FORWARD

Years 2013- 2014 were the success years of our Journal- The International Journal for Sciences and Technology- IJST. Great values were added to our Journal when it scored two international impact factors: ICV: 4.32 and SJIF:3.735. Today, IJST is coming for you all in its third issue of volume nine for year 2014, as our deep belief in continuing the steps we began since nine years ago with diversity of researches and elite experts of the Editorial Board and Advisory Group. The members of Editorial Board, the Editorial Board Secretary and I hope you will find this collection of research articles useful and informative.

The current issue comes to you while the Islamic World is celebrating Eid Al- Adha , which gives me an opportunity to send you all my deep wishes and faithful prays to Allah for peaceful times for all our beloved Arab and Islamic countries.

Finally, 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

Using active and attenuated lactic acid bacteria to accelerate ripening of Gouda cheese

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ABSTRACT

Three combinations of Lactic acid bacteria LAB cultures were used with a final ratio of 1%, with addition of attenuated freeze shocked *Lactobacillus delbrueckii subsp. bulgaricus* at different ratios (0.1%, 0.5% and 1%). Cheese ripening was carried out under the same conditions and temperature (10 ºC), with four different time courses (0, 7, 14, 21 and 28 days). Chemical analysis data showed a significant decrease ($P < 0.05$) in moisture content as ripening time increased. However, there were no significant differences in titratable acidity for all treatments compared to their controls. Primary proteolysis data using electrophoresis showed that the $\alpha s1$-casein degradation was greater than β-casein degradation. Secondary proteolysis results showed significant increase in (water soluble nitrogen) WSN and (trichloroacetic acid-soluble nitrogen) TCA-SN contents for all treatments during ripening with the highest value for the treatments that contain 1% attenuated bacteria. There was a significant decrease in lactobacilli count, at day 28, while lactococci count did not decrease during ripening. Best sensory properties were for treatments that contained 1% attenuated bacteria. Cheeses with accelerated ripening showed no bitterness.

Keywords: freeze shocked , Gouda cheese, ripening , accelerate , adjunct bacteria , proteolysis.

الملخص باللغة العربية

تم استخدام ثلاثة توليفات من بكتيريا حامض اللاكتيك وبنسبة إضافة كلية بلغت 1% مع إضافة بكتيريا *Lactobacillus delbrueckii subsp.* *Bulgaricus* المضعفة بطريقة الصدمة بالتجميد وبالنسب التالية:
(0.1% ، 0.5% ، 1%) . وتم الإنضاج تحت نفس الظروف وبدرجة حرارة 10° م لأربع فترات زمنية مختلفة (0 ، 7 ، 14 ، 21 ، 28) يوما.
أظهرت نتائج التحليل الكيمياوي انخفاضا معنويا في الرطوبة بمرور فترة الإنضاج ولم تنخفض الحموضة التسحيحية لجميع المعاملات مقارنة مع العينة الضابطة . أظهرت نتائج التحلل البروتيني الأولى باستخدام تقنية الترحيل الكهربائي تميز ألفا أس–كازين على بيتا–كازين في نطاق التحلل، كما أظهرت نتائج التحلل البروتيني الثانوي زيادة معنوية في قيمة كل من النيتروجين الذائب بالماء والنيتروجين غير البروتيني لجميع المعاملات بمرور فترة الإنضاج مع تفوق المعاملات التي استخدمت فيها 1% من البكتيريا المضعفة . وانخفض عدد بكتيريا حامض اللاكتيك العـصوية معنويا بعد 28 يوما من الإنضاج بينما لم تنخفض أعداد بكتيريا حامض اللاكتيك الكروية خلال فترة الإنضاج .
تفوقت المعاملات التي استخدمت فيها البكتيريا المضعفة بنسبة 1% حسيا وتميزت الأجبان المسرعة الإنضاج بعدم ظهور المرارة في الطعم.

INTRODUCTION

Cheese ripening is a complex balance between glycolysis, proteolysis and lipolysis of the various milk components. Proteolysis is a good indicator of the rate of ripening and flavor development in cheeses. The process is controlled by the action of the proteinase enzymes which degrade the (proteins) caseins, to oligopeptides, which are later transformed into amino acids by intracellular peptidases (1,2). Increasing attention has been given to methods, which accelerate the cheese ripening time as it is a costly and lengthy process. One of the recognized means of accelerating the process is to increase the bacterial enzyme pool in the cheese without interfering with the activity of the primary starter culture.

This can be achieved by introducing attenuated whole LAB that are unable to grow and produce significant levels of lactic acid but are still able to provide active ripening enzymes throughout the cheese ageing process. This technique has been shown to accelerate the ripening process and to improve the overall quality of the end product (3).

The objective of the current study was to investigate the influence of *Lactobacillus helviticus* and attenuated freeze shocked *Lactobacillus delbrueckii subsp. bulgaricus* on the proteolysis pattern and sensory properties of Gouda cheese.

MATERIALS AND METHODS

Active strains culture:

Lactococcus lactis subsp lactis and *Leuconostoc mesentroides subsp cremoris*, were cultivated by growing 2 times at 30 ºC overnight in 12% (w/v) sterile reconstituted skim milk (RSM), whereas *Lb.helviticus* was cultivated by growing two times at 37 ºC overnight in 12% (w/v) RSM. The count of each bacteria was estimated by plating on MRS or M17 agar before its addition as starter culture.

Attenuated strains culture:

Overnight adjunct *Lactobacillus delbrueckii subsp bulgaricus* (45 ºC for 18 hrs.) were obtained from (MRS) broth. Cells were harvested by centrifugation (at 4000 g for 30 min, 4 ºC). The cell pellet was washed twice with 0.01M potassium phosphate buffer, pH 7, and resuspended in the same buffer to obtain a total viable cell concentration of approximately 10^9 CFU/ml by using stander curve of Lb. *delbrueckii subsp bulgaricus* (4). Freeze shock technique was carried out for attenuated Lb. *delbrueckii subsp bulgaricus* by freezing the cell suspension at -18 ºC for 24 hrs followed by thawing using water bath 40 ºC for 10 min before using (5). The survival and growth of Lb. *delbrueckii subsp bulgaricus* was determined after attenuation using MRS agar (37 ºC for 24 hrs). There was about 10^9 cfu/ ml of *Lb. delbrueckii subsp bulgaricus* before attenuation which decreased by about 1 log after attenuation reaching about 10^8 cfu/ml.

Cheese manufacturing:

Cheeses were made according to (6). Twelve cheeses (Triplicates of three treatments) were manufactured. The activated starter culture was added as a mixture at a ratio of 1% v/v (80 ml starter culture in 8 liter milk) to the pasteurized milk.

The three parts treated were as follows:

Treatment A: [0.50% *Lactococus lactis subsp. Lactis* (125×10^9) + 0.50% *Leuconostoc mesentroides subsp.cremoris* (133×10^9)].

Treatment B: [0.35% *Lactococus lactis subsp. Lactis* (120×10^9) + 0.35% *Lactobacillus helviticus* (145×10^9) + 0.30% *Leuconostoc mesentroides subsp. cremoris* (130×10^9)].

Treatment C: [0.15% *Lactococus lactis subsp. lactis* (122×10^9) + 0.60% *Lactobacillus helviticus* (140×10^9) + 0.25% *Leuconostoc mesentroides subsp. cremoris* (132×10^9)] with addition of suspensions of attenuated cultures of *Lb. delbrueckii subsp. bulgaricus* $(130 \times 10^8$ cfu/ml) at levels of 0.1,0.5 and 1% v/wt) individually just before rennet (control with no attenuated cultures was made for each treatment), and named as follows Ac , A1, A2, A3 for control, 0.1, 0.5 and 1% of *Lb. delbrueckii subsp. bulgaricus* respectively and the same was followed used for the rest treatments. Cheeses stored at 10ºC to ripe for 0, 7, 14, 21 and 28 days.

Chemical analysis :

Moisture content was determined according to (7). Protein content was determined using the Kjedhal method) (8). Titratable acidity was determined as lactic acid percentage according to (9).

Proteolysis assessment:

Electrophoresis was used to assess primary proteolysis according to (10) with some modifications by (11) Kjeldahl method was used to determine WSN and TCA-SN (12-14).

Microbial analysis :

Lactobacillus and Lactococus counts during ripening was estimated by plating the bacteria on MRS and M 17 agar, respectively (15).

Sensory evaluation:

Sensory evaluation for cheese samples ripened for 30, 60, and 90 days were organoleptically assessed (16).

Statistical Analysis:

Data collected for all parameters were analyzed by two-factorial analysis in a completely randomized design (CRD). Means with significant differences were compared by Duncan's multiple range tests. SAS program was also used (17). Different treatments were given different letters.

RESULTS AND DISCUSSION

Moisture content decreased significantly (p < 0.05) as ripening time increased as shown in table (1). Table (2) shows that the concentration of lactic acid was not increased in cheeses made with the addition of attenuated FS *Lb. delbrueckii subsp. bulgaricus* at different ratios (0.1%, 0.5% and 1%) compared to their controls, which made without attenuated FS bacteria. The reason might be due to the fact that stressed cells do not produce significant amounts of acid during cheese ripening, but may retain protease and peptidase activity (18).

Assessment of proteolysis:
Figure (1) shows electrophoretogram of cheese samples taken at different time courses, which were treated with different ratios of attenuated *Lb. delbrukii subsp. bulgaricus*. Both β-casein and αs-casein showed weaker band intensities after 28 days of ripening. Band intensity is correlated with the protein breakdown. Protein degradation level of αs-casein was greater than that of β-casein. αs- casein and β-casein showed similar degradation patterns in all treatments (A1, A2, A3, B1, B2, B3, C1, C2 and C3) after 7 days and 28 days of ripening.
The pattern and the rate of proteolysis were higher in cheeses treated with attenuated *Lb. delbrueckii subsp. bulgaricus* compared to the pattern of cheeses made without attenuated *Lb. delbrueckii subsp. bulgaricus*. The increase in proteolysis might be correlated to higher peptidiolytic activity and higher rate of (19, 20).
Attenuated FS *Lb. delbrueckii subsp. bulgaricus* added at different ratios (0.1%, 0.5% and 1%) caused an increase in WSN and TCA-SN levels for all treatments compared to their controls after 28 days of ripening. This probably is related to the breakdown of proteins and peptides. While different ratios of attenuated *Lb. delbrueckii subsp. bulgaricus* were used, the rate of proteolysis in C3, C2 and C1 was higher than the rate in other treatments for all stages of ripening (Figure 2). Enhancement of proteolysis was observed in C3 after 28 days of ripening. On the contrary, the rate of proteolysis in control samples were lower for all stages of ripening (figs. 2 and 3). This might be due to the release of intracellular proteinases of lysed attenuated bacteria into surrounding cheese matrix (21). In general, it was concluded that the attenuated starter bacteria was successful in accelerating casein breakdown and it was also clear that ripening time for semi-hard cheese can be reduced to 7 days.

Microbial analysis :
The viable counts of lactobacilli in cheeses at different stages of ripening are shown in table (3). The actual numbers of lactobacilli retained at day one attenuated FS treated cheeses, and they varied from 38×10^7 to 103×10^7 cfu/g in all treatments. This variation might be due to variation in cell viability and cell retention during attenuation and cheese making. In all cases, the initial lactobacilli counts in adjunct-treated cheeses were higher than the counts in control cheeses, which reflect the positive

retention of lactobacilli, and they declined by 1-2 log after 28 day of ripening, a similar results were mentioned by (22-24) in which they demonstrated that attenuated adjuncts in cheese curds undergo a high rate of cell lysis, which consequently leads to gradual reduction in cell viability (figure 3). The decline in cell viability of attenuated lactobacilli during ripening may not necessarily mean the cells died, but they may have been injured to the extent that they could no longer grow (25). Cell injury can induce cell autolysis and permit the release of the enzyme system more rapidly into the cheese curd (19, 22,23). Also, the viability of starter lactococci in cheeses during ripening for 28 days is shown in Table (4). The counts of Starter lactococci were not decreased as the ripening period increased (P < 0.05) probably due to the favorable conditions in cheeses such as water activity, redox potential, pH, salt and survival of unfermentable carbohydrates.

Sensory analysis of the cheeses :
The sensory scores of cheeses at different time courses (0, 7, 14, 21 and 28 days) of ripening are summarized in tables (5, 6 and 7). There was no significant difference (p < 0.05) in the mean value of colour for all treatments during ripening. The taste and aroma scores were significantly higher (P <0.05) in cheeses made with attenuated FS *Lb. delbrueckii subsp. bulgaricus* which received the highest taste and aroma scores and never developed any bitter flavors as the panelists indicated. Debittering activity was noticed in several cheese trials that contain adjunct lactobacilli (20, 24, 26, 27). Cheeses made with *Lb. delbrueckii subsp. bulgaricus* were described as having creamy texture and less firmness in the early stages of ripening compared with the control. There were no significant differences in undesirable taste and aroma and salt taste during ripening (0, 7, 14, 21and 28 days) in treatments made with attenuated FS bacteria compared to their controls .The score of general acceptance in treatments made with attenuated FS bacteria were higher than their controls. Among them the highest value of general acceptance was in treatments made with 1% attenuated FS bacteria.

Table (1): Moisture (%) in treatments during ripening for 28 days

Sample types	Ripening period				
	0 day	**7 days**	**14 days**	**21 days**	**28 days**
Ac	41.00±0.01b	40.98±0.02c	40.66±0.05f	40.00±0.00h	39.65±0.03i
A1	41.00±0.01b	40.90±0.03e	39.66±0.03i	38.52±0.03l	37.40±0.02O
A2	41.33±0.02a	40.39±0.02d	39.33±0.06j	38.66±0.01k	37.43±0.01n
A3	40.33±0.01g	40.00±0.01h	39.33±0.02j	37.66±0.02m	37.00±0.01p
Bc	41.33±0.01c	41.00±0.03de	40.80±0.03ef	39.92±0.01g	38.53±0.01i
B1	41.66±0.01b	41.20±0.03cd	40.83±0.02ef	39.38±0.01g	38.53±0.02i
B2	42.00±0.01a	41.96±0.05a	40.76±0.01f	39.86±0.02g	38.86±0.33h
B3	41.31±0.01c	41.00±0.01de	40.73±0.04f	39.80±0.03g	38.50±0.03i
Cc	42.33±0.03a	41.76±0.03c	40.63±0.02g	39.23±0.01k	38.16±0.00n
C1	42.00±0.06b	41.72±0.01d	40.70±0.03f	39.56±0.00j	38.20±0.03m
C2	42.00±0.05b	41.70±0.03d	40.52±0.01h	39.20±0.03k	38.23±0.02m
C3	41.66±0.04e	40.67±0.02f	39.83±0.04i	38.33±0.02l	37.66±0.04O

Ac, Bc, Cc: cheeses made without attenuated Freeze shocked *Lb. delbrueckii subsp. bulgaricus* (control cheeses);
A1,B1,C1:cheeses made with attenuated Freeze Shocked *Lb. delbrueckii subsp. bulgaricus* added at ratio 0.1%;
A2,B2,C2:cheeses made with attenuated Freeze Shocked *Lb. delbrueckii subsp. bulgaricus* added at ratio
0.5%;A3,B3,C3:cheeses made with attenuated Freeze Shocked *Lb. delbrueckii subsp. bulgaricus* added at ratio 1%.

Table (2): Titrable acidity (%) in cheese treatments during ripening for 28 days

Sample types	Ripening period				
	0 day	**7 days**	**14 days**	**21 days**	**28 days**
Ac	0.16±0.06g	0.17±0.03f	0.18±0.03e	0.19±0.01d	0.22±0.01a
A1	0.16±0.06g	0.17±0.01f	0.18±0.01e	0.19±0.01d	0.20±0.01c
A2	0.16±0.01g	0.17±0.03f	0.18±0.01e	0.19±0.01d	0.21±0.01b
A3	0.16±0.01g	0.17±0.01f	0.18±0.06e	0.19±0.01d	0.21±0.03b
Bc	0.19±0.01f	0.20±0.01e	0.21±0.01d	0.22±0.01c	0.24±0.01a
B1	0.19±0.01f	0.20±0.01e	0.21±0.01d	0.22±0.01c	0.23±0.01b
B2	0.19±0.01f	0.20±0.01e	0.21±0.01d	0.22±0.01c	0.23±0.01b
B3	0.19±0.01f	0.20±0.01e	0.21±0.01d	0.22±0.01c	0.23±0.01b
Cc	0.19±0.12g	0.21±0.01e	0.23±0.01c	0.24±0.01b	0.25±0.27a
C1	0.19±0.01g	0.20±0.01f	0.21±0.01e	0.22±0.12d	0.23±0.21c
C2	0.19±0.01g	0.20±0.01f	0.21±0.01e	0.22±0.12d	0.23±0.29c
C3	0.19±0.12g	0.21±0.01e	0.22±0.01d	0.23±0.01c	0.24±0.02b
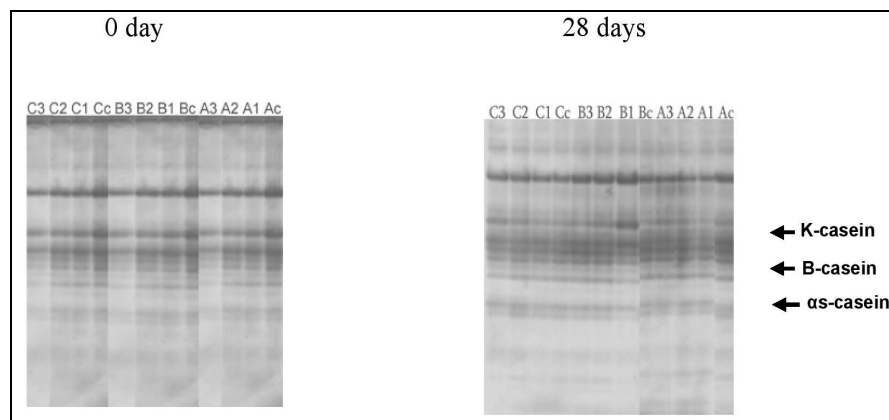
Figure (1): Urea-polyacrylamide gel electrophoretograms of cheese made without (control cheese: lane Ac, Bc and Cc) or with added different ratio attenuated (FS) *Lb. delbrueckii subsp. bulgaricus* (lane A1, B1 and C1 added at ratio 0.1%), (lane A2, B2 and C2 added at ratio 0.5%), (lane A3, B3 and C3 added at ratio 1%).
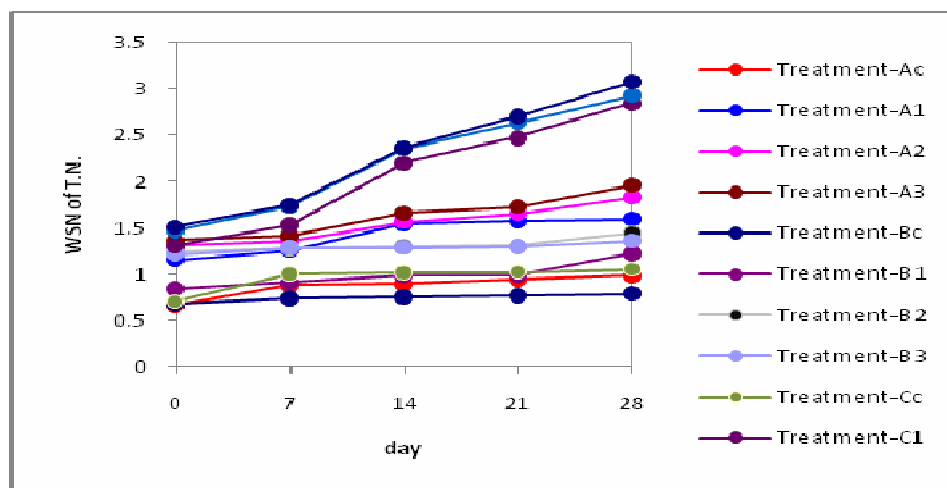
Figure (2): Changes in WSN of TN in cheese treatments during ripening for 28 days

Table (3): Lactobacilli count cfu/g in different treatments during ripening for 28 days

Sample types	Ripening period				
	0 day	7 days	14 days	21 days	28 days
Ac	$40×10^6±0.10f$	$28×10^6±0.10f$	$24×10^6±0.26f$	$20×10^6±0.26f$	$16×10^5±0.27f$
A1	$38×10^7±0.69ab$	$32×10^7±0.69bc$	$24×10^7±0.69d$	$16×10^7±0.69e$	$12×10^6±0.26f$
A2	$39×10^7±0.69ab$	$32×10^7±0.69bc$	$26×10^7±0.69cd$	$20×10^7±0.69de$	$14×10^6±0.26f$
A3	$40×10^7±0.69a$	$36×10^7±0.69ab$	$27×10^7±0.69cd$	$22×10^7±0.26de$	$15×10^6±0.69f$
Bc	$60×10^7±0.69a$	$56×10^7±0.37ab$	$52×10^7±0.38cd$	$42×10^7±0.69e$	$34×10^7±0.37f$
B1	$59×10^7±025ab$	$52×10^7±0.37cd$	$50×10^7±0.69d$	$39×10^7±0.69ef$	$38×10^6±0.01g$
B2	$57×10^7±0.25abc$	$54×10^7±0.38bcd$	$51×10^7±0.33cd$	$39×10^7±0.01ef$	$38×10^6±0.01g$
B3	$58×10^7±0.37ab$	$55×10^7±0.32abc$	$52×10^7±0.33cd$	$38×10^7±0.37ef$	$38×10^6±0.53g$
Cc	$100×10^7±0.69a$	$84×10^7±0.14bcd$	$74×10^7±0.37de$	$61×10^7±0.69f$	$50×10^7±0.69g$
C1	$102×10^7±0.37a$	$85×10^7±0.69bc$	$75×10^7±0.69cd$	$61×10^7±0.69f$	$58×10^6±0.26h$
C2	$102×10^7±0.37a$	$86×10^7±0.38bc$	$73×10^7±0.14de$	$62×10^7±0.15f$	$59×10^6±0.43h$
C3	$103×10^7±0.37a$	$88×10^7±0.37b$	$74×10^7±0.69de$	$64×10^7±0.37ef$	$61×10^6±0.26h$

Table (4): Lactococci count cfu/g in different treatments during ripening for 28 days

Sample types	Ripening period				
	0 day	7 days	14 days	21 days	28 days
Ac	$100\times10^7\pm0.69$a	$64\times10^7\pm0.69$e	$52\times10^7\pm0.37$f	$40\times10^7\pm0.69$g	$38\times10^7\pm0.69$g
A1	$80\times10^7\pm0.69$c	$66\times10^7\pm0.37$de	$50\times10^7\pm0.37$f	$39\times10^7\pm0.70$g	$37\times10^7\pm0.70$gh
A2	$84\times10^7\pm0.25$b	$67\times10^7\pm0.25$de	$50\times10^7\pm0.37$f	$40\times10^7\pm0.14$g	$35\times10^7\pm0.70$h
A3	$80\times10^7\pm0.70$c	$68\times10^7\pm0.40$d	$51\times10^7\pm0.40$f	$40\times10^7\pm0.69$g	$35\times10^7\pm0.69$h
Bc	$84\times10^7\pm0.37$c	$74\times10^7\pm0.70$e	$52\times10^7\pm0.40$g	$48\times10^7\pm0.69$h	$36\times10^7\pm0.69$l
B1	$85\times10^7\pm0.37$b	$74\times10^7\pm0.69$e	$53\times10^7\pm0.69$f	$48\times10^7\pm0.25$h	$36\times10^7\pm0.70$l
B2	$86\times10^7\pm0.33$a	$75\times10^7\pm0.69$d	$53\times10^7\pm0.70$f	$48\times10^7\pm0.70$h	$38\times10^7\pm0.69$k
B3	$84\times10^7\pm0.70$c	$74\times10^7\pm0.69$e	$52\times10^7\pm0.33$g	$47\times10^7\pm0.70$i	$39\times10^7\pm0.69$j
Cc	$60\times10^7\pm0.70$c	$54\times10^7\pm0.70$f	$50\times10^7\pm0.69$g	$46\times10^7\pm0.33$j	$38\times10^7\pm0.25$m
C1	$61\times10^7\pm0.37$b	$56\times10^7\pm0.69$e	$48\times10^7\pm0.25$i	$45\times10^7\pm0.37$k	$39\times10^7\pm0.69$n
C2	$61\times10^7\pm0.69$b	$57\times10^7\pm0.37$d	$49\times10^7\pm0.40$h	$46\times10^7\pm0.25$j	$39\times10^7\pm0.70$n
C3	$62\times10^7\pm0.69$a	$56\times10^7\pm0.69$e	$48\times10^7\pm0.70$i	$44\times10^7\pm0.70$l	$38\times10^7\pm0.70$m

Table (5): Sensory score for treatments (A) during cheese ripening for 28 days made without   (control cheese) and with attenuated FS *Lb. delbrueckii subsp. bulgaricus*

Property	Cheese samples	0day	7 days	14 days	21 days	28 days
Color	A C	8.00±0.01a	8.00±0.04a	8.00±0.02a	8.00±0.01a	8.00±0.01a
	A 0.1%	8.00±0.01a	8.00±0.01a	8.00±0.01a	8.00±0.02a	8.00±0.02a
	A 0.5%	8.00±0.02a	8.00±0.06a	8.00±0.02a	8.00±0.01a	8.00±0.03a
	A 1%	8.00±0.01a	8.00±0.01a	8.00±0.03a	8.00±0.05a	8.00±0.01a
Texture	A C	6.40±0.54f	7.40±0.60ef	7.40±0.32ef	8.00±0.60cde	8.00±0.60cde
	A 0.1%	7.60±0.58de	7.80±0.51de	8.00±0.49cde	8.00±0.20cde	8.00±0.49cde
	A 0.5%	8.00±0.40cde	8.20±0.60bcd	8.60±0.63ab	8.80±0.01ab	9.00±0.24a
	A 1%	9.00±0.01a	9.00±0.20a	9.00±0.40a	9.00±0.24a	9.00±0.22a
Taste and aroma	A C	7.20±0.40d	7.40±0.37cd	7.60±0.01bcd	7.80±0.20bcd	8.00±0.24bcd
	A 0.1%	7.40±0.40cd	8.00±0.49bcd	8.00±0.25bcd	8.00±0.63bcd	8.00±0.31bcd
	A 0.5%	7.40±0.40cd	8.00±0.37bcd	8.20±0.01abc	9.00±0.37a	9.00±0.24a
	A 1%	8.40±0.40ab	9.00±0.24a	9.00±0.25a	9.00±0.20a	9.00±0.25a
No abnormal tast and aroma	A C	8.00±0.20b	8.00±0.24b	8.00±0.25b	8.00±0.24b	8.00±0.25b
	A 0.1%	9.00±0.25a	9.00±0.24a	9.00±0.25a	9.00±0.25a	9.00±0.24a
	A 0.5%	9.00±0.25a	9.00±0.24a	9.00±0.25a	9.00±0.24a	9.00±0.25a
	A 1%	9.00±0.24a	9.00±0.25a	9.00±0.25a	9.00±0.24a	9.00±0.24a
Salt taste	A C	8.60±0.37a	8.40±0.25a	8.40±0.24a	8.20±0.25ab	7.20±0.37c
	A 0.1%	8.60±0.25a	8.40±0.24a	8.40±0.25a	8.20±0.58ab	7.40±0.25bc
	A 0.5%	8.60±0.24a	8.40±0.25a	8.40±0.20a	8.20±0.25ab	7.40±0.24bc
	A 1%	9.00±0.24a	8.60±0.25a	8.40±0.25a	8.40±0.01a	7.40±0.28bc
General acceptance	A C	6.80±0.49d	6.80±0.25d	6.80±0.37d	7.60±0.49cd	7.60±0.31cd
	A 0.1%	8.00±0.24bc	8.20±0.25abc	8.4±0.25abc	8.60±0.24ab	8.80±0.25ab
	A 0.5%	9.00±0.25a	9.00±0.37a	9.00±0.37a	9.00±0.37a	9.00±0.25a
	A 1%	9.00±0.37a	9.00±0.24a	9.00±0.37a	9.00±0.20a	9.00±0.25a

Table (6): Sensory score for treatments (B) during cheese ripening for 28 days made without   (control cheese) and with attenuated FS *Lb. delbrueckii subsp. bulgaricus*.

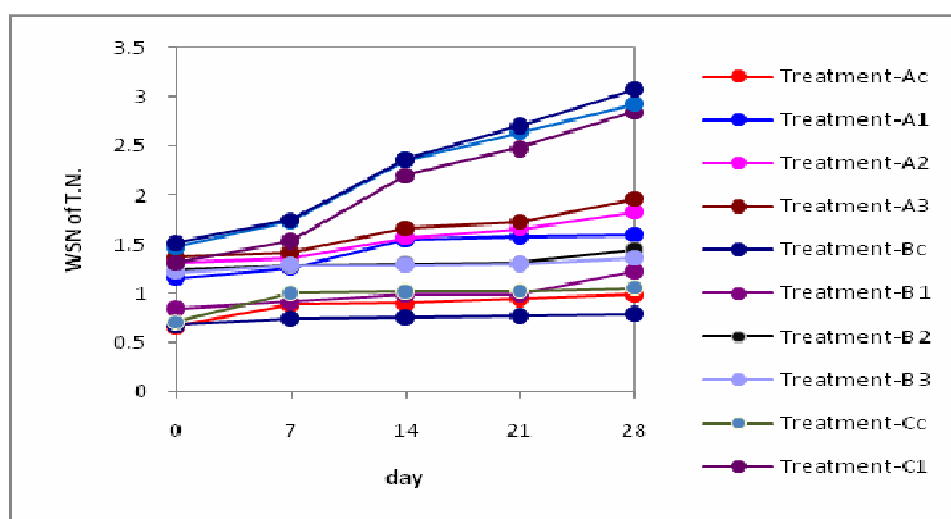| Property	Cheese samples	0 day	7 days	14 days	21 days	28 days
Color	B C	8.00±0.01a	8.00±0.02a	8.00±0.01a	8.00±0.01a	8.00±0.01a
	B 0.1%	8.00±0.02a	8.00±0.02a	8.00±0.04a	8.00±0.02a	8.00±0.04a
	B 0.5%	8.00±0.01a	8.00±0.01a	8.00±0.03a	8.00±0.01a	8.00±0.03a
	B 1%	8.00±0.03a	8.00±0.02a	8.00±0.02a	8.00±0.02a	8.00±0.05a
Texture	Bc	5.20±0.89f	5.20±0.54f	5.40±0.51f	5.80±0.49f	7.40±0.51cde
	B 0.1%	7.00±0.58e	7.20±0.60de	7.80±0.24bcde	8.00±0.44abcde	8.00±0.44abcde
	B 0.5%	7.40±0.81cde	7.80±0.73bcde	8.00±0.60abcde	8.20±0.51abcd	8.40±0.37abc
	B 1%	8.00±0.73abcde	8.20±0.54abcd	8.40±0.60abc	8.60±0.58ab	9.00±0.25a
Taste and Aroma	BC	5.20±0.81f	5.40±0.66f	6.00±0.44f	6.00±0.58f	7.40±0.58de
	B 0.1%	7.20±0.71e	7.60±0.68cde	7.80±0.32bcde	8.00±0.20bcde	8.20±0.45abcd
	B 0.5%	7.40±0.74de	7.80±0.24bcde	8.00±0.58bcde	8.2±0.24abcd	8.40±0.25abc
	B 1%	7.80±0.89bcde	8.20±0.25abcd	8.40±0.37abc	8.60±0.25ab	9.00±0.28a
No abnormal taste or odor	BC	8.00±0.24b	8.00±0.25b	8.00±0.24b	8.00±0.25b	8.00±0.24b
	B 0.1%	9.00±0.25a	9.00±0.24a	9.00±0.25a	9.00±0.20a	9.00±0.24a
	B 0.5%	9.00±0.24a	9.00±0.25a	9.00±0.25a	9.00±0.20a	9.00±0.25a
	B 1%	9.00±0.25a	9.00±0.24a	9.00±0.24a	9.00±0.20a	9.00±0.28a
Salt taste	BC	8.80±0.40a	8.40±0.25ab	8.20±0.20ab	7.80±0.24bc	7.60±0.40bc
	B 0.1%	8.40±0.25ab	8.40±0.24ab	8.40±0.37ab	7.80±0.25bc	7.60±0.24bc
	B 0.5%	8.40±0.24ab	8.40±0.24ab	8.20±0.37ab	7.80±0.24bc	7.60±0.37bc
	B 1%	8.40±0.24ab	8.40±0.40ab	8.20±0.37ab	7.60±0.40ab	7.20±0.57c
General Acceptance	BC	5.40±0.83d	5.40±0.51d	5.40±0.51d	5.40±0.51d	5.80±0.51d
	B 0.1%	7.40±0.54c	7.60±0.25bc	7.80±0.58bc	8.20±0.60abc	8.40±0.37abc
	B 0.5%	7.60±0.80bc	7.80±0.24bc	8.00±0.45abc	8.20±0.25abc	8.60±0.37ab
	B 1%	8.00±0.81abc	8.20±0.24abc	8.40±0.37abc	8.60±0.58ab	9.00±0.25a

Figure (3): Changes in TCA-SN of TN in cheese treatments during ripening for 28 days

Table (7): Sensory score for treatments (C) during cheese ripening for 28 days made without (control cheese) and with attenuated FS *Lb. delbrueckii subsp. bulgaricus*

Property	Cheese samples	0 day	7 days	14 days	21 days	28 days
Color	Cc	8.00±0.01a	8.00±0.02a	8.00±0.01a	8.00±0.02a	8.00±0.01a
	C 0.1%	8.00±0.03a	8.00±0.03a	8.00±0.01a	8.00±0.02a	8.00±0.01a
	C 0.5%	8.00±0.02a	8.00±0.04a	8.00±0.02a	8.00±0.02a	8.00±0.02a
	C 1%	8.00±0.01a	8.00±0.02a	8.00±0.01a	8.00±0.03a	8.00±0.03a
Texture	Cc	6.60±0.37e	7.00±0.86de	7.00±0.25de	7.60±0.25cd	8.00±0.20bc
	C 0.1%	7.60±0.51cd	8.00±0.37bc	8.20±0.25bc	8.20±0.24bc	8.40±0.37ab
	C 0.5%	9.00±0.40a	9.00±0.37a	9.00±0.31a	9.00±0.25a	9.00±0.31a
	C 1%	9.00±0.54a	9.00±0.51a	9.00±0.24a	9.00±0.37a	9.00±0.25a
Taste and aroma	Cc	7.00±0.51d	7.00±0.70d	7.00±0.31d	7.20±0.25d	7.80±0.37bc
	C 0.1%	7.40±0.67cd	8.00±0.37b	8.00±0.20b	8.00±0.37b	8.00±0.37b
	C 0.5%	9.00±0.66a	9.00±0.40a	9.00±0.20a	9.00±0.40a	9.00±0.22a
	C 1%	9.00±0.40a	9.00±0.54a	9.00±0.67a	9.00±0.31a	9.00±0.28a
No abnormal taste or odor	C c	8.00±0.24b	8.00±0.25b	8.00±0.25b	8.00±0.20b	8.00±0.24b
	C 0.1%	9.00±0.24a	9.00±0.25a	9.00±0.25a	9.00±0.24a	9.00±0.24a
	C 0.5%	9.00±0.24a	9.00±0.25a	9.00±0.25a	9.00±0.24a	9.00±0.25a
	C 1%	9.00±0.25a	9.00±0.24a	9.00±0.24a	9.00±0.25a	9.00±0.28a
Salt taste	C c	8.40±0.20a	7.8±0.31ab	7.4±0.30bc	7.40±0.31bc	7.20±0.20b
	C 0.1%	8.40±0.40a	8.00±0.37ab	7.60±0.20ab	7.40±0.20ab	7.20±0.40b
	C 0.5%	8.40±0.40a	8.40±0.86a	7.80±0.20ab	7.60±0.20ab	7.40±0.25ab
	C 1%	8.40±0.40a	8.40±0.51a	7.80±0.20ab	7.60±0.24ab	7.20±0.47b
General acceptance	Cc	7.40±0.73b	7.40±0.74b	7.80±0.24b	7.80±0.40b	7.80±0.20b
	C 0.1%	8.80±0.49a	8.80±0.37a	8.80±0.20a	9.00±0.49a	9.00±0.25a
	C 0.5%	9.00±0.51a	9.00±0.51a	9.00±0.20a	9.00±0.32a	9.00±0.20a
	C 1%	9.00±0.25a	9.00±0.37a	9.00±0.49a	9.00±0.29a	9.00±0.28a

CONCLUSION

Freeze shocked attenuated *Lb. delbrueckii subsp. bulgaricus* was successfully used in accelerating Gouda cheese ripening within a period of 7 days . The concentration of lactic acid was not increased in cheeses made with the addition of attenuated FS *Lb. delbrueckii subsp. bulgaricus* at different ratios compared to their controls which made without attenuated FS bacteria. electrophoretogram of cheese samples showed that the rate of proteolysis were higher in cheeses treated with attenuated *Lb. delbrueckii subsp. bulgaricus* compared to the pattern of cheeses made without attenuated *Lb. delbrueckii subsp. bulgaricus*. Attenuated FS *Lb. delbrueckii subsp. bulgaricus* added at different ratios caused an increase in WSN and TCA-SN levels for all treatments compared to their controls after 28 days of ripening. Best sensory properties were for treatments that contained 1% attenuated bacteria . Cheeses with accelerated ripening showed no bitterness.

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Effect of obesity and height in Iraqi males: a biochemical approach

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ABSTRACT

Growth of human being is usually controlled by many hormones. Some of these hormones play roles in growth during different periods of age, other continue to affect growth. A among these hormones, we have growth hormone, the thyroid hormone and sex hormone etc.. . in addition, there are other hormones which play roles in growth of human being but yet their roles in body mass index are more. These hormones are Ghrelin hormone, which is negatively correlated BMI. Also we have leptin, which regulates food intake.

The current study was conducted for 50 male students whom were selected randomly aged (8-18 years) and divided them into two groups: obese group and normal weight group, then, we studied these hormones, which are related to growth.

The results obtained revealed that there are different changes in the level of hormones between obese and normal weight people.

Key words: obstatin, leptin, Ghrelin, BMI.

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الملخص باللغة العربية

إن نمو الإنسان يخضع تحت سيطرة هرمونات الجسم، بعض هذه الهرمونات تلعب دوراً رئيساً في نمو الإنسان خلال فترة معينة مـن حياتــه ، وبعضها الآخر يلعب دوراً مهماً في نمو الانسان طيلة فترة حياته، من هذه الهرمونات هرمون النمو،هرمون الغدة الدرقية إضافة إلى الهرمونات الجنسية التي تلعب دوراً مهماً في نمو الإنسان وخاصة في فترة البلوغ.

وبالإضافة إلى هذه الهرمونات ، هناك هرمونات عرفت حديثاً تلعب دوراً غير واضح في نمو الإنسان ولكن لها دور فعال في دليل كتلة الجـسم، منها هرمون Ghrelinleptin وهرمون obstatin.

لمعرفة تأثير هذه الهرمونات على جسم الإنسان وخاصة في فترة البلوغ ، تم أخذ عينات دم عشوائية من عدد من الطلبـة الـذكور (50 طالـب) تتراوح أعمارهم من 8-18 سنة من مدارس الغد في مدينة بغداد (التي تشمل ابتدائية ، متوسطة ، وإعدادية).

تم تقسيم هذه المجموعة إلى مجموعتين اعتمادا على دليل كتلة الجسم (مجموعة البدناء، ومجموعة ذوي الأوزان الطبيعية) وتم دراسة تأثير هـذه الهرمونات على نمو الطلبة.

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INTRODUCTION

The human being passes through his all life by different stages of growth. The growth of body means increase in weight, height and maturity of organs.

When increase in weight over the threshold, it will be considered as obesity.

Obesity is a complex condition, with serious social and psychological impact affecting all age and socio-economic groups. It considers so, since it is a major contributor to global burden of chronic disease and disability (like type 2 diabetes, cardio-vascular disease, hypertension, stoke and certain forms of cancers) (1).

The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI).

BMI can be expressed as the weight in kilograms divided by square of height in meter (2).

BMI over 25 kg/m^2 defines as over weight.

BMI over 30 kg/m^2 considers as obese .

BMI below 18.5 kg/m^2 is under weight.

Many hormones play an important role in determining the body weight (BMI) either directly through food intake or indirectly through food metabolism. One of these hormones that play role in body weight is Ghrelin.

Ghrelin is the natural legend of the growth hormone secrelgogue receptor. It is a potent stimulator of growth hormones secretion (2,3). Moreover, ghrelin is also an appetite-stimulating hormone including food intake and weight gain in human (4-6) and promoting gastric emptying (7).

Ghrelin hormone is composed of 28 amino acid peptides, predominantly produced by endothetial mucosal lining of stomach (mainly the fundus) and secreted into blood stream.

Ghrelin level in plasma is negatively correlated with BMI (8) and its level decreases with weight gain and obesity (9).

The other hormone, which involves in regulation of body weight is obestatin, which was discovered recently. It is composed of 23-a.a peptide encoded by the ghrelin gene (9). This hormone has been shown to suppress food intake and decrease gastric emptying, therefore antagonized the orexigenic effect of the ghrelin (10).

Leptin is another hormone that is involved in food intake (in another word regulates body weight). It is produced by adipocyte. Leptin appears to increase in both boys and girls before the appearance of other reproduction hormones related to puberty. Leptin; in boys declines about the time when testestrone level increases (9).

The aim of the current study is to reveal the correlation between obesity and the height of Iraqi teenagers' early adolescent males with the role of different hormones.

SUBJECTS AND METHODS

Subjects:
Blood samples were obtained from 50 male students been taken randomly from Private school (Al-Khad), intermediate and secondary school (Baghdad city). Age group taken was between (8 – 18) years. This study was conducted between February 2013 to September 2013.

Methods:
Males were divided into two groups:
1. Obese group: if B.M.I is more than 30 kg\m2
2. Normal weight group: if B.M.I is between18-25 kg\m2

In each group, males were subjected to measure their weights and hights. The biochemical measurements of Ghrelin, obestatin, leptin and growth hormones were done using ELISA technique (PHOENIX PHARMSCEUTLCAL SINC).

Statistical analysis of the results was done using student T-test and p-value less than 0.05 is considered as significant.

RESULTS

Results obtained from this study are summarized in table (1).

Table (1): The comparison between the two groups involved in this study (obese and normal weight)

Parameter	Obese male mean $\pm$ SD	Normal weight male (control) $\pm$ SD
number	25	25
Age (year)	15.1 $\pm$ 7	13 $\pm$ 7.5^
Height (cm)	140 $\pm$7.3	157$\pm$15.5 **
Weight (kg)	60$\pm$10.2 **	45$\pm$12
BMI (kg/m)	34.5$\pm$3.2 **	23.2$\pm$2.5
S. leptin (ng/ml)	60$\pm$30 **	10$\pm$2.2
S. ghrelin (fmol/ml)	100$\pm$10.1	150$\pm$25.5 **
S. obestatin (ng/ml)	7$\pm$2.2	15$\pm$5.2
S. growth hormone. (ng/ml)	8 $\pm$ 3.1	15 $\pm$ 2.3

^ Non significant: P > 0.05
** Highly significant: P < 0.001

Table (2) illustrates a trial to elicit the correlation between BMI, height and age in obese male, with hormones related to obesity as S. Leptin, S.Ghrlin, S.obstatin and S. growth hormones.

Table (2): The correlation between BMI, height and age in obese male, with hormones related to obesity as S. Leptin, S.Ghrlin, S.obstatin and S. growth hormones

	BMI (p-value)	Height (p-value)	Age (p-value)
S. Leptin	+ 0.9 **	+ 0.75 **	0.35 ^
S. Ghrlin	- 0.45 ^	+ 0.3 ^	- 0.6 *
S. obstatin	- 0.65 *	+ 0.8 **	- 0.55 *
S.growth hormone	+ 0.5 *	+ 0.45 ^	- 0.56 *

** P > 0.001   * P < 0.01   ^ P > 0.05

From table (2), the following results were obtained:

1. Regarding growth hormone, there is a significant negative correlation with aging, while the correlation was significantly positive with BMI.

2. Regarding serum Ghrelin, results revealed negatively significant correlation with aging, while the correlation with height and BMI were non-significant.

3. Serum obstatin showed a significant reduction with aging and BMI, while positive correlation with height was shown.

4. Serum leptin elicited highly significant relation with BMI and height, While no significant correlation could be elicited with aging.

DISCUSSION

Actually, our growth is controlled by endocrine gland of our body. These endocrine glands are pituitary gland, thyroid gland, thymus gland and some sex hormones (11).

Pituitary gland is situated in brain and it controls the secretion of other endocrine hormones and the growth of the bone. At the age of 18, the body gets the approximately bone full maturity and after that bones stop growing up, because after this age, the glands responsible for growth, become less active (11). On the bases of this fact, the subjects of the present study were chosen with no significant difference in age between obese subjects and normal weight subject; as it is clear in table (1) (15.1 $\pm$ 7 year) vs (13 $\pm$ 7.5 year) (11,12).

The result of this study also revealed that the levels of growth hormone and serum Ghrelin in obese individual were significantly reduced (p< 0.001) (as shown in table 1). The human growth is a complex process starting at conception and completes in adolescence at the time of growth plate fusion (13). Growth can be divided into four phases (11):

1. Fetal: Where the predominant endocrine factors controlling growth are insulin and insulin-like factor.

2. Infancy: Where growth is mainly dependent upon nutrition.

3. Childhood: Where the growth hormone-insulin-like growth factor-1 (GF-1) axis and thyroid hormone are most important.

4. Puberty: Where along with the growth hormone and [IGF-1] axis.

The activation of the hypothalamus-pituitary-gonadal axis to generate sex steroid secretion, becomes vital to the completion of growth.

The results of the current study explained the fact that central Ghrelin simultaneously regulate food intake. It is well known that growth hormone is released from the pituitary gland in a pulsatile fashion under the control of growth hormone releasing hormone (G.H.R.H) Ghrelin and somatostatin (13,14).

Therefore, when serum Ghrelin increases, it will stimulate the release of growth hormone as shown in table (1), but according to the type of food intake during childhood and adipose tissue metabolism through distinct mechanism, this explains the rapid growth in obese children (early childhood) (4), while the level of growth hormone decreases with aging as shown table (2) (r= -056, p< 0.01). Ghrelin affects energy hemostasis by stimulating growth hormone secretion, therefore even if there is a feeling of hunger, the level of Ghrelin begins to decrease, causing suppression in growth hormone release (9). This explains the short status in obese individuals when compared with normal weight individuals with the same age (5).

There were also significant differences in fasting obestatin in plasma levels between two studied groups (obese and normal weight individuals) with p< 0.05.

Serum obestatin decreases in obese individuals consequently to the level of serum Ghrelin as shown in table (1). However, the role of obestatin on energy balance to elicit the role of obestatin on energy hemostasis (15).

However, obestatin has been shown to suppress food intake and decrease gastric emptying, therefore, antagonizing the orexigenic effect of Ghrelin subsequent studies on rodents had yielded controversial results (16). According to the controversy, it is important to understand how changes in energy balance affect obestatin level in plasma of human being.

The majority-but not all- of the studies showed that fasting obestatin level in plasma is reduced in obese comparing with normal weight individuals (13). The current study agreed with those studies, therefore supporting the role of obestatin in the regulation of body weight and energy hemostasis (13).

In fact, leptin is exclusively produced in the adipocyte to regulate the satiety centre in the hypothalamus causing a decrease in appetite and increase in energy expenditure (17). Serum leptin concentration is directly correlated with BMI (as shown in table 2) and the amount of fat (table 1), obese subjects showed higher level than normal subjects and normal weight subjects have extremely reduced leptin levels.

Balance between leptin and other hormones is significantly regulated by nutritional status. The balance influences many organ systems, including brain, liver, and skeletal muscles to mediate the essential adaptation process. The possible physiological functions of leptin and its signaling pathways during childhood and adolescence include control of food intake, energy regulation, growth puberty, and immunity (18). Moreover, its secretion

and possible roles in the adaptation process during different disease states (obesity, malnutrition, eating disorders, delayed puberty, congenital heart diseases and hepatic disorders).

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Electromagnetic removal of suspended solids in wastewater

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ABSTRACT

The removal of suspended solids (S.S) presented in municipal and industrial wastewater (WW) is considered to be one of the most important procedures of WW treatment. In the traditional techniques of treatment certain types of coagulants and flocculants are used for this purpose such as alum $(Al_2(SO_4)_3.18H_2O)$, $FeCl_3$ and polyamides, and due to the use of these chemicals large quantities of slugs will be produced which will form an environmental burden in order to be handled and treated in an environmentally appropriate way.

Electromagnetic field is used as a new technology in the treatment of the S.S presented in the WW as a replacement for the traditional coagulants used nowadays. The use of the electromagnetic field enhances the removal of the S.S and reduces the time required for the settling without the need for any chemicals addition. Parameters of flow rate, contact time, exposure distance, total dissolved solids (TDS), electrical conductivity (EC) and zeta potential are used to investigate their effect on the removal of S.S.

Experiments showed that removal of S.S increases as contact time, exposure distance and magnetic field increase and flow rate decrease, they also showed that TDS and EC of the WW increase with increased magnetic field and contact time, and that zeta potential decreases with increased magnetic field.

Key words: Electromagnetic, Suspended Solids, Wastewater, Treatment

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الملخص باللغة العربية

تعتبر عملية إزالة المواد الصلبة العالقة في مياه الصرف الصحي والمياه الصناعية واحدة من أهم تقنيات معالجة المياه العادمة. فـــي التقنيـــات التقليدية لمعالجة المياه، يتم استخدام أنواع محددة من المواد المكتلة والمواد المشتتة مثل مركبـــات $FeCl_3$,$(Al_2(SO_4)_3.18H_2O)$ ومركبـــات البوليميدات، هذه المركبات تستخدم بكميات كبيرة للحفاظ على البيئة، ويأتي في مقدمة تلك الطرق الحديثة استخدام المجال الكهرومغناطيسي، والذي يحفز إزالة المــــواد الصلبة للمعالجة تكون ملائمة للحفاظ على البيئة، ويأتي في مقدمة تلك الطرق الحديثة استخدام المجال الكهرومغناطيسي، والذي يحفز إزالة المــــواد الصلبة والمخلفات العالقة في المياه العادمة بأقل وقت لازم للتنفيذ ودون الحاجة لإضافة مواد كيميائية مثلما يحدث في الطرق التقليدية للمعالجة.

في هذه الدراسة، تم إجراء تجربة لاستخدام التقنية الكهرومغناطيسية لمعالجة مياه الصرف الصحي، وقد تم دراسة المؤشرات التالية: معدل التدفق، زمن المعالجة، مسافة التعرض، وإجمالي المواد الصلبة المذابة، والتوصيل الكهربائي، وقوة زيتا للكشف عن أثر التقنية الكهرومغناطيـــسية فـــي إزالة المواد الصلبة من المياه.

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INTRODUCTION

Municipal wastewater (MWW) contains different types of pollutants which are coming from many sources such as the pollutants introduced into the MWW collecting system with run-off rain water, from domestic sources and small commercial sources. The main pollutants present in the MWW are organic such as bacteria and cell debris and inorganic pollutants such as TDS and total suspended solids (TSS).

Total suspended solids (TSS) include all particles suspended in water which will not pass through a filter. Suspended solids are present in sanitary wastewater and many types of industrial wastewater. These suspended particles are treated and removed from water and wastewater by many methods and technologies such as coagulation, sedimentation and electromagnetic sedimentation.

Magnetic and Electromagnetic treatments for water and wastewater attract a special attention due to their safety, ecological purity, simplicity and low operating costs. Magnetic treatment of water is an attractively simple approach by which the water to be treated flows through a magnetic field and consequently changes some of its physicochemical properties. Many researches have been published concerning the use of the electromagnetic field in treatment of water and wastewater (1).

Electromagnetic field was used to separate dyes in wastewater with high efficiency and speed using High Gradient Magnetic Separation with superconducting magnet. The result showed that, all the organic dyes were removed with high efficiency by magnetic separation. In addition, it was shown that one of the driving forces for adsorption of organic dyes on the surface of ferromagnetic particle is electrostatic interaction between the dye and the particle, which can be control by changing pH of the slurry (2). Magnetic field was tested as an alternative to induce some improvements during the textile laundering procedure. The results with standard washing of cotton indicated that MWT modified the mineral fouling on the textile and consequently increased the whiteness. There are also some indications of improved oxidative ability (3).

The effect of magnetic field on the activity of activated sludge in wastewater treatment was investigated. The results show that magnetic field had a positive effect on the growth of mixed bacteria in the activated sludge. Also The bacteria magnetically pre-acclimated has a higher biodegradation ability than those without the same pre-acclimation (4).

The possibility of an electromagnetic field (EMF) influencing the intensification of phosphorus (P) and organic compound (COD) removal from domestic wastes was investigated. The values of the pollutants in wastes in the control samples that were not under EMF and without metal packing decreased but not in a large range. There was the decrease in COD concentration approximately from 5% to 10% after 24 hours and from 20% to 25% after 48 hours of the reaction. The decrease in the P

concentration was observed but it was different depending on the detention time. After 48 in the domestic wastes the efficiency of P_{tot} removal was about 15% (2).

EXPERIMENT AND METHODS

The samples of WW were collected from Al-Rustamia sewage treatment plant from November 2013 to June 2014 by using plastic containers of (20L). The samples were taken from the effluent of the biological treatment stage by holding the open containers in the opposite direction to the WW flow until they are completely filled, then closed and transported immediately to the laboratory within short time for experiments. Figure (1) shows the flow diagram of the work.
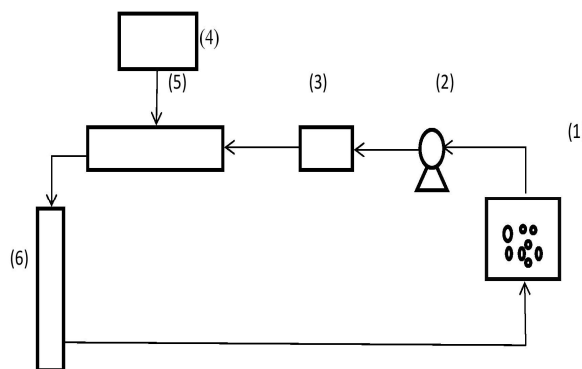
Figure (1): Experimental setup for the magnetization of the sample: (1) Open tank with air bubbling for mixing (plastic, 120L), (2) Circulation pump, (3) Flow meter, (4) DC power supply (30V, 2Am), (5) Magnetization setup, (6) Settling tank (length of 1m and 10 out lets for sampling).

The samples were used for electromagnetic treatment experiments at room temperature. The electromagnetic field was generated by using electrical relays (Finder 60.12) and the electrical current used for the production of the magnetic field was generated by using DC power supply (30V, 2Am). Table (1) shows the different values of the magnetic field used for the treatment of the suspended solids in sewage waste water.

Table (1): Values of the electromagnetic field used for the S.S treatment

| Current (Am)	Voltage(V)	Magnetic Flux at Center (G)	Magnetic Flux at Tangent (G)
0	0	0	0
0.05	2	50	150
0.06	4	70	300
0.07	6	90	350
0.1	8	120	550
0.12	10	170	700
0.14	12	185	800
0.17	14	230	900
0.19	16	240	1300
0.21	18	260	1500
0.24	20	285	1550
0.26	22	300	1650
0.27	24	325	2800

Experimental Work:

The S.S concentration of the WW is an important parameter to know in order to determine the percentage of removal of S.S by using the electromagnetic technology. The concentration of S.S in wastewater is determined by using vacuum filtration. Samples of the treated WW are filtered with qualitative No.1 filter paper. The filter papers were kept in an oven for 30 mints at a temperature of 150 ^{o}C. S.S concentration then is calculated by subtracting of filter paper weight before and after filtration after which divided by the volume of the sample. The concentration of the S.S in mg/L or ppm is evaluated as a unit of measurement (5).

Effect of Flow Rate:

Effect of flow rate (Q) on the S.S removal percentage is shown in figures. (2- 6) were the magnetization of the S.S is conducted by varying the flow rate from (1 Lpm) to (5 Lpm) consequently. It was shown that the removal of S.S is decreased as the value of the flow rate is increased. The Increase in flow rate means increase in drag force, as a result particles contained in the WW are not properly magnetized under this high flow velocity. For a lower flow rate value the removal of S.S is found to be higher. The reason is that in slower flow rates particles receives more magnetic strength therefore more suspended particles are attracted and cloaked together. Consequently this behavior would result in extra reduction in suspended solids. It was found from the figures that the optimum flow rate that gives the optimum removal of the S.S is (1) Lpm.
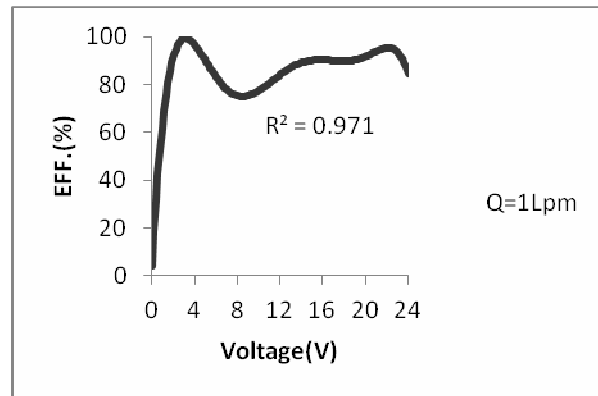
Figure (2):Removal effect at flow rate of 1Lpm
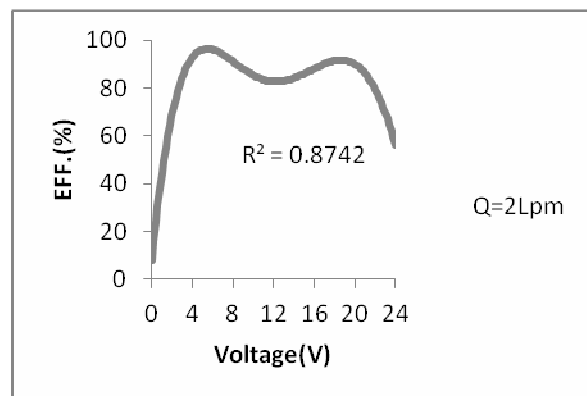
Figure (3):Removal effect at flow rate of 2Lpm
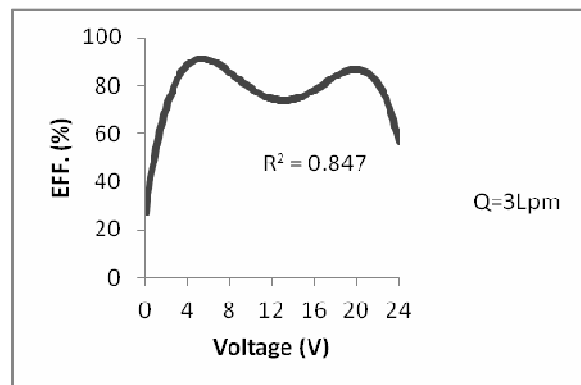
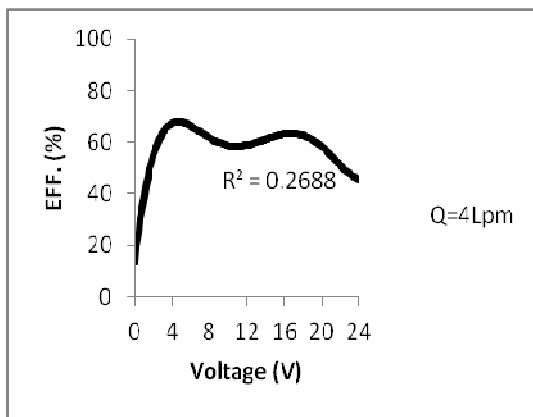**Figure (4):Removal effect at flow rate of 3Lpm**
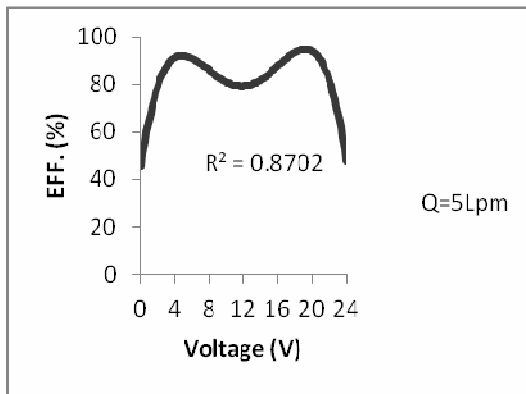
Figure (5): Removal effect at flow rate of 4Lpm

Figure (6):Removal effect at flow rate of 5Lpm

Effect of different electromagnetic field strength:

The effect of different electromagnetic field strength on the removal of the S.S is shown in figure (7). values of the electromagnetic field strengths used for the magnetization of the S.S are (50, 70, 90, 120, 170, 185, 230, 240, 260, 285, 300, 325). It was found that at flow rate of 1Lpm the removal of S.S increased with increased electromagnetic field strength.
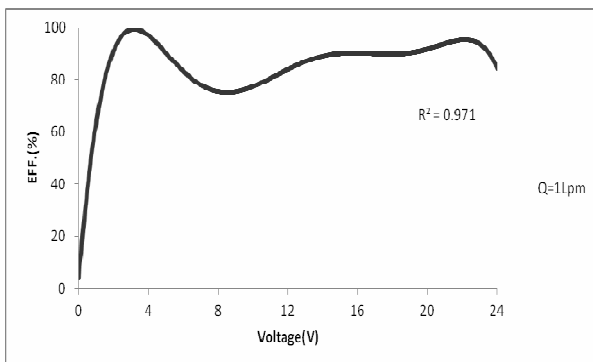
Figure (7): curve of removal of S.S at various electromagnetic field strength

Effect of Contact Time:

Several values of flow rate were used to achieve several contact time for this experiment. The contact time obtained from the flow rate is described by the following equation:

$Q = vol./t$ (1)

Where :
Q = flow rate (Lpm).
Vol. = volume of the contact distance (L).
t = contact time (mint).
By applying eq.(1) to find contact time values, table (2) was obtained as follows:

Table (2): Contact time values

Flow Rate (Lpm)	Contact Time (sec)
1	23
2	12
3	8
4	6
5	5

It was found that the removal of the S.S decreased with decreased contact time as shown in figures (8- 12). This occurs because the S.S particale that are present in the WW do not have enough time to receive more magnetic strength and to be   charged eough to be attracted and cloaked togethor. It was found from the figures that the optimum contact time that gives the optimum removal of the S.S is (23) minutes (1).
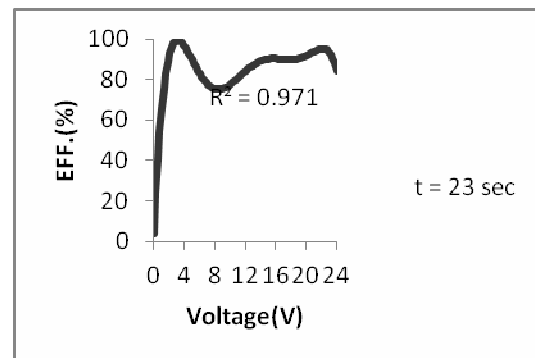
Figure (8): removal effect at contact time of 23 seconds.
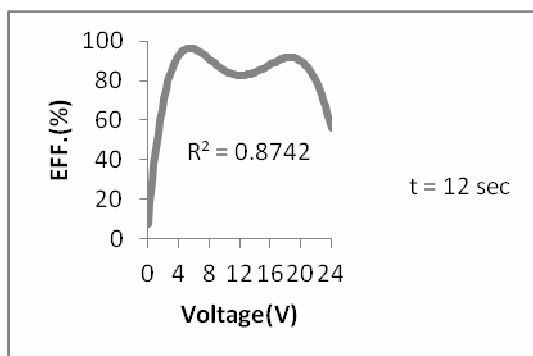
Figure (9): removal effect at contact time of 12 seconds
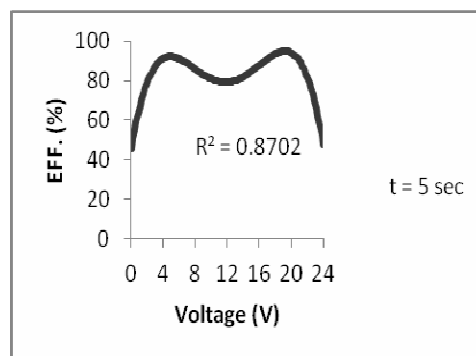
Figure (12): removal effect at contact time of 5 seconds
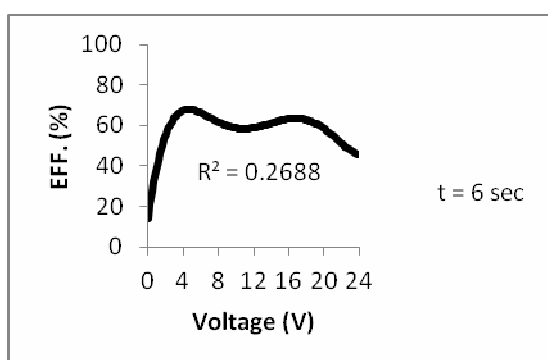
Figure (10): removal effect at contact time of 6 seconds

Effect of Exposure Distance:

Effect of the exposure distance on the removal of the S.S is shown in figures (13-16). Values of exposure distance used for the experiments are (10, 20, 30, 40) cm with fixed flow rate of 1Lpm. It was found from the figurs that the optimum exposure distance which gives the highest removal of S.S is (40) cm since this exposure distance gives the S.S sufficient contact time for the suspended particals to be charged, attracted and cloaked together.
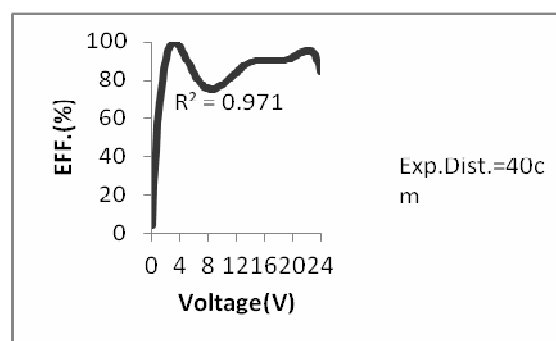
Figure (13): Removal effect at Exp. Dist. of 40cm

Figure (11): removal effect at contact time of 8 seconds
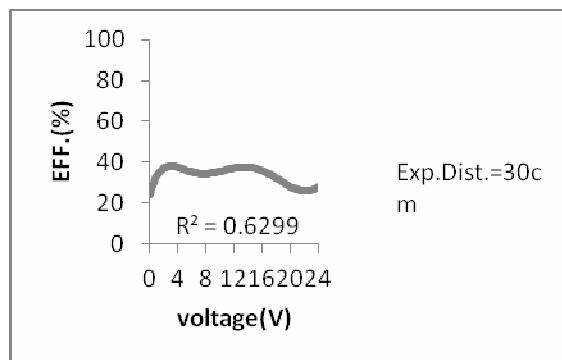
Figure (14):Removal effect at exp. Dist. of 30cm.
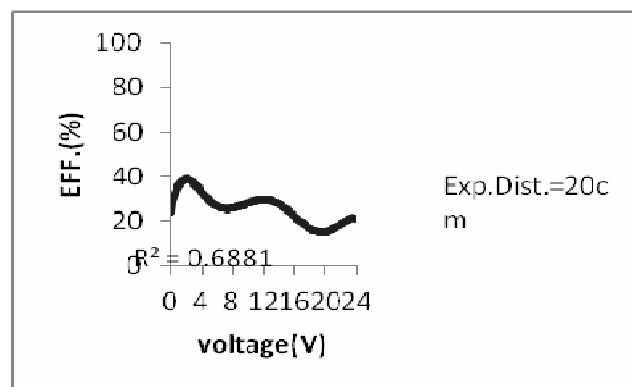
Figure (15):Removal effect at Exp. Dist. of 20cm.
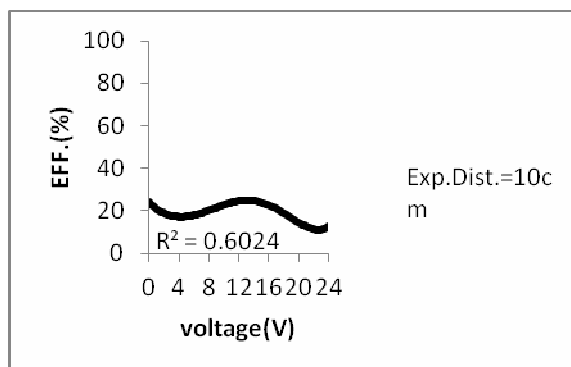
Figure (16):Removal effect at Exp. Dist. of 30cm.

Comparison of Zeta Potential between conventional coagulation and electromagnetic field:

Zeta potential is the repulsion force that occurs between charged suspended partials, which prevents these particles from being attracted and cloaked together. A comparison in zeta potential between the conventional method of coagulation by using alum and the method of electromagnetic settling is shown in figures (17, 18). It was found that zeta potential decreased with increased electromagnetic field more than the decrease in zeta potential in the conventional coagulation by using alum and this will result in better S.S clogging and settling.
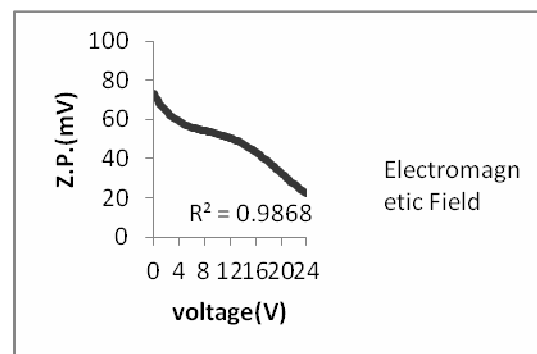
Figure (17):Z.P. at electromagnetic field.
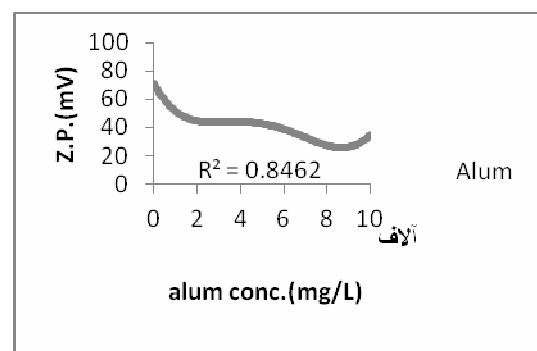
Figure (18):Z.P. at alum

Effect of electromagnetic field on TDS and EC:

The influence of electromagnetic field on the values of TDS on the MWW is shown in figures(19- 23). These figures show that the TDS values increased as increasing in electromagnetic field.
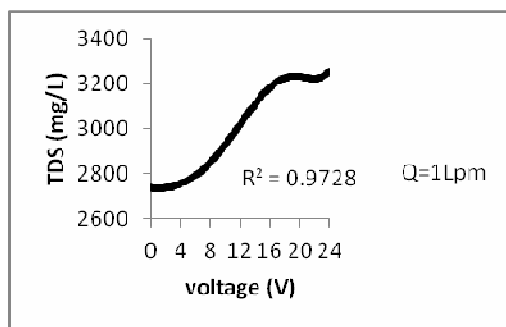
Figure (19):TDS curve at flow rate of 1Lpm
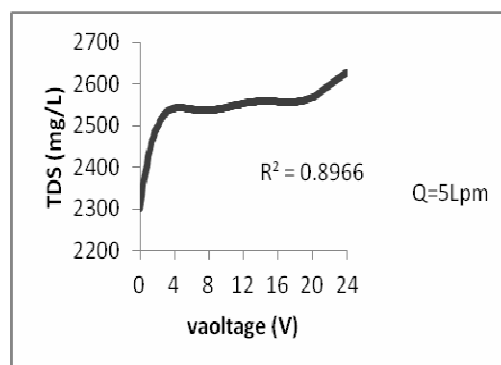
Figure (23):TDS curve at flow rate of 5Lpm
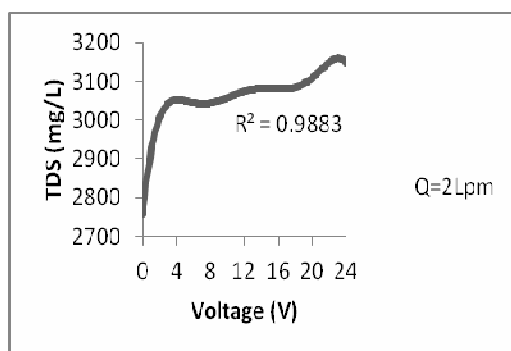
Figure (20): TDS curve at flow rate of 2Lpm

The influence of electromagnetic field on the values of EC of the MWW is shown in figures (24-28). Since EC is directly related to TDS then EC values increase with increased electromagnetic field.
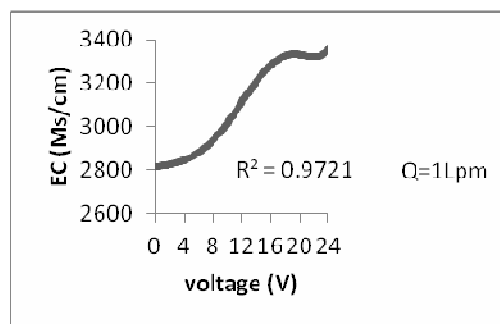
Figure (24): EC curve at flow rate of 1Lpm
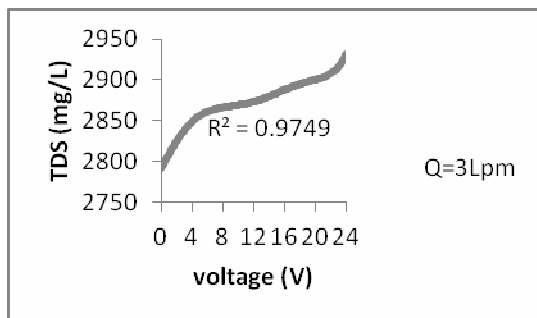
Figure (21): TDS curve at flow rate of 3Lpm
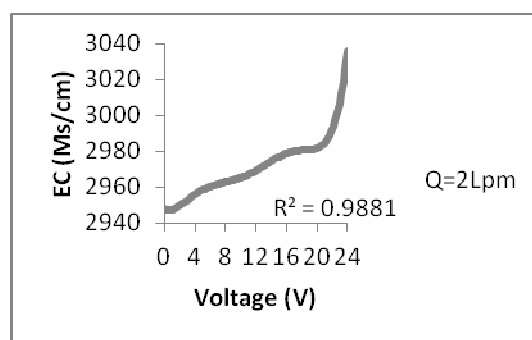
Figure (25):EC curve at flow rate of 2Lpm
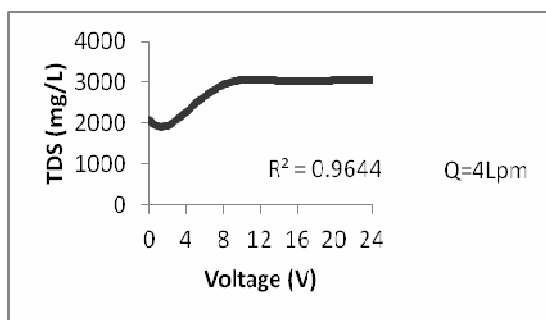
Figure (22):TDS curve at flow rate of 4Lpm
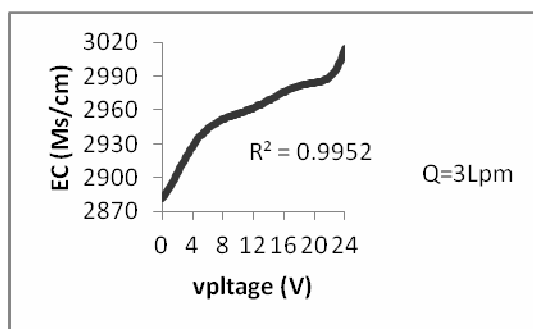
Figure (26): EC curve at flow rate of 3Lpm
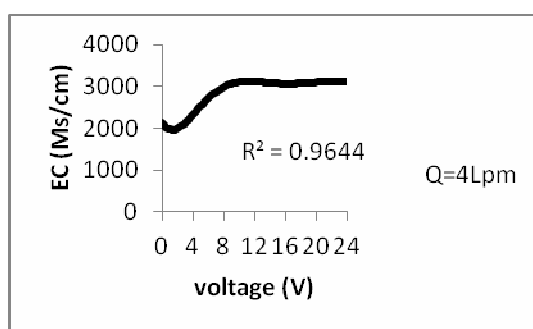
Figure (27):EC curve at flow rate of 4Lpm.
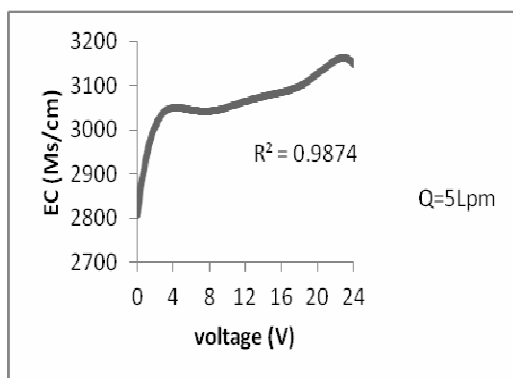
Figure (28):EC curve at flow rate of 5Lpm.

RESULTS AND DISCUSSION

This study resulted in massive reduction in suspended solids concentrations. The electromagnetic treatment of wastewater resulted in reduction in the concentration of the suspended solids of about 95% and Table (3) refers the values of these reductions:

Table (3): Values of reduction in S.S concentration

Q=5Lpm, Eff.(%)	Q=4Lpm, Eff.(%)	Q=3Lpm, Eff.(%)	Q=2Lpm. Eff.(%)	Q=1Lpm. Eff.(%)	Magnetic field(G)
0	3	3	20	20	2
50	97	81	84	92	80
70	98	90	91	86	81
90	82	84	85	91	81
120	75	95	76	83	82
170	78	81	78	79	79
185	91	99	78	78	76
230	83	87	84	85	83
240	87	72	73	91	54
260	91	89	86	94	45
285	99	99	89	98	60
300	89	79	71	96	79
325	86	55	62	97	34

Also due to the electromagnetic field zeta potential has been enormously decreased when compared with values of zeta potential for conventional alum coagulation as shown in tables (4, 5).

Table (4): Z.P. values for EMF

Magnetic field(G)	ZETA POTANTIAL(mV)
0	-72.05
50	-66
70	-60.17
90	-54.56
120	-52.58
170	-51.92
185	-51.15
230	-50.71
240	-41.36
260	-40.37
285	-30.69
300	-26.95
325	-22.88

Table(5): Z.P. values for alum

Alum (mg/L)	Z.P. (mV)
0	-72.05
2000	-40.04
4000	-52.8
6000	-29.41
8000	-31.79
10000	-34.32

The influence of electromagnetic field resulted in increased TDS concentrations with increased magnetic field as shown in table (6).

Table (6): TDS values at different flow rates

Magnetic field (G)	TDS(mg/L) at Q= 1Lpm	TDS(mg/L) at Q=2Lpm	TDS (mg/L) at Q= 3Lpm	TDS (mg/L) at Q= 4Lpm	TDS (mg/L) at Q= 5Lpm
0	2739	2739	2794	2794	2295
50	2739	2739	2816	2816	2520
70	2750	2750	2860	2860	2522
90	2783	2783	2860	2860	2522
120	2893	2893	2860	2860	2560
170	2915	2915	2871	2871	2570
1/85	2948	2948	2871	2871	2530
230	3190	3190	2882	2882	2530
240	3190	3190	2893	2893	2570
260	3190	3190	2893	2893	2600
285	3234	3234	2893	2893	2522
300	3234	3234	2915	2915	2615
325	3256	3256	2930	2930	2625

This increase in TDS values happened due to the fact that the external electromagnetic field (energy) is charging the suspended solids present in the wastewater. Due to this external energy these charged particles will be in unstable phase, and in order to reach a more stable stage in energy level these charged particles would tend to be dissociated or destructed and broken down into finer particles.

It can be concluded from this study that:

1. Wastewater that is treated with electromagnetic field would result in decrement in suspended solids.

2. The percentage of removal of suspended solids is increased with decreased flow rate.

3. The percentage of removal of suspended solids is increased with increased contact time and increased exposure distance.

4. Zeta potential is decreased with increased electromagnetic field.

5. TDS concentrations and EC values are increased with increased electromagnetic field.

6. The optimum operating parameters are Q= 1Lpm, contact time= 23 sec, exposure distance= 40cm.

7. The optimum operating electromagnetic field is ranged from (185G) to (285G).

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Properties of pyrolytic char and local adsorbent material derived from pyrolysis of waste tires

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ABSTRACT

The aim of this study was to produce a local adsorbent, using a waste tire as raw materials. Pyrolysis process has the potential of transforming used tires into useful recyclable products. Production of local adsorbent material from solid wastes is one of the most environment-friendly solutions by transforming negative valued wastes to valuable materials. Thus, in this research local adsorbent was prepared from waste tires using two-step pyrolysis method.

In the carbonization process, different nitrogen gas flow rate (0.1, 0.2, 0.3 lit/min) were studied with different carbonization temperature (400, 500, 700ºC) and studying their effects on the products yields and surface areas. Moreover, the optimum conditions were set to (500°C) for (1hr) under nitrogen gas flow rate of (0.2 lit/min). The char products were then proceed to the activation process at (850°C) under carbon dioxide (CO_2) activation flow rate of (0.6 lit/min) for (3hr). The activation method produced local adsorbent material with a surface area and total pore volume as high as (118.59m2/gm) and (0.1467cm3/gm), respectively.

Key words: pyrolysis, waste tires, activation, carbonization

الملخص باللغة العربية

الهدف من هذا البحث هو إنتاج مادة مازة محلية باستعمال الإطارات المستعملة كمادة خام، وذلك من خلال عملية التحلل الفيزيائي حيث يتم تــدوير هذه الإطارات المستعملة إلى منتج مفيد. ويعتبر إنتاج المادة المازة المحلية من مخلفات الإطارات واحدا من الحلول الصديقة للبيئة وذلك بتحويــل مخلفات سلبية الفائدة إلى مادة مفيدة، وهذه المادة تحضر بالتحلل الفيزيائي وعلى مرحلتين. في عمليه الكربنة تم استخدام معدلات جريــان مختلفة لغاز النيتروجين (0.1، 0.2، 0.3 لتر/دقيقة) وعلى درجات حرارية مختلفة (400، 500، 700 درجة سيليزية) مع دراسة كل مــن معــدل الجريان ودرجه الحرارة على إنتاج المادة والمساحة السطحية، وتم تثبيت الظروف المثلى إلى (500 درجه سيليزية) لمدة ساعة واحدة تحت معــدل جريان (0.2 لتر/دقيقة) من غاز النيتروجين. وتم تنشيط الفحم الناتج إلى (850 درجة سيليزية) وبتصريف (0.6 لتر/دقيقة) من غاز ثاني أوكسيد الكربون، وتم إنتاج المادة بمساحة سطحية قدرها (118.95 م2/غم) وبحجم المسام الكلي (0.1467 سم3/غم) على التوالي.

INTRODUCTION

Due to the continuous increase in the production and usage of automobiles, the number of waste tires will increase considerably. It was estimated that approximately (1.5×10^9) of waste tires are discarded worldwide every year (1,2)**.** For example, the total number of waste tires in Iraq, and according to the mayoralty of Baghdad statistics an approximately of (4830 ton / yr) of waste tires are discarded. An estimate for the annual generation of tires in China is around (1million tons), which brings out at least (19 million used tires per year) (3). The EU, USA and Japan together were responsible for the disposal of (5million ton/yr) of waste tires (4). As a result, the large amount of waste tires were thrown, dumped in the landfills, stockpiled, they have become a serious source of environmental pollution (5).

Pyrolysis may be an environmentally friendly process to transform used tires into useful products. This process transforms used tires into gas (5-20wt %), oil (40-60wt %), and solid char (30-40 wt %). The solid char obtained from pyrolysis can be used in adsorption process as a secondary and advanced treatment technique in environmental engineering. It is used in practice for removal of various pollutants such as soluble organics, dyes, pesticides, lignin, etc., from wastewaters and for removal of color and taste and odor-producing substances from natural waters that are to be used as potable water supplies. Therefore, in this study, waste tire pyrolysis was evaluated from an environmental perspective with a focus on the commercial utilization of the solid product. The carbonization and physical activation were carried out on the solid product to make it an adsorbent material. The removal of lead from aqueous solution was investigated.

MATERIALS AND METHODS

Materials

Waste tires were used in the present work represent a mixture of used truck tires, containing no steel or synthetic cord. Before being treated, the waste tires were shredded using an electrical drill (BOSCH 305) adopted with crushing disk and sieved to a size of (0.5mm) by using (RETSCH sieves number 35) as shown in figure (1).

Figure (1): Steps of shredding and grinding waste tires

Methods of analysis:

1. Surface area, pore size and volume: Surface area is one of the key indicators attributed to the adsorptive properties of porous materials. Pores in different sizes are important to the adsorbent. The presence of micro- and mesopores in local adsorbent material enhance the adsorption of large adsorbates such as heavy metals molecules. The surface area and pore size of the produced activated carbon were measured using (SURFACE AREA ANALYSER/THERMO USA MR 9600).

2. Microscopic investigation : A microscope investigation was made for the produced adsorbent material by using (AA 3000 Scanning Probe Microscope) in the Nano- Technology center/ University of Technology.

Experimental procedure:

The sieved waste tires particles filled in the refractory furnace, the furnace type is (BARNSTEAD/ THERMOLYNE FURNACE 62700, 1.5KW) that provided with a digital (EUROTHERM) controller in order to control the temperature. The furnace can reach a maximum temperature of (1000ºC). The furnace provided with a fan in order to evacuate air from the furnace. Two pressurized cylinders of carbon dioxide gas and nitrogen gas (that purchased from local market) adopted with flow regulator adaptor (YAMAWITE 35) in order to control the flow of the gas to the furnace chamber to ensure the inert environment during the carbonization and activation phases. The pyrolysis process passes through two stages carbonization and activation. The two consequence processes were applied to produce the local adsorbent material.

1. carbonization process: As the efficiency of the carbonization process is a function of the temperature and nitrogen gas flow rate, a sieved sample to (0.5mm) particle size was weighed using (SARTORIUS scale 7100) and put in the furnace. The sample was heated at rate of (5oC/min) in the presence of high purity nitrogen gas to (400, 500, 700 oC) typically (400-700 oC) (6) in an inert atmosphere with a gas flow of (0.1, 0.2, 0.3 lit/min) controlled by a volumetric flow meter in a typical run, where it was maintained for (1hr). The optimum chosen temperature and flow rate were depends on the solid product yields and the best surface area.

2. Physical activation process: Physical activation was performed by using carbon dioxide gas as oxidizing agent. The activation temperature is usually set to be (850 oC) for (3hrs) (according to the previous researches the activation temperature may ranged from 800-900 oC) (6) to maintain a sufficiently high reaction rate. The carbon dioxide gas was introduced during the whole process at a rate of (0.6 lit/min).

RESULTS AND DISCUSSION

As in this study, the major goal was to produce local adsorbent material from waste tires by pyrolysis process, the parameters that affecting this process was studied and investigated. It was found that the most affecting parameters that influencing the pyrolysis process is the temperature and nitrogen gas flow (7). Different carbonization temperature was conducted (400, 500, 700 ºC) for each certain flow rate of nitrogen gas (0.1, 0.2, 0.3 lit/min) while the activation process temperature and carbon dioxide gas flow was fixed at (850 ºC) and (0.6 lit/min) respectively and the corresponding yields and surface area of the produced activated carbon was investigates. Table(1) represents the results for this step.

Table (1): The yields and surface areas of the produced local adsorbent material resulted from different carbonization temperature and nitrogen flow rate

Gas flow rate lit/min	Temperature ºC					
	400		500		700	
	yield (%)	Surface area (m2/gm)	yield (%)	Surface area (m2/gm)	yield (%)	Surface area (m2/gm)
0.1	77	34.11	59	63	36	88.3
0.2	79	29.2	67	61	40	84.81
0.3	81	20.5	69.5	34.15	44	49.1

1. The burn off curve: The term burn off refers to the amount of material losses due to the activation process (8).

$$Burn\ off\ (w\ \%) = (w_1-w_2)/w_1 \times 100 \qquad (1)$$

Where w_1 and w_2 are the char mass before and after activation respectively. It should be noted that the weight loss is not totally because of activation, and part of it is because of the evaporation of hydrocarbon deposit in raw char with the increasing temperature during carbonization process which followed by activation process.

The burn off exhibited a direct proportion with the activation time at constant activation temperature of (850ºC) and carbon dioxide gas flow rate of (0.6lit/min) as shown in figure (2).
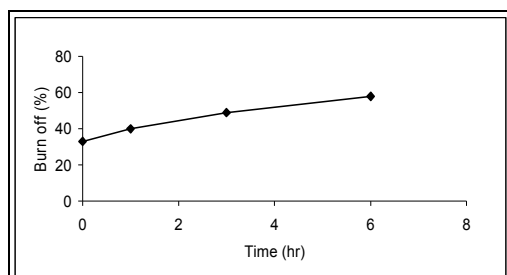
Figure (2): Effect of the activation time on the burn-off of char with an activation temperature of 850ºC and CO$_2$ flow rate of 0.6lit/min

The burn-off curves do not start at zero, because there is a substantial mass loss during the carbonization step. This initial mass loss is (33%) at (500ºC) at (1hr) under nitrogen flow, the holding time in range (0-3hrs) had the same effect on the surface area of the produced activated carbon as that of burn off. However, increasing holding time to (6 hrs) had the same effect on burn off and the inverse effect on the surface area. The effect of the burn off on the surface area of the produced activated carbon was studied and the results show in figure (3). Figure (3) shows the maximum surface area attained was (118 m2/gm) at (49%) burn off (850 ºC, 3hrs holding time), which is nearly doubled the surface area of pyrolytic waste tires (61m2/gm). The surface area at (58%) burn off was (95m2/gm) this decrease in the surface area could be explained by burning off walls between the adjacent pores and turning more microspores into macrospores.
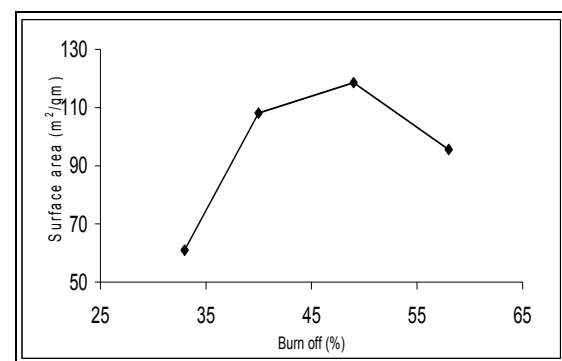
Figure (3): Surface area values of the produced local adsorbent material obtained at different burn-off levels

2. Microscopic analysis: The microscopic analysis micrographs provide information on the structural changes in the produced local adsorbent material for analysis during the activation process. Figures 4.a, 4.b and 4.c show the graduate expansion in the microscopic structure for the pores for different magnification power.

The surface of the produced material is resulted from the presence of the bonds of hydrocarbons in the raw marital without any cracks. This would account for its poor or negligible surface area. The framework development was so rapid in figure 4.c, resulting extra cavities and lead to crack formation. Due to this well developed pores, the produced material possessed high surface area. The micrograph magnifies the internal cavities, which are now clearly visible. The rate of activation (the formation of pores in the process) will influenced by the formation of oxides on the surface (small white particles which scattered on the surface of the produced local adsorbent material) (2) as shown in figure (5).
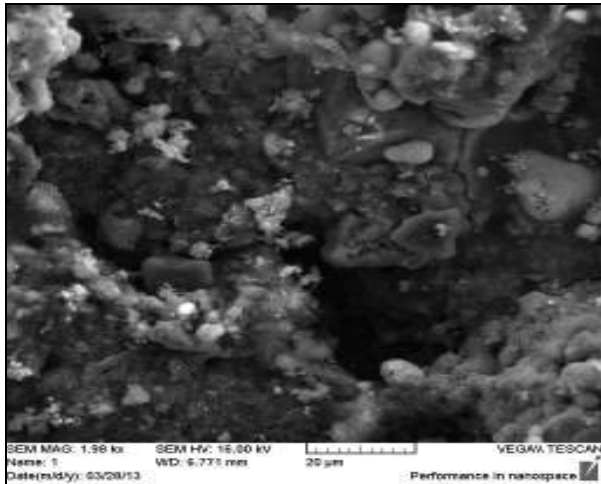
Figure (4.a): The microscopic structure of the produced local adsorbent material at magnification power (1.98 kx).
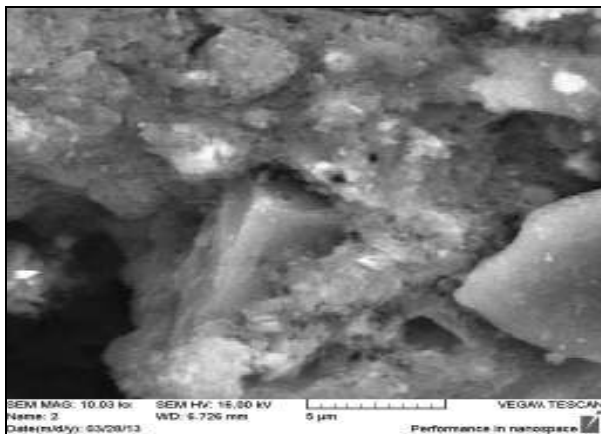
Figure (4.b): The microscopic structure of the produced local adsorbent material at magnification power (10.03 kx)
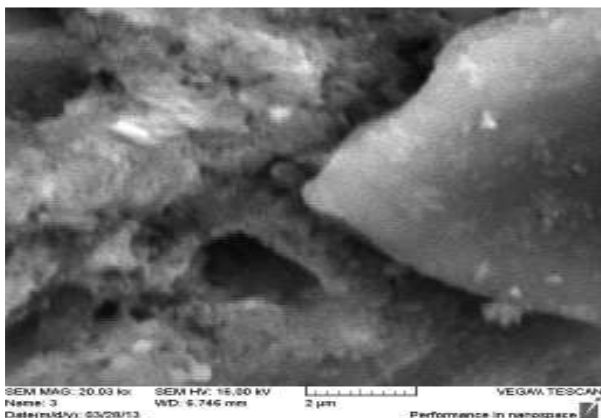
Figure (4. C): The microscopic structure of the produced local adsorbent material at magnification power (20.03 kx).

Figure (5): Formation of white scattered particles on the surface

CONCLUSION

The local adsorbent material with medium porosities can be produced from pyrolysis of waste tires at 500 ºC, followed by physical activation at 850 ºC. The surface areas and pore volume of the produced material were found to be (118.59 m2/gm) and (0.1467 cm3/gm) respectively.

The effects of different parameters during carbonization were investigated. it was found that the optimum nitrogen gas flow rate was(0.2lit/min),and the temperature of (500ºC).

The material yield found to decrease with increasing the carbonization (temperature, holding time, Nitrogen gas flow rate).

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The acute effect of commercial pesticide imidacloprid on freshwater cladoceran *Simocephalus vetulus*

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ABSTRACT

The ecological risk assessments of insecticide imidacloprid were studied, when cladoceran *Simocephalus vetulus* was exposed to it. The acute toxicity test of this insecticide was assessed by Median Lethal Concentration (LC_{50}). The Morality percentage and safe concentration were calculated. LC_{50} of imidacloprid on *S.vetulus* were 15.8, 7.2 and 4.4 mg/L during 24, 48 and 72 hr. respectively. The mortality rate of *S.vetulus* was increased proportionally with the increase concentrations of insecticide.

Key words: imidacloprid, *Simocephalus vetulus*, biological effect

الملخص باللغة العربية

تضمنت الدراسة تحديد المخاطر البيئية الناتجة من استخدام نوع من المبيدات الحشرية هو مبيد الايميداكلوبرايد (imidacloprid) وذلك من خلال دراسة تأثيره الحيوي على نوع من القشريات المائية التابعة لرتبة متفرعة اللوامس (cladocera) وهو *Simocephalus vetulus* ضمن فترات من التعريض الحاد لمبيد الايميداكلوبرايد على النوع *S.vetulus* وتضمن التعريض الحاد تحديد قيم كل من التراكيز المميتة لنصف العدد (LC50) وتحديد النسب المئوية لهلاك الأفراد كما تم ايجاد قيم التراكيز الأمينة (safe concentrations)، وقد وجد أن قيم التراكيز المميتة لنصف العدد (LC50) للنوع *S.vetulus* المعرض لمبيد الايميداكلوبرايد 15.8 ، 7.2 ، 4.4 ملغم / لتر خلال 24 ، 48 ، 72 ساعة على التوالي. وبينت الدراسة أن زيادة تراكيز المبيد تؤدي الى زيادة النسبة المئوية للهلاكات.

INTRODUCTION

Water pollution caused by agricultural pesticides is a well recognized problem, as pesticides are widely produced and released into the natural environment (1). Pesticides and other agrochemicals have been increasingly used given their benefits in controlling pests, pathogens and weeds (2). The majority of pesticides are designed to be used in a terrestrial environment, however substantial amounts end up in aquatic ecosystem, in either surface or ground water (3). Non target animal population can be affected and some need more than six months for their abundance to recover after pesticides runoff that ends up in streams (4). Imedacloprid belongs to a class of chloronicotinyl insecticides (5). It is the best known example of these highly effective new insecticides; these nicotiniod insecticides have a selective effect on nicotinic acetyl cholinesterase receptors (6). *Simocephalus vetulus* is common Cladocerans inhabiting macrophyte-rich littoral zones of fresh water and this microcrustacean show a benthic behavior and can be an important component of zooplankton system (7). *S. vetulus* was used in this study because of its' abundance in Iraqi water, as reported by (8).

The objective of this study was to determined the acute toxicity of imidacloprid in its' commercial available form under constant exposure conditions.

MATERIALS AND METHODS

Water properties of the sampling area: Water properties of collected samples from Tigris River at AL-Jadyria campus included ; water temperature, hydrogen ion concentration ,water conductivity (measured in µs/cm (Microsiemens /cm) unit), salinity and dissolved oxygen were measured by standard reported methods (8, 9).

Water quality: Filtered river water (fresh water) was used in culturing of isolated animals and preparation of toxic solution (10). This water was collected from Tigris river which it represented the natural habitat of these animals and was filtered by Dual water purification system with three filter housing which filters the water in the first stage and purifies it in the second . Filtered water was checked again for some properties like; water temperature, hydrogen ion concentration, water conductivity, salinity and dissolved oxygen.

Collection and Isolation of Crustacea:

Simocephalus vetulus was collected from the bank of Tigris River in AL-Jadyria campus by using zooplanktons net with meshed pores of 45-50 µm. The organisms were trapped in net container washed with water into a glass container and transported directly to the laboratory for isolation and identification. The collection or catchments area should be had no rural activity (10).

Identification, Classification and culturing of *S. vetulus*:

Collected animals were identified according to Edmondson (11). The cultures were continuously maintained in filtered river water (fresh water) by method described by Rousseaux *et al.* (12). The animals were reared under laboratory conditions which included 20±2° C Photoperiodicity of 16 hr. light and 8 hr. dark is sufficient to the growth and reproduction of the cladocera (13).

Nutrition of cladocera:

The juice of two plants (vegetables) which include Spinach and Celery were used as food for the cladocera as described by (14).

Preparation of *S. vetulus* for the toxicity test:

Before exposure experiments, the gravid females of *S.vetulus* were taken out and cultured individually in 120 ml glass beakers until they oviposited ,healthy neonates (about 24 hrs.) were always taken from the second and the following broods as recommended (15).

Preparation of Imidacloprid solutions for toxicity test:

Imidacloprid (trade name confidor) 200SL, packed in plastic bottle at concentration 200 g/L (manufactured by Pioneers International Trading (LTD), China). The insecticides were stored under refrigeration (4C°) to prevent dissociation (16).

The concentration of imidacloprid stock solution was 200g/L according to Song *et al.* (17).The following concentrations were prepared by added (25, 50, 75 and 100) µl from stock solution to 1L of filtered river water to obtain the tested concentrations (5, 10, 15 and 20) mg/L respectively.

Acute toxicity test:

Acute toxicity experiments were demonstrated by exposed 4 groups, each group consist of 10 animals with control group had the same number of animals according to method described by Papchenkova *et al*. (18). Ten *S. vetulus* were exposed to the imidacloprid with 0.0 (zero) control. The neonates were characterized as described by Rousseaux *et al.* (12).

Death of cladocera was defined according to Brennan *et al.* (19), as the inability to swim for more than a few strokes within 15s after gentle agitation of the test vessel and recording the presence or absence of movement:

1. Mortality Percentages: Mortality percentages were calculated for *S.vetulus* which was exposed to imidacloprid through 96hr of exposing (20).

2. Median Lethal Concentration (LC_{50}): Median lethal concentration was calculated for *S.vetulus* exposed to imidacloprid. Probit analysis (SPSS v.13 for Windows®, SPSS Inc.) was used to estimate LC_{50} values at a regular interval for each test in acute exposure with cladocera (immobilization) (15).

3. Safe Concentration (SC): Safe concentration was calculated by using the method described by Al-Obaidy (21).

Statistical Analysis:

Probit analysis (SPSS v.13 for Windows®, SPSS Inc.) was used to estimate LC_{50} values.

RESULTS AND DISCUSSION

Water properties of the sampling area and testing water quality:

Table (1) shows some physical and chemical properties of water in the sampling area in AL-Jadyria campus from which *S. vetulus* was collected during 2009. These values were similar to that reported on Tigris River of Al-Jadyria (22). Table (1) includes the measurements of the same physical and chemical parameters but for the filtered river water. The results of these measurements are very near or close to the results obtained with water properties from the sampling area and this gives a confirmation of the suitability of using this type of water in rearing the animals.

Acute toxicity test of insecticide Imidacloprid:

1. Median Lethal Concentration (LC_{50}) of *S.vetulus*: The Median Lethal Concentration (LC_{50}) values of *S. vetulus* exposed to imidacloprid during periods 24, 48, 72 hr. were 15.8 mg/L., 7.2 mg/L. and 4.4 mg/L. respectively (Figures 1, 2 and 3).
The results showed high susceptibility of *S.vetulus* toward imidacloprid as jugged by LC_{50}, were less than the standard toxicity test which may fluctuated between $10 – 85$ mg/L, 17.4 mg/L to *D. magna* at 20º C (EC_{50} or LC_{50} 48 hr) (17), whereas the value of LC_{50} 48 hr. in this study was 7.2 mg/L for *S.vetulus*. Therefore imidacloprid has toxicity value higher than those reported by standard toxicity test and this was observed from the lower values of LC_{50} which were obtained in this study for *S.vetulus* during 48 hr. Several aquatic invertebrates appear to be sensitive to imidacloprid. Tomlin reported a 48 hr. EC_{50} (immobility) 85 mg /L for *D.magna* (23), while another study was obtained value from 65 to 133 mg/L for *Daphnia* sp. (24). The LC_{50} values of the present study was near to the LC_{50} values obtained by Jemec *et al.*, (25) who determined LC_{50} in 48 hr. (as LOLC: lower observed lethal concentration) for *D. magna* 10 mg/L. LC_{50} in 48 hr. for two non-target crustacean species *Acellus aquaticus* and *Gammarus fossarum* : 8.5 mg/L , and 1 mg/L respectively was reported by Lukancic *et al.*,(10).
In summary it is likely that the different life stages ,end points used , increase in temperature ,length of exposure periods and the type of organisms are responsible for toxicity exhibition (26).

2. Mortality percentage of *S.vetulus* exposed to insecticide Imidacloprid: Figure (4) demonstrate the mortality percentage (10%) of *S. vetulus* exposed to imidacloprid in concentration of 5 mg/L and 60% in concentration of 20 mg/L after 24hr. of exposure.
The result showed that the mortality percentage increases with an increased concentration of insecticide for the species of cladocera. In previous study they found that imidocloprid had an effect on what is known as (ETS/R) ratios (ETS: Electron transport system and R: Respiration value of crustacean) (10). Therefore it had noxious effect on

these vital processes through high short term concentrations**.**
The movement behavior which was observed in *S.vetulus* included at first slowness, and then the animals became stiff and settle on the bottom of the beakers. The same thing was observed by Ren *et al.* (15), when they exposed *D. magna* to different (OPs) and they suggested that the changes in the movement behavior of cladocera could be considered as an early warning system for aquatic environmental quality. In addition, the biological response of organisms to toxicant can vary among different laboratories (27).

3. Safe concentrations of insecticide Imidacloprid to *S.vetulus*: It is observed that the safe values concentration of imidacloprid to *S.vetulus* were 1.07mg/L and 0.5mg/L. A study reported that the safe concentrations of Al-Daura Refinery waste to two types of cladocera *D.magna* and *S. exspinosus* were higher for *S. exspinosus* than for *D.magna* (21). *S.vetulus* has a comparatively smaller gut length compared to its' body size, the shorter gut passage time may save it from the toxic effects and this may give it this higher tolerant to different pollutants (28).
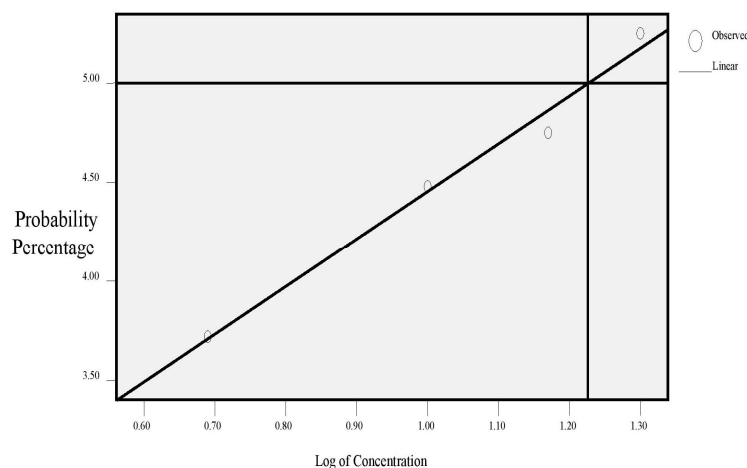
Figure (1): Toxicity curve of the insecticide imidacloprid to *S.vetulus* after 24 hr. of exposure
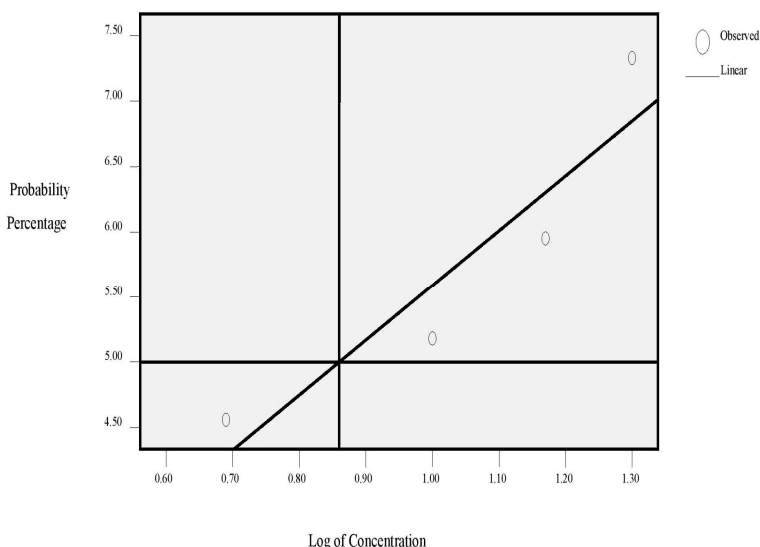
Figure (2): Toxicity curve of the insecticide imidacloprid to *S.vetulus* after 48 hr. of exposure
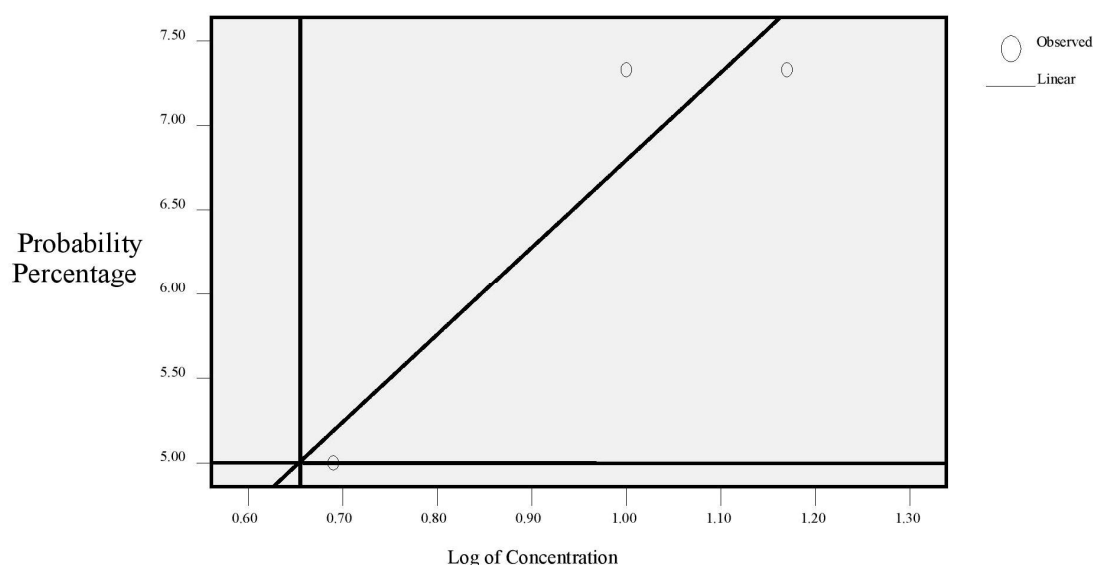
Figure (3): Toxicity curve of the insecticide imidacloprid to *S.vetulus* after 72 hr. of exposure

Table (1): physical and chemical properties of water from the sampling area in Al-Jadyria campus from which *S. vetulus* was collected and filtered river water during the year 2009

Water Sample	Temperature (C°)		Dissolved oxygen (D.O) (mg/L)		Hydrogen ion		Electrical conductivity (µs/cm)		Salinity (ppt)	
	Range	Value obtained	Range	Value obtained	Range	Value obtained	Range	Value obtained	Range	Value obtained
Water of sampling area	18-20	19	6.6-7.5	7.05	7.6-8.06	7.8	882-1098	990	0.54-0.68	0.61
Filtered River Water	18-22	20	-	-	7.9-7.97	7.93	980-1028	1004	0.6-0.63	0.61
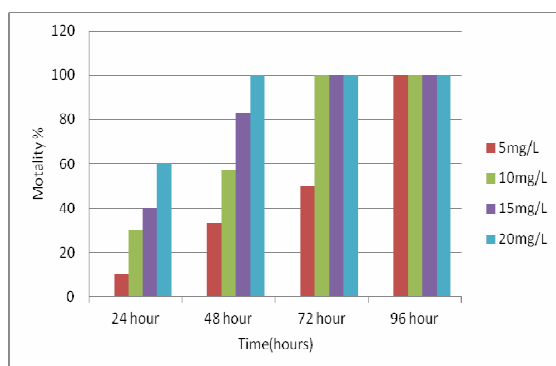
Figure (4): Mortality percentage of *S.vetulus* exposed to insecticide imidacloprid

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Association between Serum Irisin and TNFα in newly onset Diabetic type 2 in Iraqi patients

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ABSTRACT

The current study was conducted to examine the association between serum Irisin and serum TNFα in patients with new-onset T2D. In this cross-sectional study, population was selected from a population-based study and included 100 healthy group(70 male and 30 female) and 100 patient (70 male and 30 female)with new-onset T2D Serum Irisin and TNFα levels and metabolic parameters were measured. Multivariate logistic regression analysis was performed to assess the association between Irisin levels and TNFα newly diagnosed T2D.

The results showed that serum Irisin levels were significantly decreased in the new-onset T2D group compared with the NGT control group (p = < 0.001). Serum TNFα levels were significantly increased   in the new-onset T2D group compared with the NGT control group (p = < 0.001) and no association between serum Irisin and serum TNFα in patient with new-onset T2D.

In the current study, it was found that there is no correlation between serum Irisin and TNFα in patient with new-onset T2D suggesting that Irisin may play a crucial role in glucose intolerance and T2D.

Key words: Irisin , TNFα, T2D

الملخص باللغة العربية

تهدف الدراسة الحالية إلى ايجاد علاقة بين هرمون الايرسين وعامل النخر الورمي الفا في الاشخاص المصابين بداء السكري من النــوع الثــاني المشخصين حديثا.

شملت الدراسة 100 شخص (70 ذكر ، 30 انثى) من المجموعة الضابطة مع 100 مريض (70 ذكر، 30 انثى) من المصابين بداء السكري النوع الثاني، وقد أظهرت النتائج انخفاض نسبة هرمون الايرسين بصورة ملحوظة بالنسبة للأشخاص المصابين بــداء الــسكري مقارنــة مــع المجموعة الضابطة وارتفاع عامل النخر الورمي بصورة ملحوظة بالنسبة للأشخاص المصابين بداء السكري من النــوع الثــاني مقارنــة مــع المجموعة الضابطة ولاتوجد علاقة بين هرمون الايرسين وعامل النخر الورمي في الأشخاص المصابين بداء السكري من النوع الثاني.

خلال الدراسة وجدنا انه لاتوجد علاقة بين هرمون الايرسين وعامل النخر الورمي الفا وان هناك دور حاسم بالنسبة لهرمون الايرسين بالنــسبة للاشخاص المصابين بداء السكري والحساسية المفرطة تجاه الجلوكوز.

INTRODUCTION

The term diabetes mellitus (DM) describes a metabolic disorder of multiple a etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (1) Increasing evidence demonstrates that skeletal muscle produces and releases substances such as cytokines capable of modulating different metabolic processes (2). A novel peptide was recently identified by Bostrom. and has been named Irisin by the researchers after these Greek messenger goddess Iris produced by cleavage from fibronectin type III domain containing 5 (FNDC5) (3). Irisin may prove beneficial not only in monitoring and/or the treatment of obesity and diabetes, but also for a wide range of pathological conditions that are characterized by a variable imbalance of energy demand and expenditure (4,5). Tumor necrosis factor-α is a cytokine initially described as an endotoxin-induced factor causing necrosis of tumors and subsequently shown to be identical to cachexin, a factor secreted by macrophages *in vitro* (6). However, several studies assessed circulating irisin concentrations in obese and/or T2D patients, throwing controversial results and conclusion (7). Therefore, the aim of this study was to evaluate the circulating Irisin levels in T2D patients as well as to determine whether Irisin levels correlate with other commonly used biochemical parameters in clinical medicine.

MATERIALS AND METHODS

This study includes (100) patients (30 female and 70 male) with age range (38-59) year and (100) controls group (30 female and 70male) with age range (39-58) year.Serum Irisin concentrations were measured using commercial ELISA Kit (Causobio China) Serum TNFα concentrations were measured using commercial ELISA kit (demeditec Germany),and serum Insulin concentrations were measured using commercial ELISA kit(DRG company Germany) Glucose, total cholesterol, Triglyceride, urea, uric acid, and creatinine were determined by enzymatic methods and HDL-chol by precipitation (Linear Company Spain) Insulin resistance was calculated using HOMA-IR and β cell function also calculated by using HOMA-β HbA1c was determined (HbA1C kit by CLOVER A1C Korea Infopia).

RESULTS

The age ranges of diabetic patients were 38-59 years with the mean of 46±0.45 years the age range of control patients was 39-58 years with the mean of 46.3±0.43. There were no significant difference between the two groups according to age similarly; there were no significant difference between the study and control groups with respect to BMI. The range of BMI was 19.2–28.8 kg/m^2 with the mean of 23±0.29 for the control group, while the range of BMI was 19.a3–29.4 kg/m^2 with the mean of 23.1±0.27 for the diabetic patients group as shown in table (1).

Table (1): The case-control difference in mean age and BMI

	Study group		
	Healthy controls	Cases (DM)	P
Age (years)			0.57 [NS]
Range	(38-59)	(39-58)	
Mean± SE	46±0.45	46.4±0.43	
N	100	100	
BMI (kg/m²)			0.85 [NS]
Range	(19.2-28.8)	(19.3-29.4)	
Mean± SE	23±0.29	23.1±0.27	
N	100	100	

Table (2) depicts the mean±SE of HbA1c in patients with type 2 D.M & healthy individual, the mean±SE HbA1c the was significant increased mean±SE (9.23±0.08%) in patients compared with the mean±SE (4.96±0.03%) of healthy control group (P<0.001).This table revealed that there was significant elevated in mean±SE of plasma glucose levels in patients with type 2 D.M (151.7±1.05 mg/dl) as compared with mean±SE of control group (84.7±0.58 mg/dl). Also the table shows the mean±SE of plasma glucose level 2 hours after oral glucose tolerance test was significantly increase in the diabetic patients (304.2±1.54 mg/dl) as compared to the healthy individual (99.7±0.43mg/dl) (p< 0.001).The serum insulin concentration showed significant (p<0.001) higher levels in the group of type 2 diabetes mellitus compared to the group of healthy controls group mean±SE (19.05±0.21vs. 6.31±0.19 μIU/ml respectively). HOMA-2- β cell function in the diabetic patients was significantly decreased (p<0.001) in comparison to that of the healthy individuals (67% compared to 94.1%). HOMA-2-insulin resistance was significantly (p<0.001) increased in the diabetic patients compared to its value in the healthy controls (2.7 compared to 0.8).

Table (2): The case-control difference in mean of selected biochemical serum parameters related to glucose control

	Study group		
	Healthy controls	Cases(DM)	P
Blood HbA1c %			<0.001
Range	(4.23-5.77)	(7.6-11.6)	
Mean±SE	4.96±0.03	9.23±0.08	
N	100	100	
Plasma fasting glucose conc.(mg/dl)			<0.001
Range	(73.4-94.5)	(133.5-187.3)	
Mean±SE	84.7±0.58	151.7±1.05	
N	100	100	
Plasma glucose after 2hours (OGTT) (mg/dl)			<0.001
Range	(90.2-107.4)	(273.9-363.4)	
Mean±SE	99.7±0.43	304.2±1.54	
N	100	100	
Serum Insulin conc.(µIU/ml)			<0.001
Range	(2.89-9.89)	(14.4-25.3)	
Mean±SE	6.31±0.19	19.05±0.21	
N	100	100	
HOMA2-B cell function (%)			<0.001
Range	(54-146.5)	(44.3-89.5)	
Mean±SE	94.1±2.13	67±0.94	
N	100	100	
HOMA2-insulin resistance			<0.001
Range	(0.4-1.3)	(2.1-3.6)	
Mean±SE	0.8±0.02	2.7±0.03	
N	100	100	

Table (3) presents the means of serum Irisin, and TNFα in diabetic patients and control individual. The mean±SE Values of serum Irisin and serum TNFα were in diabetic patient were (8.7±0.2 ng/ml), and (11.28±0.11 pg/ml) respectively while in control group were (42.9±0.58 ng/ml) and (7.56±0.11) pg/ml statistical analysis showed significant decrease in mean of Irisin (P<0.001) and increase in mean of serum TNFα (p<0.001).

Table (3): The case-control difference in mean serum Irisin TNF α

	Study group		P
	Healthy controls	**Cases (DM)**	
Serum Irisin conc. (ng/ml)			<0.001
Range	(29.1-56.2)	(4.4-15)	
Mean ±SE	42.9±0.58	8.7±0.2	
N	100	100	
Serum TNF alpha conc. (pg/ml)			<0.001
Range	(5.02-10.23)	(8.98-14.23)	
Mean ±SE	7.56±0.13	11.28±0.13	
N	100	99	

The mean (TNF α) showed no important or statistical differences significant between the three categories of serum Irisin similarly the was no linear correlation with serum Irisin as shown in table (4).

DISCUSSION

Irisin was originally identified as a muscle-derived glycosylated polypeptide It is well established that physical exercise stimulates the transcriptional coactivator PGC-1α in skeletal muscles, resulting in health benefits such as reduction of obesity and insulin resistance. It was hypothesized that PGC-1α stimulates expression of genes involved in obesity prevention and insulin resistance (3). The present study showed that mean serum Irisin level was significantly lower in patient with diabetes mellitus type 2 compared to healthy controls. This negative association between Irisin and DM was reported in recent articlesserum Irisin level was lower in patients with diabetes mellitus type 2 compared to healthy controls. In addition, a negative correlation between the hemoglobin A1C (HbA1c) and circulating levels of Irisin has been also observed.

Thus, the blood concentration of Irisin may reflect the metabolic status of patients suffering from metabolism disorders. In addition to glycemia or HbA1c, 'irisinemia' may also become a new promising concept employed to monitor metabolic disorders such as T2DM or obesity, representing a novel and useful tool in the management of metabolic diseases in the near future (2). TNF-α is an adipocytokine that has been implicated in the development of insulin resistance. Dysregulation of TNF-α production has been implicated in a variety of human diseases including type 2 diabetes mellitusTNF-α is an adipocytokine that has been implicated in the development of insulin resistance. Dysregulation of TNF-α production has been implicated in a variety of human diseases including type 2 diabetes mellitus Serum TNFα was significantly increased in patient with T2DM when compared with healthy controls in the current study. This observation was in agreement with[8] which reported a similar pattern of elevated concentration of serum TNFα among subjects with type 2 diabetes mellitus compared to healthy control subjects There was no correlation between the serum level Irisin and serum level TNFα in patients with type 2 DM in the current study. This negative finding agreed with (8). Irisin is not related to inflammatory process reflected by TNF.

Table (4): The mean TNF α by ordered categories of serum Irisin among cases with DM only

	Serum Irisin conc. (ng/ml)-quartiles			P
	First (lowest) quartile (<=7.3)	**Average (inter-quartile range) 7.4 -10.1**	**Fourth (highest) quartile (10.2+)**	
Serum TNF alpha conc. (pg/ml)	-	-	-	0.74[NS]
Range	(9.07-14.23)	(8.98-13.2)	(9.87-13.04)	
Mean	11.31	11.31	11.21	
SD	1.29	1.04	1.01	
SE	0.25	0.15	0.21	
N	27	48	24	
r=0.011	-	-	-	0.92[NS]

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Study of CA125, HCG and seromucoid in sera of patients with ovarian cancer

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ABSTRACT

Several different types of potential markers have been investigated in ovarian cancer patients. Tumors might elaborate some hormones or other substances that could be detected in serum, urine and tissues of the patients.
The current study was conducted to evaluate the level of CA125, hCG and seromuciod in sera of patients with ovarian cancer to show the usefulness of these biochemical parameters in diagnosis of ovarian cancer.
The study was done on 50 patients with ovarian cancer, 50 healthy individuals and 50 patients with benign ovarian tumors as pathological control. Serum CA125, hCG and seromuciod had been determined for the above groups.
CA125 and hCG were significantly increased in patients with ovarian cancer when compared to healthy individuals and pathological control while seromucoid showed no difference in ovarian cancer in comparison healthy individual and pathological control.
CA125 and hCG might be useful in diagnosis of ovarian cancer. Seromucoid is of no value in diagnosis of ovarian cancer.

Key words: Ovarian cancer,CA125 , hCG

الملخص باللغة العربية

هناك عدة أنواع من معلمات السرطان قد تم تشخيصها في مريضات سرطان المبيض. إن الاورام قد تحرر هرمونات أو مركبات اخرى يتم تعيينها في مصلّ أو إدرار أو أنسجة المريضات.
أجريت الدراسة الحالية لتقييم مستوى hCG, CA125, Seromucoid في أمصال المريضات المصابات بسرطان المبيض لمعرفة أهميتها في تشخيص سرطان المبيض.
تم إجراء الدراسة على عينة شملت خمسين مريضة مصابة بسرطان المبيض وخمسين من الأشخاص الأصحاء وخمسين من المريضات اللواتي لديهن أورام حميدة في المبيض .
تم قياس مستوى hCG ,CA125, Seromucoid للمجموعات تلك وكانت هناك زيادة يعتد بها في كل من CA125 وhCG في مريضات سرطان المبيض عند المقارنة بالأشخاص الأصحاء والعينة المرضية الضابطة، فيما لم تكن هناك أي زيادة محسوسة في Seromucoid.
وبذلك يتبين أن كلا من CA125 و hCG له أهمية في تشخيص سرطان المبيض بينما Seromucoid غير مهم في تشخيص سرطان المبيض.

INTRODUCTION

CA125 is a glycoprotein found on the surface of many ovarian cancers and in small amounts in normal tissues. CA125 is used as tumor marker, which means that CA125 can help in the diagnosis of some types of cancers. Increased level of CA125 may indicate ovarian cancer. However ,there are many other conditions affect CA125 levels as ovulation, menstruation ,endometriosis, benign ovarian cysts ,liver or kidney disease, and other cancers such as breast or lung cancer (1).

hCG is a glycoprotein secreted by the syncytiotrophoblastic cells of normal placenta .It is placental hormone that is also tumor marker for gestational trophoblastic neoplasm and testicular cancer (2). Elevated serum level of hCG had been observed in more than half of patients with ovarian cancer (3).

Seromucoid is the fraction of the plasma protein comprises of glycoprotein, characterized by their solubility in certain precipitant (perchloric, sulphosalcylic and trichloroacetic acids). Seromucoid had been studied as a biochemical marker for the detection of tumors (4). Increased level in plasma seromuciod was reported in cancer in general, highest value was found in widely disseminated cancer (5).

PATIENTS AND METHODS

The study was done on 50 patients with malignant ovarian tumors, 50 patients with benign ovarian tumors as pathological control and 50 normal healthy individuals from April 2011 to June 2013 . This study was done on the Patients who were attending Al-Yarmouk Teaching Hospital. All the patients were diagnosed clinically and by histopathologicl studies.

CA125, hCG and seromuciod were determined in sera for all the above groups.

The collected blood samples were allowed to coagulate at room temperature and then centrifuged for 10 minutes at 3000 rpm. The resulting sera were separated and placed in a test tube, which was then stored in deep freeze until used.

Both CA125 and hCG were measured by Enzyme linked Fluorescent Technique (ELFA) using VIDAS instrument (biomeriux, France).The assay principle combines two-steps enzyme immune assay sandwich method with final fluorescent detection. The Solid Phases Receptacle (SRP) serve as solid phase as well as the pipetting device for the assay (6,7).

The seromucoid was precipitated by the perchloric acid, and then the orcinol reagent was added to form chromogen by reacting with seromucoid and read the absorbance spectrophotometrically at 540 nm (8).

Statistical analysis:

Data were expressed as means ± SD. Student's t-test was used to evaluate differences between the studied groups. For all tests, $p \leq 0.05$ was considered statistically significant. All calculations were made using Excel 2003 program for Windows.

RESULTS

Table (1) represents the comparison of the mean values of CA125 concentration in the sera of ovarian cancer patients, pathological controls and healthy individuals.

The CA125 level in patients with ovarian cancer was significantly increased (P=0.001) when compared to healthy individuals and pathological control.

Table (1): Levels of serum CA125 in patients with ovarian cancer, healthy individuals and pathological control

Group	No.	CA125 (u/ml) (Mean ± SD)	P value
Healthy individuals	50	27.8 ± 5.4	0.001*
Ovarian cancer	50	192 ± 141	
Pathological controls	50	33.2 ± 10.6	0.001 **

*P value for comparison of healthy individuals and ovarian cancer.
**P value for comparison pathological control and ovarian cancer

Table (2) represents the comparison of the mean values of hCG concentration in the sera of ovarian cancer patients, pathological controls and healthy individuals.

hCG values were significantly increased (P = 0.001) in patients with ovarian cancer compared to healthy individual and pathological control.

Table (2): Levels of serum HCG in patients with ovarian cancer, pathological control and healthy individuals

Group	No.	hCG (Iu/L) (Mean ± SD)	P value
Healthy individuals	50	2300 ± 2100	0.001*
Ovarian cancer	50	12700 ± 9600	
Pathological controls	50	2700 ± 2300	0.001 **

*P value: Values for comparing ovarian cancer and healthy individuals
**P value for comparing ovarian cancer and pathological controls

Table (3) shows our findings with respect to seromucoid in healthy individuals, pathological control and ovarian cancer patients. There was no significant difference in seromucoid value in ovarian cancer patients when compared to healthy individuals and pathological controls.

Table (3): Serum seromucoid in patients with ovarian cancer, healthy individual and pathological controls

Group	No.	seromucoid (mg/dl) (Mean ± SD)	P value
Healthy individuals	50	14.5 ± 2.8	NS
Ovarian cancer	50	13.9 ± 3.6	
Pathological controls	50	13.7 ± 1.7	NS

NS: non significant

DISCUSSION

CA125 is an antigen expressed by 80% of patients with epithelial ovarian cancer but less frequently by other types of gynecological malignancies (9), which is agreed with the results of the current study as the level of serum CA125 was higher in patients with ovarian cancer.

It has been postulated that other tumor marker may be elevated in sera of patients with ovarian cancer and that commitment measurement with CA125 may provide a more specific for early detection of ovarian cancer (10).

The current study is in agreement with previously reported result which indicate that serum hCG might be useful in the diagnosis of ovarian cancer (11). The applicability of hCG as tumor marker is variable, however hCG elevation have been found to correlate with poor survival in patients with epithelial ovarian cancer (12).

Increased level of seromucoid have been found in sera of many types of cancer (13).

Our data regarding seromucoid suggest that there was no difference in the level of seromucoid in ovarian cancer patients compared to healthy individuals and pathological control. Seromucoid was of no value in the diagnosis of ovarian cancer.

CONCLUSION

From the results of the current study, we conclude that CA125 and hCG measurements might be useful in diagnosis of ovarian cancer patients. While seromucoid measurement of no value in diagnosis of ovarian cancer patients.

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Torque Teno Virus (TTV) infection and genotypes in Iraqi thalassemia patients

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ABSTRACT

TTV infects patients at risk for parenteral exposure and chronic blood transfusion, such as those with β-thalassemia major. This study was designed to investigate the prevalence of TTV infection in thalassemia patients and furthermore to sequence and analyze phylogenetic of TTV. One hundred fifty four thalassemia patients (64.3% male, 35.7% female) with a mean age of 23.8 ± 6.8 years were involved in this study. TTV DNA was detected using Real time PCR. Furthermore, conventional PCR was done for sequencing and phylogenetic analysis using N22 region from open reading frame 1 (ORF1). TTV was detected in 45 of 154 (29.2%) of thalassemia patients with predominance of males than females (64.4%vs.35.6%), which approximately half (48.9 %) had TTV infection alone and 51.1% had both TTV and HCV infection. The results of genotyping in 12 randomly selected patients showed the presence of equal percentage of genogroup 1 (G1) and genogroup 2 (G2) 50% for each one, with homology between them was 79.0%. Mixed infection of the same patient with multiple TTV genotypes was observed.

Key words: Torque teno virus (TTV), Prevalence**,** HCV, Genotype; Phylogenetic analysis

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الملخص باللغة العربية

إن فيروس تي تي (TTV) يصيب المرضى المعرضين لخطر الحقن ونقل الدم المتكرر، كما في المرضى المصابين بفقر دم البحر الأبيض المتوسط (β-thalassemia major).

صممت هذه الدراسة للتحري عن مدى انتشار فيروس تي تي بين مرضى فقر دم البحرالأبيض المتوسط ودراسة تسلسل وتحليل النشوء والتطور الوراثي لفيروس تي تي . اشتملت عينة الدراسة على مئة وأربعة وخمسين مريضا (ذكور 64.3%، اناث 35.7%) بمعدل عمر 23.8±6.8 سنة، وتم الكشف عن الحامض النووي لفيروس تي تي باستعمال تفاعل البلمرة التسلسلي اللحظي(Real-time PCR)، كما تم استخدام تفاعل البلمرة التسلسلي التقليدي (Conventional PCR) لتحديد تتابعات النيوكليد وتحليل الشجرة الجينية من خلال المنطقة الجينية N22.

لوحظ من نتائج الدراسة أن فيروس تي تي قد كشف عنه في 45 مريضا من أصل 154 (29.2%) مريضا بفقر دم البحر الأبيض المتوسط مع هيمنة الذكور على الإناث(64.4% مقابل 35.6%)، وقد كان ما يقارب نصف المرضى مصابين بفيروس تي تي لوحده(48.9%) و(51.1%) مصابين بفيروس تي تي وفيروس التهاب الكبد نمط ج ، كما بينت نتائج التنميط الجيني لدى 12 مريضا تم اختيارهم عشوائيا أن نسبة وجود G1وG2 متساوية 50% لكل منهما في عينة الدراسة مع نسبة تشابه بينهما 79%.

إن الدراسة الحالية تشير إلى أن G1 و G2 هي السائدة في هذه المنطقة. كما لوحظ وجود إصابة مختلطة لعدة أنماط جينية لفيروس تي تي في ذات المريض.

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INTRODUCTION

Patients with β-thalassemia major are prone to transfusion-related hepatitis because of chronic dependency on blood transfusion, and associated transfusion-related iron overload and transmission of viruses. Some hepatitis viruses are detected. In 1997 by Japanese researchers isolated a new DNA virus by representative difference analysis from the blood of patient with non A to G hepatitis. The virus was named TT virus of the initials of the index patient (initials T.T.) (1). Later, as the main route of transmission was thought to be via blood and blood products, the name "Transfusion Transmitted virus "was also introduced. The newest designation "Torque Tenovirus" refers to the shape of the viral genome, as torque means necklace and tenuous/teno means thin (2).Human TTV is also known an orphan virus (viruses that are not associated with any disease but may cause pathogenicity) (3).TTV consists of small icosahedral, non- enveloped virionwith a diameter of 30-32 nm, single strand DNA and negative sense (4). This virus was classified as a species of the alphatorquevirus genus, anelloviridaefamily (5). TTV DNA is divided into a non-coding region (Untranslated region) of 1.2 kb and coding region of 2.6 kb. In the coding region, contains four partially overlapping open reading frames (ORFs) (6).The untranslated region (UTR) contains a rich area (89-99% of GC content) (7). TTV divided into six groups G1, G2, G3, G4, G5 and G6 and at least 30 genotypes have been described (5). Few subtypes (1a, 1b, 2a, 2b) have also been identified

TTV genotypes have higher variation at amino acid level than at the nucleotide level, but still there is a similarity in the functions of proteins encoded by different genotypes. So divergences of 47–70% have been reported at the amino acid level (8). The occurrence of mutations in ORF1 is much higher than any other proteins of TTV in which change in nucleotides (mutations) leads to amino acid changes and it occurs more frequently than in other protein parts (3). To our knowledge there were no previous study was accomplished in Iraq regarding TTV, therefore, this study has been under taken to investigate the prevalence of TTV, co-infection with HCV and genogroups of TTV infection in Iraqi thalassemia patients in thalassemia patients on chronic transfusion therapy.

PATIENTS AND METHODS

Blood samples:

Blood samples were taken from154 β- thalassemia patients (99 male; 55 female), aged (18-42 years) who received regular blood transfusions, which had been conducted between February to December 2013 from Al-Karama teaching hospital and Ibn Al-Baladi hospital maternity and children's hospital.

Screens test for hepatitis C:

All the patients were routinely screened for hepatitis C virus (HCV) infection by using ELISA and western blot to detect and confirm the presence of HCV infection (9,10).

Extraction of DNA:

The DNA genome of TT virus was extracted from all EDTA-treated blood samples of patients by Wizard Genomic DNA Purification kit (Promega, USA), and TTV DNA was detected for all the patients by real- time PCR (Liferiver, China) (11).

Genotyping:

Randomly twelve samples were selected to determine the genotypes. Primers from the ORF1 (N22 region) (Table 1), as described by Okamoto *et al*., (12), were designed for amplification of most divergent variants currently described to date by semi-nested PCR. PCR was performed in a 25 μl total volume containing 12.5 μl master mix (Go taq green master mix); 0.8 μl of each primer, DNA template 5 μl and 5.9 μl nuclease free water. Briefly, nucleic acid was extracted and amplified using standard PCR conditions by a first run of PCR performed with primers NG059 and NG063 for 35 cycles (94ºC, 30 s; 58ºC, 30 s; 72ºC, 45 s), plus an additional cycle of 72ºC for 7 minute, second run PCR NG063 with NGo61 (antisense) for 25 cycles at the same conditions (12). A 2% agarose gel electrophoresis was used to locate the protein bands and its percentages by Photo Capt Molecular Weight Software, 2001) (Figure 1) (13).

Table (1): The oligonucleotide primers used for genotyping

	Primer	Sequence
1	NG059	5′-ACA GAC AGA GGA GAA GGC AACATG-3′
2	NG063	5′- CTG GCA TTT TAC CAT TTC CAA AGT T- 3′
3	NGo61	5`-GGC AAC ATG TTA TGG ATA GAC TGG 3`

PCR sequencing:

Sequencing of PCR product was carried out by NICEM Company/ (South Korea) using an ABI 3730XL DNA Analyzer according to method described by Okamoto (12). Homology search was conducted between the sequences of standard gene BLAST program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and using BioEdit program. Evolutionary analysis was conducted in MEGA 5 (14).
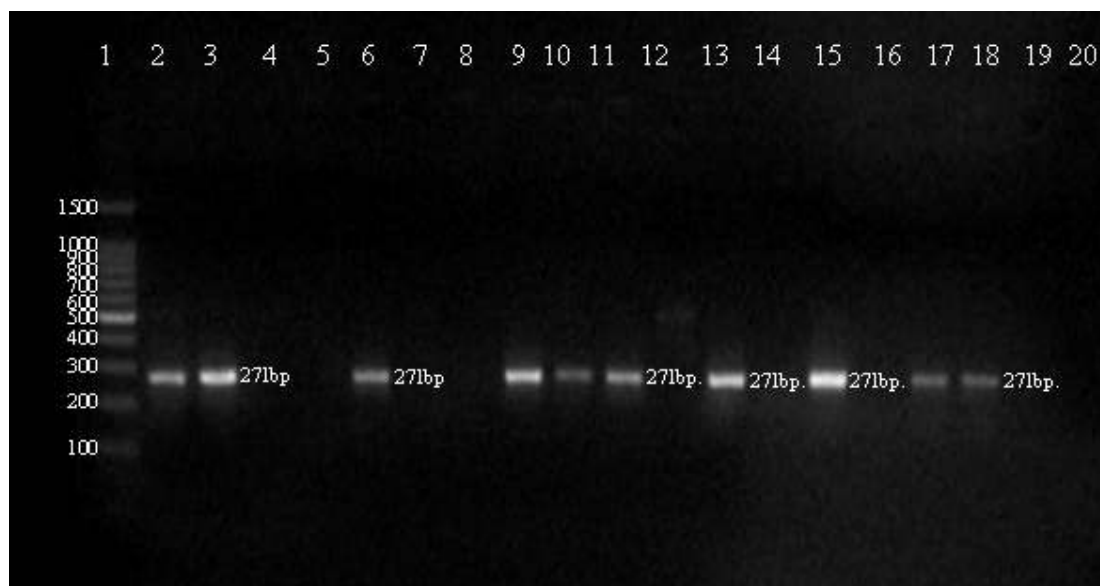
Figure (1): Detection of TTV-DNA by nested PCR. PCR products were separated on 2% agarose gel electrophoresis and were stained with ethedium bromide.Lane1 -100 base pair DNA ladder. Lanes 2,3,6,9,10,11,13,15, 17,18,show positive TTV DNA viraemia at 271bp. Lane 4, 5, 7, 8, 12, 14, 16, 19, 20, Negative TTV-DNA

Statistical analysis:

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22).

The significance of difference between percentages (qualitative data) was tested using chi-square test (χ2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

RESULTS

Out of 154 blood samples taken from thalassemia patients, 45 samples were positive to TTV. The prevalence rate was 29.2% for total samples examined in this study, and the prevalence rate of 64.4% males and 35.6% females. Figure (2).

However, there was no significant difference in prevalence rate between males and females 29.3% vs.29.1% respectively among thalassemia patients, neither with age, nor with frequency of blood transfusion. (Table 2).
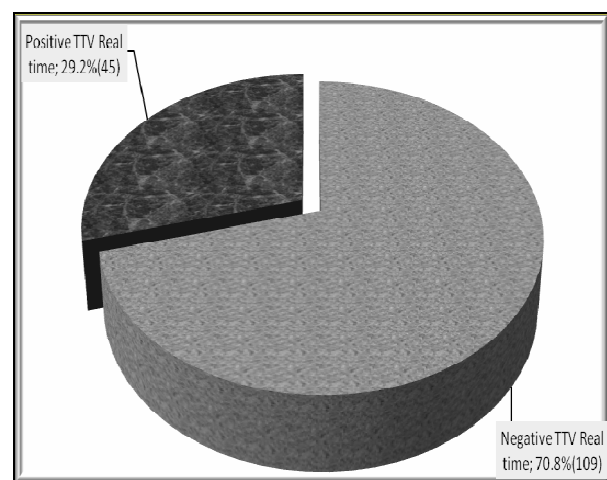
Figure (2): The prevalence of TTV in thalassemia patients

Table (2): TTV in thalassemia patients according to age, gender, and frequency of blood transfusion

		TTV (Real time PCR)				P value
		Positive		Negative		
		No.	%	No.	%	
Age (years)	<20	10	21.3	37	78.7	0.135
	20- 24	22	40.0	33	60.0	
	25 -29	6	20.7	23	79.3	
	=>30	7	30.4	16	69.6	
Blood transfusion pints	10-	3	27.3	8	72.7	0.962
	20-	10	31.3	22	68.8	
	30-	13	25.5	38	74.5	
	40-	9	32.1	19	67.9	
	=>50	10	31.3	22	68.8	
Gender	Male	29	29.3	70	70.7	0.979
	Female	16	29.1	39	70.9	

*Significant using Pearson Chi-square test at 0.05 level*

According to their HCV antibodies and DNA of TTV and TTV detection in blood patient, the patients in current study were divided into four subgroups (figure 3): (Anti –HCV Ab+ TTV+; Anti-HCV Ab+ TTV- ; Anti- HCV Ab- TTV+; Anti-HCV Ab -TTV-).Table (3) shows that there were no significant differences in prevalence of TTV infection among thalassemia patients with or without anti-HCV Abs.

Nucleotide sequence and phylogenetic analysis of 12/45 (26.67%) TTV positive isolates belong to G1(50%), G2 (50%) and similarity of these were 79.0.%.This allowed us to construct a phylogenetic tree based on a partial polymerase 271 bp fragment of N22 in ORF1.Phylogenetic tree for each sample was obtained as seen in figure (4).

Genotyping results for the amplified region of each sample are shown in table (4). Nucleotide sequence analysis showed that there are TTV genotypes in Iraqi thalassemia patients were closely related to the novel isolates that isolated from Italy, Iran and Poland that deposited in the GenBank with accession no.AY032886.1, GQ179964.1, AJ309730.1 and considered as new genotypes of the TTV family and unclassified until the time. Sequencing of TTV clones from thalassemia patients showed the presence of different variants in the same serum.
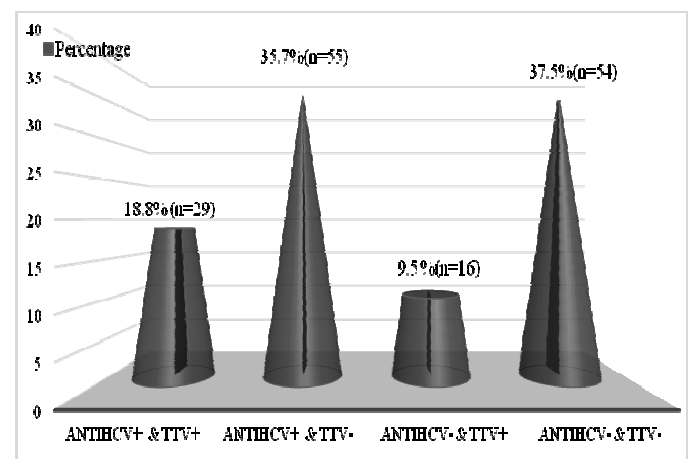
Figure (3): Demographic of 154 thalassemia patients according to TTV and HCV infection

Table (3): TTV in thalassemia patients with or without anti-HCV Abs

		TTV (Real time PCR)				P value
		Positive		Negative		
		No.	%	No.	%	
Anti-HCV Abs	Positive	29	34.5	55	65.5	0.113
	Negative	16	22.9	54	77.1	

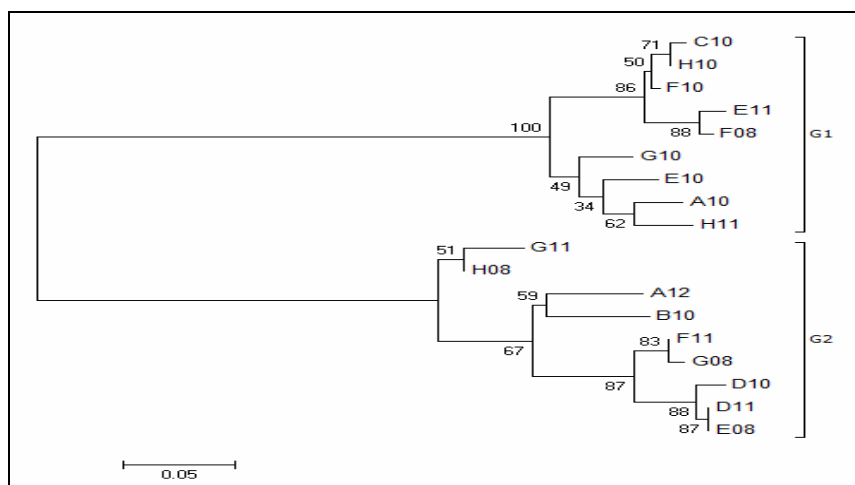*Significant using Pearson Chi-square test at 0.05 level*

Figure (4): Phylogenetic tree on the basis of the TT virus partial open reading frame 1 (ORF1) sequence as constructed by the neighbor-joining (N-J) method. Note that there are two major clusters, tentatively named G1, G2

Table (4): The characteristics of genotyped TTV -positive clinical samples

	Name of sample	Name of gene	Accession N.	Homology		Country
					%	
1	1_Primer-63_D11	TT virus isolate 7K NS-HD ORF1 gene, partial cds	AY032886.1	Homo sapiens	99%	Italy
2	1_Primer-63_E08	TT virus isolate 7K NS-HD ORF1 gene, partial cds	AY032886.1	Homo sapiens	99%	Italy
3	4_Primer-63_F11	TT virus isolate 7K NS-HD ORF1 gene, partial cds	AY032886.1	Homo sapiens	99%	Italy
4	9_Primer-63_D10	TT virus isolate 7K NS-HD ORF1 gene, partial cds	AY032886.1	Homo sapiens	99%	Italy
5	2_Primer-63_F08	TT virus clone BTRL-09 ORF1 gene, partial cds	AF397741.1	-	97%	-
6	8_Primer-63_C10	TT virus clone BTRL-09 ORF1 gene, partial cds	AF397741.1	-	99%	-
7	15_Primer-63_H10	TT virus clone BTRL-09 ORF1 gene, partial cds	AF397741.1	-	100%	-
8	11_Primer63_F10	TT virus clone BTRL-09 ORF1 gene, partial cds	AF397741.1	-	99%	-
9	2_Primer-63_E11	TT virus clone BTRL-09 ORF1 gene, partial cds	AF397741.1	-	96%	-
10	4_Primer-63_G08	TT virus isolate 7K NS-HD ORF1 gene	AY032886.1	Homo sapiens	95%	Italy
11	5_Primer-63_G11	TT virus isolate G75 long ORF gene, partial cds	AF067981.1	isolated from human serum	93%	-
12	5_Primer-63_H08	TT virus isolate G75 long ORF gene, partial cds	AF067981.1	isolated from human serum	95%	-
13	6_Primer-63_A10	Torque Teno virus isolate Isfahan MEF5 ORF1 gene, partial cds	GQ179964.1	Homo sapiens	93%	Iran
14	6_Primer-63_H11	Torque Teno virus isolate Isfahan MEF5 ORF1 gene, partial cds	GQ179964.1	Homo sapiens	90%	Iran
15	7_Primer-63_A12	TT virus ORF1, clone D-95nt	AJ309730.1	Homo sapiens	91%	Poland
16	7_Primer-63_B10	TT virus ORF1, clone D-95nt	AJ309730.1	Homo sapiens	89%	Poland
17	14_Primer-63_G10	Torque teno virus isolate Isfahan MEF5 ORF1 gene, partial cds	GQ179964.1	Homo sapiens	97%	Italy
18	10_Primer-63_E10	Torque teno virus isolate Isfahan MEF5 ORF1 gene, partial cds	GQ179964.1	Homo sapiens	96%	Iran

DISCUSSION

The prevalence of TTV infection was found to be 29.2% in a sample of Iraqi thalassemia patient by detection of nucleic acid using Real-time PCR. The prevalence of TTV among thalassemia patients was reported to vary from 50% to 100% in different studies elsewhere due to differences in diagnostic techniques, study sample size, and geographic distribution (15, 16).

Moreover, the prevalence of TTV DNA in current study is generally low compared with other studies elsewhere (16, 17) that showed prevalence of TTV was 100% in contrast to other studies which showed 39.4% in Egypt and 50.5% in Iran respectively(18,19).

The current study showed also prevalence of TTV infection in thalassemia patients co-infected with hepatitis C implying that HCV and TTV may share common modes of transmission routes such as blood transfusion (20, 21).

It could be concluded from the presented data that age, gender, and frequency of blood transfusion did not differ significantly between TTV-positive and TTV-negative thalassemia patient groups. This conclusion found to be consistent with other studies elsewhere (22,23), and inconsistent with result of previous evaluation of TTV infection in 250 thalassemia patients in Ahwaz which showed that there was a significant correlation between TTV infection, age and history of blood transfusion (24).

TTV is characterized by an unusually high degree of sequence variability compared to other DNA viruses so several distinct TTV genotypes have been described (12, 25, 26). The PCR assays developed soon after the discovery of the virus, were not able to amplify DNA of all genotypes, and the TTV prevalence in the populations of various countries have therefore been dramatically underestimated. Recently, improved PCR protocols and new sets of primers have led to increased rates of TTV DNA detection. In our study, the genotyping of TTV were G1 and G2 based on the N22 region (271 bp approx.) in ORF1. It should be noted that the primers we used do not permit the detection of all TTV genotypes, although they allow the detection of the main TTV genotypes reported in other studies. The results of the present study are in parallel with data published from other parts of the world where the TTV genotypes 1 and 2 have been described in several countries including Japan, Thailand, United Kingdom, and Germany. Other genotypes have also been reported in these regions (27- 29). So, the distribution of the major TTV genotypes, G1 and G2, was not related to their geographic distribution. This suggests that TTV, a single stranded DNA virus, probably spread all over the world a long time ago and coexisted with humans for long time without shows any signs of disease (25).

Even sequencing of TTV clones from thalassemia patients showed that one patient had multiple TTV variants (15). Mixed infections of TTV have been reported in individuals at high risk for infection with parenterally transmitted viruses, such as intravenous drug users, hemophiliacs (4), and hemodialysis patients (7), as well as in patients with liver disease (25). In our study, several clones derived from the same serum were sequenced to determine co-infection (or superinfection) with multiple TTV strains occurred .That infection by a given genotype is not protective against the superinfection by another type (30). Furthermore, the number of TTV isolates infecting an individual can be high due to the frequency of blood transfusion from an individual may be carrier of these viruses , or there are many possible reasons which suggest higher than expected rate of mutation in TTV (31).

Since there are no reports available showing the prevalence of this virus in Iraq, it is not possible to support or contradict other observations and therefore this report should be taken as accepted at this stage. The results concluded that TTV is moderately present in Iraqi thalassemia patients, with G1 and G2 were predominant. Multiple TTV variants from one patient was sequenced in this work and proved to be possible.

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Calculating some parameters of quadrupole magnet to focus ions beam from plasma source

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ABSTRACT

In the present study, a computational investigation has been introduced to calculate some parameters of quadrupole magnet and included theoretical analysis using matrices representation because it is best method to represent and tracing the charged particles beam along any optical system, using matrix representation could be analysis the motion of charged particles and calculate both of the bandwidth (envelope) and the angle of rotation in both the horizontal and vertical planes, take in consideration that the motion of the particles in each level is independent of another level. The results show good focusing properties when the value of the length of quadrupole magnet is equal to (400mm), has been get minimum value for envelope in both horizontal and vertical planes.

Key words: quadrupole, Magnetic Lenses, Transfer Matrix, Focus, Plasma

الملخص باللغة العربية

في الدراسة الحالية، تم عرض تفسير حسابي لحساب بعض المعلمات لمغناطيس رباعي و تضمن تحليلا نظريا باستخدام المصفوفات باعتبارها الطريقة الأمثل لتمثيل وتتبع حزمة الجسيمات المشحونة خلال أي منظومة بصرية، ويمكن باستخدام تمثيل المصفوفة تحليل حركة الجسيمات المشحونة وحساب كل من عرض الحزمة وزاوية الدوران في كل من المستوى الأفقي والعمودي، بافتراض أن حركة الجسيمات في كل مستوى غير معتمدة على المستوى الآخر. بينت النتائج صفات تبئير جيدة عندما تكون قيمة طول مغناطيس رباعي القطب مساوية ألى (400 mm)، التي لديها أقل قيمة لعرض الحزمة في كل من المستوى الأفقي والعمودي.

INTRODUCTION

The most obvious components of particle accelerators and beam transport systems are those that provide the beam guidance and focusing system. Whatever, the application may be, a beam of charged particles is expected by design to follow closely a prescribed path along a desired beam transport line or along a closed orbit in case of circular accelerators (1). Furthermore, ion and electron optics was extended to include the focusing of beams in circular accelerators. New types of focusing systems, such as quadruple lenses, edge focusing in sector-shaped magnets, alternating-gradient focusing, and so on, were invented and contributed to the successful development of plasma physics and accelerators with steadily increasing energies and improving performance characteristics. New interest in particle dynamics came also from space science, industrial applications of electron-ion beam devices (welding, micromachining, ion implantation, charged particle beam lithography) (2).

 At their core, these systems consist of a sequence of electromagnetic devices through which a beam of charged particles passes. There are many different types of electromagnetic devices like magnet, where it used to guide a charged particle along a predefined path, magnetic fields are used which deflect particles as determined by the equilibrium of the centrifugal force and the Lorentz force. The quadrupole magnet is important part into the system of charged particles beam transport. It works as a focusing element in one axis and defocusing in the other axis (1).

This work aimed to study the effect of length of quadrupole magnet on focus the charge particles beam by selection the different of lengths.

THEORITICAL FORMALISM

1. Particle Beam Focusing:

Similar to the properties of light rays, particle beams also have a tendency to spread out due to an inherent beam divergence. The characteristic property of such focusing lenses is that a light ray is deflected by an angle proportional to the distance of the ray from the center of the lens. With such a lens a beam of parallel rays can be focused to a point .To keep the particle beam together and to generate specifically desired beam properties at selected points along the beam transport line, focusing devices are required (1). The most suitable device that provides a material free aperture and the desired focusing field is called a quadrupole magnetic lens (3,4).

2. Magnetic Lenses:

A magnetic lens is a device for the focusing or deflection of moving charged particles, such as electrons or ions, by use of the magnetic Lorentz force. Its strength can often be varied by usage of electromagnets. Magnetic lenses are used in diverse applications, from cathode ray tubes over electron microscopy to particle accelerators. A magnetic lens typically consists of several electromagnets arranged in a quadrupole, sextuple, or higher format; the electromagnetic coils are placed at the vertices of a square or another regular polygon. From this configuration a customized magnetic field can be formed to manipulate the particle beam (5).

3. Quadrupole Magnet:

A quadrupole is a magnetic element that has four poles, two norths and two souths. They are symmetrically arranged around the centre of the magnet. There is no magnetic field along the central axis (6). These magnets are used to focus the particle beam. In quadrupole magnet the field lines all cancel each other out at the centre of the quadrupole so a particles beam feels no force as it passes through the centre. The further from the centre of the quadrupole we get the stronger the field gets so particles beam which are further off axis get focused more strongly. It can be seen that while the particles beam is being focused in the vertical direction it is simultaneously being defocused in the horizontal direction (1,2). Consequently two different types of quadrupole have to be used, first type in (x-y) plane and the other is the same thing rotated through (90 degree). This rotated version will cause the particles beam to be focused in the horizontal plane and defocused in the vertical plan. When arranged correctly a series of quadrupole can lead to net focusing in both planes (7). Generally, quadrupoles, which focus in the horizontal plane are often referred to as focusing quadrupoles (QF) whereas quadrupoles which focus in the vertical plane are often referred to as defocusing quadrupoles (QD) (6).

4. Transfer Matrix of the Quadrupole Magnet:

Transfer matrices describe changes in the transverse position and angle of particles relative to the main beam axis. If x and y are the coordinates normal to z, then a particle orbit at some axis position can be represented by the four dimensional vector (x, x^-, y, y^-), the quantities x^- and y^- are angles with respect to the axis. An optical element operates on an entrance orbit vector to generate an output orbit vector. The transfer matrix represents this operation. Orbits can therefore be represented by two independent two dimensional vectors, X=(x, x^-) and Y=(y, y^-). This separation holds for other useful optical elements, such as the

magnetic sector field (7,8). In a single plane, the trajectory of a particle at a given position s determined by its phase space coordinates:

$$X(s)=[\ x(s)\ \ x^{\cdot}(s)]\ \ ……………(1)$$

Assuming particles of nominal momentum ($\delta=0$) the equation of motion inside a quadrupole magnet takes the form of a simple harmonic oscillation:

$$x'' + kx = 0 \ …………………(2)$$

Whose solution can be written in matrix formalism (8,9):

$$X(s)=MX_0 \ ………………….............(3)$$

With $X_0=X(0)$ and

$$
M=
\begin{cases}
\begin{pmatrix} \cos(\sqrt{k}L) & \frac{1}{k}\sin(\sqrt{k}L) \\ -\sqrt{k}L\sin(\sqrt{k}L) & \cos(\sqrt{k}L) \end{pmatrix} & \text{if } k < 0 \text{ (focusing)} \\[2em]
\begin{pmatrix} 1 & s \\ 0 & 1 \end{pmatrix} & \text{if } k = 0 \text{ (drift space)} \quad (4) \\[2em]
\begin{pmatrix} \cosh(\sqrt{k}L) & \frac{1}{k}\sinh(\sqrt{k}L) \\ \sqrt{k}L\sinh(\sqrt{k}L) & \cosh(\sqrt{k}L) \end{pmatrix} & \text{if } k > 0 \text{ (defocusing)}
\end{cases}
$$

M is called the transfer matrix of the quadrupole. The two main parameters that show in equation (4) are k and L, where L represented the effective length of quadrupole magnet (in metre) and k represented the normalized gradient (k= 0.004 mm^{-2}) (7,10). The mathematical model of a quadrupole we have used is one where the field gradient is constant inside the quadrupole and zero outside, this behavior of the field is called the hard-edge model (8).

RESULTS AND DISCUSSION

The purpose of any system of charged particles beam (ion beam) focusing mostly is to obtain the minimum spot of charged particles beam, that means, minimum magnification, that is achieved to good control for any application. Table (1) represents the main configurations (parameters) of charged particles beam through quadrupole magnet, when using (Ar$^+$) ions as charged particles extracted from the source of plasma. Table (2) represents the different values of effective length of quadrupole

magnet with the values of magnifications and displacement maximum of both x and y. It is clear that, when the effective length is (400 mm) the minimum size of beam (the shape of phase space ellipse nearly waist). (1)

Table (1): The main parameters

Parameters of Source	The value
Extraction voltage (kV)	30
x_0 (mm)	10
$x_0^{\cdot}$ (mrad)	0.039
y_0 (mm)	15
$y_0^{\cdot}$ (mrad)	0.007
B_0 (Gauss)	3000

That means good properties of charged particle beam at target region. Figure (1) represents the relation between the different values of maximum displacement of x (beam envelope) as function of displacement direction (the beam path (s)) for different values of effective length of quadrupole magnet to horizontal plane. We note from this figure, the increasing in (L) causes decreasing in beam envelope, while figure (2) for vertical plane, the opposite action of quadrupole magnet appears. Also from this figures could be note the opposite action of quadrupole magnet as converge element for the horizontal plane and diverge element for vertical plane that agree with many authors (9,10). We note clearly, the minimum size of beam in (L=400 mm).

Table (2): The values of magnification and both x and y displacement max, with different values of effective length of quadrupole magnet

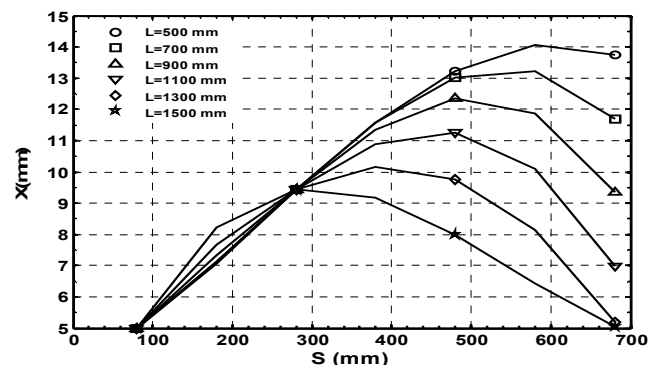| L mm	x mm	y mm	Mag.$_x$ x/x$_o$	Mag.$_y$ y/y$_o$
200	13.743	11.231	1.040	1.071
250	11.693	11.553	0.898	1.082
300	9.351	11.908	0.757	1.092
350	6.988	12.298	0.620	1.102
400	5.194	12.724	0.532	1.111
450	5.033	13.185	0.628	1.100

Figure (1): Effect of the length on beam envelope as a function of displacement direction for horizontal plane
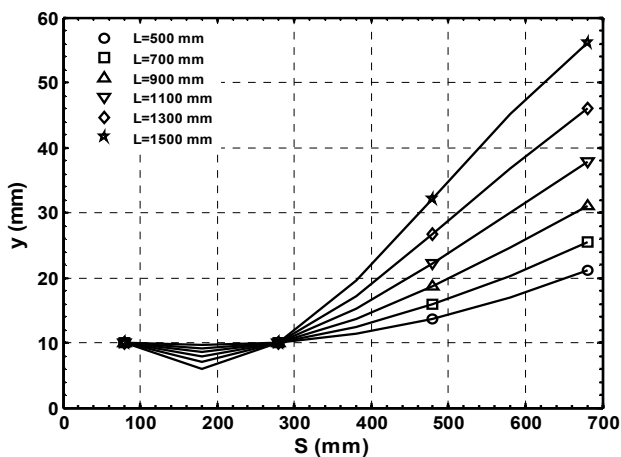
Figure (2): Effect of the length on beam envelope as a function of displacement direction for vertical plane

CONCLUSION

1. Quadrupole magnet acts as converging element for the horizontal plane while acts as diverging element for vertical plane.
2. The increasing in (L) causes decreasing in beam envelope for horizontal plane, the opposite action appears for vertical plane.
3. The best focusing properties of quadrupole magnet obtained when (L) equal to (400 mm).

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Preparation and electrical, mechanical properties of $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$ (n = 1, 2, 3) superconductors

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ABSTRACT

Multilayered cuprates of $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$, with n = 1–3 have been synthesized by using solid state reaction method in order to investigate the variation of transition temperature T_c. The temperature dependence of resistance showed that the T_c depends on n. The Vickers hardness of the observed phases obey, an increase in Cu-O layers. The superconducting transition temperature is determined by electrical resistively-temperature dependency and rises reaching a maximum value of 105 K at *n*= 3

Key words: superconducting transition temperature Tc, electrical resistivity, Vickers hardness (HV), layer structures

الملخص باللغة العربية

تم تصنيع مركب متعدد الطبقات $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$ حيث (3 -1 = *n*) بطريقة تفاعل الحالة الصلبة لبحث التغير في درجة حرارة الانتقال الحرجة T_c باعتماد المقاومة على درجة الحرارة. وأظهرت النتائج أن T_c تعتمد على (n) ، أما تغير صلادة فكرز للأطوار فقد وجد أنها تخضع إلى زيادة في طبقات Cu-O ، وأن درجة انتقال فائق التوصيل يتم تحديدها بدرجة انتقال المقاومة الكهربائية ، وترتفع وصولا إلى قيمة 105كلفن.

INTRODUCTION

Ceramic high temperature superconductivity (HTSC) has confounded many theorists (1). As Compared to normal metals these "poor metal" ceramics exhibit many transport anomalies even in their normal state, so it is scarcely surprising that their superconductive properties should be even more unexpected. All known microscopic theories of metallic superconductors are based on the BCS model of Cooper pairs formed by attractive electron-phonon interactions that overwhelm repulsive Coulomb interactions, yet many features of electron-phonon interactions in the BCS theory that are confirmed in metals, appear to be weak or absent in the infrared spectra of ceramic HTSC (2). The Bi-Sr-Ca-Cu-0 superconductor, which was discovered by Maeda *et al.* (3). It is considered as one of the important materials for device applications. Impurity substitution in the cuprate superconductors has a powerful of the basic electronic properties. For example, Pb doping of Bi-Sr-Ca-Cu–O superconducting systems induces a partially melted liquid phase that eases the diffusion of the element to form the high-Tc phases and to increase their critical temperature (4). Several alloying elements, such as MgO can be added to improve the electrical properties of polycrystalline 2212, and the addition of MgO was reported to have beneficial effects on the grain connectivity and grain alignment in fully processed samples of Bi2223 and on its critical current value (5). The effect of Pb substitution on the high-T_c superconductor Bi-Sr-Ca-Cu-O was studied. They found that the zero resistivity temperature $T_{c(offset)}$ was increased with the increase of Pb content.

Maximum $T_{c(offset)}$ value of 85.5 K was obtained for the composition $Bi_{0.5}Pb_{0.5}SrCaCu_2O_\delta$, enhancement of Pb caused a decrease in T_c. it was observed that oxygen annealing degraded the superconductivity of Bi-Pb-Ca-Sr-Cu-O as Pb≈0.3 (6), while it improves the superconductivity properties of samples with Pb≈0.4(7). samples were prepared with a nominal composition $Bi_{1.84}Pb_{0.34}Sr_{1.91}Ca_{2.03}Cu_{3.06}O_\delta$ by solid state reaction with T_c=110K. Also they studied crystal structure by XRD and their results showed that the compound have the structure for the 2223-phase (8).

Mechanical property that may be important to consider is hardness, which is a measure of a material's resistance to localized plastic deformation ,and Quantitative hardness techniques have been developed over the years in which a small indenter is forced into the surface of a material to be tested, under controlled conditions of load and rate of application. Two other hardness-testing techniques are Knoop (pronounced *nup*) and Vickers (sometimes also called diamond pyramid). For each test a very small diamond indenter having pyramidal geometry is forced into the surface of the specimen. Applied loads are much smaller than for Rockwell and Brinell, ranging between 1 and 1000 g. The resulting impression is observed under a microscope

and it is measured; this measurement is then converted into a hardness number (9) . In the present work we have successfully to prepare $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$ bulk polycrystalline for n=1, 2 and 3 by used solid state reaction process, and study the electrical ,mechanical properties of different n synthesized at the optimum conditions.

EXPERIMENT AND METHODS

The synthesis of samples with chemical formula $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$ for n=1, 2 and 3 have been performed by solid state reaction method, using appropriate weights of pure powders of Bi_2O_3, BaO, CaO, and CuO. The powders were mixed together by using agate mortar. The mixture and to form slurry during the process of grinding for about 24hrs. The mixture was dried for an oven at $250^\circ C$. The powder was pressed into disc-shaped pellets 1.5 cm in diameter and 0.21 cm thick, using hydraulic press under a pressure of 7 ton/cm^2.The pellets were put in furnace that has programmable controller, for sintering. For these pellets were heated to temperature of 750 $^\circ$C for 120 hours with a rate of $2^\circ C$ /min, then cooled to room temperature by the 0.5 $^\circ$C /min. A four-point probe is the most common method to determine the T_c of a superconductor (10). Wires are attached to a material at four points with a conductive adhesive. Through the outer two of these points a voltage is applied and if the martial is conductive a current will flow. Then, if any resistance exists in the material a voltage will appear across the inner two points in accordance with ohm's law. When the material enters a superconductive state, its resistance drops to zero and no voltage appears across the second set of points. Figure (1) shows the circuit diagram of the resistivity measurement.
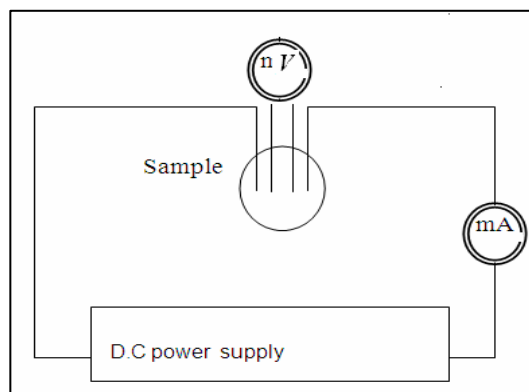

Figure (1): Circuit diagram of the sample of resistivity measurement

The cryostat system joined to a rotary pump to get a pressure of [$6*10^{-2}$ mbar] inside the cryostat, and is also joined to a sensor of a digital thermometer type [KT] and thermocouple type K near the sample position. Four wires have been connected to the cryostat. The two outer most leads for a current and two inner leads for a voltage. A small current passes through the sample, by a current source D.C power supply. The resistivity [ρ] could be found from the relation:

$$\rho = \frac{V}{I}\frac{wt}{L} \quad \textbf{(four points probe)}\ldots\ldots\ldots\ldots\ldots\ldots\text{[1]}$$

where,   I: is the current passing through the sample, V: is the voltage drop across the electrodes, t: is the thickness of the pellet ,L: is the length of the sample ,w: is the width of the sample or found from equation(1):

$$\rho = 4.5324\ \frac{V}{I} \quad \textbf{(linear-four points probe)} \ldots\ldots\ldots\text{[2]}$$

The critical temperature T_c could be found from this figure or from the relation:

$$\textbf{T}_c = \textbf{(T}_{c1}\textbf{+T}_{c2}\textbf{) / 2}\ldots\ldots\ldots\ldots\ldots\text{[3]}$$

where T_{c1}: the onset of the transition temperature, T_{c2}: the offset of the transition temperature at the zero resistivity point.(12). Vickers hardness [HV] measurements of the sample produced are performed in air by using a digital micro hardness tester at room temperature   ,where   they are determined by dividing the applied force, *F*, by this area, a, (13).

$$\textbf{(VHN= 1.854}F/a^2\textbf{)}\ldots\ldots\ldots\ldots\text{[4]}$$

RESULTS AND DISCUSSION

Figures (2,3) display the temperature dependence of the normalized resistivity ρ for $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$,with n = 1–3 phases. All samples were characterized by a metallic behavior from room temperature down to 200 K. Basically, the normal metallic behavior reflects the conduction along the CuO planes. It were found that the values of critical transition temperature at zero resistivity temperature T_c(offset) and onset T_c(onset) for grown $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$, with n = 1–3 phases are 79, 85 and 105 K respectively.
It is clear from table (1) that the increase of the Cu-O layers which leads to increase the holes in the structures thus enhanced the transition temperature from 79 K to 105 K and reduced the transition width ΔT, they mean that the increasing multiphase

2201,2212,and 2223 (14). Therefore, the ΔT are increasing as shown in figure (2).
Figure (3) shows the change of Hardness values HVn as function Cu-O layers. Hardness value decreases with increasing Cu-O layers, the possibility for very large variations in values depending on technique used and microstructure surface effects . This means   increasing the value (n) lead to increasing brittleness of the samples , because number increase Cu-O layers on length c-axis.
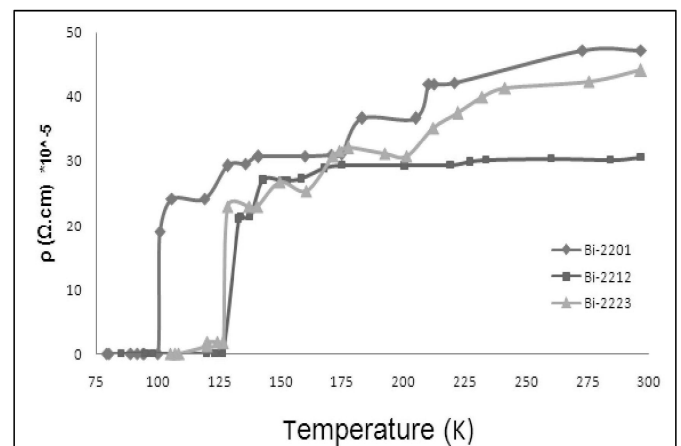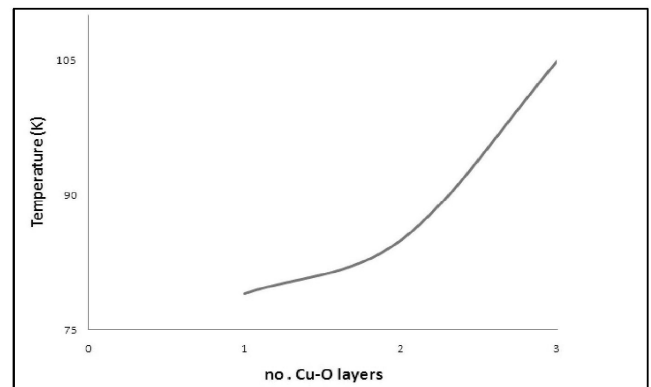

Figure (2). Resistivity versus temperature for $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$, with n = 1–3 phases

Figure(3): critical transition temperature with n = 1–3 phases for $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$.

Table (1): Values of transition temperature T_c(offset), T_c(onset), and HVn for $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$, with n = 1, 2, and 3

n	Samples	$T_{c(Offset)}$(K)	$T_{c(onset)}$(K)	ΔT	HVn
1	$Bi_2Ba_2Cu_1O_{6+\delta}$	79	100	89.5	421.05
2	$Bi_2Ba_2Ca_1Cu_2O_{8+\delta}$	85	120	102.5	404.4
3	$Bi_2Ba_2Ca_2Cu_3O_{10+\delta}$	105	133	119	395.88

CONCLUSION

Three samples of $Bi_2Ba_2Ca_{n-1}Cu_nO_{2n+4+\delta}$, with n = 1–3 were synthesized by solid-state reaction method. By increasing n, the transition temperature is increased from 79 at n=1 to 105 at n=3. The increase of Cu-O layers produced a decrease in Vickers hardness (HV), from 421.05 to 395.58 that mean the mass densities of the samples are with increasing the Cu-O layers.

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A CASE REPORT

Diagnosis of primary ES/PNET of the breast

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ABSTRACT

ES/PNETs are composed of small, malignant, undifferentiated cells and often presented as bone or soft tissue masses in the trunk or axial skeleton in adolescents and young adults. It was firstly described in 1918. Peripheral PNETs which was first described in 1979 are tumors that originated in the soft tissue of the chest wall, occasionally in bone, and, rarely, in the peripheral lung. Exceptionally these may occur in the kidney, ureter, bladder, testis and seminal vesicles, and in many other visceral sites (ie, ovary, pancreas, uterus, parotid gland, and lungs. PNETS in breast tissue have been extremely rare only nine cases were documented until 2013. At 2014 a new case was reported in Turkey.

Key words: Ewing sarcoma / Primitive neuroectodermaltumor (ES/PNETs), Breast primary, diagnosis.

الملخص باللغة العربية

مجموعة أو عائلة أورام يوينغ، والتي تسمى أيضا أورام يوينغ الغرنية هي أورام سرطانية نادرة تنشأ بالعظام، والأنسجة الرخوة (مثل العضلات، و الأنسجة الضامّة كالأوتار التي تربط العضلات بالعظام، والأنسجة الليفية و الغضاريف، والأنسجة الزُّلالية المحيطة بالمفاصل مكونة من خلايا زرقاء اللون صغيرة، وهي أورام تنشأ لدى الأطفال والمراهقين ، تم اكتشافها لاول مرة عام 1918، و قد سميت هذه الأورام نسبة إلى الطبيب جيمس يوينغالذي الذي قدم أول دراسة طبية عنها سنة1921. ولقد أفادت الأبحاث الطبية بعد ذلك عن وجود تشابه كبير في العديد من الخواص بين أورام يوينغ و بين أورام الأدمة الظاهرة العصبية الأوليّـة و هي أورام نادرة لدى الأطفـــال قد تنشأ بالأنسجة الرخـــوة و العظام، و من هنا جاءت تسمية هذه الأورام بمجموعة أو عائلة يوينغ. تجدر الإشارة إلى أن أورام الأدمة الظاهرة العصبية أو أورام يوينغ خارج العظم تسمى بأورام آسكن التي تم اكتشافها عام 1979 ،غالبا تنشأ هذه الأورام لدى المراهقين، وتظهر في الأنسجة الرخوة المرتبطة بالقفص الصدري وأحيانا بعظام القفص الصدري (الأضلاع) ومن النادر أن تنتقل إلى الرئتين. لقد تم تسجيل ظهور بعض الحالات من هذه الأورام في الأعضاء الداخلية (وليس في العظام أو الأنسجة الرخوة كما هو معروف وشائع) ، و من النادر جدا ظهور مجموعة أورام يوينغ / أورام الأدمة الظاهرة العصبية في الثدي حيث تم تسجيل 9 حالات فقط حتى عام 2013 ، وتم نشر حالة اخرى جديدة في تركيا في العام الحالي 2014.

INTRODUCTION

The ES/PNET family of tumors is part of a rare group of malignant neoplasms arising from neuroectodermal elements, with small round cell morphology (1).

This group of tumors is characterized by the presence of the typical translocation (11; 22) (q24; q12) (2). Currently, ES/ PNET and malignant small round-cell tumors of the thoraco-pulmonary region are considered different manifestations of a single tumor family. Tumors at the poorly differentiated (Ewing's) end of spectrum have scanty, pale cytoplasm and round to ovoid open nuclei with finely distributed chromatin pattern. At the other end of the spectrum the cells may have somewhat eosinophilic cytoplasm and the coarser chromatin pattern with more frequent nucleoli.

Mitotic figures are common in PNET, as is necrosis and endothelial hyperplasia. A further aid in the differential diagnosis is provided by the presence of rosettes or pseudo rosettes in PNETs. The morphological and biological characteristics of Ewing's tumors developing in soft tissues appear to be indistinguishable from those of tumors developing at bone sites (3).

ES/PNET of the Breast is an extremely rare location and has been reported only seven times as a primary tumor and one as a metastatic tumor, in a thorough search through the medical literature as described in table (1) (1).

Table (1): summary of ES/PNET tumors of breast reported in the literatures

Reference	Age (years)	Presentation	Size (cm)	Disease	Treatment	Outcome
Tamura et al.(6)	47	Breast lump	2.11.8	Primary	Mastectomy	Not available
Maxwell et al. (12)	35	Breast lump	1.8	Primary	Lumpectomy + chemotherapy	Free of disease at 2.5 years
da Silva et al.(7)	35	Breast lump	12. 7.5	Primary	Chemotherapy + radiotherapy	Local and pulmonary relapse; death at 2 years
Ko et al(14).	33	Breast lump	2.5 .2	Primary	Lumpectomy	Free of disease at 6 months
Vindal and Kakar (15)	26	Breast lump	---------	Primary	Wide local excision + adjuvant chemotherapy	Free of disease at 36 months
Kwak et al. (16)	49	Mass in the axilla	---------	Metastatic	Chemotherapy	Not available
Dhingra et al. (17)	26	Breast lump	3.5. 3	Primary	Mastectomy + chemotherapy + radiotherapy	Free of disease at 12 months
Suebwong et al. (13)	46	Breast lump	4	Primary	Chemotherapy + radiotherapy	Local and pulmonary progression
Majid et al.(4)	30	Bilateral breast lump	7 and 5 in the right and left, respectively	Metastatic	Chemotherapy	The patient's medical condition deteriorated, and she died after 2 cycles of chemotherapy
FUNDA TAŞLIetal(5)	24	Breast lump	11.10	primary	Not available	Not available

CASE REPORT

A thirty- eight years old female patient presented with a painless left breast mass 12 months ago. Lumpectomy was done at Hamad Shihab Hospital / Baghdad   at June 2013. The histopathological report proved a benign breast lesion. During follow up two months later, the patient had a painful swollen left breast with swelling of upper arm, two ultra sound reports done at August 2013 showed hypo echoic breast tissue (edematous) no mass or cyst seen. No fluid collection was seen.   The axillary L.N was enlarged with reactive center and the differential diagnosis is lymphatic obstruction or mastitis. During this period, the patient received antibiotic therapy with no response. A third ultra sound was done to the patient at September 2013 revealed a well-defined heterogeneous breast mass of lobulated out lines measures 16x13.5x10 cm seen occupying most of breast tissue suggestive of carcinoma or soft tissue tumor, few enlarged axillary lymphnodes   seen largest one M.1.3 cmx1cm , left supra clavicular LAP seen the largest one M.1 cmX0.7cm . Huge left sided pleural effusion is obviously seen .Ultrasound of right breast is normal with normal axillary tissue lymphnodes. Ultrasound of the abdomen was normal . C.T scan was done to the patient after the third U.S showed evidence of huge soft tissue mass occupying the most of left breast tissue extending to involve the chest wall and invasion of pleura of left hemi thorax with periosteal reaction of the 4th rib in axillary and anterior parts of the chest wall with evidence of multiple enlarged axillary lymph nodes. The findings were suggestive of a breast carcinoma with secondary involvement of chest wall, massive left sided pleural effusion with collapsed left lung and mediastinal shift to the right, the heart is normal and right lung is clear . Malignant FNA was done to the breast mass the result came back of clusters of mammary carcinoma, the oncologist started a palliative chemotherapy . Seven cycles of TAC (taxoter 40 unites ,   Adriamycin 80 unites and cyclophosphamide 1000 unites respectively) therapy   given to the patient starting from September , October and November 2013 (4 cycles respectively) radiological follow up by chest x ray was clear (no pleural effusion , no mediastinal LAP , no mass in the chest wall, breast mass disappeared) . Another three cycles of TAC therapy given at December 2013 and January 2014   then followed up with anotherchest x-ray which was clear, in February 17th 2014 a chest C.T scan done showed no pulmonary metastasis and no axillary LAP with clear chest. After completing the palliative chemotherapy at May 2014 , a recurrent breast mass of for which Left simple mastectomy with axillary clearance   was done to the patient at Baghdad medical city hospital and the gross was sent to the teaching laboratories / histopathology department   which showed firm overlying skin with brownish discoloration (1 cm away from upper surgical margin and 4.5 cm away from the areola at the upper lateral quadrant) , cut section showed 3 masses the largest measures 5 cm in maximum dimension located in upper lateral quadrant of breast ,the second mass measures 4 cm in maximum dimension located near the deep surgical margin with necrosis and hemorrhage extending to the axillary tail , the smallest mass measures 2.5 cm firm whitish (the report did not mention the exact site), seven lymph nodes were seen , the nipple is retracted not ulcerated.   The diagnosis was infiltrative atypical lymphoid cells with frequent mitotic figures and multifocal necrosis , the tumor cells invading skeletal muscle .positive nodal involvement. Picture is that of NHL high grade. The case was referred to central public health laboratories histopathology department to confirm the definite diagnosis by immunehistochemical stains, H. and E. sections showed solid sheets of hyperchromatic relatively monomorphic   cells divided into irregular nodules by thin and thick septa ,tumor cells have indistinct cell membrane , a characteristic pertheliomatous growth pattern seen , few pseudorosettes seen, necrosis is prominent , numerous mitotic figure seen . tumor cells infiltrate the   adjacent   skeletal   muscle   fibers Immunehistochemical panel showed positive staining for CD99 surface membrane , NSE, S100 protein , patchy synaptophysin   and negative staining for LCA , CD3, CD20 (except for the intratumoral lymphocytes) , actin , melanosome , chromograninA   and cytokeratin 18.   The differential diagnosis of lymphoma , neuroendocrine carcinoma,   rhabdomyosarcoma,   malignant melanoma ,breast carcinoma all were excluded by the negatively stained markers .

DISCUSSION AND CONCLUSION

Primitive neuroectodermal tumors are uncommon, malignant, small-round-cell tumors that arise in soft tissues or bone, most commonly in children and young adolescents. Primary PNETs demonstrate a predilection for the truncal and axial soft tissue, including the chest wall (Askin tumor), the paravertebral region (50-60% of cases), and the extremities (20-25% of cases) (6). The thoracopulmonary region (Askin tumor) is the single most common primary site. Primary PNETs of many organs of the body have been documented (11,12), but only few(nine) cases of primary PNET of the breast were documented (1). Previous reports of primary breast PNET showed common clinical findings of a growing mass, over a 2-year or 4-month follow-up (13,14). In our case, the mass grew over a 10 months period. PNETs of other visceral organs usually present with a painful mass and constitutional symptoms (3), but in this case, the patient complained of signs of mastitis and swelling of the upper arm.

Maxwell *et al.* (4) described sonographic findings of primary PNET of the breast as a superficial, circumscribed, hypoechoic mass with posterior acoustic enhancement and an apparent hypoechoic tract extending to the skin. In our case, sonographic findings wereheterogenous lobulated mass occupying most of the breast tissue which is compatible with SuebwongC. *et al* (9)**.**

Our case was an adult patient with a mass on her left breast and extending to the left hemi thorax causing massive left sided pleural suffusion and collapsed left lung with periosteal reaction of 4th rib and auxiliary LAP , suggesting that the breast is the primary site with chest wall involvement. Immunohistochemistry and histology were necessary to confirm the diagnosis. the tumor was composed of hyperchromatic relatively monomorphic cells divided into irregular nodules by thin and thick septa ,tumor cells have indistinct cell membrane , a characteristic pertheliomatous growth pattern seen , few pseudorosettes seen , necrosis is prominent , numerous mitotic figure seen. Tumor cells infiltrate the adjacent skeletal muscle fibers. Figures below described the results of the current case report.
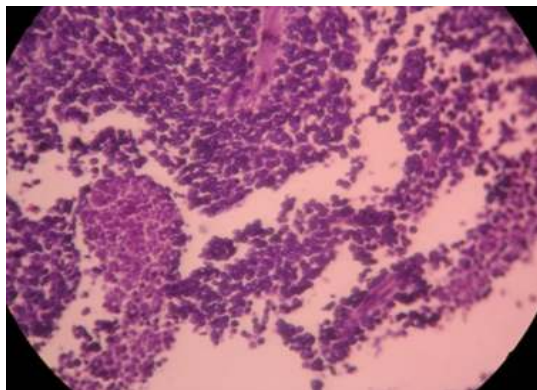
Figure (1): Histopathology of tumor mass- small hyperchromatic.
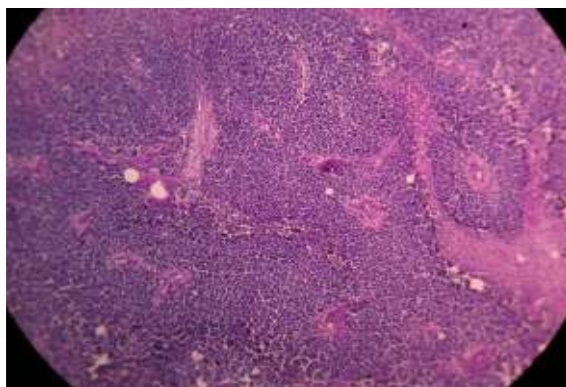

Figure (2): tumor mass- small hyperchromatic with prominent necrosis .10HPF cells with characteristic perivascular growth pattern. 4HPF
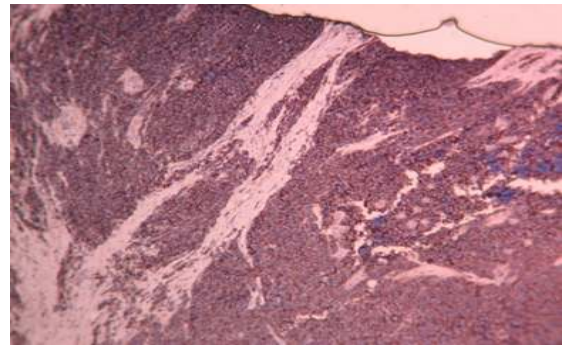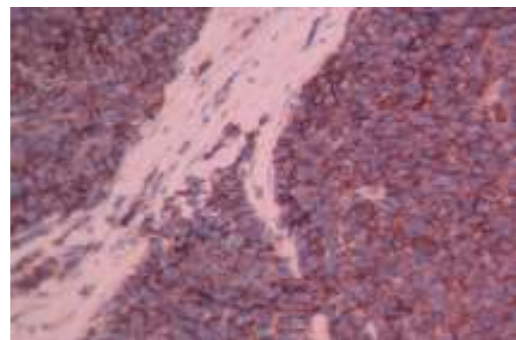

Figure (3): Low power field of CD99 .4 xPF

Figure (4): High power field of CD99 cell membrane 40x PF
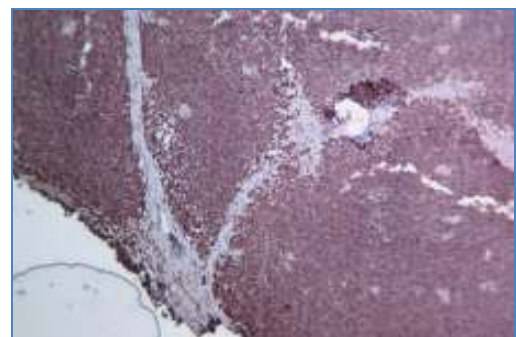
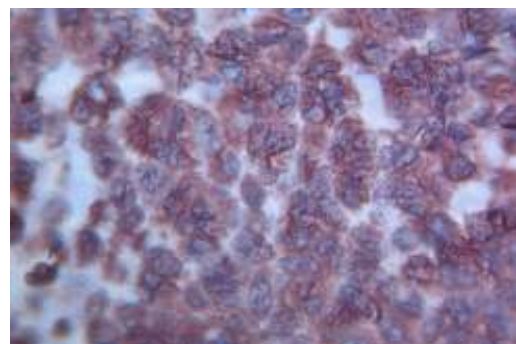

Figure (5): NSE positive staining of tumor cell ,X4PF.
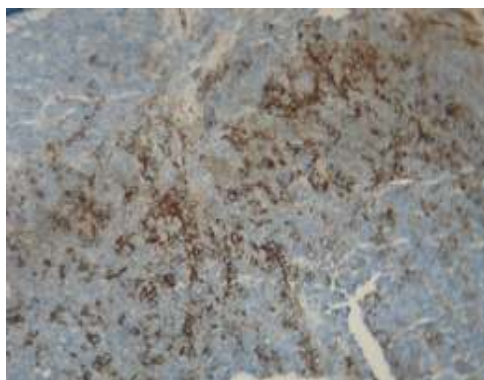

Figure (6): NSE cytoplasmic staining of tumor cells.x40HPF.

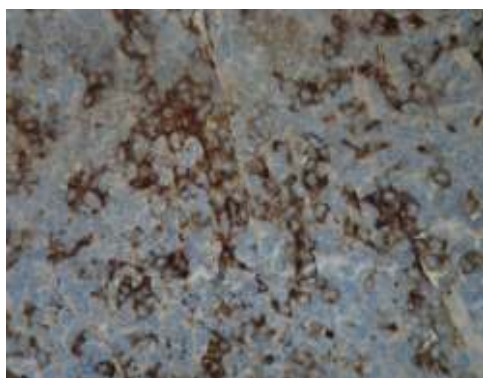**Figure (7): patchy positive synaptophysin .20XPFFig**

Figure (8): patchy positive synaptophysin . at X40 PF

In conclusion, we reported a rare case of ES/PNET presented as breast mass and missed as invasive breast carcinoma on FNA (fine needle aspirate and cytology) and NHL ,high grade on tissue section infiltrating chest wall**.**

Following up our patient since last session of chemotherapy in January 2014 the patient was in complete remission with clear chest and mediastinum yet she developed a recurrent breast mass at May 2014 after which simple mastectomy with axaillary clearance done till now patient is stable with no chest wall or contralateral breast mass**.**

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قسم الدراسات العربية

ARABIC SECTION

التأثير التطفيري لبعض المستخلصات الفطرية على كونيدات الفطر *Aspergillus amstelodami*

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الملخص باللغة العربية

تضمنت هذه الدراسة اختبار أربعة تراكيز تحت ســـامة (Sub lethal) (0.1, 0.15, 0.2, 0.25) ملغـــم/مـــل للإفـــرازات الخارجيـــة للفطـــر *Trichoderma harzianum* من حيث قابليتها على حث طفرات أمامية في كونيدات الفطر *Aspergillus amstelodami* باستخدام طـــرق التطفير الثلاثة: التضمين بالطبق, المعاملة المسبقة, النمو الوسيط. لم تظهر التراكيز الأربعة المدروسة أي تأثير تطفيـــري مقارنـــة بالطـــافرات التلقائية, وقد بين التحليل الإحصائي باستخدام اختبار t عند مستوى احتمالية 0.05 عدم وجود اي فرق معنوي بين متوســـط تكـــرار الطـــافرات المقاومة والمستحثة بالإفرازات الخارجية للفطر *Trichderma harzianum* وبين متوسط تكرار الطافرات التلقائية(السيطرة السالبة).

الكلمات المفتاحية: التأثير التطفيري، *Trichoderma harzianum* ، *Aspergillus amstelodami*

Mutagenic effect of some fungal extracts on conidia of fungus *Aspergillus amstelodami*

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ABSTRACT

This study was investigated the ability of four concentrations produced from external fungus secretions (*Trichoderma harzianum*) to induced mutation on conidia of *Aspergillus amstelodami*. Three methods were introduced, namely: Plate Incorporation method , Pretreatment method , Growth mediated method. All four concentration did not produced any mutant effect compared by spontaneous mutations, statistical analysis by using t-test at probability rate of 0.05 showed there is no significant difference between the mean repeated mutations resistant and induced by the external secretions of fungus *Trichoderma harzianum* and the mean repeated spontaneous mutations (negative control).

المقدمة

لقد بدأ التوجه في الآونة الأخيرة إلى المكافحة الحيوية بـدلا مـن استخدام المبيدات السامة والتي تسبب مشاكل كبيرة للبيئة ولصحة الإنسان حيـث أثبتـت الدراسـات أن المبيـدات تتـرك بـواقي (Residues) وتكمن خطورة هذه البواقي في احتمالية وصـولـها للإنسان عبر السلسلة الغذائية (1، 2، 3)، لذا تعتبر المستخلصات الفطرية من أنسب الطرق التي استخدمت حديثا في مكافحة الآفات لأنها مستخلصات طبيعية وليس لها أي تأثيرات سلبية (4) ، كمـا أنها تستخدم تجاريا كمبيدات فطرية حيوية Biofungicides لأن تأثيرها أكبر من تأثير المبيدات الكيمياوية (5). عرفت أجنـاس الترايكوديرما بقدرتها المثبطة لنمو الفطريات منذ عام 1930، وقد تـــم اسـتخدام العديـد مــن الـسلالات (T.harzianum,T.virens,T.viride,T.reesei,T.hamatum) في المكافحة الحيوية للفطريات الممرضة للنباتات وعلى العديد من المحاصيل الزراعية مثل الفراولة والبقوليـات والخيـار والفجـل والقطن والطماطم والشمندر (6) ، ومن الممرضـات التـي تمـت مكافحتها حيويا (Pythium Phytophthora ,Plasmopara .spp Penicillium, Aspergillus niger, Fusarium)، (7، 8)، كمـا أشـارت الدراسـات إلـى أن اسـتخدام جـنس Trichoderma harzianum مع أجناس مختلفة مـن البكتيريـا يزيد من كفاءة المكافحة الحيوية ويحسن المحصول (9، 10). إن المحاصيل التـي يـتم معاملتهـا بالمستخلـصات الفطرية للترايكوديرما تنمو أفضل من المحاصيل التي لـم تعامـل بهـذه المستخلصات وذلك باستحثاث مقاومة النبات ضد الفطر الممرض عن طريق تكوين بروتينات داخل الخلية النباتية تقوم بتثبيط نشاط الفطر الممرض، كما أنها تحفز زيادة تفرعات الجذور وتغلغلها في التربة وبذلك تزيد من مقاومة النبات للجفاف لذلك تم اسـتخدامها كأسـمدة حيويـة Biofertilizer ، وقـد لـوحظ أن عـزلات الترايكوديرما لها قدرة على إنتـاج مـواد شـبيهة بالأوكـسينات والجبرلينات المحفزة لنمو النبات ، لذا استخدمت كمخصبات للتربة Soil amendments (9، 10). إن المستخلصات الفطرية لجنس الترايكوديرما تتكون من إنزيمات خـــارج خلويـةExtracellular مثـل الـسليليز Cellulase والبروتيــــــــــز Protease والكلوكــــــــانيز Glucanase والكايتينيز Chitinase إضافة إلى مادة الاسيتونتريل Acetonitrile ومادة الترايكوستين Trichosetin (12–15)، وهذه المركبـات أعطت أهمية صيدلانية للفطر ترايكوديرما كمـضاد للعديد مـن الأحياء المجهرية (16–18)، كما أثبت كل من الرجبو والعبيـدي (19) أن الجنس Trichoderma harzianum ذو فعالية عاليـة جدا ضد أنواع مختلفة من فطر Trichophyton والمسبب لأنواع مختلفة من الأمراض الجلدية البشرية.
ونظرا لما تقدم مـن أهميـة اسـتخدام مستخلـصات الفطـر Trichoderma harzianum صيدلانيا فقد صممت هذه الدراسة للتحري عن تأثيراتها الوراثية وذلك من خلال التقصي عن قدرتها علـــى إحـداث طفـرات جينيـة فـي الفطـر Aspergillus amstelodami .

المواد وطرق العمل

كائن الاختبار:

أجريت جميع تجارب البحث على السلالة A₁(WA₁) من الفطـر الكيسي Aspergillus amstelodami والتي تم الحصول عليهـا من الاستاذ الدكتور ساهي جواد ضاحي في كلية العلوم في جامعة الموصل.

الأوساط الزرعية وظروف الزرع :

تم استخدام وسطان اساسيان لغرض نمـو الفطـر Aspergillus amstelodami وهما: الوسط الأدنى Minimal medium (M) وقد أجريت جميع الاختبارات على هذا الوسط مضافا إليـه مـادة الاختبار . وسط مستخلص الشعير وملح الطعـام -Malt extract salt medium للحصول على اكبر عـدد مـن الكونيـدات . استخدمت مادة(D)Sodium deoxycholate بتركيز نهائي قدره 400 مايكروغرام / مل لنحصل على مستعمرات منفـردة (19)، كما تم استخدام وسط مرق البطاطا(PDA) لغرض نمـو الفطـر T.harzianum (20).

التحضين والظروف المثلى للنمو:

حضنت العينات بدرجة حرارة 30°م ، وهي الحرارة المثلى لنمو الفطر Aspergillus amstelodami لمدة 4-6 أيام بالحاضـنة لعزل الطافرات ، وثلاثة أيام للحصول على الكونيدات لتحـضير العالق الكونيدي واستخدامه في الاختبارات اللاحقة (20) .

المحلول الخزين للبينوميل:

حضر المحلول الخزين بإذابة 0.02 غرام مـن المبيـد الفطـري بينوميل في 500 مل من الماء المقطر فتم الحصول على محلـول ذي تركيز مقداره 40 مايكرو غرام بينوميل/مـل أو 20 مـايكرو غرام مادة فعالة Benomyl / مل ، عقم بعد ذلك بالموصد عنـد 121°م لمدة 15 دقيقة ثم حفظ في الثلاجة لحين الاستعمال (21، 22).

اسـتخلاص الإفرازات الخارجيـة للفطر Trichoderma harzianum:

تم سحب الإفرازات الخارجية للفطر T.harzianum المأخوذ من كلية الزراعة، جامعة الموصل وبالاعتماد على طريقة Eziashi وآخرون(23) إذ استخلصت الإفرازات الخارجية لرواشح الفطـر T.harzianum بعد 14 يوما من نموها على وسط مرق البطاطا (PDA) ثم ترشيحها بقطعة شاش ووضع الراشح في قمـح فـصل وأضيف إليه نفس المقدار (حجم/حجم) من البيوتانول ورج جيدًا ثم ترك لفصل الطبقتين وسحبت طبقـة البيوتـانول الحاويـة علـى الإفرازات الخارجية للفطر وكررت الخطوة السابقة ثلاث مـرات وجمع البيوتانول الناتج وجفف ثم أذيب بحجم معلوم من (DMSO Dimethyl sulphoxide) وعقم المستخلـص بالبسترة في حمـام مائي بدرجة حرارة تتراوح بين 63-62 °م وذلك لمدة 20 دقيقة.

تحديد التركيز الأدنى المثبط (MIC) :

لقـد جـرى تحديـد التركيـز الأدنـى المثـبط لمـستخلص الفطـر T.harzianum باستعمال تراكيز متصاعدة من المادة مع وسط النمو بطريقة الوخز وذلك بعمل أربعة وخزات من الفطـر A.amstelodami في كل طبق مضافا إليـه المـادة المدروسـة وتحضن الأطباق لمدة أربعة أيام بدرجة 30°م، يتم بعدها قيـاس قطر المستعمرة النامية في كل طبق ويقارن بقطر المـستعمرات النامية على الوسط الأدنى (M) الخالي من المادة والتي تمثل قراءة السيطرة (Control) ويسجل التركيز الذي يتوقف عنده نمو الفطر تماماً حول نقطة الوخز إذ يمثل هذا التركيز الحد الأدنى المثبط (القاتل) لنمو الفطر . وكذلك تم حساب النسبة المئوية للتثبيط مـن خلال المعادلة التالية:

النسبة المئوية للتثبيط = متوسط المعاملة التلقائية (المقارنة) – متوسط المعاملة بالمادة × 100
متوسط المعاملة التلقائية (المقارنة)

وبنفس الطريقة تم تحديد التركيز الأدنى المثبط لمحلول البينوميـل بتركيز مقداره 40 مايكرو غرام بنليت/مل أو 20 مـايكرو غـرام مادة فعالة Benomyl / مل.

تحضير العالق الكونيدي:

تم تحضير العالق الكونيدي للـسلالة A₁ مـن مزرعـة حديثـة النمو(أربعة أيام) مزروعة على الوسط (CMTS). وقد جـرى تقدير عدد الكونيدات فيـه باسـتخدام شـريحة عـد الخلايـا (Heamocytometer) وظهر إنها بحدود 10⁷ كونيدة/ مل (24).

دراسة التـأثيـري التطفيـري لمـستخلص الفطر *harzianum* *Trichoderma*:

جرت دراسة أربعة تراكيز لمستخلص الفطـر.*T.harzianum* وهي (0.1، 0.15، 0.2، 0.25) ملغم/ مل وهي تراكيـز غيـر سامة فضلا عن المعاملة الخالية من المادة المدروسة أي المعاملة صفر حيث تم دراسة الأثر الطفري لهذه المادة بطريقة التـضمين بالطبق وطريقة المعاملة المسبقة وطريقة النمو الوسيط (25).

طرق دراسة الأثر الطفري لمـستخلص الفطر *harzianum* *Trichoderma* :

1. طريقة التضمين بالطبق: تتضمن هذه الطريقة زراعة كونيدات الفطر *A.amstelodami* على الوسط الغذائي(MD) الحاوي على التراكيز (0.1، 0.15، 0.2، 0.25) ملغم/ مل من مستخلص الفطر *T.harzianum* فضلا عن المادة الانتقائية (البينوميل) كل تركيز على حدا وفي الوقت نفسه حضر مـن العـالق 10^{ZERO} (10^0) تخافيف متسلسلة لغاية 10^{-6} وزرعت على أطبـاقMD لحساب العدد الحي للكونيدات.

2. طريقة المعاملة المسبقة: تتضمن هذه الطريقة معاملة العالق الكونيدي للفطر *A.amstelodami* بـالتراكيـز المدروسـة مـن مستخلص الفطر *T. harzianum* لمدة ساعة لكل تركيز علـى حدا قبل عملية الزرع حيث حضر مـن العـالق 10^0 تخافيف متسلسلة لغاية 10^{-6} وزرعها على أطباق MD لحساب العدد الحي للكونيدات ، كما تم زرع 0.1 مل من العالق غير المخفف10^0 من كل معاملة على أطباق (MDالمضاف اليه البينوميل) للتحري عن الطفرات المقاومة للبينوميل.

3. طريقة النمو الوسيط: تتضمن هذه الطريقة زراعة الفطر *A. amstelodami* على الوسط (MD) الحـاوي علـى التراكيـز المدروسة من مستخلص الفطر *T. harzianum* ثم حضنت لمدة أربعة أيام بحيث نسمح للمادة بان تتأيض داخل الكونيدات في أثناء عملية الانقسام التي يرافقها تكوين أنزيمات ايضية متعددة والتي قد يكون لها دور مهم في تنشيط التأثير التطفيري للمـادة المطفـرة (26)، بعدها حضر عالق كونيدي من كل تركيز على حدا وهـو العينة غير المخففة ذات التركيز 10^0 وزرع على أطبـاق حاويـة على MD+بينوميل ومن العالق نفسه حضر تخـافيف متسلسلة لغاية 10^{-6} وزرعت على أطبـاقMD لحسـاب العـدد الحـي للكونيدات، وكررت التجربة ثلاثة مرات .

عينات السيطرة :

تضمنت الدراسة نوعين من عينات السيطرة وهي السيطرة السالبة (Negative control) ويمثلها العالق الكونيدي المحضر بالمـاء المقطر دون إضافة أي مـادة مطفـرة وهـي المعامـلة صـفر، والطفرات الحاصلة من هذه المعاملة تمثّل الطفرات التلقائية كمـا تضمنت الدراسة إدخال سيطرة معروفة في تأثيرها التطفيري وقد استخدمت مادة حامض النتروز كسيطرة موجبة (التي حضرت من تفاعل نتريت الصوديوم$NaNo_2$ (0.2 g\ML D.W.) مـع دارئ خلات الاسيتات حيث كل النترات المستخدمة سوف تكون حامض النتروز) (27).و جرت دراسة الأثر الطفري لحـامض النتـروز بطريقة المعاملة المسبقة فقط وحسب طريقة (28).

عزل الطافرات وحساب تكرارها:

جرى عزل الطافرات التلقائية والمـستحثة بمـستخلص الفطـر *T. harzianum* المقاومة للمبيد البينوميل والمستحثة بحـامض النتروز وتم حساب متوسط تكرار حـدوثها علـى أسـاس عـدد الكونيدات الحية في العالق الكونيدي (29).

التحليل الإحصائي:

تم تنفيذ ثلاث مكررات للعينة التلقائية والعينات المعاملة بمستخلص الفطر *T. harzianum* وعينة السيطرة الموجبة، وتـم حـساب متوسط تكرار الطافرات وإيجاد الخطأ القياسي Standard Error (SE) لكل معاملة. وقد تم إجراء التحليل الإحـصائي باسـتخدام اختبار (t) لأربع درجات حرية (2+2)t عند مـستوى احتمالية 0.05 إذ جرت مقارنة متوسط الطافرات لكل عينة مـع متوسط تكرار الطافرات في العينة التلقائية لملاحظة مدى معنوية الفرق في متوسط العينة المعاملة مقارنة بمتوسط العينة التلقائية (30).

النتـــــائج والمناقشـــــة

التركيز الأدنى المثبط (MIC) :

تم تحديد التركيز المثبط الأدنى لمستخلص الفطـر*harzianum* *Trichoderma* وذلك باستخدام تراكيز متـصاعدة مـن هـذا المستخلص اعتبارا من الصفر – 0.45 ملغم/مل، ومـن النتـائج المبينة في الجدول رقم (1) حيث نلاحظ أن أقطار مـستعمرات الفطر *A.amstelodami* تأخذ بالتناقص التـدريجي مـع زيـادة تركيز المستخلص في الوسط الغذائي ،حيث تراوحت النسبة المئويـة للتثبيط بين 19.3% – 77.9% للتراكيز 0.45-0.01 ملغم/مـل على التوالي وهذا يتفق مع ما ذكره العديد من الباحثين عن الفعالية المضادة للفطريات Antifungal لمستخلص الفطر *harzianum* *.T* ومنها الفطر *Aspergillus spp.* ,حيـث أن آليـة المكافحـة الحيوية للفطر *Trichoderma* تتم عن طريق قدرة هـذا الفطـر على البقاء والعيش في ظروف شـديدة التنافسـية كـذلك قدرتـه التنافسية على العناصر الغذائية الموجودة فـي التربـة وامتلاكـه القابلية على التطفل المباشـر علـى الغـزل الفطـري للفطـر الممرض(يمتلك ضراوة عالية) وقدرته التثبيطية العالية للفعاليـة الممرضة للفطريات (31، 32).

كما نلاحظ من الجدول أنه لم يتم الحصول على تثبيط كامل لنمـو الفطر وذلك لأن التاثير التطفيري يحدث عند تركيـز أقـل مـن المستوى القاتل Sub lethal إذ إن المستوى القاتل يكون مصحوبا بتحطيم DNA (33) لذا تم اختيار التراكيـز (0.1، 0.15، 0.2، 0.25) ملغم/ مل.

وبما أن المبيد الفطري البينوميل أعطى نسبة تثبيط 100% عنـد التركيز 0.6 مايكروغرام/مل لذا فقد اعتبر هـذا التركيـز هـو التركيز السام للبينوميل واستخدم لعزل الطافرات. الجـدول رقـم (2).

جدول رقم (1): أقطار المستعمرات (سم) للفطر *A. amstelodami* المزروعة على الوسط (M) مضافا إليه تراكيز مختلفة من مستخلص الفطر*Trichoderma harzianum* بطريقة الوخز

النسبة المئوية للتثبيط	المعدل	R3	R2	R1	تركيز المادة ملغم/مل
0	4.67	4.6	4.7	4.7	0
19.3	3.77	4	3.5	3.8	0.01
22.9	3.6	3.5	3.8	3.5	0.025
34.3	3.07	3	3.2	3	0.05
43.7	2.63	2.7	2.6	2.6	0.1
47.1	2.47	2.4	2.5	2.5	0.15
52.2	2.23	2.2	2.3	2.2	0.2
55	2.1	2.1	2.2	2	0.25
58.7	1.39	2	1.8	2	0.3
66.4	1.57	1.5	1.6	1.6	0.35
70.7	1.37	1.4	1.3	1.4	0.4
77.9	1.03	1.3	1	1	0.45

R1، R2، R3 : مكررات الأطباق المستخدمة لقياس أقطار مـستعمرات الفطر *A. amstelodami* عند كل تركيز.

جدول رقم (2): أقطار مستعمرات الفطر A.amstelodami المزروعة على الوسط (M) المضاف إليه تراكيز مختلفة من المبيد الفطري البينوميل

تركيز المادة مايكروغرام / مل	تكرار أقطار المستعمرات (سم)			المتوسط	النسبة المئوية للتثبيط
	R1	R2	R3		
0	4.5	4.2	4.0	4.23	-
0.3	0.4	0.5	0.45	0.45	89.36
0.4	0.2	0.3	0.22	0.24	94.33
0.5	0.1	0.22	0.1	0.14	96.69
0.6	0.0	0.0	0.1	0.0	100

R1،R2،R3 : مكررات الأطباق المستخدمة لقياس أقطار مستعمرات الفطر A amstelodami. عند كل تركيز.

التـأثير التطفيــري لمــستخلص الفطــر harzianum Trichoderma :

من خلال معاملة كونيدات الفطــر A.amstelodami بـالتراكيز الأربعة لمستخلص الفطر T. harzianum تم حـساب متوسط تكرار الطافرات المقاومة للمبيد البينوميل والمستحثة بمستخلص الفطـر T. harzianum او المستحثة بحامض النتروز ومتوسط تكرار الطافرات التلقائية حيث نلاحظ من الجداول (3، 4، 5) أن متوسـط تكـرار الطـافرات المـستحثة بالمـستخلص الفطر T. harzianum اكبر من متوسط تكرار الطافرات التلقائية لطرق التطفير الثلاثة كما تبين نتائج الجدول (5) وجود زيادة في متوسط تكرار الطافرات المقاومة للبينوميل والمستحثة بمستخلص الفطر T. harzianum بطريقة النمو الوسيط عن معدل تكرار الطافرات المقاومة للبينوميل والمستحثة بمستخلص الفطر T. harzianum بطريقتي المعاملة المسبقة والتضمين في الطبق, إلا أنها تشير إلى أن طريقة النمو الوسيط أفضل من الطريقتين السابقتين في الكشف عن الطفرات وقد يعود السبب في زيادة تكرار الطافرات التي تم الحصول عليها بهذه الطريقة إلـى ترك الكونيدات تنمو وتنقسم بوجود مستخلص الفطر T. harzianum لمدة أربعة أيام مما أعطى الوقت الكـافي لأنزيمـات النمـو الخضري كي تايض مستخلص الفطـر T. harzianum وتنتـج مركبات وسطية قد تكون أكثر كفاءة من المستخلص في التفاعـل مع DNA (26).

أجريت المقارنات الإحصائية لنتائج الجداول (3، 4، 5) باستخدام اختبار t عند مستوى احتمالية $P \geq 0.05$ وذلك من خلال مقارنة متوسطات تكرار الطافرات المقاومة للبينوميل المستحثة بمستخلص الفطر T. harzianum مع متوسط تكرار الطافرات التلقائية تبين عدم وجود فروق معنوية، وهذا يشير إلى عدم قـدرة مـستخلص الفطر Trichoderma harzianum بجميع تراكيزه قيد الدراسة على إحداث الطفرات الوراثية فـي الفطـر A. amstelodami مقارنة بتكرار الطافرات التلقائية المقاومة للمبيد بينوميـل لطـرق التطفير الثلاثة . إذ كانت قيمة $t_{(4)}$ الجدولية 4.032 أكبر من قيمة $t_{(4)}$ المحسوبة للمعاملات الأربعة.

وعند مقارنة متوسط تكرار الطافرات المستحثة بمستخلص الفطر T. harzianum المقاومة للبينوميل مع متوسط تكرار الطـافرات المستحثة بحامض النتروز المقاومة للبينوميل (سـيطرة موجبة) لطرق التطفير الثلاثة تبين وجود فرق معنوي واضح عند مستوى احتمالية$p \geq 0.05$, وهذا يدل على أن السلالة A1 هي سلالة قابلة للطفور إذ استجابت للتـأثير التطفيري لحامض النتروز وهذا يشير إلى إمكانية استخدام هذه السلالة للكشف عـن القـدرة التطفيريـة المحتملة للمستخلص قيد الدراسة.

جدول رقم (3): تكرار الطافرات ($\times 10^{-6}$) المقاومة والمستحثة في كونيدات الفطر A.amstelodami بعد معاملتها بمستخلص الفطر Trichoderma harzianum بطريقة التضمين بالطبق

التركيز بالملغم/مل	تكرار الطافرات			المتوسط ± الخطأ القياسي	قيمة t_4 المحسوبة
	R1	R2	R3		
0	0	0.8	1.786	0.516±0.862	–
0.1	2.778	0	2.564	0.892±1.780	0.865
0.15	1.429	1.538	2.941	0.486±1.969	1.572
0.2	1.613	4.839	0	1.422±2.150	0.851
0.25	1.724	3.333	2.00	0.496±2.352	2.083
HNO$_2$	17.45	18.74	17.45	0.43±17.88	34.55*

0: بدون معاملة وتكراراتها تمثل التكرارات التلقائية. *:معنوية عند مستوى احتمالية p>0.05.
$t_{(4)}$: قيمة t لأربع درجات من درجات الحرية والتي تقارن متوسط تكرار الطافرات من كل معاملة مع متوسط المعاملة صفر.

جدول رقم (4): تكرار الطافرات ($\times 10^{-6}$) المقاومة والمستحثة في كونيدات الفطر A.amstelodami بعد معاملتها بمستخلص الفطر Trichoderma harzianum بطريقة المعاملة المسبقة

التركيز بالملغم/مل	تكرار الطافرات			المتوسط ± الخطأ القياسي	قيمة t_4 المحسوبة
	R1	R2	R3		
0	0	0.858	0	0.284±0.286	-
0.1	1.429	0	2.817	0.812±1.415	1.314
0.15	0	1.639	0	0.546±0.546	1.009
0.2	1.613	0	1.587	0.532±1.066	0.283
0.25	0	1.776	0	0.591±0.592	0.467
HNO$_2$	17.45	18.74	17.45	0.43±17.88	34.55*

0: بدون معاملة وتكراراتها تمثل التكرارات التلقائية. *:معنوية عند مستوى احتمالية p>0.05.
$t_{(4)}$: قيمة t لأربع درجات من درجات الحرية والتي تقارن متوسط تكرار الطافرات من كل معاملة مع متوسط المعاملة صفر.

جدول رقم (5): تكرار الطافرات ($\times 10^{-6}$) المقاومة والمستحثة في كونيدات الفطر A.amstelodami بعد معاملتها بمستخلص الفطر Trichoderma harzianum بطريقة النمو الوسيط

التركيز بالملغم/مل	تكرار الطافرات			المتوسط ± الخطأ القياسي	قيمة t_4 المحسوبة
	R1	R2	R3		
0	0.787	2.40	1.724	0.467±1.637	-
0.1	2.64	3.82	4.032	0.432±3.497	2.929
0.15	2.857	4.225	5.797	0.849±4.293	2.743
0.2	3.053	3.144	4.615	0.505±3.604	2.863
0.25	3.333	3.448	3.636	0.086±3.472	3.871
HNO$_2$	17.45	18.74	17.45	0.43±17.88	34.55*

0: بدون معاملة وتكراراتها تمثل التكرارات التلقائية. *:معنوية عند مستوى احتمالية p>0.05.
$t_{(4)}$: قيمة t لأربع درجات من درجات الحرية والتي تقارن متوسط تكرار الطافرات من كل معاملة مع متوسط المعاملة صفر.

ومن هذا يتضح أن مستخلص الفطر T. harzianum لم يظهر تأثيرا طفريا مهما في كونيدات الفطر A.amstelodami وهـذا يتفق مع العديد من البحوث التي أكدت أن مستخلصات الفطـر T. harzianum لم تظهر أي سمية عند تجريبها للجرذان (12) كما أنها لم تظهر أي شذوذ أو انحراف كروموسومي عند اختبارها على الخلايا اللمفية على الإنسان (11) ولم يكـن لهـا أي تـأثير تطفيري على البكتريا(12) ولها فعالية عالية جدا في تثبيط التأثير السام للافلاتوكسينات التي تفرزها أجناس الـ Aspergillus (16، 17)، وأكد كل من Marfori وMalasa (13) أن مـستخلص الفطر T. harzianum يحوي على مادة الـTrichosetin (والتي تعتبر مضادا حيويا جديدا) لم يكن لها اي تأثير سام عند تجريعها

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للدواجن. وبهذا اعتبر مستخلص الفطــر T. harzianum مــن المواد الآمنة وراثیًا.

الاستنتاجــــات

1. إن مستخلص الفطر Trichoderma harzianum له تأثير تثبيطي على نمو الفطر A.amstelodami .
2. لم تظهر التراكيز الاربعة المدروسـة (0.15, 0.2 ,0.25 ,0.1) ملغــم/مــل لمــستخلص الفطــر Trichoderma harzianum اي تأثير تطفيري فــي كونيــدات الفطر A.amstelodami مقارنة بالطــافرات التلقائيــة وبطرائــق التطفير الثلاثة المستخدمة ضمن الظروف التجريبية المتبعة.
3. إن نتائج التحليل الإحصائي لم تظهر أي فروق معنوية بــين متوسط تكرار الطافرات التلقائية وبــين متوسط تكــرار الطافرات المقاومة والمستحثة بمستخلص الفطر Trichoderma harzianum

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دراسة أولية للكشف عن تلوث حبوب اللقاح المجموعة من قبل شغالات نحل العسل Apis mellifera L.
بالعناصر الثقيلة في العراق

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الملخص باللغة العربية

جمعت سبع عشرة عينة من حبوب اللقاح المجموعة من قبل شغالات نحل العسل داخل الخلية ومن عدة محافظات في المنطقة الوسطى (بغــداد وبابل وواسط والنجف) والجنوب (ميسان والبصرة) والشمال (كركوك). أجري الكشف عن التلوث بالعناصر الثقيلة (Pb ، Cd ، Cu ، Ni و Zn) في عينات حبوب اللقاح في مختبر دائرة البيئة والمياه في وزارة العلوم والتكنولوجيا في بغداد .
أظهرت نتائج البحث أن جميع العينات ملوثة بالرصاص وتراوح تركيزه بين (0.04 – 0.535 ملغم / كغم) وكان أعلى تركيز فــي محافظــات الوسط وأقل تركيز في محافظات الجنوب والشمال . أما حبوب اللقاح المستوردة فتراوح التركيز فيها بين 0.04 – 0.215 ملغم / كغم. كما أن جميع العينات ملوثة بالكادميوم وتراوح تركيزه فيها بين (0.01 – 0.016 ملغم / كغم) وسجل أعلى تركيز في محافظات الجنوب وأقل تركيز في الشمال . أما حبوب اللقاح المستوردة فكان الصيني 1 أعلى تركيز 0.025 ملغم / كغم. أما التلوث بالنحاس فتراوح تركيزه بين 4.57 – 5.153 ملغم / كغم وأعلى تركيز في محافظات الوسط وأقل تركيز في الجنوب والشمال ، أما حبوب اللقاح المستورد فكان أعلى تركيز الــصيني 4.52 ملغ / كغم . أما التلوث بالنيكل فتراوح تركيزه بين 0.68 – 2.583 أعلى تركيز في محافظات الوسط وأقل تركيز في الشمال ، أما حبوب اللقاح المستورد فكان أعلى تركيز الصيني 0.455 ملغ / كغم. أما التلوث بالزنك فتراوح بين (4.04 – 6.18 ملغم / كغم) حيث سجل أعلى تركيز في محافظات الوسط ، وأقل تركيز في محافظات الشمال . أما حبوب اللقاح المستوردة فكان أعلى تركيز في حبوب اللقاح الــصيني (3.84 ملغــم / كغم).

الكلمات المفتاحية: حبوب اللقاح، نحل العسل، العناصر الثقيلة

Preliminary study on detection of bees pollen contamination by heavy metals in Iraq

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ABSTRACT

Seventeen samples of pollen grains collected from honeybee workers in hive from different of Governorates middle Iraq (Baghdad , Babylon , Wasit , Najef), South Iraq (Mesan , Basra) and north Iraq (Kirkuk) .
Analysis of contaminated pollen grains with (Pb , Cd , Cu , Ni and Zn) in pollen samples is done by Environment and water researches, Ministry of Science and Technology in Baghdad . The results showed that all samples were contaminated by Pb (0.04 – 0.535 mg/kg). The concentration of Pb high in middle Governorates while south and north Governorates much lower. The imported pollen grain samples (0.4-0.215).
The samples also showed high of samples contamination by Cd (0.01 – 0.016 mg/kg) The highest concentration with Cd in south Governorates and also lower in north Governorates. The imported pollen grain samples in China highest (0.025 mg/kg) The concentration of Cu (4.57 – 5.153 mg/kg) the highest concentration in middle Governorates. The imported pollen in China highest (4.52 mg/kg) .
The concentration of Ni in the samples were in the range (0.68 – 2.583 mg/kg). the highest concentration of Ni in middle Governorates but lower in north Governorates The imported pollen in China highest (0.455 mg/kg). The concentration of Zn in the collected samples (4.04 – 6.18 mg/kg) .the highest levels of Zn in middle Governorates while north was lower. The imported pollen in China highest (3.84 mg/kg).

المقدمة

يعد نحل العسل .Apis mellifera L احد الكائنات التي لها علاقة متبادلة ووثيقة بالنبات الذي يزودها بالغذاء فتمنحه البقاء من خلال عملية التلقيح ، وعند زيارة الشغالات للنباتات المزهرة للحصول على الرحيق تتعلق حبوب اللقاح بشعيرات جسمها فضلا عن وجود سلة حبوب اللقاح Corbicula التي يجمع بها ثم ينقلها الى داخل الخلية (1). حبوب اللقاح هي المصدر الرئيسي للبروتينات والدهون والفيتامينات والمعادن اذ تحتوي على الماء بنسبة 12-20% والبروتينات بنسبة 10-40% والدهون 1.5 - 13.5% والفيتامينات بنسبة قليلة وتضم مجموعة فيتامين B و C و A و E . اما المعادن فتتراوح نسبتها بين 2.5 - 6.5% والكاربوهيدرات بين 1.5 - 4.5% وعدد من الانزيمات مثل الامليز والانفرتيز والفوسفاتيز فضلا عن الصبغات التي تعطي حبوب اللقاح لونها اضافة الى المضادات الحيوية (2).

يستعمل النحل حبوب اللقاح لتغذية حضنة الشغالات والذكور ، ونظرا لاهمية حبوب اللقاح وما تحويه من عناصر فقد استعملها الانسان لاغراض طبية ، نتيجة للتقدم الصناعي وما تقذفه الصناعات المختلفة من معادن ثقيلة بهيئة نفايات غازية وسائلة وصلبة تستقر في النهاية بالبيئة (3 ، 4). وحيث ان نحل العسل يقوم بزيارة جميع مكونات البيئة من تربة ونبات وماء وهواء للحصول على غذائه من رحيق وحبوب لقاح وينقلها الى داخل الخلية لذلك يلعب النحل ومنتجاته دورا في الكشف عن التلوث البيئي بالعناصر الثقيلة والمبيدات والمواد المشعة (5 ، 6). درس (7) تلوث حبوب اللقاح بالمعادن الثقيلة Zn ، Pb ، Cu و Cd في 12 عينة من اماكن متفرقة من فلندا واظهرت النتائج ان تركيزات المعادن الثقيلة عالية في حبوب اللقاح. وفي دراسة قام بها (8) بقياس ثلاثة عناصر ثقيلة Cd ، Pb و Cr في منتجات خلية النحل وهي العسل وحبوب اللقاح والشمع والعكبر وقد سجلت اعلى تراكيز في الشمع وحبوب اللقاح والعكبر اما العسل فأقل تركيزا. درس (9) الخواص الفيزيائية والكيميائية والاس الهيدروجيني ومحتوى الماء وقياس تركيز العناصر الثقيلة Cd ، Mn ، Cr ، Ni ، Pb و Cu في ثمانية عينات من حبوب اللقاح من اماكن متفرقة من الاردن ، فوجدوا ان مكونات حبوب اللقاح وخواصها تختلف من منطقة الى اخرى وحسب نوع النبات السائد ووجدوا ان تركيزات Cd و Pb عالية في حبوب اللقاح. وفي دراسة اجراها (1) حول حبوب اللقاح المستخلصة من عينات العسل وجد ان تركيز الحديد كان عاليا تبعه الرصاص ثم النحاس. ونظرا لتلوث البيئة العراقية بالعناصر الثقيلة نتيجة الحروب واستعمال الكادميوم والرصاص والزنك والنحاس في الصناعة واستعمال المبيدات الكيمياوية بشكل غير منتظم واستعمال المواد المشعة ومناطق حرق القمامة. فقد هدفت الدراسة الى جمع عينات من حبوب اللقاح من عدة محافظات للكشف عن مدى التلوث بعناصر Cd ، Pb ، Cu ، Ni ، Zn وتحديد نسبتها .

المواد وطرق العمل

جمع العينات:
جمع سبعة عشر عينة من حبوب اللقاح المجموعة من قبل النحل داخل الخلية في انابيب بلاستيكية معقمة من مناحل من المحافظات الوسطى (بغداد وبابل وواسط والنجف) والجنوب (ميسان والبصرة) والشمال (كركوك) اضافة الى حبوب لقاح مستوردة (صيني وتركي) للفترة من 4/1 – 2013/10/1 ثم نقلت الى مختبر فحص الاغذية في دائرة البيئة والمياه التابعة لوزارة العلوم والتكنولوجيا ووضعت في المجمدة لحين العمل.

تحضير العينات وقياس العناصر الثقيلة:
وزن 5 غم من كل عينة من عينات حبوب اللقاح ووضعت في جفنة زجاجية ثم جففت النماذج على درجة 150 سليلزية ووضعت الجفنات في فرن حراري بدرجة 550 سليلزية حتى يصبح الرماد ابيض ثم بردت الجفنات واضيف 5 مل من محلول اساس مكون من حامض الهيدروكلوميك 200 مل وحامض النتريك 150 مل لاذابة الراسب الابيض ورشح بورق ترشيح Whatman No. (1) بقنينة حجمية 10 مل ثم اكمل الحجم بالماء المقطر الى 10 مل مع الرج لتجانس المحلول وتصبح العينات المحلية والمستوردة جاهزة لقياس العناصر الثقيلة بوساطة جهاز الامتصاص الذري (Atomic Absorption Spectrometry210/211 VGP(USA وعلى طول موجي تختلف باختلاف العناصر 217 nm Pb ، 228.9 nm Cd ، 213.9 nm Zn ، 324.8 nm Cu ، 232 nm Ni ، (4 ، 10).

التحليل الاحصائي:
استعمل البرنامج SAS– Statistical Analysis System (11) في التحليل الاحصائي لدراسة تأثير العينات المدروسة في الصفات ألمختلفة وقورنت الفروق المعنوية بين المتوسطات باستخدام اختبار أقل فرق معنوي (LSD).

النتائج والمناقشة

تقدير الرصاص في عينات حبوب اللقاح:
أوضحت النتائج حسب جدول رقم (1) أن جميع عينات حبوب اللقاح ملوثة بتراكيز مختلفة من عنصر الرصاص الذي يعد من العناصر الثقيلة السامة وغير موجودة في تركيب حبوب اللقاح وله تأثير على الجهاز العصبي والكلي (10). سجل أعلى تركيز في وسط العراق حيث بلغ 0.535 ملغم / كغم . اما في الجنوب والشمال بلغ اقل تركيز 0.04 ملغم / كغم ، اما حبوب اللقاح المستوردة والموجودة في الاسواق المحلية فقد بلغ اعلى تركيز في حبوب اللقاح التركية 0.215 ملغم / كغم واقل تركيز في العسل الصيني 0.04ملغم / كغم وفق دستور الاغذية (12) ، أن تركيز الرصاص المسموح به 0.1 ملغم / كغم وبمقارنة النتائج نجد ان جميع العينات ضمن الحد المسموح به دوليا. شكل رقم (1).
نستخلص من النتائج ان حبوب اللقاح تكشف عن تلوث الرصاص هو الملوث الرئيسي للهواء نتيجة انبعاثه من عادمات السيارات وقرب المراعي التي يتغذى عليها النحل من المصانع ومناطق حرق القمامة (13). اما تلوث حبوب اللقاح المستوردة قد يعود الى المنشأ الذي جمع من النحل.
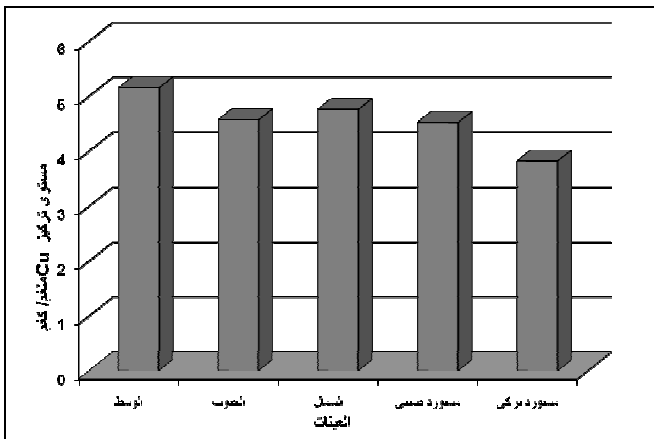
جدول رقم (1): تراكيز بعض العناصر الثقيلة في حبوب اللقاح في العينات المدروسة

الزنك (Zn)	الكادميوم (Cd)	النحاس (Cu)	النيكل (Ni)	الرصاص (Pb)	العينة
6.18	0.012	5.153	2.583	0.535	الوسط
5.37	0.016	4.57	2.81	0.04	الجنوب
4.04	0.010	4.76	0.68	0.040	الشمال
3.84	0.025	4.52	0.455	0.04	مستورد صيني
3.82	0.010	3.82	0.405	0.215	مستورد تركي
* 2.534	* 0.014	NS 3.138	* 1.792	NS 1.045	قيمة LSD

(P<0.05) *

شكل رقم (1): تقدير تركيز التلوث بعنصر الرصاص (Pb) بعينات حبوب اللقاح.

تقدير النحاس في عينات حبوب اللقاح:

أوضحت النتائج في جدول (1) وشكل (3) وجود تراكيز مختلفة من عنصر النحاس الذي يعد من العناصر الموجودة فـي حبـوب اللقاح. سجل اعلى تركيز في المحافظات الوسطى، إذ بلغ 5.153 ملغم / كغم واقل تركيز في الجنوب والشمال اذ بلغ على التـوالي 4.57 ، 4.76 ملغم / كغم ، اما العسل الصيني المـستورد فكـان تركيزه 4.52 ملغم / كغم والعسل التركي بتركيز 82..3 ملغم / كغم بينت نتائج التحليل الاحصائي عن عدم وجود فروق معنويـة في العينات اذ بلغت قيمة LSD (3.138) وبمقارنة النتـائج مـع التراكيز وفق دستور الاغذية (12) 0.15 ملغم / كغم المسموح به ، فنجد 2 عينة اعلى من الحد المسموح به وان من اهـم اسـباب التلوث بالنحاس استعماله في المبيدات والاسمدة الزراعية.
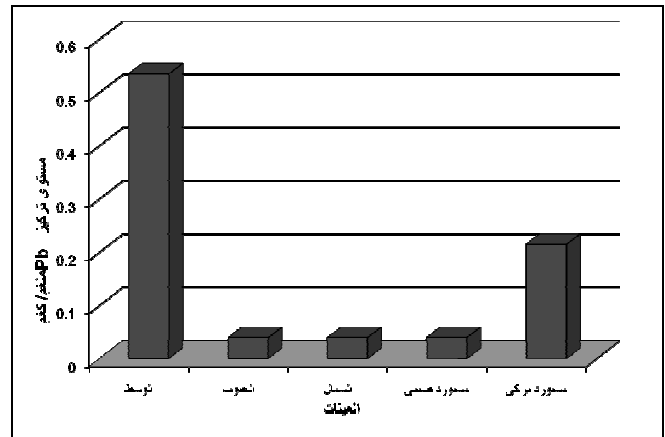

شكل رقم (3): تقدير تركيز التلوث بعنصر النحاس (Cu) بعينات حبوب اللقاح

تقدير النيكل في عينات حبوب اللقاح:

بينت النتائج في جدول (1) وشكل (4) وجود تراكيز مختلفة مـن عنصر النيكل الذي يعد من العناصر الموجودة في حبوب اللقاح ويتراوح تركيزه بين (2.81-0.68) ملغم / كغم ، سـجل اعلـى تركيز في محافظات الجنوب اذ بلغ 2.81 ملغم / كغم واقل تركيز في الشمال 0.62 ملغم / كغم ثم الوسط بلغ تركيزه 2.583 ملغم / كغم اما العسل المستورد فكان تركيـز اعلى للعـسل المـستورد الصيني 0.455 ملغم / كغم واقل تركيـز فـي العـسل التركـي 0.0405 ملغم / كغم ، اوضحت نتائج التحليل الاحصائي وجـود فروق معنويـة بـين العينـات اذ بلغـت قيمـة LSD (1.792) وبمقارنة النتائج مع ما مسموح به دولياً وفق دستور الاغذية (12) (1.9-0.3) ملغم / كغم نجد ان جميع العينات اعلى من الحـدود المسموح به دولياً.

نستنتج ان المعادن ومنها النيكل الموجودة في حبوب اللقاح تختلف باختلاف انواع النباتات وباختلاف المناطق وعامل الجفـاف وان التلوث بهذا العنصر نتيجة استعماله في الصناعات ومنها صـناعة البطاريات اضافة الى مناطق حرق القمامة (14).
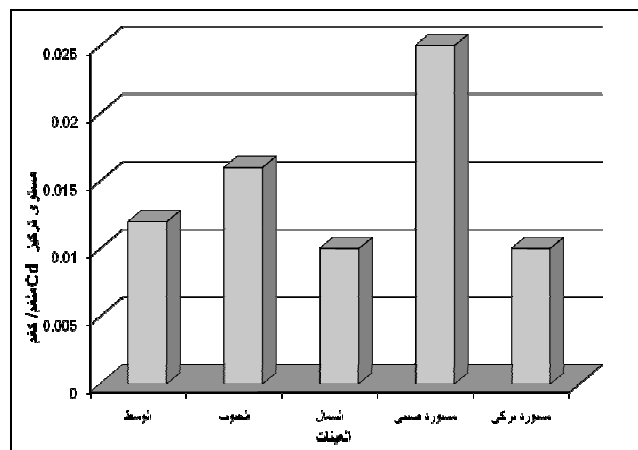
تقدير الكادميوم في عينات حبوب اللقاح:

أظهرت النتائج جدول (1) وشكل (2) ان جميع العينات المدروسة ملوثة بتراكيز مختلفة من الكادميوم الذي يعد من العناصر الثقيلـة السامة وغير موجود في تركيب حبوب اللقاح ويسبب تثبيط لنشاط عدد كبير من الانزيمات (10)، حيث سجل أعلـى تركيـز فـي محافظات الجنوب اذ بلغ 0.016 ملغـم / كغـم امـا محافظـات المناطق الوسطى فبلغ التركيز 0.012 ملغم / كغم واقل تركيز في الشمال 0.010 ملغم / كغم اما العسل المستورد الـصيني سـجل اعلى تركيز 0.025 ملغم / كغم اما العسل التركي سجل تركيـز 0.01 ملغم / كغم ، وقد بينت نتائج التحليل الاحصائي عن وجـود فروق معنوية بين العينات اذ كانت قيمة LSD هـي 0.014 . ان تركيز الكادميوم ضمن دستور الاغذية (12) 0.05 ملغم / كغم لذا فان جميع العينات ضمن الحدود المسموح بها دولياً.

نستنتج من النتائج ان حبوب اللقاح كاشف عن التلوث بالكـادميوم الذي ينتقل عن طريق التربة نتيجة قرب المناحل مـن المـصانع ومناطق حرق القمامة واستعمال المبيدات بشكل عشوائي ومكثـف (13) . كما ان تلوث حبوب اللقاح المستوردة قد يعود الى المنـشأ الذي جمعت منه.

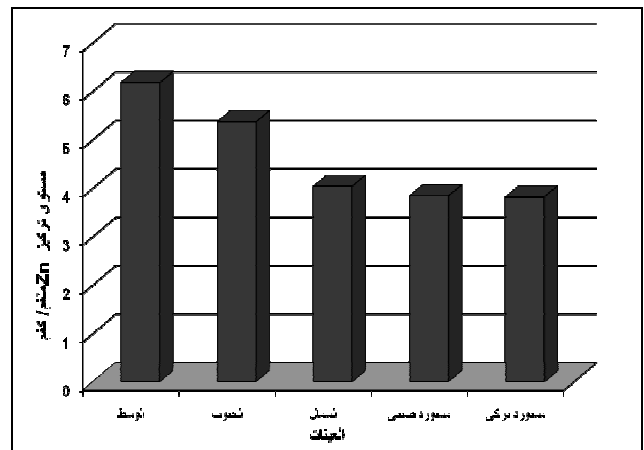شكل رقم (2): تقدير تركيز التلوث بعنصر الكادميوم (Cd) بعينات حبوب اللقاح.

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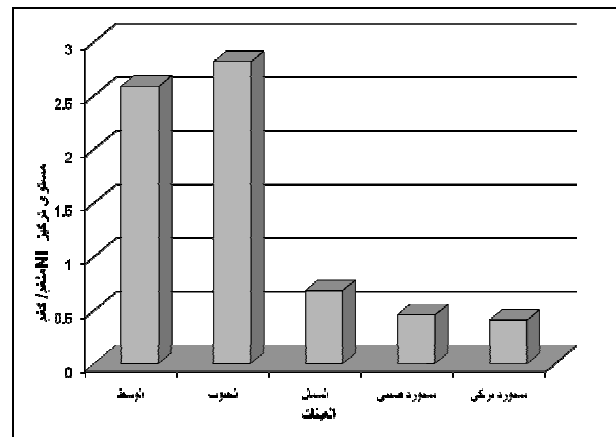
شكل رقم (4): تقدير تركيز التلوث بعنصر الكادميوم (Ni) بعينات حبوب اللقاح.

تقدير الزنك في عينات حبوب اللقاح:

يعد الزنك احد العناصر الموجودة في حبوب اللقـــاح ، واظهـــرت نتائج جدول (1) ان اعلى تركيز للزنك في المحافظات الوسطى اذ بلغ 6.18 ملغم / كغم ثم الجنوب بلغ 5.37 ملغـــم / كغـــم واقـــل تركيز في محافظات الشمال 4.04 ملغم / كغم اما اعلـــى تركيـــز كان في حبوب اللقاح الصيني المستورد 3.82 ملغم / كغم واقـــل تركيز في التركي 3.82 ملغم / كغم (شكل رقم 5)، كمـــا بينـــت نتائج التحليل الاحصائي الى وجود فروق معنوية في العينـــات اذ بلغت قيمة LSD (2.534) وبمقارنة النتائج مع ما مسموح به وفق دستور الاغذية (12) 5 ملغم / كغم نجد ان عينات المحافظـــات الوسطى اعلى من الحد المسموح استعماله في الصناعات المعدنية وصناعة البطاريات به (14).

شكل رقم (5): تقدير تركيز التلوث بعنصر الزنك (Zn) بعينات حبوب اللقاح.

الاستنتاجـــــــات

بناء على نتائج الدراسة فان جميع عينات حبوب اللقـــاح المحليـــة والمستوردة ملوثة بتراكيز مختلفة من العناصر الثقيلة (Zn, Ni, Cu, Cd, Pb) وتعد حبوب اللقاح المجموعة من قبل شغالات نحل العسل كشفاً عن التلوث البيئي بالعناصر الثقيلة.

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The Results section should be in logical order presenting the experimental results. Please do not include any interpretations, inferences, arguments or speculations in this section.

4. Discussion

The authors should interpret their results clearly and suggest what they might mean in a larger context. Please do not repeat the information provided in the Results section.

Acknowledgements

Assistance received from funding agencies and colleagues should be acknowledged in this section.

References

Published or "in press" articles may be included in the reference list. Unpublished studies should be referred to as such or as a personal communication in the text. Citations of references should be between brackets, e.g. (1,3,5-7). The lists of references, tables or figures should be numbered consecutively staring from 1. The references should be contain the last names and initials of up to four authors, year of publication, title of the paper, and the title of the journal. These should be followed by the volume and page numbers. References to books should include the title of the book, the year of publication between brackets, the publishing company and the place of publication. _Some examples are given below._

Smith PF.; Patel KR. and Al-Shammari AJN. (1980). An Alde hydro-Phosphoglycolipid from Acholeplasma granularum. Biochem.Biophys. Acta 617: 419-429

Sambrook J. and Russell DW. (2001). Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

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Example

Department of Health: The Interdepartmental Working Group on Tuberculosis 1999. The Prevention and Control of Tuberc1ilosis in the United Kingdom [Online] [accessed 2000 September]. Available from URL http://www.doh.gov.ukltbguide1.htm

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