوره على نوعية مياه الأهوار في تلك المنطقة (13) .
يبين كل من الشكل رقم (1)، ورقم (2) ارتفاع بعض الخصائص الكيميائية والفيزيائية والتي شملت (القلوية Alk والعسرة T. والتعكر) في عام 2014 والتي بلغت (603.6 و 560.4 و 43.2 ppm) مقارنة بعام 2006 والتي بلغت (309.3 و 347.3 و 29.3 ppm) كمعدل لجميع الأهوار المختارة ولنفس الخصائص المقطرة أعلاه، ويرجع ذلك إلى تدهور نوعية مياه نهري الفرات ودجلة مع مرور الزمن نتيجة عدم وجود الرقابة البيئية والصحية على مياه الصرف الصحي التي تلقى في النهرين، وهذا ما أكدته معظم المنظمات البيئية والبحوث الحديثة في العراق ، وقد انعكس هذا الواقع بدوره أيضا على انخفاض قيم الأوكسجين الذائب DO والذي بلغ 4.9 ppm في عام 2014 ، بينما كانت قيمة 6.2 ppm عام 2006 وكمعدل لجميع الأهوار المختارة ، مما يعد مؤشرا خطرا على الأحياء المائية وخاصة الثروة السمكية ، والتي أشارت المصادر إلى أن نسبة الحد الحرج للأوكسجين الذائب في المسطحات المائية هو 5 ppm .



شكل رقم (1): معدل قيم بعض الخصائص الكيميائية للأهوار المختارة عام 2014 و 2006



شكل رقم (2): قيم معدل بعض الأيونات للأهوار المختارة عام 2006 و 2014

لقد تباينت قيم درجة التعكر في جميع الأهوار من الارتفاع الشديد إلى الانخفاض، وسجلت تذبذبا ملحوظا في القيم، وقد تأثرت قيم الخصائص الكيميائية المقاسة في هذه الدراسة بنوع المياه القادمة من نهري الفرات والفرات (دجلة) (شكل رقم 3) ، وهذا يتفق مع دراسة كل من (9، 14، 15)، إذ تلعب كمية المياه القادمة من دجلة والفرات دورا أساسيا في تقلبات قيم الملوحة في جميع الأهوار المدروسة، كما أن ارتفاع قيمة الأس الهيدروجيني عن التعادل يعكس حالة تحلل كبير في الأهوار. إن أيون الكلورايد

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كفاءة الإشارة الظاهرية والضمنية المعمارية بين القديم والحديث: دراسة تحليلية لمآذن مساجد محافظة**بابل – العراق****محمود رزوقي جنجون**

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البريد الإلكتروني: mjanjun@yahoo.com**الملخص باللغة العربية**

تعتبر المئذنة مكوناً أساسياً من مكونات عمارة المساجد في الحضارة الإسلامية، رغم أنها لم تكن تتطلب شكلاً معيناً في زمن النبي محمد صلى الله عليه وآله وسلم، وقد اتخذت المآذن أشكالاً معينة نتيجة تأثيرها بالعمارة التي كانت موجودة في كل عصر من العصور الإسلامية، كما تأثرت بالطابع الجغرافي لتنسجم مع المحيط الذي كانت تعمر به، وللمآذن وظيفة أساسية هي إعلان الأذان للصلوات، وقد لوحظ عدم وجود صلة بين الأصل المعماري للمآذن في مختلف الحضارات وبين الوظيفة والرمزية التي اكتسبتها تلك المآذن لاحقاً، فقد ارتبطت أيضاً إضافة إلى المساجد بالأضرحة والمدارس الدينية كرمزية وليس كوظيفة. وفي محافظة بابل في العراق، هناك إشارة ظاهرية وضمنية معمارية استمت بها المآذن، سعت الدراسة الحالية لتناولها من خلال استعراض مقارن للمئذنة القديمة والحديثة، حيث إن العمارة الإسلامية قد أعطت لكل الأبنية والمساجد أشكالاً ومعانٍ معينة، ولغرض تحقيق أهداف الدراسة، فقد تم التركيز على دراسة وتتبع الإشارة المرسل من المئذنة لتوضيح النقص المعرفي في هذه الإشارة في بعض مآذن محافظة بابل، وعليه قدمت الدراسة تحليلاً لبعض الإشارات الظاهرية والضمنية الصادرة من شكل المئذنة وكفاءة أدائها، وقد تم تمييز مفردة أساسية لمآذن بابل تمثلت في انسياب المعلومة من خلال الإشارة الظاهرية والضمنية المرسل من المئذنة للناظر المتلقي.

الكلمات المفتاحية: المآذن، بابل، الرسالة، الدقة، التشويش

The efficiency of architectural virtual and implicit signals between ancient and modern: an analytical study of the minarets of mosques in Babylon governorate – Iraq

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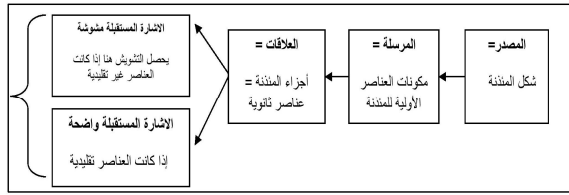
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ABSTRACT

The minaret is considered as the essential element of the constituents of architectural mosques in Islamic culture, though it does not need for particular shape during the time of the prophet Muhammad (God's blessing on him and his followers). The minaret takes given shape affected by the past architectural shape. It is also affected by its geographic property which makes it harmony with the environment of those minarets which employ in praying. In fact, there is no relation between the original architecture of the minarets in ancient culture and the function and the symbolism which the minarets get. Additionally the minaret relates to the tombs and religious schools as a symbolism but not as a function. There is apparent sing and architectural implication marked by the minarets of Babylon which the study concerns with through the comparison between the ancient and modern minarets. To achieve the goals of the study, the research focuses on the study and follows the signals of the minaret to clarify the lack of knowledge in sending sins from the minarets in Babylon. There for, the study give an analysis for the implicit and apparent signal which arise from the shape and efficiency of the minarets. As a result, it has been discriminated Babylon minarets by the term information flow through the apparent and implicit signals which send from the minaret for the viewer.

المقدمة

الضماني للمئذنة وفقدان زيادة العناصر الداخلة في إنشاء المئذنة
يسبب تشويشا للمتلقي (مخطط رقم 1).



مخطط رقم (1): يوضح مصدر الرسالة الى الجهة المقصودة وشكل
انحرافها من خلال مصدر التشويش (الباحث)

الوظيفة والرمزية:

بالنظر إلى المصادر المعمارية (3، 4) التي تتحدث عن المآذن بشكل عام ، لم يتخذ موضع أو بناء معين لرفع الآذان في زمن الرسول صلى الله عليه وآله وسلم عندما شرع الآذان سنة هجرية ، واستمر الحال هكذا في زمن الخلفاء، ولكن في دمشق كانت هنالك أول محاولة لبناء أسطوانات في الجامع الأموي الذي كان بالأساس كنيسة ولها ثلاث منائر للإشعال النار للمسافرين لسيلا حورت لأن تكون مآذن لاحقاً في زمن الخليفة الأموي الوليد ثم أنتشر بناء المآذن إلى بقية العالم الإسلامي .أذن أول بداية للمآذن هو في العصر الأموي ولا يوجد اتفاق حول تبرير ظهورها ، هل هي لأغراض دينية بحتة أم لأسباب رمزية ووظيفية أخرى، فالمآذن في العمارة الإسلامية مثلاً قد استغلت في وظائف كثيرة إضافة إلى وظيفتها في إيصال صوت الآذان أوقات الصلاة، ففي العراق نرى المنارة الملوية قد استغلت لأداء أكثر من وظيفة سواء كانت أدائية أو رمزية، وفي حقيقة الأمر إن الجانب الشكلي كانت له أولوية أكثر من الوظيفة نفسها وهذا ما يفسر موقعها خارج نطاق المسجد عند المدخل وبمحورية مع اتجاه القبلة وشكلها الفريد من نوعه الذي طغى على الوظيفة فمنارة جامع أبو دلف تقع شمالي المدينة مما يصعب سماع صوت الآذان في القسم الجنوبي حيث يسكن عامة الناس إضافة إلى الارتفاعات العالية حيث بلغ ارتفاع منارة جامع سامراء الكبير 40 متراً . المنارة هي موضع النور ولهذه اللفظة استعمالات عديدة قبل الإسلام فالمنارة هي الشمعة ذات السراج والموضع الذي يوضع عليه السراج والمنار علماً لطريق وهو ما يبنى لهداية المسافرين وأرشاد القوافل والسفن ومنار الأرض هو ما يوضع لتحديد حدود الأرض والمنار هو موضع الرصد والمراقبة (8) .

إن لفظة المنارة لها أصول وظيفية حيث أطلق أسم المنارات على مآذن جامع عمرو بن العاص بالفسطاط عند إعادة بنائه سنة 92هـ وتعني المنارة هي إضافة وظيفة أخرى للمئذنة وهي الإنارة أي الهداية إلى المسجد في الليل للإعلان عن وقت الإفطار والسحور في شهر رمضان من خلال استعمال القناديل في رأسها، ومآذن المساجد على طول الساحل الإفريقي فيما وراء الإسكندرية غرباً كانت تقوم بوظيفة الحراسة والإنذار من خطر العدو البحري من خلال الرسائل الضوئية من جانب وبين وظيفتي المئذنة والصومعة (9). والجدول رقم (1) يبين بشكل تفصيلي وظائف العناصر الأساسية والثانوية للمئذنة.

أصبحت المئذنة من معالم حضارة المدن الإسلامية سابقاً ، وفي هذه الأيام تعتبر جزءاً من التكوين المعماري لعمارة المساجد بشكل عام، واصطبغت بطابع معماري أخذ شكله وتكويناته من تراث المنطقة من ناحية، ومن طرز دخلت وإفادته مع مختلف العهود الإسلامية التي تركت بصماتها في كل حواضر المدن الإسلامية من ناحية أخرى، وبشكل أو بآخر، اتخذت منائر محافظة بابل شكلاً تقليدياً سائداً كما في بقية محافظات العراق، وهي طراز معماري نعتز به وهو بلا شك حضارة وقيم روحية نفتخر بها كتراث أول ولا عمارة ثانياً تزينت بها حارات ومناطق وضواحي كل بلادنا الإسلامية وعلى الخصوص في محافظة بابل، ولكن هنالك مآذن حديثة شيدت بشكل أو بآخر حرفت وشوهت الإشارة والرمزية من شكلها، وأصبحت مآثر تساؤل لماذا هذا التكوين الجديد والغريب عن الطراز المعهود؟ ولذا سوف يتناول البحث استقراء تحليلياً لمآذن محافظة بابل بين القديم التقليدي والحديث وذلك لتأصيل المئذنة من الناحية المعمارية.

مردفات المئذنة هي الصومعة والمنارة بحسب ما جاءت به المصادر (1-4)، وملخص ذلك أن المئذنة لغة وهي موضع الآذان ، وارتبط الآذان بالسنة الأولى الهجرية وهي مصطلح إسلامي مستحدث وكل موقف يقف فيه المؤذن أو عنده يسمى مئذنة حتى لو لم يكن بناء ، وقد ارتبطت المئذنة بالرمزية السياسية التي تشير إلى قوة الحاكم ورمزية ثقافية تشير إلى ثقافة منطقة معينة ورمزية ، حيث ربط الناس بعض الأحداث بالمئذنة ويقفون ببناء مئذنة جديدة.

تشكل المئذنة عنصراً معمارياً عمودياً له شرفات ويرتبط بالمساجد وظيفته رفع الآذان من فوقها (5). ويمكن تمييز ثلاثة أجزاء رئيسية للمئذنة وهي القاعدة والبدن والقمة ، حيث إن لكل عنصر معماري جزءاً رئيسياً له وأجزاء مميزة تربطه أو تفصله مع ما يجاوره من أجزاء أخرى ، وهكذا يمكن اعتبار بدن المئذنة الجزء الأساسي فيها والقاعدة الجزء الذي يربطها أو يفصلها مع الأرض، في حين تمثل الجزء الذي يربطها أو يفصلها مع ما فوقها أي السماء، أما الشرفة فهي ما يميز المئذنة كعنصر معماري عمودي، فهي جزء رئيسي آخر لها وهي محمولة على البدن وتفصل جزءاً متميزاً آخر عنه وهو العنق، إذ إن الأجزاء الرئيسية للمئذنة هي خمسة: القاعدة ، البدن، الشرفة، العنق، والقمة. ويمكن تقسيم هذه الأجزاء الرئيسية إلى أجزاء أصغر تتضمنها وهي فتحة المدخل في القاعدة، فتحات الشبائيك في البدن والقمة، وكذلك تحتوي هذه الأجزاء الخمسة على عناصر تزيينية مثل أشرطة الكتابة أو الزخارف الهندسية والنباتية. وتتميز بعض العناصر بالتعدد مثل الشرفة (5).

الصومعة هي صفة للنشء العالي المستدق ولها استخدامات قبل الإسلام ، وهي بيت لعبادة المسيحيين لكونها تبنى في مناطق جبلية خوفاً من الاضطهاد ، ثم عمم اللفظ على كل بناء لعبادة المسيحيين ، وفي العصر الإسلامي، انتقلت اللفظة للدلالة على المئذنة إما لمشابهة الشكل العالي المستدق أو بسبب وجود بعض الصوامع المهجورة في العصر الأموي كما في الجامع الأموي (6)، (4) التي استخدمت للآذان ومثلها في اليمن . ويرى الباحث أن المئذنة لفظ مستحدث بعد تشريع الآذان، في حين أن المنارة والصومعة كان لها استعمالات أخرى قبل الإسلام، وأن المئذنة تدل على موضع الآذان حتى لو لم يكن بناء، في حين أن المنارة والصومعة تدلان على أبنية قد لا تكون مآذن.

الرسالة: هي طلب يراد إرساله إلى وجهة معينة وهي هنا المتلقي. دقة المعلومة: ويعني بها مدى مطابقة الإشارة المستقبلية للرسالة المرسلة، ويُعبّر عنها بالنسبة بين قوة الإشارة ومستوى التشويش. (7) وهي هنا وصول الرسالة واضحة للمتلقى عبر الطراز القديم وهنالك مقدار من التشويش الحاصل في طريقة الإنشاء ومواد الإنشاء واختفاء بعض العناصر من الطراز القديم في المئذنة الحديثة. التشويش ويقصد به انحراف الرسالة عن مسارها وتغير خواصها، كما يشير إلى الالامعنى. (8) . وهو أي شيء يتداخل مع أو يشوه الرسالة مقللاً المعلومات المستقبلية من المئذنة من خلال زيادة أو نقصان في الشكل والهيئة والمادة المستعملة. ويرى الباحث أن مقدار التشويش الحاصل عبر تغير الشكل الظاهري أو

جدول رقم (1): الإشارة المرسلية عبر العناصر الأساسية وأجزاء العناصر الثانوية في المئذنة (الباحث)

العناصر الأساسية للمئذنة	(1) عنصر أساسي	(2) عنصر أساسي	(3) عنصر ثانوي	(4) عنصر ثانوي	(5) عنصر ثانوي
	القاعدة	البدن	الشرفة	العنق	القمة
1- العناصر الثانوية للمئذنة	فتحة المدخل أشرطة الكتابة والزخارف والمقرنصات	شبابيك أشرطة الكتابة والزخارف والمقرنصات	أشرطة الكتابة والزخارف والمقرنصات	شبابيك ومدخل وأعمدة كما في مئذنة الحرم المكي	تاج يحمل أما لفظ الجلالة أو هلال
2- وظيفة استخدام العناصر	تستخدم القاعدة لحمل بدن المئذنة	يستخدم البدن لحمل هيكل المئذنة	للأذان أو الإنارة أو الحراسة أو بث الرسائل	جزء مكمل لبدن المئذنة	وظيفة هذا التاج هي كشف اتجاه القبلة من خلال لفظ الجلالة أو الهلال
3- وظيفية ورمزية دقة المعلومة Information Accuracy.	دقة المعلومة هنا نسبية في شكل القاعدة وضوح في المعلومة المرسلية عبر شكل قاعدة المئذنة التي قد تكون مربعة أو مضلعة	دقة المعلومة في البدن نسبية وضوح في المعلومة من خلال بدن المئذنة وزخارفها أو أشرطة الكتابة و انسيابيتها في الفضاء ومن خلال شكل البدن الذي يكون أسطوانياً أو مضلعاً أو مربعاً ويكون أما مزخرفاً أو به أشرطة كتابة	دقة المعلومة المرسلية تتأكد كلياً من خلال الصوت المنبعث حين الأذان من الشرفة الواحدة وتتأكد من خلال تعددها من خلال الشرفة الواحدة أو الشرفتين أو الثلاث كما في الحرم المكي الشريف	دقة المعلومة في القمة نسبية وضوح في المعلومة من خلال العنق والمقرنصات السفلية لها وقد تكون بها أعمدة لحمل إجراء ثانوية أخرى تغلوا بوضوح	تتأكد دقة المعلومة في شكل نهاية القمة قمة المئذنة التي قد تكون مخروطية أو بصلية أو بصلية مفصصة أو مدببة كما في مآذن تركيا تعطي للمعلومة المرسلية من خلال لفظ الجلالة أو من خلال شكل الهلال أو التاج

رسائل الإشارة وظيفية ورمزية للمئذنة:

وكذلك يصف (12) في دراسته عن العلاقة بين الوظيفة والإشارة كيف يمكن للشكل المعماري أن يكتسب وظيفته الدلالية من وظيفته الأساس مستعينا بالكهف كمثال على ذلك إذ يذكر كيفية لجوء الإنسان القديم إلى فجوات وحفر في الجبال (كهوف) محمية من الرياح والمطر يقوم باختيارها وتفحصها في ضوء النهار متخذاً منها مأوى له، إذ لاحظ الإنسان إذ ذاك نطاق الكهف وفهم ذلك على أنه حد الفضاء الخارجي، والذي يكون معزولاً ويعدّ كبداية للفضاء الداخلي الذي يثير بداخله شعوراً ربما بالحنين والحماية، شعوراً يشوبه الغموض بالنسبة له من خلال مراقبته للظل والضوء، ومتى ما انتهت العاصفة يكون بإمكانه الخروج ودراسة الكهف من الخارج، وهكذا فإن فكرة الكهف سجدت طريقها إلى ذاكرته كإمكانية للإيواء، ويمكن له إيصال هذه الفكرة ومشاركتها مع أناس آخرين بوساطة الإشارات التخطيطية، وهكذا فإن رسم صورة للكهف يصبح بمثابة اتصال أو تبليغ لفكرة وظيفة ممكنة حتى لو لم يكن هناك إنجاز لتلك الوظيفة والرغبة في إنجازها. وهكذا بالنسبة إلى النداء للصلاة من المئذنة أو بصوت الأذان المنبعث من برجها سابقاً أو بصوت مكبرات الصوت حالياً ، والجدول رقم (2) يبين تطبيق رسائل الإشارة.

يعتبر الشكل المعماري رسالة، وقد ظهرت مثل هذه الأفكار في النصف الثاني من القرن العشرين، ومن هذه المحاولات العديدة فسرت العمارة على أنها نظام لغوي يعبر عن الأفكار والمعاني. فعدت الشكل المعماري رسالة، وأن دور الرسالة المعمارية مشابه لدور الرسالة اللغوية والمتمثل بنقل المعنى والدلالة (10)، ويشير (11) إلى فكرة Walter Gropius بأن العمارة تعد أداة لنقل الثقافة والحضارة والإيمان. وتمثل العمارة كما وصفها Schulz Schulz التعبير الواضح لأفكار الإنسان، وحماسه، وإنسانيته، وإيمانه، ودينه، وإنها حتى في أدنى مستوياتها تبقى محتفظة بالبعد الميتافيزيقي. والعمارة كما يراها (11) مؤلفة من شفرات منفصلة، قابلة لتغيير مؤشرات دلالتها فضلاً عن شفراتها (فالأفكار، والنماذج الاجتماعية، واللغة كلها قابلة للتغيير) ومع ذلك فإن مجموعة متنوعة من الشفرات تحدث سنوياً أو تظهر معا في أي زمن لتجعل من تجربة العمارة تجربة متماسكة ومعمره. (11). وبذا يمكن عد العمارة استعمالاً لدلائل شكلية مواد وأنطقه لإظهار الأشياء المشار إليها كمدلول (11) (طرق الحياة، والقيم، والوظائف) بالاستفادة من وسائل معينة (تركيبية، واقتصادية، وتقنية، وألية)، وكما يرى الباحث، فمن خلال الشفرات المتنوعة، تتركب الإشارة المعمارية الظاهرية والضمنية.

جدول رقم (2): تطبيق رسائل الإشارة الظاهرية والضمنية للشكل المعماري (المئذنة) كما صنفها الباحثون (الباحث)

الإشارة المعمارية الصادرة من المئذنة (الظاهرية والضمنية)	تصنيف Walter Gropius	تصنيف Jencks Schulz	تصنيف Eco
1- معنى دال ظاهري ومدلول ضمني	معنى ودلالة للثقافة اتصال أو تبليغ إشارة الشكل (والأذان)	الحضارة التي تنتمي إليها المئذنة ومؤشراتها وأنواعها بعد ميتافيزيقي شفرات قابلة للتغيير	دلائل شكلية دال ومدلول شفرة قابلة للتحدث التطورات التي حصلت في شكل المئذنة
2- شكل الكتلة	-	-	شكل، فضاء، سطح، حجم
3- العلاقات	ثقافة الشكل وتبليغ الإشارة التي أصبحت مهمة، إن وجد الشكل فلا يرقى إلى المستوى المطلوب (الباحث)	تعبير مازال قائماً وحسب الظروف المادي وآخر مشفر وتغير و اكتفى بشكل مجرد من الحديد والزجاج والالمنيوم (الباحث)	يتمثل في شكل المئذنة العمودي (الباحث)
	علاقات قديمة تراثية تعطي نكهة الماضي الرصين وحاضر يتمثل ببساطة الشكل ولاختزال إلى حد العبثية في الشكل (الباحث)	علاقات مفككة غيرت من الطراز وهذه ديناميكية الشكل حسب استخدام مواد إنشائية جديدة في بناء المئذنة واستحداث إشكال غير منسجمة مع الحالة التراثية (الباحث)	ليقاع، ولون، وتركيب، ونسيج، وكثافة... إلخ في المئذنة ويتمثل في أشرطة الكتابة والزخارف واللون المستخدم في المئذنة (الباحث)

الضمني وتشير الدلائل حول الكتلة (شكل، نسبة، و حجم، ملمس) والعلاقات (إيقاع، ولون، وحدة، تناظر، ونسيج، وهذا يتمثل في الشكل والمظهر الخارجي للمبنة وبالعناصر الثانوية لها) (الباحث). ومع هذا يؤكد (11) إن العمارة لا يمكن أن تبلغ بتعبيرها دقة اللغة، مفسراً بهذا لجوء أكثر المماريين إقناعاً مثل Le Corbusier إلى إسناد الفكرة التي تطرحها أبنيتهم بالكلمات (11). من خلال ما تم عرض في الجدول السابق (2)، يتم استقبال الإشارة المعمارية الظاهرية والضمنية من قبل المتلقي عن طريق (الدلالة وشكل الكتلة والعلاقات) حيث يتم تفسير وتأويل هذه الإشارات المعمارية حسب الجدول أعلاه الذي يتضمن وضوح كامل إلى معنى ودلالة وشفرات قابلة للتغيير والتحديث لذا أرى أن المبنة تعطي حسب هذه الطروحات النظرية والعملية مستوى فهم الإشارة التي ترسلها المبنة إلى المتلقي سواء كانت بشكلها وبأجزائها كعناصر ثانوية في غير وقت الأذان ولذا فإن الطراز المعماري هو تلك الأشكال والخصائص والصفات والمفردات الظاهرية أو ضمنية. هذه المفردات تعمل على ظهور أعمال معمارية من مجموعة أخرى أفضل متولدة من قبل فرد أو مجموعة وتعود لفترة مضت. والطراز في العمارة هو المعنى الكلي للشكل الفيزيائي الذي نحصل عليه من وجود عناصر وسمات ثابتة في العمل الفردي أو الجماعي (مخطط رقم 2).

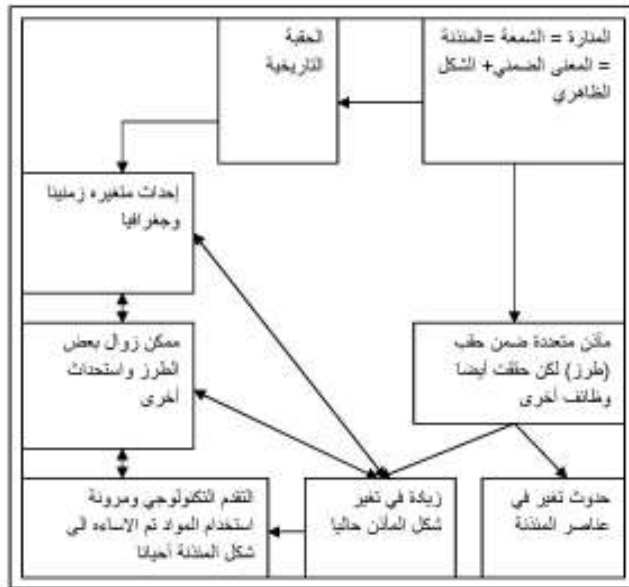
ولا ندري لعل المستقبل يختزل الكثير من ما موجود حالياً فيعطي لنا صوت عبر وسيلة أخرى سواء أكان صوت أو شعاع ليزري وغير ذلك كما أستحدث ذلك في مبنة جامع محمد الخامس في الدار البيضاء المغرب التي تمتاز بشعاعها الذي يمتد نحو 30 كم باتجاه الكعبة الشريفة بيت الله الحرام كما في الصورة رقم (1)، المركز الإسلامي في جزيرة السعديات أبو ظبي، مسجد يقوم على أساس الاستدامة مأذنه تصدر ضوء ليزري مكتوب به فقرات الأذان كما في الصورة رقم (2).



صورة رقم (1): جامع محمد الخامس الدار البيضاء (16)



صورة رقم (2): المركز الاسلامي في السعديات في ابو ظبي (17)



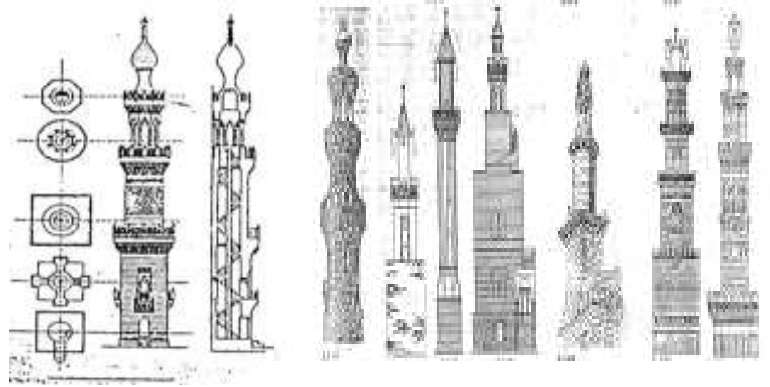
مخطط رقم (2): مدى تأثر شكل المبنة بالمكان والزمان واثـر التكنولوجيا إذا تم استخدامها بشكل سبيى أحياناً (الباحث)

الإطار التحليلي الخاص بمبنة محافظة بابل:

يعتمد التحليل على اخذ عينة ومقارنتها مع ما موجود من أمثلة من المصادر التاريخية لذا تم الاقتصار على النموذج في الرسم التوضيحي رقم (1) واختيار النموذج شكل (بغداد TH) الموجود لكون هذا الشكل هو الطراز التقليدي المحلي في واقع محافظة بابل والقياس عليه كدليل للتحليل.

الإشارة الظاهرية والضمنية في المبنة:

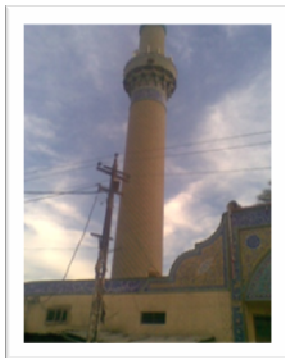
يمكن القول إن المبنة رسالة تعبيرية ثقافية بشكلها المعماري القديم والحديث وهي قراءة صحيحة حسب Walter Gropius وهي تعبير عن شفرات قابلة للتغيير والتحديث ومكونون من الميتافيزيقية حسب Jencks ومن خلال الشفرات المتنوعة تتركب الإشارة الظاهرية والضمنية المعمارية حسب (12). وعليه، فإن الإشارة المعمارية تمثل ثنائية الأولى (الدال) الظاهري والثاني (المدلول)



رسم توضيحي رقم (1): أشكال تعتبر ذات فرز متعددة (17)

الحالة الدراسية: تحليل العينات:

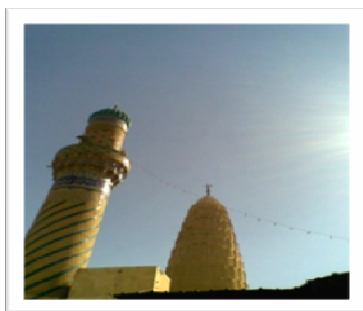
تم رسم نموذج تقليدي لمئذنين العراق ومن اسفل الرسم التوضيحي رقم (2) يوجد نموذج تحت اسم (بغداد TH) تم القياس عليه كنموذج للحالة الدراسية.



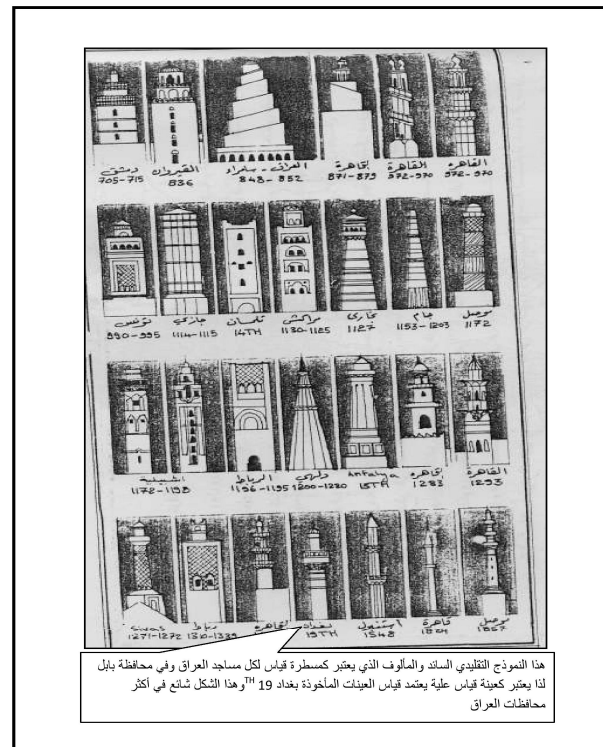
صورة رقم (3): مسجد ابن إدريس الحلي في وسط الحلة (طراز قديم يحمل كل الجمالية والنسب الصحيحة وظيفيا ورمزيا)



صورة رقم (4): مسجد مشهد الشمس في باب الحسين (طراز قديم يحمل كل الجمالية والنسب الصحيحة وظيفيا ورمزيا)



صورة رقم (5): مسجد السوق الكبير في سوق الحلة الكبير (طراز قديم يحمل كل الجمالية والنسب الصحيحة وظيفيا ورمزيا)



رسم توضيحي رقم (2): تنوع المآذن التقليدية في العالم الإسلامي

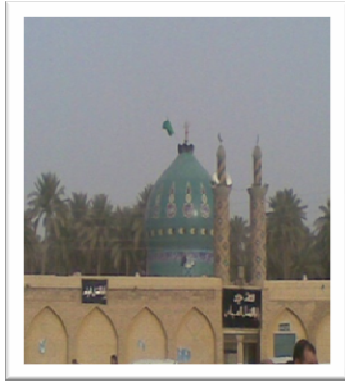
وقد جرى تطبيق الدراسة على مجموعة من المآذن الموجودة في مدينة بابل، حيث شملت مآذن المساجد التالية: (الصور أرقام 3-15).



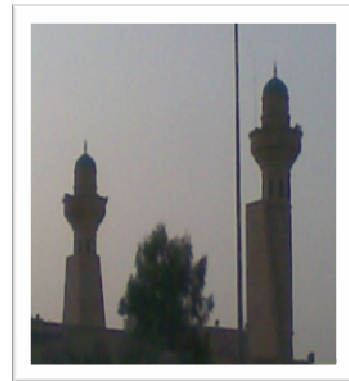
صورة رقم (9): مسجد الاحمدية في حي القاضية (مازال هناك شي من الطراز القديم الإشارة والرسالة واضحة)



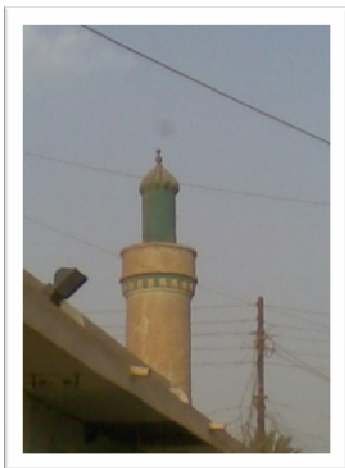
صورة رقم (6): مسجد ابن نما الحلي في القصبة القديمة (طراز قديم يحمل كل الجمالية والنسب الصحيحة وظيفيا ورمزيا)



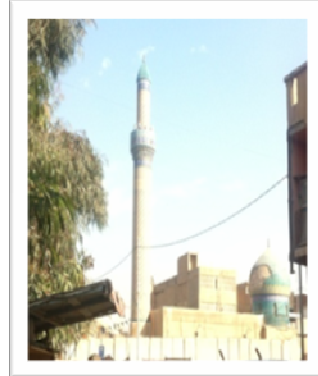
صورة رقم (10): قبة ومنارة الإمام محمد الأسمر في ناحية الإمام (طراز قديم ولكن لا توجد نسبة بين القبة والمنذنة ولكن الإشارة و الرسالة واضحة)



صورة رقم (7): مسجد الحلة الكبير شارع أربعين عناصر قديمة بشكل تقني حديث ذو رمزية ووظيفية (



صورة رقم (11): مسجد قرية فزع جنوب بابل (مزيج من القديم والحديث الإشارة و الرسالة واضحة)



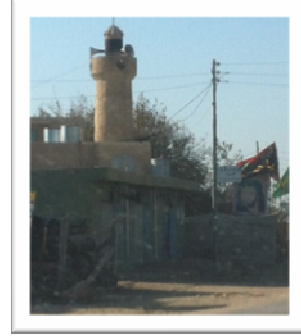
صورة رقم (8): مسجد حي المهندسين (عناصر قديمة ولكن النسبة خطأ في الشرفة الرسالة هنا يوجد بها خلل)

بعد ذلك، تم إعداد وتجهيز مسطرة القياس ثم إجراء استبيان بهدف التأكد من فحص مجمل التفاصيل الموجودة في المئذنة وذلك عبر استمارة أعدت لهذا الغرض في محاولة لكشف المئذنة التي ترسل إشارات ظاهرية وضمنية وقد وضع الباحث أسئلة وتم توزيعها على مجموعة من المهندسين المعماريين ثم تم تحليل هذه العينات والتوصل إلى أن الطراز البغدادي التقليدي (TH19) القديم هو المفترض وجوده في محافظة بابل ويعطي أكثر اشارته من السائد حاليا والمنشأ من الحديد وغيره والذي لا يعطي اشارة واضحة بل إشارة مشوشة.

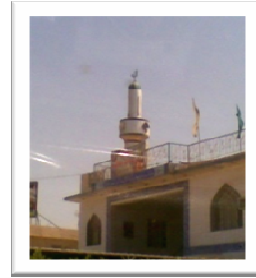
بعد أن تم جمع البيانات وإدخالها في برنامج الإكسل وتم جمع كل الإجابات واخذ المعدل العام لكل مفردة من المتغيرات ، وتم التوصل إلى نتيجة عامة.

وقد تم وضع مخطط عام يوضح دوال معدلات النسب لكل مفردة من مفردات المئذنة بحسب الإجابات (شكل رقم 1، 2).

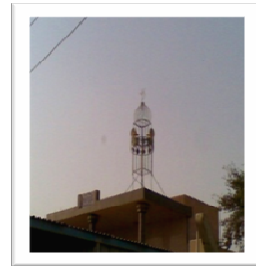
والمئذنة الحديثة في محافظة بابل والتي لا تنتمي إلى الطراز القديم بل إلى التصاميم العصرية يمكن توضيحها بالصورة التالية (12-15).



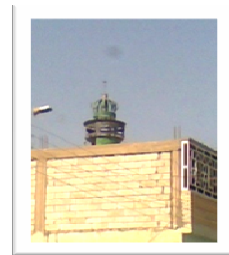
صورة رقم (12): مسجد الإمامين العسكريين الحى العسكري (هيكل مجرد كل القديم بعناصر من الحديد الإشارة والرسالة مشوشة)



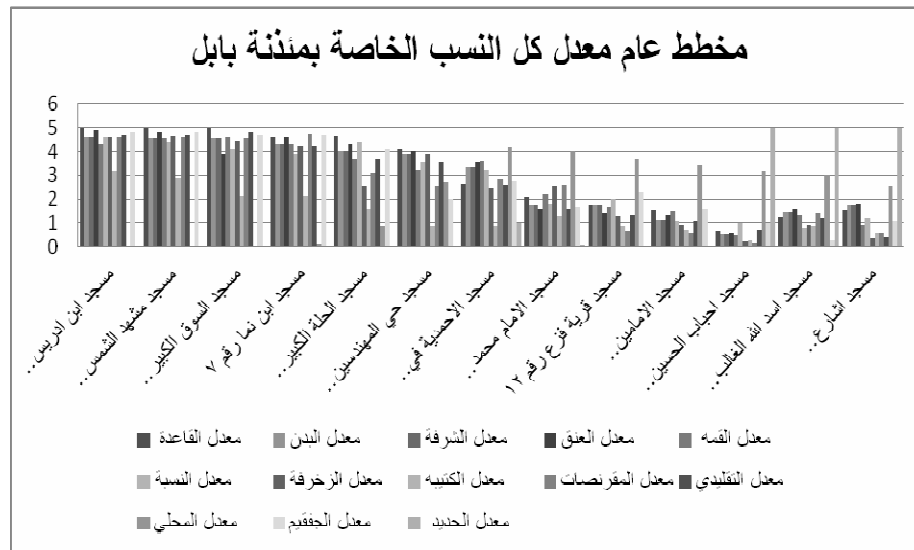
صورة رقم (13): مسجد أحباب الحسين (ع) حى الإمام (هيكل مجرد كل القديم بعناصر من الحديد الإشارة والرسالة مشوشة)



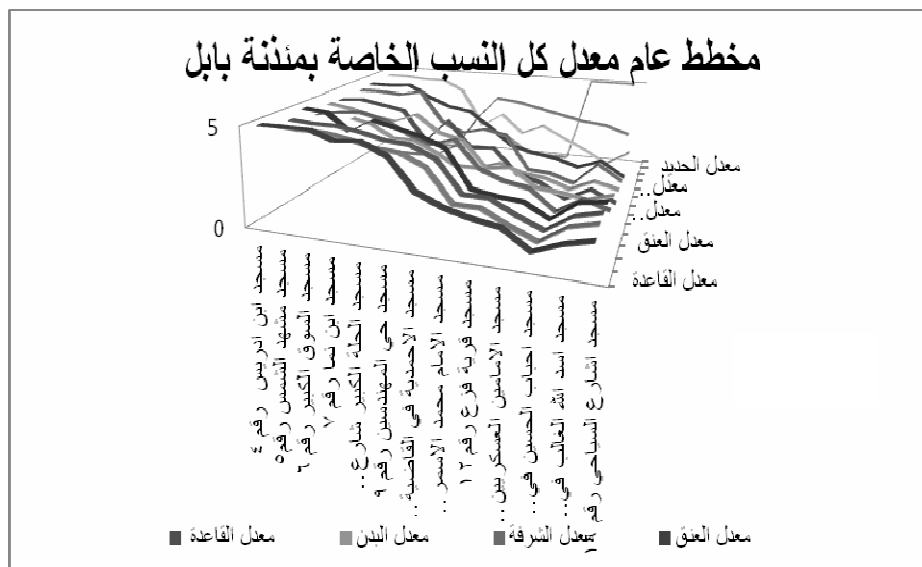
صورة رقم (14): مسجد أسد الله الغالب الإمام علي في حى الإمام (هيكل من الحديد ولكن احتفظ بالطراز القديم الإشارة والرسالة واضحة)



صورة رقم (15): مسجد شارع السباحي (قريب من الطراز التقليدي ولكن مجرد من كل العناصر الثانوية)



شكل رقم (1): المخطط النهائي لمعدلات كل النسب الخاصة بالعناصر الأولية والثانوية



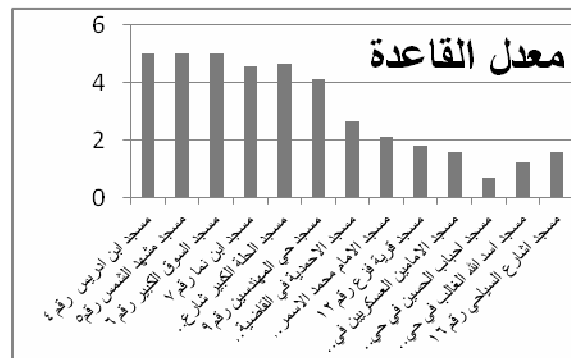
شكل رقم (2): دالة المنزلة التقليدية بشكل مرتفع يدل على ان الشكل التقليدي اكتسب اهتمام المهندسين

يشكل في الواقع جيود الاشارة عن خط سيرها الصحيح وايضا يعطي أكثر من علامة استفهام للمتلقى لماذا هذا الشكل وما الداعي له ولربما عامل السياسة ما قبل 2003م ، ونتيجة للحصار المفروض سعى المحسنون قدر إمكاناتهم في ذلك الظرف من اتخاذ إشكالا غريبة عن واقع المنزلة التقليدية والحيود عنها إلى المنزلة المحلية الصنع والأداء والبناء.

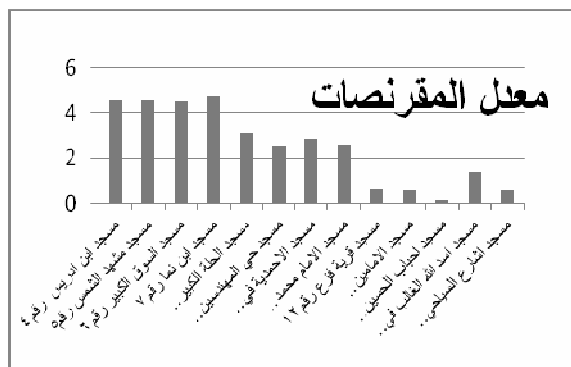
وإذا تم أخذ شكل رقم (3) كعينة لعنصر أساسي يلاحظ أن معدل قاعدة المنزلة في حالة ارتفاع و مؤشر الهبوط في باقي المساجد ، وبالمثل لباقي عناصر المنزلة.

ومن خلال الملاحظة الدقيقة لشكل رقم (1) يتبين وعلى سبيل الفرض ومن جهة اليسار معدل دوال إجابات المهندسين المعماريين حيث يلاحظ وضوح ارتفاع الدوال إلى مستوى رقم (5) ابتداء من مسجد ابن ادریس الحلبي مسجد الاحمدية حيث إن الاشارة واضحة تماما وتبدأ الدوال بقراءة باقي المساجد من حيث أنها لاتحمل خصائص المنزلة التقليدية.

أما في الشكل رقم (2) فيلاحظ ايضا أن الاشارة بدأت تتضح بشكل أكثر من الشكل السابق عبر هبوط المؤشر ابتداء من مسجد الاحمدية ايضا أن استخدام مواد غير تقليدية والخروج على المألوف



شكل رقم (3): معدل القاعدة



شكل رقم (4): معدل المقرنصات

حاليا سواء من الحديد او الالمنيوم او الصفيح او الزجاج لاتكاد تلبي الاشارة الضمنية والظاهرية لصعوبة بناء المئذنة التقليدية أولا، وثانيا عدم الاهتمام بالطراز التقليدي ولذا اختصرت على شكل أجوف يوحي لشكل المئذنة التقليدية، كما إن ابتعاد ونقل بعض من عناصرها الجمالية وجزء من وظيفتها وجزء من شكلها الإنشائي والمعماري يحرف مسار الإشارة و يوشوشها.

3. تناول البحث موضوع دقة المعلومة عن طريق الإحساس بالمئذنة شكلا وطرازاً تقليدي سائد ومعنى ورسالة قاصداً بناء قاعدة معلوماتية تخص البحث في عملية استقبال الإشارة التي ترسلها المئذنة بأشكالها وعناصرها الأساسية والثانوية ، وذلك من خلال تعريف أهم المفاهيم والمفردات التي ارتبطت بموضوع الإشارة استنادا إلى نظريات المنظرين السالفة الذكر. ويقودنا هذا الهدف إلى تحديد المشكلة العامة للبحث والمتمثلة بالنقص المعرفي حول دور المئذنة وعناصرها المؤثرة في كفاءة أدائها، أكدت على أهمية الدور الذي تلعبه الإشارة في المئذنة في استخلاص السلوك المطلوب للمتلقي، مبرزة أهمية الواجهة العمودية للمئذنة بعناصرها و محدداً لكتلتها ومعرف للفضاء ومولد للمعلومات. واتضح من مراجعتها وجود نقص في المعرفة المتوفرة السابقة بدا واضحا أهمية المئذنة وبرز دورها بشكلها القديم والحديث تقوم بنقل المعنى من المصمم (المرسل) إلى المشاهد (المتلقي) بمشكلة بحثية في نوعية الطرز المستخدمة ، ومن النقص المعرفي الموجود تركزت إشكال المآذن في محافظة بابل على نوعين: - النوع التقليدي من الطراز المنتخب في كل العراق تقريبا المعروف بشكلها وعناصرها منذ عصر الإسلام والتي تعتبر تقليدية بشكلها وعناصرها وجوده إشارتها إلى المتلقي وهي أيضا تعتبر نقطة دلالة وتعريف وجذب للمكان.

وبالنظر إلى شكل رقم (4) كعينة لعنصر مثال آخر عن العناصر الثانوية يلاحظ مثلا أن المقرنصات تتجه نحو أدنى المستويات لعدم التزام هذه المآذن بالشكل التقليدي وجنوحها إلى التوجه المحلي. ومن خلال هاتين العينتين يتضح أن تطبيقهما على باقي المخططات التفصيلية تأتي لنا بنفس الجواب. يتبين فيما سبق إن أي عملية تطور ونمو في الواقع الجديد يرجع إلى انتخاب تقاليد من حقبتين مختلفتين زمنيا قديم وحديث واعتماد إحدى الحقيقتين كأساس للانطلاق في العمل مع الاحتفاظ بالموروث سواء كان ذلك التطور شكلي ظاهري أو ضمني مفاهيمي واكتساب الشكل بفضل هذا المفهوم أمكانية الاستمرار في تكوينه وتطوره وعلى مستويين هما ظاهري وضميني، ثم أن الرسالة بالإشارة تتطلب مما سبق شكل التصميم ومقدار دقة المعلومة إلى المتلقي بشكل واضح خصوصا فيما يتعلق بالمئذنة في محافظة بابل وشكل الطراز التقليدي السائد تقريبا في عموم العراق ومحافظة بابل على الخصوص فهي تلك الإشكال والعناصر الثابتة والتي يمكن تحديدها ضمنيا أو ظاهريا من خلال مجموعة من الأعمال المعمارية. ومن هذه العناصر تعمل على تميز مجموعة مآذن من مجموعة أخرى. وكما يرى الباحث أن كل هذه العناصر التي تنتج من قبل فرد أو مجموعة وتعود إلى فترة زمنية معينة.

الاستنتاجات

1. تكوين الشكل يمثل أنواع التقنية المستعملة ويعبر عن طريقة الإنشاء، وكذلك يعتمد على الإمكانيات التكنولوجية التي تحول الأفكار إلى مادة ملموسة وتنتقل المعنى إلى الآخرين عبر الإشارة المرسل.
2. من خلال تحليل العينات تبين إن الأساليب التكنولوجية تلبي المتطلبات الوظيفية والرمزية والنفعية والجمالية والبيئية من خلال الشكل وفقا لترتيب العناصر، كما إن التقنية تخلق الشكل. ولكن في مئذنة بابل غير التقليدية التي تنشأ من التقنيات المستخدمة

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- النوع الذي غلب عليه التجريد من القديم التقليدي حيث فقد بعض الإشارات الضمنية التي تميزت بها المئذنة التقليدية وأدخل عليها بعض التقنيات الحديثة وتم تغيير شكلها وفقد أغلب عناصرها بحيث يبدو شكلها غريباً عن المألوف والإشارة هنا ضعيفة وغريبة ولا تعتبر تعريفية ولا حتى نقطة جذب للمكان.

التوصيات

بما أن العمارة الإسلامية ادخرت لنا كل هذا التراث التقليدي لشكل ووظيفة المئذنة التي تعتبر وسيلة اتصال وإشارة وشكل وجمالية بشكلها وعناصرها وتعدد طرزها من حيث الشكل والجمالية بالزخارف والكتيبات والشرافات والطاقيات وما تحمل من لفظ الجلالة أو الأهلة وما تضمنت من إشارات مختلفة كانت وما زالت تغطي للحاضر كما أعطت للماضي وطالما حكمت قصص عن من جاووها فهي تاريخ ولها جغرافية بموقعها حيث كانت تعبر عن شكلها من حيث أداؤها في زمانها ووقعت لها شكلاً أصبح توقيعها طراز ومثل قد يكرر هنا أو هناك عبر المكان والزمان تتجدد برسائلها التي تنشرها عبر الأثير إلى كل أطراف الحي يصدق من شرفاتها صوت الأذان في أوقات الصلوات، وفي ذلك توصي الدراسة بما يلي:

1. باعتماد الشكل التقليدي السائد ذو العناصر الإنشائية والجمالية الذي مازال يرسل إشارات واضحة شكلية ظاهرة وضمنية معنوية مستوحاة من فلسفة الإسلام في تجرد الأشياء نحو المطلق من خلال الارتفاع العمودي والاستدقاق النهائي نحو السماء.
2. المئذنة الحديثة بشكلها وعناصرها اختزلت كثير من القديم بمجرد صنعها من مادة الحديد أو الألمنيوم حيث أثرت هذه العناصر على شكلها الحديث ولم تعطي بتقدير الباحث إشارة واضحة وتبقى قيد الاستفهام عن شكلها ومضمونها لأنها على عدة أشكال ولا يوجد هنالك توقيع لشكل يحتذي به ولذا يوصي الباحث على عدم تكرار هكذا أمثلة غريبة عن الواقعية التي ألفناها من الأشكال التقليدية القديمة ذات الطرز الموقعة عبر الزمن.
3. استخدام التقنيات الحديثة لما يخدم حاجتنا إلى الطرز الموقعة معمارياً في المحافظة وأن لا نخرج عن المألوف وأن ندخل ما أمكن بالتقنيات كل العناصر الأولية والثانوية لمئذنة بابل. ومن الأمثلة الواضحة لمئذنة بابل مئذنة جامع ابن إدريس الحلي ومئذنة ابن نما، ومئذنة جامع مشهد الشمس وتعد هذه المئذنان نموذج موقع معمارياً حسب المعايير والنسب وأشكال ولذا يوصي الباحث بها من حيث الحفاظ عليها والتكثير من مفرداتها التقليدية وطرز جمالية ووظيفية وظاهرية وقديمة وحديثة.
4. على من يتقدم بتقديم للحصول على إجازة بناء مسجد يحتوي على مئذنة في بابل أن يلتزم بالمعايير الواردة ضمن هذا البحث حتى لا تخرج من أصول التراث وندخل في صلب العملية التقليدية مستخدمين كل ما أمكن من تقنيات حديثة تحاكي الموقع من المآذن التقليدية.

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